Making Sense of G Proteins: Genetic analysis of sensory G protein signaling in the nematode *C. elegans*

Making Sense of G Proteins: Genetic analysis of sensory G protein signaling in the nematode *C. elegans*

Zin in G eiwitten:
Genetische analyse van G eiwit signaaltransductie
betrokken bij perceptie in de nematode *C. elegans*

Proefschrift ter verkrijging van de graad van doctor aan de Erasmus Universiteit Rotterdam op gezag van de rector magnificus Prof.dr. S.W.J. Lamberts en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
woensdag 5 oktober 2005 om 15.45 uur door

Willem Johannes Lans

geboren te Rotterdam

Promotiecommissie

Promotor:	Prof.dr. F.G. Grosveld
Overige leden:	Prof.dr. C.P. Verrijzer Prof.dr. J.H.J. Hoeijmakers Dr.ir. N.J. Galjart
Copromotor:	Dr. G. Jansen

Dit proefschrift kwam tot stand binnen de vakgroep Celbiologie en Genetica van de faculteit der Geneeskunde en Gezondheidswetenschappen van de Erasmus Universiteit Rotterdam. De vakgroep maakt deel uit van het Medisch Genetisch Centrum Zuid-West Nederland. Het onderzoek is financieel ondersteund door het Center for Biomedical Genetics, de Koninklijke Nederlandse Akademie van Wetenschappen en de Nederlandse Organisatie voor Wetenschappelijk Onderzoek.

if I have prophecy and know all the secrets and all the knowledge,
and if I have all the faith so as to remove mountains,
and have not love, I am nothing.
(Paul and Sosthenes)

voor Judith en mijn ouders

Contents

Aim of the thesis

Life is impossible without the ability to interact with the environment. Therefore, perception of the environment is essential for every organism. It enables an organism to find food and potential mates and to avoid harmful environmental conditions and predators. Furthermore, organisms adjust their development and behavior in response to environmental changes such that they are optimally adapted to survive. Hence, the study of the molecular pathways that govern sensory signaling is of major importance.

Multiple forms of sensory perception exist in all organisms, such as vision, hearing, smell, taste and touch. Some senses may appear to be more important than others, but this is not necessarily true. For instance, to humans the sense of smell would seem to be less important, but its significance is evident from the fact that as much as 4% of the genome of many higher eukaryotes encodes for olfactory proteins (Firestein 2001). In the last decades, part of the molecular mechanisms that underlie sensory perception has been elucidated. Still, most mechanisms are far from being understood. Much of what we know shows that sensory signaling is enormously complex. Sensory systems must be highly specialized to cope with the various stimuli that an organism encounters. For instance, the olfactory system must be able to detect and discriminate between countless related or unrelated odorants. Not only the odorant itself, but also its quantitative and temporal features are informative. High odorant concentrations that are continually present must be ignored. In addition, the appropriate response must be initiated, whether it be a behavioral or developmental change.

The nematode *C. elegans* is an excellent model to study the molecular mechanisms involved in sensory signaling and the effects of sensory perception on behavior and development. *C. elegans* can smell, taste and feel, but cannot see and hear. In many ways sensory signal transduction in *C. elegans* resembles that in higher eukaryotes. For example, a mammalian olfactory receptor can be functionally expressed in *C. elegans* and a *C. elegans* olfactory receptor can be functionally expressed in mammalian cells (Zhang et al. 1997; Milani et al. 2002). Furthermore, the mammalian cation channel TRPV4, which is thought to sense osmotic pressure, can substitute for the *C. elegans* TRPV-related channel OSM-9 in the perception of osmotic strength (Liedtke et al. 2003). These findings show that similar molecules and mechanisms underlie sensory signal transduction in diverse organisms such as nematodes and mammals.

Among the key molecules involved in sensory perception are G proteins, which act in every cell to activate a cascade of signaling molecules in response to certain environmental cues. In this thesis, several studies on the role of G proteins in the sensory system of *C. elegans* are described. First, in Chapter 1, a brief overview of the biology of *C. elegans* and of G protein signaling in general and in *C. elegans* is presented. Next, in Chapter 2, the sensory system of *C. elegans* is discussed in more (molecular) detail. In Chapter 3, the impact of sensory signaling on the regulation of dauer formation and longevity is discussed. Chapter 4 deals

with the role of G protein signaling in the detection of attractive odorants by *C. elegans*. Data is presented which indicate that olfaction in *C. elegans* is regulated by a complex signaling network involving five G proteins. In Chapter 5, the regulation of olfactory receptor gene expression by G proteins is described. Both cell autonomous as well as non-cell autonomous G protein signals regulate *str-2* receptor gene expression, in cooperation with Ca^{2+}/MAPK signaling molecules. Chapter 6 shows that G protein signaling in the sensory neurons also modulates longevity in *C. elegans.* Finally, in Chapter 7, future directions are provided.

Chapter 1

C. elegans as a model system to study G protein signaling

1. *C. elegans* as a model system to study G protein signaling

1.1 A brief introduction to the biology of *C. elegans*

The 1 mm large soil nematode *C. elegans* (Figure 1A, B) was first used by Sydney Brenner to study the genetics of development and behavior (Brenner 1974). Since then, extensive research using the animal has provided many valuable insights into the molecular mechanisms that govern various biological processes in *C. elegans*, but also in other organisms (Riddle et al. 1997). In the laboratory, *C. elegans* is cultured on Petri dishes containing buffered agar and OP50, a uracil biosynthesis defective *E. coli* strain that serves as food. The standard *C. elegans* strain, referred to as wild type, is a natural isolate from Bristol in England. It has been cultured in the laboratory for decades and is considered to be homozygous for all genes. *C. elegans* develops through four larval stages (L1-4), in three days at 20°C, to become either a self fertilizing hermaphrodite or a male. The hermaphrodite lays about 300-350 eggs and can live for approximately 2 weeks. Males are only found in 0.05% of a wild type population. In addition, under adverse environmental conditions, *C. elegans* L2 larvae can develop into an alternative third larval stage, which is called dauer. Dauer larvae are resistant to harsh conditions and show an increased life span. When environmental conditions improve, dauer larvae develop through the L4 stage to become normal adults. In this way, *C. elegans* can postpone reproduction until it encounters a well suited environment.

The *C. elegans* hermaphrodite consists of 959 somatic cells. These form organs and tissues like the cuticle, the muscles, the pharynx, the gut, the reproductive and the nervous system. Of all cells, the complete embryonic lineage, which is mostly invariant, has been determined (Sulston et al. 1983; Schnabel et al. 1997). Furthermore, the positions, morphologies, synapses and gap junctions of all 302 neurons have been described (White et al. 1986, 1988). Many neurons, including the chemosensory neurons (Chapter 2), are located around the pharynx where their processes form a ring called the nerve ring. The extensive knowledge of the cell lineage and neural wiring is a major advantage in understanding how the function of a certain gene in a particular cell affects the biology of the entire animal. This makes *C. elegans* very well suited for developmental and behavioral studies.

Above all, *C. elegans* is an ideal genetic model system. Firstly, its complete genome, consisting of five pairs of autosomal and one pair of sex chromosomes, has been sequenced and annotated (The *C. elegans* Sequencing Consortium, 1998) and is easily accessible and searchable via internet (www.wormbase.com). Secondly, due to the short generation time, self fertilization and small size of *C. elegans,* forward and reverse genetic screens are fast, easy, cheap and can be easily scaled up. Thirdly, *C. elegans* is susceptible to manipulation using a range of genetic techniques. Germ line transformation is easy and fast, generating transgenic animals within a few days (Mello et al. 1991). Mutations in genes can be obtained using chemical mutagenesis or transposons and, via targeted selected gene inactivation, mutant libraries covering the whole genome can be established

(Zwaal et al. 1993; Jansen et al. 1997; Liu et al. 1999). Furthermore, most somatic cells are highly susceptible to RNAi, applied by injection or by feeding, enabling genome wide RNAi screens (Kamath et al. 2003). Lastly, due to its transparency, *C. elegans* is well suited for *in vivo* imaging studies.

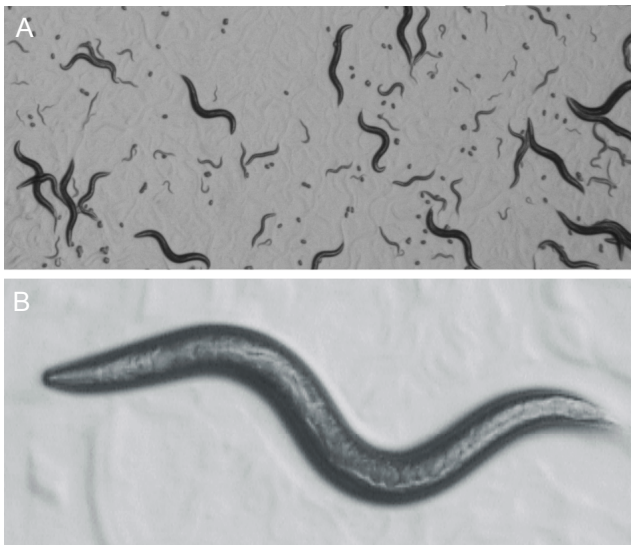

Figure 1. *C. elegans*
(A) *C. elegans* crawling on an agar plate.
(B) An adult *C. elegans* (400x magnification).

1.2 G protein signaling

Signal transduction via heterotrimeric G proteins is one of the most widely used mechanisms of eukaryotic cells to deal with signals from the environment (Hamm 1998; Neves et al. 2002). Many signals, ranging from hormones and neurotransmitters to light and odorants, activate G protein coupled receptors (GPCRs), which signal to one or more G proteins to activate a downstream cascade of effectors. G proteins function in many different physiological processes, controlling cell growth, differentiation, gene expression, sensory signaling, learning and mitosis (Wettschurek et al. 2004; Hampoelz and Knoblich 2004). It is therefore not surprising that G protein signaling has been implicated in a variety of diseases, including cancer (Spiegel et al. 1996; Marinissen and Gutkind 2001).

Heterotrimeric G proteins consist of a Gα, a Gβ and a Gγ subunit (Hamm 1998). In the inactive form, the three subunits are tightly associated and bound to the plasma membrane. A GDP molecule is bound to the Gα subunit. Activation of a G protein occurs by an activated GPCR, which catalyzes the exchange of GDP for GTP. Additionally, a G protein might be activated independently of membrane bound GPCRs (Gotta and Ahringer 2001; Schaefer et al. 2001; Gotta et al. 2003; see below). Upon binding of a GTP molecule, the Gα subunit can activate multiple downstream effectors, like ion channels and enzymes that generate second messengers. Traditionally, the activated Gα subunit is thought to dissociate from the Gβ and Gγ subunit, but there are indications that the G protein complex only

adopts a more open conformation (Rebois et al. 1997; Klein et al. 2000). The Gβ and Gγ subunits form a dimeric complex that can also activate multiple target molecules. In this way, a signal is amplified by the activation of a cascade of signaling molecules. Eventually, signaling ceases when the GTP on the Gα subunit is hydrolyzed to GDP, due to the slow intrinsic GTPase activity of the Gα subunit. Gα-GDP then reassociates with the Gβγ complex.

G protein signaling in mammals

In mammals, genes encoding 16 Gα, 5 Gβ and 12 Gγ subunits have been identified (Hurowitz et al. 2000; Schwindinger and Robishaw 2001; Wettschurek et al. 2004). These subunits can combine to form different G protein complexes, with specific functions, although not all combinations are found *in vivo.* Gα subunits are subdivided in four different classes, based on sequence similarity and function (Neves et al. 2002). The first class, G_s, consists of $G\alpha_s$ and G_{olf}, which both stimulate adenylyl cyclases to generate the second messenger cAMP. $G\alpha_s$ is ubiquitously expressed and regulates many physiological processes including insulin sensitivity (Yu et al. 1998, 2000). G_{olf} functions in the olfactory epithelium to mediate odorant detection (Belluscio et al. 1998). The second class, $G_{i/o}$, consists of eight Gα subunits. Its members inhibit adenylyl cyclase activity and activate cGMP phosphodiesterases to downregulate cGMP levels. Well known examples are $G\alpha_{t\text{-}r}$ and $G\alpha_{gust}$, also called transducin and gustducin. $G\alpha_{t\text{-}r}$ functions in phototransduction in the rod photoreceptor cells (Lerea et al. 1986; Calvert et al. 2000). $G\alpha_{t\text{-}r}$ is also expressed in the taste cells, together with $G\alpha_{gust}$. $G\alpha_{gust}$, and possibly also $G\alpha_{t\text{-}r}$, mediates the perception of bitter, sweet and umami taste (Wong et al. 1996; He et al. 2004). The third class, consisting of four Gα subunits, is called the G_q class. Members of this class act by stimulating the production of 1,4,5-triphosphate and the increase of intracellular Ca^{2+} levels through phospholipase Cβ. A well known example is the ubiquitously expressed $G\alpha_q$, which regulates diverse processes such as platelet aggregation in the hematopoietic system and Purkinje cell innervation in the nervous system (Offermanns 1997a, 1997b). The fourth class, G_{12}, consists of $G\alpha_{12}$ and $G\alpha_{13}$, whose expression patterns overlap. Both Gα subunits are involved in embryonic development (Offermanns et al. 1997c; Gu et al. 2002). Members of the G_{12} class can activate a range of effectors, including small G proteins (Hart et al. 1998; Kozasa et al. 1998; Jiang et al. 1998).

Like the Gα subunits, the Gβγ dimers can activate multiple effector molecules. Of the 5 Gβ subunits found in mammals, the $G\beta_1$, $G\beta_2$, $G\beta_3$ and $G\beta_4$ subunits are highly homologous and widely expressed. The fifth, $G\beta_5$, differs significantly from the other four and is predominantly expressed in the central nervous system and retinal photoreceptor cells (Watson et al. 1994, 1996). The 12 Gγ subunits are more diverse than the Gβ subunits and can be classified according to sequence similarity or to posttranslational modifications. Most Gγ subunits are widely expressed, but some show a restricted expression pattern. For example, the

$G\gamma_1$ subunit is expressed in rod photoreceptor cells, where it probably functions together with transducin in phototransduction (Kisselev and Gautam 1993). Furthermore, $G\gamma_{13}$ is expressed in taste cells and functions together with gustducin and probably $G\beta_1$ and/or $G\beta_3$ in bitter perception (Huang et al. 1999). The proteins that are regulated by $G\beta\gamma$ dimers include G protein coupled Receptor Kinases (GRKs), adenylyl cyclases, PLCβ, inward rectifier G protein gated potassium channels (GIRKs), N and P/Q type Ca^{2+} channels, MAPK pathways and PI_3K (Clapham and Neer 1997; Schwindinger and Robishaw 2001). The $G\beta\gamma$ dimer also targets the $G\alpha$ subunit to the plasma membrane (Evanko et al. 2000, 2001).

Signaling via $G\alpha$ subunits and $G\beta\gamma$ dimers often interacts with other signaling pathways. Much research focuses on the regulation of the JNK, ERK and p38 MAPK pathways by G proteins. These MAPK pathways are cascades of serine/threonine protein kinases which transduce a large variety of extracellular signals to the nucleus, thereby generating a cellular response. Through these pathways, GPCRs can signal to the nucleus and regulate cell proliferation, differentiation and apoptosis (Marinissen and Goodkind 2001; Belcheva and Coscia 2002). G proteins have been found to stimulate all three MAPK pathways, but the precise mechanisms are still not completely understood. In addition, G proteins might interact with or even function in the Wnt signaling pathway, which is involved in cell polarity and intercellular signaling during development. Recently, a role for the *Drosophila* $G\alpha_o$ was suggested in transducing signals from the Frizzled receptor, a principal protein of the Wnt pathway (Katanaev et al. 2005). However, whether this role is direct or indirect, is still under debate (Bejsovec 2005).

Mechanisms of regulating G protein activity

The activity of G proteins is not only regulated by GPCRs, but can also be regulated by many other proteins. For example, the Regulators of G protein signaling (RGS proteins) contain an RGS domain which can accelerate the intrinsic GTPase activity of $G\alpha$ subunits and thus inhibit $G\alpha$ signaling (Koelle and Horvitz 1996; Watson et al. 1996). Many RGS proteins harbor additional domains, like $G\gamma$ like (GGL) domains and Goloco domains, through which they can interact with G proteins. Because of their sequence similarity to $G\gamma$ subunits, GGL domains enable proteins to bind $G\beta$ subunits (Snow et al. 1998). In this way, GGL domain containing proteins can bind and inhibit $G\alpha$ subunits. For example, the mammalian $G\beta_5$ associates with the GGL domain containing RGS9 *in vivo* to regulate the activity of transducin in photoreceptor cells (Makino et al. 1999; Chen et al. 2003). Furthermore, in *C. elegans*, the $G\beta_5$ homologue GPB-2 regulates the activity of the $G\alpha_q$ homologue EGL-30, through associating with the GGL domain containing RGS proteins EAT-16 and EGL-10 (Chase et al. 2001; van der Linden et al. 2001). Goloco domains facilitate binding of proteins to inactive, GDP bound $G\alpha$ subunits, thus preventing binding to $G\beta\gamma$ dimers (Kimple et al. 2002). In this manner, $G\alpha$ subunit activation is inhibited while $G\beta\gamma$ dimers are still active. This kind of regulation of $G\alpha$ subunits is thought to occur during mitotic spindle orientation in

different organisms (Schaefer et al. 2000, 2001; Du et al. 2001; Gotta et al. 2003; Srinivasan et al. 2003). In this process, also the Ric-8/Synembryn regulator of G protein signaling functions. Like GPCRs, this protein stimulates Gα function by acting as guanine nucleotide exchange factor, stimulating the exchange of GDP for GTP on Gα subunits (Miller and Rand 2000; Tall et al. 2003; Afshar et al. 2004; Couwenbergs et al. 2004). In this way, G proteins might be activated independently of GPCRs.

Not only the activity of Gα subunits is under control of regulatory proteins, also the activity of Gβγ dimers and GPCRs can be extensively regulated. For instance, the function of Gβγ dimers is inhibited by binding to proteins containing a pleckstrin homology domain, like G protein coupled Receptor Kinases (GRKs) (Koch et al. 1993, 1994). In addition, the interaction of Gβγ dimers and GRKs targets GRKs to the membrane, where they inhibit signal transduction via GPCRs (Daaka et al. 1997; Pitcher et al. 1998). This mechanism underlies receptor desensitization and adaptation following prolonged exposure to a signal. Some GRKs also contain an RGS domain through which they can bind and inhibit Gα subunits (Carman et al. 1999; Day et al. 2003).

Research of the last two decades has shown that G protein signaling entails a network of many interacting proteins. GPCRs might activate multiple different G protein complexes that activate different effector molecules. Furthermore, the activity of GPCRs, G proteins and their effectors is subject to regulation and there is extensive cross talk with other signaling pathways. Therefore, it is of interest to study how specificity and cross talk of G protein signaling pathways is regulated. *C. elegans* serves as an excellent model system to study the activity and interaction of multiple G proteins simultaneously *in vivo*. As described below, *C. elegans* contains multiple G proteins that function in complex networks which control locomotion, egg laying and chemosensory signaling.

G protein signaling in C. elegans

The genome of *C. elegans* encodes 21 Gα, 2 Gβ and 2 Gγ subunits (Table 1). Using forward and reverse genetic approaches, a functional characterization of most subunits has been performed. Each of the mammalian classes of Gα subunits is represented by one member in *C. elegans*: *gsa-1* for G_s, *goa-1* for $G_{i/o}$, *egl-30* for G_q and *gpa-12* for G_{12} (Lochrie et al. 1991; Brundage et al. 1996; Park et al. 1997; Jansen et al. 1999). These conserved Gα subunits are expressed widely in muscle and neuronal cells and mostly regulate general behavior. GSA-1 is essential for survival, since *gsa-1* loss-of-function mutants developmentally arrest as L1 larvae (Korswagen et al. 1997). Furthermore, GSA-1 is involved in regulating locomotion, egg laying, necrotic neuronal cell death and several other developmental processes (Korswagen et al. 1997; Berger et al. 1998; Simmer et al. 2003; Schade et al. 2005). GOA-1 and EGL-30 have opposite functions in regulating locomotion, egg laying and feeding (Segalat et al. 1995; Mendel et al. 1995; Brundage et al. 1996). Extensive genetic research has shown that GOA-1 and EGL-30 antagonize each other in a complex signaling network that regulates

acetylcholine release at the neuromuscular junctions that control locomotion (Koelle and Horvitz 1996; Hadju-Cronin et al. 1999; Nurrish et al. 1999; Lackner et al. 1999; Reiner et al. 1999; Miller et al. 1999, 2000; Robatzek and Thomas 2000; van der Linden et al. 2001; Chase et al. 2001; Robatzek et al. 2001; Bandyopadhyay et al. 2002; Lee et al. 2004). A similar network probably also regulates egg laying (Dong et al. 2000; Bastiani 2003) and is involved in plasticity of salt perception (Hukema et al. *submitted*; Chapter 2). Furthermore, GOA-1 and EGL-30 mediate the serotonin controlled migration of the descendants of Q neuroblasts (Kindt et al. 2002). GOA-1 also acts redundantly to GPA-16 in positioning the mitotic spindle during early cell divisions (Gotta and Ahringer 2001)

Table 1. G proteins of *C. elegans*

	expression pattern	**function**
Gα subunits		
GSA-1	ubiquitous	viability, locomotion, egg laying, neuronal cell death
GOA-1	ubiquitous	locomotion, egg laying, feeding, cell migration, mitotic spindle orientation
EGL-30	ubiquitous	locomotion, egg laying, feeding, cell migration, vulva development
GPA-1	sensory neurons (ADL, ASH, ASI, ASJ, PHA, PHB), male tail	taste, longevity
GPA-2	sensory neurons (AWC, PHA, PHB, IL1L, IL2L), OLL PVT, anal sphincter muscle, M1, M5, I5	pheromone detection, olfaction, receptor expression
GPA-3	sensory neurons (all except AWB and ADF), PVT, AIZ	taste, olfaction, nociception, pheromone detection, receptor expression
GPA-4	sensory neuron ASI	
GPA-5	sensory neurons (AWA, ADL)	olfaction, receptor expression
GPA-6	sensory neurons (AWA, AWB, ASH, ADL)	receptor expression
GPA-7	ubiquitous	egg laying, nociception
GPA-8	URX, AQR, PQR	
GPA-9	sensory neurons (ASJ, PHB), PVQ, pharynx muscle, spermatheca	longevity
GPA-10	sensory neurons (ADF, ASI, ASJ), ALN, CAN, LUA, spermatheca	
GPA-11	sensory neurons (ADL, ASH)	food/serotonin signal, longevity
GPA-12	pharyngeal muscle, hypodermis	feeding, locomotion, embryogenesis, touch sensitivity
GPA-13	sensory neurons (ADF, ASH, AWC, PHA, PHB)	olfaction, receptor expression
GPA-14	sensory neurons (ASI, ASJ, ASH, ASK, PHA, PHB, ADE), ALA, AVA, CAN, DVA, PVQ, RIA,vulva muscle	
GPA-15	sensory neurons (ADL, ASH, ASK, PHA, PHB), distal tip cell, anchor cell, male specific neurons	
GPA-16	AVM, PDE, PLM BDU, PVC, RIP, pharynx, body wall muscle, vulva muscle	mitotic spindle orientation
GPA-17	intestine	
ODR-3	sensory neurons (AWA, AWB, AWC, ASH, ADF, PHA and/or PHB)	taste, olfaction, nociception, receptor expression, longevity
Gβ subunits		
GPB-1	ubiquitous	mitotic spindle orientation
GPB-2	ubiquitous	locomotion, egg laying
Gγ subunits		
GPC-1	sensory neurons (ADL, ASH, ASJ, ASI, AFD, AWB, PHB)	taste, longevity
GPC-2	ubiquitous	mitotic spindle orientation

and EGL-30 promotes vulva development under certain environmental conditions (Moghal et al. 2003). The G_{12} homologue GPA-12 controls feeding behavior, as overexpression of a constitutive active form of GPA-12 inhibits pharyngeal pumping, and may also be involved in locomotion, embryogenesis and touch sensitivity (van der Linden et al. 2003; Yau et al. 2003). It is expressed predominantly in pharyngeal muscle and hypodermal cells.

In addition to the conserved Gα subunits, *C. elegans* has 17 Gα subunits that do not belong to any of the mammalian classes of Gα subunits (*gpa-1* to *gpa-11, gpa-13* to *gpa-17* and *odr-3*; Table 1; Fino Silva and Plasterk 1990; Zwaal et al. 1997; Roayaie et al. 1998; Jansen et al. 1999; Cuppen et al. 2003; J. Burghoorn and G. Jansen, personal communication). 14 of these show specific expression in subsets of chemosensory neurons, suggesting that they have specific roles in sensory signaling (Jansen et al. 1999). Using target selected gene inactivation, mutants with loss-of-function alleles for all Gα subunits were isolated and characterized. This showed that many of them are involved in sensory signaling or the development of the sensory system (Jansen et al. 1999, 2002; Lans et al. 2004; Hukema et al. *submitted*; Chapters 2-7).

One of the Gβ subunits of *C. elegans*, *gpb-1*, resembles the mammalian $G\beta_{1-4}$ subunits and is ubiquitously expressed (van der Voorn et al. 1990; Zwaal et al. 1996). *gpb-1* loss-of-function mutations are embryonic lethal, due to defects in centrosome positioning during early cell divisions (Zwaal et al. 1996; Gotta and Ahringer 2001). The second Gβ subunit, *gpb-2,* is most similar to the mammalian $G\beta_5$ (van der Linden et al. 2001). GPB-2 is also widely expressed in neuronal and muscle tissues. It has been found to interact genetically with GOA-1 and EGL-30 in regulating egg laying, locomotion and pharyngeal pumping. Interestingly, GPB-2 probably regulates the activity of these genes by associating with the GGL-domain containing RGS proteins EGL-10 and EAT-16 and not by binding to Gγ subunits (van der Linden et al. 2001; Chase et al. 2001; Robatzek et al. 2001). The two Gγ subunits *gpc-1* and *gpc-2* do not strongly resemble any of the mammalian Gγ subunits. GPC-1 is specifically expressed in a subset of chemosensory neurons, like many of the Gα subunits, where it is involved in regulating the response to salts (Jansen et al. 2002; Chapter 2). GPC-2 is broadly expressed and associates with GPB-1 and GPA-16 and/or GOA-1 to regulate spindle orientation during embryonic development (Gotta and Ahringer 2001; Jansen et al. 2002).

The identification of the complete family of G proteins of *C. elegans* has provided a great opportunity to study G protein coupled signal transduction. This thesis focuses on the role that G proteins play in sensory signaling. In the following chapters, the mechanisms of sensory signaling and the functions of sensory perception in behavior and development of *C. elegans* are described. Subsequently, three comprehensive studies on the role of G proteins in sensory signaling are presented.

Chapter 2

Chemosensory signaling in *C. elegans*: olfaction and taste

2. Chemosensory signaling in *C. elegans*: olfaction and taste

2.1 Chemosensation in *C. elegans*

In order to survive in nature, *C. elegans* can detect and respond to many different environmental signals. These include a wide variety of volatile and nonvolatile chemicals, but also touch, osmotic strength, temperature and oxygen levels. When exposed to an attractive or repulsive chemical on an agar plate, *C. elegans* will move towards or away from that chemical. Using chemotaxis assays, laser ablation and genetic screens, the cells and many of the genes that *C. elegans* uses to explore its environment have been identified.

Structure and development of the amphid neurons

In *C. elegans*, 12 pairs of bilateral amphid neurons, located in the head of the animal (Figure 1, A-C), and 2 pairs of bilateral phasmid neurons, located in the tail, have been shown to be involved in the perception of the environment (Table 1; Bargmann and Horvitz 1990, 1991a, 1991b; Bargmann et al. 1993; Kaplan and Horvitz 1993; Mori and Ohshima 1995; Hilliard et al. 2002). Each amphid neuron has an axon, which contacts other neurons in the nerve ring, and a dendrite extending to tip of the nose, which ends in a sensory ciliary structure (Ward et al. 1975; Ware et al. 1975; White et al. 1986). The cilia of the ASE, ADF, ASG, ASH, ASI, ASJ, ASK and ADL amphid neurons are exposed to the environment through the amphid channel, which is formed by the glial like socket cell. Each of these

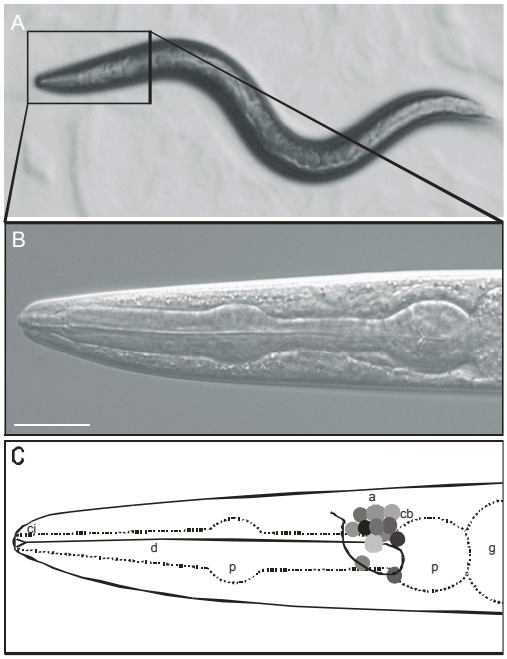

Figure 1. Amphid neurons of *C. elegans*
(A) An adult *C. elegans* (400x magnification).
(B) and (C) Head region of an adult *C. elegans* (1000x magnification). Between the first and second bulb of the pharynx (p), the cell bodies (cb) of the sensory amphid neurons are located. Each amphid neuron extends a dendrite (d) anteriorly, which ends in a cilium (ci) exposed to the environment, and an axon (a) which innervates the nerve ring. The gut (g) is also indicated.

cells has a single cilium, except ADL and ADF which have two cilia. The AWA, AWB, AWC and AFD neurons have elaborate cilia, which are not directly exposed to the environment. All sensory endings are enveloped and supported by the socket cell and another glial like cell called the sheath cell. In a similar manner, the phasmid neurons are exposed to the environment through ciliated dendrites. Due to differences in cilia morphology and position of the cell body, each amphid neuron can be easily recognized.

Table 1. Amphid and phasmid neurons

	neuron	sensitivity
olfaction	AWA	diacetyl, pyrazine, 2,4,5-trimethylthiazole
	AWC	benzaldehyde, butanone, isoamylalcohol, 2,3-pentanedione, 2,4,5-trimethylthiazole
taste	ASE	Na^+, Cl^-, K^+, cAMP, biotin, lysine, Cu^+, Cd^{2+}, protons
	ADF	dauer pheromone, Na^+, Cl^-, cAMP, biotin, protons
	ASG	dauer pheromone, Na^+, Cl^-, cAMP, biotin, lysine
	ASI	dauer pheromone, Na^+, Cl^-, cAMP, biotin, lysine
	ASJ	dauer pheromone
	ASK	lysine, protons
nociception	AWB	2-nonanone
	ADL	octanol, Cu^+, Cd^{2+}
	ASH	osmolarity, touch, odorants, heavy metals, protons and many other repellents
	PHA	SDS
	PHB	SDS
thermosensation	AFD	temperature

Function of the amphid and phasmid neurons in *C. elegans*. See text for details and references.

The amphid neurons arise from multiple, separate embryonic cell lineages. They are formed at different time points after approximately 270 minutes following the first cell cleavage (Sulston et al. 1983; Schnabel et al. 1997). Approximately 130 minutes later, they migrate towards the tip of the head where they form a rudimentary sensillum. As they migrate posteriorly to their final position, they lay down dendritic processes along the way. Finally, an axon is formed. Sensory neuron development requires specific organization of the cytoskeleton, specific (intraflagellar) transport and expression of sensory signaling genes. In addition, the morphology and gene expression of some sensory neurons can change in different developmental stages or in response to environmental signals (Albert and Riddle 1983; Peckol et al. 1999, 2001). Many genes have been identified that regulate the development and morphology of each neuron (Lanjuin and Sengupta 2004; Melkman and Sengupta 2004). These include nuclear hormone receptors and several types of homeodomain genes, which control the expression of a unique set

of signaling genes that defines the identity and function of each neuron. For instance, the nuclear hormone receptor ODR-7 is necessary for the expression of all AWA specific genes (Sengupta et al. 1994). Consequently, animals with a loss-of-function mutation in the *odr-7* gene show behavior defects associated with loss of AWA function. Each class of amphid and phasmid neurons develops into a left and a right member which share similar functions.

Thus far, only the left and right members of the AWC and ASE neuron pairs have been shown to be functionally different and to express different genes (Yu et al. 1997; Troemel et al. 1999; Wes and Bargmann et al. 2001; Pierce-Shimomura et al. 2001). The left ASE neuron (ASEL) expresses the guanylyl cyclases *gcy-6* and *gcy-7* and the right ASE neuron (ASER) expresses *gcy-5* (Yu et al. 1997). Several regulators of ASE asymmetry have been identified. These include the homeobox gene *cog-1* and the Groucho homologue *unc-37*, which act in ASER to repress the ASEL fate, and the PHD-finger protein *lin-49* and homeobox genes *lim-6* and *ceh-36*, which promote the expression of ASEL specific genes (Hobert et al. 1999; Pierce-Shimomura et al. 2001; Chang et al. 2003). In addition, a microRNA called *lsy-6*, has been identified that specifically represses *cog-1* expression (Johnston and Hobert 2003).

Unlike the ASE neurons, the fate of the left and right AWC neurons is not invariant, but randomly determined. The GPCR *str-2* is randomly expressed in either the left or the right AWC neuron (Troemel et al. 1999). In a screen for animals with defective asymmetric *str-2* expression, mutations in the α1 and α2 subunits of a N/P-type voltage-gated Ca^{2+} channel, called *unc-2* and *unc-36,* in the Ca^{2+}/calmodulin dependent protein kinase II (CaMKII) *unc-43* and in three new genes called *nsy-1, nsy-2* and *nsy-3* (for *n*eural *sy*mmetry) were isolated. *nsy-1*

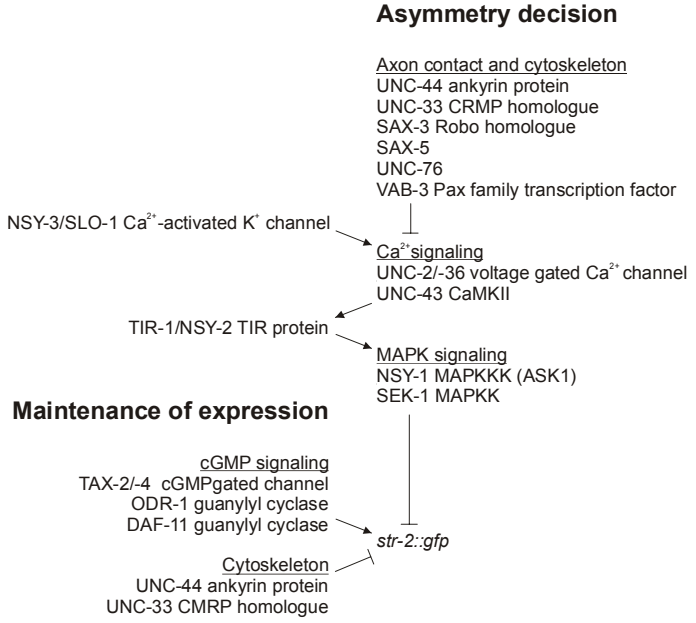

Figure 2.
Model for regulation of *str-2* repression in the AWC neurons
Early in development, *str-2* expression is repressed in one of the AWC neurons by lateral axon contact and a Ca^{2+}/MAPK signaling pathway.
During later stages, *str-2* expression is maintained by cGMP signaling. See text for details and references.

was shown to encode a homologue of the human ASK1 MAPKK kinase and *nsy-2* the Toll Interleukin-1 Receptor (TIR) protein TIR-1 (Sagasti et al. 2001; Chuang and Bargmann 2005). *nsy-3* is also known under the name *slo-1* and encodes a Ca^{2+} activated K^+ channel (Wang et al. 2001; Davies et al. 2003). Epistasis and cell specific rescue experiments have suggested that in the late embryo, *str-2* expression is suppressed in one of the AWC neurons by Ca^{2+} signaling, via the UNC-2/UNC-36 channel and the UNC-43 CaMKII, which activates a MAPK pathway consisting of the MAPKK kinase NSY-1, the MAPK kinase SEK-1 and possibly the p38 MAPK homologues PMK-1 and PMK-2 (Figure 2; Troemel et al. 1999; Sagasti et al. 2001; Tanaka-Hino et al. 2002). NSY-2/TIR-1 binds UNC-43 and probably also NSY-1 and localizes both proteins to the AWC synapses (Chuang and Bargmann 2005). The NSY-3/SLO-1 K^+ channel functions upstream of Ca^{2+} signaling (M. Vanhoven and C. Bargmann, personal communication). Interestingly, *str-2* is not expressed in either one of the AWC neurons when the other one is killed early in development, suggesting that communication between the AWC neurons is necessary to generate asymmetric *str-2* expression (Troemel et al. 1999). This is supported by the fact that mutations in genes involved in axon guidance also disrupt *str-2* asymmetry.

General properties of chemosensation and chemotaxis

Cell killing experiments using a laser have identified the functions of the amphid neurons (Table 1; see below). The AWA, AWB, AWC, ASH and ADL neurons detect volatile chemicals and the ASE, ASG, ASH, ASI, ASJ, ADF and ASK neurons nonvolatile chemicals (Bargmann and Horvitz 1990, 1991a, 1991b; Bargmann et al. 1993). In addition, the ASH neurons detect mechanical stimuli (Kaplan and Horvitz 1993) and the AFD neurons detect temperature changes (Mori and Ohshima 1995). In the cilia of each sensory neuron, the detection of signals is mediated by different molecular mechanisms (see below) and eventually leads to an increase in intracellular Ca^{2+} levels. Because the genome of *C. elegans* lacks voltage gated Na^+ channels (Bargmann 1998), other channels must generate the action potentials in response to Ca^{2+} increase. Whole cell patch clamp recordings of the ASE and several unidentified neurons have shown that the neurons fire by means of an outward K^+ current, which is activated by depolarization (Goodman et al. 1998; Pierce-Shimomura et al. 2001). In support of these results, it was found that the voltage gated K^+ channels *egl-2* and *kvs-1* and the K^+ channel associated MiRP related protein *mps-1* are expressed in several amphid neurons and involved in chemotaxis (Weinshenker et al. 1999; Bianchi et al. 2003).

The amphid neurons transmit environmental signals via classical neurotransmitters, including glutamate, dopamine and serotonin (Hart et al.1995; Maricq et al. 1995; Duerr et al. 1999; Sawin et al. 2000; Sze et al. 2000). They synapse onto several interneurons, like AIA, AIR, AIY, AIZ and PVQ, which in turn synapse onto other interneurons or directly onto motoneurons (White et al. 1986, 1988). Some amphid neurons also synapse onto other amphid neurons. Because all neural connections are known, the neural network involved in certain sensory

behaviors can be identified (Ishihara et al. 2002; Sze and Ruvkun 2003; Gray et al. 2005). In addition, some amphid neurons produce endocrine signals, such as TGFβ, insulins, FLP and NLP neuropeptides, which regulate development and behavior (Ren et al. 1996; Schackwitz et al. 1996; Nelson et al. 1998; Li et al. 1999; Nathoo et al. 2001; Pierce et al. 2001; Li et al. 2003; Kim and Li 2004).

Eventually, the integration of sensory information leads to behavior. Chemotaxis behavior results from typical exploration behavior, during which *C. elegans* randomly moves forward (run) and occasionally reverses (reversal) or turns around (omega turn). When the concentration of an attractive chemical increases, gradually or stepwise, the probability of reversals and omega turns decreases but that of runs increases, resulting in net movement in the direction of the chemical (Pierce-Shimomura et al. 1999; Miller et al. 2005). This behavior is consistent with the idea that *C. elegans* chemotaxes by comparing differences in the concentration of a chemical over time. In addition, *C. elegans,* compares sensory inputs from neurons in the head and tail (Hilliard et al. 2002).

GPCR expression

Sensory cues are detected by specialized receptors on the outer surface of the cell membrane. Most sensory cues, like odorants and tastants, are detected by GPCRs. In mammals, some GPCRs responsible for the detection of odorants, amino acids, sweet and bitter compounds have been identified (Scott 2004; Reed 2004). The detection of other cues, like salt and sour taste, is mediated by ion channels, including the family of Degenerin (Deg)/Epithelial Sodium Channel (ENaC) Na^+ channels, or by members of the Transient Receptor Potential (TRP) channel family (Lindemann 2001; Lyall et al. 2004, 2005). Furthermore, heat and cold or chemicals that produce a similar sensation, like capsaicin and menthol, and osmolarity are also detected by TRP channels (Clapham 2003). The genome of *C. elegans* encodes several hundreds of GPCRs, at least 11 TRP channel homologues and 22 degenerin channels (Bargmann 1998), suggesting that chemosensory signaling in *C. elegans* could be mediated by similar mechanisms as in higher organisms.

Most GPCRs of *C. elegans* are orphan receptors without clear homologues in other species. Analysis of the complete genome sequence of *C. elegans* has identified multiple chemoreceptor families, based on sequence similarity (Troemel et al. 1995, 1997; Bargmann 1998; Robertson 1998, 2000, 2001). Thus far, the only chemosensory receptor in *C. elegans* of which the ligand is known, is the high affinity GPCR for the odorant diacetyl, called ODR-10 (Sengupta et al. 1996, see below). Like ODR-10, many GPCRs are specifically expressed in one or more amphid neurons, at variable expression levels, and are localized to the cilia. Additionally, some receptors show hermaphrodite or male specific expression patterns. Probably many receptors are expressed by each neuron, enabling *C. elegans* to respond to multiple, sometimes unrelated chemicals, using only one or few cells. For a long time, this property was thought to distinguish the sensory neurons of *C. elegans* from those of other organisms, like *Drosophila* and

mammals, in which each neuron class was thought to express only one type of receptor. This is, however, difficult to demonstrate because of the large number of sensory neurons and receptors of most organisms. In addition, in *Drosophila* a neuron class that expresses two receptors has been described recently (Goldman et al. 2005).

Several genes have been identified that control receptor expression. These include homeobox genes, nuclear hormone receptors and other transcription factors that function in development to induce cell specific gene expression (Lanjuin and Sengupta 2004; Melkman and Sengupta 2004). Furthermore, these include many signaling genes. For instance, the candidate odorant receptor *str-2* is asymmetrically expressed in one of the two AWC neurons and weakly in the ASI neurons (Troemel et al. 1999). During embryonic development, its expression is controlled by Ca^{2+}, MAPK and axon guidance genes (Figure 2). During later stages, sensory signaling genes such as the guanylyl cyclases *odr-1* and *daf-11* and the cGMP gated cation channel subunits *tax-2* and *tax-4* are necessary to maintain *str-2* expression. All these genes function together with several G proteins in a signaling network, encompassing signals from multiple neurons, that regulates the expression of *str-2* in the AWC and ASI neurons. This network is extensively discussed in Chapter 5.

Other mechanisms exist that regulate receptor expression in response to developmental or environmental changes. For example, whereas *str-2* is strongly expressed in the AWC neurons and weakly in the ASI neurons in adult animals, it is strongly expressed in the ASI neurons but not in the AWC neurons in dauer animals (Peckol et al. 2001). Several genes involved in dauer development have been found to control receptor expression. Mutations in TGFβ signaling genes, which regulate entry into the dauer stage, and in the ser/thr kinase *kin-29* result in altered receptor expression patterns (Nolan et al. 2002; Lanjuin and Sengupta 2002). Furthermore, the expression of some receptors is under direct control of environmental signals. Expression of the receptors *srd-1, str-2* and *str-3* in the ASI neurons is repressed when *C. elegans* is exposed to dauer pheromone and *srd-1* expression is abolished in mutants that lack functional cilia (Peckol et al. 2001). Additionally, the same pathway that mediates olfaction in the AWA neurons (see below) also regulates expression of the ODR-10 receptor (Tobin et al. 2002; A. Kahn, E. Peckol and C.B., personal communication; H. Lans and G. Jansen, unpublished results).

2.2 Olfaction

Olfaction is used in nature to locate food sources or other organisms over a long distance. *C. elegans* responds to a wide variety of volatile chemicals using five pairs of amphid neurons. The AWA and AWC cells mediate the detection of attractive odorants and the AWB, ASH and ADL cells mediate the detection of repulsive odorants (Bargmann et al. 1993; Troemel et al. 1995, 1997). Generally, low odorant concentrations are specifically detected by one neuron pair, whereas high odorant concentrations can be detected by multiple neuron pairs (Bargmann

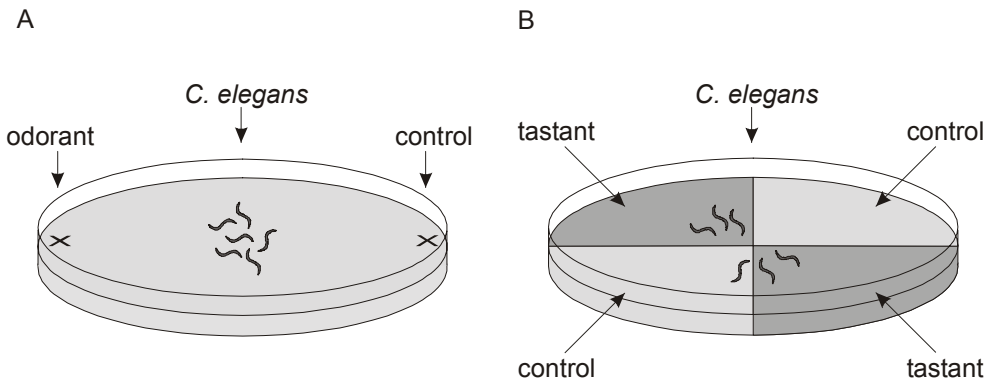

Figure 3. Olfaction and taste chemotaxis assays
A. Volatile chemotaxis assay (Bargmann et al. 1993)
Approximately 100-200 adult animals are placed in the middle of a 9 cm Petri dish containing 10 ml of buffered agar. On one side of the dish 1 μl of odorant diluted in ethanol and on the other side 1 μl ethanol, as a control, is spotted. In addition, on both sides 1 μl NaN_3 is spotted to anesthetize animals that reach the spot. Animals are allowed to move freely for at least an hour and are then counted. A chemotaxis index is calculated as the number of animals at the odorant spot minus the number of animals at the control ethanol spot, divided by the total number of animals.
B. Soluble compound assay (Wicks et al. 2000)
Approximately 100-200 adult animals are placed in the middle of a quadrant plate containing buffered agar and, in two opposite quadrants, a dissolved test compound. Animals are allowed to move freely for several minutes, after which their distribution over the quadrants is determined. A chemotaxis index is calculated as in A.

et al. 1993; Chou et al. 2001; Chao et al. 2004). The detection of odorants is studied by testing the chemotaxis of animals towards or away from a point source of an odorant on an agar plate (Bargmann et al. 1993; Troemel et al. 1997; Figure 3A). The detection of repulsive odorants can also be tested by measuring the avoidance response to a hair dipped in the odorant and placed directly in front of an animal (Troemel et al. 1995). Most research has focused on the function of the AWA and AWC neurons. Surprisingly, this has shown that, although both neuron types mediate the detection of attractive odorants, they use different transduction machineries.

Odorant detection by the AWA neurons

The AWA neurons specifically detect dilutions of the odorants diacetyl, pyrazine and 2,4,5-trimethylthiazole (Bargmann et al. 1993). This process involves G protein mediated activation of TRP channels (Figure 4A). The AWA neurons express at least two GPCRs, *odr-10* and *sra-13*. ODR-10 is the high affinity diacetyl receptor and is localized to the cilia of the AWA neurons (Sengupta et al. 1996). The ligand of SRA-13 is not known (Battu et al. 2003; see below). Furthermore, the AWA neurons express four Gα subunits, *odr-3, gpa-3, gpa-5* and *gpa-6,* which may be activated by GPCRs such as ODR-10 (Roayaie et al. 1998; Jansen et al. 1999; Lans et. al 2004; Chapter 4). ODR-3 is localized to the cilia and

required for all odorant responses mediated by the AWA neurons (Bargmann et al. 1993; Roayaie et al. 1998). In addition, ODR-3 is sufficient for AWA mediated odorant detection and thus represents the major G protein signaling pathway in the AWA neurons (Lans et al. 2004; Chapter 4). GPA-3 mediates the residual odorant response of *odr-3* mutants and can be sufficient for AWA mediated odorant detection as well. In contrast, loss-of-function mutations in *gpa-5* restore the reduced odorant response of *odr-3* mutants. GPA-3 and GPA-5 are both localized to the cilia. These results suggest that GPA-3 acts redundantly to ODR-3 but that GPA-5 inhibits signaling. The roles of Gα subunits in odorant detection by the AWA and AWC neurons are extensively discussed in Chapter 4.

The TRPV related channels OSM-9 and OCR-2 probably function downstream of the G proteins. OSM-9 and OCR-2 are localized to the cilia and necessary for all AWA mediated odorant perception (Colbert et al. 1997; Tobin et al. 2002). Interestingly, in *osm-9* mutants OCR-2 is not localized to the cilia, while in *ocr-2* mutants OSM-9 is not localized to the cilia. These results suggest that the two channel proteins act in a complex to mediate the detection of odorant signals. In addition, the TRPV channel OCR-1 is expressed in the AWA neurons but not essential for olfaction (Tobin et al. 2002). Together with OSM-9 and OCR-2, however, OCR-1 regulates the expression of ODR-10 in the AWA neurons.

A major question is how the TRP channels are activated and controlled by G protein signaling. Several lines of evidence suggest that multiple mechanisms exist, which involve signaling via phospholipase C (PLC) and the second messenger diacylglycerol (DAG). First of all, DAG is a precursor of polyunsaturated fatty acids (PUFAs). In *C. elegans*, chemotaxis to AWA sensed odorants is defective in animals with mutations in the genes *fat-1*, *fat-3* and *fat-4* (Kahn-Kirby et al. 2004). These genes encode lipid desaturase enzymes necessary for the synthesis of long chain PUFAs, like arachidonic acid (AA), eicosapentaenoic acid (EPA) and omega-3 arachidonic acid (O3AA) (Watts and Browse 2002). The defective odorant response of *fat-3* mutants can be rescued by exogenous AA and EPA, suggesting that PUFAs are necessary and may modulate the OSM-9/OCR-2 TRP channels (Kahn-Kirby et al. 2004). Such a mechanism resembles *Drosophila* phototransduction, in which PUFAs can directly activate TRP channels (Chyb et al. 1999). Secondly, DAG can activate protein kinase C (PKC). Recently, it was shown that the nPKCε/η TTX-4 and the nPKC-δ/θ TPA-1 are required for AWA mediated olfaction and can be activated by the DAG-analog PMA (Okochi et al. 2005). In mammals, the OSM-9/OCR-2 related TRPV1 channel is modulated by nPKCε (Numazaki et al. 2002; Premkumar and Ahern 2000), suggesting a similar mechanism could exist in the AWA neurons of *C. elegans.* Thirdly, the involvement of DAG is confirmed by the finding that *ppk-2* (Type II phosphatidylinositol phosphate kinase), one of the enzymes that generates a precursor of DAG, phosphatidylinositol-4,5-bisphosphate ($PI(4,5)P_2$), is required for strong AWA mediated odorant responses (H.L. and G.J., unpublished results). Interestingly, $PI(4,5)P_2$ can also directly modulate the activity of the mammalian TRPV1 channel (Chuang et al. 2001; Prescott and Julius 2003). Although DAG is produced by G

protein activated PLC, thus far, no specific PLC has been implicated in AWA mediated olfaction.

In summary, the current data suggest that binding of an odorant to a GPCR in the AWA cell membrane leads to the activation of the Gα subunits ODR-3 and GPA-3, which in turn activate an unknown PLC, resulting in the production of DAG (Figure 4A). Subsequently, DAG can activate the nPKCs TTX-4 and TPA-1 and can be used to produce PUFAs, which, probably by regulating the OSM-9/OCR-2 TRP channels, leads to a Ca^{2+} influx. GPA-5 most likely inhibits signaling upstream of OSM-9, because a *gpa-5* loss-of-function mutation only restores the impaired odorant response of *odr-3* mutants but not of *osm-9* mutants (H.L. and G.J., unpublished results).

Odorant detection by the AWC neurons

The AWC neurons specifically respond to dilutions of the odorants benzaldehyde, butanone, isoamyl alcohol, 2,4,5-trimethylthiazole and 2,3-pentanedione (Bargmann et al. 1993; Chou et al. 2001). This response is mediated by cGMP signaling (Figure 4B). Thus far, several GPCRs, including *str-2* and *sra-13,* have been found to be expressed in the AWC neurons, but their ligands are unknown (Troemel et al. 1999; Battu et al. 2003; Colosimo et al. 2004). *str-2* and *sra-13* mutants have no obvious olfactory defects (H.L. and G.J., unpublished results; see below). In the AWC neurons, four Gα subunits are localized to the cilia: ODR-3, GPA-2, GPA-3 and GPA-13 (Zwaal et al. 1997; Roayaie et al. 1998; Jansen et al. 1999; Lans et al. 2004; Chapter 4). Of these, only mutations in *odr-3* result in a severely impaired response to all AWC sensed odorants, suggesting that ODR-3 forms the major transduction route for odorant detection. Mutations in *gpa-3* and *gpa-13* further impair the response of *odr-3* animals to some odorants, suggesting that these Gα subunits act redundantly to ODR-3. Both ODR-3 and GPA-3 can be sufficient for odorant detection. GPA-2, on the other hand, has an inhibitory and stimulatory function. A mutation in *gpa-2* restores the impaired odorant response of *odr-3* mutants to some odorants (Lans et al. 2004; Chapter 4), but at the same time it reduces the response of *odr-3* mutants to butanone (Roayaie et al. 1998). The functions of the Gα subunits are extensively discussed in Chapter 4.

The guanylyl cyclases ODR-1 and DAF-11 are required for the strong response of *C. elegans* to all AWC sensed odorants (Bargmann et al. 1993; Vowels and Thomas 1994; Birnby et al. 2000; L'Etoile and Bargmann 2000). Both guanylyl cyclases are localized to the cilia, suggesting that they are directly involved in odorant detection and may be activated by G protein signaling. Interestingly, a mutation in the heat shock gene *daf-21* also impairs odorant detection, similarly to *daf-11* mutations. This impairment can be rescued by addition of exogenous cGMP, indicating that DAF-21 regulates cGMP levels in the AWC neurons (Vowels and Thomas 1994; Birnby et al. 2000). These results make it likely that cGMP acts as second messenger in AWC mediated olfaction. Consistently, the α and β subunits of a cGMP gated channel, *tax-4* and *tax-2*, were

found to be necessary for the response to AWC sensed odorants (Bargmann et al.1993; Coburn and Bargmann 1996; Komatsu et al. 1996). When expressed together in HEK293 cells, the TAX-2 and TAX-4 proteins form a functional channel

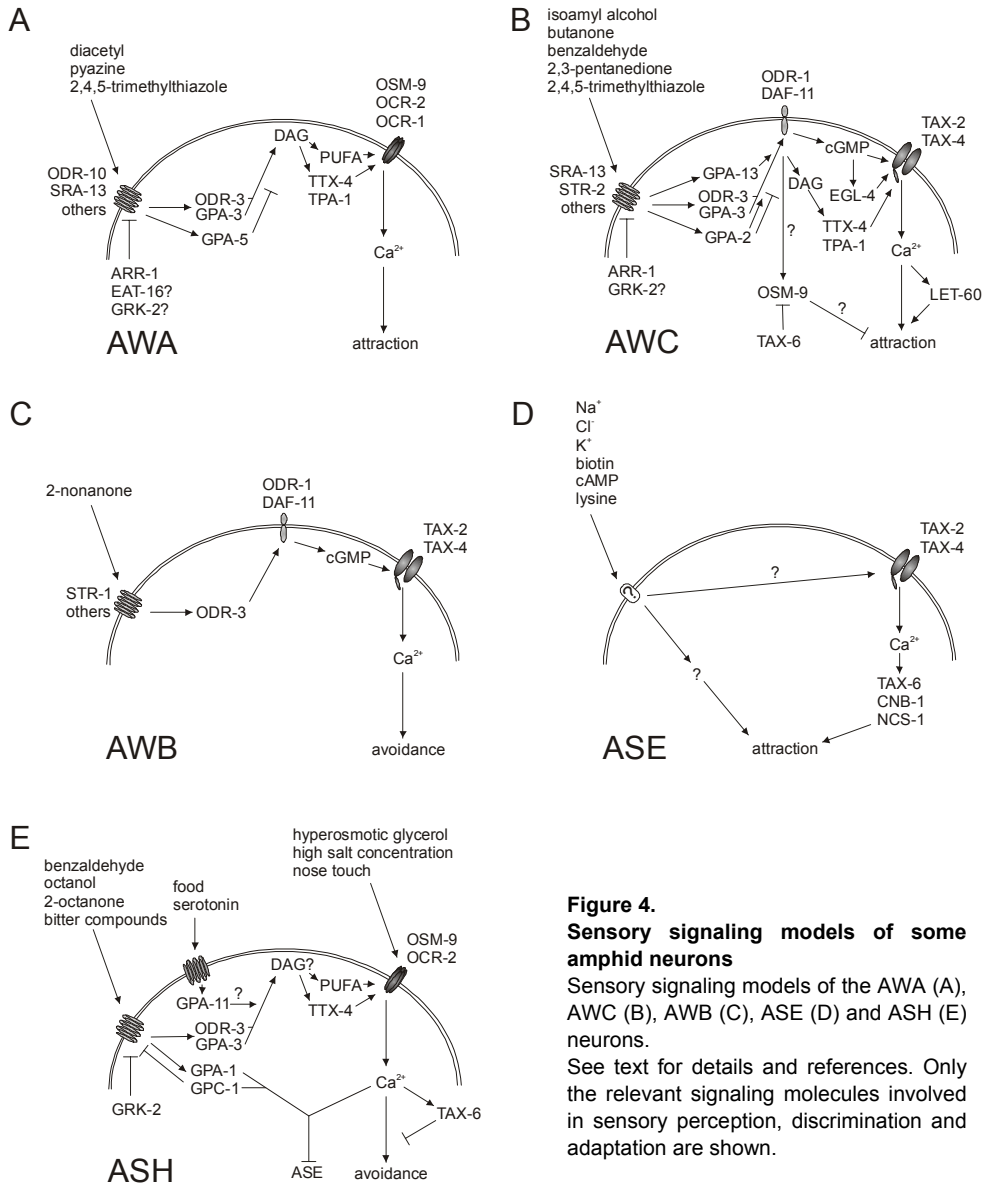

Figure 4.
Sensory signaling models of some amphid neurons
Sensory signaling models of the AWA (A), AWC (B), AWB (C), ASE (D) and ASH (E) neurons.
See text for details and references. Only the relevant signaling molecules involved in sensory perception, discrimination and adaptation are shown.

that is sensitive to cGMP and permeable to Ca^{2+}. TAX-4 proteins alone can also form a functional homomultimeric channel (Komatsu et al. 1996, 1999). However, TAX-2 and TAX-4 colocalize to the cilia of several amphid cells, including the AWC neurons, making it likely that the two proteins form a heterodimeric cGMP sensitive Ca^{2+} channel *in vivo*. Taken together, odorants detected by the AWC neurons probably activate ODR-3, GPA-2, GPA-3 and GPA-13, which signal to the guanylyl cyclases ODR-1 and DAF-11, leading to an increase in intracellular cGMP (Figure 4B). cGMP then activates the TAX-2/TAX-4 channel, resulting in an increase in intracellular Ca^{2+} levels.

In the AWC neurons, downstream of the cGMP pathway, a Ras/MAPK pathway might function. Mutations in *let-60* Ras and its downstream targets *lin-45* Raf MAPKK kinase, *mek-2* MAPK kinase and *mpk-1* MAP kinase and a kinase suppressor of Ras, called *ksr-1*, were found to cause defects in AWA and AWC mediated responses to low odorant concentrations (Hirotsu et al. 2000). Activated MAPK immunoreactivity was observed within 10 seconds of exposure to isoamyl alcohol in the AWC cytoplasm. Interestingly, this immunoreactivity was absent in *odr-3, tax-2* and *tax-4* animals and in animals with a mutation in *unc-2,* the α subunit of a voltage gated Ca^{2+} channel. This suggests that the LET-60 Ras pathway is activated by electrical activity downstream of the cGMP pathway. The GPCR SRA-13 was found to suppress the LET-60 Ras pathway (Battu et al. 2003). In addition, overexpression of *sra-13* partially impairs the response to isoamyl alcohol and diacetyl and this defect is restored by a mutation in *gpa-5*. These results seem to suggest that SRA-13, through GPA-5, negatively regulates the LET-60 Ras pathway during olfaction. However, a major concern of these studies is how GPA-5 could function in the AWC neurons. Furthermore, the results obtained using the *let-60* gain-of-function mutations (Hirotsu et al. 2000; Battu et al. 2003), should be considered with care, as the strain that carries this mutation carries one or more side mutations that impair chemotaxis to isoamyl alcohol (corrigendum Hirotsu et al. 2000). In addition, some results could not be reproduced (H.L. and G.J., unpublished results).

Several other genes have been found to affect AWC mediated odorant detection. For instance, the Ly-6 related membrane protein ODR-2 was found to be required for all AWC mediated odorant responses (Chou et al. 2001). Interestingly, ODR-2 expression in AWC could not be demonstrated and AWC specific ODR-2 expression could not rescue the olfactory defects of *odr-2* mutant. Only expression of ODR-2 in the interneurons AIB and AIZ, two major targets of the AWC neurons, restored olfaction. ODR-2 is localized to axons, suggesting a neuromodulatory role. Mutations in the cGMP dependent protein kinase *egl-4* impair detection of butanone and isoamyl alcohol by the AWC cells (Daniels et al. 2000). Strangely, this defect is suppressed by mutations in the CoSMAD transcriptional regulator *daf-3*, which is also involved in dauer formation. This suggests that EGL-4 has a regulatory rather than an essential function in odorant detection, which fits well with its role in AWC adaptation (L'Etoile et al. 2002; see below). Recently, also the nPKCε/η TTX-4 and the nPKC-δ/θ TPA-1 were shown to regulate odorant

detection in the AWC neurons, possibly in response to activation by DAG (Okochi et al. 2005).

Odorant detection by the AWB, ASH and ADL neurons

Compared to the detection of attractive odorants, little is known about the detection of repulsive odorants. The AWB neurons detect the repulsive odorant 2-nonanone (Troemel et al. 1997). In AWB, the Gα subunits ODR-3 and GPA-6, the guanylyl cyclases ODR-1 and DAF-11 and the cGMP gated channel proteins TAX-2 and TAX-4 are expressed (Coburn and Bargmann 1996; Komatsu et al. 1996; Roayaie et al. 1998; L'Etoile and Bargmann 2000; Birnby et al. 2000; Lans et al. 2004; Chapter 4). Avoidance of 2-nonanone is defective in *odr-3, odr-1, daf-11* and *tax-2* animals. These results suggest that in the AWB neurons a similar cGMP mediated transduction pathway exists as in the AWC neurons (Figure 4C). The function of GPA-6 is still unclear, but it might function redundantly to ODR-3, because in *odr-3* animals 2-nonanone avoidance is only mildly impaired (Troemel et al. 1997).

The repulsive odorants 2-octanone and undiluted benzaldehyde are detected by the ASH neurons and undiluted octanol by the ASH and ADL neurons (Troemel et al. 1995, 1997; Tobin et al. 2002). Probably, a TRP channel pathway similar to the one in the AWA neurons functions in these neurons (Figure 4E). Avoidance of 2-octanone and benzaldehyde is impaired in *osm-9*, *ocr-2* and *fat-3* mutants (Colbert et al. 1997; Tobin et al. 2002; Kahn-Kirby et al. 2004). Furthermore, GPA-3 and ODR-3 are both implicated in the response of ASH to other stimuli (see below) and seem to be necessary for the detection of undiluted benzaldehyde (H.L. and G.J., unpublished results). Interestingly, avoidance of diluted octanol is specifically mediated by the ASH neurons (Chao et al. 2004). This avoidance is modulated by the presence of food or the addition of serotonin, which is thought to mimic the presence of food. Animals on food or serotonin respond faster to diluted octanol than animals off food. In *gpa-11* animals, this modulation is not observed, suggesting that GPA-11 mediates a food/serotonin signal in the ASH neurons that modulates the response to diluted octanol. In addition, cell ablation experiments showed that only the ASH neurons are involved in the avoidance response to octanol when the animals are on food, whereas the ASH, ADL and AWB neurons are all able to mediate the octanol avoidance response in the absence of food. These findings show that environmental signals can regulate the sensitivity of neuron and suggest the possibility that other chemosensory responses are dependent on the presence of food as well.

Odorant discrimination and adaptation

In nature, animals must be able to discriminate between new, informative signals and signals that can be neglected. *C. elegans* can discriminate between odorants and specifically adapt to odorants in behavioral assays. When *C. elegans* is placed in a uniform field of an odorant, its response to a point source of that odorant is abolished but its response to other odorants is not (Bargmann et al.

1993; L'Etoile and Bargmann 2000). This behavior is called discrimination and is rapidly reversible. Removing *C. elegans* from the uniform field restores the response to the same odorant immediately. Prolonged exposure to an odorant causes *C. elegans* to adapt specifically to that odorant, while it remains responsive to other odorants (Colbert and Bargmann 1995). This behavior is termed adaptation and requires a longer recovery time, proportional to the adaptation time. Adaptation can be tested by measuring chemotaxis of animals exposed to an undiluted odorant for an hour, in a tube or on a plate. The molecular mechanisms that underlie both behaviors are only partially understood.

The ability of *C. elegans* to discriminate between odorants is to some extent due to the existence of functionally different cells. First of all, the AWA and AWC neurons are sensitive to different odorants and concentrations (Bargmann et al. 1993). Mutants that lack the function of AWA or AWC fail to discriminate diacetyl from 2,3-pentanedione, suggesting that the AWA and AWC neurons are necessary to discriminate between these two odorants (Chou et al. 1996). Furthermore, the two AWC neurons are functionally different. In wild type *C. elegans*, one AWC neuron expresses the *str-2* GPCR (the AWC^{ON} cell) and the other one does not (the AWC^{OFF} cell). Mutations in genes of the Ca^{2+}/MAPK pathway that regulates AWC asymmetry, like the MAPKK kinase *nsy-1*, result in two AWC^{ON} cells (Troemel et al. 1999; Sagasti et al. 2001). A *nsy-1* mutation specifically impairs the response to 2,3-pentanedione and the ability to discriminate between benzaldehyde and butanone (Wes and Bargmann 2001). Laserkilling the AWC^{OFF} cell in wild type animals results in a similar phenotype. Killing the AWC^{ON} cell, on the other hand, only abolishes the response to butanone. These results are in line with a model in which 2,3-pentanedione is detected by the AWC^{OFF} cell, butanone by the AWC^{ON} cell and benzaldehyde by both AWC cells. In addition, this model can explain the observation that mutants which overexpress the guanylyl cyclase *odr-1* have butanone discrimination defects, since 50% of these animals have two AWC^{ON} neurons (L'Etoile and Bargmann 2000; Wes and Bargmann 2001). Nevertheless, this model cannot explain the ability of *C. elegans* to discriminate between other odorants (Bargmann et al. 1993; Wes and Bargmann 2001). Therefore, other mechanisms must exist.

Which other mechanisms contribute to the ability to discriminate between odorants? The ASH, ADL and AWB neurons could contribute to discrimination by negatively regulating the response to certain odorants, since undiluted benzaldehyde is detected by the ASH neurons (Troemel et al. 1995). Still, *osm-3 odr-1* mutants, which lack the function of most amphid neurons, except AWA, AWB and AFD, can discriminate between diacetyl, pyrazine and 2,4,5-trimethylthiazole (Chou et al. 1996). Likewise, *osm-3 odr-7* mutants, which only retain AWC, AWB and AFD function, can discriminate all AWC sensed odorants. Therefore, it is likely that intracellular mechanisms exist that enable discrimination between certain odorants (Colbert and Bargmann 1997). One proposed mechanism involves spatial segregation of different, odorant specific signaling pathways downstream of GPCRs (Carlson 2000). Such a mechanism could explain the discrimination defect

caused by overexpression of *odr-1*, in addition to the model mentioned before. ODR-1, when overexpressed, specifically inhibits discrimination of odorants in a uniform field of butanone, which can be suppressed by loss of GPA-2 (L'Etoile and Bargmann 2000). Possibly, ODR-1 abolishes spatial insulation due to excessive cGMP production, stimulated by GPA-2. Another mechanism for discrimination could be the involvement of multiple G proteins. However, preliminary evidence suggests that *C. elegans* can discriminate between AWC sensed odorants using only ODR-3 (M. Dekkers and G.J., personal communication).

The molecular mechanisms that facilitate adaptation are far from understood. Adaptation can be subdivided into two forms. The first is induced by exposure to an undiluted odorant and cannot be rapidly reversed (Colbert and Bargmann 1995). The second, more precisely termed habituation, is induced by exposure to diluted (≥1000x) odorant and can be rapidly reversed (dishabituated) by harsh treatment, such as centrifugation or vortexing (Bernhard and van der Kooy 2000; Morrison and van der Kooy 2001). The effects of habituation are often very mild in comparison to those of adaptation. It is difficult to distinguish both behaviors, because adaptation to benzaldehyde but not to other odorants can also be partially dishabituated by centrifugation (Nuttley et al. 2001), suggesting that adaptation to different odorants involves different mechanisms. Indeed, adaptation can be odorant specific, as is the case for butanone and diacetyl, or it can cross-adapt the response to another odorant, as is the case for benzaldehyde and isoamyl alcohol (Colbert and Bargmann 1995). Intriguingly, the CB4856 natural isolate of *C. elegans* shows normal chemotaxis towards benzaldehyde but not to isoamyl alcohol following adaptation to benzaldehyde (Atkinson-Leadbeater et al. 2004). The odorant specificity of adaptation implies that intracellular mechanisms must exist that distinguish between different odorants.

Thus far, few genes have been found to be involved in adaptation. Mutations in *osm-9* disrupt adaptation to isoamyl alcohol and butanone, although they do not affect AWC mediated odorant detection (Colbert and Bargmann, 1995). OSM-9 is localized to the cytoplasm of the AWC neurons and not to the cilia, in agreement with an indirect role in AWC function (Colbert et al. 1997; Tobin et al. 2002). Because *osm-9* mutations also interfere with cross adaptation between benzaldehyde and isoamyl alcohol, OSM-9 might confer part of the odorant specificity of adaptation. OSM-9 signaling during adaptation depends on the activity of calcineurin. Mutations in *tax-6*, the A subunit of the Ca^{2+} activated phosphatase calcineurin, affect olfaction, osmotic avoidance and thermotaxis (Dusenbery et al. 1975; Hedgecock and Russell 1975; Kuhara et al. 2002). TAX-6 is expressed predominantly in sensory neurons, including the AWA, AWC, ASE and ASH neurons. *tax-6* mutants adapt to isoamyl alcohol within 10 minutes, suggesting that they are hypersensitive to odorant stimulation. This hypersensitivity is suppressed by an *osm-9* mutation. Therefore, TAX-6 might negatively regulate the response to odorants by regulating OSM-9 dependent adaptation, possibly in response to Ca^{2+} influx through the TAX-2/TAX-4 channel or through OSM-9 itself. Next, the uncharacterized mutation called *adp-1* specifically affects benzaldehyde

and butanone adaptation (Colbert and Bargmann 1995). *adp-1* mutants only show impaired benzaldehyde adaptation when they are transferred to a new chemotaxis plate following adaptation (Nuttley et al. 2001), suggesting they are hypersensitive to a dishabituating stimulus. Finally, the arrestin ARR-1 has recently been found to be required for adaptation and recovery from adaptation to isoamylalcohol, benzaldehyde and diacetyl (Palmitessa et al. 2005). The involvement of ARR-1 suggests that one of the mechanism of adaptation is classical receptor desensitization (Pitcher et al. 1998).

The cGMP dependent protein kinase *egl-4* is required for normal responses to diacetyl, butanone and isoamyl alcohol (Daniels et al. 2000). A specific mutation in *egl-4*, however, only impairs adaptation and not chemotaxis to AWC sensed odorants (L'Etoile et al. 2002). One of its target molecules might be TAX-2. Animals carrying a mutation in a putative phosphorylation site in TAX-2 are specifically defective in adaptation to odorants after short pre-exposure times (30-60 minutes). Mutating a putative NLS in the EGL-4 protein specifically interferes with adaptation after long pre-exposure times (>1hr). These results support a model in which, upon initial odorant exposure, EGL-4 first phosphorylates signaling proteins like TAX-2 to downregulate the odorant response, but, following prolonged exposure, translocates to the nucleus to regulate gene transcription and adaptation. This model seems to be confirmed by the finding that mutations in the Tbx2/Tbx3 T-box transcription factor *sdf-13* specifically abolish adaptation to AWC sensed odorants, but not odorant detection itself (Miyahara et al. 2004). However, as the adaptation defects of *sdf-13* mutants are already apparent after short pre-exposure, SDF-13 might also be required to transcribe genes essential for the onset of adaptation.

Adaptation and discrimination behaviors are modulated by sensory experience. For instance, animals deprived of food discriminate between isoamyl alcohol and benzaldehyde, but well fed animals lose this ability (Colbert and Bargmann 1997). In addition, animals deprived of food show stronger adaptation to benzaldehyde than well fed animals or animals exposed to serotonin, which mimics a food cue. These results might be explained by assuming that *C. elegans* associates the presence of an odorant with the absence of food. Several assays developed to test the plasticity of the odorant response confirm this notion. Animals exposed to benzaldehyde in the presence of food do not adapt to the odorant (Nuttley et al. 2002). Furthermore, animals exposed to an odorant in the presence of ethanol or a salt only show an adapted response when ethanol or the salt is present (Bettinger and McIntire 2004; Law et al. 2004). Associative learning is observed in other assays as well. Following the paired presentation of diacetyl and acetic acid, animals display a diminished response to diacetyl, even after 24 hours (Morrison et al. 1999). Three mutants, *lrn-1, lrn-2* and *glr-1,* were found to be impaired in this form of associative learning (Morrison and van der Kooy 2001). The *lrn-1* and *lrn-2* mutations have not been characterized (Wen et al. 1997), but *glr-1* encodes an AMPA type glutamate receptor. This demonstrates that glutamate signaling is important for olfactory associative learning in *C. elegans*.

GRK and RGS

GRK and RGS proteins have a prominent position among the many modulators of G protein signaling. *C. elegans* has two GRK genes, which both contain an RGS domain, and at least 13 RGS proteins (Koelle and Horvitz 1996; Hajdu-Cronin et al. 1999; Dong et al. 2000; Fukuto et al. 2004). A loss-of-function mutation in the GRK *grk-2* was found to impair AWA and AWC mediated olfaction and ASH mediated octanol avoidance. Although GRKs are usually involved in receptor desensitization, *grk-2* mutants show no sign of hypersensitivity. Surprisingly, the octanol avoidance defect of *grk-2* mutants can be partially restored by overexpression of ODR-3, indicating that in these mutants ODR-3 signaling is repressed. Furthermore, diacetyl chemotaxis of *grk-2* mutants can be restored by a mutation in the RGS *eat-16*, consistent with the idea that ODR-3 might be suppressed by this RGS protein. Diacetyl chemotaxis can also be restored by expression of GRK-2 in the ASH and ADL neurons (G.J. unpublished results), suggesting that these neurons are hypersensitive in *grk-2* animals, resulting in avoidance of otherwise attractive odorants. Still, it could be possible that GRK-2 has an unexpected stimulatory function in olfaction. Thus far, the precise molecular mechanisms of its function remain unclear. Besides EAT-16, other RGS proteins could be involved in olfaction as well. Mutations in the RGS genes *rgs-1* and *rgs-2* have no effect on olfaction (H.L. and G.J., unpublished results), but the involvement of other RGS genes has not been tested yet.

2.3 Taste

C. elegans responds to various nonvolatile cues, like salts, cAMP, biotin, basic pH and some amino acids (Ward 1973; Dusenbery 1974; Bargmann and Horvitz 1990, 1991a). In addition, *C. elegans* avoids hazardous environments, like high salt or osmotic concentrations, acid pH, Cu^{+} ions and bitter substances (Dusenbery 1974; Culotti and Russel 1978; Bargmann and Horvitz 1990, 1991a; Hilliard et al. 2004). The detection of attractive compounds is mainly mediated by the ASE neurons, whereas the detection of aversive stimuli is mainly mediated by the ASH neurons. Chemotaxis to or away from a chemical can be tested by placing worms on an agar plate containing a gradient of the chemical (Ward 1973; Bargmann and Horvitz 1991a) or on a plate divided in four quadrants of which two contain the chemical (Figure 3B; Wicks et al. 2000; Jansen et al. 2002). Avoidance behavior can also be tested by measuring backward movement in different assays, depending on the stimulus. Nose touch avoidance can be determined by tapping with an eyelash on the tip of the nose (Kaplan and Horvitz 1993). Osmotic and copper avoidance can be analyzed by determining whether animals cross a barrier of hyperosmotic glycerol or fructose or $CuSO_4$ (Culotti and Russell 1978; Wicks et al. 2000). Avoidance of volatile repellents can be tested by placing an eyelash or a capillary tube dipped in the odorant in front of the animal (Troemel et al. 1995). Avoidance of soluble compounds can be studied by placing a drop of the repellent on the animal itself (Hilliard et al. 2002). Recently, also Ca^{2+} imaging has been

used to characterize the function of the ASH neurons (Fukuto et al. 2004; Kahn-Kirby et al. 2004; Hilliard et al. 2005).

Salt detection by the ASE neurons

Perception of the attractive compounds NaCl, biotin, cAMP and lysine is primarily mediated by the ASE neurons (Bargmann and Horvitz 1991a). In addition, the ASI, ASG and ADF neurons contribute to the detection of NaCl, biotin and cAMP and the ASI, ASG and ASK neurons contribute to the detection of lysine. There are indications that the ASE neurons detect decreases in the concentration of a chemical and, by increasing the probability of reversals and omega turns during movement, direct chemotaxis in the direction of the chemical (Miller et al. 2005). Although both ASE neurons appear morphologically similar, they are not functionally identical. First of all, several genes have been found to be asymmetrically expressed. The ASEL neuron expresses the guanylyl cyclases *gcy-6* and *gcy-7* and the homeobox gene *lim-6*, whereas the ASER neuron expresses *gcy-5* (Yu et al. 1997; Hobert et al. 1999). Secondly, laser ablation experiments have shown that the ASER neurons are mainly sensitive to Cl^- and K^+ ions and the ASEL neurons to Na^+ ions (Pierce-Shimomura et al. 2001). Consistent with these findings, *lim-6* mutants, which express *gcy-5* in both ASE neurons, show reduced Na^+ chemotaxis, as if two ASER neurons are present.

Which molecular mechanisms enable the ASE neurons to detect salts and other chemicals? G proteins are not expected to be involved in salt perception, because, in mammals, salts are detected by amiloride sensitive ion channels or by TRP channels (Lindemann 2001; Lyall et al. 2004, 2005). However, salt perception by *C. elegans* is not sensitive to amiloride and is not impaired by mutations in TRP channels (Jansen et al. 2002; Hukema et al. *submitted*; R. Hukema and G.J., personal communication). Only one Gα subunit, GPA-3, is expressed in the ASE neurons, but *gpa-3* mutants have no defects in NaCl detection (Hukema et al. *submitted*). Furthermore, few other genes have been found to be involved in NaCl detection (Figure 4D). *tax-2* and *tax-4* animals are defective in chemotaxis up a NaCl gradient (Dusenbery et al. 1975; Komatsu et al. 1996; Coburn and Bargmann 1996). When these animals are tested using a quadrant assay (Figure 3B), they also show a chemotaxis defect, but they are able to detect high NaCl concentrations (Hukema et al. *submitted*). These results suggest that the TAX-2/TAX-4 channel is involved NaCl detection and that an additional pathway exists as well. It is possible that the cGMP gated Ca^{2+} channel formed by TAX-2 and TAX-4 is activated by one of the guanylyl cyclases expressed in the ASE neurons, either *gcy-5, gcy-6* or *gcy-7*. Thus far, only *gcy-5* mutants have been tested, but they showed wild type NaCl detection (R.H. and G.J., personal communication). In addition, the guanylyl cyclase *daf-11* has been reported to be involved in chemotaxis towards Cl^- ions, cAMP and biotin, but *daf-11* expression was not observed in the ASE neurons (Vowels and Thomas 1994; Birnby et al. 2000) and these results could not be confirmed using the quadrant plate assay (Hukema et al. *submitted*). Therefore, it remains unclear how TAX-2 and TAX-4 are activated.

Several additional genes are involved in NaCl detection. Mutations in *tax-6* and *cnb-1*, which encode the A and B subunits of calcineurin, reduce chemotaxis to NaCl (Dusenbery et al. 1975; Kuhara et al. 2002; Hukema et al. *submitted*). TAX-6 is expressed in the ASE neurons and CNB-1 in the majority of head neurons. Furthermore, the neuronal Ca^{2+} sensor *ncs-1* and the nPKCε/η TTX-4 are also expressed in the ASE neurons and necessary for NaCl detection (Gomez et al. 2001; Okochi et al. 2005; Hukema et al. *submitted*). In addition, mutations in the vesicular glutamate transporter *eat-4* and the α type subunit of a glutamate gated Cl^- channel *avr-14* reduce chemotaxis to NaCl, suggesting that glutamate acts as neurotransmitter in NaCl detection (Hukema et al. *submitted*)

Detection of repellents by the polymodal ASH neuron

Many repulsive stimuli, including volatile repellents, nose touch, osmotic strength, high salt concentrations, bitter compounds and heavy metal ions, are predominantly detected by the ASH neurons (Bargmann et al. 1990; Kaplan and Horvitz 1993; Troemel et al. 1995; Sambongi et al. 1999, 2000; Hilliard et al. 2002, 2004, 2005; Hukema et al. *submitted*). The ASH neurons are polymodal because they can detect and discriminate between many of these stimuli. Avoidance behavior mediated by ASH and other neurons is part of a sensory network in which sensory information from the head is modulated by information from the tail. The avoidance of SDS and quinine, which depends on the amphid neurons ASH, ASK and probably ADL, is negatively regulated by the phasmid neurons PHA and PHB (Hilliard et al. 2002, 2004). In addition, other neurons assist ASH in mediating the response to certain stimuli. For instance, Cu^+ and Cd^{2+} ions are sensed by the ASH, ADL and ASE neurons and protons by the ASH, ASK, ADF and ASE neurons (Sambongi et al. 1999, 2000). Light touch to the nose is sensed by the ASH, FLP and OLQ cells (Kaplan and Horvitz 1993). Touch is also sensed by the body touch mechanosensory neurons ALM, PLM, AVM and PVM (Driscoll and Kaplan 1997). These neurons use degenerin channels like MEC-4 and MEC-10 to detect touch. However, *mec-4* and *mec-10* mutants show normal avoidance of nose touch (Hart et al. 1999), indicating that different molecular mechanisms exist in the ASH neurons as compared to the mechanosensory neurons.

Several GPCRs have been found to be expressed in the ASH neurons, but it is not known which signals they transduce (Troemel et al. 1995; Colosimo et al. 2004). It is likely that the majority of volatile and nonvolatile repellents are detected by GPCRs, but that nose touch and hyperosmolarity are detected by TRP channels, as is the case in mammals (Lindemann 2001; Clapham 2003). Furthermore, at least eight Gα subunits are expressed in the ASH neurons: *gpa-1, gpa-3, gpa-6, gpa-11, gpa-13, gpa-14, gpa-15* and *odr-3* (Zwaal et al. 1997; Roayaie et al. 1998; Jansen et al. 1999; Lans et al. 2004). Of these, ODR-3 is necessary for the response to osmotic strength, nose touch and high salt concentrations (Roayaie et al. 1998; Hukema et al. *submitted*). GPA-3 is necessary for the avoidance of quinine, in which ODR-3 has a minor redundant role (Hilliard et al. 2004). Both ODR-3 and GPA-3 are necessary for the avoidance of Cu^{2+} ions.

In addition, the TRP channels OSM-9 and OCR-2 are involved in the avoidance of osmotic strength and nose touch (Colbert et al. 1997; Tobin et al. 2002) and TAX-6 negatively regulates osmotic avoidance (Kuhara et al. 2002). These results suggest that signaling in the ASH neurons resembles signaling in the AWA neurons (Figure 4E).

The idea that TRP channels in the ASH neurons act as receptors for osmolarity and nose touch is supported by the finding that the OSM-9/OCR-2 related vertebrate TRPV4 channel, which senses osmotic stimuli, can substitute for OSM-9 in the avoidance of these stimuli (Liedtke and Friedman 2003; Liedtke et al. 2003). Still, TRPV4 mediated detection of osmolarity and nose touch depends on ODR-3, making it unclear in what way G proteins, or GPCRs, are involved. It might be that G proteins modulate the activity of the OSM-9 and OCR-2 channels by regulating the levels of PUFAs. The avoidance of hyperosmolarity, nose touch and heavy metal ions is strongly impaired in *fat-3* mutants and mildly in *fat-1* and *fat-4* mutants, showing that PUFAs are involved in ASH mediated behaviors (Kahn-Kirby et al. 2004). Using the Ca^{2+} sensor G-CaMP, it was shown that stimulation of the ASH neurons with a hyperosmotic glycerol solution induces a Ca^{2+} increase in the ASH neurons of wild type animals, but a diminished increase in *osm-9* and *fat-3* mutants. Furthermore, the exposure of animals to EPA induced a strong avoidance response and an increase in intracellular Ca^{2+} levels in wild type animals but not in *osm-9* and *ocr-2* mutants. These results make it likely that OSM-9 and OCR-2 are directly activated by EPA. Another way for G proteins to regulate OSM-9 and OCR-2 activity might be by activating the nPKCε/η TTX-4, which regulates the sensitivity of the ASH neurons to hyperosmotic glycerol (Okochi et al. 2005).

Intracellular Ca^{2+} levels in the ASH neurons have also been tested using the Ca^{2+} signaling indicator cameleon. Ca^{2+} currents were demonstrated in the ASH cells upon stimulation with a wide range of stimuli, including the bitter substances quinine and denationium, heavy metal ions, SDS and a hyperosmotic glycerol solution (Hilliard et al. 2005). As a confirmation of previous results, the responses to all stimuli were reduced or eliminated in *odr-3, osm-9* and *gpa-3 odr-3* mutants. Adaptation within ASH was also observed, as repeated exposure to Cu^{2+} ions resulted in diminished intracellular Ca^{2+} levels over time. Interestingly, repeated Cu^{2+} exposure did not strongly interfere with the response to hyperosmotic glycerol. In *gpc-1* animals, adaptation to Cu^{2+} ions was weaker, suggesting a role for the Gγ subunit GPC-1 in ASH adaptation (see below). Ca^{2+} imaging further showed that the ASH response is under control of modulatory cues, because nose touch only elicited a response in the presence of serotonin.

The ability of the ASH neurons to detect and discriminate between different, often unrelated signals can be explained by the fact that some proteins are specifically involved in the response to one type of signal. *osm-10* animals show an impaired avoidance of osmotic strength, but react normal to nose touch, bitter and volatile repellents (Hart et al. 1999; Hilliard 2004). OSM-10 encodes a novel cytoplasmic protein with unknown function. Furthermore, *grk-2* mutants are partially defective in avoiding hyperosmotic glycerol, completely defective in

avoiding quinine, but show no defect in avoiding high salt concentrations (Fukuto et al. 2004; Hukema et al. *submitted*). Lastly, animals with a mutation in the novel WD-40 domain containing protein QUI-1 are impaired in their response to quinine, SDS and low pH, but not in avoiding osmotic strength (Hilliard et al. 2004). In addition to the specificity of these proteins, the polymodality of the ASH neurons is probably facilitated by the activation of different postsynaptic neurotransmitters in response to different stimuli. The EAT-4 vesicular glutamate transporter is required for all ASH mediated behaviors, suggesting that ASH uses glutamate as neurotransmitter (Berger et al. 1998; Lee et al. 1999; Bellochio et al. 2000; Takamori et al. 2000). Three glutamate receptor subunits have been found to be expressed by interneurons to which ASH projects: the NMDA glutamate receptor subunit NMR-1 and the non-NMDA glutamate receptors GLR-1 and GLR-2. Mutations in *glr-1* and *glr-2* cause defects in nose touch and osmotic avoidance (Hart et al. 1995; Maricq et al. 1995; Mellem et al. 2002), whereas a mutation in *nmr-1* only affects osmotic avoidance (Brockie et al. 2001). Furthermore, the avoidance of quinine only partially requires *eat-4* and *glr-1* (Hilliard et al. 2004). These results show that different stimuli activate different sets of postsynaptic glutamate receptors and that other neurotransmitters than glutamate must be involved as well.

Plasticity of taste

Several forms of behavioral plasticity of the taste response have been described. One of these, called gustatory plasticity, involves the exposure of animals to high salt concentrations (100 mM) for 15 minutes, after which the animals do not chemotax to or even avoid salt concentrations that are otherwise attractive (Jansen et al. 2002; Hukema et al. *submitted*). Experiments using this assay have shown this response is partly salt specific. For instance, animals pre-exposed to NaCl do not chemotax to NaAc, but animals pre-exposed to NaAc, still chemotax to NaCl. Thus far, 48 genes have been found to be involved in this form of plasticity. Among these are the G proteins *gpa-1, odr-3, goa-1, egl-30, gpb-2* and *gpc-1*, the TRP channels *osm-9, ocr-1* and *ocr-2,* the RGS proteins *eat-16* and *egl-10* and the guanylyl cyclase *gcy-35*. Gustatory plasticity is probably the result of the integration of antagonistic cues from multiple sensory neurons. First of all, the ASE neurons are necessary to detect salt. Secondly, cell specific rescue experiments indicate that *gpc-1* functions in the ASH, ASI and possibly the ADL neurons and *odr-3* in the ADF neurons to regulate gustatory plasticity. Thirdly, disrupting the function of the AQR, PQR and URX body cavity neurons using a gain-of-function allele of a K^+ channel impairs gustatory plasticity. Finally, *grk-2* mutants do not chemotax to NaCl, but do avoid high NaCl concentrations, demonstrating that their ability to detect NaCl is not abolished. When GRK-2 is expressed in the ASH and ADL neurons, chemotaxis is restored. One way to explain this is by assuming that the ASH and ADL neurons of *grk-2* animals are hypersensitive to salt, resulting in excessive salt avoidance. This would mean that the behavioral response to NaCl results from the integration of attraction signals

from the ASE neurons and avoidance signals from ASH and other neurons. Another clue that gustatory plasticity is more than the inability to detect NaCl comes from the fact that animals exposed to high salt concentrations do not chemotax to salt but can still associate the presence of salt with the presence of an odorant (Law et al. 2004).

Other plasticity assays seem to be more focused on associative learning tasks. For instance, animals cultured without food for several hours, in the presence of standard concentrations of NaCl (50 mM), lose their ability to chemotax to or even avoid NaCl and other chemicals detected by ASE (Saeki et al. 2001). Although this behavior is called associative learning and takes a long time to acquire, to some extent it might be similar to gustatory plasticity. Both behaviors require HEN-1, a LDL receptor motif containing protein that is localized to the synapses of the ASE and AIY neurons (Ishihara et al. 2002; Hukema et al. *submitted*). Another assay uses NaAc or NH_4Cl in combination with or without food to condition animals (Wen et al. 1997). Animals conditioned on food in the presence of either Na^+ or Cl^- ions learn to prefer the ions that predict food. Two mutants, *lrn-1* and *lrn-2*, were identified in a genetic screen, but have not been characterized up to now.

C. elegans as model to study sensory signaling and behavior

The features of the olfactory and taste systems of *C. elegans* demonstrate that, in spite of its small size and limited number of cells, *C. elegans* is able to engage in very complex behaviors. Underlying these behaviors are many interwoven signaling pathways that are reminiscent of the signaling pathways found in other (higher) organisms. In addition to the sensory cues described in this chapter, *C. elegans* shows complex behavior in response to touch (Driscoll and Kaplan 1997), temperature (Mori 1999) and oxygen (Gray et al. 2004; Cheung et al. 2005). This makes *C. elegans* a good model to unravel the molecular mechanisms of sensory perception.

Chapter 3

Regulation of development by sensory signaling: dauer development and longevity

3. Regulation of development by sensory signaling: dauer development and longevity

3.1 Developmental and behavioral impact of sensory perception

C. elegans adjusts many of its developmental and behavioral processes in response to environmental cues. For instance, in the sensory neurons, sensory perception controls the maintenance of receptor gene expression and axon structure (Peckol et al. 1999, 2001; Tobin et al. 2002). Furthermore, food signals regulate the sensitivity of sensory neurons, resulting in altered behavior towards sensory stimuli (Colbert and Bargmann 1997; Wen et al. 1997; Nuttley et al. 2002; Chao et al. 2004). Many other behaviors are also altered by food exposure, such as egg laying (Trent et al. 1983), locomotion (Croll 1975), feeding (Avery and Horvitz 1990), defecation (Thomas 1990; Liu and Thomas 1994) and social behavior (de Bono et al. 2002). In addition, sensory perception regulates decisions made during development, such as dauer development during the L1 larval stage and longevity during adulthood (Golden and Riddle 1984a; Apfeld and Kenyon 1999; Alcedo and Kenyon 2004). These latter subjects will be briefly discussed in this chapter.

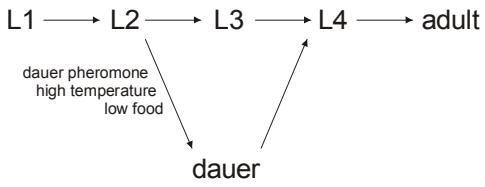

Figure 1. Life cycle of *C. elegans*
C. elegans develops through four larval stages to become an adult. To survive under adverse conditions, *C. elegans* can form dauer larvae, which resume development into adulthood only when environmental conditions improve.

3.2 Regulation of dauer development

Under ideal circumstances, *C. elegans* develops into adulthood through four larval stages called L1, L2, L3 and L4, separated by molts (Figure 1). Under adverse conditions, however, such as overcrowding and low food availability, *C. elegans* enters an alternative third larval stage called dauer (Cassada and Russell 1975; Golden and Riddle 1984a; Riddle and Albert 1997). Because of extensive anatomical and physiological changes, dauer larvae can survive without food for several months. When environmental conditions improve, dauer larvae recover and resume development as L4 larvae. The ability to form dauer larvae gives *C. elegans* the ability to postpone reproduction until a suited environment is found. Three types of sensory cues, food availability, temperature and a dauer pheromone, influence the decision to form dauer larvae (Golden and Riddle 1984a). Of these, dauer pheromone is the most important. Its chemical structure has recently been identified (Jeong et al. 2005). Dauer pheromone is a nonvolatile, fatty acid like substance constitutively secreted by each animal, thus serving as a measure of population density (Golden and Riddle 1982, 1984b). When high pheromone levels are present, many second stage larvae will develop into dauer

animals.

Sensory amphid neurons regulate dauer development

Dauer regulating cues are detected by the amphid sensory neurons. Animals with structural defects of the amphid cilia do not form dauer larvae under dauer inducing conditions and recover poorly from the dauer stage (Albert et al. 1981; Starich et al. 1995). Dauer formation and recovery are controlled by the ADF, ASI, ASG and ASJ neurons. Animals in which the ADF, ASI and ASG neurons are killed constitutively form dauer larvae (Bargmann and Horvitz 1991b). Therefore, these neurons probably function to prevent dauer formation. Their activity might be suppressed by dauer pheromone. In addition, killing the ASJ neurons diminishes dauer formation induced by dauer pheromone, showing that the ASJ neurons promote dauer formation (Schackwitz et al. 1996). Killing the ASJ neurons also prevents recovery from the dauer stage, implicating the ASJ neurons in recovery as well (Bargmann and Horvitz 1991b). Some mutants can recover from the dauer stage independently of the ASJ neurons, suggesting the involvement of additional neurons (Coburn et al. 1998).

Signaling genes that regulate dauer formation

A complex network of genes has been identified that regulates dauer development (Thomas et al. 1993; Riddle and Albert 1997). Mutations in genes of this network either cause a dauer constitutive (daf-c) or a dauer defective (daf-d) phenotype. Many of the genes can be arranged in three parallel pathways of which two converge on the DAF-12 nuclear hormone receptor and one on the FOXO family transcription factor DAF-16 (Figure 2). However, these pathways are not fully separate since certain genes suppress or regulate genes of multiple pathways (Thomas et al. 1993; Zwaal et al. 1997; Murakami et al. 2001; Li et al. 2003).

One pathway, involving TGFβ signaling, includes the TGF-β homologue *daf-7* and its Type I and Type II receptors *daf-1* and *daf-4* (Georgi et al. 1990; Estevez et al. 1993; Ren et al. 1996; Schackwitz et al. 1996; Riddle and Albert 1997). Mutations in these genes confer a daf-c phenotype that is suppressed by mutations in the transcriptional regulators *daf-3* and *daf-5* (Patterson et al. 1997; da Graca et al. 2004). The second pathway, involving cGMP signaling, includes the guanylyl cyclase *daf-11* and the heat shock protein *daf-21* (Vowels and Thomas 1994; Riddle and Albert 1997; Birnby et al. 2000). The daf-c phenotype caused by mutations in these genes is suppressed by mutations that disrupt the amphid cilia (Vowels and Thomas 1992). The third pathway is an insulin/insulin-like growth factor (IGF-1) pathway. This pathway includes the insulin/IGF-1 receptor DAF-2 and regulates dauer development as well as longevity (see below; Kenyon et al. 1993; Gottlieb and Ruvkun 1994; Larsen et al. 1995; Kimura et al. 1997; Lin et al. 1997; Ogg et al. 1997).

Although little is known about the exact molecular mechanisms that function in the amphid neurons to control dauer development, sensory perception is known to affect all three pathways. Animals with mutations in *daf-3, daf-5* or *daf-*

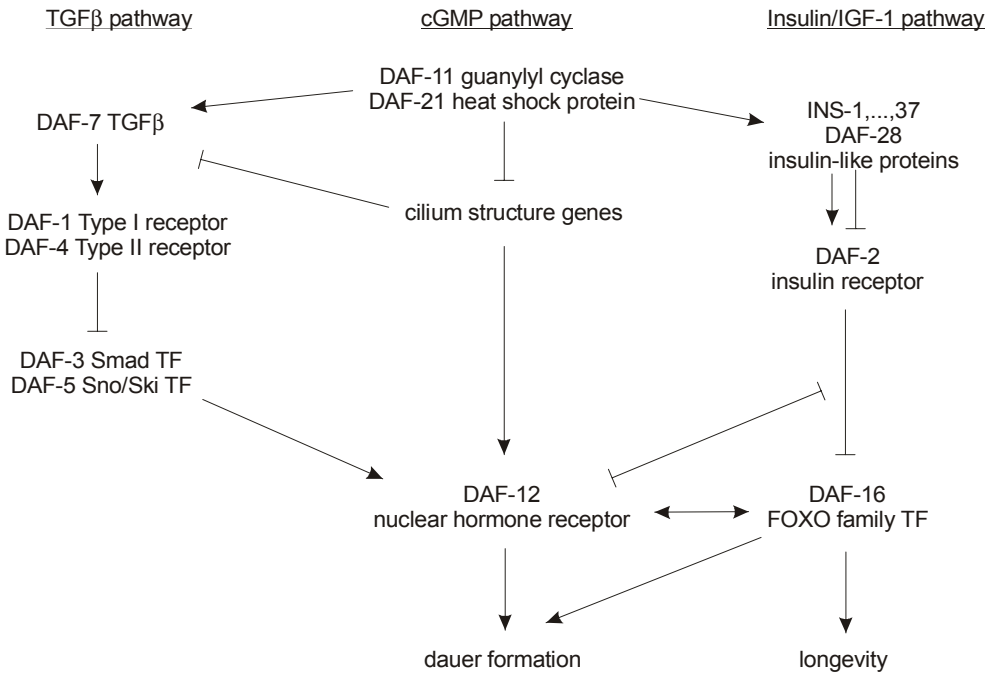

Figure 2. Genetic pathways that control dauer formation and life span
Three genetic pathways regulate dauer formation and longevity. Not all known genes of the pathways are shown. All three pathways or individual genes of the pathways also regulate other developmental processes. TF denotes Transcription Factor. See text for references and details.

12 of which the ADF, ASI and ASG neurons are killed do not develop into dauer larvae (Schackwitz et al. 1996). This suggests that the TGFβ pathway is a major target of sensory signaling. The TGFβ homologue *daf-7* is thought to be secreted by the ASI neurons to promote nondauer development, by binding of the widely expressed DAF-1 type I and DAF-4 type II TGFβ receptors (Ren et al. 1996; Schackwitz et al. 1996; Gunther et al. 2000). *daf-7* expression in the ASI neurons is suppressed by dauer pheromone and increasing temperature, but stimulated in the presence of food (Ren et al. 1996; Schackwitz et al. 1996). Therefore, regulation of DAF-7 expression might be a means to control dauer development.

Besides the TGFβ pathway, sensory signaling seems to control dauer development through endocrine signaling via the insulin/IGF-1 pathway. A semidominant mutation in the insulin-like gene *daf-28,* which is expressed in the ASI and ASJ neurons, causes constitutive dauer formation (Malone and Thomas 1994; Malone et al. 1996; Li et al. 2003). *daf-28* expression is repressed by dauer pheromone and by mutations in the *daf-7* and *daf-11* genes. Furthermore, overexpression of two other insulin-like genes, *ins-1* and *ins-18*, which are expressed in the ASI and ASJ neurons and in unidentified amphid neurons, respectively, also causes constitutive dauer formation (Pierce et al. 2001). Secreted insulin-like proteins are thought to function as ligands of the DAF-2 insulin/IGF-1 receptor.

The receptor that recognizes dauer pheromone has not yet been found, but it is probably a GPCR. Loss-of-function mutations in the Gγ subunit *gpc-1* and several Gα subunits, such as *gpa-1, gpa-2, gpa-3* and *odr-3*, which are expressed in the amphid neurons, cause reduced sensitivity to dauer pheromone (Zwaal et al. 1997; J. Burghoorn and G. Jansen, unpublished results; Chapter 6). Only the roles of *gpa-2* and *gpa-3* have been studied in detail (Zwaal et al. 1997). Animals with constitutively activating mutations in *gpa-2* and *gpa-3* show a daf-c phenotype. In addition, *gpa-2* dauer mutants recover from the dauer stage in the presence of dauer pheromone, whereas wild type dauer larvae do not. These results suggest that GPA-2 and GPA-3 transduce the dauer pheromone signal. However, only GPA-3 is expressed in the ASI, ADF, ASG and ASJ neurons implicated in dauer formation. GPA-2 is expressed in the AWC neurons and in several other neurons including the AIA interneurons, to which some amphid neurons project. Therefore, GPA-2 might have an indirect, regulatory function.

GPA-2 and GPA-3 seem to function in the TGFβ as well as in the cGMP pathway (Zwaal et al. 1997). Mutations in *gpa-2* and *gpa-3* partially suppress the daf-c phenotype of *daf-11* mutants, while at the same time the daf-c phenotype of a constitutively activating mutation in *gpa-3* is suppressed by a *daf-5* mutation. Therefore, the exact way in which both Gα subunits control dauer development is unclear. Because none of the Gα mutations tested so far completely blocks the response to dauer pheromone, probably extensive redundancy exists among the Gα subunits in the transduction of dauer pheromone.

A likely candidate to be activated by G protein signaling in dauer formation is the guanylyl cyclase DAF-11. Loss-of-function mutations in *daf-11* cause a strong daf-c phenotype, which is suppressed by mutations that cause cilia defects (Riddle et al. 1981; Vowels and Thomas 1992, 1994; Birnby et al. 2000). Therefore, DAF-11 probably functions in the cilia to transduce the dauer pheromone signal. In addition, the daf-c phenotype is partially suppressed by mutations in the SMAD transcription factor *daf-3* and the FOXO family transcription factor *daf-16*, suggesting that DAF-11 signals via the TGFβ and the insulin pathway (Vowels and Thomas 1992; Thomas et al. 1993). Several lines of evidence suggest that the daf-c phenotype of *daf-11* mutants is caused by downregulation of *daf-7* (Murakami et al. 2001). First of all, the expression of *daf-7* in the ASI neurons of *daf-11* mutants is reduced. Next, overexpression of *daf-7* in some amphid neurons, including the ASI neurons, suppresses the daf-c phenotype of *daf-11* mutants. Finally, cilia mutations that suppress the daf-c phenotype of *daf-11* mutants also restore *daf-7* expression.

Loss-of-function of *daf-11* also affects the expression of the insulin-like gene *daf-28* (Li et al. 2003). The involvement of DAF-11 in multiple pathways might be explained by the fact that DAF-11 functions in multiple sensory neurons. Cell specific expression of *daf-11* in both the ASJ as well as the ASI neurons suppresses dauer formation (Murakami et al. 2001). Furthermore, expression of *daf-11* in the ASI neurons restores *daf-7* expression (Murakami et al. 2001), but the ASJ neurons are necessary to confer a daf-c phenotype in *daf-11* mutants

(Schackwitz et al. 1996). Therefore, DAF-11 probably functions in the ASI and ASJ neurons to regulate dauer formation.

The cGMP gated channel formed by TAX-2 and TAX-4 could function downstream of DAF-11. *tax-2* and *tax-4* are expressed in the ASI and ASJ neurons (Coburn and Bargmann 1996; Komatsu et al. 1996). Furthermore, *tax-2* and *tax-4* mutants have a weak daf-c phenotype and are defective in dauer recovery (Coburn et al. 1998). These results suggest that the TAX-2/TAX-4 channel prevents dauer formation and promotes dauer recovery. However, mutations in *tax-2* and *tax-4* suppress the daf-c phenotype and the dauer recovery defect of *daf-11* mutants, indicating that the channel promotes dauer formation and prevents dauer recovery as well, downstream of DAF-11 (Coburn et al. 1998) . Thus, DAF-11 and the TAX-2/TAX-4 channel seem to act antagonistically and, in dauer formation, the TAX-2/TAX-4 channel has dual, opposing functions.

Thus, it is very well possible that G protein signaling, involving multiple Gα subunits and cGMP signaling, mediates the detection of dauer pheromone and/or regulates dauer formation in response to sensory cues. The importance of cGMP signaling is also evident from the fact that animals with a mutation in the cGMP dependent protein kinase *egl-4* are hypersensitive to dauer pheromone (Daniels et al. 2000). Although *egl-4* mutants show some phenotypes characteristic of the DAF-11 pathway, EGL-4 likely functions in the TGFβ pathway. Future experiments, perhaps utilizing the recently chemically synthesized dauer pheromone (Jeong et al. 2005), will be needed to fully understand the sensory signaling pathways that govern dauer formation.

3.3 Regulation of longevity

In the laboratory, at 20°C, wild type animals have a life span of two to three weeks. In contrast, dauer larvae can survive more than two months. This suggests that life span in *C. elegans* is actively controlled and that the rate of aging can be slowed down if needed. Indeed, mutations in a number of genes have been found to extend life span, making the aging process of *C. elegans* an attractive focus of genetic research. The control of life span in adult *C. elegans* is independent from the control of dauer formation, although some genes are involved in both processes. The primary pathway that regulates longevity in *C. elegans* is the insulin/IGF-1 pathway. One of the key genes of this pathway, the insulin/IGF-1 receptor DAF-2, functions prior to the L3 larval stage to regulate dauer formation, but exclusively in adult animals to regulate longevity (Dillin et al. 2002). Interestingly, as in dauer formation, the pathways that regulate longevity are modulated by sensory perception.

Pathways that regulate longevity

Many genes have been identified that somehow affect the life span of *C. elegans*. Mutations in some genes extend life span by affecting the intake of food. This probably causes caloric restriction, which can extend the life span of many organisms (Lakowski and Hekimi 1998). Mutations in other genes influence life

span by disrupting mitochondrial function (Felkai et al. 1999; Feng et al. 2001). By far the best studied pathway that regulates longevity is the insulin/IGF-1 pathway (Figure 2). In this pathway, the insulin/IGF-1 receptor DAF-2 suppresses longevity via the PI_3K AGE-1, the 3-phosphoinositide dependent kinase PDK-1, the serine/threonine kinases AKT-1 and AKT-2 and the FOXO family transcription factor DAF-16 (Kenyon et al. 1993; Morris et al. 1996; Kimura et al. 1997; Ogg et al. 1997; Paradis and Ruvkun 1998; Paradis et al. 1999). Reduction-of-function mutations in members of this pathway extend the life span of *C. elegans*, which is suppressed by mutations in *daf-16*. Therefore, DAF-16 is thought to promote longevity. Its activity is dependent on phosphorylation by AKT-1 and AKT-2, which causes its exclusion from the nucleus (Lin et al. 2001). In wild type animals, DAF-16 is present predominantly throughout the cytoplasm, but in *daf-2* mutants DAF-16 accumulates in the nucleus.

In addition to the insulin/IGF-1 mediated pathway, other pathways that influence longevity also rely on the function of DAF-16. Signals from the reproductive system influence life span in a DAF-2 and DAF-16 dependent and independent manner (Hsin and Kenyon 1999). Ablation of the germ line, by killing the germ line precursor cells Z2 and Z3, extends life span, indicating that the germ line produces a signal that shortens life span. Ablation of the somatic gonad and the germ line, by killing the somatic gonad precursors Z1 and Z4, has no effect on life span, indicating that the somatic gonad produces a signal that counterbalances the germ line signal. In *daf-16* mutants, ablation of the germ line precursors has no effect, but ablation of the somatic gonad precursors shortens life span. Therefore, the germ line signal seems to depend on DAF-16, but the somatic gonad seems to be at least partially DAF-16 independent. Furthermore, the germ line signal is independent of DAF-2, because killing the germ line precursors extends the life span of *daf-2* mutants. The somatic gonad signal, however, depends partially on DAF-2, since killing the somatic gonad precursors in *daf-2* mutants yields different results with different *daf-2* reduction-of-function alleles.

In addition to the reproductive system, sensory perception and a Jun N-terminal kinase (JNK) pathway regulate longevity in a DAF-16 dependent, but DAF-2 independent, manner. Interestingly, sensory perception is required for the somatic gonad signal (see below). The JNK pathway seems to promote DAF-16 activity, in contrast to the insulin/IGF-1 pathway, and acts in response to environmental stress signals (Oh et al. 2005).

Regulation of longevity by sensory perception

Environmental cues influence the life span of *C. elegans.* For instance, the life span of *C. elegans* shortens with increasing temperature (Klass 1977). Sensory perception through the amphid neurons controls the rate of aging as well. Mutants with structural defects of the amphid cilia, like *daf-6, osm-3, daf-10* and *osm-5* mutants, have an extended lifespan (Apfeld and Kenyon 1999). This lifespan extension is dependent on the function of DAF-16, but not completely, and is probably induced by nuclear accumulation of DAF-16 (Lin et al. 2001). Mutants

with cilia defects show normal feeding behavior, suggesting that the increase in life span is not caused by caloric restriction. Using laser cell ablation, it was shown that the same sensory neurons that regulate dauer formation, also regulate longevity (Alcedo and Kenyon 2004). Killing the ASI or the ASG neurons slightly increases life span, which is suppressed by additional killing of the ASJ or ASK neurons. In addition, killing the olfactory AWA and AWC neurons extends life span synergistically to killing the ASI neurons, suggesting that they function in parallel. Consistently, the ASI neurons seem to act in a pathway with DAF-2 and DAF-16, while the AWA and AWC neurons seem to act partially independent of DAF-16. Unfortunately, the involvement of other amphid neurons (except ADF and ASE) has not been tested by laser ablation. Life span experiments using *gpa-11* mutants suggest that the ADL and ASH neurons regulate life span as well (see below; Chapter 6).

Killing the germ line precursor cells extends the life span of *daf-6, osm-3, daf-10* and *osm-5* cilia mutants, suggesting that the germ line signal regulates life span separately from sensory signals (Apfeld and Kenyon 1999). However, killing the somatic gonad precursors also extends the life span of *daf-10* and *osm-5* mutants, but not that of *daf-6* and *osm-3.* This suggests that in *daf-10* and *osm-5* mutants the somatic gonad signal is silenced. Using animals with a mutation in the nuclear receptor *odr-7*, which is required for the function of the AWA neurons, it was shown that the somatic gonad signal partially depends on the function of the AWA neurons (Alcedo and Kenyon 2004). Possibly, the AWA neurons secrete a hormone that is necessary for the somatic gonad to regulate longevity. Alternatively, the somatic gonad might signal via the AWA neurons.

Although the sensory neurons appear to regulate longevity through DAF-16, it is not completely clear if and how DAF-2 is involved. Mutations in the cilia genes *daf-6* and *osm-5* have no effect on *daf-2* mutants, but, strangely, mutations in the cilia genes *daf-10* and *osm-3* shorten the life span of *daf-2* mutants (Apfeld and Kenyon 1999). The life span of *daf-2* mutants is also reduced when the ASJ and ASK neurons are killed (Alcedo and Kenyon 2004), when the Ca^{2+} dependent activator protein for secretion (CAPS) *unc-31,* which likely regulates neuronal insulin secretion, is mutated (Ailion et al. 1999) and when *gpa-11* is overexpressed (Chapter 6). These results are startling because mutations in *daf-10, osm-3,* and *unc-31* and overexpression of *gpa-11* extend the life span of wild type animals. The most likely explanation is that two antagonistic signals are involved. One possibility is that both signals are ligands of DAF-2, but only one signal still functions as ligand of *daf-2* reduction-of-function alleles (Apfeld and Kenyon 1999). Another possibility is that one signal acts as DAF-2 ligand and another, weaker signal functions independently of DAF-2 (Chapter 6). Only in *daf-2* animals mutations that affect the weaker signal shorten life span.

The involvement of the sensory neurons suggests that sensory cues regulate longevity, but so far no candidate cues have been identified. Exposure to dauer pheromone does not extend life span (Alcedo and Kenyon 2004). It is likely that the sensory signals that influence lifespan are transduced by GPCRs and G

proteins. Loss-of-function mutations in the Gα subunits *odr-3, gpa-1, gpa-9* and the Gγ subunit *gpc-1* and overexpression of *gpa-11* extend lifespan (Chapter 6; Alcedo and Kenyon 2004). Regulation of life span by ODR-3 and GPA-11 depends on DAF-16 and appears to be partially independent of DAF-2 (Chapter 6). Furthermore, mutations in the cGMP-gated channel α subunit *tax-4,* but not in the β subunit *tax-2,* were found to extend life span (Apfeld and Kenyon 1999). Finally, RNAi experiments indicated that the GPCR *str-2* might transduce a signal that reduces life span (Alcedo and Kenyon 2004), but these results could not be confirmed with a *str-2* loss-of-function mutation (H. Lans and G.J., unpublished results).

How do the sensory neurons control the physiological changes that enable an animal to survive longer? The involvement of the insulin/IGF-1 receptor DAF-2 shows that insulin signaling is important. This is further substantiated by the fact that mutations in the CAPS *unc-31* and in the syntaxin homologue *unc-64*, which might be necessary for neural insulin secretion, increase life span (Ailion et al. 1999). Furthermore, several insulin-like peptides have been found to regulate longevity. The genome of *C. elegans* encodes at least 38 insulins, *ins-1* to *ins-37* and *daf-28*, of which many are expressed in the amphid neurons (Gregoire et al. 1998; Kawano et al. 2000; Pierce et al. 2001; Li et al. 2003). For instance, *ins-1* is expressed in the ASI, ASH and the ASJ neurons and *ins-9* in the ASI and ASJ neurons (Pierce et al. 2001). RNAi against *ins-7* and *ins-18* extends life span (Kawano et al. 2000; Murphy et al. 2003). In addition, a semidominant mutation in *daf-28*, which is expressed in the ASI and ASJ neurons, extends life span probably through nuclear accumulation of DAF-16 (Malone and Thomas 1994; Malone et al. 1996; Li et al. 2003). Interestingly, DAF-28 expression is upregulated in long-lived sensory mutants. These findings suggest that insulin-like peptides secreted by the sensory neurons function as endocrine regulators of longevity.

References

Afshar, K., F.S. Willard, K. Colombo, C.A. Johnston, C.R. McCudden, D.P. Siderovski, and P. Gonczy. 2004. RIC-8 is required for GPR-1/2-dependent Galpha function during asymmetric division of *C. elegans* embryos. *Cell* **119**: 219-30.

Ailion, M., T. Inoue, C.I. Weaver, R.W. Holdcraft, and J.H. Thomas. 1999. Neurosecretory control of aging in *Caenorhabditis elegans*. *Proc Natl Acad Sci U S A* **96**: 7394-7.

Albert, P.S., S.J. Brown, and D.L. Riddle. 1981. Sensory control of dauer larva formation in *Caenorhabditis elegans*. *J Comp Neurol* **198**: 435-51.

Albert, P.S. and D.L. Riddle. 1983. Developmental alterations in sensory neuroanatomy of the *Caenorhabditis elegans* dauer larva. *J Comp Neurol* **219**: 461-81.

Alcedo, J. and C. Kenyon. 2004. Regulation of *C. elegans* Longevity by Specific Gustatory and Olfactory Neurons. *Neuron* **41**: 45-55.

Apfeld, J. and C. Kenyon. 1999. Regulation of lifespan by sensory perception in *Caenorhabditis elegans*. *Nature* **402**: 804-9.

Atkinson-Leadbeater, K., W.M. Nuttley, and D. van der Kooy. 2004. A genetic dissociation of learning and recall in *Caenorhabditis elegans*. *Behav Neurosci* **118**: 1206-13.

Avery, L. and H.R. Horvitz. 1990. Effects of starvation and neuroactive drugs on feeding in *Caenorhabditis elegans*. *J Exp Zool* **253**: 263-70.

Bandyopadhyay, J., J. Lee, J.I. Lee, J.R. Yu, C. Jee, J.H. Cho, S. Jung, M.H. Lee, S. Zannoni, A. Singson, H. Kim do, H.S. Koo, and J. Ahnn. 2002. Calcineurin, a calcium/calmodulin-dependent protein phosphatase, is involved in movement, fertility, egg laying, and growth in *Caenorhabditis elegans*. *Mol Biol Cell* **13**: 3281-93.

Bargmann, C.I., J.H. Thomas, and H.R. Horvitz. 1990. Chemosensory cell function in the behavior and development of *Caenorhabditis elegans*. *Cold Spring Harb Symp Quant Biol* **55**: 529-38.

Bargmann, C.I. and H.R. Horvitz. 1991a. Chemosensory neurons with overlapping functions direct chemotaxis to multiple chemicals in *C. elegans*. *Neuron* **7**: 729-42.

Bargmann, C.I. and H.R. Horvitz. 1991b. Control of larval development by chemosensory neurons in *Caenorhabditis elegans*. *Science* **251**: 1243-6.

Bargmann, C.I., E. Hartwieg, and H.R. Horvitz. 1993. Odorant-selective genes and neurons mediate olfaction in *C. elegans*. *Cell* **74**: 515-27.

Bargmann, C.I. 1998. Neurobiology of the *Caenorhabditis elegans* genome. *Science* **282**: 2028-33.

Bastiani, C.A., S. Gharib, M.I. Simon, and P.W. Sternberg. 2003. *Caenorhabditis elegans* Galphaq regulates egg-laying behavior via a PLCbeta-independent and serotonin-dependent signaling pathway and likely functions both in the nervous system and in muscle. *Genetics* **165**: 1805-22.

Battu, G., E.F. Hoier, and A. Hajnal. 2003. The *C. elegans* G-protein-coupled receptor SRA-13 inhibits RAS/MAPK signalling during olfaction and vulval development. *Development* **130**: 2567-77.

Bejsovec, A. 2005. Wnt pathway activation: new relations and locations. *Cell* **120**: 11-4.

Belcheva, M.M. and C.J. Coscia. 2002. Diversity of G protein-coupled receptor signaling pathways to ERK/MAP kinase. *Neurosignals* **11**: 34-44.

Bellocchio, E.E., R.J. Reimer, R.T. Fremeau, Jr., and R.H. Edwards. 2000. Uptake of glutamate into synaptic vesicles by an inorganic phosphate transporter. *Science* **289**: 957-60.

Belluscio, L., G.H. Gold, A. Nemes, and R. Axel. 1998. Mice deficient in G(olf) are anosmic. *Neuron* **20**: 69-81.

Berger, A.J., A.C. Hart, and J.M. Kaplan. 1998. G alphas-induced neurodegeneration in *Caenorhabditis elegans*. *J Neurosci* **18**: 2871-80.

Bernhard, N. and D. van der Kooy. 2000. A behavioral and genetic dissection of two forms of olfactory plasticity in *Caenorhabditis elegans*: adaptation and habituation. *Learn Mem* **7**: 199-212.

Bettinger, J.C. and S.L. McIntire. 2004. State-dependency in *C. elegans*. *Genes Brain Behav* **3**: 266-72.

Bianchi, L., S.M. Kwok, M. Driscoll, and F. Sesti. 2003. A potassium channel-MiRP complex controls neurosensory function in *Caenorhabditis elegans*. *J Biol Chem* **278**: 12415-24.

Birnby, D.A., E.M. Link, J.J. Vowels, H. Tian, P.L. Colacurcio, and J.H. Thomas. 2000. A transmembrane guanylyl cyclase (DAF-11) and Hsp90 (DAF-21) regulate a common set of chemosensory behaviors in *Caenorhabditis elegans*. *Genetics* **155**: 85-104.

Brenner, S. 1974. The genetics of *Caenorhabditis elegans*. *Genetics* **77**: 71-94.

Brockie, P.J., J.E. Mellem, T. Hills, D.M. Madsen, and A.V. Maricq. 2001. The *C. elegans* glutamate receptor subunit NMR-1 is required for slow NMDA-activated currents that regulate reversal frequency during locomotion. *Neuron* **31**: 617-30.

Brundage, L., L. Avery, A. Katz, U.J. Kim, J.E. Mendel, P.W. Sternberg, and M.I. Simon. 1996. Mutations in a *C. elegans* Gqalpha gene disrupt movement, egg laying, and viability. *Neuron* **16**: 999-1009.

Calvert, P.D., N.V. Krasnoperova, A.L. Lyubarsky, T. Isayama, M. Nicolo, B. Kosaras, G. Wong, K.S. Gannon, R.F. Margolskee, R.L. Sidman, E.N. Pugh, Jr., C.L. Makino, and J. Lem. 2000. Phototransduction in transgenic mice after targeted deletion of the rod transducin alpha -subunit. *Proc Natl Acad Sci U S A* **97**: 13913-8.

Carlson, J.R. 2000. Insulation of signaling pathways: odor discrimination via olfactosomes? *Neuron* **25**: 503-4.

Carman, C.V., J.L. Parent, P.W. Day, A.N. Pronin, P.M. Sternweis, P.B. Wedegaertner, A.G. Gilman, J.L. Benovic, and T. Kozasa. 1999. Selective regulation of Galpha(q/11) by an RGS domain in the G protein-coupled receptor kinase, GRK2. *J Biol Chem* **274**: 34483-92.

Cassada, R.C. and R.L. Russell. 1975. The dauerlarva, a post-embryonic developmental variant of the nematode *Caenorhabditis elegans*. *Dev Biol* **46**: 326-42.

Chang, S., R.J. Johnston, Jr., and O. Hobert. 2003. A transcriptional regulatory cascade that controls left/right asymmetry in chemosensory neurons of *C. elegans*. *Genes Dev* **17**: 2123-37.

Chao, M.Y., H. Komatsu, H.S. Fukuto, H.M. Dionne, and A.C. Hart. 2004. Feeding status and serotonin rapidly and reversibly modulate a *Caenorhabditis elegans* chemosensory circuit. *Proc Natl Acad Sci U S A* **101**: 15512-7.

Chase, D.L., G.A. Patikoglou, and M.R. Koelle. 2001. Two RGS proteins that inhibit Galpha(o) and Galpha(q) signaling in *C. elegans* neurons require a Gbeta(5)-like subunit for function. *Curr Biol* **11**: 222-31.

Chen, C.K., P. Eversole-Cire, H. Zhang, V. Mancino, Y.J. Chen, W. He, T.G. Wensel, and M.I. Simon. 2003. Instability of GGL domain-containing RGS proteins in mice lacking the G protein beta-subunit Gbeta5. *Proc Natl Acad Sci U S A* **100**: 6604-9.

Cheung, B.H.H., M. Cohen, C. Rogers, O. Albayram, and M. de Bono. 2005. Experience-Dependent Modulation of *C. elegans* behavior by ambient oxygen. *Curr Biol* **15**:905-17

Chou, J.H., C.I. Bargmann, and P. Sengupta. 2001. The *Caenorhabditis elegans odr-2* gene encodes a novel Ly-6-related protein required for olfaction. *Genetics* **157**: 211-24.

Chou, J.H., E.R. Troemel, P. Sengupta, H.A. Colbert, L. Tong, D.M. Tobin, K. Roayaie, J.G. Crump, N.D. Dwyer, and C.I. Bargmann. 1996. Olfactory recognition and discrimination in *Caenorhabditis elegans*. *Cold Spring Harb Symp Quant Biol* **61**: 157-64.

Chuang, H.H., E.D. Prescott, H. Kong, S. Shields, S.E. Jordt, A.I. Basbaum, M.V. Chao, and D. Julius. 2001. Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns(4,5)P2-mediated inhibition. *Nature* **411**: 957-62.

Chuang, C.F. and C.I. Bargmann. 2005. A Toll-interleukin 1 repeat protein at the synapse specifies asymmetric odorant receptor expression via ASK1 MAPKKK signaling. *Genes Dev* **19**: 270-81.

Chyb, S., P. Raghu, and R.C. Hardie. 1999. Polyunsaturated fatty acids activate the Drosophila light-sensitive channels TRP and TRPL. *Nature* **397**: 255-9.

Clapham, D.E. and E.J. Neer. 1997. G protein beta gamma subunits. *Annu Rev Pharmacol Toxicol* **37**: 167-203.

Clapham, D.E. 2003. TRP channels as cellular sensors. *Nature* **426**: 517-24.

Coburn, C.M. and C.I. Bargmann. 1996. A putative cyclic nucleotide-gated channel is required for sensory development and function in *C. elegans*. *Neuron* **17**: 695-706.

Coburn, C.M., I. Mori, Y. Ohshima, and C.I. Bargmann. 1998. A cyclic nucleotide-gated channel inhibits sensory axon outgrowth in larval and adult *Caenorhabditis elegans*: a distinct pathway for maintenance of sensory axon structure. *Development* **125**: 249-58.

Colbert, H.A. and C.I. Bargmann. 1995. Odorant-specific adaptation pathways generate olfactory plasticity in *C. elegans*. *Neuron* **14**: 803-12.

Colbert, H.A. and C.I. Bargmann. 1997. Environmental signals modulate olfactory acuity, discrimination, and memory in *Caenorhabditis elegans*. *Learn Mem* **4**: 179-91.

Colbert, H.A., T.L. Smith, and C.I. Bargmann. 1997. OSM-9, a novel protein with structural similarity to channels, is required for olfaction, mechanosensation, and olfactory adaptation in *Caenorhabditis elegans*. *J Neurosci* **17**: 8259-69.

Colosimo, M.E., A. Brown, S. Mukhopadhyay, C. Gabel, A.E. Lanjuin, A.D. Samuel, and P. Sengupta. 2004. Identification of thermosensory and olfactory neuron-specific genes via expression profiling of single neuron types. *Curr Biol* **14**: 2245-51.

Couwenbergs, C., A.C. Spilker, and M. Gotta. 2004. Control of embryonic spindle positioning and Galpha activity by *C. elegans* RIC-8. *Curr Biol* **14**: 1871-6.

Croll, N.A. 1975. Behavioural analysis of nematode movement. *Adv Parasitol* **13**: 71-122.

Culotti, J.G. and R.L. Russell. 1978. Osmotic avoidance defective mutants of the nematode *Caenorhabditis elegans*. *Genetics* **90**: 243-56.
Cuppen, E., A. M. van der Linden, G. Jansen and R. H. Plasterk. 2003. Proteins interacting with *Caenorhabditis elegans* Gα subunits. *Comp Funct Genomics* **4**: 479–91.
Daaka, Y., J.A. Pitcher, M. Richardson, R.H. Stoffel, J.D. Robishaw, and R.J. Lefkowitz. 1997. Receptor and G betagamma isoform-specific interactions with G protein-coupled receptor kinases. *Proc Natl Acad Sci U S A* **94**: 2180-5.
da Graca, L.S., K.K. Zimmerman, M.C. Mitchell, M. Kozhan-Gorodetska, K. Sekiewicz, Y. Morales, and G.I. Patterson. 2004. DAF-5 is a Ski oncoprotein homolog that functions in a neuronal TGF beta pathway to regulate *C. elegans* dauer development. *Development* **131**: 435-46.
Daniels, S.A., M. Ailion, J.H. Thomas, and P. Sengupta. 2000. *egl-4* acts through a transforming growth factor-beta/SMAD pathway in *Caenorhabditis elegans* to regulate multiple neuronal circuits in response to sensory cues. *Genetics* **156**: 123-41.
Davies, A.G., J.T. Pierce-Shimomura, H. Kim, M.K. VanHoven, T.R. Thiele, A. Bonci, C.I. Bargmann, and S.L. McIntire. 2003. A central role of the BK potassium channel in behavioral responses to ethanol in *C. elegans*. *Cell* **115**: 655-66.
Day, P.W., C.V. Carman, R. Sterne-Marr, J.L. Benovic, and P.B. Wedegaertner. 2003. Differential interaction of GRK2 with members of the G alpha q family. *Biochemistry* **42**: 9176-84.
de Bono, M., D.M. Tobin, M.W. Davis, L. Avery, and C.I. Bargmann. 2002. Social feeding in *Caenorhabditis elegans* is induced by neurons that detect aversive stimuli. *Nature* **419**: 899-903.
Dillin, A., D.K. Crawford, and C. Kenyon. 2002. Timing requirements for insulin/IGF-1 signaling in *C. elegans*. *Science* **298**: 830-4.
Dong, M.Q., D. Chase, G.A. Patikoglou, and M.R. Koelle. 2000. Multiple RGS proteins alter neural G protein signaling to allow *C. elegans* to rapidly change behavior when fed. *Genes Dev* **14**: 2003-14.
Driscoll, M. and J. Kaplan. 1997. Mechanotransduction. In *C. elegans II* (eds. D.S. Riddle et al.), pp. 645-78. Cold Spring Harbor Press, Cold Spring Harbor, NY.
Du, Q., L. Taylor, D.A. Compton, and I.G. Macara. 2002. LGN blocks the ability of NuMA to bind and stabilize microtubules. A mechanism for mitotic spindle assembly regulation. *Curr Biol* **12**: 1928-33.
Duerr, J.S., D.L. Frisby, J. Gaskin, A. Duke, K. Asermely, D. Huddleston, L.E. Eiden, and J.B. Rand. 1999. The cat-1 gene of *Caenorhabditis elegans* encodes a vesicular monoamine transporter required for specific monoamine-dependent behaviors. *J Neurosci* **19**: 72-84.
Dusenbery, D.B. 1974. Analysis of chemotaxis in the nematode *Caenorhabditis elegans* by countercurrent separation. *J Exp Zool* **188**: 41-7.
Dusenbery, D.B., R.E. Sheridan, and R.L. Russell. 1975. Chemotaxis-defective mutants of the nematode *Caenorhabditis elegans*. *Genetics* **80**: 297-309.
Estevez, M., L. Attisano, J.L. Wrana, P.S. Albert, J. Massague, and D.L. Riddle. 1993. The *daf-4* gene encodes a bone morphogenetic protein receptor controlling *C. elegans* dauer larva development. *Nature* **365**: 644-9.
Evanko, D.S., M.M. Thiyagarajan, and P.B. Wedegaertner. 2000. Interaction with Gbetagamma is required for membrane targeting and palmitoylation of Galpha(s) and Galpha(q). *J Biol Chem* **275**: 1327-36.
Evanko, D.S., M.M. Thiyagarajan, D.P. Siderovski, and P.B. Wedegaertner. 2001. Gbeta gamma isoforms selectively rescue plasma membrane localization and palmitoylation of mutant Galphas and Galphaq. *J Biol Chem* **276**: 23945-53.
Felkai, S., J.J. Ewbank, J. Lemieux, J.C. Labbe, G.G. Brown, and S. Hekimi. 1999. CLK-1 controls respiration, behavior and aging in the nematode *Caenorhabditis elegans*. *EMBO J* **18**: 1783-92.
Feng, J., F. Bussiere, and S. Hekimi. 2001. Mitochondrial electron transport is a key determinant of life span in *Caenorhabditis elegans*. *Dev Cell* **1**: 633-44.
Fino Silva, I. and R.H. Plasterk. 1990. Characterization of a G-protein alpha-subunit gene from the nematode *Caenorhabditis elegans*. *J Mol Biol* **215**: 483-7.
Firestein, S. 2001. How the olfactory system makes sense of scents. *Nature* **413**: 211-8.
Fukuto, H.S., D.M. Ferkey, A.J. Apicella, H. Lans, T. Sharmeen, W. Chen, R.J. Lefkowitz, G. Jansen, W.R. Schafer, and A.C. Hart. 2004. G protein-coupled receptor kinase function is essential for chemosensation in *C. elegans*. *Neuron* **42**: 581-93.
Georgi, L.L., P.S. Albert, and D.L. Riddle. 1990. *daf-1*, a *C. elegans* gene controlling dauer larva development, encodes a novel receptor protein kinase. *Cell* **61**: 635-45.

Golden, J.W. and D.L. Riddle. 1982. A pheromone influences larval development in the nematode *Caenorhabditis elegans*. *Science* **218**: 578-80.
Golden, J.W. and D.L. Riddle. 1984a. The *Caenorhabditis elegans* dauer larva: developmental effects of pheromone, food, and temperature. *Dev Biol* **102**: 368-78.
Golden, J.W. and D.L. Riddle. 1984b. A *Caenorhabditis elegans* dauer-inducing pheromone and an antagonistic component of the food supply. J. Chem. Ecol. **10**: 1265-1280
Goldman, A.L., W. van der Goes van Naters, D. Lessing, C.G. Warr, and J.R. Carlson. 2005. Coexpression of two functional odor receptors in one neuron. *Neuron* **45**: 661-6.
Gomez, M., E. De Castro, E. Guarin, H. Sasakura, A. Kuhara, I. Mori, T. Bartfai, C.I. Bargmann, and P. Nef. 2001. Ca2+ signaling via the neuronal calcium sensor-1 regulates associative learning and memory in *C. elegans*. *Neuron* **30**: 241-8.
Goodman, M.B., D.H. Hall, L. Avery, and S.R. Lockery. 1998. Active currents regulate sensitivity and dynamic range in *C. elegans* neurons. *Neuron* **20**: 763-72.
Gotta, M. and J. Ahringer. 2001. Distinct roles for Galpha and Gbetagamma in regulating spindle position and orientation in *Caenorhabditis elegans* embryos. *Nat Cell Biol* **3**: 297-300.
Gotta, M., Y. Dong, Y.K. Peterson, S.M. Lanier, and J. Ahringer. 2003. Asymmetrically distributed *C. elegans* homologs of AGS3/PINS control spindle position in the early embryo. *Curr Biol* **13**: 1029-37.
Gottlieb, S. and G. Ruvkun. 1994. *daf-2, daf-16* and *daf-23*: genetically interacting genes controlling Dauer formation in *Caenorhabditis elegans*. *Genetics* **137**: 107-20.
Gray, J.M., D.S. Karow, H. Lu, A.J. Chang, J.S. Chang, R.E. Ellis, M.A. Marletta, and C.I. Bargmann. 2004. Oxygen sensation and social feeding mediated by a *C. elegans* guanylate cyclase homologue. *Nature* **430**: 317-22
Gray, J.M., J.J. Hill, and C.I. Bargmann. 2005. A circuit for navigation in *Caenorhabditis elegans*. *Proc Natl Acad Sci U S A* **102**: 3184-91.
Gregoire, F.M., N. Chomiki, D. Kachinskas, and C.H. Warden. 1998. Cloning and developmental regulation of a novel member of the insulin-like gene family in *Caenorhabditis elegans*. *Biochem Biophys Res Commun* **249**: 385-90.
Gu, J.L., S. Muller, V. Mancino, S. Offermanns, and M.I. Simon. 2002. Interaction of G alpha(12) with G alpha(13) and G alpha(q) signaling pathways. *Proc Natl Acad Sci U S A* **99**: 9352-7.
Gunther C.V., L.L. Georgi, and D.L. Riddle. 2000. A *Caenorhabditis elegans* type I TGF beta receptor can function in the absence of type II kinase to promote larval development. *Development* **127**:3337-47.
Hajdu-Cronin, Y.M., W.J. Chen, G. Patikoglou, M.R. Koelle, and P.W. Sternberg. 1999. Antagonism between G(o)alpha and G(q)alpha in *Caenorhabditis elegans*: the RGS protein EAT-16 is necessary for G(o)alpha signaling and regulates G(q)alpha activity. *Genes Dev* **13**: 1780-93.
Hamm, H.E. 1998. The many faces of G protein signaling. *J Biol Chem* **273**: 669-72.
Hampoelz, B. and J.A. Knoblich. 2004. Heterotrimeric G proteins: new tricks for an old dog. *Cell* **119**: 453-6.
Hart, A.C., S. Sims, and J.M. Kaplan. 1995. Synaptic code for sensory modalities revealed by *C. elegans* GLR-1 glutamate receptor. *Nature* **378**: 82-5.
Hart, M.J., X. Jiang, T. Kozasa, W. Roscoe, W.D. Singer, A.G. Gilman, P.C. Sternweis, and G. Bollag. 1998. Direct stimulation of the guanine nucleotide exchange activity of p115 RhoGEF by Galpha13. *Science* **280**: 2112-4.
Hart, A.C., J. Kass, J.E. Shapiro, and J.M. Kaplan. 1999. Distinct signaling pathways mediate touch and osmosensory responses in a polymodal sensory neuron. *J Neurosci* **19**: 1952-8.
He, W., K. Yasumatsu, V. Varadarajan, A. Yamada, J. Lem, Y. Ninomiya, R.F. Margolskee, and S. Damak. 2004. Umami taste responses are mediated by alpha-transducin and alpha-gustducin. *J Neurosci* **24**: 7674-80.
Hedgecock, E.M. and R.L. Russell. 1975. Normal and mutant thermotaxis in the nematode *Caenorhabditis elegans*. *Proc Natl Acad Sci U S A* **72**: 4061-5.
Hilliard, M.A., C.I. Bargmann, and P. Bazzicalupo. 2002. *C. elegans* responds to chemical repellents by integrating sensory inputs from the head and the tail. *Curr Biol* **12**: 730-4.
Hilliard, M.A., C. Bergamasco, S. Arbucci, R.H. Plasterk, and P. Bazzicalupo. 2004. Worms taste bitter: ASH neurons, QUI-1, GPA-3 and ODR-3 mediate quinine avoidance in *Caenorhabditis elegans*. *EMBO J* **23**: 1101-1111.
Hilliard, M.A., A.J. Apicella, R. Kerr, H. Suzuki, P. Bazzicalupo, and W.R. Schafer. 2005. In vivo imaging of *C. elegans* ASH neurons: cellular response and adaptation to chemical repellents. *EMBO J* **24**: 63-72.
Hirotsu, T., S. Saeki, M. Yamamoto, and Y. Iino. 2000. The Ras-MAPK pathway is important for olfaction in *Caenorhabditis elegans*. *Nature* **404**: 289-93.

Hobert, O., K. Tessmar, and G. Ruvkun. 1999. The *Caenorhabditis elegans* lim-6 LIM homeobox gene regulates neurite outgrowth and function of particular GABAergic neurons. *Development* **126**: 1547-62.

Hsin, H. and C. Kenyon. 1999. Signals from the reproductive system regulate the lifespan of *C. elegans*. *Nature* **399**: 362-6.

Huang, L., Y.G. Shanker, J. Dubauskaite, J.Z. Zheng, W. Yan, S. Rosenzweig, A.I. Spielman, M. Max, and R.F. Margolskee. 1999. Ggamma13 colocalizes with gustducin in taste receptor cells and mediates IP3 responses to bitter denatonium. *Nat Neurosci* **2**: 1055-62.

Hurowitz, E.H., J.M. Melnyk, Y. Chen, H. Kouros-Mehr, M.I. Simon, and H. Shizuya. 2000. Genomic characterization of the human heterotrimeric G protein α, β and γ subunit genes. *DNA Research* **7**: 111-20

Ishihara, T., Y. Iino, A. Mohri, I. Mori, K. Gengyo-Ando, S. Mitani, and I. Katsura. 2002. HEN-1, a secretory protein with an LDL receptor motif, regulates sensory integration and learning in *Caenorhabditis elegans*. *Cell* **109**: 639-49.

Jansen, G., E. Hazendonk, K.L. Thijssen, and R.H. Plasterk. 1997. Reverse genetics by chemical mutagenesis in *Caenorhabditis elegans*. *Nat Genet* **17**: 119-21.

Jansen, G., K.L. Thijssen, P. Werner, M. van der Horst, E. Hazendonk, and R.H. Plasterk. 1999. The complete family of genes encoding G proteins of *Caenorhabditis elegans*. *Nat Genet* **21**: 414-9.

Jansen, G., D. Weinkove, and R.H. Plasterk. 2002. The G-protein gamma subunit gpc-1 of the nematode *C. elegans* is involved in taste adaptation. *EMBO J* **21**: 986-94.

Jeong, P.Y., M. Jung, Y.H. Yim, H. Kim, M. Park, E. Hong, W. Lee, Y.H. Kim, K. Kim, and Y.K. Paik. 2005. Chemical structure and biological activity of the *Caenorhabditis elegans* dauer-inducing pheromone. *Nature* **433**: 541-5.

Jiang, Y., W. Ma, Y. Wan, T. Kozasa, S. Hattori, and X.Y. Huang. 1998. The G protein G alpha12 stimulates Bruton's tyrosine kinase and a rasGAP through a conserved PH/BM domain. *Nature* **395**: 808-13.

Johnston, R.J. and O. Hobert. 2003. A microRNA controlling left/right neuronal asymmetry in *Caenorhabditis elegans*. *Nature* **426**: 845-9.

Kahn-Kirby, A.H., J.L. Dantzker, A.J. Apicella, W.R. Schafer, J. Browse, C.I. Bargmann, and J.L. Watts. 2004. Specific polyunsaturated fatty acids drive TRPV-dependent sensory signaling in vivo. *Cell* **119**: 889-900.

Kamath, R.S., A.G. Fraser, Y. Dong, G. Poulin, R. Durbin, M. Gotta, A. Kanapin, N. Le Bot, S. Moreno, M. Sohrmann, D.P. Welchman, P. Zipperlen, and J. Ahringer. 2003. Systematic functional analysis of the *Caenorhabditis elegans* genome using RNAi. *Nature* **421**: 231-7.

Kaplan, J.M. and H.R. Horvitz. 1993. A dual mechanosensory and chemosensory neuron in *Caenorhabditis elegans*. *Proc Natl Acad Sci U S A* **90**: 2227-31.

Katanaev, V.L., R. Ponzielli, M. Semeriva, and A. Tomlinson. 2005. Trimeric G protein-dependent frizzled signaling in Drosophila. *Cell* **120**: 111-22.

Kawano, T., Y. Ito, M. Ishiguro, K. Takuwa, T. Nakajima, and Y. Kimura. 2000. Molecular cloning and characterization of a new insulin/IGF-like peptide of the nematode *Caenorhabditis elegans*. *Biochem Biophys Res Commun* **273**: 431-6.

Kenyon, C., J. Chang, E. Gensch, A. Rudner, and R. Tabtiang. 1993. A *C. elegans* mutant that lives twice as long as wild type. *Nature* **366**: 461-4.

Kim, K. and C. Li. 2004. Expression and regulation of an FMRFamide-related neuropeptide gene family in *Caenorhabditis elegans*. *J Comp Neurol* **475**: 540-50.

Kimple, R.J., M.E. Kimple, L. Betts, J. Sondek, and D.P. Siderovski. 2002. Structural determinants for GoLoco-induced inhibition of nucleotide release by Galpha subunits. *Nature* **416**: 878-81.

Kimura, K.D., H.A. Tissenbaum, Y. Liu, and G. Ruvkun. 1997. *daf-2*, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. *Science* **277**: 942-6.

Kindt, K.S., T. Tam, S. Whiteman, and W.R. Schafer. 2002. Serotonin promotes G(o)-dependent neuronal migration in *Caenorhabditis elegans*. *Curr Biol* **12**: 1738-47.

Kisselev, O. and N. Gautam. 1993. Specific interaction with rhodopsin is dependent on the gamma subunit type in a G protein. *J Biol Chem* **268**: 24519-22.

Klass, M.R. 1977. Aging in the nematode *Caenorhabditis elegans*: major biological and environmental factors influencing life span. *Mech Ageing Dev* **6**: 413-29.

Klein, S., H. Reuveni, and A. Levitzki. 2000. Signal transduction by a nondissociable heterotrimeric yeast G protein. *Proc Natl Acad Sci U S A* **97**: 3219-23.

Koch, W.J., J. Inglese, W.C. Stone, and R.J. Lefkowitz. 1993. The binding site for the beta gamma subunits of heterotrimeric G proteins on the beta-adrenergic receptor kinase. *J Biol Chem* **268**: 8256-60.

Koch, W.J., B.E. Hawes, J. Inglese, L.M. Luttrell, and R.J. Lefkowitz. 1994. Cellular expression of the carboxyl terminus of a G protein-coupled receptor kinase attenuates G beta gamma-mediated signaling. *J Biol Chem* **269**: 6193-7.

Koelle, M.R. and H.R. Horvitz. 1996. EGL-10 regulates G protein signaling in the *C. elegans* nervous system and shares a conserved domain with many mammalian proteins. *Cell* **84**: 115-25.

Komatsu, H., I. Mori, J.S. Rhee, N. Akaike, and Y. Ohshima. 1996. Mutations in a cyclic nucleotide-gated channel lead to abnormal thermosensation and chemosensation in *C. elegans*. *Neuron* **17**: 707-18.

Komatsu, H., Y.H. Jin, N. L'Etoile, I. Mori, C.I. Bargmann, N. Akaike, and Y. Ohshima. 1999. Functional reconstitution of a heteromeric cyclic nucleotide-gated channel of *Caenorhabditis elegans* in cultured cells. *Brain Res* **821**: 160-8.

Korswagen, H.C., J.H. Park, Y. Ohshima, and R.H. Plasterk. 1997. An activating mutation in a *Caenorhabditis elegans* Gs protein induces neural degeneration. *Genes Dev* **11**: 1493-503.

Kozasa, T., X. Jiang, M.J. Hart, P.M. Sternweis, W.D. Singer, A.G. Gilman, G. Bollag, and P.C. Sternweis. 1998. p115 RhoGEF, a GTPase activating protein for Galpha12 and Galpha13. *Science* **280**: 2109-11.

Kuhara, A., H. Inada, I. Katsura, and I. Mori. 2002. Negative regulation and gain control of sensory neurons by the *C. elegans* calcineurin TAX-6. *Neuron* **33**: 751-63.

Lackner, M.R., S.J. Nurrish, and J.M. Kaplan. 1999. Facilitation of synaptic transmission by EGL-30 Gqalpha and EGL-8 PLCbeta: DAG binding to UNC-13 is required to stimulate acetylcholine release. *Neuron* **24**: 335-46.

Lakowski, B. and S. Hekimi. 1998. The genetics of caloric restriction in *Caenorhabditis elegans*. *Proc Natl Acad Sci U S A* **95**: 13091-6.

Lanjuin, A. and P. Sengupta. 2002. Regulation of chemosensory receptor expression and sensory signaling by the KIN-29 Ser/Thr kinase. *Neuron* **33**: 369-81.

Lanjuin, A. and P. Sengupta. 2004. Specification of chemosensory neuron subtype identities in *Caenorhabditis elegans*. *Curr Opin Neurobiol* **14**: 22-30.

Lans, H., S. Rademakers, and G. Jansen. 2004. A network of stimulatory and inhibitory Galpha-subunits regulates olfaction in *Caenorhabditis elegans*. *Genetics* **167**: 1677-87.

Larsen, P.L., P.S. Albert, and D.L. Riddle. 1995. Genes that regulate both development and longevity in *Caenorhabditis elegans*. *Genetics* **139**: 1567-83.

Law, E., W.M. Nuttley, and D. van der Kooy. 2004. Contextual taste cues modulate olfactory learning in *C. elegans* by an occasion-setting mechanism. *Curr Biol* **14**: 1303-8.

Lee, R.Y., E.R. Sawin, M. Chalfie, H.R. Horvitz, and L. Avery. 1999. EAT-4, a homolog of a mammalian sodium-dependent inorganic phosphate cotransporter, is necessary for glutamatergic neurotransmission in *Caenorhabditis elegans*. *J Neurosci* **19**: 159-67.

Lee, J., C. Jee, H.O. Song, J. Bandyopadhyay, J.I. Lee, J.R. Yu, B.J. Park, and J. Ahnn. 2004. Opposing functions of calcineurin and CaMKII regulate G-protein signaling in egg-laying behavior of *C. elegans*. *J Mol Biol* **344**: 585-95.

Lerea, C.L., D.E. Somers, J.B. Hurley, I.B. Klock, and A.H. Bunt-Milam. 1986. Identification of specific transducin alpha subunits in retinal rod and cone photoreceptors. *Science* **234**: 77-80.

L'Etoile, N.D. and C.I. Bargmann. 2000. Olfaction and odor discrimination are mediated by the *C. elegans* guanylyl cyclase ODR-1. *Neuron* **25**: 575-86.

L'Etoile, N.D., C.M. Coburn, J. Eastham, A. Kistler, G. Gallegos, and C.I. Bargmann. 2002. The cyclic GMP-dependent protein kinase EGL-4 regulates olfactory adaptation in *C. elegans*. *Neuron* **36**: 1079-89.

Li C., K. Kim, L.S. Nelson. 1999. FMRFamide-related neuropeptide gene family in *Caenorhabditis elegans*. *Brain Res* **848**:26–34.

Li, W., S.G. Kennedy, and G. Ruvkun. 2003. *daf-28* encodes a *C. elegans* insulin superfamily member that is regulated by environmental cues and acts in the DAF-2 signaling pathway. *Genes Dev* **17**: 844-58.

Liedtke, W. and J.M. Friedman. 2003. Abnormal osmotic regulation in trpv4-/- mice. *Proc Natl Acad Sci U S A* **100**: 13698-703.

Liedtke, W., D.M. Tobin, C.I. Bargmann, and J.M. Friedman. 2003. Mammalian TRPV4 (VR-OAC) directs behavioral responses to osmotic and mechanical stimuli in *Caenorhabditis elegans*. *Proc Natl Acad Sci U S A* **100 Suppl 2**: 14531-6.

Lin, K., J.B. Dorman, A. Rodan, and C. Kenyon. 1997. *daf-16*: An HNF-3/forkhead family member that can function to double the life-span of *Caenorhabditis elegans*. *Science* **278**: 1319-22.

Lin, K., H. Hsin, N. Libina, and C. Kenyon. 2001. Regulation of the *Caenorhabditis elegans* longevity protein DAF-16 by insulin/IGF-1 and germline signaling. *Nat Genet* **28**: 139-45.

Lindemann, B. 2001. Receptors and transduction in taste. *Nature* **413**: 219-25.

Liu, D.W. and J.H. Thomas. 1994. Regulation of a periodic motor program in *C. elegans*. *J Neurosci* **14**: 1953-62.
Liu, L.X., J.M. Spoerke, E.L. Mulligan, J. Chen, B. Reardon, B. Westlund, L. Sun, K. Abel, B. Armstrong, G. Hardiman, J. King, L. McCague, M. Basson, R. Clover, C.D. Johnson. 1999. High-throughput isolation of *Caenorhabditis elegans* deletion mutants. *Genome Res* **9**:859-67
Lochrie, M.A., J.E. Mendel, P.W. Sternberg, and M.I. Simon. 1991. Homologous and unique G protein alpha subunits in the nematode *Caenorhabditis elegans*. *Cell Regul* **2**: 135-54.
Lyall, V., G.L. Heck, A.K. Vinnikova, S. Ghosh, T.H. Phan, R.I. Alam, O.F. Russell, S.A. Malik, J.W. Bigbee, and J.A. DeSimone. 2004. The mammalian amiloride-insensitive non-specific salt taste receptor is a vanilloid receptor-1 variant. *J Physiol* **558**: 147-59.
Lyall, V., G.L. Heck, A.K. Vinnikova, S. Ghosh, T.H. Phan, and J.A. Desimone. 2005. A Novel Vanilloid Receptor-1 (VR-1) Variant Mammalian Salt Taste Receptor. *Chem Senses* **30 Suppl 1**: i42-i43.
Makino, E.R., J.W. Handy, T. Li, and V.Y. Arshavsky. 1999. The GTPase activating factor for transducin in rod photoreceptors is the complex between RGS9 and type 5 G protein beta subunit. *Proc Natl Acad Sci U S A* **96**: 1947-52.
Malone, E.A. and J.H. Thomas. 1994. A screen for nonconditional dauer-constitutive mutations in *Caenorhabditis elegans*. *Genetics* **136**: 879–86.
Malone, E.A., T. Inoue, and J.H. Thomas. 1996. Genetic analysis of the roles of *daf-28* and *age-1* in regulating *Caenorhabditis elegans* dauer formation. *Genetics* **143**: 1193–1205.
Maricq, A.V., E. Peckol, M. Driscoll, and C.I. Bargmann. 1995. Mechanosensory signalling in *C. elegans* mediated by the GLR-1 glutamate receptor. *Nature* **378**: 78-81.
Marinissen, M.J. and J.S. Gutkind. 2001. G-protein-coupled receptors and signaling networks: emerging paradigms. *Trends Pharmacol Sci* **22**: 368-76.
Melkman, T. and P. Sengupta. 2004. The worm's sense of smell. Development of functional diversity in the chemosensory system of *Caenorhabditis elegans*. *Dev Biol* **265**: 302-19.
Mellem, J.E., P.J. Brockie, Y. Zheng, D.M. Madsen, and A.V. Maricq. 2002. Decoding of polymodal sensory stimuli by postsynaptic glutamate receptors in *C. elegans*. *Neuron* **36**: 933-44.
Mello, C.C., J.M. Kramer D. Stinchcomb, and V. Ambros. 1991. Efficient gene transfer in *C. elegans*: extrachromosomal maintenance and integration of transforming sequences. *EMBO J* **10**: 3959-70.
Mendel, J.E., H.C. Korswagen, K.S. Liu, Y.M. Hajdu-Cronin, M.I. Simon, R.H. Plasterk, and P.W. Sternberg. 1995. Participation of the protein Go in multiple aspects of behavior in *C. elegans*. *Science* **267**: 1652-5.
Milani, N., E. Guarin, E. Renfer, P. Nef, and P.J. Andres-Barquin. 2002. Functional expression of a mammalian olfactory receptor in *Caenorhabditis elegans*. *Neuroreport* **13**: 2515-20.
Miller, K.G., M.D. Emerson, and J.B. Rand. 1999. Goalpha and diacylglycerol kinase negatively regulate the Gqalpha pathway in *C. elegans*. *Neuron* **24**: 323-33.
Miller, K.G., M.D. Emerson, J.R. McManus, and J.B. Rand. 2000. RIC-8 (Synembryn): a novel conserved protein that is required for G(q)alpha signaling in the *C. elegans* nervous system. *Neuron* **27**: 289-99.
Miller, K.G. and J.B. Rand. 2000. A role for RIC-8 (Synembryn) and GOA-1 (G(o)alpha) in regulating a subset of centrosome movements during early embryogenesis in *Caenorhabditis elegans*. *Genetics* **156**: 1649-60.
Miller, A.C., T.R. Thiele, S. Faumont, M.L. Moravec, and S.R. Lockery. 2005. Step-response analysis of chemotaxis in *Caenorhabditis elegans*. *J Neurosci* **25**: 3369-78.
Miyahara, K., N. Suzuki, T. Ishihara, E. Tsuchiya, and I. Katsura. 2004. TBX2/TBX3 transcriptional factor homologue controls olfactory adaptation in *Caenorhabditis elegans*. *J Neurobiol* **58**: 392-402.
Moghal, N., L.R. Garcia, L.A. Khan, K. Iwasaki, and P.W. Sternberg. 2003. Modulation of EGF receptor-mediated vulva development by the heterotrimeric G-protein Galphaq and excitable cells in *C. elegans*. *Development* **130**: 4553-66.
Mori, I. and Y. Ohshima. 1995. Neural regulation of thermotaxis in *Caenorhabditis elegans*. *Nature* **376**: 344-8.
Mori, I. 1999. Genetics of chemotaxis and thermotaxis in the nematode *Caenorhabditis elegans*. *Annu Rev Genet* **33**:399-422
Morris, J.Z., H.A. Tissenbaum, and G. Ruvkun. 1996. A phosphatidylinositol-3-OH kinase family member regulating longevity and diapause in *Caenorhabditis elegans*. *Nature* **382**: 536-9.
Morrison, G.E., J.Y. Wen, S. Runciman, and D. van der Kooy. 1999. Olfactory associative learning in *Caenorhabditis elegans* is impaired in lrn-1 and lrn-2 mutants. *Behav Neurosci* **113**: 358-67.

Morrison, G.E. and D. van der Kooy. 2001. A mutation in the AMPA-type glutamate receptor, glr-1, blocks olfactory associative and nonassociative learning in *Caenorhabditis elegans*. *Behav Neurosci* **115**: 640-9.
Murakami, M., M. Koga, and Y. Ohshima. 2001. DAF-7/TGF-beta expression required for the normal larval development in *C. elegans* is controlled by a presumed guanylyl cyclase DAF-11. *Mech Dev* **109**: 27-35.
Murphy, C.T., S.A. McCarroll, C.I. Bargmann, A. Fraser, R.S. Kamath, J. Ahringer, H. Li, and C. Kenyon. 2003. Genes that act downstream of DAF-16 to influence the lifespan of *Caenorhabditis elegans*. *Nature* **424**: 277-83.
Nathoo A.N., R.A. Moeller, B.A. Westlund, A.C. Hart. 2001. Identification of neuropeptide-like protein gene families in *Caenorhabditis elegans* and other species. *Proc Natl Acad Sci U S A* **98**:14000-5
Nelson L.S., K. Kim, J.E. Memmott, C. Li. 1998. FMRFamide-related gene family in the nematode, *Caenorhabditis elegans*. *Brain Res Mol Brain Res* **58**:103–11.
Neves, S.R., P.T. Ram, and R. Iyengar. 2002. G protein pathways. *Science* **296**: 1636-9.
Nolan, K.M., T.R. Sarafi-Reinach, J.G. Horne, A.M. Saffer, and P. Sengupta. 2002. The DAF-7 TGF-beta signaling pathway regulates chemosensory receptor gene expression in *C. elegans*. *Genes Dev* **16**: 3061-73.
Numazaki M., T. Tominaga, H. Toyooka, and M. Tominaga. 2002. Direct phosphorylation of capsaicin receptor VR1 by Protein Kinase Cε and identification of two target serine residues. *J Biol Chem* **277**:13375-78
Nurrish, S., L. Segalat, and J.M. Kaplan. 1999. Serotonin inhibition of synaptic transmission: Galpha(0) decreases the abundance of UNC-13 at release sites. *Neuron* **24**: 231-42.
Nuttley, W.M., S. Harbinder, and D. van der Kooy. 2001. Regulation of distinct attractive and aversive mechanisms mediating benzaldehyde chemotaxis in *Caenorhabditis elegans*. *Learn Mem* **8**: 170-81.
Nuttley, W.M., K.P. Atkinson-Leadbeater, and D. van der Kooy. 2002. Serotonin mediates food-odor associative learning in the nematode *Caenorhabditis elegans*. *Proc Natl Acad Sci U S A* **99**: 12449-54.
Offermanns, S., C.F. Toombs, Y.H. Hu, and M.I. Simon. 1997a. Defective platelet activation in G alpha(q)-deficient mice. *Nature* **389**: 183-6.
Offermanns, S., K. Hashimoto, M. Watanabe, W. Sun, H. Kurihara, R.F. Thompson, Y. Inoue, M. Kano, and M.I. Simon. 1997b. Impaired motor coordination and persistent multiple climbing fiber innervation of cerebellar Purkinje cells in mice lacking Galphaq. *Proc Natl Acad Sci U S A* **94**: 14089-94.
Offermanns, S., V. Mancino, J.P. Revel, and M.I. Simon. 1997c. Vascular system defects and impaired cell chemokinesis as a result of Galpha13 deficiency. *Science* **275**: 533-6.
Ogg, S., S. Paradis, S. Gottlieb, G.I. Patterson, L. Lee, H.A. Tissenbaum, and G. Ruvkun. 1997. The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in *C. elegans*. *Nature* **389**: 994-9.
Oh, S.W., A. Mukhopadhyay, N. Svrzikapa, F. Jiang, R.J. Davis, and H.A. Tissenbaum. 2005. JNK regulates lifespan in *Caenorhabditis elegans* by modulating nuclear translocation of forkhead transcription factor/DAF-16. *Proc Natl Acad Sci U S A* **102**: 4494-9.
Okochi, Y., K.D. Kimura, A. Ohta, and I. Mori. 2005. Diverse regulation of sensory signaling by *C. elegans* nPKC-epsilon/eta TTX-4. *EMBO* **24**: 2127-37
Palmitessa, A., H.A. Hess, I. A. Bany, Y. Kim, M.R. Koelle, J.L. Benovic. 2005. *C. elegans* arrestin regulates neural G protein signaling and olfactory adaptation and recovery. *J Biol Chem* 280: 24649-62
Paradis, S., M. Ailion, A. Toker, J.H. Thomas, and G. Ruvkun. 1999. A PDK1 homolog is necessary and sufficient to transduce AGE-1 PI3 kinase signals that regulate diapause in *Caenorhabditis elegans*. *Genes Dev* **13**: 1438-52.
Paradis, S. and G. Ruvkun. 1998. *Caenorhabditis elegans* Akt/PKB transduces insulin receptor-like signals from AGE-1 PI3 kinase to the DAF-16 transcription factor. *Genes Dev* **12**: 2488-98.
Park, J.H., S. Ohshima, T. Tani, and Y. Ohshima. 1997. Structure and expression of the gsa-1 gene encoding a G protein alpha(s) subunit in *C. elegans*. *Gene* **194**: 183-90.
Patterson, G.I., A. Koweek, A. Wong, Y. Liu, and G. Ruvkun. 1997. The DAF-3 Smad protein antagonizes TGF-beta-related receptor signaling in the *Caenorhabditis elegans* dauer pathway. *Genes Dev* **11**: 2679-90.
Peckol, E.L., J.A. Zallen, J.C. Yarrow, and C.I. Bargmann. 1999. Sensory activity affects sensory axon development in *C. elegans*. *Development* **126**: 1891-902.

Peckol, E.L., E.R. Troemel, and C.I. Bargmann. 2001. Sensory experience and sensory activity regulate chemosensory receptor gene expression in *Caenorhabditis elegans*. *Proc Natl Acad Sci U S A* **98**: 11032-8.

Pierce, S.B., M. Costa, R. Wisotzkey, S. Devadhar, S.A. Homburger, A.R. Buchman, K.C. Ferguson, J. Heller, D.M. Platt, A.A. Pasquinelli, L.X. Liu, S.K. Doberstein, and G. Ruvkun. 2001. Regulation of DAF-2 receptor signaling by human insulin and *ins-1*, a member of the unusually large and diverse *C. elegans* insulin gene family. *Genes Dev* **15**: 672-86.

Pierce-Shimomura, J.T., T.M. Morse, and S.R. Lockery. 1999. The fundamental role of pirouettes in *Caenorhabditis elegans* chemotaxis. *J Neurosci* **19**: 9557-69.

Pierce-Shimomura, J.T., S. Faumont, M.R. Gaston, B.J. Pearson, and S.R. Lockery. 2001. The homeobox gene lim-6 is required for distinct chemosensory representations in *C. elegans*. *Nature* **410**: 694-8.

Pitcher, J.A., N.J. Freedman, and R.J. Lefkowitz. 1998. G protein-coupled receptor kinases. *Annu Rev Biochem* **67**: 653-92.

Premkumar, L.S., and G.P. Ahern. 2000. Induction of vanilloid receptor channel activity by protein kinase C. *Nature* **408**:985-90.

Prescott, E.D. and D. Julius. 2003. A modular PIP2 binding site as a determinant of capsaicin receptor sensitivity. *Science* **300**: 1284-8.

Rebois, R.V., D.R. Warner, and N.S. Basi. 1997. Does subunit dissociation necessarily accompany the activation of all heterotrimeric G proteins? *Cell Signal* **9**: 141-51.

Reed, R.R. 2004. After the holy grail: establishing a molecular basis for Mammalian olfaction. *Cell* **116**: 329-36.

Reiner, D.J., E.M. Newton, H. Tian, and J.H. Thomas. 1999. Diverse behavioural defects caused by mutations in *Caenorhabditis elegans* unc-43 CaM kinase II. *Nature* **402**: 199-203.

Ren, P., C.S. Lim, R. Johnsen, P.S. Albert, D. Pilgrim, and D.L. Riddle. 1996. Control of *C. elegans* larval development by neuronal expression of a TGF-beta homolog. *Science* **274**: 1389-91.

Riddle, D.L., M.M. Swanson, and P.S. Albert. 1981. Interacting genes in nematode dauer larva formation. *Nature* **290**: 668-71.

Riddle, D.L., T. Blumenthal, B.J. Meyer, and J. R. Priess. 1997. Introduction to *C. elegans*. In *C. elegans II* (eds. D.S. Riddle et al.), pp. 1-22. Cold Spring Harbor Press, Cold Spring Harbor, NY.

Riddle, D.L. and P.S. Albert. 1997. Genetic and environmental regulation of dauer larva development. In *C. elegans* II (eds. D.S. Riddle et al.) pp.739-68. Cold Spring Harbor, NY.

Roayaie, K., J.G. Crump, A. Sagasti, and C.I. Bargmann. 1998. The G alpha protein ODR-3 mediates olfactory and nociceptive function and controls cilium morphogenesis in *C. elegans* olfactory neurons. *Neuron* **20**: 55-67.

Robatzek, M. and J.H. Thomas. 2000. Calcium/calmodulin-dependent protein kinase II regulates *Caenorhabditis elegans* locomotion in concert with a G(o)/G(q) signaling network. *Genetics* **156**: 1069-82.

Robatzek, M., T. Niacaris, K. Steger, L. Avery, and J.H. Thomas. 2001. eat-11 encodes GPB-2, a Gbeta(5) ortholog that interacts with G(o)alpha and G(q)alpha to regulate *C. elegans* behavior. *Curr Biol* **11**: 288-93.

Robertson, H.M. 1998. Two large families of chemoreceptor genes in the nematodes *Caenorhabditis elegans* and Caenorhabditis briggsae reveal extensive gene duplication, diversification, movement, and intron loss. *Genome Res* **8**: 449-63.

Robertson, H.M. 2000. The large srh family of chemoreceptor genes in *Caenorhabditis* nematodes reveals processes of genome evolution involving large duplications and deletions and intron gains and losses. *Genome Res* **10**: 192-203.

Robertson, H.M. 2001. Updating the str and srj (stl) families of chemoreceptors in *Caenorhabditis* nematodes reveals frequent gene movement within and between chromosomes. *Chem Senses* **26**: 151-9.

Saeki, S., M. Yamamoto, and Y. Iino. 2001. Plasticity of chemotaxis revealed by paired presentation of a chemoattractant and starvation in the nematode *Caenorhabditis elegans*. *J Exp Biol* **204**: 1757-64.

Sagasti, A., N. Hisamoto, J. Hyodo, M. Tanaka-Hino, K. Matsumoto, and C.I. Bargmann. 2001. The CaMKII UNC-43 activates the MAPKKK NSY-1 to execute a lateral signaling decision required for asymmetric olfactory neuron fates. *Cell* **105**: 221-32.

Sambongi, Y., T. Nagae, Y. Liu, T. Yoshimizu, K. Takeda, Y. Wada, and M. Futai. 1999. Sensing of cadmium and copper ions by externally exposed ADL, ASE, and ASH neurons elicits avoidance response in *Caenorhabditis elegans*. *Neuroreport* **10**: 753-7.

Sambongi, Y., K. Takeda, T. Wakabayashi, I. Ueda, Y. Wada, and M. Futai. 2000. *Caenorhabditis elegans* senses protons through amphid chemosensory neurons: proton signals elicit avoidance behavior. *Neuroreport* **11**: 2229-32.

Sawin, E.R., R. Ranganathan, and H.R. Horvitz. 2000. *C. elegans* locomotory rate is modulated by the environment through a dopaminergic pathway and by experience through a serotonergic pathway. *Neuron* **26**: 619-31.

Schackwitz, W.S., T. Inoue, and J.H. Thomas. 1996. Chemosensory neurons function in parallel to mediate a pheromone response in *C. elegans*. *Neuron* **17**: 719-28.

Schade, M.A., N.K. Reynolds, C.M. Dollins, and K.G. Miller. 2005. Mutations That Rescue the Paralysis of *Caenorhabditis elegans* ric-8 (Synembryn) Mutants Activate the G{alpha}s Pathway and Define a Third Major Branch of the Synaptic Signaling Network. *Genetics* **169**: 631-49.

Schaefer, M., A. Shevchenko, and J.A. Knoblich. 2000. A protein complex containing Inscuteable and the Galpha-binding protein Pins orients asymmetric cell divisions in Drosophila. *Curr Biol* **10**: 353-62.

Schaefer, M., M. Petronczki, D. Dorner, M. Forte, and J.A. Knoblich. 2001. Heterotrimeric G proteins direct two modes of asymmetric cell division in the Drosophila nervous system. *Cell* **107**: 183-94.

Schnabel, R., H. Hutter, D. Moerman, and H. Schnabel. 1997. Assessing normal embryogenesis in *Caenorhabditis elegans* using a 4D microscope: variability of development and regional specification. *Dev Biol* **184**: 234-65.

Schwindinger, W.F. and J.D. Robishaw. 2001. Heterotrimeric G-protein betagamma-dimers in growth and differentiation. *Oncogene* **20**: 1653-60.

Scott, K. 2004. The sweet and the bitter of mammalian taste. *Curr Opin Neurobiol* **14**: 423-7.

Segalat, L., D.A. Elkes, and J.M. Kaplan. 1995. Modulation of serotonin-controlled behaviors by Go in *Caenorhabditis elegans*. *Science* **267**: 1648-51.

Sengupta, P., H.A. Colbert, and C.I. Bargmann. 1994. The *C. elegans* gene odr-7 encodes an olfactory-specific member of the nuclear receptor superfamily. *Cell* **79**: 971-80.

Sengupta, P., J.H. Chou, and C.I. Bargmann. 1996. odr-10 encodes a seven transmembrane domain olfactory receptor required for responses to the odorant diacetyl. *Cell* **84**: 899-909.

Simmer, F., C. Moorman, A.M. van der Linden, E. Kuijk, P.V. van den Berghe, R.S. Kamath, A.G. Fraser, J. Ahringer, and R.H. Plasterk. 2003. Genome-wide RNAi of *C. elegans* using the hypersensitive rrf-3 strain reveals novel gene functions. *PLoS Biol* **1**: E12.

Snow, B.E., A.M. Krumins, G.M. Brothers, S.F. Lee, M.A. Wall, S. Chung, J. Mangion, S. Arya, A.G. Gilman, and D.P. Siderovski. 1998. A G protein gamma subunit-like domain shared between RGS11 and other RGS proteins specifies binding to Gbeta5 subunits. *Proc Natl Acad Sci U S A* **95**: 13307-12.

Spiegel, A.M. 1996. Defects in G protein-coupled signal transduction in human disease. *Annu Rev Physiol* **58**: 143-70.

Srinivasan, D.G., R.M. Fisk, H. Xu, and S. van den Heuvel. 2003. A complex of LIN-5 and GPR proteins regulates G protein signaling and spindle function in *C. elegans*. *Genes Dev* **17**: 1225-39.

Starich, T.A., R.K. Herman, C.K. Kari, W. Yeh, W.S. Schackwitz, M.W. Schuyler, J. Collet, J.H. Thomas, and D.L. Riddle (1995) Mutations affecting the chemosensory neurons of *Caenorhabditis elegans*. *Genetics* **139**: 171-88.

Sulston, J.E., E. Schierenberg, J.G. White, and J.N. Thomson. 1983. The embryonic cell lineage of the nematode *Caenorhabditis elegans*. *Dev Biol* **100**: 64-119.

Sze, J.Y., M. Victor, C. Loer, Y. Shi, and G. Ruvkun. 2000. Food and metabolic signalling defects in a *Caenorhabditis elegans* serotonin-synthesis mutant. *Nature* **403**: 560-4.

Sze, J.Y. and G. Ruvkun. 2003. Activity of the *Caenorhabditis elegans* UNC-86 POU transcription factor modulates olfactory sensitivity. *Proc Natl Acad Sci U S A* **100**: 9560-5.

Takamori, S., J.S. Rhee, C. Rosenmund, and R. Jahn. 2000. Identification of a vesicular glutamate transporter that defines a glutamatergic phenotype in neurons. *Nature* **407**: 189-94.

Tall, G.G., A.M. Krumins, and A.G. Gilman. 2003. Mammalian Ric-8A (synembryn) is a heterotrimeric Galpha protein guanine nucleotide exchange factor. *J Biol Chem* **278**: 8356-62.

Tanaka-Hino, M., A. Sagasti, N. Hisamoto, M. Kawasaki, S. Nakano, J. Ninomiya-Tsuji, C.I. Bargmann, and K. Matsumoto. 2002. SEK-1 MAPKK mediates Ca2+ signaling to determine neuronal asymmetric development in *Caenorhabditis elegans*. *EMBO Rep* **3**: 56-62.

The *C. elegans* Sequencing Consortium. 1998. Genome sequence of the nematode *C. elegans*: a platform for investigating biology. *Science* **282**: 2012-8.

Thomas, J.H. 1990. Genetic analysis of defecation in *Caenorhabditis elegans*. *Genetics* **124**: 855-72.

Thomas, J.H., D.A. Birnby, and J.J. Vowels. 1993. Evidence for parallel processing of sensory information controlling dauer formation in *Caenorhabditis elegans*. *Genetics* **134**: 1105-17.

Tobin, D., D. Madsen, A. Kahn-Kirby, E. Peckol, G. Moulder, R. Barstead, A. Maricq, and C. Bargmann. 2002. Combinatorial expression of TRPV channel proteins defines their sensory functions and subcellular localization in *C. elegans* neurons. *Neuron* **35**: 307-18.

Trent, C., N. Tsuing, and H.R. Horvitz. 1983. Egg-laying defective mutants of the nematode *Caenorhabditis elegans*. *Genetics* **104**: 619-47.

Troemel, E.R., J.H. Chou, N.D. Dwyer, H.A. Colbert, and C.I. Bargmann. 1995. Divergent seven transmembrane receptors are candidate chemosensory receptors in *C. elegans*. *Cell* **83**: 207-18.

Troemel, E.R., B.E. Kimmel, and C.I. Bargmann. 1997. Reprogramming chemotaxis responses: sensory neurons define olfactory preferences in *C. elegans*. *Cell* **91**: 161-9.

Troemel, E.R., A. Sagasti, and C.I. Bargmann. 1999. Lateral signaling mediated by axon contact and calcium entry regulates asymmetric odorant receptor expression in *C. elegans*. *Cell* **99**: 387-98.

van der Linden, A.M., F. Simmer, E. Cuppen, and R.H. Plasterk. 2001. The G-protein beta-subunit GPB-2 in *Caenorhabditis elegans* regulates the G(o)alpha-G(q)alpha signaling network through interactions with the regulator of G-protein signaling proteins EGL-10 and EAT-16. *Genetics* **158**: 221-35.

van der Linden, A.M., C. Moorman, E. Cuppen, H.C. Korswagen, and R.H. Plasterk. 2003. Hyperactivation of the G12-mediated signaling pathway in *Caenorhabditis elegans* induces a developmental growth arrest via protein kinase C. *Curr Biol* **13**: 516-21.

van der Voorn, L., M. Gebbink, R.H. Plasterk, and H.L. Ploegh. 1990. Characterization of a G-protein beta-subunit gene from the nematode *Caenorhabditis elegans*. *J Mol Biol* **213**: 17-26.

Vowels, J.J. and J.H. Thomas. 1992. Genetic analysis of chemosensory control of dauer formation in *Caenorhabditis elegans*. *Genetics* **130**: 105-23.

Vowels, J.J. and J.H. Thomas. 1994. Multiple chemosensory defects in *daf-11* and *daf-21* mutants of *Caenorhabditis elegans*. *Genetics* **138**: 303-16.

Wang, Z.W., O. Saifee, M.L. Nonet, and L. Salkoff. 2001. SLO-1 potassium channels control quantal content of neurotransmitter release at the *C. elegans* neuromuscular junction. *Neuron* **32**: 867-81.

Ward, S. 1973. Chemotaxis by the nematode *Caenorhabditis elegans*: identification of attractants and analysis of the response by use of mutants. *Proc Natl Acad Sci U S A* **70**: 817-21.

Ward, S., N. Thomson, J.G. White, and S. Brenner. 1975. Electron microscopical reconstruction of the anterior sensory anatomy of the nematode *Caenorhabditis elegans*. *J Comp Neurol* **160**: 313-37.

Ware, R. W., D. Clark, K. Crossland and R. L. Russell. 1975. The nerve ring of the nematode *C. elegans*: sensory input and motor output. *J. Comp. Neurol.* **162**: 71–110.

Watson, A.J., A. Katz, and M.I. Simon. 1994. A fifth member of the mammalian G-protein beta-subunit family. Expression in brain and activation of the beta 2 isotype of phospholipase C. *J Biol Chem* **269**: 22150-6.

Watson, A.J., A.M. Aragay, V.Z. Slepak, and M.I. Simon. 1996. A novel form of the G protein beta subunit Gbeta5 is specifically expressed in the vertebrate retina. *J Biol Chem* **271**: 28154-60.

Watson, N., M.E. Linder, K.M. Druey, J.H. Kehrl, and K.J. Blumer. 1996. RGS family members: GTPase-activating proteins for heterotrimeric G-protein alpha-subunits. *Nature* **383**: 172-5.

Watts, J.L. and J. Browse. 2002. Genetic dissection of polyunsaturated fatty acid synthesis in *Caenorhabditis elegans*. *Proc Natl Acad Sci U S A* **99**: 5854-9.

Weinshenker, D., A. Wei, L. Salkoff, and J.H. Thomas. 1999. Block of an ether-a-go-go-like K(+) channel by imipramine rescues egl-2 excitation defects in *Caenorhabditis elegans*. *J Neurosci* **19**: 9831-40.

Wen, J.Y., N. Kumar, G. Morrison, G. Rambaldini, S. Runciman, J. Rousseau, and D. van der Kooy. 1997. Mutations that prevent associative learning in *C. elegans*. *Behav Neurosci* **111**: 354-68.

Wes, P.D. and C.I. Bargmann. 2001. *C. elegans* odour discrimination requires asymmetric diversity in olfactory neurons. *Nature* **410**: 698-701.

Wettschureck, N., A. Moers, and S. Offermanns. 2004. Mouse models to study G-protein-mediated signaling. *Pharmacol Ther* **101**: 75-89.

White, J. G., Southgate, E., Thomson, J. N., and Brenner, S. 1986. The structure of the nervous system of the nematode *Caenorhabditis elegans*. *Philos. Trans. R. Soc. Lond. B Biol. Sci*. **314:** 1-340.

White, J., E. Southgate, R. Durbin. 1988. Appendix 2 Neuroanatomy. In The Nematode *Caenorhabditis elegans* (eds. W.B. Wood and community of *C. elegans* researchers), pp. 433-55. Cold spring Harbor Press, Cold Spring Harbor, NY

Wicks, S.R., C.J. de Vries, H.G. van Luenen, and R.H. Plasterk. 2000. CHE-3, a cytosolic dynein heavy chain, is required for sensory cilia structure and function in *Caenorhabditis elegans*. *Dev Biol* **221**: 295-307.

Wong, G.T., L. Ruiz-Avila, D. Ming, K.S. Gannon, and R.F. Margolskee. 1996. Biochemical and transgenic analysis of gustducin's role in bitter and sweet transduction. *Cold Spring Harb Symp Quant Biol* **61**: 173-84.

Yau, D.M., N. Yokoyama, Y. Goshima, Z.K. Siddiqui, S.S. Siddiqui, and T. Kozasa. 2003. Identification and molecular characterization of the G alpha12-Rho guanine nucleotide exchange factor pathway in *Caenorhabditis elegans*. *Proc Natl Acad Sci U S A* **100**: 14748-53.

Yu, S., L. Avery, E. Baude, and D.L. Garbers. 1997. Guanylyl cyclase expression in specific sensory neurons: a new family of chemosensory receptors. *Proc Natl Acad Sci U S A* **94**: 3384-7.

Yu, S., D. Yu, E. Lee, M. Eckhaus, R. Lee, Z. Corria, D. Accili, H. Westphal, and L.S. Weinstein. 1998. Variable and tissue-specific hormone resistance in heterotrimeric Gs protein alpha-subunit (Gsalpha) knockout mice is due to tissue-specific imprinting of the gsalpha gene. *Proc Natl Acad Sci U S A* **95**: 8715-20.

Yu, S., O. Gavrilova, H. Chen, R. Lee, J. Liu, K. Pacak, A.F. Parlow, M.J. Quon, M.L. Reitman, and L.S. Weinstein. 2000. Paternal versus maternal transmission of a stimulatory G-protein alpha subunit knockout produces opposite effects on energy metabolism. *J Clin Invest* **105**: 615-23.

Zhang, Y., J.H. Chou, J. Bradley, C.I. Bargmann, and K. Zinn. 1997. The *Caenorhabditis elegans* seven-transmembrane protein ODR-10 functions as an odorant receptor in mammalian cells. *Proc Natl Acad Sci U S A* **94**: 12162-7.

Zwaal, R.R., A. Broeks, J. van Meurs, J.T. Groenen, and R.H. Plasterk. 1993. Target-selected gene inactivation in *Caenorhabditis elegans* by using a frozen transposon insertion mutant bank. *Proc Natl Acad Sci U S A* **90**: 7431-5.

Zwaal, R.R., J. Ahringer, H.G. van Luenen, A. Rushforth, P. Anderson, and R.H. Plasterk. 1996. G proteins are required for spatial orientation of early cell cleavages in *C. elegans* embryos. *Cell* **86**: 619-29.

Zwaal, R.R., J.E. Mendel, P.W. Sternberg, and R.H. Plasterk. 1997. Two neuronal G proteins are involved in chemosensation of the *Caenorhabditis elegans* dauer-inducing pheromone. *Genetics* **145**: 715-27.

Chapter 4

A network of stimulatory and inhibitory Gα subunits regulates olfaction in *Caenorhabditis elegans*

Hannes Lans, Suzanne Rademakers and Gert Jansen
published in *Genetics* 167: 1677–1687 (2004)

4. A network of stimulatory and inhibitory Gα subunits regulates olfaction in *Caenorhabditis elegans*

Abstract

The two pairs of sensory neurons of *C. elegans*, AWA and AWC, that mediate odorant attraction, express six Gα subunits, suggesting that olfaction is regulated by a complex signaling network. Here, we describe the cellular localization and functions of the six olfactory Gα subunits: GPA-2, GPA-3, GPA-5, GPA-6, GPA-13 and ODR-3. All except GPA-6 localize to sensory cilia, suggesting a direct role in sensory transduction. GPA-2, GPA-3, GPA-5, and GPA-6 are also present in cell bodies and axons and GPA-5 specifically localizes to synaptic sites. Analysis of animals with single- to sixfold loss-of-function mutations shows that olfaction involves a balance between multiple stimulatory and inhibitory signals. ODR-3 constitutes the main stimulatory signal and is sufficient for the detection of odorants. GPA-3 forms a second stimulatory signal in the AWA and AWC neurons, also sufficient for odorant detection. In AWA, signaling is suppressed by GPA-5. In AWC, GPA-2 and GPA-13 negatively and positively regulate signaling, respectively. Finally, we show that only ODR-3 plays a role in cilia morphogenesis. Defects in this process are, however, independent of olfactory behavior. Our findings reveal the existence of a complex signaling network that controls odorant detection by *C. elegans*.

Introduction

Heterotrimeric G proteins transduce diverse signals, varying from intercellular mediators to environmental stimuli (Hamm 1998). Activation of the G protein complex, consisting of an α, a β, and γ subunit, results in exchange of GDP for GTP and release of the GTP-bound Gα and the Gβγ dimer. Both entities can activate effector molecules. In many species, ranging from yeast to mammals, multiple Gα, β, and γ genes, which may function simultaneously in the same cells, have been identified. It is often unclear how many and which G protein pathways can be activated by a certain signal. Parallel activation of multiple pathways implies that specificity and cross-talk must be precisely regulated.

The olfactory system of *Caenorhabditis elegans* is adequately suited for studying G protein signaling and its specificity. Five pairs of neurons, AWA, AWB, AWC, ASH, and ADL, are involved in olfaction, but only two of these, the AWA and AWC cells, detect attractive odorants (Bargmann et al. 1993; Troemel et al. 1995, 1997). Both AWA and AWC neurons express many G protein coupled receptors (GPCRs) and at least three Gα subunits (Troemel et al. 1995; Zwaal et al. 1997; Roayaie et al. 1998; Jansen et al. 1999). Using these four cells, *C. elegans* can detect and discriminate many odorants (Bargmann et al. 1993) and adapt to an odorant while remaining responsive to another (Colbert and Bargmann 1995). Part of the odorant specificity arises from the ability of the AWA and AWC cells to detect different odorants (Bargmann et al. 1993). A functional asymmetry between the left

and the right AWC cell provides a further means to discriminate between odorants (Wes and Bargmann 2001). Still, even functional differences between all four AWA and AWC cells cannot account for all observed odorant discrimination (Wes and Bargmann 2001), suggesting that additional intracellular mechanisms exist that establish odorant specificity.

Genetic screens have identified several genes involved in odorant detection (Table 1). Surprisingly, signal transduction in the AWA and AWC neurons is not identical. In both cell types, binding of an odorant to a GPCR is thought to activate the Gα protein ODR-3 (Roayaie et al. 1998). In the AWA cells, ODR-3 probably activates a TRPV channel consisting of the subunits OSM-9, OCR-1 and/or OCR-2 (Colbert et al. 1997; Tobin et al. 2002). In the AWC cells, ODR-3 induces an increase in intracellular cGMP, mediated by the guanylyl cyclases ODR-1 and DAF-11, leading to opening of the cyclic nucleotide gated channel TAX-2/TAX-4 (Coburn and Bargmann 1996; Komatsu et al. 1996; Birnby et al. 2000; L'Etoile and Bargmann 2000). In addition to ODR-3, the Gα proteins GPA-2 and GPA-5 also are involved in regulating odorant responses (Roayaie et al. 1998; Jansen et al. 1999). However, their exact function remains unclear. The functions of other olfactory Gα subunits have not been determined.

Previously, we have shown that, in addition to ODR-3 and GPA-2, the Gα proteins GPA-3, GPA-5, GPA-6, and GPA-13 function in the AWA and AWC neurons (Jansen et al. 1999). In this study, we report a further characterization of all six olfactory Gα genes. Using antibodies, we show that they all, except GPA-6, localize to the ciliated endings of the sensory neurons. GPA-2, -3, -5 and -6 are also localized to the cell bodies and axons, GPA-5 is enriched at synaptic sites. Next, we show that, in the AWA neurons, ODR-3 and GPA-3 stimulate olfaction, whereas GPA-5 suppresses signaling. In the AWC neurons, olfaction is regulated by a balance between the stimulatory Gα subunits ODR-3, GPA-3, and GPA-13 and the inhibitory Gα GPA-2. Finally, we show that only ODR-3 is involved in cilia morphogenesis. Although *odr-3* mutants have flattened AWC cilia, this does not affect olfaction. Our analysis suggests that odorant perception requires a precisely balanced signaling network, consisting of both stimulatory and inhibitory signaling routes.

Tabel 1. Signaling molecules involved in odorant detection by AWA and AWC

cells	Gα subunits and signaling pathway
AWA	ODR-3 GPA-3 GPA-5, GPA-6 (Gα subunits) OSM-9, OCR-1, OCR-2 (TRPV channel subunits)
AWC	ODR-3, GPA-2, GPA-3, GPA-13 (Gα subunits) ODR-1, DAF-11 (guanylyl cyclases) TAX-2, TAX-4 (cGMP gated channel subunits)

Gα subunits expressed in AWA and AWC with their downstream signaling targets. See text for references.

Materials and methods

Strains and plasmids

Nematodes were grown at 20° or 25° on *Escherichia coli* strain *OP50* using standard methods (Brenner 1974). Wild type animals were *C. elegans* variety Bristol, strain N2. Alleles used in this study were *gpa-2(pk16)* and *gpa-3 (pk35)* (Zwaal et al. 1997); *gpa-3XS(pkIs508)*, *gpa-5(pk376)*, *gpa-5(pk377)*, *gpa-5XS (pkIs379)*, *gpa-6(pk480)*, *gpa-6::GFP(pkIs583)*, *gpa-6XS(pkIs519)*, and *gpa-13(pk1270)* (Jansen et al. 1999); *odr-1(n1936)* (L'Etoile and Bargmann 2000); *odr-3(n1605)* (Roayaie et al. 1998); *odr-7(ky4)* (Sengupta et al. 1994); and *str-2::GFP (kyIs140)* (Troemel et al. 1999). Injection of transgenes, at 5–50 ng/µl, was performed according to standard methods (Mello et al. 1991). Extrachromosomal transgenes were *gpa-5::snb-1::GFP*, *gpa-6::GFP* (Jansen et al. 1999), *gpa-13::GFP* (Jansen et al. 1999), and *gpc-1::GFP* (Jansen et al. 2002). *gpa-5::snb-1::GFP* was created by fusing a 1385-bp *Nae*I-*Eco*RI *snb-1::GFP* fragment from pSB120 (a gift from M. Nonet; Nonet 1999) to a 3724-bp *Pst*I-*Sma*I fragment of the *gpa-5* promoter in vector pPD95.79 (a gift from A. Fire).

Chemotaxis assays and statistical analysis

Olfactory chemotaxis was tested at least four times on two separate days, as described (Bargmann et al. 1993). Odorants used were diacetyl, pyrazine, benzaldehyde, 2,3-pentanedione, isoamyl alcohol, 2,4,5-trimethylthiazole, and butanone (Sigma Chemie, Acros Organics, and Fluka Chemie). A chemotaxis index was calculated as the number of animals at the odorant minus the number of animals at the control, divided by the total number of animals. The data obtained were analyzed using a one-way ANOVA test.

Immunofluorescence

Animals were permeabilized, fixed, and stained according to standard methods (Finney and Ruvkun 1990). Polyclonal antibodies were generated by collecting serum from rabbits immunized with full-length, *E. coli* produced, SDS-PAGE-purified Gα proteins fused to glutathione *S*-transferase. Staining was performed using dilutions of crude sera, or, if necessary, sera that were first affinity purified or precleared with acetone-fixed mutant animals. Secondary antibodies were goat-anti-rabbit Alexa-594-conjugated (Molecular Probes, Eugene, OR). Specificity of all antibodies was confirmed by the absence of immunoreactivity in Gα null mutants.

Microscopy

Antibody staining and cilia morphology were examined using a Leica DMRBE microscope, equipped with a Hamamatsu C4880 camera (Figure 1) and a Zeiss Axiovert 200, equipped with a Hamamatsu ORCA-ER camera (Figure 4). Individual cells were identified by using a combination of their position and

morphology (White et al. 1986) and the *gpc-1::GFP* construct (Jansen et al. 2002). AWA and AWC cilia were identified by using *gpa-6::GFP* and *gpa-13::GFP* (Jansen et al. 1999) constructs, respectively. While observing cells and cilia, we did not observe obvious defects in axon morphology or outgrowth in Gα-mutants.

Results

Six Gα subunits localize to the cilia and axons of the AWA and AWC neurons

Of the 21 Gα genes of *C. elegans*, 14 are specifically expressed in the amphid sensory neurons (Zwaal et al. 1997; Roayaie et al. 1998; Jansen et al. 1999; Cuppen et al. 2003). We focused on Gα function in two pairs of amphid neurons, AWA and AWC. Each pair senses a different set of odorants (Bargmann et al. 1993) and expresses at least three Gα genes. The AWC cells express *gpa-2*, *gpa-13*, and *odr-3*, while the AWA cells express *gpa-5*, *gpa-6*, and *odr-3*, as visualized using *LacZ* and *GFP* fusion constructs (Zwaal et al. 1997; Roayaie et al. 1998; Jansen et al. 1999). To determine the subcellular localization of the Gα subunits, we generated polyclonal antibodies and immunostained nematodes. The overall expression patterns of the Gα subunits, as observed with the antibodies, correlated well with the described expression patterns (Table 2).

The AWA and AWC cells each have an axon innervating the nerve ring, contacting other neurons, and a dendrite ending in elaborate cilia structures at the tip of the nose (Ward et al. 1975; Ware et al. 1975; White et al. 1986). The AWA and AWC cilia can be recognized due to their widespread, wing-like morphology (see Figure 4, A and E), in contrast to the single or double rod-like cilia of most of the other amphid neurons. In agreement with previous data (Roayaie et al. 1998), anti-ODR-3 antibodies were detected in the cilia of amphid neurons, while only faint staining of the dendrites and cell bodies was observed (Table 2). At high magnification, wing-shaped cilia, probably of the AWA, AWB, and AWC neurons, and the rod-like cilia of other amphid neurons, probably ASH and ADF, could be discerned (results not shown). In addition, our anti-ODR-3 serum also stained the cilia of one or both of the phasmid neurons PHA and PHB (Table 2).

Staining of the amphid cilia was also observed with anti-GPA-2, GPA-13, and GPA-5 antibodies (Figure 1, C–E). Anti-GPA-2 antibodies stained many cilia, cell bodies, and axons in the head (Figure 1C; results not shown). Two cell bodies in the anterior ganglion, two pharyngeal muscle cells, and seven cell bodies in the lateral and ventral ganglia showed staining, indicating that GPA-2 is expressed in more cells than initially identified (Zwaal et al. 1997; Table 2). Importantly, in addition to rod-like cilia, the typical wing shape of the AWC cilia could be distinguished at high magnification (results not shown). This confirms that GPA-2 is expressed in the AWC cells. We found no indications of GPA-2 expression in the AWA cells.

Anti-GPA-13 antibodies stained the cilia of amphid and phasmid neurons (Figure 1D; Table 2). At high magnification, the AWC cilia and two slender cilia that probably belong to the ASH and ADF neurons could be observed (Jansen et al. 1999; results not shown).

Anti-GPA-5 antibodies were detected not only in the cilia, but also in the cell bodies and axons of the AWA neurons (Figure 1, E and F). Often, although faintly, staining was observed in the ADL cell bodies, but not in the ASI cells (Jansen et al. 1999; Table 2). A punctate staining pattern of the axon with anti-GPA-5 antibodies suggested that GPA-5 might be located at the synaptic sites of the AWA axons. Therefore, we generated animals that expressed a *snb-1::GFP* fusion construct specifically in AWA. Due to its fusion with SNB-1 synaptobrevin, green fluorescent protein (GFP) localizes to synaptic vesicles in these animals (Nonet 1999). Staining with anti-GPA-5 serum showed a punctate localization of GPA-5 close to SNB-1::GFP (Figure 1F), suggesting that GPA-5 might function at the periactive zones of the AWA synapses.

Table 2. Gα expression patterns and intracellular localization

gene	amphid cells	other cells	intracellular localization
gpa-2	AWC	PHA, PHB, IL1L, IL2L, OLL or URB, PVT, anal sphincter muscle, M1, M5, I5, AIA	cilia, cell bodies, axons
gpa-3	**AWA**, **AWC**, ADF, ADL, ASE, ASG, ASH, ASI, ASJ, ASK	PHA, PHB, PVT, AIZ	cilia, cell bodies, axons
gpa-5	AWA, ADL		cilia, cell bodies, axons
gpa-6	AWA, **AWB**, **ADL**, **ASH**	**PHA**, PHB	dendrites, cell bodies, axons
gpa-13	ADF, ASH, AWC	PHA, PHB	cilia
odr-3	AWA, AWB, AWC, ASH, ADF	**PHA** or **PHB**	cilia, dendrites, cell bodies

Expression patterns of GPA-2, GPA -3, GPA-5, GPA-6, GPA-13 and ODR-3 are shown as observed with polyclonal antibodies. Staining was performed on wild type animals, except for anti-GPA-6 antibodies, which were applied to *gpa-6XS* animals. Expression patterns based on GFP fusion constructs have been described previously (Zwaal et al. 1997; Roayaie et al. 1998; Jansen et al. 1999). Cells that were previously not identified are shown in bold typeface.

The only Gα subunit that was not detected in the amphid cilia was GPA-6. In wild type animals, no GPA-6 immunoreactivity could be detected. However, in animals overexpressing GPA-6, the dendrites, cell bodies and axons of four pairs of amphid neurons, but not the cilia, showed staining (Figure 1G; Table 2). The positions of the cell bodies and colocalization experiments suggested that these

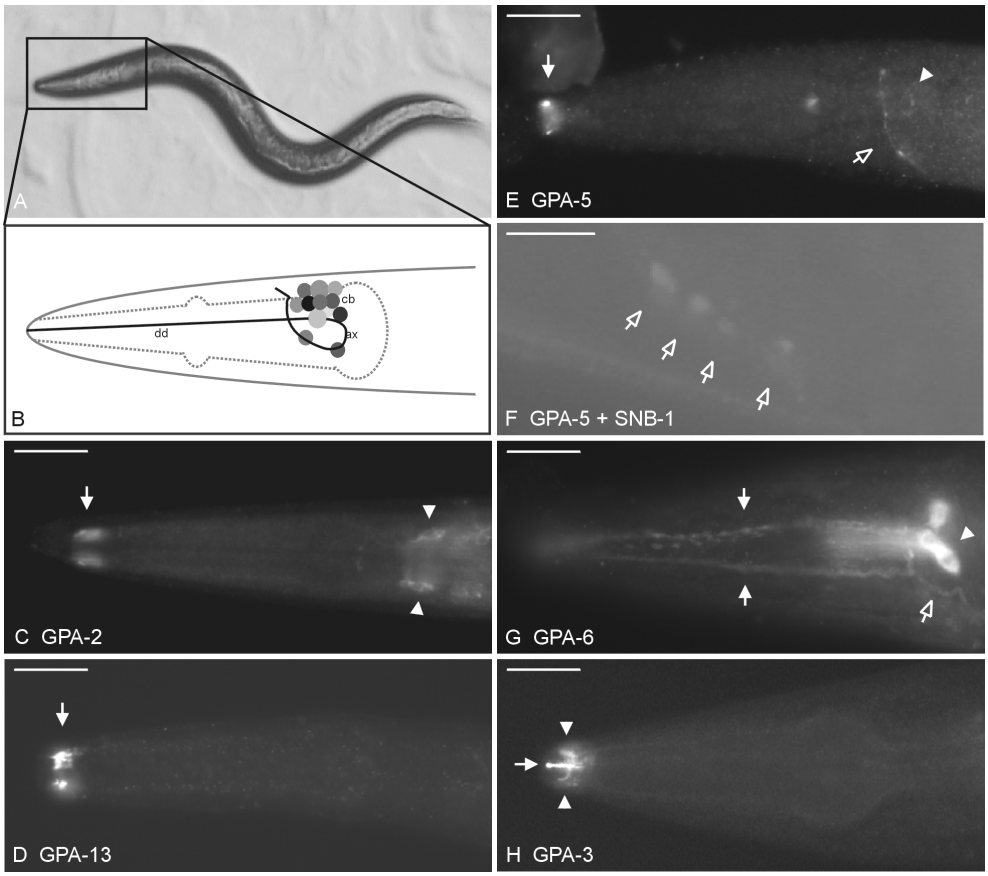

Figure 1. Intracellular localization of olfactory Gα subunits
In adult *C. elegans (*A), the olfactory Gα subunits locate to specific compartments of the amphid neurons. (B) The location and structures (dd, dendrite; cb, cell body; ax, axon) of the amphid neurons. GPA-2 (C) localizes to cilia (arrow) and cell bodies (arrowheads) of amphid neurons. GPA-13 (D) localizes to amphid cilia (arrow). GPA-5 (red) can be observed in the cilia (arrow), axons (open arrow), and cell bodies (arrowhead) of the AWA cells (E) and is localized nearby *snb-1::GFP* (green) in the axons (F, open arrows). GPA-6, in *gpa-6XS* animals, was not detected in the cilia, but in the dendrites (arrows), cell bodies (arrowhead), and axons (open arrow) of four pairs of amphid cells (G). GPA-3 localizes to the cilia of many amphid cells (H). GPA-3 staining in the rod-like cilia is clearly visible (arrow), surrounded by the more fuzzy wing-shaped AWA and AWC cilia staining (arrowheads). Bars: C, D, E, G, and H, 20 µm; F, 5 µm. (A–H) x400.

cells are AWA, AWB, ADL and ASH. No staining was observed in the ASI cells (Jansen et al. 1999). The two pairs of phasmid neurons, PHA and PHB, and a third unidentified cell, posterior to these neurons, were also visible.

Previously, we found impaired odorant responses in nematodes overexpressing constitutively active GPA-3 (GPA-3QL; Jansen et al. 1999). However, *gpa-3::lacZ* and *GFP* expression could not be detected in the AWA or AWC cells (Zwaal et al. 1997; results not shown). We found anti-GPA-3 antibody staining in the cilia, cell bodies, and axons of many amphid cells (Figure 1H) and

the two phasmid cells PHA and PHB (Table 2). The amphid cell bodies were faintly visible, but the typical wing shape of the AWC cilia could be recognized. Application of anti-GPA-3 antibodies to *gpa-6::GFP* animals showed GPA-3 colocalization with GPA-6::GFP in the AWA cilia and cell bodies (results not shown).

Taken together, our results suggest that ODR-3, GPA-2, GPA-3, GPA-5, and GPA-13 may be directly involved in chemosensory signaling in the amphid cilia, unlike GPA-6, and that GPA-5 may also function at the synapse.

ODR-3 and GPA-3 act redundantly in olfactory signaling

To determine whether the Gα subunits are involved in odorant detection, we tested animals with loss-of-function (l.o.f.) mutations in one or more Gα-subunits (Zwaal et al. 1997; Roayaie et al. 1998; Jansen et al. 1999) in odorant chemotaxis assays. First, the optimal chemotaxis conditions in our laboratory were determined. We obtained strong, reproducible chemoattraction with 1:10 and 1:100 dilutions of benzaldehyde, butanone, and isoamyl alcohol, whereas diacetyl, 2,4,5-trimethylthiazole and 2,3-pentanedione gave reliable results at dilutions ranging from 1:10 to 1:1000. Optimal pyrazine chemotaxis was achieved with 10 and 100 mg/ml concentrations. Low odorant concentrations are specifically detected by either the AWA or the AWC cells and higher concentrations by both (Bargmann et al. 1993; Chou et al. 2001). Therefore, we determined the cellular specificity of the odorant response, using *odr-7* and *odr-1* animals, which are defective in AWA and AWC olfaction, respectively (Bargmann et al. 1993; Sengupta et al. 1994). Chemotaxis to butanone and isoamyl alcohol almost completely depended on AWC, whereas only low concentrations of benzaldehyde were specific to AWC. Low concentrations of diacetyl and pyrazine were specifically detected by the AWA cells, but high concentrations involved both AWA and AWC. The responses to 2,4,5-trimethylthiazole and 2,3-pentanedione seemed not specific to the AWA or AWC cells at the concentrations that gave strong, reproducible results (Table 3 and results not shown).

Table 3. Cellular specificity of odorant detection

odorant	wild type	*odr-7* AWA	*odr-1* *AWC*
pyrazine 10 mg/ml	0.78 ± 0.03	**0.21 ± 0.16**	0.80 ± 0.05
diacetyl 1:1000	0.65 ± 0.04	**0.20 ± 0.18**	0.76 ± 0.06
trimethylthiazole 1:1000	0.66 ± 0.05	0.54 ± 0.17	0.75 ± 0.07
pentanedione 1:1000	0.80 ± 0.04	0.83 ± 0.07	0.56 ± 0.08
benzaldehyde 1:100	0.83 ± 0.06	0.71 ± 0.12	**0.29 ± 0.09**
isoamyl alcohol 1:100	0.77 ± 0.06	0.85 ± 0.06	**0.06 ± 0.06**
butanone 1:100	0.61 ± 0.05	0.94 ± 0.02	**0.24 ± 0.08**

Shown are the mean chemotaxis indexes (±s.e.m) of wild type, *odr-7* and *odr-1* animals. *odr-7* and *odr-1* animals lack functional AWA and AWC, respectively. AWA or AWC specific responses are indicated in boldface.

We tested the *odr-3(n1605)* l.o.f. mutant and confirmed that *odr-3* is necessary for the detection of all odorants, at all concentrations tested (Roayaie et al. 1998; Figure 2, A–C; results not shown). In our assays, the response to butanone and low concentrations of diacetyl and benzaldehyde seemed to depend solely on *odr-3*. In contrast, the response to 2,3-pentanedione was only slightly affected. Other odorants gave intermediate phenotypes.

To identify the Gα responsible for the residual response of *odr-3* animals, we also tested other Gα l.o.f. mutants, but observed no or only very mild effects (results not shown). This suggested that ODR-3 alone is sufficient for odorant detection. To test this, we generated animals with mutations in all six Gα subunits except *odr-3*. These *gpa-2 gpa-3 gpa-5 gpa-6 gpa-13* mutants showed wild type levels of chemotaxis to all odorants at all concentrations tested (Figure 2, A–C; results not shown), validating the idea that ODR-3 constitutes the major signaling pathway in AWA and in AWC. Additional loss of *odr-3* abrogated chemotaxis to all odorants, except to pyrazine and high concentrations of 2,4,5-trimethylthiazole (Figure 2, A–C; results not shown). These odorants may also be sensed by other cells (Bargmann et al. 1993).

Subsequently, to identify the Gα mediating the residual response in *odr-3* animals, double mutants between *odr-3* and *gpa-2*, *gpa-3*, *gpa-5*, *gpa-6*, or *gpa-13* were tested. Chemotaxis to all odorants was completely abolished in *gpa-3 odr-3* mutants, at all concentrations tested (Figure 2, A–C; results not shown), indicating that GPA-3 also has a stimulatory role in odorant detection, redundant to ODR-3. This phenotype could be rescued by the introduction of a *gpa-3* transgene (Figure 2D). To confirm that GPA-3 is functionally redundant to ODR-3, animals were generated with mutations in all six Gα subunits except *gpa-3*. Surprisingly, these *gpa-2 gpa-5 gpa-6 gpa-13 odr-3* animals showed strong chemotaxis to all odorants, except to butanone (Figure 2, A–C; results not shown). Especially, chemotaxis to AWA-detected odorants was very strong. These results indicate that GPA-3 also is sufficient for detection of most odorants.

GPA-2, GPA-5, and GPA-13 modulate signaling via ODR-3 and GPA-3

Previously, we have shown that GPA-5 negatively influences the response to 2,4,5-trimethylthiazole (Jansen et al. 1999; Figure 3, A–C), suggesting that GPA-5 might have an inhibitory function. Further analysis of the olfactory response of *gpa-5 odr-3* mutants showed that loss of GPA-5 suppressed the chemotaxis defect of *odr-3* animals for all odorants detected by the AWA cells (Figure 3, A and B; results not shown). Suppression was strongest when using high odorant concentrations. The impaired chemotaxis of *odr-3* animals to isoamyl alcohol, butanone, and low concentrations of benzaldehyde could not be suppressed by loss of GPA-5, in agreement with the fact that these odorants are detected by AWC, in which *gpa-5* is not expressed (Figure 3C; results not shown). To confirm that GPA-5 caused the suppression of the *odr-3* phenotype, we tested an independent *gpa-5* l.o.f. allele (pk377), which gave similar results (results not shown).

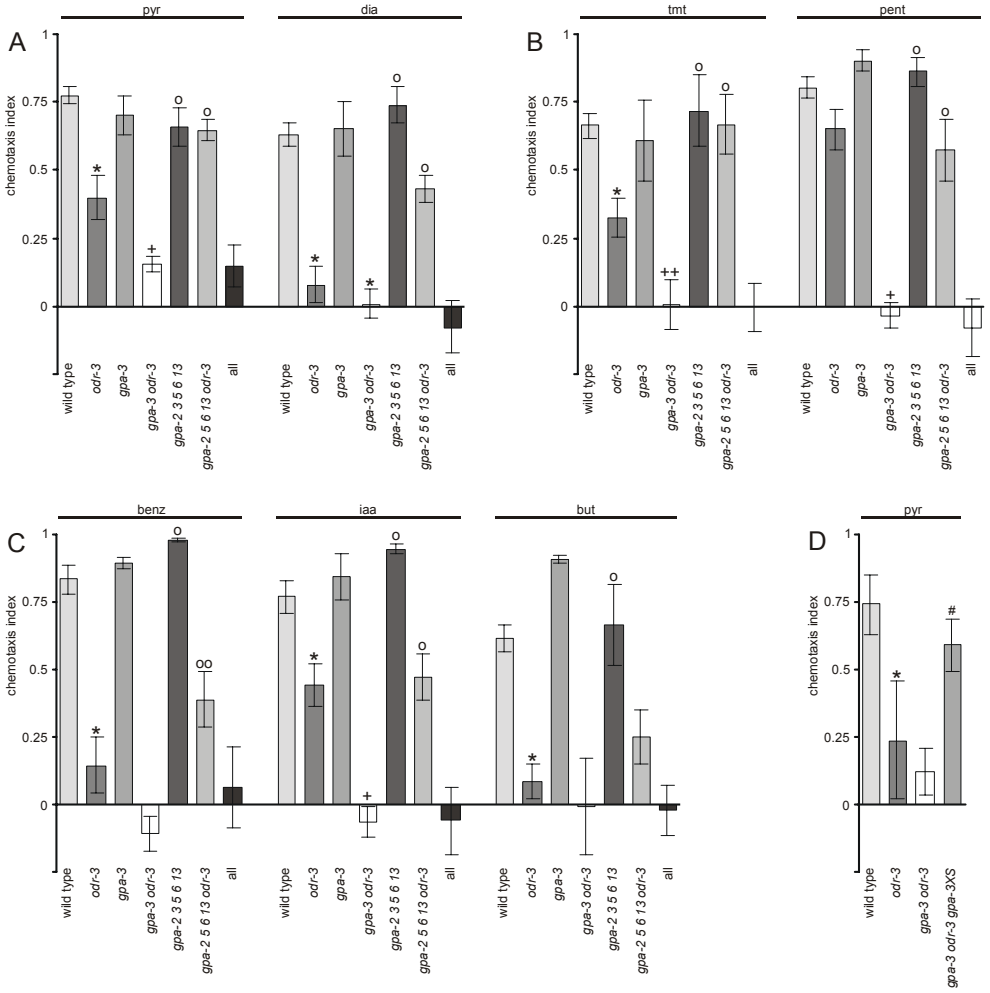

Figure 2. ODR-3 and GPA-3 form the major stimulatory signals in odorant detection

Chemotaxis of *odr-3* animals and *gpa-2 gpa-3 gpa-5 gpa-6 gpa-13* animals (shown as *gpa-2 3 5 6 13*) shows that ODR-3 is necessary and sufficient for chemotaxis to all odorants (see also Roayaie et al. 1998). GPA-3 is redundant to ODR-3 and is sufficient for chemotaxis at almost wild type levels, as is evident from chemotaxis of *gpa-3* animals, *gpa-3 odr-3* double mutants and *gpa-2 gpa-5 gpa-6 gpa-13 odr-3* mutants (shown as *gpa-2 5 6 13 odr-3*). Mutants that lack all six olfactory Gα subunits (shown as 'all') display no odorant attraction.

Shown are the responses to odorants primarily sensed by AWA (A, 10 mg/ml pyrazine (pyr) and 1:1000 diacetyl (dia)), by AWA and AWC (B, 1:1000 2,4,5-trimethylthiazole (tmt) and 2,3-pentanedione (pent)) and by AWC (C, 1:100 benzaldehyde (benz), isoamyl alcohol (iaa) and butanone (but)). D shows that chemotaxis to 50 mg/ml pyrazine of *gpa-3 odr-3* mutants can be restored by introducing a *gpa-3* transgene (shown as *gpa-3 odr-3 gpa-3XS)*.

Error bars denote standard error of the mean. Significant differences of *odr-3* and *gpa-3* animals compared to wild type (*: p≤0.01), *gpa-3 odr-3* compared to *odr-3* animals (+: p≤0.01, ++: p≤0.05) and *gpa-2 gpa-3 gpa-5 gpa-6 gpa-13* and *gpa-2 gpa-5 gpa-6 gpa-13 odr-3* animals compared to *gpa-2 gpa-3 gpa-5 gpa-6 gpa-13 odr-3* mutants (°: p≤0.01, °°: p≤0.05) are shown. In D, # denotes significant difference from *gpa-3 odr-3* (p≤0.01).

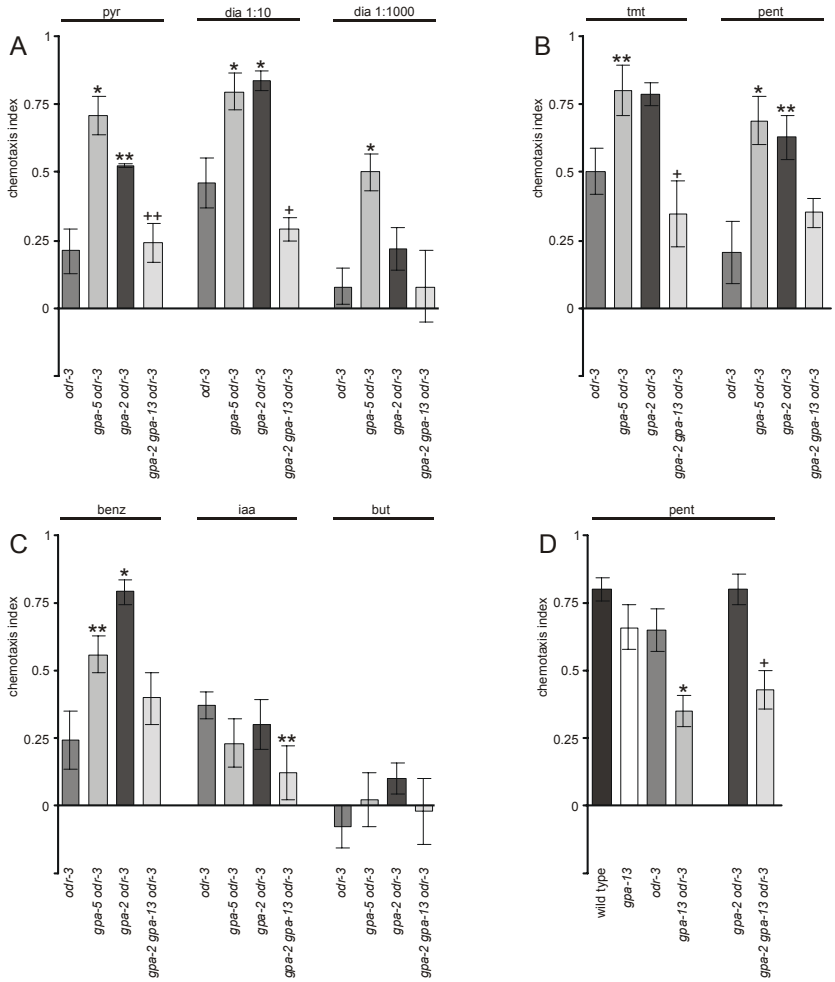

Figure 3. GPA-5 and GPA-2 negatively regulate chemotaxis to most odorants, GPA-13 has a stimulatory function

Chemotaxis of animals that lack ODR-3 and GPA-5 (*gpa-5 odr-3*) or GPA-2 *(gpa-2 odr-3)* reveals that GPA-5 and GPA-2 inhibit odorant detection in *odr-3* animals. Furthermore, GPA-13 has a stimulatory function, which becomes apparent when *gpa-2* is inactivated in an *odr-3* background (compare *gpa-2 odr-3* with *gpa-2 gpa-13 odr-3*).

Shown are the responses to high concentrations of odorants primarily sensed by AWA (A, 100 mg/ml pyrazine (pyr) and 1:10 diacetyl (dia)), by AWA and AWC (B, 1:10 2,4,5-trimethylthiazole (tmt) and 2,3-pentanedione (pent)) and by AWC (C, 1:10 benzaldehyde (benz), isoamyl alcohol (iaa) and butanone (but)). Low concentrations of diacetyl (1:1000, A) are also shown. D shows that GPA-13 is specifically involved in 2,3-pentanedione (1:1000 diluted; pent) signaling.

Error bars denote standard error of the mean. Significant differences of *gpa-5 odr-3, gpa-2 odr-3* and *gpa-13 odr-3* animals compared to *odr*-3 animals (*: p≤0.01, **: p≤0.05), *gpa-2 gpa-13 odr-3* compared to *gpa-2 odr-3* animals (+: p≤0.01, ++: p≤0.05).

Next, we tested chemotaxis of *gpa-2 odr-3* animals. Previously, a stimulatory function for GPA-2 was found in butanone perception (Roayaie et al. 1998). We found that GPA-2 negatively affects odorant detection, since *gpa-2 odr-3* animals showed improved odorant responses as compared to *odr-3* animals (Figure 3, A–C; results not shown). Like GPA-5, the inhibitory function of GPA-2 was clearest when using high odorant concentrations. Only isoamyl alcohol and butanone responses seemed not affected. Since the butanone response was completely abolished in *odr-3* animals, a redundant stimulatory function for GPA-2 in butanone detection could not be analyzed (Roayaie et al. 1998).

Chemotaxis of *gpa-6 odr-3* and *gpa-13 odr-3* mutants also was tested. A function for GPA-6 in odorant detection could not be found, which is in agreement with its absence from the cilia. The behavior of *gpa-13 odr-3* animals was indistinguishable from that of *odr-3* animals, except for the response to low concentrations of 2,3-pentanedione (Figure 3D). In addition to this phenotype, we observed that inactivation of *gpa-13* in *gpa-2 odr-3* or *gpa-5 odr-3* animals significantly reduced chemotaxis responses (Figure 3; results not shown), suggesting a minor stimulatory role for GPA-13 in olfactory signaling.

Taken together, our results confirm that ODR-3 constitutes the main stimulatory olfactory signaling pathway. Furthermore, our findings suggest that the olfactory defect of *odr-3* mutants, for most odorants, is caused by the inhibitory action of GPA-2 and GPA-5. GPA-3 can fully substitute for ODR-3, but only when the function of GPA-2 or GPA-5 is lost and with the aid of GPA-13 or when all five other Gα subunits have been inactivated. Our data suggest that the olfactory response in *C. elegans* is regulated by a complex signaling network, which is activated upon odorant stimulation and may be necessary to obtain a precisely tuned response.

Olfactory defects are not caused by altered cilia morphology

The odorant chemotaxis defects of Gα subunit mutants could be the result of developmental or structural defects of the sensory neurons. Such defects have been described for *gpa-3* and *odr-3* animals. The amphid neurons of *gpa-3QL* animals have lost the ability to take up fluorescent dyes (Zwaal et al. 1997), probably because of structural defects of the cilia (J. Burghoorn and G. Jansen, unpublished results). Furthermore, the level of *odr-3* expression controls AWA and AWC cilia morphology (Roayaie et al. 1998). Low levels of ODR-3 define flattened, filamentous cilia, but this is probably not the cause of the olfactory defects of *odr-3* mutants. To rule out the possibility that altered cilia morphology causes the olfactory differences seen in Gα mutants, we analyzed the AWA and AWC cilia of these mutants in more detail. For this purpose, we used the Gα specific antibodies and the AWA-expressed *gpa-6::GFP* and AWC-expressed s*tr-2::GFP* and *gpa-13::GFP* transgenes.

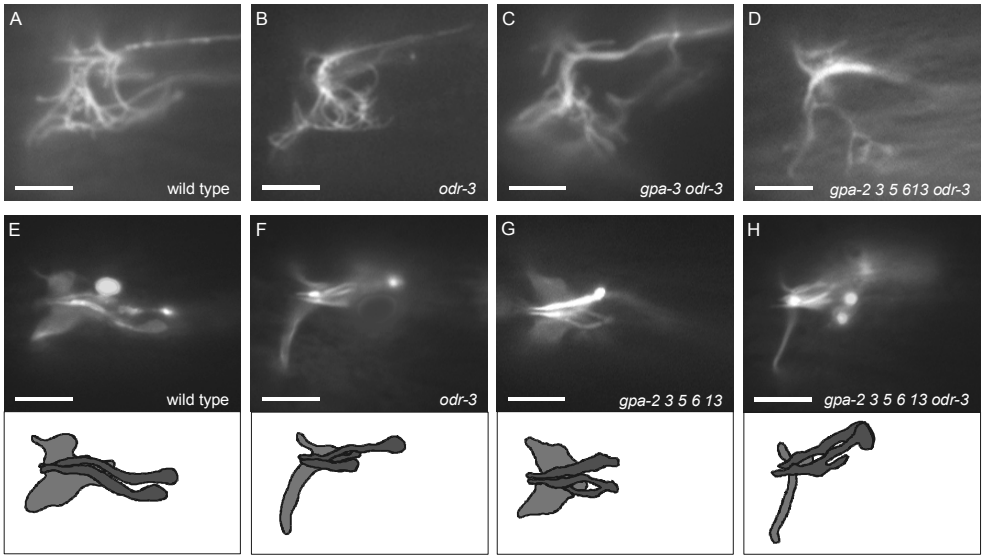

Figure 4. ODR-3 plays a role in cilia morphogenesis
(A–D) *gpa-6::GFP* expression in the AWA cilia. (E–H) *gpa-13::GFP* in the AWC and ADF/ASH cilia. The branched, filamentous structure of the AWA cilia is apparent from A–D. Because the structure of the AWC cilia is less apparent in E–H, a schematic of the cilia of AWC (blue) and ADF and ASH (red) is provided. The AWA cilia of wild type (A), *odr-3* (B), *gpa-3 odr-3* (C), and *gpa-2 gpa-3 gpa-5 gpa-6 gpa-13 odr-3* sixfold mutants (D) are all extensively branched, despite olfactory differences. The AWA cilia of *gpa-2 gpa-3 gpa-5 gpa-6 gpa-13 odr-3* mutants were difficult to visualize properly, because *gpa-6::GFP* is poorly expressed in these mutants. The AWC cilia of *odr-3* (F) and *gpa-2 gpa-3 gpa-5 gpa-6 gpa-13 odr-3* sixfold mutants (H) are less well differentiated than the AWC cilia of wild-type (E) and *gpa-2 gpa-3 gpa-5 gpa-6 gpa-13* animals (G). For *odr-3* cilia, see also Roayaie *et al.* (1998). Left is anterior. Bars, 5 μm. (A–H) x630.

The AWA cilia have a branched, filamentous shape, while the AWC cilia have a wide, wing-like structure (Ward et al. 1975; Figure 4, A and E). Unlike the majority of the amphid cilia, these cilia are not exposed to the environment (Ward et al. 1975; Ware et al. 1975).

First, single mutant animals were analyzed. All mutants, except *odr-3* animals, had normal axon, dendrite and cilia structures (Figure 4, B and F; results not shown). As previously described (Roayaie et al. 1998), the AWC cilia of *odr-3* animals were flatter and less well differentiated, whereas the AWA cilia appeared normal. Next, *gpa-3 odr-3* double mutants were analyzed. Although these mutants are more severely defective than *odr-3* animals, their AWA and AWC cilia resembled those of *odr-3* animals (Figure 4C; results not shown). Likewise, the cilia of animals with combinations of l.o.f. mutations in *gpa-2*, *gpa-5*, *gpa-6*, and *gpa-13*, some of which chemotax better than *odr-3* animals (Figures 2 and 3), were comparable to *odr-3* mutants (results not shown). These observations suggest that altered cilia morphology, as observed in *odr-3* animals, does not cause the apparent olfactory defects or that impaired sensory signaling causes cilia degradation.

Next, five- and sixfold Gα mutants were examined. The AWA cilia appeared as wild type in animals that expressed only *gpa-3*, *gpa-5*, *gpa-6*, or *odr-3*, although these mutants show varying chemotaxis (Figure 2; results not shown). Also, animals that had lost all six Gα subunits showed wild type AWA cilia (Figure 4D). With regard to the AWC cilia, nematodes that expressed only *gpa-2*, *gpa-3*, or *gpa-13* and animals with l.o.f. mutations in all six olfactory Gα subunits had flattened AWC cilia, similar to *odr-3* animals (Figure 4H; results not shown). In contrast, animals that expressed only *odr-3*, *i.e.*, *gpa-2 gpa-3 gpa-5 gpa-6 gpa-13* mutants, had wild type AWC cilia (Figure 4G). These results indicate that, of the four AWC-expressed Gα subunits, only ODR-3 is necessary to establish normal AWC cilia morphology. The other five olfactory Gα subunits are not involved in AWA or AWC cilia development.

Discussion

Olfaction using four cells requires signaling via five Gα subunits

Our results show that olfactory signaling in *C. elegans* is more complex than initially realized. Five Gα subunits regulate the response of *C. elegans* to attractive odorants (Figure 5). ODR-3 constitutes the main stimulatory signal in the AWA and AWC cells. GPA-3 also provides a stimulatory signal in both neuron pairs. GPA-5 and GPA-2 have inhibitory functions in the AWA and the AWC cells, respectively, and GPA-13 has a minor stimulatory role in the AWC cells. Although the odorant concentrations used in this study are not all detected specifically by either AWA or AWC, it is highly likely that the observed regulation by Gα subunits occurs only in these cells. First of all, no other cells have been reported to be involved in odorant attraction in *C. elegans*. Second, GPA-5 is expressed only in AWA and faintly in ADL, whereas AWC is the only sensory amphid cell in which GPA-2 is expressed. Finally, AWA-specific expression of GPA-3 rescues AWA-mediated olfaction in a *gpa-3 odr-3* background (H. Lans and G. Jansen, unpublished results).

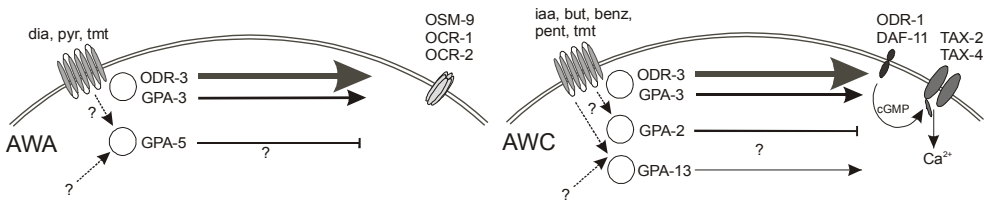

Figure 5. Model of Gα signaling during odorant detection via the AWA and AWC cells
In AWA, both ODR-3 and GPA-3 can transduce the odorant signal to the TRPV channel OSM-9/OCR-1/OCR-2, but ODR-3 forms the major pathway. GPA-5, at an unknown effector, cross-interacts with this signal to inhibit signaling. GPA-5 might be activated by the same receptor as ODR-3 and GPA-3 or by another receptor. In AWC, mainly ODR-3, but also GPA-3, transduces the odorant signal to the guanylyl cyclases ODR-1 and DAF-11, leading to the opening of the TAX-2/TAX-4 ion channel. GPA-2 and GPA-13 inhibit and stimulate this signal, respectively.

We cannot exclude that other ubiquitously expressed Gα subunits, like *goa-1* (Mendel et al. 1995; Segalat et al. 1995), *gsa-1* (Korswagen et al. 1997), *egl-30* (Lackner et al. 1999), and *gpa-7* (Jansen et al. 1999), also function in the AWA and AWC cells. However, on the basis of the data presented in this study, it is not likely that one of these is required for olfactory signaling. It is striking that signaling in AWA and AWC, the only cells required for odorant attraction (Bargmann et al. 1993), involves different Gα subunits. Interestingly, this correlates well with the existence of dissimilar downstream signaling pathways in the two neuron pairs (Table 1; Figure 5). In AWA, signaling is reminiscent of Drosophila phototransduction, due to its dependence on TRP channel proteins (Colbert et al. 1997; Hardie and Raghu 2001; Tobin et al. 2002). AWC signaling, on the other hand, resembles that in the mammalian main olfactory epithelium, where all signals converge on a cyclic nucleotide-gated channel (Coburn and Bargmann 1996; Komatsu et al. 1996; Firestein 2001). Signaling in this system also involves a separate modulatory pathway and multiple Gα proteins (Jones and Reed 1989; Belluscio et al. 1998; Wekesa and Anholt 1999; Luo et al. 2002; Spehr et al. 2002).

Why would *C. elegans* need several Gα subunits with overlapping or antagonizing functions to modulate olfaction? We hypothesize that such a balanced signaling network is necessary to allow adjustment of the response to an odorant when odorant concentrations or other environmental conditions change. The inhibitory effects of GPA-2 and GPA-5 were most obvious when the animals were exposed to high odorant concentrations. In these circumstances, desensitization or adaptation mechanisms may become activated. Following prolonged exposure to high odorant concentrations, *C. elegans* shows a diminished response to that odorant, but not to other odorants (Colbert and Bargmann 1995). This process, which is called adaptation, involves the unknown *adp-1* gene, the TRPV channel subunit OSM-9, and the cGMP-dependent protein kinase EGL-4 (Colbert and Bargmann 1995; L'Etoile et al. 2002). Since odorant adaptation is triggered by calcium and cGMP levels, it seems likely that this response is regulated by stimulating and inhibiting Gα subunits. Furthermore, odorant-specific adaptation is modulated by other environmental cues, which are probably transduced by G proteins. For example, the absence of food stimulates benzaldehyde adaptation, but not isoamyl alcohol adaptation (Colbert and Bargmann 1997). Similarly, the presence of food suppresses benzaldehyde adaptation (Nuttley et al. 2002). Although it is uncertain where the integration of signals like food availability and odorants occurs, multiple G proteins within one cell might be essential to control the proper response of an animal to several simultaneous cues.

In addition, overlapping G protein pathways could facilitate discrimination between odorants detected by the same neuron (Wes and Bargmann 2001). Our finding that two Gα subunits, ODR-3 and GPA-3, can mediate the detection of all odorants provides a basis for this idea. Inhibition of ODR-3 and GPA-3 signaling by GPA-2 and GPA-5 could also be a means for odorant discrimination. Both adaptation and discrimination assays could shed more light on the involvement of

G proteins in adaptation and discrimination.

Finally, another hint at the biological significance of the G protein network could be provided by the finding that vulval induction by the Ras-mitogen-activated protein kinase (MAPK) pathway is negatively regulated by GPA-5 and the GPCR SRA-13, depending on food conditions (Battu et al. 2003). This indicates that olfactory signaling not only serves to direct movement toward food, but also may regulate developmental processes.

Specificity of G protein signaling pathways

Our genetic analysis of G protein function in olfactory signaling shows that the Gα subunits have specific functions, despite their shared localization in the cell. This raises the question of how specificity is organized and maintained.

First of all, specificity may be defined by specific interactions with receptors, Gβγ subunits, and effectors. It is likely that ODR-3 and GPA-3 are activated by the same receptors during odorant detection. GPA-2 and GPA-5 might inhibit signaling by competing for these receptors, but could also be activated by different receptors or in a receptor-independent fashion. Recently, GPA-5 and SRA-13 have been found to negatively regulate a Ras/MAPK pathway downstream of ODR-3 in the AWC cells (Hirotsu et al. 2000; Battu et al. 2003). Although this could provide a mechanism through which ODR-3 and GPA-3 signaling is inhibited, it is unclear how GPA-5 could function in the AWC cells. Furthermore, it is striking that no effect of GPA-2 on the Ras/MAPK pathway has been observed (Battu et al. 2003).

Another determinant is provided by the βγ dimer that associates with the Gα-subunits. *C. elegans* has two Gβ and two Gγ subunits, GPB-1 and -2 and GPC-1 and -2 (van der Voorn et al. 1990; Zwaal et al. 1996; Jansen et al. 1999). GPC-1 functions specifically in a subset of sensory cells, but not in AWA and AWC (Jansen et al. 2002). Therefore, the ubiquitously expressed GPC-2 (Jansen et al. 2002) is the likely candidate to interact with the olfactory Gα subunits. In AWA and AWC, GPC-2 may dimerize with either GPB-1 or GPB-2, which are both widely expressed (Chase et al. 2001; van der Linden et al. 2001). Alternatives to GPC-2, however, could be the Gγ-like domain containing RGS proteins EAT-16 and EGL-10, which can interact with GPB-2 (Chase et al. 2001; Robatzek et al. 2001; van der Linden et al. 2001). Thus, together there are four different Gβγ(-like) partners. It is difficult to test which of these partners functions *in vivo* because both *gpb-1* and *gpc-2* mutations are lethal (Zwaal et al. 1996; Gotta and Ahringer 2001).

A spatial separation of different pathways is a further means to confer specificity. Within the cilia, compartmentalization could separate the different Gα-subunits, as was previously suggested to insulate cGMP signaling during odorant discrimination and adaptation (L'Etoile and Bargmann 2000; L'Etoile et al. 2002).

A third method to provide specificity could be a temporal induction of G protein signaling. For example, the translocation of the cGMP-dependent protein ki nase EGL-4 to the nucleus of the AWC cells, following long-term adaptation to odorants (L'Etoile et al. 2002), may induce the expression of genes necessary for

adaptation that would otherwise interfere with olfactory signaling.

It is becoming increasingly clear that most signaling pathways are part of complex, intracellular signaling networks. Our characterization of the olfactory system of *C. elegans* provides us with the possibility of identifying the molecular mechanisms that regulate specificity in vivo.

Acknowledgements

We thank Ronald Plasterk in whose lab this project was started. We acknowledge the assistance of Marieke van der Horst and Gert van Cappellen. We thank Cori Bargmann and the Caenorhabditis Genetics Center for strains and Andy Fire and Michael Nonet for plasmids. This work was supported by the Netherlands Organization for Scientific Research (NWO, grant ALW 805-48-009), the Royal Dutch Academy of Sciences (KNAW), and the Center for Biomedical Genetics, Rotterdam, The Netherlands.

Literature cited

Bargmann, C. I., E. Hartwieg and H. R. Horvitz, 1993 Odorant-selective genes and neurons mediate olfaction in C. elegans. Cell **74:** 515-527.

Battu, G., E. F. Hoier and A. Hajnal, 2003 The *C. elegans* G-protein-coupled receptor SRA-13 inhibits RAS/MAPK signalling during olfaction and vulval development. Development **130:** 2567-2577.

Belluscio, L., G. H. Gold, A. Nemes and R. Axel, 1998 Mice deficient in G(olf) are anosmic. Neuron **20:** 69-81.

Birnby, D. A., E. M. Link, J. J. Vowels, H. Tian, P. L. Colacurcio *et al.*, 2000 A transmembrane guanylyl cyclase (DAF-11) and Hsp90 (DAF-21) regulate a common set of chemosensory behaviors in *Caenorhabditis elegans*. Genetics **155:** 85-104.

Brenner, S., 1974 The genetics of *Caenorhabditis elegans*. Genetics **77:** 71-94.

Chase, D. L., G. A. Patikoglou and M. R. Koelle, 2001 Two RGS proteins that inhibit Galpha(o) and Galpha(q) signaling in *C. elegans* neurons require a Gbeta(5)-like subunit for function. Curr. Biol. **11:** 222-231.

Chou, J. H., C. I. Bargmann and P. Sengupta, 2001 The *Caenorhabditis elegans odr-2* gene encodes a novel Ly-6-related protein required for olfaction. Genetics **157:** 211-224.

Coburn, C. M., and C. I. Bargmann, 1996 A putative cyclic nucleotide-gated channel is required for sensory development and function in *C. elegans*. Neuron **17:** 695-706.

Colbert, H. A., and C. I. Bargmann, 1995 Odorant-specific adaptation pathways generate olfactory plasticity in *C. elegans*. Neuron **14:** 803-812.

Colbert, H. A., and C. I. Bargmann, 1997a Environmental signals modulate olfactory acuity, discrimination, and memory in *Caenorhabditis elegans*. Learn Mem **4:** 179-191.

Colbert, H. A., T. L. Smith and C. I. Bargmann, 1997b OSM-9, a novel protein with structural similarity to channels, is required for olfaction, mechanosensation, and olfactory adaptation in *Caenorhabditis elegans*. J. Neurosci. **17:** 8259-8269.

Cuppen, E., A. M. van der Linden, G. Jansen and R. H. A. Plasterk, 2003 Proteins interacting with *Caenorhabditis elegans* Gα subunits. Comp. Funct. Genom. **4:** 479-491.

Finney, M., and G. Ruvkun, 1990 The *unc-86* gene product couples cell lineage and cell identity in *C. elegans*. Cell **63:** 895-905.

Firestein, S., 2001 How the olfactory system makes sense of scents. Nature **413:** 211-218.

Gotta, M., and J. Ahringer, 2001 Distinct roles for Galpha and Gbetagamma in regulating spindle position and orientation in *Caenorhabditis elegans* embryos. Nat. Cell. Biol. **3:** 297-300.

Hamm, H. E., 1998 The many faces of G protein signaling. J. Biol. Chem. **273:** 669-672.

Hardie, R. C., and P. Raghu, 2001 Visual transduction in *Drosophila*. Nature **413:** 186-193.

Hirotsu, T., S. Saeki, M. Yamamoto and Y. Iino, 2000 The Ras-MAPK pathway is important for olfaction in *Caenorhabditis elegans*. Nature **404:** 289-293.

Jansen, G., K. L. Thijssen, P. Werner, M. van der Horst, E. Hazendonk *et al.*, 1999 The complete family of genes encoding G proteins of *Caenorhabditis elegans*. Nat. Genet. **21:** 414-419.

Jansen, G., D. Weinkove and R. H. Plastterk, 2002 The G-protein gamma subunit *gpc-1* of the nematode *C. elegans* is involved in taste adaptation. EMBO J. **21:** 986-994.

Jones, D. T., and R. R. Reed, 1989 Golf: an olfactory neuron specific-G protein involved in odorant signal transduction. Science **244:** 790-795.
Komatsu, H., I. Mori, J. S. Rhee, N. Akaike and Y. Ohshima, 1996 Mutations in a cyclic nucleotide-gated channel lead to abnormal thermosensation and chemosensation in *C. elegans.* Neuron **17:** 707-718.
Korswagen, H. C., J. H. Park, Y. Ohshima and R. H. Plasterk, 1997 An activating mutation in a *Caenorhabditis elegans* Gs protein induces neural degeneration. Genes Dev. **11:** 1493-1503.
Lackner, M. R., S. J. Nurrish and J. M. Kaplan, 1999 Facilitation of synaptic transmission by EGL-30 Gqalpha and EGL-8 PLCbeta: DAG binding to UNC-13 is required to stimulate acetylcholine release. Neuron **24:** 335-346.
L'Etoile, N. D., and C. I. Bargmann, 2000 Olfaction and odor discrimination are mediated by the *C. elegans* guanylyl cyclase ODR-1. Neuron **25:** 575-586.
L'Etoile, N. D., C. M. Coburn, J. Eastham, A. Kistler, G. Gallegos *et al.*, 2002 The cyclic GMP-dependent protein kinase EGL-4 regulates olfactory adaptation in *C. elegans*. Neuron **36:** 1079-1089.
Luo, A. H., E. H. Cannon, K. S. Wekesa, R. F. Lyman, J. G. Vandenbergh *et al.*, 2002 Impaired olfactory behavior in mice deficient in the alpha subunit of G(o). Brain Res. **941:** 62-71.
Mello, C. C., J. M. Kramer, D. Stinchcomb and V. Ambros, 1991 Efficient gene transfer in *C. elegans*: Extrachromosomal maintenance and integration of transforming sequences. EMBO J. **10:** 3959-3970.
Mendel, J. E., H. C. Korswagen, K. S. Liu, Y. M. Hajdu-Cronin, M. I. Simon *et al.*, 1995 Participation of the protein Go in multiple aspects of behavior in *C. elegans*. Science **267:** 1652-1655.
Nonet, M. L., 1999 Visualization of synaptic specializations in live *C. elegans* with synaptic vesicle protein-GFP fusions. J. Neurosci. Methods **89:** 33-40.
Nuttley, W. M., K. P. Atkinson-Leadbeater and D. van der Kooy, 2002 Serotonin mediates food-odor associative learning in the nematode *Caenorhabditis elegans*. Proc Natl Acad Sci U S A **99:** 12449-12454.
Roayaie, K., J. G. Crump, A. Sagasti and C. I. Bargmann, 1998 The G alpha protein ODR-3 mediates olfactory and nociceptive function and controls cilium morphogenesis in *C. elegans* olfactory neurons. Neuron **20:** 55-67.
Robatzek, M., T. Niacaris, K. Steger, L. Avery and J. H. Thomas, 2001 *eat-11* encodes GPB-2, a Gbeta(5) ortholog that interacts with G(o)alpha and G(q)alpha to regulate *C. elegans* behavior. Curr. Biol. **11:** 288-293.
Segalat, L., D. A. Elkes and J. M. Kaplan, 1995 Modulation of serotonin-controlled behaviors by Go in *Caenorhabditis elegans*. Science **267:** 1648-1651.
Sengupta, P., H. A. Colbert and C. I. Bargmann, 1994 The C. elegans gene *odr-7* encodes an olfactory-specific member of the nuclear receptor superfamily. Cell **79:** 971-980.
Spehr, M., C. H. Wetzel, H. Hatt and B. W. Ache, 2002 3-phosphoinositides modulate cyclic nucleotide signaling in olfactory receptor neurons. Neuron **33:** 731-739.
Tobin, D., D. Madsen, A. Kahn-Kirby, E. Peckol, G. Moulder *et al.*, 2002 Combinatorial expression of TRPV channel proteins defines their sensory functions and subcellular localization in *C. elegans* neurons. Neuron **35:** 307-318.
Troemel, E. R., J. H. Chou, N. D. Dwyer, H. A. Colbert and C. I. Bargmann, 1995 Divergent seven transmembrane receptors are candidate chemosensory receptors in *C. elegans*. Cell **83:** 207-218.
Troemel, E. R., B. E. Kimmer and C. I. Bargmann, 1997 Reprogramming chemotaxis responses: sensory neurons define olfactory preferences in *C. elegans*. Cell **91**: 161-169.
Troemel, E. R., A. Sagasti and C. I. Bargmann, 1999 Lateral signaling mediated by axon contact and calcium entry regulates asymmetric odorant receptor expression in *C. elegans*. Cell **99**: 387–398.
van der Linden, A. M., F. Simmer, E. Cuppen and R. H. Plasterk, 2001 The G-protein beta-subunit GPB-2 in *Caenorhabditis elegans* regulates the G(o)alpha-G(q)alpha signaling network through interactions with the regulator of G-protein signaling proteins EGL-10 and EAT-16. Genetics **158:** 221-235.
van der Voorn, L., M. Gebbink, R. H. Plasterk and H. L. Ploegh, 1990 Characterization of a G-protein beta-subunit gene from the nematode *Caenorhabditis elegans*. J. Mol. Biol. **213:** 17-26.
Ward, S., N. Thomson, J. G. White and S. Brenner, 1975 Electron microscopical reconstruction of the anterior sensory anatomy of the nematode *Caenorhabditis elegans*. J. Comp. Neurol. **160:** 313-337.
Ware, R. W., D. Clark, K. Crossland and R. L. Russell, 1975 The nerve ring of the nematode *C. elegans*: Sensory input and motor output. J. Comp. Neurol. **162:** 71-110.

Wekesa, K. S., and R. R. Anholt, 1999 Differential expression of G proteins in the mouse olfactory system. Brain Res. **837:** 117-126.
Wes, P. D., and C. I. Bargmann, 2001 *C. elegans* odour discrimination requires asymmetric diversity in olfactory neurons. Nature **410:** 698-701.
White, J. G., E. Southgate, J. N. Thomson and S. Brenner, 1986 The structure of the nervous system of the nematode *Caenorhabditis elegans*. Phil. Trans. Royal Soc. London. Series B **314:** 1-340.
Zwaal, R. R., J. Ahringer, H. G. van Luenen, A. Rushforth, P. Anderson *et al.*, 1996 G proteins are required for spatial orientation of early cell cleavages in *C. elegans* embryos. Cell **86:** 619-629.
Zwaal, R. R., J. E. Mendel, P. W. Sternberg and R. H. Plasterk, 1997 Two neuronal G proteins are involved in chemosensation of the *Caenorhabditis elegans* Dauer-inducing pheromone. Genetics **145:** 715-727.

Chapter 5

Non-cell autonomous G protein signals converge with a Ca^{2+}/MAPK pathway to regulate *str-2* receptor gene expression in the AWC and ASI neurons of *C. elegans*

Hannes Lans and Gert Jansen

manuscript submitted for publication

5. Non-cell autonomous G protein signals converge with a Ca^{2+}/MAPK pathway to regulate *str-2* receptor gene expression in the AWC and ASI neurons of *C. elegans*

Abstract

In the sensory system of *C. elegans* the candidate odorant receptor *str-2* is strongly expressed in one of the two AWC neurons and weakly in both ASI neurons. Asymmetric AWC expression results from suppression of *str-2* expression by a Ca^{2+}/MAPK signaling pathway in one of the AWC neurons early in development. Here we show that the same Ca^{2+}/MAPK pathway promotes *str-2* expression in the AWC and ASI neurons together with multiple non-cell autonomous G protein signaling pathways. In first stage larvae and adult animals, signals mediated by the Gα subunits ODR-3, GPA-2, GPA-5 and GPA-6 and a Ca^{2+}/MAPK pathway involving the Ca^{2+} channel subunit UNC-36, the CaMKII UNC-43 and the MAPKK kinase NSY-1 induce strong *str-2* expression. Cell specific rescue experiments suggest that ODR-3 and the Ca^{2+}/MAPK genes function in the AWC neurons, but that GPA-5 and GPA-6 function in the AWA and ADL neurons, respectively. In dauer larvae, the same network of genes promotes strong *str-2* expression in the ASI neurons, but ODR-3 functions in AWB and ASH and GPA-6 in AWB. Our results reveal a complex signaling network, encompassing signals from multiple cells, that controls the level of receptor gene expression at different developmental stages.

Introduction

It is essential that a sensory neuron expresses the correct chemoreceptors at the required level, since this defines to which stimuli a neuron can respond. As such, receptor expression defines the functional identity of a neuron. In addition, by changing receptor expression, an animal is able to modify its behavior in response to developmental or environmental changes. The expression of receptors seems to be regulated precisely in terms of spatial and temporal organization. Probably, developmental decisions establish a predefined chemoreceptor repertoire, which is subject to sensory activity dependent refinements, enabling optimal survival in nature. However, much of the underlying regulatory mechanisms are still poorly understood.

The nematode *C. elegans* senses chemical cues in its environment using 11 bilateral pairs of chemosensory amphid neurons. Cell ablation experiments have shown that these cells are involved in the response to specific sensory cues (reviewed in Bargmann and Mori 1998). The AWA and AWC neurons detect attractive odorants, whereas the AWB, ASH and ADL neurons detect repulsive odorants. The ASE, ADF, ASK, ASG, ASI, ADL and ASH neurons respond to nonvolatile attractive and repulsive chemicals. In addition, the ADF, ASI, ASG and ASJ neurons are involved in the response to dauer pheromone, which regulates development of the non-reproductive dauer larval stage.

The genome of *C. elegans* encodes approximately 800 functional receptor genes, many of which are specifically expressed in the amphid neurons (Troemel et al. 1995; Bargmann 1998; Robertson 1998, 2000). Binding of a ligand to a receptor activates one or more heterotrimeric G proteins, consisting of Gα, Gß and Gγ subunits, leading to the activation of a cascade of effectors. The sensory neurons of *C. elegans* express 14 Gα subunits, several of which have been shown to be involved in olfaction, pheromone perception and taste (Zwaal et al. 1997; Roayaie et al. 1998; Jansen et al. 1999; Hilliard et al. 2004; Lans et al. 2004). For example, odorant detection by the AWA and AWC neurons involves at least five Gα subunits (Roayaie et al. 1998; Lans et al. 2004). The Gα subunit ODR-3 constitutes the major signaling route and is sufficient for odorant detection. The Gα subunit GPA-3 is redundant to ODR-3 and also sufficient for most odorant signals. Signaling via ODR-3 and GPA-3 is inhibited by GPA-5 and GPA-2 in the AWA and AWC neurons, respectively, and stimulated by GPA-13 in the AWC neurons. The presence of multiple receptors and G proteins per cell probably enables *C. elegans* to respond to a wide variety of sensory cues, using only few cells.

In *C. elegans*, receptor expression is regulated by developmental and experience based signals. Receptor expression can vary from embryos to adults or from males to hermaphrodites (Troemel et al. 1995, 1999). Furthermore, receptor expression can change when *C. elegans* develops into the non-reproductive, dauer larval stage (Peckol et al. 2001). In addition, sensory experience regulates receptor expression, allowing *C. elegans* to adjust behavior in changing environments. For instance, dauer pheromone represses expression of the *srd-1, str-2* and *str-3* receptors in adult animals. Also, *srd-1* expression is abolished in the absence of sensory signaling (Peckol et al. 2001).

In well-fed adult animals, the candidate odorant receptor *str-2* is strongly expressed in either the left or the right AWC neuron and weakly in both ASI neurons (Troemel et al. 1999). In dauer animals, *str-2* is repressed in the AWC neurons and strongly expressed in both ASI neurons (Peckol et al. 2001). Early in development, probably in the late embryo, a stochastic choice is made to repress *str-2* expression in one of the two AWC neurons. This is accomplished by Ca^{2+} signaling via the N/P-type voltage-gated Ca^{2+} channel UNC-2/UNC-36 and the Ca^{2+}/calmodulin dependent protein kinase II (CaMKII) UNC-43, which activates a MAPK pathway consisting of the MAPKK kinase NSY-1 and the MAPK kinase SEK-1 (Troemel et al. 1999; Sagasti et al. 2001; Tanaka-Hino et al. 2002). Also, the Ca^{2+}-activated K^{+}-channel NSY-3/SLO-1, upstream of or parallel to UNC-43, and the TIR protein TIR-1, which binds UNC-43 and probably also NSY-1, are involved (Troemel et al. 1999; Sagasti et al. 2001; Wang et al. 2001; Davies et al. 2003; Chuang and Bargmann 2005). Furthermore, mutations that disrupt axon guidance impair asymmetric *str-2* expression and killing one AWC neuron in the embryo abolishes *str-2* expression, suggesting that the asymmetry is initiated by lateral AWC axon contact (Troemel et al. 1999). Once asymmetry is established, *str-2* expression is maintained by the activity of several olfactory signaling molecules, such as the guanylyl cyclases ODR-1 and DAF-11 and the cyclic

nucleotide-gated channel TAX-2/TAX-4 (Troemel et al. 1999). The regulation of *str-2* expression in the ASI neurons seems to involve different mechanisms, because no effects of *unc-36* and *tax-2/-4* mutations have been observed (Peckol et al. 2001). ASI expression is suppressed by dauer pheromone and under control of genes of the TGFß signaling pathway, which also regulates dauer development (Peckol et al. 2001; Nolan et al. 2002).

Since several lines of evidence suggest that receptor expression is regulated by sensory signaling, we studied the involvement of the olfactory Gα subunits in regulating *str-2* expression. We show that G proteins regulate *str-2* gene expression, but do not affect the developmental choice to express *str-2* asymmetrically. Four Gα subunits, ODR-3, GPA-2, GPA-5 and GPA-6, together with Ca^{2+} signaling through UNC-36, UNC-43 and NSY-1, promote *str-2* expression in the AWC and ASI neurons of young larvae, dauer larvae and adults. Interestingly, this regulation involves G protein signaling in other neurons than AWC and ASI and does not seem to require sensory cues.

Results

str-2 expression is reduced in animals with Gα subunit mutations

C. elegans expresses six Gα subunits in its olfactory neurons AWA and AWC: GPA-2, GPA-3, GPA-5, GPA-6, GPA-13 and ODR-3 (Zwaal et al. 1997; Roayaie et al. 1998; Jansen et al. 1999; Lans et al. 2004). All, except GPA-6, play a role in the detection of odorants. To determine their involvement in regulating gene expression, we examined the asymmetric expression of *str-2* in the AWC cells (Fig. 1A) of several Gα loss-of-function mutants. For this purpose, the *kyIs140[str-2::gfp]* allele (Troemel et al. 1999) was introduced in *gpa-2, gpa-3, gpa-5, gpa-6, gpa-13* and *odr-3* mutants. No change in the asymmetry of *str-2* expression was observed in these mutants, as was already found for *odr-3* (results not shown; Troemel et al. 1999).

In the Gα mutants, we observed only very limited variation in *str-2* expression levels (Table 1). This was unexpected, since mutations in the cGMP-signaling genes *odr-1*, *daf-11*, *tax-2* and *tax-4,* which are likely activated through G protein signaling, dramatically reduce *str-2* expression (Table 1; Troemel et al. 1999). To test whether this discrepancy resulted from functional redundancy of the Gα subunits, *str-2* expression was examined in animals with mutations in two to six Gα subunits. Surprisingly, in most mutants the strength of expression was close to normal and no effect on the asymmetry of *str-2* expression was observed (Table 1; results not shown). Only *gpa-6 odr-3* double mutants displayed a strong reduction, which was partially (*gpa-3* or *gpa-5*) or completely *(gpa-2* or *gpa-13)* restored by additional mutations in other Gα subunits (Fig 1B; Table 1). Animals that had lost the function of all six Gα subunits also displayed a clear reduction of *str-2::gfp* strength, but not as strong as *gpa-6 odr-3* animals (Fig. 1C; Table 1). These results show that the six olfactory Gα subunits regulate *str-2* expression in a redundant

fashion, but do not influence the asymmetry of *str-2* expression.

To some extent, the effects of Gα mutations on *str-2* expression resemble the effects of mutations in *odr-1*, *daf-11, tax-2* and *tax-4.* These genes do not affect the decision for asymmetric *str-2* expression, but maintain *str-2* expression once asymmetry is established (Troemel et al. 1999). In *tax-4* and *odr-1* L1 larvae, *str-2* is expressed strongly, but during later stages expression is reduced or even disappears (Table 1; Troemel et al. 1999). To determine whether the olfactory Gα subunits function to maintain expression, we determined *str-2::gfp* strength in the L1 larvae of several Gα mutants (Table 1; results not shown). In *gpa-6 odr-3* larvae, we observed relatively higher *str-2* expression than in adults. However, these and *gpa-2 gpa-3 gpa-5 gpa-6 gpa-13 odr-3* larvae still showed a reduction in *str-2::gfp* strength when compared to wild type L1 larvae. These results suggest that the Gα subunits function predominantly to maintain *str-2* expression, but also contribute to the initiation of *str-2* expression.

Table 1. *str-2* expression is reduced in certain Gα mutants

genotype	strong	weak	off	*n*
wild type	100	0	0	112
gpa-2(pk16)	100	0	0	32
gpa-3(pk35)	100	0	0	61
gpa-5(pk376)	100	0	0	54
gpa-6(pk480)	98	2	0	57
gpa-13(pk1270)	95	4	2	55
odr-3(n1605)	100	0	0	21
gpa-6 odr-3	45	35	21	92
gpa-2 gpa-6 odr-3	100	0	0	29
gpa-3 gpa-6 odr-3	88	1	10	67
gpa-5 gpa-6 odr-3	77	23	0	60
gpa-6 gpa-13 odr-3	100	0	0	25
gpa-2 gpa-5 gpa-6 odr-3	96	2	2	90
gpa-2 gpa-3 gpa-5 gpa-6 gpa-13 odr-3	81	18	1	80
odr-1(n1936)	0	7	93	56
tax-4(p678)	21	34	45	56
dyf-8(m539)	100	0	0	82
osm-6(p811)	100	0	0	80
osm-6 unc-36	89	4	7	161
wild type (L1)	98	2	0	101
gpa-6 odr-3 (L1)	80	8	12	50
gpa-2 gpa-3 gpa-5 gpa-6 gpa-13 odr-3 (L1)	76	16	8	50
odr-1 (L1)	94	6	0	79
tax-4 (L1)	100	0	0	50

Percentage of animals that show strong (visible at 100x magnification), weak (visible at 400x but not at 100x) or no (off; undetectable at 400x) *str-2::gfp* expression. L1 larvae (L1) were scored at 160 and 400x magnification.

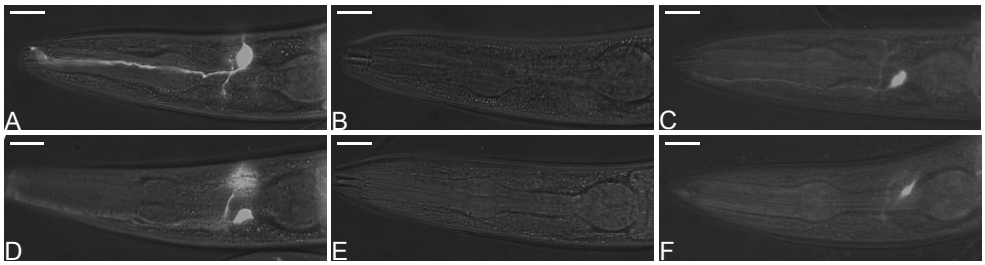

Figure 1. *str-2* expression is reduced in several Gα mutants
In wild type animals, *str-2::gfp* is expressed at high levels in one AWC neuron (*A*), whereas more than half of the *gpa-6 odr-3* mutants show reduced *str-2::gfp* expression (*B*). Expression is partially restored in *gpa-2 gpa-3 gpa-5 gpa-6 gpa-13 odr-3* mutants (*C*). *unc-36* mutants show strong *str-2::gfp* expression in both AWC neurons (*D*; Troemel et al. 1999). This expression is lost in *gpa-5 gpa-6 odr-3 unc-36* animals (*E*), but restored in *gpa-2 gpa-3 gpa-5 gpa-6 gpa-13 odr-3 unc-36* mutants (*F*). Scale bars denote 20 μm. All panels are at 400x magnification.

G proteins cooperate with Ca^{2+}/MAPK signaling to regulate str-2 *expression*

Early in development, the decision to express *str-2* asymmetrically is executed by Ca^{2+} and MAPK signaling (Troemel et al. 1999; Sagasti et al. 2001; Tanaka-Hino et al. 2002; Chuang and Bargmann 2005). We tested the involvement of G proteins by examining *str-2* expression in animals with mutations in Ca^{2+}/MAPK signaling genes and Gα subunits. Mutations in the Ca^{2+} channel subunit *unc-36* result in strong *str-2* expression in both AWC neurons, indicating that in wild type animals UNC-36 represses *str-2* in one neuron (Troemel et al. 1999; Fig. 1D). Additional mutations in Gα subunits never changed this symmetric expression (results not shown), but, surprisingly, affected the strength of *str-2* expression in *unc-36* mutants (Fig. 2A; results not shown). A mild reduction in *str-2* expression was observed in *odr-3 unc-36* and *gpa-6 odr-3 unc-36* mutants. However, an *unc-36* mutation together with different combinations of three or four Gα subunit mutations, including *odr-3*, *gpa-2*, *gpa-5* or *gpa-6*, severely reduced expression (Fig. 1E, 2A). Reduction of *str-2* expression was strongest in *gpa-5 gpa-6 odr-3 unc-36* animals, in which also at 400x magnification almost no fluorescence could be observed (results not shown). Interestingly, expression was restored to wild type levels when all six olfactory Gα subunits were inactivated (Fig. 1F). These results suggest that ODR-3, GPA-2, GPA-5 and GPA-6 cooperate with UNC-36 to promote *str-2* expression, but that GPA-3 and GPA-13 inhibit *str-2* expression.

Several genes have been identified that function in a pathway with UNC-36 to regulate asymmetric *str-2* expression. Genetic epistasis experiments place the CaMKII UNC-43, the TIR protein TIR-1, the ASK1 MAPKKK homologue NSY-1 and the MAPKK SEK-1 in a linear pathway downstream of UNC-36 and the Ca^{2+} activated K^+ channel NSY-3/SLO-1 upstream of or parallel to UNC-43 (Troemel et al. 1999; Sagasti et al. 2001; Wang et al. 2001; Tanaka-Hino et al. 2002; Davies et al. 2003; Chuang and Bargmann 2005). Mutations in these genes all disrupt *str-2* asymmetry. We determined if these genes function in the same pathway to

regulate *str-2* expression together with G proteins. Mutations in *gpa-5, gpa-6* and *odr-3* in an *unc-43* background reduced *str-2* expression levels (Fig. 2B). Additional loss of *gpa-2* even further reduced *str-2* expression. Similarly to *unc-36,* mutations in all six Gα subunits restored *str-2* expression to wild type levels. In *nsy-1* mutants, loss of *gpa-5, gpa-6* and *odr-3* had no effect, but loss of *gpa-2, gpa-5, gpa-6* and *odr-3* reduced *str-2* expression levels severely (Fig. 2B; results not shown). Again, loss of all olfactory Gα subunits restored *str-2* expression levels. Unexpectedly, in *tir-1* and *nsy-3* animals, no strong reduction in expression was observed (Fig. 2C), although TIR-1 is necessary to localize the NSY-1 correctly to postsynaptic sites (Chuang and Bargmann 2005). *sek-1* mutants already showed a mild reduction in *str-2::gfp* strength, which was not enhanced by additional Gα mutations (Fig 2C; results not shown). This shows that, in parallel to part of the Ca^{2+}/MAPK pathway, several G protein signaling pathways exist that regulate *str-2* expression. Furthermore, these results suggest dual functions for *unc-36, unc-43* and *nsy-1* in the AWC neurons: suppression of *str-2* expression in one AWC cell to establish *str-2* asymmetry and promotion of *str-2* expression together with G protein signaling.

To determine if the signaling network of G proteins and Ca^{2+}/MAPK genes is required when *str-2* expression is initiated or to maintain expression, *str-2* expression in several *Gα/unc-36* mutants was determined at different time points during development (Fig. 2D). As comparison, we also examined *odr-1* animals. In *gpa-5 gpa-6 odr-3 unc-36* L1 larvae, *str-2* expression was weak, but increased considerably in successive larval stages. In adults, *str-2* expression diminished again. In contrast, *str-2* expression in the AWC neurons of *odr-1* animals disappeared completely after the L1 stage. These results suggest that *str-2* expression levels are controlled by the G protein and Ca^{2+}/MAPK signaling network early during development and in adults. Other mechanisms might promote *str-2* expression from the L2 to L4 stage.

str-2 *expression does not require sensory cues*

Because *str-2* expression maintenance requires genes that are essential for olfaction (Coburn and Bargmann 1996; Komatsu et al. 1996; Troemel et al. 1999; Birnby et al. 2000; L'Etoile and Bargmann 2000; this study), it was suggested that *str-2* expression is regulated by sensory cues. To test this, we introduced *str-2::gfp* in *osm-6* and *dyf-8* mutants, which have impaired chemosensory responses due to structural defects of the cilia (Starich et al. 1995; Collet et al. 1998). Surprisingly, *osm-6* and *dyf-8* animals showed wild type levels of *str-2* expression in the AWC neurons (Table 1), indicating that expression of *str-2* does not require sensory activity per se. Also, *osm-6 unc-36* double mutants showed almost wild type *str-2* expression levels (Table 1). Expression of *str-2* in the ASI neurons had previously been shown to be unaffected by structural defects of the cilia (Peckol et al. 2001). These results hint at developmental rather than sensory activity dependent regulation of *str-2* expression.

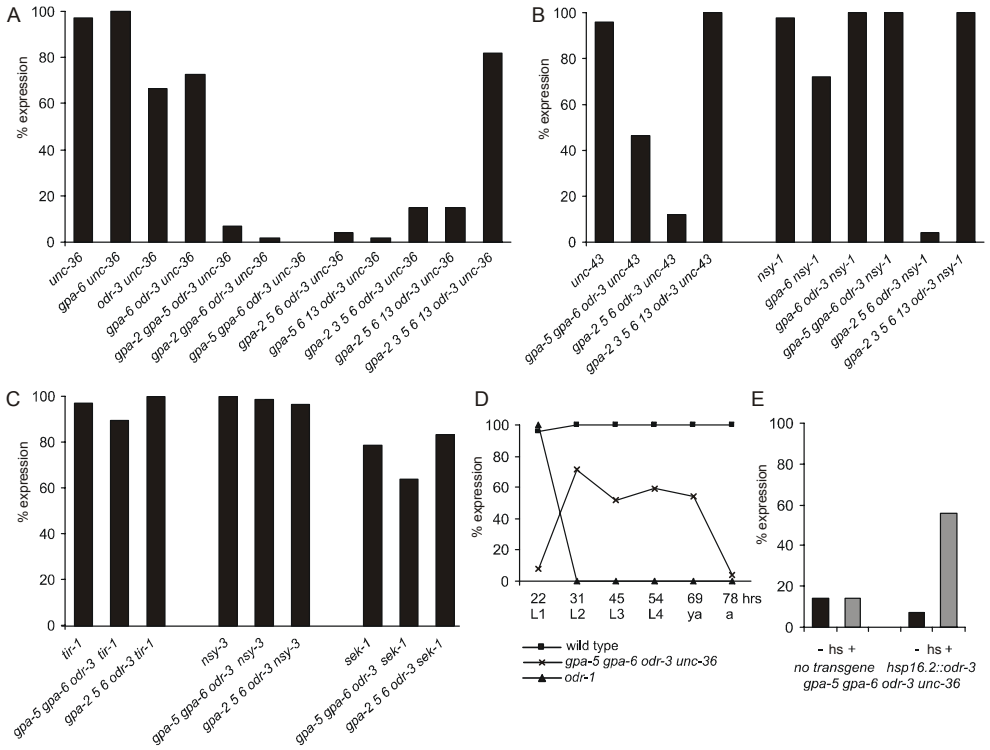

Figure 2. G protein and Ca^{2+}/MAPK genes together regulate *str-2* expression in the AWC neurons

Shown are the percentages of animals with strong *str-2* expression (detectable at 100x in *A*, *B*, *C* and *E* and 160x in *D*), in the AWC neurons. (*A, B* and *C*) In adult animals, ODR-3 and two or three other Gα subunits (GPA-2, GPA-5 and GPA-6) and UNC-36, UNC-43 or NSY-1 are required for strong *str-2* expression. GPA-3 and GPA-13 have inhibiting functions. Each bar represents at least 50 animals, except *tir-1* (*n*=32) and *nsy-3* (*n*=40). *2=gpa-2*, *3=gpa-3*, *5=gpa-5*, *6=gpa-6* and *13=gpa-13*. (*D*) *str-2* expression at different time points after egg laying, at 20°C. The corresponding developmental stage is indicated: the four larval stages, young adult (ya) and adult (a). Wild type animals were indistinguishable from *unc-36* mutants (not shown) and show strong expression throughout development, whereas *gpa-5 gpa-6 odr-3 unc-36* mutants show delayed *str-2* expression in L2 larvae, which is abolished in adult animals. *odr-1* mutants lose expression following the L1 stage. Each data point represents at least 27 animals. (*E*) In adults, *str-2* expression is restored when ODR-3 is reintroduced following heat shock (+) of *gpa-5 gpa-6 odr-3*

AWC cell fate is unaltered in gpa-5 gpa-6 odr-3 unc-36 mutants

Reduction of *str-2* expression could be caused by general developmental or structural defects caused by loss of G protein signaling. ODR-3 has a function in regulating the morphology of the AWC cilia, but this is independent of sensory signaling (Roayaie et al. 1998; Lans et al. 2004). We observed no abnormalities of the axons or dendrites of *gpa-5 gpa-6 odr-3 unc-36* mutants. Also, these mutants showed normal fluorescent dye filling of the amphid neurons, suggesting that their cilia are intact (results not shown). To test whether any unnoticed structural defects caused the reduction of *str-2* expression, we tested if *str-2* expression could be

restored by reintroduction of ODR-3. Following heat shock of adult *gpa-5 gpa-6 odr-3 unc-36* animals carrying an *odr-3* gene under control of a heat shock promoter, *str-2* expression was significantly upregulated (Fig. 2E). This shows that *str-2* can be expressed immediately in response to an activating signal, suggesting that the AWC neurons develop correctly.

The function and morphology of a sensory neuron can be defined by the sensory signaling and structural genes that it expresses. Therefore, reduction of *str-2* expression might be caused by partial or complete loss of AWC identity. This is the case for animals with mutations in the Otx homeodomain gene *ceh-36*, which do not express *odr-1* and *str-2* in their AWC neurons and do not respond to AWC sensed odorants (Lanjuin et al. 2003). To test if expression of additional AWC specific genes was affected in *gpa-5 gpa-6 odr-3 unc-36* animals, we introduced *gfp* fusions of *gpa-13* and *odr-1* in these mutants (Jansen et al. 1999; L'Etoile and Bargmann 2000). Both genes were strongly expressed in the AWC neurons, confirming that basal AWC neurons develop normally in *gpa-5 gpa-6 odr-3 unc-36* animals.

Next, we determined whether GPA-5, GPA-6, ODR-3 and UNC-36 only regulate the expression of *str-2* or also that of other receptors. To this end, we examined the expression of *odr-10*, expressed in AWA (Sengupta et al. 1996), *str-1*, expressed in AWB (Troemel et al. 1997), *srh-142*, expressed in ADF (Sagasti et al. 1999), *sra-6*, expressed in ASH, and *sro-1,* expressed in ADL (Troemel et al. 1995). All receptors showed comparable expression levels in wild type animals and in *gpa-5 gpa-6 odr-3 unc-36* animals, in the appropriate cells (results not shown). These results suggest that the signaling network under study specifically regulates the expression of *str-2*.

ODR-3 and UNC-36 act in AWC, GPA-5 in AWA and GPA-6 in ADL to regulate str-2 expression in AWC

Intriguingly, GPA-5 and GPA-6 are not expressed in the AWC neurons (Jansen et al. 1999; Lans et al. 2004), indicating that signals from other sensory neurons regulate *str-2* expression in AWC. We decided to identify the neurons in which ODR-3, GPA-5, GPA-6 and UNC-36 function using cell specific rescue constructs. ODR-3 is expressed in the amphid neurons AWA, AWB, AWC, ASH and ADF (Roayaie et al. 1998). Introduction of the wild type *odr-3* gene in *gpa-5 gpa-6 odr-3 unc-36* animals fully restored *str-2* expression (Fig. 3A). Full rescue was also obtained when ODR-3 was expressed under the *gpa-13* promoter, which drives expression in AWC and in the ASH and ADF cells (Jansen et al. 1999). These results are consistent with a function of ODR-3 in the AWC neurons. In agreement, specific expression of ODR-3 in the AWA, AWB or ASH neurons, using the *odr-10, str-1* and *sra-6* promoter, did not restore *str-2* expression (Fig. 3A). However, a partial restoration of *str-2* expression was observed when ODR-3 was expressed in the ADF neurons, using the *srh-142* promoter (Fig. 3A). Simultaneous expression of *odr-3* in the ASH and ADF neurons, but not AWC, had no additional effect (results not shown). These results suggest that ODR-3 functions

predominantly in AWC and, to a lesser extend, in the ADF neurons, to regulate *str-2* expression.

GPA-5 is expressed in the AWA and ADL neurons (Jansen et al. 1999; Lans et al. 2004). Introduction of extrachromosomal copies of the *gpa-5* gene partially restored *str-2* expression in *gpa-5 gpa-6 odr-3 unc-36* mutants, while an integrated *gpa-5* allele fully restored *str-2* expression (Fig. 3B). Expression of GPA-5 in the AWA neurons, using the *odr-10* promoter, also restored *str-2* expression, while expression in the ADL cells, using the *gpa-11* promoter, had no effect (Fig. 3B). Therefore, GPA-5 likely acts in the AWA neurons to regulate *str-2* expression in AWC.

GPA-6 is expressed in the amphid neurons AWA, AWB, ADL and ASH (Jansen et al. 1999; Lans et al. 2004). *str-2* expression could be restored by an integrated and an extrachromosomal transgene of *gpa-6* (Fig. 3C). To find out in which neurons GPA-6 functions, GPA-6 was expressed in the AWA, AWB, ASH and ADL neurons using cell specific promoters. Surprisingly, only expression in the ADL neurons, using the *gpa-11* and *sro-1* promoters, noticeably restored *str-2* expression (Fig. 3D). These data show that GPA-6 acts predominantly in the ADL neurons to regulate *str-2* expression in the AWC neurons.

UNC-36 is broadly expressed in neuronal and probably also muscle tissue (Schafer et al. 1996). In the embryo, it is predicted to act in one of the two AWC neurons to repress *str-2* expression in response to lateral cell contact (Troemel et

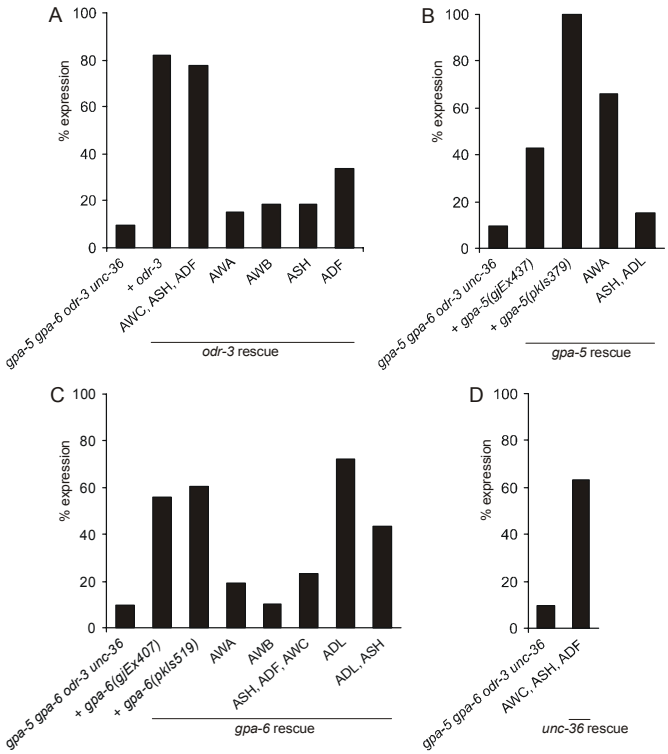

Figure 3.
Cell specific expression of ODR-3, GPA-5, GPA-6 and UNC-36 restores *str-2* expression in *gpa-5 gpa-6 odr-3 unc-36* mutants
Shown are the percentages of adult animals in which *str-2::gfp* could be detected in the AWC neurons at 100x magnification. Each bar represents at least 73 animals, except *gpa-6(pkIs519)* (*n*=31) (*A*) ODR-3 restores *str-2* expression when expressed in the AWC and ADF neurons. (*B*) GPA-5 restores *str-2* expression when expressed in the AWA neurons. (*C*) GPA-6 restores *str-2* expression when expressed in the ADL neurons. (*D*) UNC-36 expression in the neurons AWC strongly increases *str-2* expression.

al. 1999). Furthermore, the downstream genes *unc-43, nsy-1* and *sek-1*, have all been shown to act in AWC to regulate functional asymmetry (Sagasti et al. 2001; Tanaka-Hino et al. 2002). We expressed UNC-36 in the AWC neurons using the *gpa-13* promoter, which resulted in strong *str-2* expression in more than 60% of the animals (Fig. 3D). Although we cannot rule out that UNC-36 functions in the ASH or ADF neurons, it seems plausible that UNC-36 functions in AWC to promote *str-2* expression. This implies that in one cell type, AWC, UNC-36 has a dual, paradoxical role and both suppresses and promotes *str-2* expression.

ASI expression of str-2 *is regulated by the same genes that regulate AWC expression, but involves different neurons*

Thus far, we considered *str-2* expression in the AWC neurons. Adult animals also express *str-2* weakly in both ASI neurons and dauer larvae express *str-2* strongly in the ASI neurons. ASI expression in adults is regulated by dauer pheromone and a TGFß signaling pathway, which regulates entry in the dauer stage (Peckol et al. 2001; Nolan et al. 2002). Mutations in the TGFß homologue *daf-7*, the SMAD transcriptional regulator *daf-3* and the nuclear hormone receptor *daf-12* affect the expression of *str-2* and other receptors in the ASI neurons, in all developmental stages (Ren et al. 1996; Schackwitz et al. 1996; Riddle and Albert 1997; Patterson et al. 1997). In young *gpa-5 gpa-6 odr-3 unc-36* larvae, faint ASI expression was detectable, but this disappeared in adult animals (results not shown). To test if the Gα/Ca^{2+}/MAPK network regulates *str-2* expression in the ASI neurons, we examined *str-2* expression in dauer larvae.

str-2 expression in *gpa-5 gpa-6 odr-3 unc-36* dauer larvae was strongly reduced in the ASI neurons and mostly off in the AWC cells (Fig. 4A; results not shown). This suggests that the same network of G proteins and Ca^{2+}/MAPK signaling genes regulates *str-2* expression in the ASI neurons of dauer larvae. Next, we determined in which neurons the Gα subunits and UNC-36 function, by introduction of cell specific rescue constructs in *gpa-5 gpa-6 odr-3 unc-36* dauer larvae. Unexpectedly, *str-2* expression was partially restored when *odr-3* was expressed in the AWB or in the ASH neurons (Fig. 4B). Specific expression of *odr-3* in the AWA, AWC or ADF neurons did not restore *str-2* expression in ASI (results not shown). Furthermore, *str-2* expression was partially restored when GPA-6 was expressed in the AWB neurons, but was not or only weakly restored when GPA-6 was expressed in the AWA, ASH or ADL neurons (Fig. 4C; results not shown). Finally, *str-2* expression could be restored by expressing GPA-5 in the AWA neurons and UNC-36 in the AWC, ASH and ADF neurons (Fig. 4D, E). These results suggest that, although the same genes regulate *str-2* expression in the ASI neurons as in the AWC neurons, they do not all function in the same cells. ASI expression seems to require ODR-3 in the AWB and ASH neurons, instead of AWC, and GPA-6 in the AWB neurons, instead of ADL. The site of action of GPA-5 and UNC-36 seems unaltered.

Next, we determined whether the Gα/Ca^{2+}/MAPK genes interact with the TGFß pathway that regulates *str-2* expression in the ASI neurons. In *daf-7* adult

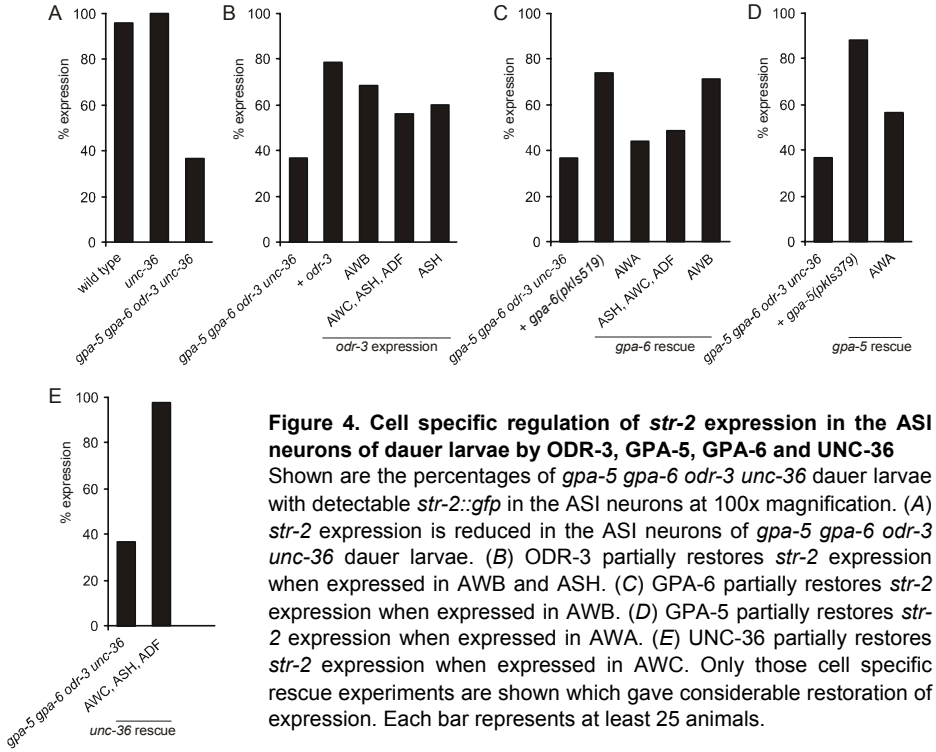

Figure 4. Cell specific regulation of *str-2* expression in the ASI neurons of dauer larvae by ODR-3, GPA-5, GPA-6 and UNC-36
Shown are the percentages of *gpa-5 gpa-6 odr-3 unc-36* dauer larvae with detectable *str-2::gfp* in the ASI neurons at 100x magnification. (*A*) *str-2* expression is reduced in the ASI neurons of *gpa-5 gpa-6 odr-3 unc-36* dauer larvae. (*B*) ODR-3 partially restores *str-2* expression when expressed in AWB and ASH. (*C*) GPA-6 partially restores *str-2* expression when expressed in AWB. (*D*) GPA-5 partially restores *str-2* expression when expressed in AWA. (*E*) UNC-36 partially restores *str-2* expression when expressed in AWC. Only those cell specific rescue experiments are shown which gave considerable restoration of expression. Each bar represents at least 25 animals.

animals, *str-2* is upregulated in the ASI neurons (Table 2; Nolan et al. 2002). In *daf-7* dauer animals, we observed that *str-2* expression was reduced, in contrast to what has been reported (results not shown; Nolan et al. 2002). In *gpa-5 gpa-6 odr-3 unc-36 daf-7* animals *str-2* expression was strongly reduced in the AWC and ASI neurons of adults and dauer animals, similar to *gpa-5 gpa-6 odr-3 unc-36* animals (Table 2; results not shown). Mutations in *daf-3* and *daf-12* suppress the upregulated ASI expression of *daf-7* animals, but a mutation in *daf-12* also impairs *str-2* expression in AWC and ASI by itself (Table 2; Nolan et al. 2002). We observed no effect on *str-2* expression by an additional mutation in *daf-12* in *gpa-5 gpa-6 odr-3 unc-36* animals (Table 2). However, *str-2* expression in the AWC and ASI neurons of *gpa-5 gpa-6 odr-3 unc-36* mutants was fully restored by an additional mutation in *daf-3* (Table 2), suggesting that *daf-3* represses *str-2* expression in these mutants. In contrast, *daf-7* and *daf-12* show no apparent interaction with the Gα/Ca^{2+}/MAPK network and therefore might signal upstream of the Gα/Ca^{2+} signals.

odr-1 *and* osm-9 *are downstream targets of the Gα/Ca^{2+}/MAPK network*

tax-2, tax-4, *odr-1* and *daf-11* have been shown to regulate the level of *str-2* expression and are also likely to interact with the Gα/Ca^{2+}/MAPK network (Troemel et al. 1999). In olfactory signaling, ODR-1 and DAF-11 probably function downstream of ODR-3, GPA-3, GPA-13 and GPA-2, but upstream of TAX-2 and

TAX-4 (Coburn and Bargmann 1996; Komatsu et al. 1996; Roayaie et al. 1998; Birnby et al. 2000; L'Etoile and Bargmann 2000; Lans et al. 2004). However, in regulating *str-2* expression, *odr-1* is epistatic to *tax-4* (Troemel et al. 1999). We tested whether *odr-1* is also epistatic to the Gα subunits in regulating *str-2* expression. Loss of *str-2* expression in *gpa-5 gpa-6 odr-3 unc-36* animals is suppressed by additional mutations in *gpa-2, gpa-3* and *gpa-13* (Fig. 2A). To test if these mutations also suppressed the *str-2* phenotype of *odr-1* mutants, we generated *gpa-2 gpa-3 gpa-13 odr-1* animals. In these mutants *str-2* expression was off (Table 2), suggesting that *odr-1* is epistatic to all six Gα subunits in regulating *str-2* expression.

In the AWA and ASH neurons, TRPV channels are thought to act downstream of G protein sensory signaling (Colbert et al. 1997; Roayaie et al. 1998). Three TRPV channels have been shown to regulate *odr-10* receptor gene expression in AWA (Tobin et al. 2002). Of these, the OSM-9 protein is also expressed in the AWC cells, where it is required for adaptation to certain odorants (Colbert and Bargmann 1995). Thus far, no involvement of OSM-9 in regulating receptor expression in AWC has been described. Therefore, we examined *str-2* expression in *osm-9* mutants. In *osm-9* single and *osm-9 unc-36* double mutants,

Table 2. *str-2* expression in TGF-ß and several signaling mutants

	AWC				ASI			
	strong	**weak**	**off**	***n***	**strong**	**weak**	**off**	***n***
wild type	100	0	0	112	68	23	9	191
unc-36(e251)	97	2	1	98	25	33	42	73
gpa-5 6 odr-3 unc-36	4	71	24	115	0	0	100	115
daf-7(e1372)	100	0	0	90	96	4	0	52
gpa-5 6 odr-3 unc-36 daf-7	18	65	16	49	8	35	57	49
daf-3(mgDf90)	100	0	0	60	57	37	7	60
gpa-5 6 odr-3 unc-36 daf-3	100	0	0	114	70	12	18	114
daf-12(ok493)	83	15	2	92	38	47	15	97
gpa-5 6 odr-3 unc-36 daf-12	3	51	46	112	nd			
odr-1(n1936)	0	2	98	176	99	1	0	120
gpa-2 gpa-3 gpa-13 odr-1	0	5	95	74	nd			
osm-9(n1603)	94	4	2	53	nd			
osm-9 unc-36	100	0	0	78	nd			
gpa-5 osm-9 unc-36	0	38	62	45	nd			
gpa-6 osm-9 unc-36	19	45	36	47	nd			
odr-3 osm-9 unc-36	4	71	24	49	nd			
gpa-5 gpa-6 osm-9 unc-36	48	38	13	52	nd			
gpa-5 odr-3 osm-9 unc-36	2	56	42	48	nd			
gpa-5 gpa-6 odr-3 osm-9 unc-36	78	16	5	55	nd			

Percentage of adult animals that show strong (visible at 100x magnification), weak (visible at 400x but not at 100x) or no (off; undetectable at 400x) *str-2::gfp* expression in the AWC and ASI neurons. nd denotes not determined

no change in *str-2* expression was observed (Table 2). However, mutations in *unc-36, osm-9* and either *odr-3*, *gpa-5* or *gpa-6* severely reduced *str-2* expression, while mutations in all five genes restored expression to almost wild type levels (Table 2). This shows that OSM-9 regulates *str-2* expression in a complex manner and possibly functions in both stimulatory and inhibitory G protein pathways to regulate *str-2* expression.

Discussion

str-2 *expression is regulated by a network of G protein and Ca^{2+}/MAPK genes*

Our results identify a signaling network consisting of multiple different and redundant pathways that cooperate to control the expression of a single receptor. First, we find that maintenance of *str-2* expression is stimulated by ODR-3 and GPA-6 and inhibited by four other Gα subunits, in a redundant fashion. Second, we find that a signaling network involving signals from several sensory neurons, mediated by GPA-2, GPA-5, GPA-6, ODR-3 and the UNC-36/UNC-43/NSY-1 pathway, promotes *str-2* expression. GPA-3 and GPA-13 suppress *str-2* expression in this network.

We propose a model in which signals from different neurons converge in the AWC neurons in adults to regulate *str-2* expression (Fig. 5A). Our results confirm previous cell specific rescue experiments (Sagasti et al. 2001; Tanaka-Hino et al. 2002; Chuang and Bargmann 2003), which suggest that the Ca^{2+}/MAPK pathway functions in the AWC neurons. In addition, ODR-3 and probably GPA-2 function in the AWC neurons. AWC is the only amphid neuron in which GPA-2 is expressed, but we cannot exclude a function in an interneuron (Zwaal et al. 1997). Furthermore, *str-2* expression in the AWC neurons is stimulated by ODR-3 signaling in the ADF neurons, GPA-5 signaling in the AWA neurons and GPA-6 signaling in the ADL neurons.

A similar Gα/Ca^{2+}/MAPK network regulates *str-2* expression in the ASI neurons in dauer larvae (Fig. 5B), but there are striking differences. In dauer larvae, the G protein signals seem to originate from different cells: ODR-3 seems to function in the ASH and AWB neurons and GPA-6 in the AWB neurons, whereas in adults these Gα subunits function in the AWC, ADF and ADL neurons. Furthermore, *str-2* expression in the ASI neurons in adults does not seem to involve *tax-2* and *tax-4* (Peckol et al. 2001) or *odr-1*. It is unclear what these differences mean. We propose that *str-2* gene expression in the AWC and ASI neurons is regulated by different developmental signals, originating from different sensory neurons, but mediated by the same signaling molecules.

Thus far, we have not identified any other receptor regulated by the same network. However, preliminary data suggest that expression of the diacetyl receptor *odr-10* is regulated by G proteins via different mechanisms (H.L, G.J, Amanda Kahn and Cori Bargmann, unpublished results). We suspect that additional receptors are regulated by signaling networks involving different G proteins and other signaling genes.

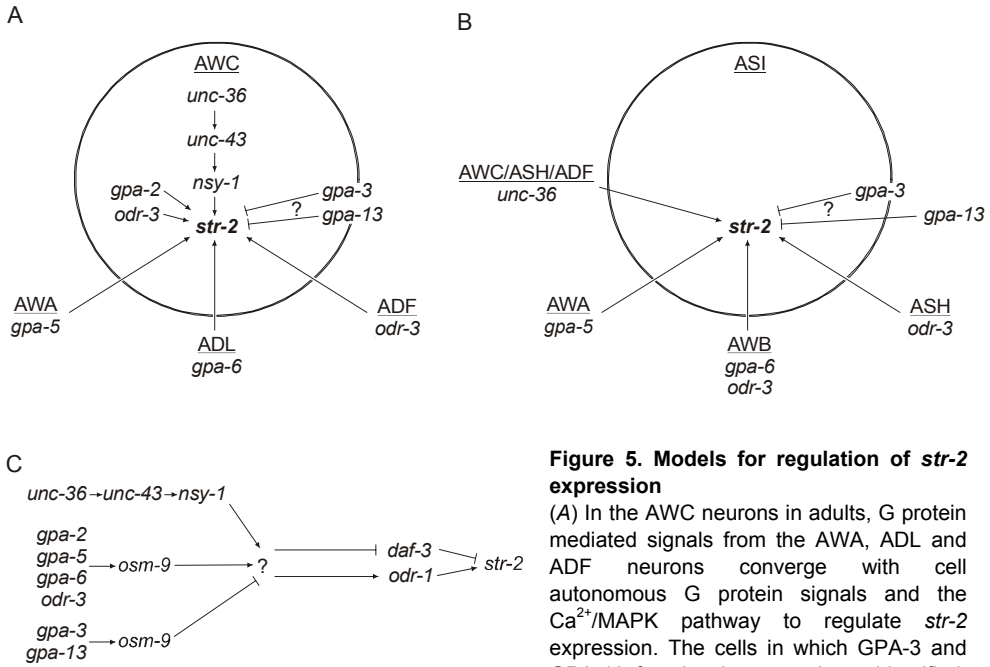

Figure 5. Models for regulation of *str-2* expression

(*A*) In the AWC neurons in adults, G protein mediated signals from the AWA, ADL and ADF neurons converge with cell autonomous G protein signals and the Ca^{2+}/MAPK pathway to regulate *str-2* expression. The cells in which GPA-3 and GPA-13 function have not been identified, but the AWC neurons are likely candidates. (*B*) In dauer larvae, signals from the AWA, AWB, ASH and possibly AWC and/or ADF neurons regulate *str-2* expression in the ASI neurons. (*C*) Shown is a model of the genetic pathway that regulates *str-2* expression. The Gα/Ca^{2+}/MAPK network probably signals partially via *daf-3*. *odr-1* is epistatic to the Gα/Ca^{2+}/MAPK network indicating a parallel or downstream function. The position of *osm-9* has been extrapolated from its presumed cellular function.

Neural signaling

A major question that emerges is how signals from the AWA, AWB, ASH, ADL and ADF neurons influence gene expression in the AWC and ASI neurons. One possibility is that these signals are transmitted via synaptic signaling. However, the AWC neurons are only innervated by the ADL, ASE and ASI neurons (White et al. 1986; Durbin 1987). Thus, only the GPA-6 signal originating from the ADL neurons can be transmitted directly to the AWC neurons. The ASI neurons are not innervated by any of the other amphid neurons. Therefore, we think that signaling via synapses does not play a major role, unless indirect signaling via interneurons is involved.

Paracrine or endocrine signaling might be a better mechanism to explain the non-cell autonomous regulation of *str-2* expression. In *C. elegans*, endocrine regulation is probably a common way through which the sensory neurons influence behavior and development. The sensory neurons control dauer development, longevity, body size and social feeding probably by secreting endocrine signals such as TGFβ homologues, insulin-like peptides and various neuropeptides (Ren et al. 1996; Schackwitz et al. 1996; de Bono et al. 2002; Fukiwara et al. 2002;

Rogers et al. 2003; Li et al. 2003). The genome of *C. elegans* contains at least 5 TGFβ homologues, 38 insulin-like genes, 23 FMRFamide-related (FaRP) and 151 non-FaRP neuropeptides, many of which are expressed in the amphid sensory neurons (Ren et al. 1996; Schackwitz et al. 1996; Morita et al. 1999; Nathoo et al. 1999; Pierce et al. 2001; Li et al. 2003; Kim and Li 2004). Importantly, *str-2* expression itself is regulated by the TGFβ homologue DAF-7, which is secreted by the ASI neurons (Nolan et al. 2002).

It will be interesting to determine which other endocrine signals contribute to the non-cell autonomous regulation of *str-2* expression in the AWC and ASI neurons. In this respect, it may be of importance that the asymmetric expression of *str-2* in the AWC neurons, which is thought to require lateral axon contact between the two AWC neurons, is unaffected by mutations that impair synaptic signaling and gap junction formation (Troemel et al. 1999).

Downstream effector molecules

In this study, we identify three genes that regulate *str-2* expression probably downstream of the G protein and Ca^{2+}/MAPK signaling network (Fig. 5C). First, all G proteins, including GPA-3 and GPA-13, could signal via the TRPV channel OMS-9, as loss of *osm-9* in different Gα mutant backgrounds either reduced or restored *str-2* expression. Second, double mutant analysis suggests that ODR-1 is epistatic to *unc-36, unc-43, tax-4* (Troemel et al. 1999) and the Gα subunits. Therefore, *odr-1* acts downstream of or in parallel to the Gα/Ca^{2+}/MAPK network, though at present it is unclear how *odr-1* regulates *str-2* expression. Third, a *daf-3* mutation suppressed the loss of *str-2* expression in *gpa-5 gpa-6 odr-3 unc-36* animals, suggesting *daf-3* acts downstream of the Gα/Ca^{2+}/MAPK network. *daf-3* is a coSMAD transcription factor that is negatively regulated by the TGFβ pathway to suppress dauer formation (Patterson et al. 1997). In addition, DAF-3 suppresses gene transcription in the pharynx, probably by direct binding to DNA (Thatcher et al. 1999). At this point, it is unclear where *daf-3* functions in relation to *odr-1*.

str-2 *expression does not require sensory signaling*

Previous studies have shown that, in the sensory neurons of *C. elegans,* genes which are required for the detection of sensory cues also regulate maintenance of cilia, axon morphology and receptor gene expression (Roayaie et al. 1998; Troemel et al. 1999; Peckol et al. 1999, 2001; Tobin et al. 2002). For example, mutations in the cyclic nucleotide-gated channel subunits *tax-2* and *tax-4*, which are essential for sensory signal transduction in several amphid neurons, and mutations in genes that disrupt cilia function cause ectopic axon outgrowth (Peckol et al. 1999). Furthermore, the TRPV channels OSM-9 and OCR-2, which mediate olfaction in the AWA neurons, are necessary for *odr-10* receptor gene expression (Tobin et al. 2002). Therefore, axon maintenance and gene expression are thought to be regulated by sensory activity.

In this study, we show that the Gα subunits that mediate odorant detection

also regulate *str-2* receptor gene expression in the AWC and ASI neurons. The response to most odorants depends on signaling via ODR-3 and GPA-3, which is modulated by stimulatory signaling via GPA-13 and inhibitory signaling via GPA-5 and GPA-2 (Roayaie et al. 1998; Lans et al. 2004). No clear function for GPA-6 was found in olfaction. Regulation of *str-2* expression, however, involves different mechanisms, suggesting that *str-2* expression is not regulated by olfactory cues. Consistently, mutations in *osm-6* and *dyf-8*, which disrupt sensory cilia formation and thus sensory perception, do not affect *str-2* expression. These results suggest that sensory activity is not required for *str-2* expression.

The Ca^{2+}/MAPK pathway has dual, opposite functions in regulating str-2 *expression*

Previous genetic analysis suggests that in the embryo the two AWC neurons develop into functionally different cells due to the activity of the Ca^{2+} channel UNC-2/UNC-36 and the Ca^{2+}/calmodulin dependent protein kinase II (CaMKII) UNC-43, which activate the MAPKK kinase NSY-1 and the MAPK kinase SEK-1 via the TIR protein TIR-1 (Troemel et al. 1999; Wes and Bargmann 2000; Sagasti et al. 2001; Tanaka-Hino et al. 2002; Chuang and Bargmann 2005). As a result, *str-2* is expressed in only one of the two AWC neurons. Mutations in these genes cause *str-2* expression in both AWC neurons, demonstrating that they repress *str-2* expression. In this study, we show that mutations in *unc-36, unc-43* and *nsy-1*, but not in *sek-1* or *tir-1*, in combination with mutations in *gpa-2, gpa-5, gpa-6* and *odr-3* cause a severe loss of *str-2* expression. Thus, part of the Ca^{2+}/MAPK pathway also promotes *str-2* expression together with G protein signaling. We postulate that in the embryo UNC-36, UNC-43 and NSY-1 mediated signaling activates SEK-1 via TIR-1 to define AWC asymmetry, while later, once asymmetry is established, these proteins activate other downstream effectors to regulate and maintain the expression of specific genes, including *str-2*. Interestingly, in embryos, *str-2* is expressed in approximately 10 cells, whereas in adults only in AWC and ASI (Troemel et al. 1999), suggesting that additional mechanisms exist that turn *str-2* expression on and off.

Gα subunits modulate sensory signaling

Thus far, for nine of the thirteen Gα subunits that are expressed in the amphid sensory neurons (Jansen et al. 1999) a function has been found. The response to the majority of signals detected by the amphid neurons requires only ODR-3 and/or GPA-3 (Troemel et al. 1997; Roayaie et al.1998; Hilliard et al. 2004; Lans et al. 2004), suggesting that these two Gα subunits are directly involved in sensory transduction. The other Gα subunits, including GPA-1, GPA-2, GPA-5, GPA-6, GPA-9, GPA-11 and GPA-13 seem to have a modulatory rather than a mediatory function (Zwaal et al. 1997; Chao et al. 2004; Lans et al. 2004; this study; H.L, R. Hukema and G.J., unpublished results), although GPA-2 might specifically mediate butanone perception (Roayaie et al. 1998). Therefore, the expression of multiple, regulatory Gα subunits could be a way for *C. elegans* to specifically fine-tune its

sensory responses. The Gα/Ca^{2+}/MAPK pathway identified in this study might be one of the mechanisms by which this is accomplished.

Materials and methods

Strains

Nematodes were grown at 20°C or 25°C on E. coli strain *OP50* using standard methods (Brenner 1974). Wild type animals were *C. elegans* variety Bristol, strain N2. Alleles used in this study were: *daf-3(mgDf90), daf-4(m63), daf-7(e1372), daf-12(ok493), dyf-8(m539), gpa-2(pk16), gpa-3(pk35), gpa-5(pk376), gpa-5XS (pkIs379), gpa-6(pk480), gpa-6XS(pkIs519), gpa-13(pk1270), nsy-1(ky397), nsy-2/tir-1(ky388), nsy-3/slo-1(ky389), odr-1(n1936), odr-3(n1605), osm-6(p811), osm-9(n1603), sek-1(km4), str-2::gfp(kyIs140), tax-4(p678), unc-36(e251), unc-43(e408).*

Transgenes and germ line transformation

To examine *str-2* and *odr-10* expression, the integrated alleles *kyIs140[str-2::gfp]* (Troemel et al. 1999) and *kyIs37[odr-10::gfp]* (Sengupta et al. 1996) were used. All other transgenes were generated by standard germ line transformation (5-100 ng/μl; Mello et al. 1991) and maintained extrachromosomally using the *elt-2::gfp* construct as co-injection marker (Fukushige et al. 1999).

Promoters used were *gpa-11* (ADL, ASH; Jansen et al. 1999), *gpa-13* (ADF, ASH, AWC; Jansen et al. 1999), *hsp-16.2* (a gift from A. Fire), *odr-10* (AWA, Sengupta et al. 1996), *sra-6* (ASH, ASI, PVQ, SPDm/SPVm; Troemel et al. 1995), *srh-142* (ADF; Sagasti et al. 1999), *sro-1* (ADL, SIA; Troemel et al. 1995) and *str-1* (AWB; Troemel et al. 1997). In addition, we examined expression of the receptor *sra-13*, but we could not confirm AWC expression as previously reported (Battu et al. 2003). All other expression patterns were confirmed using *promoter::gfp* fusion constructs (in pPD95.79, a gift from A. Fire), except *sro-1::gfp*, which was observed in additional cells but not in other sensory neurons. We realize that the cellular specificity of some of the promoters used might be altered in dauer animals. However, *sra-6* expression is unaltered in dauer larvae and expression of *odr-10*, *str-1* and *srh-142* is unaffected by mutations in the TGFβ pathway (Nolan et al. 2002).

Details of plasmid construction are available on request. The *odr-3* gene was derived from plasmid *pODR3.1*, a gift from C. Bargmann (Royaie et al. 1998). Antibody staining (Lans et al. 2004) showed that ODR-3 was expressed at wild type levels under the *sra-6* promoter and stronger under the *gpa-13* and *srh-142* promoters. As expected, ODR-3 was localized mainly to the cilia.

Microscopy

To examine *str-2* expression, animals were allowed to lay eggs for 2-5 hrs at 20°C and then removed. Their offspring was picked at different developmental stages to score *str-2::gfp* levels (3-4 days for adults). *str-2::gfp* was scored and

images were acquired using a Leica Aristoplan microscope, equipped with a Sony DXC-9508 3CCD camera. Expression was scored as strong if GFP fluorescence was detected at 100x magnification, regardless of brightness, weak if GFP was only detected at 400x and off if GFP could not be detected at 400x.

Dauer larvae

Dauer larvae were generated using a crude extract of dauer pheromone (Golden and Riddle 1984). Adults were allowed to lay eggs for ~3 hrs on plates containing 40 µl of pheromone, the minimum required to induce 80% wild type dauer formation. The plates were incubated at 25°C for three days, after which *str-2* expression was scored.

Acknowledgements

We thank Amanda Kahn-Kirby and Cori Bargmann for communication of unpublished results, critical reading of the manuscript, plasmids and strains, the *Caenorhabditis* Genetics Center and the *C. elegans* Gene Knockout Consortium for strains and Andy Fire for plasmids. This work was financially supported by the Netherlands Organization for Scientific Research (NWO, grant ALW 805-48-009), the Royal Dutch Academy of Sciences (KNAW), and the Center for Biomedical Genetics.

References

Bargmann, C.I., Hartwieg, E., and Horvitz, H.R. 1993. Odorant-selective genes and neurons mediate olfaction in *C. elegans*. *Cell* **74:** 515-527.

Bargmann, C.I. and Mori, I. 1997. Chemotaxis and thermotaxis. in *C. elegans II* (eds. D.S. Riddle et al.), pp. 717–737. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Bargmann, C.I. 1998. Neurobiology of the *Caenorhabditis elegans* genome. *Science* **282:** 2028-2033.

Battu, G., Hoier, E.F., and Hajnal, A. 2003. The *C. elegans* G-protein-coupled receptor SRA-13 inhibits RAS/MAPK signalling during olfaction and vulval development. *Development* **130:** 2567-2577.

Birnby, D.A., Link, E.M., Vowels, J.J., Tian, H., Colacurcio, P.L., and Thomas, J.H. 2000. A transmembrane guanylyl cyclase (DAF-11) and Hsp90 (DAF-21) regulate a common set of chemosensory behaviors in C*aenorhabditis elegans*. *Genetics* **155:** 85-104.

Brenner, S. 1974. The genetics of *Caenorhabditis elegans*. *Genetics* **77:** 71-94.

Chao, M.Y., H. Komatsu, H.S. Fukuto, H.M. Dionne, and A.C. Hart. 2004. Feeding status and serotonin rapidly and reversibly modulate a *Caenorhabditis elegans* chemosensory circuit. *Proc Natl Acad Sci U S A* **101**: 15512-7.

Chuang, C.F. and Bargmann, C.I. 2005. A Toll-interleukin 1 repeat protein at the synapse specifies asymmetric odorant receptor expression via ASK1 MAPKKK signaling. *Genes Dev.* **19:** 270-281.

Coburn, C.M. and Bargmann, C.I. 1996. A putative cyclic nucleotide-gated channel is required for sensory development and function in *C. elegans*. *Neuron* **17:** 695-706.

Colbert, H.A. and Bargmann, C.I. 1995. Odorant-specific adaptation pathways generate olfactory plasticity in *C. elegans*. *Neuron* **14:** 803-812.

Colbert, H.A., Smith, T.L., and Bargmann, C.I. 1997. OSM-9, a novel protein with structural similarity to channels, is required for olfaction, mechanosensation, and olfactory adaptation in *Caenorhabditis elegans*. *J. Neurosci.* **17:** 8259-8269.

Collet, J., Spike, C.A., Lundquist, E.A., Shaw, J.E., and Herman, R.K. 1998. Analysis of *osm-6*, a gene that affects sensory cilium structure and sensory neuron function in *Caenorhabditis elegans*. *Genetics* **148:** 187-200.

Davies, A.G., Pierce-Shimomura, J.T., Kim, H., VanHoven, M.K., Thiele, T.R., Bonci, A., Bargmann, C.I., and McIntire, S.L. 2003. A central role of the BK potassium channel in behavioral responses to ethanol in *C. elegans*. *Cell* **115:** 655-666.

de Bono, M., Tobin, D.M., Davis, M.W., Avery, L., and Bargmann, C.I. 2002. Social feeding in *Caenorhabditis elegans* is induced by neurons that detect aversive stimuli. *Nature* **419**: 899-903.

Durbin, R.M. 1987. Studies on the development and organisation of the nervous system of *Caenorhabditis elegans.* Doctoral thesis, University of Cambridge

Fujiwara, M., Sengupta, P., and McIntire, S.L. 2002. Regulation of body size and behavioral state of *C. elegans* by sensory perception and the EGL-4 cGMP-dependent protein kinase. *Neuron* **36**: 1091–1102.

Fukushige, T., Hendzel, M.J., Bazett-Jones, D.P., and McGhee, J.D. 1999. Direct visualization of the *elt-2* gut-specific GATA factor binding to a target promoter inside the living *Caenorhabditis elegans* embryo. *Proc. Natl. Acad. Sci.* **96:** 11883-11888.

Golden, J.W. and Riddle, D.L. 1984. The *Caenorhabditis elegans* dauer larva: developmental effects of pheromone, food, and temperature. *Dev. Biol.* **102:** 368-378.

Hilliard, M.A., Bergamasco, C., Arbucci, S., Plasterk, R.H., and Bazzicalupo, P. 2004. Worms taste bitter: ASH neurons, QUI-1, GPA-3 and ODR-3 mediate quinine avoidance in *Caenorhabditis elegans*. *EMBO J.* **23:** 1101-1111.

Jansen, G., Thijssen, K.L., Werner, P., van der Horst, M., Hazendonk, E., and Plasterk, R.H. 1999. The complete family of genes encoding G proteins of *Caenorhabditis elegans*. *Nat. Genet.* **21:** 414-419.

Kim, K. and C. Li. 2004. Expression and regulation of an FMRFamide-related neuropeptide gene family in *Caenorhabditis elegans*. *J Comp Neurol* **475**: 540-50.

Komatsu, H., Mori, I., Rhee, J.S., Akaike, N., and Ohshima, Y. 1996. Mutations in a cyclic nucleotide-gated channel lead to abnormal thermosensation and chemosensation in *C. elegans. Neuron* **17:** 707-718.

L'Etoile, N.D. and Bargmann, C.I. 2000. Olfaction and odor discrimination are mediated by the *C. elegans* guanylyl cyclase ODR-1. *Neuron* **25:** 575-586.

Lanjuin, A., VanHoven, M.K., Bargmann, C.I., Thompson, J.K., and Sengupta, P. 2003. Otx/otd homeobox genes specify distinct sensory neuron identities in *C. elegans*. *Dev. Cell* **5:** 621-633.

Lans, H., Rademakers, S., and Jansen, G. 2004. A network of stimulatory and inhibitory Galpha-subunits regulates olfaction in *Caenorhabditis elegans*. *Genetics* **167:** 1677-1687.

Li, W., S.G. Kennedy, and G. Ruvkun. 2003. daf-28 encodes a *C. elegans* insulin superfamily member that is regulated by environmental cues and acts in the DAF-2 signaling pathway. *Genes Dev* **17**: 844-58.

Mello, C.C., Kramer, J.M., Stinchcomb, D., and Ambros, V. 1991. Efficient gene transfer in *C. elegans*: extrachromosomal maintenance and integration of transforming sequences. *EMBO J.* **10:** 3959-3970.

Morita, K., Chow, K.L., and Ueno, N. 1999. Regulation of body length and male tail ray pattern formation of *Caenorhabditis elegans* by a member of TGF-beta family. *Development* **126**: 1337–1347.

Nathoo A.N., Moeller, R.A., Westlund, B.A., Hart., A.C. 2001. Identification of neuropeptide-like protein gene families in *Caenorhabditis elegans* and other species. *Proc Natl Acad Sci U S A* **98**:14000-5

Nolan, K.M., Sarafi-Reinach, T.R., Horne, J.G., Saffer, A.M., and Sengupta, P. 2002. The DAF-7 TGF-beta signaling pathway regulates chemosensory receptor gene expression in *C. elegans*. *Genes Dev*. **16:** 3061-3073.

Patterson, G.I., Koweek, A., Wong, A., Liu, Y., and Ruvkun, G. 1997. The DAF-3 Smad protein antagonizes TGF-beta-related receptor signaling in the *Caenorhabditis elegans* dauer pathway. *Genes Dev.* **11:** 2679-2690.

Peckol, E.L., J.A. Zallen, J.C. Yarrow, and C.I. Bargmann. 1999. Sensory activity affects sensory axon development in *C. elegans*. *Development* **126**: 1891-902.

Peckol, E.L., Troemel, E.R., and Bargmann, C.I. 2001. Sensory experience and sensory activity regulate chemosensory receptor gene expression in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci.* **98:** 11032-11038.

Pierce, S.B., M. Costa, R. Wisotzkey, S. Devadhar, S.A. Homburger, A.R. Buchman, K.C. Ferguson, J. Heller, D.M. Platt, A.A. Pasquinelli, L.X. Liu, S.K. Doberstein, and G. Ruvkun. 2001. Regulation of DAF-2 receptor signaling by human insulin and *ins-1*, a member of the unusually large and diverse *C. elegans* insulin gene family. *Genes Dev* **15**: 672-86.

Ren, P., Lim, C.S., Johnsen, R., Albert, P.S., Pilgrim, D., and Riddle, D.L. 1996. Control of *C. elegans* larval development by neuronal expression of a TGF-beta homolog. *Science* **274:** 1389-1391.

Riddle, D.L. and Albert, P.S. 1997. Genetic and environmental regulation of dauer larva development. In *C. elegans II* (eds. D.S. Riddle et al.), pp. 739–768. Cold Spring Harbor Press, Cold Spring Harbor, NY.

Roayaie, K., Crump, J.G., Sagasti, A., and Bargmann, C.I. 1998. The G alpha protein ODR-3 mediates olfactory and nociceptive function and controls cilium morphogenesis in *C. elegans* olfactory neurons. *Neuron* **20:** 55-67.

Robertson, H.M. 1998. Two large families of chemoreceptor genes in the nematodes *Caenorhabditis elegans* and *Caenorhabditis briggsae* reveal extensive gene duplication, diversification, movement, and intron loss. *Genome Res.* **8:** 449-463.

Robertson, H.M. 2000. The large srh family of chemoreceptor genes in *Caenorhabditis nematodes* reveals processes of genome evolution involving large duplications and deletions and intron gains and losses. *Genome Res.* **10:** 192-203.

Rogers, C., Reale, V., Kim, K., Chatwin, H., Li, C., Evans, P. and de Bono, M. 2003. Inhibition of *Caenorhabditis elegans* social feeding by FMRFamide-related peptide activation of NPR-1. *Nat Neurosci* **6**: 1178-85.

Sagasti, A., Hobert, O., Troemel, E.R., Ruvkun, G., and Bargmann, C.I. 1999. Alternative olfactory neuron fates are specified by the LIM homeobox gene lim-4. *Genes Dev.* **13:** 1794-1806.

Sagasti, A., Hisamoto, N., Hyodo, J., Tanaka-Hino, M., Matsumoto, K., and Bargmann, C.I. 2001. The CaMKII UNC-43 activates the MAPKKK NSY-1 to execute a lateral signaling decision required for asymmetric olfactory neuron fates. *Cell* **105:** 221-232.

Schackwitz, W.S., Inoue, T., and Thomas, J.H. 1996. Chemosensory neurons function in parallel to mediate a pheromone response in *C. elegans*. *Neuron* **17:** 719-728.

Schafer, W.R., Sanchez, B.M., and Kenyon, C.J. 1996. Genes affecting sensitivity to serotonin in *Caenorhabditis elegans. Genetics* **143:** 1219-1230.

Sengupta, P., Chou, J.H., and Bargmann, C.I. 1996. *odr-10* encodes a seven transmembrane domain olfactory receptor required for responses to the odorant diacetyl. *Cell* **84:** 899-909.

Starich, T.A., Herman, R.K., Kari, C.K., Yeh, W.H., Schackwitz, W.S., Schuyler, M.W., Collet, J., Thomas, J.H., and Riddle, D.L. 1995. Mutations affecting the chemosensory neurons of *Caenorhabditis elegans*. *Genetics* **139:** 171-188.

Tanaka-Hino, M., Sagasti, A., Hisamoto, N., Kawasaki, M., Nakano, S., Ninomiya-Tsuji, J., Bargmann, C.I., and Matsumoto, K. 2002. SEK-1 MAPKK mediates Ca2+ signaling to determine neuronal asymmetric development in *Caenorhabditis elegans*. *EMBO Rep.* **3:** 56-62.

Thatcher, J.D., C. Haun, and P.G. Okkema. 1999. The DAF-3 Smad binds DNA and represses gene expression in the *Caenorhabditis elegans* pharynx. *Development* **126**: 97-107.

Tobin, D., Madsen, D., Kahn-Kirby, A., Peckol, E., Moulder, G., Barstead, R., Maricq, A., and Bargmann, C. 2002. Combinatorial expression of TRPV channel proteins defines their sensory functions and subcellular localization in *C. elegans* neurons. *Neuron* **35:** 307-318.

Troemel, E.R., Chou, J.H., Dwyer, N.D., Colbert, H.A., and Bargmann, C.I. 1995. Divergent seven transmembrane receptors are candidate chemosensory receptors in *C. elegans. Cell* **83:** 207-218.

Troemel, E.R., Kimmel, B.E., and Bargmann, C.I. 1997. Reprogramming chemotaxis responses: sensory neurons define olfactory preferences in *C. elegans*. *Cell* **91:** 161-169.

Troemel, E.R., Sagasti, A., and Bargmann, C.I. 1999. Lateral signaling mediated by axon contact and calcium entry regulates asymmetric odorant receptor expression in *C. elegans. Cell* **99:** 387-398.

Wang, Z.W., Saifee, O., Nonet, M.L., and Salkoff, L. 2001. SLO-1 potassium channels control quantal content of neurotransmitter release at the *C. elegans* neuromuscular junction. *Neuron* **32:** 867-881.

Wes, P.D. and Bargmann, C.I. 2001. *C. elegans* odour discrimination requires asymmetric diversity in olfactory neurons. *Nature* **410:** 698-701.

White, J. G., Southgate, E., Thomson, J. N., and Brenner, S. 1986. The structure of the nervous system of the nematode Caenorhabditis elegans. Philos. Trans. R. Soc. Lond. B Biol. Sci. 314: 1-340.

Zwaal, R.R., Mendel, J.E., Sternberg, P.W., and Plasterk, R.H. 1997. Two neuronal G proteins are involved in chemosensation of the *Caenorhabditis elegans* Dauer-inducing pheromone. *Genetics* **145:** 715-727.

Chapter 6

Sensory G proteins modulate longevity in *Caenorhabditis elegans*

Hannes Lans and Gert Jansen

6. Sensory G proteins modulate longevity in *Caenorhabditis elegans*

Summary

The life span of the nematode *C. elegans* is under control of sensory signals detected by the amphid neurons. In these neurons, *C. elegans* expresses at least 13 Gα subunits and a Gγ subunit, which are involved in the transduction and modulation of sensory signals. Here, we show that loss-of-function mutations in the Gα subunits *odr-3, gpa-1* and *gpa-9*, in the Gγ subunit *gpc-1* and overexpression of the Gα subunit *gpa-11* extend the life span of *C. elegans.* We show that *odr-3* and *gpa-11* synergistically regulate life span in a *daf-16* dependent manner, but partially independent of *daf-2*. In addition, our results suggest that the previously unanticipated ASH and/or ADL neurons regulate longevity. The implication of specific G proteins may eventually lead to the identification of the sensory cues that determine the rate of aging in *C. elegans.*

Results and Discussion

C. elegans perceives its environment through 12 bilateral pairs of ciliated amphid neurons. Mutations that disrupt the cilia of these neurons, resulting in the inability to respond to sensory cues, increase the life span of *C. elegans* [1]. Furthermore, laser ablation of the some of the neurons that detect volatile and nonvolatile cues extends life span [2], suggesting that in *C. elegans* longevity is regulated by sensory perception. Most sensory cues are thought to be detected by chemosensory G protein coupled receptors that activate several G protein mediated signaling cascades [3, 4]. The genome of *C. elegans* contains 21 Gα subunits, 2 Gβ subunits and 2 Gγ subunits [4, 5]. Of these, 13 Gα subunits and 1 Gγ subunit were shown to be specifically expressed in the amphid sensory neurons (Table 1; [4, 6-8]). Thus far, for seven Gα subunits and the Gγ subunit a role in sensory perception has been demonstrated ([7-10], R. Hukema, S. Rademakers, J. Burghoorn and G. Jansen, personal communication).

To investigate the involvement of sensory G proteins in regulating longevity, we determined the life span of animals carrying loss-of-function mutations and overexpression alleles of 16 Gα subunits and the Gγ subunit, at 25°C (Table 1). This analysis showed that mutations in the Gα subunits *gpa-1*, *gpa-5, gpa-9* and *odr-3*, in the Gγ subunit *gpc-1* and overexpression of the Gα subunits *gpa-2*, *gpa-9* and *gpa-11* and *gpc-1* significantly extended life span. A mutation in *odr-3* has been previously reported to increase lifespan [2]. To confirm that *gpa-11* overexpression extended life span, we obtained similar results with two independent alleles, *pkIs539* and *pkIs540* (henceforth referred to as *gpa-11XS* and *gpa-11XS(pkIs540*)). Most of the other mutations in Gα subunits had either no effect or seemed to decrease life span. These results suggest that specific sensory signals mediated by only a few Gα subunits regulate longevity.

Table 1. Life spans of G protein mutants at 25°C

strain	mean ± s.e.m. (days)	75th perc. (days)	*n*	p value	amphid neuron expression	sensory function
Amsterdam						
wild type	13.4 ± 0.2	15	203			
gpa-1	14.4 ± 0.3	16	78	0.007	ADL, ASH, ASI, ASJ	salt perception
gpa-1XS	12.3 ± 0.4	14	44	0.004		
gpa-2	9.5 ± 0.6	12	40	<0.0001	AWC	olfaction, dauer pheromone
gpa-2XS	15.1 ± 0.4	17	73	<0.0001		
gpa-3	11.4 ± 0.5	14	40	0.001	AWA, AWC, ADF, ADL, ASE, ASG, ASH, ASI, ASJ, ASK	nociception, olfaction, dauer pheromone
gpa-3XS	13.1 ± 0.3	15	39	0.144		
gpa-3QL	11.6 ± 1.2	14	28	0.432		
gpa-4	14.1 ± 0.4	16	43	0.053	ASI	
gpa-4XS	12.7 ± 0.6	15	44	0.981		
gpa-5	14.2 ± 0.4	16	43	0.036	AWA, ADL	olfaction
gpa-5XS	12.1 ± 0.5	14	42	0.052		
gpa-6	12.0 ± 0.5	13	41	0.036	AWA, AWB, ASH, ADL	
gpa-6XS	13.0 ± 0.5	15	46	0.912		
gpa-7	13.4 ± 0.5	15	36	0.976	probably all	
gpa-7XS	14.1 ± 0.4	16	22	0.465		
gpa-8	10.7 ± 0.5	13	35	<0.001	none	
gpa-8XS	11.5 ± 0.5	13	42	0.003		
gpa-9	17.5 ± 0.4	19	42	<0.0001	ASJ	
gpa-9XS	11.0 ± 0.6	14	41	0.001		
gpa-10	11.0 ± 0.3	12	50	<0.0001	ADF, ASI, ASJ	
gpa-11	12.8 ± 0.4	18	46	0.278	ADL, ASH	serotonin/food
gpa-11XS	19.5 ± 0.7	26	65	<0.0001		
gpa-12	13.2 ± 0.5	16	37	0.906	undetermined	
gpa-13	12.9 ± 0.4	16	38	0.261	ADF, ASH, AWC	olfaction
gpa-13XS	12.4 ± 0.7	16	46	0.727		
gpa-14	13.7 ± 0.4	16	44	0.668	ASI, ASJ, ASH, ASK	
gpa-14XS	12.9 ± 0.3	13	44	0.118		
gpa-15	13.3 ± 0.4	16	46	0.907	ADL, ASH, ASK	
gpa-15XS	11.1 ± 0.6	13	21	<0.0001		
odr-3	18.0 ± 0.4	19	80	<0.0001	AWA, AWB, AWC, ADF, ASH	olfaction, salt, nociception
gpc-1	14.7 ± 0.4	17	45	0.002	AWB, ADL, ASH, ASI, ASJ, AFD	salt perception
gpc-1XS	15.2 ± 0.5	17	33	0.0001		
gpa-11 odr-3	14.3 ± 0.5	17	35	*0.037	**<0.0001	
Rotterdam						
wild type	17.5 ± 0.3	20	89			
gpa-9	19.6 ± 0.3	22	87	<0.0001	ASJ	
gpa-9XS	19.4 ± 0.4	21	71	0.0001		
gpa-11	17.0 ± 0.6	19	37	0.843	ADL, ASH	serotonin/food
gpa-11XS	21.1 ± 0.5	24	90	<0.0001		
gpa-11XS(pkIs540)	22.8 ± 0.6	24	93	<0.0001		
odr-3	19.7 ± 0.5	24	84	<0.0001	AWA, AWB, AWC, ADF, ASH	olfaction, salt, nociception
gpa-11 odr-3	17.2 ± 0.4	19	69	*0.823	**<0.0001	
odr-3 gpa-11XS	34.7 ± 1.6	42	48	**<0.0001	***<0.0001	
odr-3 gpa-11XS(pkIs540)	25.4 ± 1.1	32	42	**<0.0001	****0.132	

Results of life span analysis at 25°C. During the course of our experiments, our lab moved from the city of Amsterdam to Rotterdam. We found consistent results, but animals survived significantly longer in Rotterdam. Two alleles of *gpa-11XS* were tested, to confirm that *gpa-11* caused life span extension. All assays in Amsterdam were performed once, except assays with *gpa-1, gpa-2XS, gpa-4XS,* which were performed twice and with wild type, which was performed six times. All assays in Rotterdam were performed twice except assays with *gpa-11, odr-3 gpa-11XS* and *odr-3 gpa-11XS(pkIs540),* which were performed once.
XS denotes overexpression, QL a constitutive activating mutation. The 75th perc. (percentile) is the age when the fraction of animals alive reaches 0.25. *n* denotes number of animals tested. Expression patterns and functions were published previously ([4, 6-11, 27], R. H., S. R., J. B. and G. J., personal communication). Strains that have significant extended life span are indicated in bold. p values are compared to wild type, except *compared to *gpa-11*, **compared to *odr-3*, ***compared to *gpa-11XS* and ****compared to *gpa-11XS(pkIs540).*

To be better able to compare our results with previous publications and to standardize our approach, we repeated some of the experiments at 20°C. Again, we found that loss-of-function of *gpa-1*, *gpa-9, odr-3* and *gpc-1* and overexpression of *gpa-1*, *gpa-9* and *gpa-11* extended lifespan (Figure 1A-D). Each of these G protein subunits are expressed in one or more amphid neurons (Table 1). Their expression patterns partially overlap, suggesting they could function in the same signaling pathway to regulate longevity. To test this, we constructed double mutants carrying *gpa-1, odr-3, gpc-1* loss-of-function or *gpa-11XS* alleles. If two G protein subunits function in the same pathway, we would expect a similar life span extension in a double mutant as in either single mutant. Alternatively, if two subunits act in separate pathways, we would expect a synergistic life span extension.

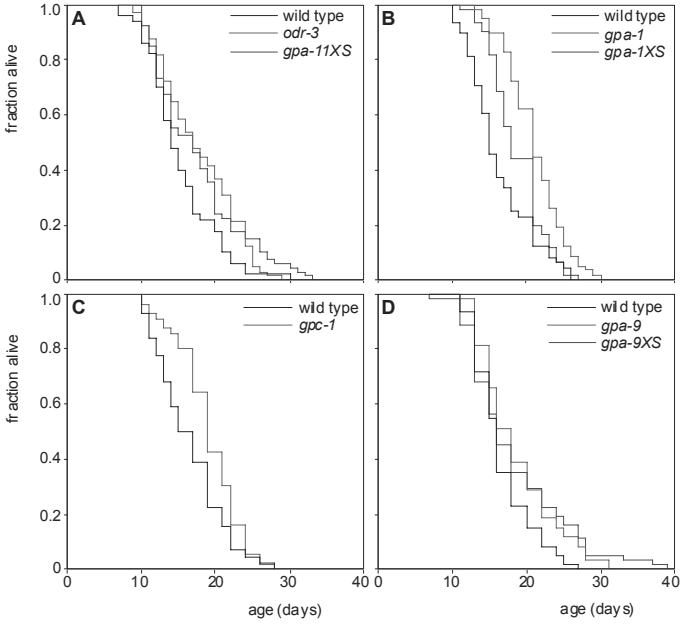

Figure 1. Sensory G protein subunits regulate life span
Mutations in *gpa-1, gpa-9, odr-3, gpc-1* and overexpression of *gpa-1, gpa-9* and *gpa-11* extend life span. Shown are the results of individual life span assays at 20°C. Numbers of animals (n) and p values comparing differences with wild type are (A) wild type (n=50), *odr-3* (n=65, p=0.013), *gpa-11XS* (n=67, p=0.027), (B) wild type (n=48), *gpa-1* (n=62, p<0.0001), *gpa-1XS* (n=61, p=0.015), (C) wild type (n=44), *gpc-1* (n=56, p= 0.017), (D) wild type (n=60), *gpa-9* (n=59, p= 0.011) and *gpa-9XS* (n=62, p= 0.036).

The life span extension in *gpc-1 odr-3* and *gpc-1 gpa-11XS* mutants showed no signs of synergism, but was similar to that in *gpc-1* single mutants (Figure 2A, B). These results suggest that *odr-3* and *gpa-11* both function in a pathway with *gpc-1*, but that *gpc-1* has an additional role in regulating longevity. Furthermore, the life span extension in *gpa-1 gpc-1* double mutants was similar to that in either single mutant (Figure 2C), suggesting that *gpa-1* also functions in a pathway with *gpc-1.* Interestingly, both genes also seem to act in the same pathway in the ASI, ASH or ADL neurons in the response to salts (R.H., S.R., J.B. and G.J., personal communication). Next, we found that the life span of *gpa-1 odr-3* double mutants was similar to that of *gpa-1* single mutants (Figure 2D). Thus, *gpa-1* might function in the same pathway as *odr-3* and in an additional pathway, just like *gpc-1*. In contrast, the life span of *gpa-1 gpa-11XS* mutants was similar to

that of *gpa-11XS* mutants, but shorter than that of *gpa-1* mutants (Figure 2E). This suggests that *gpa-1* and *gpc-1* have different functions in regulating life span. A possible explanation is that *gpa-11* mediates an additional signal that suppresses life span extension in *gpa-1* mutants (see Figure 5B and below).

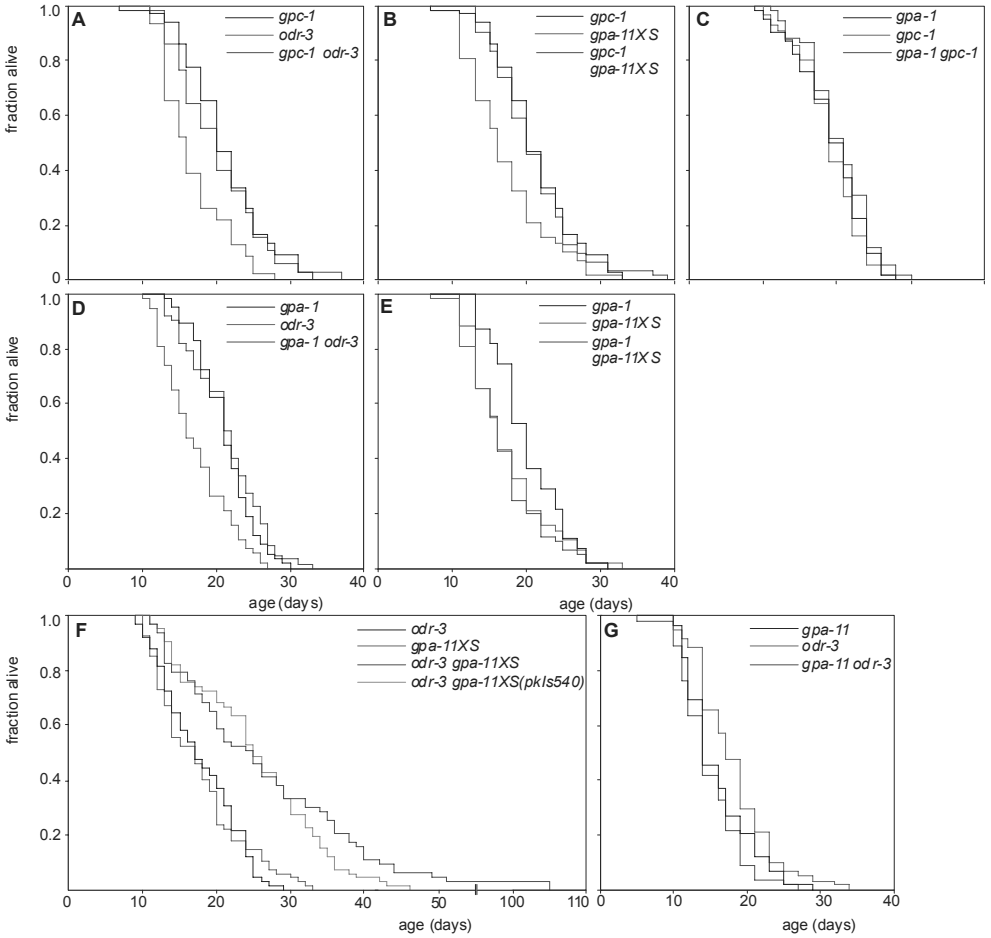

Figure 2. Sensory G protein subunits redundantly and synergistically regulate longevity
(A, B, C) Life spans of *gpc-1 odr*-3, *gpc-1 gpa-11XS* and *gpa-1 gpc-1* double mutants are similar to life spans of *gpc-1* single mutants. (D, E) Life spans of *gpa-1 odr-3* mutants are similar to life spans of *gpa-1* single mutants, but life spans of *gpa-1 gpa-11XS* mutants are similar to *gpa-11XS* single mutants. (F) *odr-3 gpa-11XS* mutants show synergistic life span extension. (D) *gpa-11 odr-3* mutants live as long as *gpa-11* single mutants. Shown are the results of individual life span assays performed at 20°C. Numbers of animals (n) and p values comparing differences between single mutant and double mutant were (A) *gpc-1* (n=66, p=0.563), *odr-3* (n=46, p=0.001), *gpc-1 odr-3* (n=65), (B) *gpc-1* (n=66, p=0.764), *gpa-11XS* (n=58, p=0.004), *gpc-1 gpa-11XS* (n=61), (C) *gpa-1*(n=62, p=0.289), *gpc-1* (n=56, p=0.086), *gpa-1 gpc-1* (n=52), (D) *gpa-1* (n=58, p=0.339), *odr-3* (n=57, p<0.0001), *gpa-1 odr-3* (n=62), (E) *gpa-1*(n=55, p=0.005), *gpa-11XS* (n=58, p=0.671), *gpa-1 gpa-11XS* (n=61), (F) *odr-3* (n=65, p<0.0001 compared to *odr-3 11XS* and to *odr-3 11XS(pkIs540)*), *gpa-11XS* (n=67, p<0.0001), *odr-3 gpa-11XS* (n=63), *odr-3 gpa-11XS(pkIs540)* (n=66), (G) *gpa-11* (n=59, p=0.194), *odr-3* (n=61, p=0.0003) and *gpa-11 odr-3* (n=55).

At 25°C (Table 1) and at 20°C (Figure 2F), we found that loss-of-function of *odr-3* and overexpression of *gpa-11* synergistically extended life span. These results suggest that, in wild type animals, *gpa-11* promotes longevity whereas *odr-3* inhibits longevity. To test if the two Gα subunits antagonize each other's function, we constructed a *gpa-11 odr-3* loss-of-function double mutant. We found that *gpa-11 odr-3* mutants had a lifespan shorter than *odr-3* mutants but similar to *gpa-11* mutants (Figure 2G), suggesting that the extended life span of *odr-3* mutants requires the function of *gpa-11*. *odr-3* might inhibit longevity by signaling via *gpa-11* or via an antagonistic, parallel pathway. If *odr-3* functions in a parallel pathway, we would expect that *gpa-11* mutants have a shortened life span as compared to wild type or *gpa-11 odr-3* double mutants, which is not the case (Figure 2G). Therefore, we favor the idea that *odr-3* inhibits longevity by signaling via *gpa-11*.

Interestingly, *gpa-11* is only expressed in the ASH and ADL neurons [8]. Thus far, these neurons have not been implicated in regulating longevity. *odr-3* is also expressed in the ASH neurons, in addition to the AWA, AWB, AWC, and ADF neurons [7]. In the ASH neurons, *gpa-11* mediates the transduction of a food/serotonin signal, which modulates the avoidance response to the odorant octanol [11]. Therefore, *gpa-11* might regulate longevity by mediating a food/serotonin signal. Exogenous serotonin, however, had no clear effect on the life span of *odr-3, gpa-11* or *odr-3 gpa-11XS* mutants (results not shown). Also *bas-1* mutants, which have reduced dopamine and serotonin levels [12], do not live longer than wild type animals (results not shown). Unlike *gpa-11*, *odr-3* is important for the detection of most sensory stimuli tested so far, including attractive odorants by the AWA and AWC neurons and nociceptive stimuli by the ASH neurons [7, 9]. Therefore, a wide variety of cues could influence longevity via *odr-3*. Because killing the AWA and AWC neurons was found to increase life span [2], it is very well possible that *odr-3* functions in these neurons to regulate longevity.

Longevity in *C. elegans* and other organisms is under control of insulin/IGF-1 signaling (reviewed in [13]). Mutations in insulin signaling genes, including reduction-of-function mutations in the insulin/IGF-1 receptor homologue *daf-2* [14, 15], greatly extend life span. This life span extension depends on the activity of the FOXO family transcription factor *daf-16*, which translocates to the nucleus to promote longevity in *daf-2* mutants [14, 16-18]. In addition, life span extension caused by cilia mutations or ablation of the ASI amphid neurons largely depends on *daf-16* [1, 2]. To investigate if the regulation of longevity by *odr-3* and *gpa-11* involves insulin/IGF-1 signaling, we tested the involvement of *daf-16*. To this end, we generated *odr-3 daf-16*, *gpa-11XS daf-16* and *odr-3 gpa-11XS daf-16* mutants and determined their life span. This showed that life span extension caused by loss-of-function of *odr-3* and overexpression of *gpa-11* completely depends on *daf-16* (Figure 3A-C). *odr-3 daf-16* and *gpa-11XS daf-16* even showed a slightly shorter life span than *daf-16* single mutants. This is in contrast to life span extension caused by cilia mutations, which is, to some extent, *daf-16* independent [1].

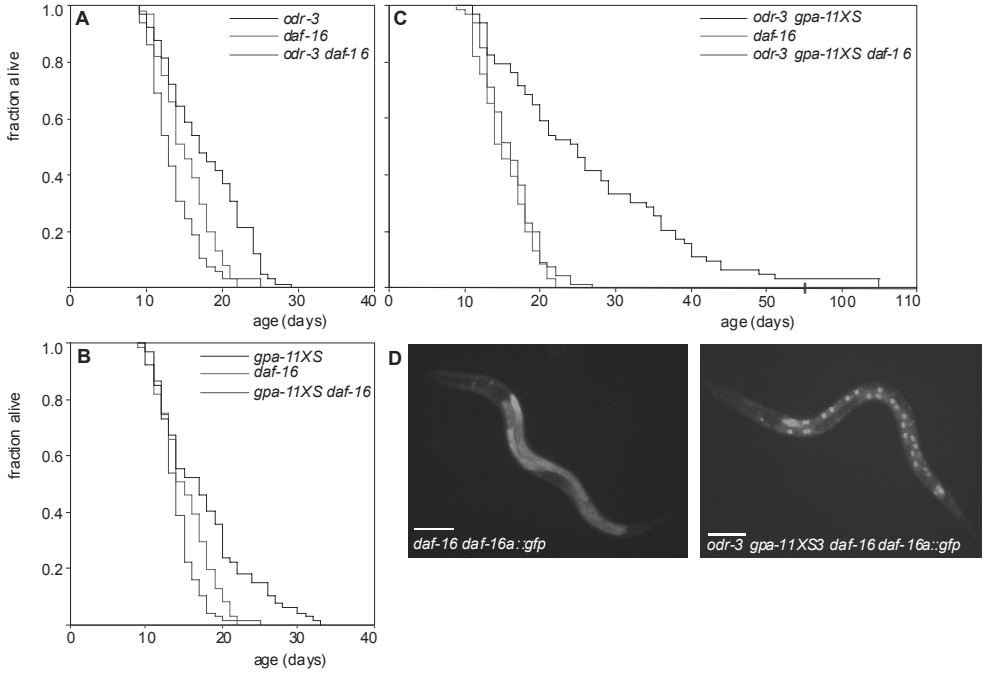

Figure 3. Life span extension in *gpa-11XS odr-3* mutants is *daf-16* dependent
Life span extension due to loss-of-function of *odr-3* (A), overexpression of *gpa-11* (B) or both (C) is fully dependent on *daf-16.* (D) DAF-16::GFP accumulates in the nucleus in most *odr-3 gpa-11XS daf-16* L2 larvae, but not in *daf-16* L2 larvae. Numbers of animals (n) and p values comparing differences between single mutants and double mutants or between double and triple mutants were (A) *odr-3* (n=65, p<0.0001)*, daf-16* (n=61, p=0.015), *odr-3 daf-16* (n=65). (B) *gpa-11XS* (n=67, p<0.0001), *daf-16* (n=61, p=0.017), *gpa-11XS daf-16* (n=67), (C) *odr-3 gpa-11XS* (n=63, p<0.0001), *daf-16* (n=61, p=0.176), *odr-3 gpa-11XS daf-16* (n=66). Bar: 0,1 mm.

Cilia mutations induce the translocation of DAF-16 to the nucleus, where it promotes longevity [18]. To test if this also occurs in *odr-3 gpa-11XS* animals, we expressed GFP fused to *daf-16* in these mutants. As expected, approximately 76% of *gpa-11XS odr-3* mutants (*n*=29) showed nuclear accumulation of DAF-16, versus 19% of control animals (*n*=36) (Figure 3D).

Sensory neurons are thought to regulate longevity by influencing *daf-2* activity, probably through the secretion of insulin-like DAF-2 ligands [1, 2, 19, 20]. For instance, killing the ASI neurons does not further extend the life span of *daf-2* mutants, suggesting that the ASI neurons signal through *daf-2*. We determined if *odr-3* and *gpa-11* function in a pathway with *daf-2* by measuring the life span of *odr-3* and *gpa-11XS daf-2* double mutants. Surprisingly, *odr-3 daf-2* mutants lived longer than either single mutant (Figure 4A), indicating that *odr-3* and *daf-2* function in separate pathways. In contrast, overexpression of *gpa-11* shortened the life span of *daf-2* and *odr-3 daf-2* mutants (Figure 4B, C), indicating that in *daf-2* mutants *gpa-11* has an inhibitory function. To verify this, we measured the life span of *gpa-11 daf-2* loss-of-function mutants. As expected, *gpa-11* loss-of-function in *daf-2* mutants further extended life span (Figure 4D). Thus, like *odr-3*, *gpa-11*

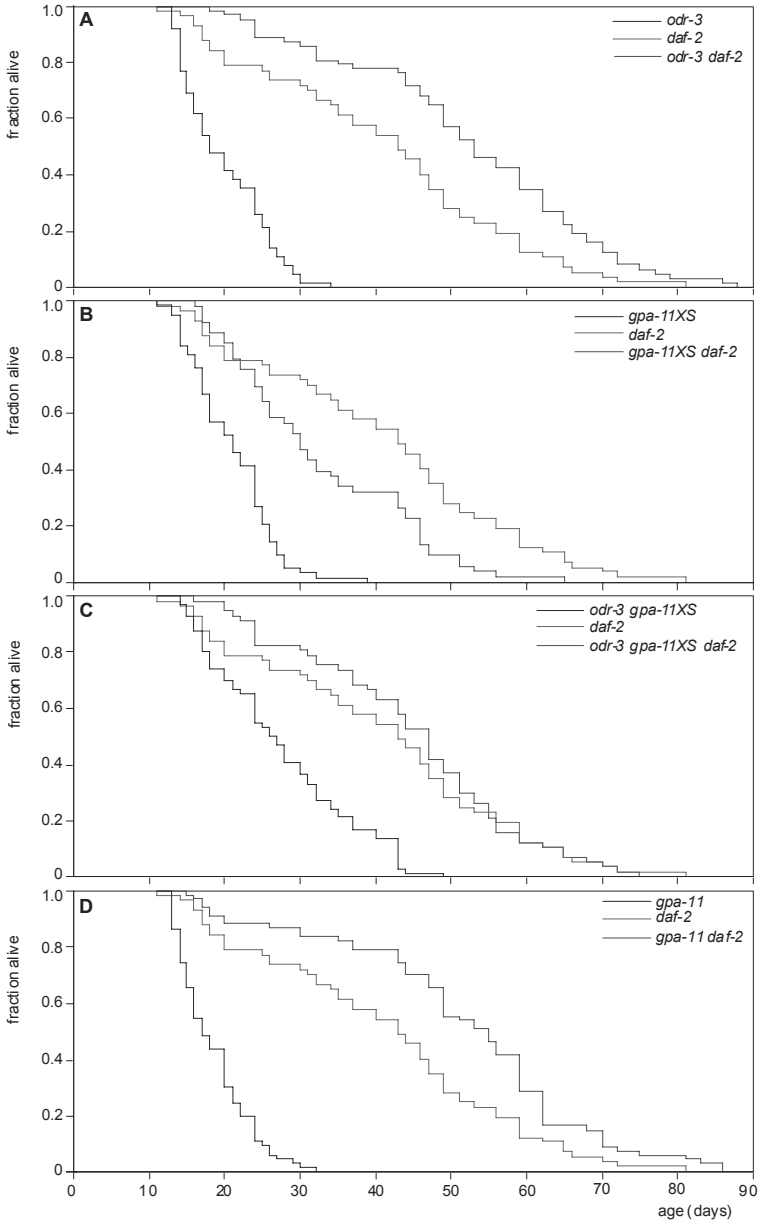

Figure 4.
ODR-3 and GPA-11 signal independently of *daf-2*
Loss-of-function of *odr-3* increases the lifespan of *daf-2* mutants (A), whereas overexpression of *gpa-11* shortens the life span of *daf-2* mutants (B) and of *odr-3 daf-2* mutants (C compared with A). Similar to *odr-3,* loss-of-function of *gpa-11* increases the lifespan of *daf-2* mutants. Numbers of animals and p values comparing differences between single and double mutants or between double and triple mutants were (A) *odr-3* (n=65, p<0.0001), *daf-2* (n=57, p=0.001), *odr-3 daf-2* (n=63), (B) *gpa-11XS* (n=63, p<0.0001), *daf-2* (n=57, p=0.001), *gpa-11XS daf-2* (n=53), (C) *odr-3 gpa-11XS* (n=66, p<0.0001), *daf-2* (n=57, p=0.593), *odr-3 gpa-11XS daf-2* (n=67), (D) *gpa-11* (n=66, p<0.0001), *daf-2* (n=57, p=0.001), *gpa-11 daf-2* (n=57).

inhibits longevity independently of *daf-2*.

Our finding that *gpa-11* promotes longevity in wild type animals, while it suppresses longevity in *daf-2* mutants, seems a paradox. One explanation could be that *gpa-11* regulates two signals that affect longevity. One signal promotes longevity by inhibiting *daf-2* activity. The other signal, which is weaker and *daf-2* independent, suppresses longevity. Interestingly, also mutations in *daf-10* and *osm-3,* which cause cilia defects, and in *unc-31,* which impairs dense core vesicle

neurosecretion, extend the life span of wild type animals, but shorten the life span of *daf-2* animals [1, 21]. We found no indications that overexpression of *gpa-11* causes cilia defects. *gpa-11XS* mutants show no sensory deficits and their sensory neurons take up fluorescent dye, a feature which requires intact cilia [4].

At 27°C, animals that overexpress *gpa-11* constitutively develop into dauer larvae [22]. This phenotype is also observed in cilia mutants, *unc-31* mutants and in many other insulin signaling mutants [21-23]. Dauer larvae have an extended life span and can survive under adverse environmental conditions. Dauer formation is induced by sensory cues, such as a constitutively secreted dauer pheromone, which serves as a signal of overcrowding, high temperature and food. Like longevity, dauer formation is under control of insulin/IGF-1 signaling (reviewed in [24]). Many mutants with increased longevity also show abnormal dauer formation. Therefore, we tested the ability of the G protein mutants with increased longevity to form dauer larvae in response to dauer pheromone. Mutations in *odr-3, gpa-1* and *gpc-1* caused reduced sensitivity to dauer pheromone, whereas overexpression of *gpa-11* caused hypersensitivity (Table 2). *gpa-9* and *gpa-11* loss-of-function had no effect. These results suggest that the same G proteins that regulate longevity also regulate dauer formation, possibly via comparable mechanisms. Some G proteins might even mediate the detection of dauer pheromone, although dauer pheromone by itself does not affect life span [2].

Thus far, six sensory amphid neurons have been shown to regulate longevity. Killing the ASI, ASG, AWA and AWC neurons extends life span, whereas ablation of ASJ or ASK suppresses the extended life span of ASI ablated animals [2]. The ASI and ASG neurons seem to function in parallel to the AWA and AWC

Table 2. Dauer sensitivity of G protein mutants at 25°C

strain	% dauers (±s.e.m.)	*n*
wild type	87.9 ± 3.5	301
gpa-1	66.8 ± 1.5	333
gpa-9	89.8 ± 2.9	427
gpa-11	86.6 ± 5.0	398
odr-3	65.8 ± 0.1	184
gpc-1	25.8 ± 4.5	307
gpa-11XS	100.0 ± 0.0	168
odr-3 gpa-11XS	96.0 ± 0.9	73

Percentages of animals that developed into dauer larvae at 25°C in the presence of dauer pheromone, within 48 hours following egg laying. L2 and some L3 *gpa-11XS* and *odr-3 gpa-11XS* larvae, which were present after 48 hours, possibly due to a growth arrest, were not scored. Percentages are the combined results of two test plates.

neurons. Our results demonstrate that G protein signaling in these neurons, probably in response to sensory cues, regulates longevity in *C. elegans.* In addition, the involvement of *gpa-11* implies that the ASH and/or ADL neurons influence longevity. In the sensory neurons, *odr-3* likely mediates a *daf-2* independent signal that inhibits longevity (Figure 5A). Furthermore, *gpa-11* signals via *daf-2* to promote longevity and independently of *daf-2* to suppress longevity (Figure 5A). Our results suggest that *odr-3* inhibits the activity of *gpa-11*, which in turn stimulates longevity by suppressing *gpa-1* and *gpc-1* activity (Figure 5B). In addition, a parallel pathway could exist, in which *gpa-11* inhibits longevity by activating *gpc-1*. Our model does not fully explain the different interactions found with *gpc-1* and *gpa-1*, but this could be due to the dominant effects of overexpression of *gpa-11* and because *gpc-1* might function as the Gγ subunit of both *gpa-1* and *gpa-11*. In this study, the role of *gpa-9* was not examined thoroughly. As more details of the sensory signaling pathways that regulate longevity are being revealed, ultimately, the environmental signals that control the life span of *C. elegans* and other organisms could be identified.

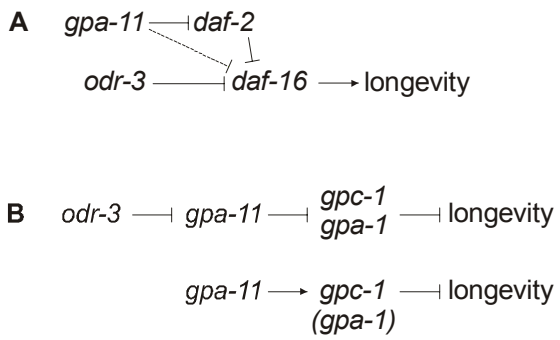

Figure 5. Models of the genetic interactions that modulate life span
(A) We find that *odr-3* and *gpa-11* signal independently of *daf-2* to inhibit longevity via *daf-16*. However, the *gpa-11* life span extending activity is *daf-2* dependent. (B) In addition, we propose a model in which *odr-3*, *gpa-11*, *gpa-1* and *gpc-1* function in a linear pathway that inhibits longevity, in parallel to a pathway in which *odr-3* is not involved and in which *gpa-11* has an opposite function. At present, the two models cannot easily be unified, possibly because G proteins function in several cells and might mediate environmental signals that either extend or shorten life

Experimental procedures

Strains

Strains were maintained using standard methods [25]. Alleles used in this study were *daf-2(e1370), daf-16(mu86), gpa-1(pk15), pkIs503[gpa-1XS], gpa-2(pk16), gpa-3(pk35), pkIs508[gpa-3XS], gpa-4(pk381), pkIs515[gpa-4XS], gpa-5(pk376), pkIs379[gpa-5XS], gpa-6(pk480), pkIs519[gpa-6XS], gpa-7(pk610), pkIs523[gpa-7XS], gpa-8(pk345), pkIs527[gpa-8XS], gpa-9(pk438), pkIs531[gpa-9XS], gpa-10(pk362), gpa-11(pk349), pkIs539[gpa-11XS], pkIs540[gpa-11XS], gpa-12(pk322te), gpa-13(pk1270), pkIs1269[gpa-13XS], gpa-14(pk347), pkIs552[gpa-14XS], gpa-15(pk477), pkIs555[gpa-15XS], gpc-1(pk298te), pkIs571[gpc-1XS], muEx128[pKL79(daf-16a::gfp)], odr-3(n1605) and syIs25[gpa-3QL].*

Life span analysis and dauer pheromone sensitivity

Life span was determined at 20°C or 25°C, as described [14]. Day 1 was defined as the day on which the animals were laid as eggs. Animals were scored as dead when they stopped moving and did not respond to prodding. Animals that crawled of the plate, had an extruded gonad or internally hatched larvae were censored. For life span analysis of all *daf-2* mutants, animals were grown at 15°C and transferred to 20°C at the L3/L4 stage, to prevent dauer development. p values were determined using the Log Rank test. $p<0.05$ was considered significant. Sensitivity to dauer pheromone was tested using a crude dauer pheromone extract [26]. Animals were allowed to lay eggs for two hours on plates containing 20 µl dauer pheromone and 20 µl 4% OP50 bacteria. After 48 hours, the percentage of dauer larvae was determined.

Acknowledgements

We thank Ronald Plasterk, in whose lab this study was started, and the *Caenorhabditis* Genetics Center for strains. This work was financially supported by the Netherlands Organization for Scientific Research (NWO, grant ALW 805-48-009), the Royal Dutch Academy of Sciences (KNAW), and the Center for Biomedical Genetics.

References

1. Apfeld, J., and Kenyon, C. (1999). Regulation of lifespan by sensory perception in *Caenorhabditis elegans*. Nature *402*, 804-809.
2. Alcedo, J., and Kenyon, C. (2004). Regulation of *C. elegans* longevity by specific gustatory and olfactory neurons. Neuron *41*, 45-55.
3. Troemel, E.R., Chou, J.H., Dwyer, N.D., Colbert, H.A., and Bargmann, C.I. (1995). Divergent seven transmembrane receptors are candidate chemosensory receptors in *C. elegans*. Cell *83*, 207-218.
4. Jansen, G., Thijssen, K.L., Werner, P., van der Horst, M., Hazendonk, E., and Plasterk, R.H. (1999). The complete family of genes encoding G proteins of *Caenorhabditis elegans*. Nat Genet *21*, 414-419.
5. Cuppen, E., van der Linden, A.M., Jansen, G., and Plasterk, R.H. (2003). Proteins interacting with *Caenorhabditis elegans* Gα subunits. Comp. Funct. Genom. *4,* 479-491.
6. Zwaal, R.R., Mendel, J.E., Sternberg, P.W., and Plasterk, R.H. (1997). Two neuronal G proteins are involved in chemosensation of the *Caenorhabditis elegans* dauer-inducing pheromone. Genetics *145*, 715-727.
7. Roayaie, K., Crump, J.G., Sagasti, A., and Bargmann, C.I. (1998). The G alpha protein ODR-3 mediates olfactory and nociceptive function and controls cilium morphogenesis in *C. elegans* olfactory neurons. Neuron *20*, 55-67.
8. Jansen, G., Weinkove, D., and Plasterk, R.H. (2002). The G-protein gamma subunit *gpc-1* of the nematode *C. elegans* is involved in taste adaptation. EMBO J *21*, 986-994.
9. Hilliard, M.A., Bergamasco, C., Arbucci, S., Plasterk, R.H., and Bazzicalupo, P. (2004). Worms taste bitter: ASH neurons, QUI-1, GPA-3 and ODR-3 mediate quinine avoidance in *Caenorhabditis elegans*. EMBO J *23*, 1101-1111.
10. Lans, H., Rademakers, S., and Jansen, G. (2004). A network of stimulatory and inhibitory Galpha-subunits regulates olfaction in *Caenorhabditis elegans*. Genetics *167*, 1677-1687.
11. Chao, M.Y., Komatsu, H., Fukuto, H.S., Dionne, H.M., and Hart, A.C. (2004). Feeding status and serotonin rapidly and reversibly modulate a *Caenorhabditis elegans* chemosensory circuit. Proc Natl Acad Sci U S A *101*, 15512-15517.
12. Loer, C.M., and Kenyon, C.J. (1993). Serotonin-deficient mutants and male mating behavior in the nematode *Caenorhabditis elegans*. J Neurosci *13*, 5407-5417.
13. Kenyon, C. (2005). The plasticity of aging: insights from long-lived mutants. Cell *120*, 449-460.

14. Kenyon, C., Chang, J., Gensch, E., Rudner, A., and Tabtiang, R. (1993). A *C. elegans* mutant that lives twice as long as wild type. Nature *366*, 461-464.
15. Kimura, K.D., Tissenbaum, H.A., Liu, Y., and Ruvkun, G. (1997). daf-2, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. Science *277*, 942-946.
16. Ogg, S., Paradis, S., Gottlieb, S., Patterson, G.I., Lee, L., Tissenbaum, H.A., and Ruvkun, G. (1997). The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in *C. elegans*. Nature *389*, 994-999.
17. Lin, K., Dorman, J.B., Rodan, A., and Kenyon, C. (1997). daf-16: An HNF-3/forkhead family member that can function to double the life-span of *Caenorhabditis elegans*. Science *278*, 1319-1322.
18. Lin, K., Hsin, H., Libina, N., and Kenyon, C. (2001). Regulation of the *Caenorhabditis elegans* longevity protein DAF-16 by insulin/IGF-1 and germline signaling. Nat Genet *28*, 139-145.
19. Pierce, S.B., Costa, M., Wisotzkey, R., Devadhar, S., Homburger, S.A., Buchman, A.R., Ferguson, K.C., Heller, J., Platt, D.M., Pasquinelli, A.A., Liu, L.X., Doberstein, S.K., and Ruvkun, G. (2001). Regulation of DAF-2 receptor signaling by human insulin and *ins-1*, a member of the unusually large and diverse *C. elegans* insulin gene family. Genes Dev *15*, 672-686.
20. Li, W., Kennedy, S.G., and Ruvkun, G. (2003) *daf-28* encodes a *C. elegans* insulin superfamily member that is regulated by environmental cues and acts in the DAF-2 signaling pathway. Genes Dev *17*, 844-858.
21. Ailion, M., Inoue, T., Weaver, C.I., Holdcraft, R.W., and Thomas, J.H. (1999). Neurosecretory control of aging in *Caenorhabditis elegans*. Proc Natl Acad Sci U S A *96*, 7394-7397.
22. Ailion, M., and Thomas, J.H. (2003) Isolation and characterization of high-temperature-induced Dauer formation mutants in *Caenorhabditis elegans*. Genetics *165*, 127-144.
23. Ailion, M., and Thomas, J.H. (2000) Dauer formation induced by high temperatures in *Caenorhabditis elegans*. Genetics *156,*1047-1067.
24. Riddle, D.L., and Albert, P.S. (1997). Genetic and environmental regulation of dauer larva development. In *C. elegans* II, D.S. Riddle et al. eds. (Cold Spring Harbor Press, NY), pp. 739–768.
25. Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. Genetics *77*, 71-94.
26. Golden, J.W., and Riddle, D.L., (1984) The *Caenorhabditis elegans* dauer larva: developmental effects of pheromone, food, and temperature. Dev Biol *102,* 368-378.
27. Yau, D.M., Yokoyama, N., Goshima, Y., Siddiqui, Z.K., Siddiqui, S.S., and Kozasa, T. (2003). Identification and molecular characterization of the Galpha12-Rho guanine nucleotide exchange factor pathway in *Caenorhabditis elegans*. Proc Natl Acad Sci U S A *100*, 14748-14753.

Chapter 7

Future prospects and directions

7. Future prospects and directions

7.1 Complex sensory signaling

C. elegans expresses 13 Gα subunits and 1 Gγ subunit specifically in its chemosensory neurons (Jansen et al. 1999, 2002). This thesis focuses on the role of G proteins in olfaction, *str-2* expression and longevity. Other studies have identified roles for G proteins in sensory perception (Roayaie et al. 1998; Jansen et al. 1999, 2002; Chao et al. 2004; Hilliard et al. 2004; Hukema et al. *submitted*), dauer formation (Zwaal et al. 1997) and cilia development (J. Burghoorn and G. Jansen, personal communication). These experiments illustrate the tremendous complexity of sensory perception and the large influence of sensory signaling on development and behavior of an organism. This is illustrated by G protein signaling in the AWC neurons, in which at least four Gα subunits function in odorant detection (Lans et al. 2004; Chapter 4) and regulation of gene expression (Chapter 5). Furthermore, one of these Gα subunits, ODR-3, also specifies cilia morphology (Roayaie et al. 1998) and might regulate longevity via these neurons (Alcedo and Kenyon 2004; Chapter 6). As far as can be determined, at least three of these processes are independently regulated. It is not exactly known whether all Gα subunits in the AWC neurons signal via the same downstream pathway, but the known major effector molecules, like ODR-1, DAF-11, TAX-2, TAX-4 and OSM-9, are involved in most processes as well.

Gradually, along with their functional characterization, the rationale of the existence of multiple sensory G proteins is being uncovered. A remaining question is how the specificity of G protein signaling networks is organized, since G proteins act simultaneously in the same cell in different processes. A clue might come from the selective involvement of some G proteins in the detection of certain chemicals, such as GPA-3 in quinine (Hilliard et al. 2004), GPA-2 in butanone (Roayaie et al. 1998) and GPA-13 in 2,3-pentanedione detection (Lans et al. 2004; Chapter 4), suggesting that GPCRs can specifically activate certain Gα subunits.

7.2 Olfaction (Chapter 4)

It is remarkable that the olfactory neurons of *C. elegans* use different signal transduction routes. Signaling in the AWA, ASH and ADL neurons converges on TRP channels, whereas signaling in the AWB and AWC cells converges on cGMP gated channels. Not one of the olfactory neurons expresses the same set of Gα subunits, although ODR-3 and/or GPA-3 seem to be most important for odorant detection in each neuron (Troemel et al. 1997; Jansen et al. 1999; Lans et al. 2004; Chapter 4; H. Lans and G.J., unpublished results). The olfactory system of *C. elegans* is a good, representative model for other sensory systems for several reasons. First of all, signaling via TRP channels and cyclic nucleotide gated channels is also observed in the sensory systems of other organisms, including mammals. For instance, signaling via TRP channel functions in *Drosophila* phototransduction (Hardie and Raghu 2001) and pheromone sensation in mammals (Clapham 2003). Furthermore, signaling via cyclic nucleotide gated

channels is found in odorant, bitter and sweet taste perception in mammals (Firestein 2001; Lindemann 2001). Secondly, in many sensory systems, signaling involves multiple G proteins. Examples are the olfactory and vomeronasal systems of fish (Hansen et al. 2003, 2004), snakes (Luo et al. 1994), rodents (Jones and Reed, 1989; Berghard and Buck 1996; Wekesa and Anholt 1997, 1999; Luo et al. 2002), dogs (Dennis et al. 2003) and the taste system of mice (He et al. 2004). The significance of multiple G protein mediated pathways is often unclear. In the past, there has been much debate whether additional pathways existed in vertebrate olfaction (Morales and Bacigalupo 1996; Gold 1999). However, nowadays, in rats, at least one additional pathway has been described that modulates the main cAMP mediated pathway during olfaction (Spehr et al. 2002). The modulatory role of some Gα subunits in *C. elegans* also illustrates the significance of multiple pathways.

Many signaling molecules that play a role in olfaction in *C. elegans* are still unknown. This is true for the vast majority of GPCRs, but probably also for many modulatory proteins whose function will be evident or required only during specific or complex olfactory behavior. In Chapter 4, standard olfactory chemotaxis assays are used to study the function of the olfactory Gα subunits. Several additional assays exist to test the ability of *C. elegans* to discriminate, adapt and learn when exposed to odorants (described in Chapter 2). The use of such assays, in combination with forward and reverse genetic screens, will undoubtedly lead to the discovery of more olfactory signaling genes. Also, the role of the olfactory Gα subunits in the various complex olfactory behaviors has not yet been fully determined, except for discrimination. In addition, the olfactory function of certain genes is likely concealed due to redundancy, similar to some Gα subunits described in Chapters 4 and 5 and the RGS EAT-16, whose function is only evident in a *grk-2* mutant (Fukuto et al. 2004). Therefore, testing the effect of mutations in candidate genes in different genetic backgrounds is essential.

Fluorescent imaging could be applied to study the involvement of genes in olfaction. First of all, translational GFP fusion constructs could reveal the site of action of certain molecules. For instance, the function of GPA-5 at synaptic sites (Chapter 4) or the nuclear translocation of genes such as EGL-4 during adaptation could be studied using this approach (L'Etoile et al. 2002). In addition, using techniques such as fluorescence resonance energy transfer, protein-protein interactions could be visualized. Secondly, visualizing signaling by means of Ca^{2+} indicators, such cameleon, or other indicators is necessary to study the involvement of genes, which have a phenotype unsuitable for chemotaxis assays. Examples are the Ca^{2+} signaling genes *unc-36* and *unc-43*, which function in the AWC neurons but also in locomotion (Brenner 1974; Reiner et al. 1999; Troemel et al. 1999; Sagasti et al. 2001) and many other synaptic signaling molecules. Additionally, using fluorescent imaging the kinetics of olfactory signaling could be studied. Moreover, by visualizing signaling in interneurons, the neural circuitry involved in the response to a stimulus could be determined.

7.3 *str-2* gene expression (Chapter 5)

The regulation of *str-2* by G protein and Ca^{2+}/MAPK signaling seems even more complex than olfactory signaling. The same G proteins that are involved in olfactory signaling regulate *str-2* expression, but in a different way. The significance of this regulation is still ambiguous and several questions remain unanswered.

First of all, it is unknown what kind of signals activates the G proteins to regulate *str-2* expression. It is not likely that the G proteins regulate *str-2* expression in response to environmental signals. Therefore, they might act in response to developmental signals. This is substantiated by the observation that the G proteins influence *str-2* expression differently during different development stages and similarly in the AWC and ASI neurons.

Secondly, it is unclear how G proteins function in a particular neuron to regulate gene expression in another neuron. As it seems unlikely that synaptic signaling is involved, endocrine signaling appears to be the most obvious explanation. The simplest way to test this would be to use mutants with general or specific defects in synaptic signaling or neurosecretion. Identification of the signaling pathways involved could possibly also point the way to the signals that activate the G proteins in the first place.

The third question is why and how certain G proteins function in other neurons in dauer larvae than in adults. Because much of the function of the amphid neurons in dauer animals is unknown, it is difficult to understand why GPA-6 and ODR-3 function in the AWB neurons instead of the ADL and AWC neurons. In addition, the mechanisms that reduce AWC expression but promote ASI expression in dauer larvae are unknown.

The fourth unanswered question is which molecules function downstream of the G proteins to regulate *str-2* expression. At present, this question is relevant for most of the sensory G proteins in *C. elegans*. In Chapter 5, several molecules that might act in a pathway with one or more Gα subunits have been identified by testing candidate genes. Forward genetic screens for suppressors of the reduced *str-2* expression in *gpa-5 gpa-6 odr-3 unc-36* animals could be conducted to identify additional genes. One such screen has already been performed and has yielded one suppressor mutation (S. Rademakers and G. J., personal communication).

The next question, related to the fourth, is how the G proteins interact with the Ca^{2+}/MAPK pathway. In the literature, many examples exist of G protein signaling via Ca^{2+} channels and MAPK pathways (Wickman and Clapham 1995; Marinissen and Goodkind 2001; Belcheva and Coscia 2002), but in regulating *str-2* expression G protein signaling seems to largely function in parallel to Ca^{2+}/MAPK signaling.

Finally, it remains to be investigated whether more G proteins are involved in regulating *str-2* expression and whether other GPCRs or even different genes are regulated by similar or other G protein networks.

7.4 Longevity (Chapter 6)

The regulation of longevity by sensory G proteins adequately demonstrates an unanticipated function of sensory signaling. At the moment, it is largely unknown which signals and other molecules are involved in the G protein regulation of longevity. An obvious way to identify these signals and molecules would be to test the life span of animals with mutations in candidate genes. In this way, it has already been found that TAX-4 and STR-2 are involved in regulating life span, but TAX-2 and OSM-9 are not (Apfeld and Kenyon 1999; Alcedo and Kenyon 2004; H.L. and G.J., unpublished results). In addition, the neurons in which the G protein subunits function to influence life span remain to be identified. Some guesses can be made from previous cell ablation experiments (Alcedo and Kenyon 2004), but the involvement of the ASH and ADL neurons, in which GPA-11 functions, has never been tested. Using cell specific rescue experiments, the neurons in which the Gα subunits function could be identified. An alternative approach would be to use genetic mutations that impair the function of one or more neurons or other cells. For instance, animals with a mutation in *odr-7*, a nuclear hormone receptor required for AWA function, show an extended life span (Alcedo and Kenyon 2004). In addition, using this approach, the involvement of signals from the somatic gonad or the germ line in regulating longevity via G proteins could be tested. For instance, *mes-1* and *glp-1* mutants lack germ cells and show an extended life span (Arantes-Oliveira et al. 2002). Lack of germ line as well as ODR-3 signaling extends the life span of long-lived *daf-2* mutants. Therefore, it would be interesting to test whether ODR-3, or another G protein subunit, and germ line signaling act in the same pathway or in parallel.

It has not yet been determined how sensory signaling influences longevity, but the involvement of endocrine signals is very likely. Whether this is the case, could be tested genetically using mutations in the CAPS *unc-31* and in the syntaxin homologue *unc-64*, which impair Ca^{2+} regulated neurosecretion and extend life span (Ailion et al. 1999). In addition, the precise endocrine signaling molecules, which are probably one or more insulins (Pierce et al. 2001; Li et al. 2003), could be identified genetically or using fluorescent imaging. For example, overexpression of the insulin *ins-1* causes a slight life span extension, similar to overexpression of *gpa-11* (Pierce et al. 2001; Chapter 6). Both genes are expressed in the ASH neurons and they are thought to act as DAF-2 antagonists. Therefore, they might function in the same pathway. This could be tested using double mutant analysis and GFP fused to the *ins-1* promoter. In this way, it has been found that the insulin *daf-28* is differently regulated by DAF-11 and TAX-4 (Li et al. 2003).

Is the study of aging in *C. elegans* useful for understanding aging in higher eukaryotes such as humans? Evidence for many forms ('theories') of aging in mammals also exists in *C. elegans*, including accumulation of damage, telomere shortening, (oxidative) stress resistance, metabolic control and a genetic aging program (Kenyon 1997, 2005). In addition, evidence that aging in *C. elegans* is actively controlled, is provided by the experiments described in Chapter 6 and by other experiments that describe the influence of environmental and reproductive

signals (Hsin and Kenyon 1999; Apfeld and Kenyon 1999; Alcedo and Kenyon 2004; Oh et al. 2005). This control involves insulin/IGF-1 signaling, which, importantly, also controls longevity in several other organisms. Among these are yeast, flies, rodents and perhaps even humans (Tatar et al. 2003; Katic and Kahn 2005). Thus far, a role for sensory perception in regulating longevity in these organisms has not been found, but it is conceivable that sensory perception, like the smell of food, influences insulin levels. Because *C. elegans* is ideally suited for genetic dissection of the aging process, studying aging in *C. elegans* could especially be useful to understand aging at the molecular genetic level.

References

Ailion, M., T. Inoue, C.I. Weaver, R.W. Holdcraft, and J.H. Thomas. 1999. Neurosecretory control of aging in *Caenorhabditis elegans*. *Proc Natl Acad Sci U S A* 96: 7394-7.

Alcedo, J. and C. Kenyon. 2004. Regulation of *C. elegans* Longevity by Specific Gustatory and Olfactory Neurons. *Neuron* 41: 45-55.

Apfeld, J. and C. Kenyon. 1999. Regulation of lifespan by sensory perception in *Caenorhabditis elegans*. *Nature* 402: 804-9.

Arantes-Oliveira, N., J. Apfeld, A. Dillin, and C. Kenyon. 2002. Regulation of life-span by germ-line stem cells in *Caenorhabditis elegans*. *Science* 295: 502-5.

Belcheva, M.M. and C.J. Coscia. 2002. Diversity of G protein-coupled receptor signaling pathways to ERK/MAP kinase. *Neurosignals* 11: 34-44.

Berghard, A. and L.B. Buck. 1996. Sensory transduction in vomeronasal neurons: evidence for G alpha o, G alpha i2, and adenylyl cyclase II as major components of a pheromone signaling cascade. *J Neurosci* 16: 909-18.

Brenner, S. 1974. The genetics of *Caenorhabditis elegans*. *Genetics* 77: 71-94.

Chao, M.Y., H. Komatsu, H.S. Fukuto, H.M. Dionne, and A.C. Hart. 2004. Feeding status and serotonin rapidly and reversibly modulate a *Caenorhabditis elegans* chemosensory circuit. *Proc Natl Acad Sci U S A* 101: 15512-7.

Clapham, D.E. 2003. TRP channels as cellular sensors. *Nature* 426: 517-24

Dennis, J.C., J.G. Allgier, L.S. Desouza, W.C. Eward, and E.E. Morrison. 2003. Immunohistochemistry of the canine vomeronasal organ. *J Anat* 202: 515-24.

Firestein, S. 2001. How the olfactory system makes sense of scents. *Nature* 413: 211-8.

Fukuto, H.S., D.M. Ferkey, A.J. Apicella, H. Lans, T. Sharmeen, W. Chen, R.J. Lefkowitz, G. Jansen, W.R. Schafer, and A.C. Hart. 2004. G protein-coupled receptor kinase function is essential for chemosensation in *C. elegans*. *Neuron* 42: 581-93.

Gold, G.H. 1999. Controversial issues in vertebrate olfactory transduction. *Annu Rev Physiol* 61: 857-71.

Hansen, A., S.H. Rolen, K. Anderson, Y. Morita, J. Caprio, and T.E. Finger. 2003. Correlation between olfactory receptor cell type and function in the channel catfish. *J Neurosci* 23: 9328-39.

Hansen, A., K.T. Anderson, and T.E. Finger. 2004. Differential distribution of olfactory receptor neurons in goldfish: structural and molecular correlates. *J Comp Neurol* 477: 347-59.

Hardie, R.C., and P. Raghu. 2001. Visual transduction in Drosophila. *Nature* 413: 186–93.

He, W., K. Yasumatsu, V. Varadarajan, A. Yamada, J. Lem, Y. Ninomiya, R.F. Margolskee, and S. Damak. 2004. Umami taste responses are mediated by alpha-transducin and alpha-gustducin. *J Neurosci* 24: 7674-80.

Hilliard, M.A., C. Bergamasco, S. Arbucci, R.H. Plasterk, and P. Bazzicalupo. 2004. Worms taste bitter: ASH neurons, QUI-1, GPA-3 and ODR-3 mediate quinine avoidance in *Caenorhabditis elegans*. *Embo J* 23: 1101-1111.

Hsin, H. and C. Kenyon. 1999. Signals from the reproductive system regulate the lifespan of *C. elegans*. *Nature* 399: 362-6.

Jansen, G., K.L. Thijssen, P. Werner, M. van der Horst, E. Hazendonk, and R.H. Plasterk. 1999. The complete family of genes encoding G proteins of *Caenorhabditis elegans*. *Nat Genet* 21: 414-9.

Jansen, G., D. Weinkove, and R.H. Plasterk. 2002. The G-protein gamma subunit gpc-1 of the nematode *C.elegans* is involved in taste adaptation. *Embo J* 21: 986-94.

Jones, D.T. and R.R. Reed. 1989. Golf: an olfactory neuron specific-G protein involved in odorant signal transduction. *Science* 244: 790-5.

Kenyon, C. 1997 Environmental factors and gene activities that influence life span. In *C. elegans II* (eds. D.S. Riddle et al.), pp.791-813. Cold Spring Harbor Press, Cold Spring Harbor, NY.
Kenyon, C. 2005. The plasticity of aging: insights from long-lived mutants. *Cell* 120: 449-60.
Katic, M. and C.R. Kahn. 2005. The role of insulin and IGF-1 signaling in longevity. *Cell Mol Life Sci* 62: 320-43.
Lans, H., S. Rademakers, and G. Jansen. 2004. A network of stimulatory and inhibitory Galpha-subunits regulates olfaction in *Caenorhabditis elegans*. *Genetics* 167: 1677-87.
L'Etoile, N.D., C.M. Coburn, J. Eastham, A. Kistler, G. Gallegos, and C.I. Bargmann. 2002. The cyclic GMP-dependent protein kinase EGL-4 regulates olfactory adaptation in *C. elegans*. *Neuron* 36: 1079-89.
Li, W., S.G. Kennedy, and G. Ruvkun. 2003. daf-28 encodes a *C. elegans* insulin superfamily member that is regulated by environmental cues and acts in the DAF-2 signaling pathway. *Genes Dev* 17: 844-58.
Lindemann, B. 2001. Receptors and transduction in taste. *Nature* 413: 219-25.
Luo, Y., S. Lu, P. Chen, D. Wang, and M. Halpern. 1994. Identification of chemoattractant receptors and g-proteins in the vomeronasal system of garter snakes. *J Biol Chem* 269:16867-77
Luo, A.H., E.H. Cannon, K.S. Wekesa, R.F. Lyman, J.G. Vandenbergh, and R.R. Anholt. 2002. Impaired olfactory behavior in mice deficient in the alpha subunit of G(o). *Brain Res* 941: 62-71.
Marinissen, M.J. and J.S. Gutkind. 2001. G-protein-coupled receptors and signaling networks: emerging paradigms. *Trends Pharmacol Sci* 22: 368-76.
Morales, B. and J. Bacigalupo. 1996. Chemical reception in vertebrate olfaction: evidence for multiple transduction pathways. *Biol Res* 29: 333-41.
Oh, S.W., A. Mukhopadhyay, N. Svrzikapa, F. Jiang, R.J. Davis, and H.A. Tissenbaum. 2005. JNK regulates lifespan in *Caenorhabditis elegans* by modulating nuclear translocation of forkhead transcription factor/DAF-16. *Proc Natl Acad Sci U S A* 102: 4494-9.
Pierce, S.B., M. Costa, R. Wisotzkey, S. Devadhar, S.A. Homburger, A.R. Buchman, K.C. Ferguson, J. Heller, D.M. Platt, A.A. Pasquinelli, L.X. Liu, S.K. Doberstein, and G. Ruvkun. 2001. Regulation of DAF-2 receptor signaling by human insulin and ins-1, a member of the unusually large and diverse *C. elegans* insulin gene family. *Genes Dev* 15: 672-86.
Reiner, D.J., E.M. Newton, H. Tian, and J.H. Thomas. 1999. Diverse behavioural defects caused by mutations in *Caenorhabditis elegans* unc-43 CaM kinase II. *Nature* 402: 199-203.
Roayaie, K., J.G. Crump, A. Sagasti, and C.I. Bargmann. 1998. The G alpha protein ODR-3 mediates olfactory and nociceptive function and controls cilium morphogenesis in *C. elegans* olfactory neurons. *Neuron* 20: 55-67.
Sagasti, A., N. Hisamoto, J. Hyodo, M. Tanaka-Hino, K. Matsumoto, and C.I. Bargmann. 2001. The CaMKII UNC-43 activates the MAPKKK NSY-1 to execute a lateral signaling decision required for asymmetric olfactory neuron fates. *Cell* 105: 221-32.
Spehr, M., C.H. Wetzel, H. Hatt, and B.W. Ache. 2002. 3-phosphoinositides modulate cyclic nucleotide signaling in olfactory receptor neurons. *Neuron* 33: 731-9.
Tatar, M., A. Bartke, and A. Antebi. 2003. The endocrine regulation of aging by insulin-like signals. *Science* 299: 1346-51.
Troemel, E.R., B.E. Kimmel, and C.I. Bargmann. 1997. Reprogramming chemotaxis responses: sensory neurons define olfactory preferences in *C. elegans*. *Cell* 91: 161-9.
Troemel, E.R., A. Sagasti, and C.I. Bargmann. 1999. Lateral signaling mediated by axon contact and calcium entry regulates asymmetric odorant receptor expression in *C. elegans*. *Cell* 99: 387-98.
Wekesa, K.S. and R.R. Anholt. 1997. Pheromone regulated production of inositol-(1, 4, 5)-trisphosphate in the mammalian vomeronasal organ. *Endocrinology* 138: 3497-504.
Wekesa, K.S. and R.R. Anholt . 1999. Differential expression of G proteins in the mouse olfactory system. *Brain Res* 837: 117-26.
Wickman, K.D. and D.E. Clapham. 1995. G-protein regulation of ion channels. *Curr Opin Neurobiol* 5: 278-85.
Zwaal, R.R., J.E. Mendel, P.W. Sternberg, and R.H. Plasterk. 1997. Two neuronal G proteins are involved in chemosensation of the *Caenorhabditis elegans* Dauer-inducing pheromone. *Genetics* 145: 715-27.

Final chapter

Summary
Samenvatting voor familie en vrienden
List of abbreviations
Curriculum vitae
This is the end….

Summary

Each organism must be able to detect signals from its environment. Among the many molecules that are involved in sensory perception, G proteins have a prominent place. Many environmental signals activate G proteins by binding to the extracellular region of G protein coupled receptors (GPCRs), which stick through the membrane of specialized sensory cells. Inactive G proteins are bound to the intracellular regions of GPCRs. Upon binding of a signal to the GPCR, G proteins are activated and, in turn, activate several other molecules, leading to a cellular response. In this thesis, the role of G proteins in sensory signaling in the nematode *C. elegans* is described.

In the first three chapters, *C. elegans*, G proteins and sensory signaling are introduced. *C. elegans* is a small soil nematode, which is well suited to study in the laboratory. Firstly, it is small, can self fertilize and has a short generation time. Secondly, the positions, morphology and neural connections of all 959 somatic cells of *C. elegans* are known. Thirdly, many easy but powerful genetic techniques can be employed using *C. elegans*. All genes of *C. elegans* are sequenced, annotated and can be searched on the internet.

G proteins consist of three subunits, a Gα, a Gβ and a Gγ subunit. When inactive, the three subunits form a heterotrimeric complex, but when actived, the Gα subunit and a dimer formed by the Gβ and Gγ subunits separately activate many target molecules. In mammals, 16 Gα, 5 Gβ and 12 Gγ subunits and many G protein regulators have been identified. These are involved in a variety of physiological processes, including olfaction and taste. In *C. elegans,* 21 Gα, 2 Gβ and 2 Gγ subunits have been identified. Four Gα subunits and the two Gβ subunits resemble the mammalian G proteins, whereas 17 Gα subunits appear different. Of these, 14 are specifically expressed in the chemosensory neurons.

Using its 12 pairs of chemosensory neurons, *C. elegans* can detect, discriminate between and adapt to a wide variety of environmental signals, including many volatile and nonvolatile cues. This is tested in specific assays in which *C. elegans* moves either towards or away from a chemical. The AWA and AWC neurons detect attractive odorants, while the AWB, ASH and ADL neurons detect repulsive odorants. In addition, the ASE neurons detect attractive nonvolatile chemicals and the ASH neurons detect a wide variety of repulsive signals. In each neuron, sensory signaling starts with the binding of an environmental signal to a GPCR, which then activates one or more G proteins. Subsequently, in the AWA, ASH and ADL neurons, G proteins signal to TRP channels and in the AWC, AWB and possibly the ASE neurons, G proteins signal to cGMP gated Ca^{2+} channels. The main G proteins involved in sensory signaling are ODR-3 and GPA-3.

Sensory perception not only regulates behavior, but also influences development. In *C. elegans,* this is exemplified by the regulation of dauer formation and longevity. The environmental determinants for dauer formation are food, temperature and a constitutively secreted dauer pheromone. The ASJ, ADF, ASI

and ASG sensory neurons control dauer development via G protein signaling and three partially parallel signaling pathways: a TGFβ pathway, a cGMP pathway and an insulin/IGF-1 pathway. In addition, the sensory neurons regulate longevity via G protein signaling and the insulin/IGF-1 pathway. The ASI, ASG, AWA and AWC neurons suppress longevity, whereas the ASJ and ASK neurons promote longevity.

In Chapter 4, the function of G proteins in olfaction in *C. elegans* is described. Attractive odorant detection is mediated by the AWA and AWC neurons. In the AWA neurons the Gα subunits ODR-3, GPA-3, GPA-5 and GPA-6 and in the AWC neurons ODR-3, GPA-2, GPA-3 and GPA-13 are expressed. All localize to the cilia, except GPA-6, and GPA-5 also localizes to synaptic sites. ODR-3 is necessary and sufficient for odorant detection by the AWA and AWC neurons. GPA-3 is redundant to ODR-3 and can also mediate the detection of odorants. In the AWA neurons, GPA-5 inhibits odorant detection. In the AWC neurons, GPA-2 inhibits and GPA-13 promotes odorant detection. In addition, ODR-3 regulates the morphology of the ciliated endings of the AWC neurons.

Chapter 5 describes the role of Gα subunits regulating *str-2* expression. The candidate odorant receptor *str-2* is randomly expressed in either the left or the right AWC neuron and in the ASI neurons. The AWC asymmetry is established early in development by lateral axon contact and Ca^{2+}/MAPK signaling involving the voltage gated Ca^{2+} channel UNC-36, the CaMKII UNC-43, the MAPKKK NSY-1, the TIR protein TIR-1 and the MAPKK SEK-1. Maintenance of *str-2* expression depends on cGMP signaling genes and the Gα subunits GPA-6 and ODR-3. In addition, GPA-2, GPA-5, GPA-6 and ODR-3 function in a signaling network with UNC-36, UNC-43 and NSY-1, but not TIR-1 and SEK-1, to regulate *str-2* expression in first stage larvae, dauer larvae and adults. All genes function in the AWC neurons, except GPA-5 and GPA-6, which function in the AWA and ADL neurons of adults to control *str-2* expression in the AWC neurons. Furthermore, in dauer larvae, ODR-3 and GPA-6 function in the AWB neurons to regulate *str-2* expression in the ASI neurons.

Chapter 6 addresses the involvement of G proteins in regulating longevity. The life span of *C. elegans* is influenced by sensory signals transduced via the sensory neurons. These signals are probably mediated by G proteins. The Gα subunits GPA-1, GPA-9 and ODR-3 and the Gγ subunit GPC-1 suppress longevity, whereas the Gα subunit GPA-11 promotes longevity. GPA-11 probably signals via the insulin receptor DAF-2, but might also suppress longevity via a separate pathway. ODR-3 also signals independently of DAF-2. Both Gα subunits signal via the FOXO family transcription factor DAF-16, which promotes longevity. In addition, the involvement of GPA-11 shows that the previously unanticipated ASH and ADL neurons regulate longevity.

Finally, in Chapter 7, prospects and directions for the future are discussed. The studies presented in this thesis contribute to the understanding that sensory signaling is enormously complex, involving many redundant and antagonistic signaling pathways. This is probably necessary to generate a precisely tuned

response to the many different environmental signals. In addition, the studies presented here illustrate the large influence of sensory signaling on the development and behavior of an organism.

Samenvatting voor familie en vrienden

Om te kunnen overleven moet elk organisme reageren op signalen uit de omgeving. Voor het waarnemen van omgevingssignalen zoals licht, geur- en smaakstoffen, zijn G eiwitten erg belangrijk. Veel omgevingssignalen worden waargenomen doordat ze binden aan speciale receptoreiwitten die door de celmembraan van gespecialiseerde zenuwcellen steken. Als een signaal een receptoreiwit bindt, wordt dit signaal via de receptor doorgegeven aan G eiwitten binnen in de cel. De op deze manier geactiveerde G eiwitten activeren op hun beurt weer andere moleculen, die weer andere moleculen activeren. Dit doorgeven van het signaal heet signaaltransductie en leidt uiteindelijk tot een reactie van de cel, bijvoorbeeld het activeren van een volgende hersencel. In dit proefschrift is de functie van G eiwitten bij het doorgeven van omgevingssignalen in de nematode *C. elegans* beschreven.

In de eerste drie hoofdstukken worden *C. elegans,* G eiwitten en transductie van omgevingsignalen geïntroduceerd. *C. elegans* is een kleine grondworm die goed te gebruiken is voor onderzoek in een laboratorium. Ten eerste is *C. elegans* gemakkelijk te kweken. Ten tweede zijn de posities en neurale connecties van alle 959 lichaamscellen bekend. Ten derde is *C. elegans* erg gebruiksvriendelijk voor genetisch onderzoek, bijvoorbeeld omdat bijna alle *C. elegans* genen bekend zijn.

G eiwitten vormen een complex dat is opgebouwd uit drie afzonderlijke eiwitten: een Gα, een Gβ en een Gγ eiwit. Zowel het Gα eiwit als de Gβ en Gγ eiwitten samen kunnen, eenmaal geactiveerd, veel verschillende moleculen activeren. In zoogdieren zijn in totaal 16 Gα, 5 Gβ en 12 Gγ eiwitten geindentificeerd. Deze zijn betrokken bij verschillende fysiologische processen, inclusief reuk en smaak. In *C. elegans* zijn 21 Gα, 2 Gβ en 2 Gγ eiwitten geidentificeerd. 14 Gα eiwitten en 1 Gγ eiwit functioneren specifiek in de zenuwcellen die betrokken zijn bij het waarnemen van signalen uit de omgeving. Deze zenuwcellen worden ook wel chemoperceptie cellen genoemd.

C. elegans kan, gebruikmakend van zijn 22 chemoperceptie cellen, veel verschillende omgevingssignalen waarnemen en van elkaar onderscheidden. Dit wordt bestudeerd door te testen of *C. elegans* naar een chemische stof toe of van een chemische stof weg kruipt. Zo is gevonden dat de AWA en AWC cellen aantrekkelijke geurstoffen detecteren, terwijl de AWB, ASH en ADL cellen afstotelijke geurstoffen detecteren. Tevens detecteren de ASE cellen aantrekkelijke smaakstoffen en de ASH cellen veel verschillende afstotelijke chemicaliën. In elke chemoperceptie cel zijn één of meerdere G eiwitten betrokken bij de detectie van omgevingssignalen.

Omgevingssignalen hebben grote invloed op de ontwikkeling van *C. elegans*, bijvoorbeeld de ontwikkeling van dauer larven of de levensduur. Dauer is een gespecialiseerd larvaal stadium van *C. elegans* dat gedurende lange tijd en onder extreme omstandigheden kan overleven. De omgevingssigalen die dauer ontwikkeling reguleren zijn voedsel, temperatuur en een continu uitgescheiden

dauer feromoon. Een aantal chemoperceptie cellen reguleren dauer ontwikkeling via G eiwit signaaltransductie, het groeihormoon TGFβ en insuline-achtige hormonen. Tegenwoordig wordt veel onderzoek verricht naar de regulatie van de levensduur van *C. elegans.* Normaal wordt *C. elegans* gemiddeld ongeveer 2 tot 3 weken oud, maar er zijn mutante wormen gevonden die gemiddeld ouder dan 10 weken worden. Omgevingssignalen die door de chemoperceptie cellen worden waargenomen reguleren onder andere levensduur. De ASI, ASG, AWA en AWC cellen remmen een lange levensduur, terwijl de ASJ en ASK cellen een lange levensduur stimuleren, waarschijnlijk via G eiwit signaaltransductie en insuline-achtige hormonen.

In hoofdstuk 4 worden de functies van Gα eiwitten bij de detectie van aantrekkelijke geurstoffen door de AWA en AWC cellen beschreven. De Gα eiwitten ODR-3, GPA-2, GPA-3, GPA-5, GPA-6 en GPA-13 functioneren in de AWA en AWC cellen. Allemaal, behalve GPA-6, bevinden ze zich in het puntje van de neus waar detectie van omgevingssignalen plaatsvind. GPA-5 bevindt zich ook in de synapsen. Zowel in de AWA als de AWC cellen is ODR-3 het belangrijkst voor geurdetectie, maar GPA-3 kan de functie van ODR-3 overnemen. In de AWA cellen wordt geurdetectie geremd door GPA-5. In de AWC cellen wordt geurdetectie geremd door GPA-2 en gestimuleerd door GPA-13. Deze vindingen suggereren dat een complex netwerk van signalen heel precies de reactie op geurstoffen regelt.

In hoofdstuk 5 wordt de rol van Gα eiwitten bij het aan- en uitzetten van het *str-2* gen besproken, hetgeen regulatie van (gen)expressie wordt genoemd. Het *str-2* gen staat of in de rechter of in de linker AWC cel en in beide ASI cellen aan. De asymmetrische AWC expressie wordt vroeg in de ontwikkeling bepaald door signaaltransductie via de moleculen UNC-36, UNC-43 en NSY-1. Het onderhouden van *str-2* expressie is afhankelijk van andere signaaltransductiemoleculen en de Gα eiwitten GPA-6 en ODR-3. Tevens wordt *str-2* expressie in jonge larven, dauer larven en volwassenen gereguleerd door de Gα eiwitten GPA-2, GPA-5, GPA-6 en ODR-3, samen met UNC-36, UNC-43 en NSY-1. Bijzonder is dat in volwassenen GPA-5 en GPA-6 in de AWA en ADL cellen de *str-2* expressie in de AWC cellen reguleren. In dauer larven reguleren ODR-3 en GPA-6 in de AWB cellen de *str-2* expressie in de ASI cellen. Hoe dit mogelijk is, is nog niet duidelijk. Deze resultaten laten zien dat signalen in een cel en signalen van buiten een cel kunnen regelen welke eiwitten in die cel worden gemaakt.

Hoofdstuk 6 beschrijft de regulatie van levensduur door G eiwitten in de chemoperceptie cellen. De levensduur van *C. elegans* wordt beïnvloed door omgevingssignalen die mogelijk via G eiwit signaaltransductie worden gedetecteerd. Signaaltransductie via de Gα eiwitten GPA-1, GPA-9, ODR-3 en het Gγ eiwit GPC-1 remt een lange levensduur, terwijl transductie via het Gα eiwit GPA-11 een lange levensduur stimuleert. GPA-11 signaleert waarschijnlijk via een standaard insuline-achtige route, maar waarschijnlijk ook op een andere manier, evenals ODR-3. De rol van GPA-11 toont tevens aan dat de ASH en ADL cellen de levensduur van *C. elegans* reguleren, hetgeen onverwacht was.

Tot slot worden in hoofdstuk 7 enige vooruitzichten besproken. De studies in dit proefschrift dragen bij aan het inzicht dat het waarnemen van omgevingssignalen enorm complex is en dat vele overlappende en tegengestelde signaaltransductieroutes betrokken zijn. De complexiteit is waarschijnlijk nodig om een precies uitgebalanceerde reactie te bewerkstelligen op de vele verschillende omgevingsignalen. Verder illustreren de studies de enorme invloed van de omgeving op het gedrag en de ontwikkeling van een organisme.

List of abbreviations

AA	Arachidonic Acid
ASEL	left ASE cell
ASER	right ASE cell
AWC^{ON}	AWC cell that expresses *str-2*
AWC^{OFF}	AWC cell that does not express *str-2*
CaMKII	Ca^{2+}/calmodulin dependent protein kinase II
CAPS	Ca^{2+} dependent Activator Protein for Secretion
daf-c	Dauer formation constitutive
daf-d	Dauer formation defective
Deg	Degenerin
DAG	DiAcylGlycerol
ENaC	Epithelial Sodium Channel
EPA	EicosaPentaenoic Acid
GGL	G Gamma (γ) like
GPCR	G protein Coupled Receptor
GRK	G protein coupled Receptor Kinase
IGF-1	Insulin-like Growth Factor
JNK	c-Jun N-terminal kinase
MAPK	Mitogen Activated Protein Kinase
O3AA	Omega-3 Arachidonic Acid
PI_3K	Phosphotidylinositol-3-kinase
$PI(4,5)P_2$	Phosphatidylinositol-4,5-bisphosphate
PLC	PhosphoLipase C
PUFA	PolyUnsaturated Fatty Acid
RGS	Regulator of G protein Signaling
TRP	Transient Receptor Potential
TIR	Toll Interleukin-1 Receptor

Curriculum vitae

naam:	Willem Johannes Lans
geboren:	18 juli 1975

1987 -1993	Gymnasium, Comenius College te Rotterdam
1993 -1994	Geofysica, Universiteit Utrecht
1994 -1999	Biomedische Wetenschappen, Rijksuniversiteit Leiden
	stages: Klinische Genetica, Erasmus MC te Rotterdam
	Klinische Oncologie, Rijksuniversiteit Leiden
	Moleculaire Celbiologie, Rijksuniversiteit Leiden
2000 -2005	Promotieonderzoek
	afdeling Celbiologie en Genetica, Erasmus MC Rotterdam
	promotor: Prof. dr. F.G. Grosveld
	copromotor: Dr. G. Jansen
2005	Postdoc
	afdeling Celbiologie en Genetica, Erasmus MC Rotterdam

Publicaties

Bontekoe, C.J., Bakker, C.E., Nieuwenhuizen, I.M., van der Linde, H., Lans, H., de Lange, D., Hirst, M.C. and Oostra, B.A. 2001. Instability of a $(CGG)_{98}$ repeat in the *Fmr1* promoter. Hum Mol Gen **10**:1693-99

Fukuto, H.S., Ferkey, D.M., Apicella, A.J., Lans, H., Sharmeen, T., Chen, W., Lefkowitz, R.J., Jansen, G., Schafer, W.R., Hart, A.C. 2004. G protein-coupled receptor kinase function is essential for chemosensation in *C. elegans*. Neuron **42**:581-93

Lans, H., Rademakers, S., Jansen, G. 2004. A network of stimulatory and inhibitory Galpha-subunits regulates olfaction in *Caenorhabditis elegans*. Genetics **167**:1677-87

Lans, H., Jansen, G. 2005. Non-cell autonomous G protein signals converge with a Ca^{2+}/MAPK pathway to regulate *str-2* receptor gene expression in the AWC and ASI neurons of *C. elegans*. *manuscript submitted for publication*

Lans, H., Jansen, G. 2005. Sensory G proteins modulate longevity in *Caenorhabditis elegans*. *manuscript in preparation*

This is the end….

Natuurlijk heb ik dit boekje niet in mijn eentje bij elkaar gepipetteerd. Heel wat mensen hebben me op diverse manieren geholpen en er bovendien voor gezorgd dat ik het naar mijn zin heb gehad de afgelopen 5 jaar. Daarvoor wil ik iedereen bedanken. Om niet alles en iedereen te hoeven noemen, volgt hieronder een samenvatting.

Gert, bedankt voor de goede begeleiding en de leuke samenwerking. Je stond aan de basis van de drie onderzoeken die in dit boekje zijn beschreven. Tussen die onderzoeken door, hebben we ook heel wat gelachen.

Suzanne, bedankt, want met jou heb ik, zeker in het begin, veel en leuk samengewerkt. Jouw pipetteertechnische inbreng was essentieel voor veel van de proeven die tot dit proefschrift hebben geleid.

Jan, samen met jou heb ik enkele twijfelachtige en zelfs onwetenschappelijke experimenten uitgevoerd. Bedankt, want die waren ook leuk. Daarnaast konden we het altijd goed met elkaar vinden op het lab en tijdens congressen. Succes met je eigen boekje.

Martijn en Renate, met jullie heb ik minder samengewerkt of minder vaak droogijs in een epje gedaan, maar ik heb het met jullie niet minder naar mijn zin gehad. Ik denk aan de vele keren dat we over het werk of het leven hebben gepraat, de vele leuke discussies en jullie up-to-date muziek die ik heb leren waarderen. Bedankt en succes met jullie eigen promotie.

Karin, Begoña, Judith, Gregor en Jeroen, voor jullie geldt hetzelfde. Ook al was jullie muzikale inbreng minder (behalve een chronische fluittoon), jullie niet-muzikale inbreng was dat zeker niet. Bedankt voor jullie gezelligheid en de vele zinnige en onzinnige gesprekken.

De rest ook bedankt. Wat betreft gezelligheid en dergelijke denk ik aan recente en minder recente ex-labcollega's, zoals Laura, Jessica, Zeli, Najat, Rowin en Noemí (er zijn er meer maar die noem ik hier niet). Wat betreft het autoclaveren van mijn flessen denk ik aan iedereen die mijn flessen weleens heeft geautoclaveerd. Wat betreft appelflappen denk ik aan Niels en ieder ander die mij er een heeft gegeven. Wat betreft promoveren denk ik aan Frank en de overige commissieleden. Ik denk ook aan Melle en Marike. Als laatste denk ik aan Jim, John, Ray, Robby, Paul, John, George, Ringo, Stevie, Neil, Peter, Tony, Phil, Steve, Mike, Donald, Walter en vele, vele anderen.

Pa en ma, bedankt dat jullie me genoeg mogelijkheden hebben gegeven.
Judith, bedankt voor alle niet-wetenschappelijke inbreng.