Molecular Mechanisms of Chemotaxis to Sodium Chloride in Caenorhabditis elegans

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Molecular Mechanisms of Chemotaxis to Sodium Chloride in *Caenorhabditis elegans*

Moleculaire mechanismen van chemotaxis naar natriumchloride in *Caenorhabditis elegans*

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CHAPTER 1

Introduction

Scope of this thesis

To explore and navigate through their environments, many organisms have evolved a strategy called chemotaxis. During chemotaxis, organisms sense and use the concentration gradient of a substance or signal to navigate and move up the gradient of an attractive compound or down the gradient of a repellent one. These general principles also apply to the cellular processes *within* a multicellular organism, such as in guided cell migration and axon guidance.

To detect such environmental cues, cells display receptors and channels on their plasma membranes. These membrane proteins bind extracellular ligands and transduce this cue downstream, usually via second messengers and effector proteins. Alternatively, ion channels, which can be constitutively open or for example ligand or pH gated, allow for and direct influx of extracellular signals. These signaling proteins can be distributed randomly over the plasma membrane or clustered in microdomains, e.g. associated with lipid rafts, to increase local density of signaling proteins (Raghunathan and Kenworthy, 2018). Furthermore, most metazoan cells possess an antenna-like organelle, the cilium, in which signaling proteins can also congregate.

The cilium is based around an axoneme, a microtubule core that pushes the cell membrane outwards, creating an antenna-like structure (Satir and Christensen, 2007; Ward et al., 1975; Ware et al., 1975). The cilium and its membrane are compartmentalized from the cell by the transition zone (TZ), a diffusion barrier that links the ciliary membrane to the microtubule core (Perkins et al., 1986; Szymanska and Johnson, 2012). The TZ is organized by MKS and NPHP modules, named after the diseases Meckel Gruber Syndrome and Nephronophthisis, which are linked to mutations in genes encoding components of these modules (Li et al., 2016; Reiter and Leroux, 2017; Williams et al., 2011). MKS and NPHP proteins assemble around characteristic Y-link structures that link the cilium membrane to the axoneme and together form a diffusion barrier that prevents (membrane) proteins from freely diffusing into and out of the cilium (Garcia et al., 2018; Jensen et al., 2015). By compartmentalizing the cilium from the rest of the cell, signaling proteins can be concentrated and a distinct signaling organelle is formed that internalizes environmental cues.

The free-living nematode *Caenorhabditis elegans* (Figure 1A) also uses cilia to detect cues from its environment and is capable of chemotaxis to various water-soluble compounds and odors (Bargmann, 2006). We used the response of this invertebrate to NaCl to study three aspects of chemotaxis, focusing on one neuron pair central to the animal's response. First, we studied the mechanisms controlling the cilium localization of a putative NaCl receptor. Second, we analyzed a genetic switch governing the development of this neuron pair and the maintenance of its function. Finally, we studied the response of the nematode to NaCl and correlated behavioral variation with differences in gene expression. Together, these three studies provide general insights in cilium signaling, cell fate maintenance, and behavioral variability, while using the same functional readout, i.e. the response of *C. elegans* to NaCl.

C. elegans is a highly suitable animal model to study various biological subjects, ranging from development to neurobiology, including the three aspects of chemotaxis described in this thesis. Adult *C. elegans* is approximately 1 mm in length and has a short lifecycle, despite its 4 larval stages preceding the adult stage, with a mean generation time of 90 hours. In addition, its eutelic feature (959 somatic cells in the adult animal) and the completely resolved cell lineage, including the nervous system, are very strong assets (Kimble and Hirsh, 1979; Sulston et al., 1983; Sulston and Horvitz, 1977). Due to the roundworm's transparent cuticle, neurons and their cilia can be visualized using fluorescence microscopy in intact animals. Its genome has been completely sequenced and annotated and recent advances in gene editing with CRISPR/Cas9 make endogenous tagging of genes with fluorophores or other tags straightforward. Combined with a cuticle that is permeable to some compounds, potent techniques such as inducible degradation of endogenously tagged proteins are also possible. Finally, its chemotaxis response is quantifiable in several assays.

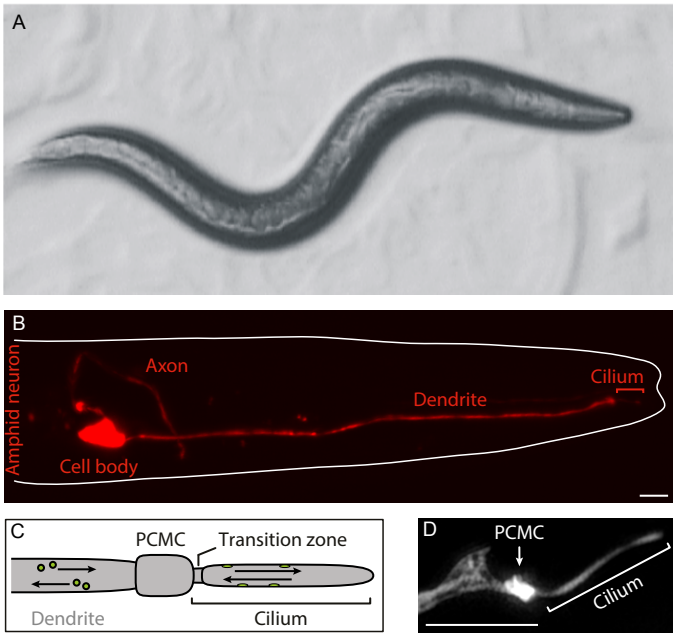

Figure 1. *C. elegans* and its ciliated amphid ASE neurons. **(A)** Photograph of a young-adult *Caenorhabditis elegans*. **(B)** ASE neuron in the head of the animal expressing mCherry (red). **(C)** Schematic of the cilium of ASE depicting the dendritic terminus and vesicular transport, periciliary membrane compartment (PCMC), transition zone, and intraflagellar transport in the cilium. **(D)** Cilium of an ASER neuron expressing mCherry (white), with the periciliary membrane compartment (PCMC), transition zone, and cilium indicated. Scale bars indicate 5 µm.

Ciliary cGMP signaling

The nervous system of *C. elegans* is comprised of 302 neurons. Based on genetic and laser-ablation studies, 32 of these neurons are presumed chemosensory (Bargmann and Horvitz, 1991; Ward, 1973; Ware et al., 1975). With the exception of one pair, the dendrites of these neurons end in a cilium (Figure 1B-D). Several of these cilia are bundled together and embedded in a channel, which has an opening to the outside environment of the animal (Perkins et al., 1986; Ware et al., 1975). It is through this channel that these neurons can detect soluble compounds and odors with their cilia. Of these sensory neurons, 28 amphid neurons have their cell bodies located in the head of the animal (Figure 1B) and together they

form the amphid and inner labial sensilla. In the tail of the animal, 4 neurons form the phasmid sensillum.

One pair of amphid neurons is chiefly responsible for chemotaxis to NaCl. These are the Amphid neurons Single ciliated ending E Left and Right, abbreviated as ASEL and ASER, respectively (Figure 1B). Although bilaterally symmetrical in structure, these ASE neurons are functionally different: ASEL responds to increases in Na^+, whereas ASER responds to a decrease in Cl^- (Ortiz et al., 2009, 2006; Smith et al., 2013). This is reflected in their differences in several receptor proteins. Most important for detecting NaCl is the difference in expression of the receptor-type guanylate cyclases (rGCs), with *gcy-14* expressed in ASEL, and *gcy-22* in ASER. Genetic evidence has shown that these receptor proteins are involved in detecting NaCl in the environment of the animal, with GCY-14 detecting Na^+ and GCY-22 detecting Cl^- ions (Ortiz et al., 2009, 2006; Smith et al., 2013). However, whether these proteins are the direct receptors of NaCl remains an open question.

The *C. elegans* genome encodes 27 rGCs, which are incorporated in the signaling cascades of a wide range of sensory modalities (Maruyama, 2017), ranging from thermosensing to gustation, including detection of NaCl. In mice and *Drosophila*, epithelium sodium channels (ENaCs) appear to be the main proteins involved in detecting NaCl (Chandrashekar et al., 2010; Liu et al., 2003). These channels can be blocked using the antagonist amiloride, thereby attenuating the response of these organisms to NaCl (Heck et al., 1984; Jenkins and Tompkins, 1990; Vandenbeuch et al., 2008). Surprisingly, amiloride is less effective in humans (Feldman et al., 2003), hinting at an ENaC-independent pathway, which might involve rGCs. Although *C. elegans* uses 28 ENaC subunits in various signaling pathways (Bianchi and Driscoll, 2002; Goodman and Schwarz, 2003; Rhoades et al., 2018), an effect of amiloride on chemotaxis to NaCl has not been found (Hukema & Jansen, unpublished results). *C. elegans* therefore presents us with an opportunity to study an ENaC independent, rGC-based NaCl detection pathway.

In contrast to *C. elegans*, the rGC gene family in mammals comprises 7 rGCs only, GC-A through GC-G, which are involved in various functions such as the regulation of blood pressure and skeletal growth, but also in vision and olfaction (Kuhn, 2016). However, a role for GCs in gustation has not been identified. Most mammalian rGCs are activated by peptides (GC-A through D), and/or CO_2 (GC-D and G) (Brenner et al., 1990; Chao et al., 2014; Fan et al., 1997; Koller et al., 1991; Leinders-Zufall et al., 2007). GC-E and F are, however, activated by guanylate cyclase activating proteins (GCAPs) in photoreceptor cells (Duda et al., 1998). To date, GCAPs have not been identified in *C. elegans*, although its rGCs have a kinase homology domain on which GCAPs could act.

Receptor-type guanylate cyclases comprise several domains (Figure 2A), including the aforementioned kinase-homology domain, which when phosphorylated in the mammalian

rGC GC-A desensitizes the receptor (Joubert et al., 2001; Potter and Garbers, 1992; Schröter et al., 2010). In addition, rGCs include an extracellular domain, a single transmembrane domain, a guanylate cyclase domain, a dimerization domain and other intracytoplasmic domain sequences (C-terminal tail). The extracellular domain provides specificity for the compounds which the respective rGCs can detect (Smith et al., 2013). Upon binding of a ligand, the transmembrane domain changes conformation and the intracellular guanylate cyclase domain becomes catalytically active, converting GTP into the second-messenger cGMP. To function, rGCs form homo- or heterodimers through their dimerization domain (Murayama et al., 2013).

In the response of *C. elegans* to NaCl, the rise in cGMP opens cyclic nucleotide-gated channels (CNGs), resulting in an influx of Ca^{2+} and activation of the cell (Figure 2B) (Komatsu et al., 1999; Li et al., 2017). The genome of *C. elegans* encodes 5 CNG subunits, of which TAX-2 and TAX-4 are the most important in chemotaxis (Coburn and Bargmann, 1996; Komatsu et al., 1996). These subunits localize to a sub-compartment of the cilium, distal to the TZ (Wojtyniak et al., 2013), and function at the start of the signal propagation down the dendrite of the neuron (Shindou et al., 2019).

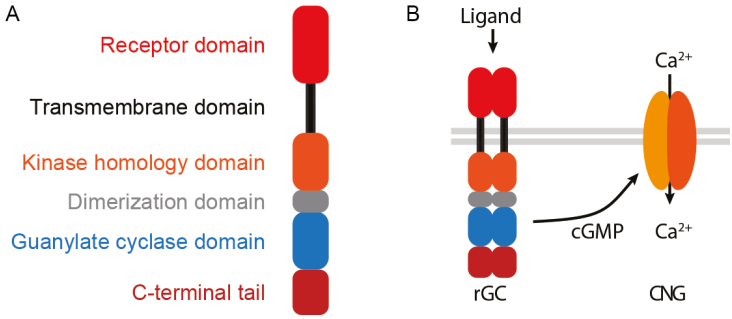

Figure 2. Schematic of a receptor-type guanylate cyclase and cGMP signaling pathway. (A) Schematic of a receptor-type guanylate cyclase (rGC) with its various domains. **(B)** cGMP signaling pathway. Upon binding of a ligand, the rGC converts GTP to cGMP which opens the cyclic nucleotide-gated Ca^{2+} channel (CNG).

Besides cGMP signaling in the ASE neurons, other pathways and neurons also play a role in chemotaxis. Ablation of the ASE neurons has shown that a residual chemotaxis response can be mediated by the ADF, ASG, and ASI neuron pairs (Bargmann and Horvitz, 1991). Avoidance of NaCl concentrations of 200 mM and higher is mainly mediated by the ASH neuron pair and involves the Gα-subunit ODR-3 and the TRPV channel OSM-9 (Colbert et al., 1997; Roayaie et al., 1998). Both proteins also function in chemotaxis to NaCl (Hukema and Jansen, unpublished results), but it is unclear how they fit in the cGMP pathway, or whether they function in parallel.

For their proper function, the signaling proteins of the cGMP pathway need to reach their correct destination in the cilium. These proteins are sorted in the Golgi-apparatus and transported along the dendrite to the cilium (Harterink et al., 2018; Martínez-Velázquez and Ringstad, 2018; Mondal et al., 2011). Once ciliary signaling proteins reach the end of the dendrite, they are stored in the periciliary membrane compartment

(PCMC). The PCMC appears to be separated from the dendritic membrane by a belt of adherens junctions (Blacque and Sanders, 2014), presumably preventing proteins from diffusing into the dendrite. At the cilium side, the transition zone (TZ) keeps proteins from diffusing out of the PCMC and into the cilium (Chih et al., 2011; Jensen et al., 2015; Williams et al., 2011). However, other mechanisms might also play a role in sequestering proteins, such as a difference in membrane composition between cellular compartments. For example, PIP2 is enriched in the PCMC, but is excluded from the cilium (Jensen et al., 2015).

To enter the cilium, proteins must be imported across the TZ. This import mechanism is selective and highly regulated, allowing only certain proteins and quantities thereof to enter. The mechanism behind this import is not completely understood but involves the intraflagellar transport machinery (Ludington et al., 2013; Prevo et al., 2015). Additionally, in *C. elegans* the ANKMY2 homolog DAF-25 is required for correct cilium localization of several classes of signaling protein (Brear et al., 2014; Jensen et al., 2010; Wojtyniak et al., 2013).

Once imported, these proteins are transported by the intraflagellar transport (IFT) system, a bidirectional transport mechanism that carries cargo along the ciliary axoneme (Rosenbaum and Witman, 2002; Scholey, 2008). In *C. elegans*, the motor complexes kinesin-II (formed by KLP-20, KLP-11 and KAP-1) and OSM-3 mediate anterograde transport (Evans et al., 2006; Snow et al., 2004), i.e. from base to ciliary tip. Transport along the proximal segment is achieved by both motor complexes but kinesin-II gradually disengages and OSM-3 based transport continues along the distal segment (Prevo et al., 2015). Retrograde transport, from tip to base, is mediated by cytoplasmic dynein (Hao et al., 2011) (Figure 3).

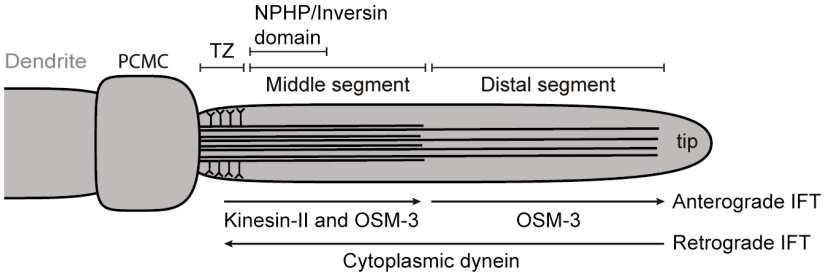

Figure 3. Schematic of a cilium of the ASE neurons. Indicated, from left to right, are the dendritic terminus (Dendrite) and periciliary membrane compartment (PCMC), the transition zone (TZ) with its Y-links which connect the microtube axoneme to the membrane of the cilium, the middle segment with microtubule doublets and NPHP/Inversin domain, and the distal segment with microtubule singlets. Anterograde intraflagellar transport is indicated with kinesin-II and OSM-3 transporting cargo along the axoneme in the middle segment and OSM-3 in the distal segment. Cytoplasmic dynein transports cargo from tip to the base in retrograde transport.

IFT particles are composed of two major complexes, IFT-A and IFT-B, believed to be linked together by the BBSome (Cole et al., 1998; Nakayama and Katoh, 2018; Ou et al., 2005). In animals mutant for BBSome subunits, the IFT-A and B complexes move separately and at different velocities. Based on these velocities, IFT-A is thought to be propelled by kinesin-II, and IFT-B by OSM-3 (Ou et al., 2005; Pan et al., 2006). Besides binding both IFT complexes,

the BBSome also functions as a bridge, linking the IFT machinery to its cargo (Klink et al., 2017; Liu and Lechtreck, 2018).

Inside the cilium, different compartments have been identified where signaling proteins can reside (Blacque and Sanders, 2014) (Figure 3). The cilium can be divided in three main sections: the TZ mentioned above and the middle and distal segments. The middle and distal segments are characterized by the microtubules of the axoneme. In the middle segment the axoneme is built up by 9 doublet microtubules which continue as single microtubules into the distal segment (Ward et al., 1975; Ware et al., 1975). Additionally, there is the inversin domain named after Inversin/NPHP2 in the middle segment, just distally to the TZ (Cevik et al., 2013; Shiba et al., 2009; Warburton-Pitt et al., 2014). Several CNGs localize to the middle segment and Inversin domain (Mukhopadhyay et al., 2008; Wojtyniak et al., 2013). Other signaling proteins, such as the TRPV channel subunits OSM-9 and OCR-2 and several GPCRs, can be found along the length of the cilium (Kim et al., 2009; McGrath et al., 2011; Qin et al., 2005; Wojtyniak et al., 2013). In mammals, components of Hedgehog signaling also localize along the length of the axoneme, but some accumulate at the very tip of the cilium (Endoh-Yamagami et al., 2009; He et al., 2014; Liem et al., 2009), a relatively unexplored ciliary compartment. The way in which the composition of these sub-ciliary domains is regulated is poorly understood. In Chapter 2 we report on a cGMP signaling compartment at the tip of the cilium and the mechanisms that govern its formation and maintenance.

Neuronal cell fate maintenance

The development of *C. elegans*, from zygote to fully-formed animal of 959 somatic cells has been extensively studied resulting in a complete linage map detailing each cell division (Sulston et al., 1983). The ASE chemosensory neurons are the final cell fates in two different lineage branches, however both ASEL and R cell fates are determined by the same terminal selector gene, *che-1* (Chang et al., 2003; Uchida et al., 2003). Terminal selector genes form the final step in a differentiation pathway and induce the expression of a multitude of target genes, which together give a cell its identity and function. Induction of neuronal cell fate through terminal selector genes is a common mechanism in neuronal differentiation and has so far been identified in mice (Monahan et al., 2017), *Drosophila melanogaster* (Konstantinides et al., 2018), the marine chordate *Ciona intestinalis* (of the subphylum Tunicata) (Horie et al., 2018), and *C. elegans* (Hobert, 2008).

CHE-1 is a zinc-finger transcription factor that contains 4 zinc-fingers which together bind a 12-base-pair DNA motif, found in the promoters of most ASE-expressed genes (Etchberger et al., 2007). It is through this motif that CHE-1 induces ASE-specific expression of its target genes. Although *che-1* expression is essential for ASE cell fate, it is not sufficient for ASE cell fate induction. Animals with a loss-of-function mutation in *che-1* show no response to NaCl, although a chemosensory neuron is present where the ASE cells are to be expected (Uchida

et al., 2003). Induction of ASE terminal cell fate in other cells also depends on the chromatin state; knock-down of members of the Polycomb repressive complex 2 (Prc2), in combination with ectopic expression of *che-1*, is required to induce ASE cell fate in germ cells in the gonad of *C. elegans* (Patel et al., 2012; Tursun et al., 2011).

In addition to driving the expression of the target genes that give the ASE neurons their function, the *che-1* terminal selector also regulates its own mRNA expression (Etchberger et al., 2007). In particular, the promoter of *che-1* contains an ASE motif, allowing *che-1* to induce its own expression. During embryonic development, expression of *che-1* is induced by the nuclear hormone receptor NHR-67 (Sarin et al., 2009). After hatching however, *nhr-67* expression is lost and the continued expression of *che-1* and its target genes is dependent on *che-1* autoregulation. This regulatory architecture, induction by *nhr-67* and subsequent autoregulation by CHE-1, is known as a bistable genetic switch (Figure 4A,B).

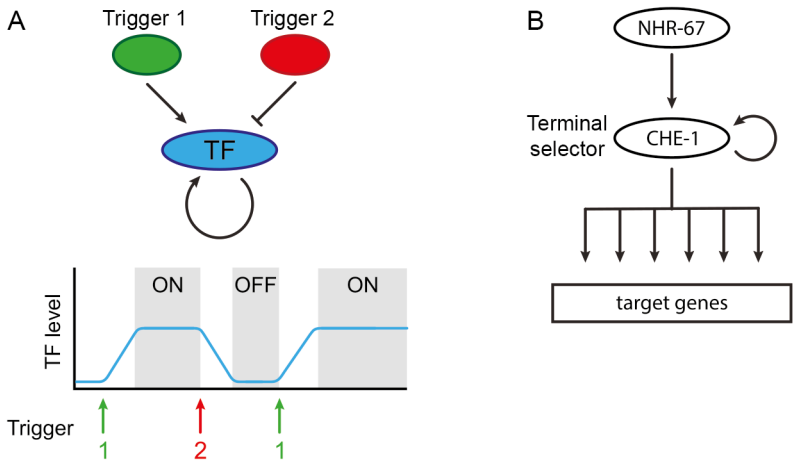

Figure 4. Schematics of a theoretical and the CHE-1 bistable genetic switch. (A) The transcription factor (TF) forms a bistable genetic switch by positively regulating its own expression. Trigger 1 (green) induces expression of TF resulting in a stable high expression or ON state. To switch to the OFF state, the inhibition of TF's auto-induction by trigger 2 (red) is required. **(B)** A minimal model of the CHE-1 genetic switch. NHR-67 induces expression of *che-1*. CHE-1 subsequently induces the expression of its own mRNA, generating a stable ON state, and the expression of its target genes.

Bistability of a genetic switch means that both the high expression or 'ON' state and the low/no expression or 'OFF' state are stable states in which a switch can be sustained (Figure 4) (Alon, 2007; Ferrell, 2002). To switch to the ON state, an outside trigger is required to induce expression of the core transcription factor. When this transcription factor subsequently induces its own expression, the ON state can be maintained, i.e. the trigger is no longer required. To switch back to the OFF state, a second trigger is needed to inhibit expression of the core transcription factor and reduce its concentration. Eventually, this inhibition will lead to termination of its auto-induction, resulting in a stable OFF state.

A classic example of a bistable switch is the lysogenic-lytic switch in bacteriophage lambda. In this system, the lambda repressor CI keeps the switch stably in the lysogenic state by inhibiting its lytic development, i.e. by blocking the OR1 and OR2 operators and expression of the regulatory protein CRO. Upon bacterial DNA damage, i.e. a trigger as described above, RecA cleaves CI, resulting in a loss of repression of the *cro* gene. Subsequently, expression of *cro* induces the lytic state of the switch via the operator OR3 but also stabilizes this state by preventing the expression of the repressor *ci* (Eisen et al., 1970; Svenningsen et al., 2005).

Besides gene regulation networks, signaling pathways can also contain bistable switch elements, for example in the regulation of the Rab GTPase Rab5. Activity of Rab5 is regulated by exchange of GDP (inactive state) to GTP (active state) by guanine nucleotide exchange factors (GEFs) or vice versa by hydrolysis of GTP to GDP, induced by GTPase activating proteins (GAPs). Because Rab5 recruits its GEF Rabex5 and effector Rabaptin5, its active state is stimulated and bistability arises (Bezeljak et al., 2020; Wandinger-Ness and Zerial, 2014; Zhang et al., 2014).

However, bistable genetic switches are known to be vulnerable to fluctuations in expression caused by molecular noise (Ozbudak et al., 2004; Süel et al., 2006). Random fluctuations in the expression of a genetic switch transcription factor can lead to instability of the switch, i.e. if the level of CHE-1 drops below a certain level, its auto-induction may fail and the switch can spontaneously turn off. As a result, expression of its target genes, and thus neuronal function, is lost. This vulnerability is aggravated by the competition of the promoters of *che-1* and its target genes for the same and limited reservoir of CHE-1 protein. How genetic switches cope with variation in gene expression is not fully understood.

However, the stability of genetic switches is vital for the long-term maintenance of neuronal fate and function. In contrast to most mammalian tissues, which show turn-over rates of 2-4 days in case of small intestine epithelium or a 15-year turn-over rate of certain muscle cells (Darwich et al., 2014; Spalding et al., 2005), most cells in the nervous system are maintained for the lifetime of the organism (Ming and Song, 2005; Spalding et al., 2005). In case of the human central nervous system, cell identity needs to be maintained over multiple decades. How neurons stabilize genetic switches and maintain the gene expression required for their identity and function is as of yet unresolved.

ASE cell fate determination is an excellent model to study genetic switch stability. First, while most switches and regulatory networks are complex, the *che-1* genetic switch is simple in architecture: there is only one trigger (*nhr-67*) to switch to the "ON" state and *che-1* auto-induction seemingly relies on CHE-1 only. Second, many targets genes are known and loss of expression of these target genes is easily identified by fluorescence microscopy or smFISH. Additionally, loss of target gene expression can be linked to ASE function via chemotaxis assays. Finally, for continued function of the ASE neurons, the 'ON' state of the switch needs

to be maintained for the lifetime of the animal, hinting at the presence of stabilization mechanisms. Together, the CHE-1 genetic switch and ASE cell fate maintenance gives us ample opportunity to study the dynamics and mechanism of this bistable genetic switch. In Chapter 3 we identify a novel mechanism which stabilizes the CHE-1 switch.

Variation in Behavior

Animals continuously respond to cues from their environment, however, these responses can vary greatly between individuals. For example, some members of a flock of red knots (*Calidris canutus*) check for nearby predators more often than others and some funnel-web spiders show more variation in their defensive behavior when presented with an intrusion in their habitat than others (Mathot et al., 2011; Riechert, 1978). This variation, or phenotypic plasticity, provides opportunities for evolution to select for fitness (Dingemanse and Wolf, 2013).

Variation in behavior can also vary within the same individual. The sources of such variation can be both external, e.g. time-of-day or temperature, or internal, such as variation in gene expression or the state of a neuronal circuit (Flores et al., 2012; Gordus et al., 2015; Iwanir et al., 2019; Korobkova et al., 2004; Munsky et al., 2012). Differences in behavioral variation can even be found in cloned—and therefore genetically identical—pigs when compared to their natural-born siblings (Archer et al., 2003).

The chemotaxis response to NaCl of isogenic populations of *C. elegans* also shows variation (Figure 5.). When presented with a choice between an attractive concentration of NaCl or no NaCl, some animals make the seemingly counterintuitive decision to move away from the attractant. Why *C. elegans* shows variation in its chemotaxis response is not known but one can imagine that it increases its chances to find additional resources.

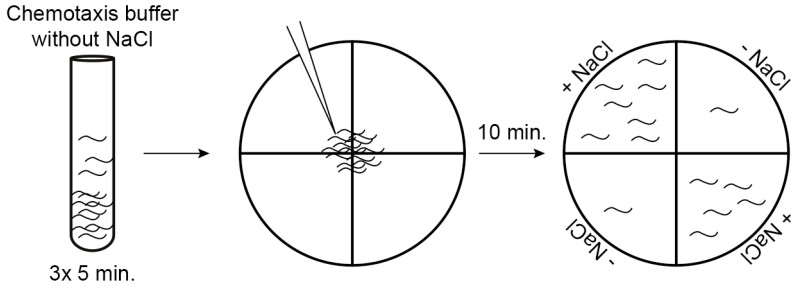

Figure 5. Quadrant chemotaxis assay and the response of *C. elegans*. Age-synchronized *C. elegans* populations consisting of genetically identical animals are washed for 15 minutes in a chemotaxis buffer without NaCl. Subsequently, approximately 100 animals are placed in the middle of a segmented petri dish with 4 quadrants filled with agar, only 2 diagonally opposite quadrants contain NaCl. After 10 minutes the animals on each quadrant are counted. Approximately 90% of the animals move to the salt containing quadrants.

C. elegans follows a boom and bust life cycle. When favorable environments are encountered, a population grows fast, but when the resources become scarce again, the population collapses. *C. elegans* employs several strategies to survive periods of limited food supply. For example, animals can go into a developmental arrest state called *dauer* (Cassada and Russell, 1975) and wait for more favorable conditions, or use a phoretic host to reach new habitats (Barrière and Félix, 2005). Behavioral variation, in which some animals of a population behave antagonistically, could also be a strategy. In this scenario, a few individuals move opposite from the main population, improving the odds of finding additional resources. Whether behavioral variation is beneficial to a species and is evolutionary conserved is still a matter of debate.

Studying behavior and its variation at an organismal level can be difficult due to the different internal and external sources of said variation. For example, differences between individuals in the genetic background, life-history, or the state of an underlying neuronal circuit can all contribute to differences in behavioral responses between individuals. Additionally, external sources such as inter-observer differences or environmental factors can play a role. However, such factors cannot be selected for by natural selection. Instead, a system that tolerates temporary differences in gene expression, presumably resulting from the underlying molecular noise, as a source of variation would be more likely if variation is evolutionarily conserved.

Because *C. elegans* hermaphrodites can reproduce asexually, its populations are essentially isogenic, eliminating genetic differences as a source of variability. Additionally, *C. elegans* populations can be age-synchronized and cultured under controlled conditions, limiting differences in life-history between individuals. Finally, chemotaxis assays have been well developed and the neurons and signaling cascades have been, at least partially, identified. Taken together, *C. elegans* is an excellent model to study behavioral variability and its possible molecular origins. In Chapter 4 we investigate if differences in gene expression between individuals can cause behavioral variation.

SCOPE OF THIS THESIS

This thesis covers three aspects of (cell) biology, which are explored using the nematode *C. elegans* and its chemotaxis response to NaCl, answering three questions: How are sub-ciliary compartments formed and what is their function in detection of NaCl? How is the cell identity and function of the NaCl detecting neurons maintained over the lifetime of *C. elegans*? Can we explain behavioral differences between individuals with variation in gene expression?

In Chapter 2 the mechanisms that regulate the localization of the receptor-type guanylate cyclase GCY-22 in a cilium tip compartment are investigated. We found that intraflagellar transport is the main driving-force behind this cilium tip compartment. We show that proper cilium import and IFT cargo loading involves DAF-25, BBS-8, and MKS-5 and we identify two protein domains of GCY-22 essential to its import into the cilium.

Chapter 3 explores a genetic switch, formed by the transcription factor CHE-1, which induces ASE cell-fate and maintains its function. We measured parameters of this switch and used *in silico* modeling to predict a mechanism that can stabilize genetic switches. We used the auxin inducible protein degradation system to validate our model. Finally, we identified an OTX-like DNA motif in the promoter of *che-1* involved in the stabilization of its expression.

In Chapter 4 we set out to correlate gene expression differences between individual animals with their response in our chemotaxis assay. We used single-worm RNA-sequencing and cluster analysis to identify possible candidate genes. We were able to verify some of our results using RT-qPCR.

REFERENCES

Alon, U., 2007. Network motifs: theory and experimental approaches. Nat Rev Genet 8, 450–461.

Archer, G.S., Friend, T.H., Piedrahita, J., Nevill, C.H., Walker, S., 2003. Behavioral variation among cloned pigs. Appl Anim Behav Sci 81, 321–331.

Bargmann, C., 2006. Chemosensation in *C. elegans*. Wormbook 1–29.

Bargmann, C.I., Horvitz, H.R., 1991. Chemosensory neurons with overlapping functions direct chemotaxis to multiple chemicals in *C. elegans*. Neuron 7, 729-742.

Barrière, A., Félix, M.A., 2005. High local genetic diversity and low outcrossing rate in *Caenorhabditis elegans* natural populations. Curr Biol 15, 1176–1184.

Bezeljak, U., Loya, H., Kaczmarek, B., Saunders, T.E., Loose, M., 2020. Stochastic activation and bistability in a Rab GTPase regulatory network. P Natl Acad Sci Usa 117, 6540–6549.

Blacque, O.E., Sanders, A.A., 2014. Compartments within a compartment. Organogenesis 10, 126–137.

Brear, A.G., Yoon, J., Wojtyniak, M., Sengupta, P., 2014. Diverse Cell Type-Specific Mechanisms Localize G Protein-Coupled Receptors to *Caenorhabditis elegans* Sensory Cilia. Genetics 197, 667–684.

Cassada, R.C., Russell, R.L., 1975. The dauerlarva, a post-embryonic developmental variant of the nematode *Caenorhabditis elegans*. Dev Biol 46, 326–342.

Cevik, S., Sanders, A.A.W.M., Wijk, E.V., Boldt, K., Clarke, L., Reeuwijk, J. van, Hori, Y., Horn, N., Hetterschijt, L., Wdowicz, A., Mullins, A., Kida, K., Kaplan, O.I., Beersum, S.E.C. van, Wu, K.M., Letteboer, S.J.F., Mans, D.A., Katada, T., Kontani, K., Ueffing, M., Roepman, R., Kremer, H., Blacque, O.E., 2013. Active Transport and Diffusion Barriers Restrict Joubert Syndrome-Associated ARL13B/ARL-13 to an Inv-like Ciliary Membrane Subdomain. Plos Genet 9, e1003977.

Chadha, A., Volland, S., Baliaouri, N.V., Tran, E.M., Williams, D.S., 2019. The route of the visual receptor, rhodopsin, along the cilium. J Cell Sci jcs.229526.

Chandrashekar, J., Kuhn, C., Oka, Y., Yarmolinsky, D.A., Hummler, E., Ryba, N.J.P., Zuker, C.S., 2010. The cells and peripheral representation of sodium taste in mice. Nature 464, 297-301.

Chang, S., Johnston, R.J., Hobert, O., 2003. A transcriptional regulatory cascade that controls left/right asymmetry in chemosensory neurons of *C. elegans*. Gene Dev 17, 2123–2137.

Chih, B., Liu, P., Chinn, Y., Chalouni, C., Komuves, L.G., Hass, P.E., Sandoval, W., Peterson, A.S., 2011. A ciliopathy complex at the transition zone protects the cilia as a privileged membrane domain. Nat Cell Biol 14, 61–72.

Coburn, C.M., Bargmann, C.I., 1996. A Putative Cyclic Nucleotide–Gated Channel Is Required for Sensory Development and Function in *C. elegans*. Neuron 17, 695–706.

Cole, D.G., Diener, D.R., Himelblau, A.L., Beech, P.L., Fuster, J.C., Rosenbaum, J.L., 1998. *Chlamydomonas* Kinesin-II–dependent Intraflagellar Transport (IFT): IFT Particles Contain Proteins Required for Ciliary Assembly in *Caenorhabditis elegans* Sensory Neurons. J Cell Biology 141, 993–1008.

Darwich, A.S., Aslam, U., Ashcroft, D.M., Rostami Hodjegan, A., 2014. Meta-analysis of the turnover of intestinal epithelia in preclinical animal species and humans. Drug Metabolism Dispos Biological Fate Chem 42, 2016–22.

Dingemanse, N.J., Wolf, M., 2013. Between individual differences in behavioural plasticity within populations: causes and consequences. Anim Behav 85, 1031–1039.

Eisen, H., Brachet, P., Silva, L.P. d, Jacob, F., 1970. Regulation of Repressor Expression in. Proc National Acad Sci 66, 855–862.

Endoh-Yamagami, S., Evangelista, M., Wilson, D., Wen, X., Theunissen, J.-W., Phamluong, K., Davis, M., Scales, S.J., Solloway, M.J., Sauvage, F.J. de, Peterson, A.S., 2009. The Mammalian *Cos2* Homolog *Kif7* Plays an Essential Role in Modulating Hh Signal Transduction during Development. Current Biology 19, 1320–1326.

Etchberger, J.F., Lorch, A., Sleumer, M.C., Zapf, R., Jones, S.J., Marra, M.A., Holt, R.A., Moerman, D.G., Hobert, O., 2007. The molecular signature and *cis*-regulatory architecture of a *C. elegans* gustatory neuron. Gene Dev 21, 1653–1674.

Evans, J.E., Snow, J.J., Gunnarson, A.L., cell, G.O.T.J. of, 2006. Functional modulation of IFT kinesins extends the sensory repertoire of ciliated neurons in *Caenorhabditis elegans*. J Cell Biology 172, 663-669.

Feldman, G.M., Mogyorósi, A., Heck, G.L., DeSimone, J.A., Santos, C.R., Clary, R.A., Lyall, V., 2003. Salt-Evoked Lingual Surface Potential in

Humans. J Neurophysiol 90, 2060–2064.
Ferrell, J.E., 2002. Self-perpetuating states in signal transduction: positive feedback, double-negative feedback and bistability. Curr Opin Cell Biol 14, 140–148.
Flores, M., Shimizu, T.S., Wolde, P.R. ten, Tostevin, F., 2012. Signaling Noise Enhances Chemotactic Drift of *E. coli*. Phys Rev Lett 109, 148101.
Garcia, G., Raleigh, D.R., Reiter, J.F., 2018. How the Ciliary Membrane Is Organized Inside-Out to Communicate Outside-In. Curr Biology Cb 28, R421–R434.
Gordus, A., Pokala, N., Levy, S., Flavell, S.W., Bargmann, C.I., 2015. Feedback from Network States Generates Variability in a Probabilistic Olfactory Circuit. Cell 161, 215–227.
Hao, L., Efimenko, E., Swoboda, P., Scholey, J.M., 2011. The Retrograde IFT Machinery of *C. elegans* Cilia: Two IFT Dynein Complexes? Plos One 6, e20995.
He, M., Subramanian, R., Bangs, F., Omelchenko, T., Liem, K.F., Kapoor, T.M., Anderson, K.V., 2014. The kinesin-4 protein Kif7 regulates mammalian Hedgehog signalling by organizing the cilium tip compartment. Nat Cell Biol 16, 663–72.
Heck, G.L., Mierson, S., DeSimone, J.A., 1984. Salttaste transduction occurs through an amiloride-sensitive sodium transport pathway. Science 223, 403 405.
Iwanir, S., Ruach, R., Itskovits, E., Pritz, C.O., Bokman, E., Zaslaver, A., 2019. Irrational behavior in *C. elegans* arises from asymmetric modulatory effects within single sensory neurons. Nat Commun 10, 3202.
Jenkins, J.B., Tompkins, L., 1990. Effects of amiloride on taste responses of *Drosophila melanogaster* adults and larvae. Journal of Insect Physiology 36, 613 618.
Jensen, V.L., Bialas, N.J., Bishop-Hurley, S.L., Molday, L.L., Kida, K., Nguyen, P.A.T., Blacque, O.E., Molday, R.S., Leroux, M.R., Riddle, D.L., 2010. Localization of a Guanylyl Cyclase to Chemosensory Cilia Requires the Novel Ciliary MYND Domain Protein DAF-25. Plos Genet 6, e1001199.
Jensen, V.L., Li, C., Bowie, R.V., Clarke, L., Mohan, S., Blacque, O.E., Leroux, M.R., 2015. Formation of the transition zone by Mks5/Rpgrip1L establishes a ciliary zone of exclusion (CIZE) that compartmentalises ciliary signalling proteins and controls PIP2 ciliary abundance. Embo J 34, 2537–2556.
Joubert, S., Labrecque, J., Léan, A., 2001. Reduced Activity of the NPR-A Kinase Triggers Dephosphorylation and Homologous Desensitization of the Receptor. Biochemistry 40, 11096–11105.
Kim, K., Sato, K., Shibuya, M., Zeiger, D.M., Butcher, R.A., Ragains, J.R., Clardy, J., Touhara, K., Sengupta, P., 2009. Two Chemoreceptors Mediate Developmental Effects of Dauer Pheromone in *C. elegans*. Science 326, 994–998.
Klink, B.U., Zent, E., Juneja, P., Kuhlee, A., Raunser, S., Wittinghofer, A., 2017. A recombinant BBSome core complex and how it interacts with ciliary cargo. Elife 6, e27434.
Komatsu, H., Jin, Y.-H., L'Etoile, N., Mori, I., Bargmann, C.I., Akaike, N., Ohshima, Y., 1999. Functional reconstitution of a heteromeric cyclic nucleotide-gated channel of *Caenorhabditis elegans* in cultured cells. Brain Res 821, 160–168.
Komatsu, H., Mori, I., Rhee, J.-S., Akaike, N., Ohshima, Y., 1996. Mutations in a Cyclic Nucleotide–Gated Channel Lead to Abnormal Thermosensation and Chemosensation in *C. elegans*. Neuron 17, 707–718.
Korobkova, E., Emonet, T., Vilar, J.M.G., Shimizu, T.S., Cluzel, P., 2004. From molecular noise to behavioural variability in a single bacterium. Nature 428, 574 578.
Li, C., Jensen, V.L., Park, K., Kennedy, J., Garcia Gonzalo, F.R., Romani, M., Mori, R.D., Bruel, A.-L., Gaillard, D., Doray, B., Lopez, E., Rivière, J.-B., Faivre, L., Thauvin-Robinet, C., Reiter, J.F., Blacque, O.E., Valente, E.M., Leroux, M.R., 2016. MKS5 and CEP290 Dependent Assembly Pathway of the Ciliary Transition Zone. Plos Biol 14, e1002416.
Li, M., Zhou, X., Wang, S., Michailidis, I., Gong, Y., Su, D., Li, H., Li, X., Yang, J., 2017. Structure of a eukaryotic cyclic-nucleotide-gated channel. Nature 542, 60–65.
Liem, K.F., He, M., Ocbina, P.J.R., Anderson, K.V., 2009. Mouse Kif7/Costal2 is a cilia-associated protein that regulates Sonic hedgehog signaling. Proceedings of the National Academy of Sciences 106, 13377–13382.
Liu, L., Leonard, A.S., Motto, D.G., Feller, M.A., Price, M.P., Johnson, W.A., Welsh, M.J., 2003. Contribution of *Drosophila* DEG/ENaC Genes to Salt Taste. Neuron 39, 133 146.
Liu, P., Lechtreck, K.F., 2018. The Bardet–Biedl syndrome protein complex is an adapter expanding the cargo range of intraflagellar

transport trains for ciliary export. PNAS 121, 201713226 E943.

Martínez-Velázquez, L.A., Ringstad, N., 2018. Antagonistic regulation of trafficking to *Caenorhabditis elegans* sensory cilia by a Retinal Degeneration 3 homolog and retromer. Proc Natl Acad Sci 115, E438–E447.

Maruyama, I.N., 2017. Receptor Guanylyl Cyclases in Sensory Processing. Frontiers in Endocrinology 7, 173.

Mathot, K.J., Hout, P.J. van den, Piersma, T., Kempenaers, B., Réale, D., Dingemanse, N.J., 2011. Disentangling the roles of frequency-vs. state-dependence in generating individual differences in behavioural plasticity. Ecol Lett 14, 1254–62.

McGrath, P.T., Xu, Y., Ailion, M., Garrison, J.L., Butcher, R.A., Bargmann, C.I., 2011. Parallel evolution of domesticated *Caenorhabditis* species targets pheromone receptor genes. Nature 477, 321.

Ming, G., Song, H., 2005. Adult neurogenesis in the mammalian central nervous system. Annu Rev Neurosci 28, 223–250.

Mondal, S., Ahlawat, S., Rau, K., Venkataraman, V., Koushika, S.P., 2011. Imaging in vivo Neuronal Transport in Genetic Model Organisms Using Microfluidic Devices. Traffic 12, 372–385.

Mukhopadhyay, S., Lu, Y., Shaham, S., Sengupta, P., 2008. Sensory signaling-dependent remodeling of olfactory cilia architecture in *C. elegans*. Dev Cell 14, 762–74.

Munsky, B., Neuert, G., Oudenaarden, A. van, 2012. Using Gene Expression Noise to Understand Gene Regulation. Science 336, 183–187.

Murayama, T., Takayama, J., Fujiwara, M., Maruyama, I.N., 2013. Environmental Alkalinity Sensing Mediated by the Transmembrane Guanylyl Cyclase GCY-14 in *C. elegans*. Curr Biol 23, 1007–1012.

Nakayama, K., Katoh, Y., 2018. Ciliary protein trafficking mediated by IFT and BBSome complexes with the aid of kinesin-2 and dynein-2 motors. J Biochem 163, 155–164.

Ortiz, C.O., Etchberger, J.F., Posy, S.L., Frøkjær Jensen, C., Lockery, S., Honig, B., Hobert, O., 2006. Searching for Neuronal Left/Right Asymmetry: Genomewide Analysis of Nematode Receptor-Type Guanylyl Cyclases. Genetics 173, 131–149.

Ortiz, C.O., Faumont, S., Takayama, J., Ahmed, H.K., Goldsmith, A.D., Pocock, R., McCormick, K.E., Kunimoto, H., Iino, Y., Lockery, S., Hobert, O., 2009. Lateralized Gustatory Behavior of *C. elegans* Is Controlled by Specific Receptor-Type Guanylyl Cyclases. Curr Biol 19, 996–1004.

Ou, G., Blacque, O.E., Snow, J.J., Leroux, M.R., Scholey, J.M., 2005. Functional coordination of intraflagellar transport motors. Nature 436, 583–587.

Ozbudak, E.M., Thattai, M., Lim, H.N., Shraiman, B.I., Oudenaarden, A. van, 2004. Multistability in the lactose utilization network of Escherichia coli. Nature 427, 737–740.

Pan, X., Ou, G., Civelekoglu-Scholey, G., Blacque, O.E., Endres, N.F., Tao, L., Mogilner, A., Leroux, M.R., Vale, R.D., Scholey, J.M., 2006. Mechanism of transport of IFT particles in *C. elegans* cilia by the concerted action of kinesin-II and OSM-3 motors. J Cell Biology 174, 1035–1045.

Patel, T., Tursun, B., Rahe, D.P., Hobert, O., 2012. Removal of Polycomb Repressive Complex 2 Makes *C. elegans* Germ Cells Susceptible to Direct Conversion into Specific Somatic Cell Types. Cell Reports 2, 1178–1186.

Perkins, L.A., Hedgecock, E.M., Thomson, J.N., Culotti, J.G., 1986. Mutant sensory cilia in the nematode *Caenorhabditis elegans*. Dev Biol 117, 456–487.

Potter, L.R., Garbers, D.L., 1992. Dephosphorylation of the guanylyl cyclase-A receptor causes desensitization. J Biological Chem 267, 14531–4.

Prevo, B., Mangeol, P., Oswald, F., Scholey, J.M., Peterman, E.J.G., 2015. Functional differentiation of cooperating kinesin-2 motors orchestrates cargo import and transport in *C. elegans* cilia. Nat Cell Biol 17, 1536–1545.

Qin, H., Burnette, D.T., Bae, Y.-K., Forscher, P., Barr, M.M., Rosenbaum, J.L., 2005. Intraflagellar Transport Is Required for the Vectorial Movement of TRPV Channels in the Ciliary Membrane. Current Biology 15, 1695–1699.

Raghunathan, K., Kenworthy, A.K., 2018. Dynamic pattern generation in cell membranes: Current insights into membrane organization. Biochim Biophys Acta 1860, 2018–2031.

Reiter, J.F., Leroux, M.R., 2017. Genes and molecular pathways underpinning ciliopathies. Nat. Rev. Mol. Cell Biol. 18, 533.

Riechert, S.E., 1978. Games spiders play: Behavioral variability in territorial disputes. Behav Ecol Sociobiol 3, 135–162.

Sarin, S., Antonio, C., Tursun, B., Hobert, O., 2009. The *C. elegans* Tailless/TLX transcription factor *nhr-67* controls neuronal identity and left/right asymmetric fate diversification. Development 136, 2933–2944.

Schröter, J., Zahedi, R.P., Hartmann, M., Gaßner, B., Gazinski, A., Waschke, J., Sickmann, A., Kuhn, M., 2010. Homologous desensitization of guanylyl cyclase A, the receptor for atrial natriuretic peptide, is associated with a complex phosphorylation pattern. FEBS Journal 277, 2440–2453.

Shiba, D., Yamaoka, Y., Hagiwara, H., Takamatsu, T., Hamada, H., Yokoyama, T., 2009. Localization of Inv in a distinctive intraciliary compartment requires the C-terminal ninein-homolog-containing region. J Cell Sci 122, 44–54.

Shindou, T., Ochi-Shindou, M., Murayama, T., Saita, E., Momohara, Y., Wickens, J.R., Maruyama, I.N., 2019. Active propagation of dendritic electrical signals in *C. elegans*. Scientific Reports 9, 3430.

Smith, H.K., Luo, L., O'Halloran, D., Guo, D., Huang, X.-Y., Samuel, A.D., Hobert, O., 2013. Defining Specificity Determinants of cGMP Mediated Gustatory Sensory Transduction in *Caenorhabditis elegans*. Genetics 194, 885–901.

Snow, J.J., Ou, G., Gunnarson, A.L., Walker, M.R.S., Zhou, H.M., Brust-Mascher, I., Scholey, J.M., 2004. Two anterograde intraflagellar transport motors cooperate to build sensory cilia on *C. elegans* neurons. Nat Cell Biol 6, 1109–1113.

Spalding, K.L., Bhardwaj, R.D., Buchholz, B.A., Druid, H., Frisén, J., 2005. Retrospective Birth Dating of Cells in Humans. Cell 122, 133–143.

Süel, G.M., Garcia-Ojalvo, J., Liberman, L.M., Elowitz, M.B., 2006. An excitable gene regulatory circuit induces transient cellular differentiation. Nature 440, 545–550.

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

Svenningsen, S.L., Costantino, N., Court, D.L., Adhya, S., 2005. On the role of Cro in prophage induction. Proc National Acad Sci 102, 4465–4469.

Tursun, B., Patel, T., Kratsios, P., Hobert, O., 2011. Direct conversion of *C. elegans* germ cells into specific neuron types. Science 331, 304–8.

Uchida, O., Nakano, H., Koga, M., Ohshima, Y., 2003. The *C. elegans che-1* gene encodes a zinc finger transcription factor required for specification of the ASE chemosensory neurons. Development 130, 1215–1224.

Vandenbeuch, A., Clapp, T.R., Kinnamon, S.C., 2008. Amiloride-sensitive channels in type I fungiform taste cells in mouse. BMC Neurosci 9, 1.

Warburton-Pitt, S.R.F., Silva, M., Nguyen, K.C.Q., Hall, D.H., Barr, M.M., 2014. The *nphp-2* and *arl-13* Genetic Modules Interact to Regulate Ciliogenesis and Ciliary Microtubule Patterning in *C. elegans*. PLoS Genetics 10, e1004866.

Ward, S., 1973. Chemotaxis by the Nematode *Caenorhabditis elegans*: Identification of Attractants and Analysis of the Response by Use of Mutants. Proc National Acad Sci 70, 817–821.

Ward, S., Thomson, N., White, J.G., Brenner, S., 1975. Electron microscopical reconstruction of the anterior sensory anatomy of the nematode *Caenorhabditis elegans*. J Comp Neurology 160, 313–337.

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

Williams, C.L., Li, C., Kida, K., Inglis, P.N., Mohan, S., Semenec, L., Bialas, N.J., Stupay, R.M., Chen, N., Blacque, O.E., Yoder, B.K., Leroux, M.R., 2011. MKS and NPHP modules cooperate to establish basal body/transition zone membrane associations and ciliary gate function during ciliogenesis. J Cell Biology 192, 1023–1041.

Wojtyniak, M., Brear, A.G., O'Halloran, D.M., Sengupta, P., 2013. Cell- and subunit-specific mechanisms of CNG channel ciliary trafficking and localization in *C. elegans*. J Cell Sci 126, 4381–95.

Zhang, Z., Zhang, T., Wang, S., Gong, Z., Tang, C., Chen, J., Ding, J., 2014. Molecular mechanism for Rabex-5 GEF activation by Rabaptin-5. Elife 3, e02687.