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Regulation of gonadotropin receptor gene expression

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Summary

The receptors for the gonadotropins differ from the other G protein-coupled receptors by having a large extracellular hormone-binding domain, encoded by nine or ten exons. Alternative splicing of the large pre-mRNA of approximately 100 kb can result in mRNA species that encode truncated receptor proteins. In this review we discuss the regulation of gonadotropin receptor mRNA expression and the possible roles of alternative splicing in gonadotropin receptor function.

Introduction

In less than a decade, starting with the cloning of the β_2-adrenergic receptor (Dixon et al., 1986), it has become apparent that all receptors that couple to the production of intracellular second messengers through GTP-binding proteins are members of a large gene family, with more than 100 members (Savarese and Fraser, 1992). The receptors of this superfamily are characterised by a hydrophobic domain that includes seven transmembrane segments. With the isolation of the cDNAs encoding the LH/CG, TSH and FSH receptors (McFarland et al., 1989; Parmentier et al., 1989; Sprengel et al., 1990), a distinct subfamily of this superfamily manifested itself. The binding domain for the large dimeric hormone molecules is encoded by the first nine or ten exons of the respective genes, followed by a last exon that encodes the transmembrane and G protein-coupling domain. This domain consists of seven transmembrane segments connected by three extra- and three intracellular loops and ends in a cytoplasmic tail. This gene structure allows for alternative splicing of the primary transcripts, which is not possible for the transcripts encoding the other G protein-coupled receptors, because almost all other members of the receptor superfamily are encoded by a single exon.

We will focus on the regulation of the expression of the gonadotropin receptor genes, emphasizing the possible role of alternative splicing of primary transcripts the expression and function of the receptors.

The gonadotropin receptors

The gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are members of a distinct family of glycoprotein hormones, which also includes thyroid-stimulating hormone (TSH) and chorionic gonadotropin (CG). Synthesized in the pituitary gland, or in the placenta in case of CG, these hormones consist of a common α-subunit and a hormone-specific β-subunit, both of which are glycosylated (Gharib et al., 1990). The β-subunit is the major determinant of the hormone specificity of receptor binding.

Cloning of the gonadotropin receptors revealed that they are members of the G protein-coupled receptor family (Loosfelt et al., 1989; McFarland et al., 1989; Minegish et al., 1991; Sprengel et al., 1990). They share the transmembrane/intracellular domain with the many other G protein-coupled receptors, but in addition possess a large extracellular domain which is responsible for most, if not all, aspects of hormone

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Abbreviations: LH: luteinizing hormone, lutropin; FSH: follicle-stimulating hormone, follitropin; TSH: thyroid-stimulating hormone, thyrotropin; CG: chorionic gonadotropin.

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binding and specificity of the receptor. In particular the presence of this extracellular domain places the LH/CG and FSH receptors in a distinct three-membered subfamily, together with the TSH receptor which will not be discussed here. Most of the 100 or so G protein-coupled receptor genes now known consist of only a single exon containing all necessary coding information: the open reading frame and the 5'- and 3'-untranslated regions. In the case of the gonadotropin receptors, however, the receptor is encoded by ten or eleven exons separated by large introns, resulting in a large gene of about 100 kb. The extracellular binding domain is encoded by the first nine (FSH receptor) or ten (LH receptor) exons, but the transmembrane domain still is encoded by a single exon, numbered 10 or 11 (Heckert et al., 1992; Koo et al., 1991; Tsai-Morris et al., 1991).

Regulation

FSH and LH receptors are expressed mainly if not exclusively in the gonads. There is little information available about the DNA elements in the receptor genes that confer this marked tissue specificity, although consensus response elements for growth factors, second messengers and steroid hormones are present in the 5'-flanking regions of the LH and FSH receptor genes (Heckert et al., 1992; Wang et al., 1992). Within the gonads, the receptors are expressed in distinct cell types: in the testis, FSH receptors can only be found in Sertoli cells (Heckert and Griswold, 1991), whereas LH receptors are expressed in the Leydig cells (Segaloff and Ascoli, 1993). In the ovary the situation is slightly more complex: FSH receptors are found in the granulosa cells, while LH receptors are present in both granulosa cells and theca cell layers (Camp et al., 1991).

There is some information available about the expression of LH and FSH receptors in the fetal and postnatal gonads. During testis development in immature rats, LH receptor mRNA expression in the testis increases continuously, both as a fraction of total RNA and per total testis RNA until a constant level is reached in adult animals (Vihko et al., 1992). FSH receptor mRNA level per Sertoli cell decreases from birth until the rats are 21 days of age, when Sertoli cells have stopped dividing. Thereafter, total testicular FSH receptor mRNA expression does not change (our unpublished observations). However, in the adult testis several components of the FSH signaling pathway are clearly regulated: different levels of FSH receptor mRNA expression, FSH-binding, FSH-dependent cAMP production, and CRE-binding protein mRNA expression in Sertoli cells were found to be associated with different stages of the cycle of the spermatogenic epithelium (Heckert and Griswold, 1991; Kliesch et al., 1992; Parvinen, 1993).

In the ovary, some LH receptor mRNA species appear to be constantly expressed during fetal life, although mRNAs that encode functional receptor molecules are expressed starting from day 7 of postnatal life only – see discussion of splice variants below (Sokka et al., 1992).

Hormonal regulation of expression of gonadotropin receptors has been studied both in vivo and in vitro, in cell cultures. In the testis, there are differential effects of hormone administration on LH receptor mRNA levels in fetal/neonatal versus adult rats: hCG treatment of intact animals resulted in up-regulation of LH receptor mRNA expression in fetal/neonatal testis, but in down-regulation in adult testis (Pakarinen et al., 1990). In the mouse Leydig tumour cell line MA-10, treatment of the cells with epidermal growth factor, phorbol ester (activation of protein kinase C) or with hCG or 8-Br-cAMP resulted in rapid loss of LH receptor mRNA (Segaloff and Ascoli, 1993; Wang et al., 1991b; Wang et al., 1991c). This loss was not a result of decreased stability of LH receptor mRNA induced by any of the compounds that were used, but rather was caused by a decrease in LH receptor gene transcription rate (Nelson and Ascoli, 1992). FSH receptor mRNA expression in Sertoli cells in vivo also is down-regulated rapidly by its homologous hormone, or by FSH, dibutyryl-cAMP or forskolin in cultured Sertoli cells (Themmen et al., 1991). However, in this case no decrease in the rate of FSH receptor gene transcription could be found, and it was inferred that FSH receptor mRNA stability is decreased by FSH action.

In the ovary, studies of hormone effects on gonadotropin receptor regulation are confounded by the fact that both LH and FSH receptors are expressed in the same cell type, the granulosa cells. In cultured granulosa cells isolated from estrogen-primed rats, both LH and FSH up-regulate their respective receptor mRNAs (Piquette et al., 1991; Tilly et al., 1992). When follicular growth is induced in immature female rats by treatment with pregnant mare serum gonadotropin, LH receptor mRNA expression in the growing follicles is increased, presumably through activation of FSH receptors. This action can be mimicked in vitro, by incubation of cultured granulosa cells with FSH (Piquette et al., 1991). Immediately after the ovulatory LH peak, either in intact cycling rats or when mimicked by injection with hCG, both FSH and LH receptor mRNA levels are decreased significantly (Camp et al., 1991). With the appearance of corpora lutea LH receptor mRNA expression again increases (LaPolt et al., 1990).

Thus, both in the testis and ovary, mechanisms exist that result in either up- or down-regulation of gonadotropin receptor mRNA expression, depending on

cyclic changes or on the developmental stage of the gonads. It must be kept in mind that short-term changes in mRNA expression may not cause changes in receptor protein levels, independent of ligand-induced receptor internalization. This is illustrated by the down-regulation of FSH receptor mRNA expression with dibutyryl-cAMP in cultured Sertoli cells, where disappearance of mRNA was not immediately followed by a decrease in hormone-binding (Themmen et al., 1991). Furthermore, the in vitro conditions under which cultured cells are treated with relatively high concentrations of hormone can result in an exaggerated and unchecked response. However, the responses of cells in vivo, such as for example the responses of Sertoli cells in developing testis or granulosa cells in growing follicles to FSH, probably occur under conditions of moderate stimulation (sub ED_{50}). They may be kept in check by paracrine mechanisms.

Multiple mRNA species and splice variants

Northern blot analysis of LH receptor mRNAs has resulted in the detection of several mRNAs with distinct sizes. LH receptor cDNA probes revealed at least four LH receptor mRNA species: 6.5, 4.3, 2.6 and 1.2 kb (Wang et al., 1991a). Similar sizes were reported by other investigators (Camp et al., 1991; Piquette et al., 1991). These mRNA species can be differentially regulated. In the developing rat testis, the smaller transcripts were apparent at five days after birth, whereas all mRNA species were present from day 25 onwards (Vihko et al., 1992). Treatment of pseudopregnant rats with hCG resulted in down-regulation of all detectable LH receptor mRNA species. However, in the testis of hCG-treated rats, the 1.2 kb mRNA persisted, while the other mRNAs were down-regulated (LaPolt et al., 1991). Reprobing the same Northern blot with a transmembrane/intracellular domain-specific probe revealed that this 1.2 kb mRNA did not contain a portion of the transmembrane/intracellular domain (LaPolt et al., 1991). Possibly, the 1.2 kb LH receptor mRNA encodes a truncated receptor protein (see below).

The existence of aberrant mRNA species should not be surprising. Aberrant expression could result from

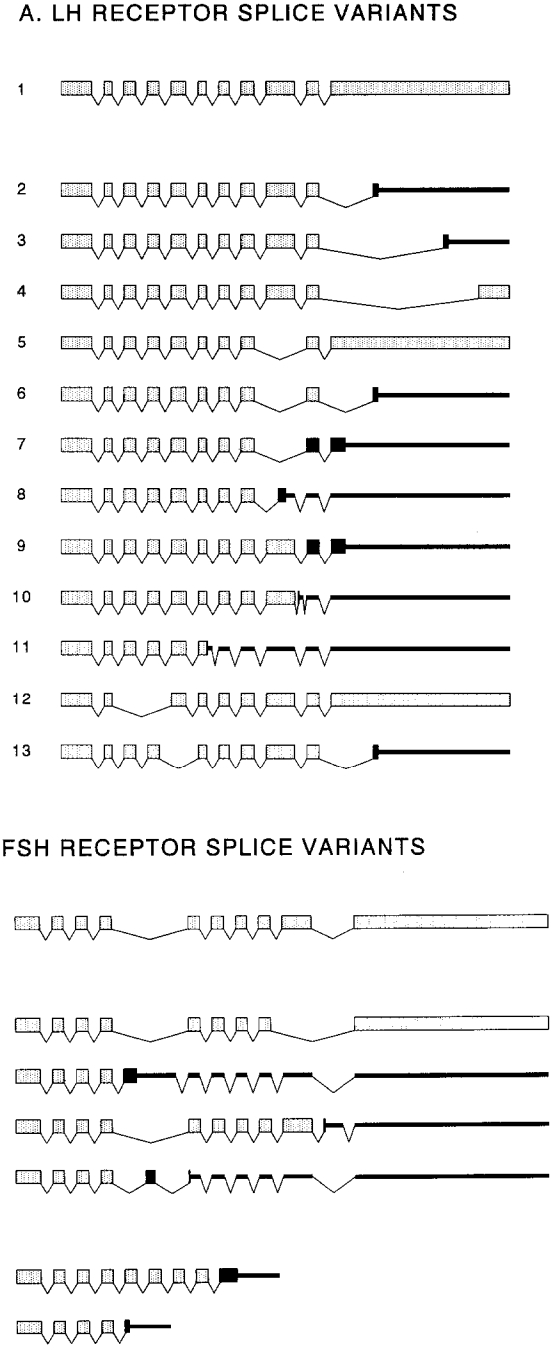

Fig. 1. LH receptor (A) and FSH receptor (B) gene splice variants. Stippled box: original open reading frame; black box: translated intron or exon sequences with a different reading frame; black line: untranslated exon or intron sequences. (A) 1: full-length LH receptor; 2: porcine LHR-B (Loosfelt et al., 1989), rat LHR-1234 (Aatsinki et al., 1992), rat LHR-B (Bernard et al., 1990), rat LHR-B4 (Segaloff et al., 1990; Tsai-Morris et al., 1990); 3: porcine LHR-C (Loosfelt et al., 1989); 4: porcine LHR-D (Loosfelt et al., 1989); 5: rat LHR-E (Bernard et al., 1990), rat LHR-A2 (Segaloff et al., 1990); 6: rat LHR-EB (Bernard et al., 1990); 7: rat LHR-B1 (Segaloff et al., 1990); 8: rat LHR-B2 (Segaloff et al., 1990), rat LHR-2075 (Aatsinki et al., 1992); 9: rat LHR-B3 (Segaloff et al., 1990); 10: rat LHR-C1 (Segaloff et al., 1990); 11: rat LHR-C2 (Segaloff et al., 1990); 12: rat LHR-1950 (Aatsinki et al., 1992); 13: rat LHR-1759 (Aatsinki et al., 1992). (B) 1: full-length FSH receptor; 2: human FSHR-E (Gromoll et al., 1992); 3: human FSHR-5–10 (Kelton et al., 1992); 4: rat FSHR-1 (Kraaij et al., unpublished observations); 5: rat FSHR-2 (Kraaij et al., unpublished observations); 6: ovine FSHR-HK18 (Khan et al., 1993); 7: ovine FSHR-151A1 (Khan et al., 1993). 6 and 7 are the result of both alternative splicing and alternative polyadenylation.

mistakes made by the splicing machinery during the conversion of the long primary transcript (100 kb) to the mature mRNA. Indeed, when the LH receptor cDNA was first reported, investigators showed cDNA variants that are probably the result of alternative splicing (McFarland et al., 1989). The list of LH receptor mRNA splice variants is growing (see Fig. 1A). Some of the variants have been found when cDNA libraries were screened for the full-length receptor cDNAs, while other research groups have used reverse transcriptase-polymerase chain reaction strategies in a more targeted effort to find other divergent LH receptor mRNAs (Aatsinki et al., 1992).

Many of the gonadotropin receptor splice variants encode at least a partially intact hormone-binding domain. Indeed, translation of a cloned cDNA resulted in a truncated protein without a transmembrane/intracellular domain, but which was able to bind hCG (Tsai-Morris et al., 1990). Similar observations have been reported for pig LH receptor cDNAs (VuHai-LuuThi et al., 1992). The latter authors reported secretion of a portion of the truncated LH receptor molecules, but other investigators studying similarly truncated cDNAs have not confirmed this observation (Ji and Ji, 1991; Xie et al., 1990). Actually, there are no indications that mRNAs encoding truncated, but hormone-binding, receptors are expressed at significant levels. If they are, these mRNAs may not be translated under physiological conditions. In the ovary, Sokka et al. (1992) have shown the existence of alternatively spliced LH receptor transcripts missing the transmembrane/intracellular domain encoding exon. They propose that the expression of these transcripts represents a default pathway which may have a physiological role in the developmental regulation of LH receptor expression in the ovary. This hypothesis implies that the LH receptor gene is constantly turned on, but complete mRNAs encoding intact LH receptor molecules are generated not until after day 7 of neonatal life, in the rat. However, the expression of the splice variants is very low at all stages of development (Sokka et al., 1992). Further information on the expression and translation of truncated LH receptor mRNAs is eagerly awaited, to try to clarify the possible role of truncated receptor proteins in the regulation of physiological responses to LH.

With regard to mRNA splice variants of the FSH receptor, similar results have been obtained (Fig. 1B). Several splice variants have been identified (Gromoll et al., 1992; Kelton et al., 1992; Khan et al., 1993). We also have cloned two FSH receptor mRNA splice variants from rat Sertoli cells (Fig. 1B), and studied their expression in testis and ovary using transcript-specific RNase protection probes. At different developmental stages (testis) and stages of the cycle (ovary), expression of the splice variants closely followed expression of the full-length FSH receptor mRNA, as indicated by a constant ratio of splice variant over intact mRNA levels (Kraaij et al., unpublished observations).

Receptor mRNAs encoding truncated receptor forms without a transmembrane/intracellular domain are not limited to the gonadotropin receptors. They have also been found for the TSH, IL-4 and IL-7 receptor genes (Goodwin et al., 1990; Mosley et al., 1989; Pleiman et al., 1991; Takeshita et al., 1992). Yet, the possible function of expressed splice variants remains an enigma. If secreted, soluble receptor ligand-binding domains might act as inhibitors of hormone action by sequestering the ligand away from the full-length receptor. In this respect it is of interest that soluble IL-4 receptors, when transfected into cell lines, are secreted and inhibit the IL-4 effects that require the full-length receptor (Mosley et al., 1989). For the growth hormone receptor gene, a circulating growth hormone-binding protein has been identified in mouse and rat, that is encoded by an alternatively spliced receptor mRNA (Baumbach et al., 1989; Smith and Talamantes, 1987). In contrast to such inhibitory effects, a free ligand-binding domain of truncated receptors might act to present the hormone to the receptor resulting in a more efficient signal. Other mechanisms, such as an intracellular role of non-secreted truncated receptor, cannot be excluded.

Recently, two studies were reported which point to interaction between full-length LH receptors and truncated forms. Remy et al. (1993) showed that co-expression of the extracellular binding domain and the transmembrane/intracellular domain resulted in reconstitution of a high-affinity LH receptor, which was able to bind hCG and to transduce the hormonal signal to adenylyl cyclase. Although the mechanism is unclear, this observation would seem to suggest that domains of the receptor can function and cooperate as separate entities. Another intriguing observation was made in a study using co-expression of full-length and truncated LH receptor cDNAs (VuHai-LuuThi et al., 1992). Expression of cDNA lacking the transmembrane/intracellular domain in transfected COS cells resulted in high-affinity binding, but no stimulation of cAMP production. However, co-expression of both full-length and truncated receptors resulted in enhanced stimulation of adenylyl cyclase by hCG. This enhancement was the result of an increase in the maximal hormone-induced cAMP level, and not from a decrease in the ED_{50} for hCG, invalidating a model in which hormone is presented to the intact receptor by a soluble truncated form.

The cloning of the gonadotropin receptors has provided the tools to study the molecular regulation of receptor expression. The finding of splice variants has left us with many intriguing questions, but also may open up new frontiers concerning the regulation of hormonal transmembrane signaling.

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References

Aatsinki, J.T., Pietila, E.M., Lakkakorpi, J.T. and Rajaniemi, H.J. (1992) Mol. Cell. Endocrinol. 84, 127–135.

Baumbach, W.R., Horner, D.I. and Logan, J.S. (1989) Genes Dev. 3, 1199–1205.

Bernard, M.P., Myers, R.V. and Moyle, W.R. (1990) Mol. Cell. Endocrinol. 71, 19–23.

Camp, T.A., Rahal, J.O. and Mayo, K.E. (1991) Mol. Endocrinol. 5, 1405–1417.

Dixon, R.A., Kobilka, B.K., Strader, D.J., Benovic, J.L., Dohlman, H.G., Frielle, T., Bolanowski, M.A., Bennett, C.D., Rands, E., Diehl, R.E., Mumford, R.A., Slater, E.E., Sigal, I.S., Caron, M.G., Lefkowitz, R.J. and Strader, C.D. (1986) Nature 321, 75–79.

Gharib, S.D., Wierman, M.E., Shupnik, M.A. and Chin, W.W. (1990) Endocr. Rev. 11, 177–199.

Goodwin, R.G., Friend, D., Ziegler, S.F., Jerzy, R., Falk, B.A., Gimpel, S., Cosman, D., Dower, S.K., March, C.J., Namen, A.E. and Park, L.S. (1990) Cell 60, 941–951.

Gromoll, J., Gudermann, T. and Nieschlag, E. (1992) Biochem. Biophys. Res. Commun. 188, 1077–1083.

Heckert, L.L. and Griswold, M.D. (1991) Mol. Endocrinol. 5, 670–677.

Heckert, L.L., Daley, I.J. and Griswold, M.D. (1992) Mol. Endocrinol. 6, 70–80.

Ji, I. and Ji, T. (1991) Endocrinology 128, 2648–2650.

Kelton, C.A., Cheng, S.V., Nugent, N.P., Schweickhardt, R.L., Rosenthal, J.L., Overton, S.A., Wands, G.D., Kuzeja, J.B., Luchette, C.A. and Chappel, S.C. (1992) Mol. Cell. Endocrinol. 89, 141–151.

Khan, H., Yarney, T.A. and Sairam, M.R. (1993) Biochem. Biophys. Res. Commun. 190, 888–894.

Kliesch, S., Penttila, T.L., Gromoll, J., Saunders, P.T., Nieschlag, E. and Parvinen, M. (1992) Mol. Cell. Endocrinol. 84, 45–49.

Koo, Y.B., Ji, I., Slaughter, R.G. and Ji, T.H. (1991) Endocrinology 128, 2297–2308.

LaPolt, P.S., Oikawa, M., Jia, X.C., Dargan, C. and Hsueh, A.J. (1990) Endocrinology 126, 3277–3279.

LaPolt, P.S., Jia, X.C., Sincich, C. and Hsueh, A.J. (1991) Mol. Endocrinol. 5, 397–403.

Loosfelt, H., Misrahi, M., Atger, M., Salesse, R., Vu Hai Luu Thi, M.T., Jolivet, A., Guiochon Mantel, A., Sar, S., Jallal, B., Garnier, J. and Milgrom, E. (1989) Science 245, 525–528.

McFarland, K.C., Sprengel, R., Phillips, H.S., Kohler, M., Rosemblit, N., Nikolics, K., Segaloff, D.L. and Seeburg, P.H. (1989) Science 245, 494–499.

Minegish, T., Nakamura, K., Takakura, Y., Ibuki, Y. and Igarashi, M. (1991) Biochem. Biophys. Res. Commun. 175, 1125–1130.

Mosley, B., Beckmann, M.P., March, C.J., Idzerda, R.L., Gimpel, S.D., VandenBos, T., Friend, D., Alpert, A., Anderson, D., Jackson, J., Wignall, J.M., Smith, C., Gallis, B., Sims, J.E., Urdal, D., Widmer, M.B., Cosman, D. and Park, L.S. (1989) Cell 59, 335–348.

Nelson, S. and Ascoli, M. (1992) Endocrinology 131, 615–620.

Pakarinen, P., Vihko, K.K., Voutilainen, R. and Huhtaniemi, I. (1990) Endocrinology. 127, 2469–2474.

Parmentier, M., Libert, F., Maenhaut, C., Lefort, A., Gerard, C., Perret, J., VanSande, J., Dumont, J.E. and Vassart, G. (1989) Science 246, 1620–1622.

Parvinen, M. (1993) In The Sertoli cell (Russell, L.D. and Griswold, M.D., eds.), pp. 331–348, Cache River Press, Clearwater, FL, U.S.A.

Piquette, G.N., LaPolt, P.S., Oikawa, M. and Hsueh, A.J. (1991) Endocrinology 128, 2449–2456.

Pleiman, C.M., Gimpel, S.D., Park, L.S., Harada, H., Taniguchi, T. and Ziegler, S.F. (1991) Mol. Cell. Biol. 11, 3052–3059.

Remy, J.J., Bozon, V., Couture, L., Goxe, B., Salesse, R. and Garnier, J. (1993) Biochem. Biophys. Res. Commun. 193, 1023–1030.

Savarese, T.M. and Fraser, C.M. (1992) Biochem. J. 283, 1–19.

Segaloff, D.L., Sprengel, R., Nikolics, K. and Ascoli, M. (1990) Recent Prog. Horm. Res. 46, 261–301.

Segaloff, D.L. and Ascoli, M. (1993) Endocr. Rev. 14, 324–347.

Smith, W.C. and Talamantes, L.F. (1987) J. Biol. Chem. 262, 2213–2219.

Sokka, T., Hamalainen, T. and Huhtaniemi, L. (1992) Endocrinology 130, 1738–1740.

Sprengel, R., Braun, T., Nikolics, K., Segaloff, D.L. and Seeburg, P.H. (1990) Mol. Endocrinol. 4, 525–530.

Takeshita, A., Nagayama, Y., Fujiyama, K., Yokoyama, N., Namba, H., Yamashita, S., Izumi, M. and Nagataki, S. (1992) Biochem. Biophys. Res. Commun. 188, 1214–1219.

Themmen, A.P.N, Blok, L.J., Post, M., Baarends, W.M., Hoogerbrugge, J.W., Parmentier, M., Vassart, G. and Grootegoed, J.A. (1991) Mol. Cell. Endocrinol. 78, R7–R13.

Tilly, J.L., LaPolt, P.S. and Hsueh, A.J. (1992) Endocrinology 130, 1296–1302.

Tsai-Morris, C.H., Buczko, E., Wang, W. and Dufau, M.L. (1990) J. Biol. Chem. 265, 19385–19388.

Tsai-Morris, C.H., Buczko, E., Wang, W., Xie, X.Z. and Dufau, M.L. (1991) J. Biol. Chem. 266, 11355–11359.

Vihko, K.K., Nishimori, K., LaPolt, P.S. and Hsueh, A.J.W. (1992) Biol. Reprod. 46, 1016–1020.

VuHai-LuuThi, M.T., Misrahi, M., Houllier, A., Jolivet, A. and Milgrom, E. (1992) Biochemistry 31, 8377–8383.

Wang, H., Ascoli, M. and Segaloff, D.L. (1991a) Endocrinology 129, 133–138.

Wang, H., Segaloff, D.L. and Ascoli, M. (1991b) Endocrinology 128, 2651–2653.

Wang, H., Segaloff, D.L. and Ascoli, M. (1991c) J. Biol. Chem. 266, 780–785.

Wang, H., Nelson, S., Ascoli, M. and Segaloff, D.L. (1992) Mol. Endocrinol. 6, 320–326.

Xie, Y.B., Wang, H. and Segaloff, D.L. (1990) J. Biol. Chem. 265, 21411–21414.