Orphan nuclear receptor regulation of reproduction

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Abstract

Orphan nuclear receptors, those without known ligands, were discovered because of their structural similarity to the ligand-driven steroid and thyroid receptors. Since their characterization, many of the orphan receptors have been adopted, i.e., ligands, usually lipids or derived lipids, have been discovered. The orphan receptors are transcriptional regulators, functioning in the reproductive context to upregulate or suppress gene expression. By this means, the orphan receptors regulate a plethora of reproductive events. In the majority of cases, the effects are stimulatory, indeed, members of the NR2 family promote Leydig cell differentiation and testicular steroidogenesis, while those of the NR4 family regulate early gestation and placental formation. The NR5 family has two members, steroidogenic factor-1 (SF-1, NR5A1) and liver receptor homolog-1 (LRH-1, NR5A2). These receptors interact with the same DNA sequence and are believed to be constitutive transcription factors. Their effects are modulated by the repressive effects of the NR0 family of orphan receptors that comprise the short heterodimeric partner (SHP, NR0B2) and dosage-sensitive sex reversal adrenal hypoplasia congenital region on the X chromosome, gene 1 (DAX1, NROB1). SHP and DAX1 inhibit the interaction of LRH-1 and SF-1 with coactivators, thereby reducing their constitutive transcriptional effects. Overall, the orphan nuclear receptors are essential regulators of reproductive function in mammals.

Keywords: NR5A1, NR5A2, orphan nuclear receptor, ovary, ovulation, testis.

Introduction

The nuclear receptors are a superfamily of protein transcription factors and cofactors defined by similarity in structure of a series of their functional domains. The first indication of their presence came from the work of Elwood Jensen, who isolated the estrogen receptor in 1961 (Jensen *et al.*, 2009). The two domains that were first defined as hallmarks of the family were the conserved zinc finger-like DNA binding domain and a ligand binding domain (Mangelsdorf *et al.*, 1995). Subsequent investigations revealed the presence of domains now known to interact with the cofactors that modulate the transcriptional activity of the nuclear receptors (Steinmetz *et al.*, 2001). Nuclear receptors have been classified into six phylogenetic families, based on structural characteristics. They have further been divided on functional grounds into the receptors for which the ligand is known (e.g. ESR1 or estrogen receptor-α, ERα, PRG, receptors for progesterone and AR, the androgen receptors) and the so-called orphan nuclear receptors. The latter are further subdivided into those with no known ligands and those for which ligands were discovered after the characterization of the receptor (the adopted nuclear receptors; Hummasti and Tontonoz, 2008). Target gene studies and mouse gene deletion models have revealed that a number of orphan nuclear receptors influence reproductive processes. Some are described in this discussion, with emphasis on two that have essential roles in the ovary, steroidogenic factor-1 (NR5A1, hereafter designated SF-1) and liver receptor homolog-1 (NR5A2, LRH-1).

Several orphan nuclear receptors influence reproduction

The estrogen-related receptors, ERRα, ERRß and ERRγ (NR3B1, 2 and 3, respectively) show structural similarity to the estrogen receptors, but have no known ligand (Villena and Kralli, 2008). Surprisingly, although they interact with the estrogen response element in target genes, they appear to play very different from than do the estrogens in reproduction. Null mutation of ERRα has no reproductive consequences (Villena and Kralli, 2008), while ERRß knockout results in infertility due to anomalies in the fetal component of the placenta, specifically, the overabundance of the trophoblast giant cells derived from the fetal chorion cells (Luo *et al.*, 1997). ERRγ is an important regulator of cardiac function (Alaynick *et al.*, 2007), while its effect on reproduction remains to be defined.

The NR2F family comprises three orphan receptors, the chicken ovalbumin upstream promoter-transcription factors (COUP-TFI or NR2F1 and COUP-TFII or NR2F2) and the V-erb related gene (EAR-2, NR2F6). Knockout strategies indicate that deletion of either of the COUP-TF genes results in abnormal fetal development. The conceptuses of mice bearing deletion of COUP-TFII are lost prior to day 10.5 of gestation, due to cardiovascular defects (Pereira *et al.*, 1999). Tissue specific knockout of COUP-TFII in the testis interdicts Leydig cell differentiation, testosterone synthesis, and thus results in hypogonadism and infertility (Qin *et al.*, 2008). Deletion of the gene in the uterus impairs placental function, with a phenotype of trophoblast giant

cell overgrowth and embryo lethality at day 10.5 (Petit *et al.*, 2007), remarkably similar to that seen with ERRß. More recent investigation indicates that the placental problems are related to over expression of ERα and consequent effects of estrogen on implantation and decidualization (Lee *et al.*, 2010). Indeed, rescue of implantation and decidualization could be achieved by pharmacological blockade of the estrogen receptors, although no pups were born, indicating that uterine COUP-TFII is necessary for successful postimplantation gestation (Lee *et al.*, 2010).

Orphan receptors of subfamily 4, NR4A1 (Nerve growth factor-1B, NGF1B), NR4A2 (Nuclear receptor related-1 or NURR1) and NR4A3 (Neuron-derived orphan receptor-1 or NOR1) are all strongly expressed in granulosa cells following the ovulatory signal (Park *et al.*, 2003). NURR1 regulates steroidogenic enzyme expression (Havelock *et al.*, 2005), and appears involved in gonadotropin secretion (Hamid *et al.*, 2008) and Leydig cell function (Martin and Tremblay, 2010). While ablation of NURR1 appears to have no reproductive consequences (Zetterstrom *et al.*, 1997), deletion of NOR1 results in embryo death during gastrulation and early implantation (DeYoung *et al.*, 2003). Similarly, NGF1 null mutation does not prevent successful reproduction (Lee *et al.*, 1995), but has been shown to regulate steroidogenesis (Li *et al.*, 2010). It remains possible that members of this subfamily of orphan receptors are redundant (Martin and Tremblay, 2010), requiring double knockout to establish their significance to reproduction.

The NR5 orphan receptors

Studies by the late Keith Parker of the promoter regions of a number of the P450 steroid hydrolylases revealed common motifs, and his consequent experiments demonstrated the presence of a cellular protein that regulated the expression of these genes (Parker and Schimmer, 1997). The cDNA that was cloned coded for the protein is now known as SF-1 (NR5A1), and it proved to be the mammalian ortholog of the *Drosophila* Fushi tarazu factor-1 (Lala *et al.*, 1992). A second factor, now known as LRH-1 (NR5A2), related to the Ftz-F1 gene, was later shown to interact with the same DNA sequence (Fayard *et al.*, 2004). Since the first identification of these two factors, numerous studies have demonstrated that these two nuclear receptors of Subfamily 5 are essential for the success of diverse reproductive processes in mammals.

SF-1: an essential regulator at multiple levels

Functionally, SF-1 coordinates endocrine tissue development, male sexual differentiation and controls steroid biosynthesis (Parker and Schimmer, 1997). SF-1 has a central role in regulating adrenal development, gonadal sex determination, and hypothalamic-pituitary control of reproduction and metabolism. In mice and in humans, SF-1 is expressed in a large number of tissues, including the hypothalamus, pituitary, adrenal, testis and ovary, and plays a role in the developing adrenal gland and gonad (Lin and Achermann, 2008). This nuclear receptor is a master controller of the tissue-specific gene expression of reproductive and steroidogenic enzyme-encoding genes, regulating the expression of the genes *STAR*, *HSD3B2*, *CYP19A1*, *CYP17A1*, *CYP11A1* (Parker *et al.*, 2002) and, in luteinizing bovine theca cells, the insulin-like factor 3 (*INSL3*; Walther *et al.*, 2006). Its expression in the ovary is associated with growth of antral follicles. It is found in both granulosa and theca cells, is strongest in the theca compartment, and is maintained in the corpus luteum and regressing in the corpora albicans (Parker and Schimmer, 1997; Hinshelwood *et al.*, 2005).

The generation of germline knockout of SF-1 mice some years ago demonstrated that this protein was required for development of both the adrenal cortex and gonads (Luo *et al.*, 1994). Although there was some evidence of early urogenital ridge formation in these mice, the adrenocortical and gonadal progenitor cells rapidly regressed through apoptosis. The mice died within 1 week after birth due to severe adrenal insufficiency, but could be rescued by administration of exogenous steroids. Subsequent studies demonstrated that SF-1 knockout mice have abnormal development of the ventromedial nucleus in the hypothalamus, reduced gonadotropin secretion and reduced spleen size (Lin and Achermann, 2008).

Given the early demise of the SF-1 germline knockout mice, further studies required the development of a time and/or tissue specific model. Insertion of loxP sites 5' and 3' of the last exon of the mouse SF-1 gene set the stage for conditional inactivation of the gene using Cre/lox strategies (Jeyasuria *et al.*, 2004). In the first of these, knockout specific to granulosa cells in the ovary resulted in aberrations in follicle development and an anovulatory condition (Jeyasuria *et al.*, 2004). Further investigation revealed that the follicular defect was characterized by compromised granulosa cell proliferation and decreased expression of steroidogenic enzymes (Pelusi *et al.*, 2008). The animals could nonetheless ovulate, when stimulated with exogenous gonadotropins, albeit less efficiently than their wildtype counterparts (Pelusi *et al.*, 2008), pointing to phenotypic disruption of neural and hypophyseal processes. NR5A1 plays a role in mammalian sex differentiation. In Sertoli cells, NR5A1 activates expression of Müllerian inhibiting substance (MIS; AMH, anti-müllerian hormone), with the consequence of regression of Müllerian (paramesonephric) structures in the developing male fetus. The deletion of this gene leads to a persistent Müllerian structures in males and a female phenotype (Lin and Achermann, 2008). By activating the expression of steroidogenic enzyme systems in Leydig cells, NR5A1 is responsible for the androgenization of the male external genitalia, and participates in spermatogenesis and germ cell

maturation (Lin and Achermann, 2008). Indeed, deletion of the NR5A1 gene from Leydig cells, using the Cre/lox strategy, resulted in hypoplastic testes with compromised steroidogenesis and spermatogenesis (Jeyasuria *et al.*, 2004).

Targeted disruption of SF-1 in the pituitary gland demonstrated that reproductive function requires SF-1 expression in gonadotropes (Zhao *et al.*, 2001). Investigation has since focused on neural expression, using Cre specific abrogation of SF-1 expression in the ventromedial hypothalamus (Kim *et al.*, 2010). This resulted in a failure to ovulate that could be reversed by gonadotropic hormone treatment, indicating problems with hypothalamic control of the pituitary.

LRH-1 also regulates reproductive processes

Given the importance of LRH-1 to induction of steroidogenic enzymes and factors (Fayard *et al.,* 2004) it is not surprising that it is highly expressed in the Leydig cells of the testis (Pezzi *et al.*, 2004). It appears to be only weakly expressed in Sertoli cells, where SF-1 predominates (Pezzi *et al.*, 2004). Mice heterozygous for LRH-1 mutation, i.e. where only a single functional allele is present, have circulating testosterone levels that are less than half their wildtype littermates (Volle *et al.*, 2007). Precocious expression of LRH-1 in mice leads to early induction of androgen synthesis and early puberty (Volle *et al.,* 2007).

The most abundant information about LRH-1 regulation derives from studies of the ovary. The gene is expressed exclusively in granulosa cells and corpora lutea, and is present in primary follicles and at all later stages of folliculogenesis (Hinshelwood *et al.*, 2005). A number of studies over the last decade have described LRH-1 regulation of target genes, including steroidogenic enzymes and proteins and inhibin-α (Zhao *et al.*, 2007 for review). The germline knockout of LRH-1 is lethal in early embryogenesis, because the gene is necessary for primitive streak formation (Labelle-Dumais *et al.*, 2006). As seen in the testis (Volle *et al.*, 2007), deletion of one allele compromises steroidogenesis, manifest in the female as luteal progesterone deficiency (Labelle-Dumais *et al.*, 2007). Our recent studies have focused on tissue specific knockout of the LRH-1 gene using Cre specific to granulosa cells and a mouse line with loxP sites flanking the third and fourth exon of the gene (Duggavathi *et al.*, 2008). This approach deletes the gene from primary through preovulatory follicles, and results in infertility. The principal cause for sterility is the total failure to ovulate that cannot be redressed by gonadotropin stimulation. The normal expression of a number of genes known necessary for steroidogenesis is disrupted, including the steroidogenic genes *CYP11A1, CYP19* and *STAR*, the rate limiting gene in prostaglandin synthesis (*PTGS2*) and genes associated with cholesterol transfer, such as scavenger receptor-B1. Although the cumulus oophorus fails to expand, it is not yet known whether the oocytes from mice in which LRH-1 has been deleted from granulosa cells are fertilizable. As LRH-1 regulates steroidogenic genes in the corpus luteum (Peng *et al.*, 2003), it would be of interest to establish its role by Cre-directed ablation in this ovarian compartment. This is currently under investigation.

The hypothalamo-hypophyseal axis is another potential site at which LRH-1 might regulate reproductive processes. A study using in situ hybridization identified the LRH-1 transcript throughout the mouse brain, including the hypothalamus (Grgurevic *et al.*, 2005). LRH-1 is expressed in the pituitary gland and in gonadotropic cell lines, and transcriptional studies have shown that it upregulates promoter activity of the follicles timulating hormone-ß, gonadotropin-releasing hormone the luteinizing hormone-ß (LHß) genes (Zheng *et al.*, 2007). The strongest response observed was with the LH ß gene. The relative importance of LRH-1 with respect to SF-1 in regulation of the pituitary remains to be established. Mice bearing the pituitary specific knockout, using the same Cre recombinase employed to delete SF-1 (Zhao *et al.*, 2001) appear to reproduce normally (D. Bernard, personal communication), suggesting that, of the two nuclear receptors that interact with the same cis-element on the promoters of target genes, SF-1 is the more significant regulator of gonadotropins in vivo. Further investigation is required to clarify this issue.

Orphan nuclear receptors regulate orphan nuclear receptors

There is compelling evidence that some nuclear receptors, including members of the ERR subfamily, are constitutively expressed. In addition, ERRγ has high constitutive transcriptional activity (Sanyal *et al.*, 2002). Both SF-1 and LRH-1 have transcriptionally active conformations, suggesting that they are constitutively active as well (Parker and Schimmer, 1997). This notwithstanding, their transactivational activity appears to be regulated by multiple factors that include posttranscriptional mechanisms, binding of other orphan nuclear receptors and the action of transcriptional coactivators and corepressors (Ohno *et al.*, 2010).

Phosphorylation of LRH-1, brought on by activation of the protein kinase-C pathway, increases its transcriptional activity (Lee *et al.*, 2006). Activation of SF-1 by phosphorylation, with similar consequences, appears to be accomplished by the mitogen-activated protein kinase (MAPK; Fowkes and Burrin, 2003) and a cyclin dependent kinase (Lewis *et al.*, 2008). A second posttranscriptional modification is the conjugation of SF-1 (Campbell et al., 2008) or LRH-1 (Yang *et al.*, 2009) to the small ubiquitin-like modifier (SUMO), at lysine residues (sumoyation). This epigenetic event reduces the transcriptional activity of both nuclear receptors, by reducing binding of the receptors to their cognate DNA sequences (Campbell *et al.*, 2008), or by

restricting translocation of LRH-1 into nuclear bodies (Yang *et al.*, 2009).

There are two further orphan nuclear receptors, short heterodimeric partner (SHP, NR0B2) and dosage-sensitive sex reversal adrenal hypoplasia congenital region on the X chromosome, gene 1 (DAX1, NROB1) that play important roles in the expression of SF-1 and LRH-1 (Fig. 1). These two receptors are unique among the nuclear receptor family in that they lack DNA binding domains (Giguere, 1999), and DAX1 has no ligand binding pocket (Sablin *et al.,* 2008). DAX1 heterodimerizes with nuclear receptors via n-terminal LXXLL motifs and the AF-2 domain, with the overall effect of reducing SF-1-induced transcription (Iyer and McCabe, 2004). This is achieved by interference with the SF-1 partnering with other stimulatory transcription cofactors including CREB, GATA-1 and 6, and SRC-1. DAX1 also binds to LRH-1 (Suzuki *et al.,* 2003), specifically in the coactivator groove, indicating competitive inhibition as the repressive mechanism (Sablin *et al.*, 2008). There is also evidence that DAX1 recruits corepressors, such as NCOR and ALIEN,

providing a further mechanism of inhibition of NR5 nuclear receptor activity (Iyer and McCabe, 2004). SHP inhibits not only the transcriptional activity of a number of nuclear receptors, including both SF-1 and LRH-1 (Iyer *et al.*, 2006), but also appears to suppress expression of both NR5 orphan nuclear receptors (Goodwin *et al.*, 2000). Its mechanism of inhibition of LRH-1-induced transcription resembles that of DAX1, in that it appears to compete with coactivators for binding to LRH-1 (Lee and Moore, 2002). Although LRH-1 can function unliganded, a phospholipid, phosphotidylserine, has been shown to interact with its ligand binding pocket (Burendahl *et al.*, 2008). These authors report that, in contrast to other nuclear receptors, ligand binding does not effect a conformational change, rather it reduces the affinity of LRH-1 for SHP. Ligand binding may thus increase transcription by reducing inhibition. The SHP-LRH-1 interaction has biological significance, as studies of SHP in the testis reveal that its inhibition of LRH-1 is a mechanism that prevents precocious puberty (Volle *et al.*, 2007).
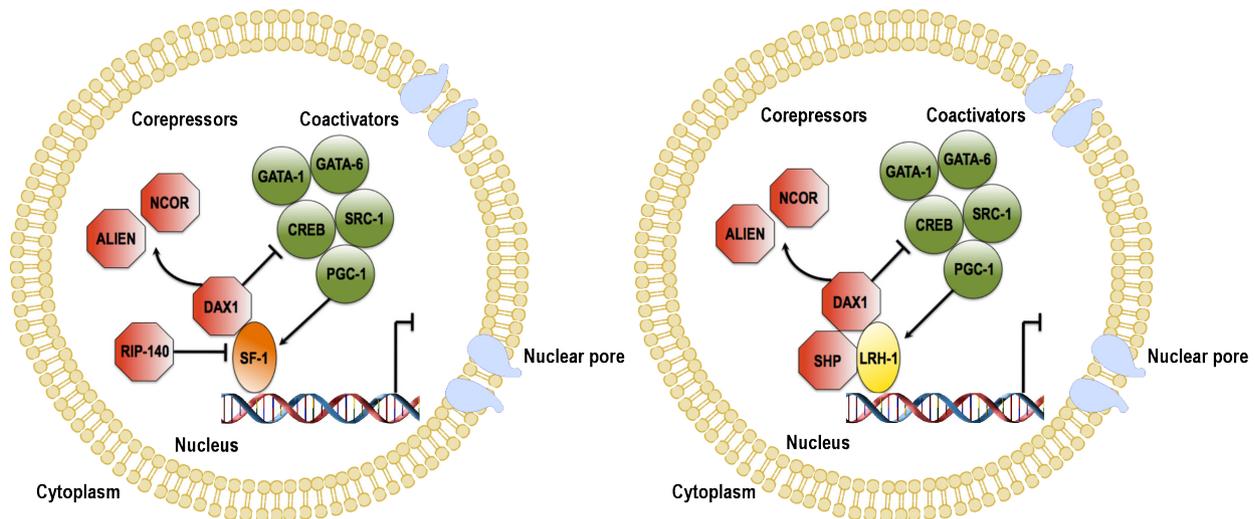
Figure 1. Schematic representation of the role of coactivators and corepressors in modulating the constitutive transcriptional activity of SF-1 and LRH-1. A number of transcription factors, including those of the GATA family and CREB act in synergy with SF-1 and LRH-1 to induce transcription of target genes. Coactivators such as SRC-1 and PGC-1 interact directly with SF-1 or LRH-1 to upregulate transcription. The orphan nuclear receptors of the NR0 family, DAX1 and SHP repress transcription by competitively inhibiting the interaction of SF-1 and LRH-1 with coactivators.

In addition to the DAX1 and SHP, other coactivators and corepressors participate in regulating SF-1 and LRH-1 transactivation of target genes. The repressor interacting protein 140 (RIP140; Fig. 1) is a negative transcriptional regulator of nuclear receptor action (Feige and Auwerx, 2007). RIP140 is required for ovulation, and its null mutation in the mouse phenocopies aspects of ovarian disruption seen in the granulosa specific knockout of LRH-1 mouse, in particular, the failure of cumulus expansion and of

follicle rupture (Duggavathi *et al.*, 2008). RIP140 is a potent inhibitor SF1-induced transcription (Mellgren *et al.*, 2003). It interacts with several other nuclear receptors, including ERRα (White *et al.*, 2008), and is therefore considered likely to regulate NR5A2 as well. Steroid receptor coactivators, such as SRC-1 (Xu *et al.*, 2004) and the PPARα-coactivator-1a (Yazawa *et al.*, 2010) potentiate the activity of both NR5A1 and NR5A2, indicating that inhibition is not the sole regulatory mechanism of transactivation by these nuclear receptors.

How can SF-1 and LRH-1 differentially regulate reproductive events, given that they interact with the same promoter sequence?

In the ovary, SF-1 is expressed in multiple cell types, including granulosa, theca and luteal cells while LRH-1 has a less extensive pattern of expression, being restricted to granulosa and luteal cells (Hinshelwood *et al.*, 2005). Both nuclear receptors are expressed in the gonadotropin cells of the pituitary (Zheng *et al.*, 2007). Although these nuclear receptors transactivate some of the same genes (Saxena *et al.*, 2007), there is evidence that they differentially regulate others (Weck and Mayo, 2006; Yazawa *et al.*, 2010). Weck *et al.* (Weck and Mayo 2006), studied the promoter region of a SF-1/LRH-1 target gene, the inhibin-α subunit, in granulosa cells. They concluded that stimulation with gonadotropins caused rapid reduction in the association of SF-1 and increased the interaction of LRH-1 with their shared response element on the promoter. Multiple, and presumably interrelated mechanisms are involved in this shift. These include the stimulatory effects of the MAP kinase and PI3 kinase pathways, as pharmacological abrogation of MAPK-MEK/ERK arm or blockade of PI3 kinase prevents SF-1 from vacating the inhibin-α subunit promoter and LRH-1 recruitment to this site. It is expected that further investigation will reveal coactivator and corepressor-based mechanisms for the selective action of these two nuclear receptors.
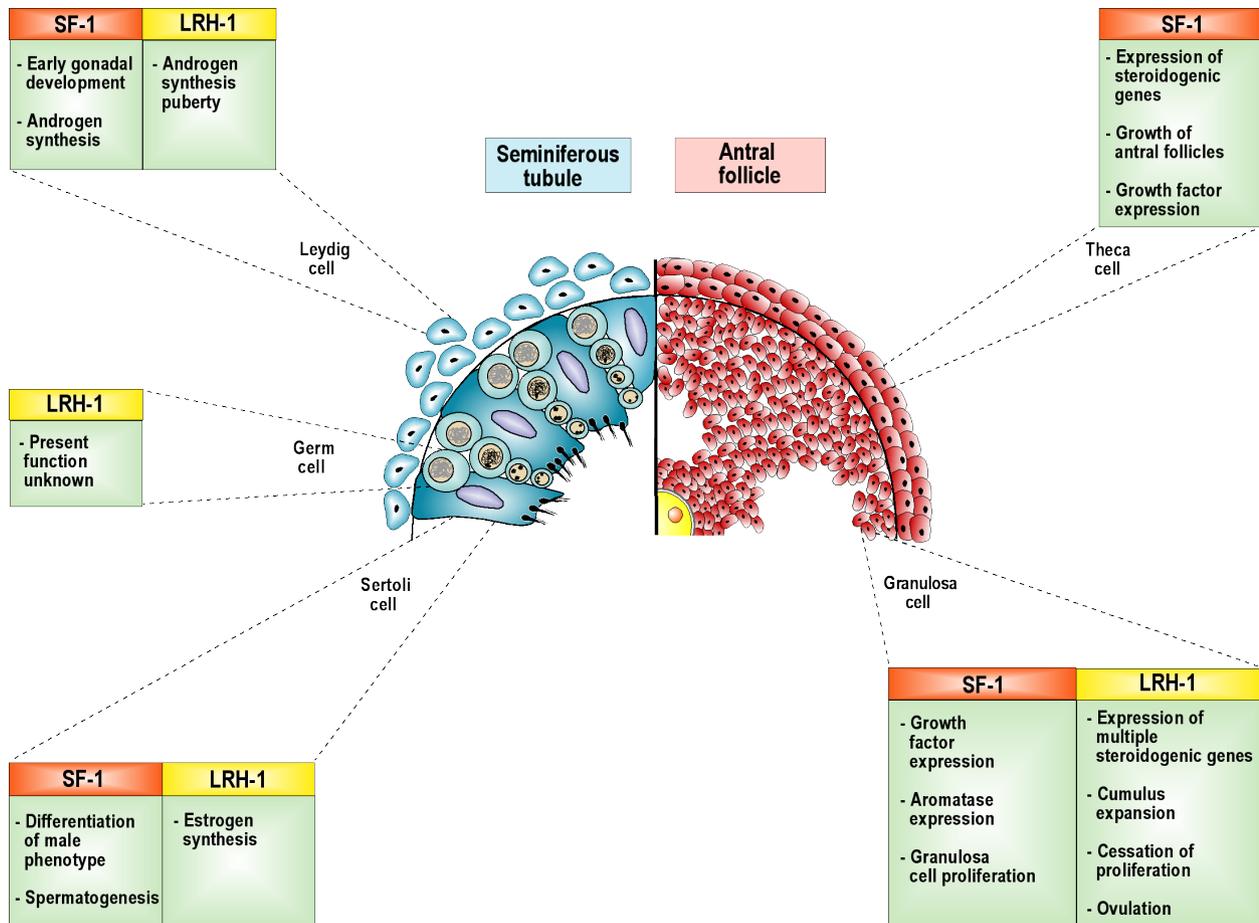
Figure 2. Overview of the effects of SF-1 and LRH-1 on the constituents of the seminiferous tubule in the male and the antral follicle in the female. Both nuclear receptors are found in Leydig and Sertoli cells where they regulate steroidogenesis, and perhaps, as a consequence, sex differentiation. LRH-1 has been shown to be present in spermatogonia, but its function in that site is obscure. In the ovary, LRH-1 is restricted to granulosa cells and the corpus luteum, while SF-1 is most strongly expressed in theca cells. It is likewise present in granulosa and luteal cells. The nuclear receptors regulate steroidogenesis and other complex events in the ovary such as expansion of the cumulus oophorus and ovulation.

Conclusions

This treatise briefly addresses the reproductive consequences of expression of a series of orphan nuclear receptors in the NR0, NR2, NR3, NR4 and NR5 families. It is clear that other nuclear receptors, both orphan and liganded, can affect reproductive processes. We have further focused on SF-1 and LRH-1 as exemplary of the pleiotropic and sometimes overlapping effects of orphan nuclear receptors on the gonads and other reproductive tissues. Fig. 2 summarizes the common and differential roles of SF-1 and LRH-1 on the testis and the ovary, with the seminiferous tubule and the antral follicles as examples. The preponderance of information to date describes positive effects on stimulation of steroidogenesis. Both receptors bring about, directly or indirectly, more complex processes, such as expansion of the cumulus oophorus and somatic cell proliferation. It is expected that the tissue specific knockout models currently available will yield considerable new information over the next years.

Acknowledgments

References

Alaynick WA, Kondo RP, Xie W, He W, Dufour CR, Downes M, Jonker JW, Giles W, Naviaux RK, Giguère V, Evans RM. 2007. ERRgamma directs and maintains the transition to oxidative metabolism in the postnatal heart. *Cell Metab*, 6:13-24.

Burendahl S, Treuter E, Nilsson L. 2008. Molecular dynamics simulations of human LRH-1: the impact of ligand binding in a constitutively active nuclear receptor. *Biochemistry*, 47:5205-5215.

Campbell LA, Faivre EJ, Show MD, Ingraham JG, Flinders J, Gross JD, Ingraham HA. 2008. Decreased recognition of SUMO-sensitive target genes following modification of SF-1 (NR5A1). *Mol Cell Biol*, 28:7476-7486.

DeYoung RA, Baker JC, Cado D, Winoto A. 2003. The orphan steroid receptor Nur77 family member Nor-1 is essential for early mouse embryogenesis. *J Biol Chem*, 278:47104-47109.

Duggavathi R, Volle DH, Mataki C, Antal MC, Messaddeq N, Auwerx J, Murphy BD, Schoonjans K. 2008. Liver receptor homolog 1 is essential for ovulation. *Genes Dev*, 22:1871-1876.

Fayard E, Auwerx J, Schoonjans K. 2004. LRH-1: an orphan nuclear receptor involved in development, metabolism and steroidogenesis. *Trends Cell Biol*, 14:250-260.

Feige JN, Auwerx J. 2007. Transcriptional coregulators in the control of energy homeostasis. *Trends Cell Biol*, 17:292-301.

Fowkes RC, Burrin JM. 2003. Steroidogenic factor-1: a key regulator of gonadotroph gene expression. *J Endocrinol*, 177:345-350.

Giguere V. 1999. Orphan nuclear receptors: from gene to function. *Endocr Rev*, 20:689-725.

Goodwin B, Jones SA, Price RR, Watson MA, McKee DD, Moore LB, Galardi C, Wilson JG, Lewis MC, Roth ME, Maloney PR, Willson TM, Kliewer SA. 2000. A regulatory cascade of the nuclear receptors FXR, SHP-1, and LRH-1 represses bile acid biosynthesis. *Mol Cell,* 6:517-526.

Grgurevic N, Tobet S, Majdic G. 2005. Widespread expression of liver receptor homolog 1 in mouse brain. *Neuro Endocrinol Lett*, 26:541-547.

Hamid T, Malik MT, Millar RP, Kakar SS. 2008. Protein kinase A serves as a primary pathway in activation of Nur77 expression by gonadotropin-releasing hormone in the LbetaT2 mouse pituitary gonadotroph tumor cell line. *Int J Oncol*, 33:1055-1064.

Havelock JC, Smith AL, Seely JB, Dooley CA, Rodgers RJ, Rainey WE, Carr BR. 2005. The NGFI-B family of transcription factors regulates expression of 3-beta-hydroxysteroid-dehydrogenase type 2 in the human ovary. *Mol Hum Reprod*, 11:79-85.

Hinshelwood MM, Shelton JM, Richardson JA, Mendelson CR. 2005. Temporal and spatial expression of liver receptor homologue-1 (LRH-1) during embryogenesis suggests a potential role in gonadal development. *Dev Dyn*, 234:159-168.

Hummasti S, Tontonoz P. 2008. Adopting new orphans into the family of metabolic regulators. *Mol Endocrinol*, 22:1743-1753.

Iyer AK, McCabe ER. 2004. Molecular mechanisms of DAX1 action. *Mol Genet Metab*, 83:60-73.

Iyer AK, Zhang YH, McCabe ER. 2006. Dosage-sensitive sex reversal adrenal hypoplasia congenita critical region on the X chromosome, gene 1 (DAX1) (NR0B1) and small heterodimer partner (SHP) (NR0B2) form homodimers individually, as well as DAX1-SHP heterodimers. *Mol Endocrinol*, 20:2326-2342.

Jensen EV, Jacobson HI, Walf AA, Frye CA. 2009. Estrogen action: a historic perspective on the implications of considering alternative approaches. *Physiol Behav*, 99:151-162.

Jeyasuria P, Ikeda Y, Jamin SP, Zhao L, De Rooij DG, Themmen AP, Behringer RR, Parker KL. 2004. Cell-specific knockout of steroidogenic factor 1 reveals its essential roles in gonadal function. *Mol Endocrinol*, 18:1610-1619.

Kim KW, Li S, Zhao H, Peng B, Tobet SA, Elmquist

JK, Parker KL, Zhao L. 2010. CNS-specific ablation of steroidogenic factor 1 results in impaired female reproductive function. *Mol Endocrinol*, 24:1240-1250.

Labelle-Dumais C, Jacob-Wagner M, Pare JF, Belanger L, Dufort D. 2006. Nuclear receptor NR5A2 is required for proper primitive streak morphogenesis. *Dev Dyn*, 235:3359-3369.

Labelle-Dumais C, Pare JF, Belanger L, Farookhi R, Dufort D. 2007. Impaired progesterone production in Nr5a2+/- mice leads to a reduction in female reproductive function. *Biol Reprod*, 77:217-225.

Lala DS, Rice DA, Parker KL. 1992. Steroidogenic factor I, a key regulator of steroidogenic enzyme expression, is the mouse homolog of fushi tarazu-factor I. *Mol Endocrinol*, 6:1249-1258.

Lee DK, Kurihara I, Jeong JW, Lydon JP, DeMayo FJ, Tsai MJ, Tsai SY. 2010. Suppression of ERalpha activity by COUP-TFII is essential for successful implantation and decidualization. *Mol Endocrinol*, 24:930-940.

Lee SL, Wesselschmidt RL, Linette GP, Kanagawa O, Russell JH, Milbrandt J. 1995. Unimpaired thymic and peripheral T cell death in mice lacking the nuclear receptor NGFI-B (Nur77). *Science*, 269:532-535.

Lee YK, Moore DD. 2002. Dual mechanisms for repression of the monomeric orphan receptor liver receptor homologous protein-1 by the orphan small heterodimer partner. *J Biol Chem*, 277:2463-2467.

Lee YK, Choi YH, Chua S, Park YJ, Moore DD. 2006. Phosphorylation of the hinge domain of the nuclear hormone receptor LRH-1 stimulates transactivation. *J Biol Chem,* 281:7850-7855.

Lewis AE, Rusten M, Hoivik EA, Vikse EL, Hansson ML, Wallberg AE, Bakke M. 2008. Phosphorylation of steroidogenic factor-1 is mediated by cyclin-dependent kinase-7. *Mol Endocrinol*, 22:91-104.

Li M, Xue K, Ling J, Diao FY, Cui YG, Liu JY. 2010. The orphan nuclear receptor NR4A1 regulates transcription of key steroidogenic enzymes in ovarian theca cells. *Mol Cell Endocrinol*, 319:39-46.

Lin L, Achermann JC. 2008. Steroidogenic factor-1 (SF-1, Ad4BP, NR5A1) and disorders of testis development. *Sex Dev*, 2:200-209.

Luo J, Sladek R, Bader JA, Matthyssen A, Rossant J, Giguere V. 1997. Placental abnormalities in mouse embryos lacking the orphan nuclear receptor ERR-beta. *Nature*, 388:778-782.

Luo X, Ikeda Y, Parker KL. 1994. A cell-specific nuclear receptor is essential for adrenal and gonadal development and sexual differentiation. *Cell*, 77:481-490.

Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schütz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P, Evans RM. 1995. The nuclear receptor superfamily: the second decade. *Cell*, 83:835-839.

Martin LJ, Tremblay JJ. 2010. Nuclear receptors in Leydig cell gene expression and function. *Biol Reprod*, 83:3-14

Mellgren G, Borud B, Hoang T, Yri OE, Fladeby C, Lien EA, Lund J. 2003. Characterization of receptor-interacting protein RIP140 in the regulation of SF-1 responsive target genes. *Mol Cell Endocrinol*, 203:91-103.

Ohno M, Komakine J, Suzuki E, Nishizuka M, Osada S, Imagawa M. 2010. Repression of the promoter activity mediated by liver receptor homolog-1 through interaction with ku proteins. *Biol Pharm Bull*, 33:784-791.

Park JI, Park HJ, Lee YI, Seo YM, Chun SY. 2003. Regulation of NGFI-B expression during the ovulatory process. *Mol Cell Endocrinol*, 202:25-29.

Parker KL, Schimmer BP. 1997. Steroidogenic factor-1: a key determinant of endocrine development and function. *Endocr Rev*, 18:361-377.

Parker KL, Rice DA, Lala DS, Ikeda Y, Luo X, Wong M, Bakke M, Zhao L, Frigeri C, Hanley NA, Stallings N, Schimmer BP. 2002. Steroidogenic factor-1: an essential mediator of endocrine development. *Recent Prog Horm Res*, 57:19-36.

Pelusi C, Ikeda Y, Zubair M, Parker KL. 2008. Impaired follicle development and infertility in female mice lacking steroidogenic factor-1 in ovarian granulosa cells. *Biol Reprod*, 79:1074-1083.

Peng N, Kim JW, Rainey WE, Carr BR, Attia GR. 2003. The role of the orphan nuclear receptor, liver receptor homologue-1, in the regulation of human corpus luteum 3-beta hydroxysteroid-dehydrogenase type II. *J Clin Endocrinol Metab*, 88:6020-6028.

Pereira FA, Qiu Y, Zhou G, Tsai MJ, Tsai SY. 1999. The orphan nuclear receptor COUP-TFII is required for angiogenesis and heart development. *Genes Dev*, 13:1037-1049.

Petit FG, Jamin SP, Kurihara I, Behringer RR, DeMayo FJ, Tsai MJ, Tsai SY. 2007. Deletion of the orphan nuclear receptor COUP-TFII in uterus leads to placental deficiency. *Proc Natl Acad Sci USA*, 104:6293-6298.

Pezzi V, Sirianni R, Chimento A, Maggiolini M, Bourguiba S, Delalande C, Carreau S, Andò S, Simpson ER, Clyne CD. 2004. Differential expression of steroidogenic factor-1/adrenal 4 binding protein and liver receptor homolog-1 (LRH-1)/fetoprotein transcription factor in the rat testis: LRH-1 as a potential regulator of testicular aromatase expression. *Endocrinology*, 145:2186-2196.

Qin J, Tsai MJ, Tsai SY. 2008. Essential roles of COUP-TFII in Leydig cell differentiation and male fertility. *PLoS ONE*, 3:e3285.

Sablin EP, Woods A, Krylova IN, Hwang P, Ingraham HA, Fletterick RJ. 2008. The structure of corepressor Dax-1 bound to its target nuclear receptor LRH-1. *Proc Natl Acad Sci USA*, 105:18390-18395.

Sanyal S, Kim JY, Kim HJ, Takeda J, Lee YK, Moore DD, Choi HS. 2002. Differential regulation of the orphan nuclear receptor small heterodimer partner

152

Anim. Reprod, v.7, n.3, p.146-153, Jul./Sept. 2010

(SHP) gene promoter by orphan nuclear receptor ERR isoforms. *J Biol Chem*, 277:1739-1748.

Saxena D, Escamilla-Hernandez R, Little-Ihrig L, Zeleznik AJ. 2007. Liver receptor homolog-1 and steroidogenic factor-1 have similar actions on rat granulosa cell steroidogenesis. *Endocrinology*, 148:726-734.

Steinmetz AC, Renaud JP, Moras D. 2001. Binding of ligands and activation of transcription by nuclear receptors. *Annu Rev Biophys Biomol Struct*, 30:329-359.

Suzuki T, Kasahara M, Yoshioka H, Morohashi K, Umesono K. 2003. LXXLL-related motifs in Dax-1 have target specificity for the orphan nuclear receptors Ad4BP/SF-1 and LRH-1. *Mol Cell Biol*, 23:238-249.

Villena JA, Kralli A. 2008. ERRalpha: a metabolic function for the oldest orphan. *Trends Endocrinol Metab*, 19:269-276.

Volle DH, Duggavathi R, Magnier BC, Houten SM, Cummins CL, Lobaccaro JM, Verhoeven G, Schoonjans K, Auwerx J. 2007. The small heterodimer partner is a gonadal gatekeeper of sexual maturation in male mice. *Genes Dev*, 21:303-315.

Walther N, Jansen M, Akbary W, Ivell R. 2006. Differentiation-specific action of orphan nuclear receptor NR5A1 (SF-1): transcriptional regulation in luteinizing bovine theca cells. *Reprod Biol Endocrinol*, 4:64.

Weck J, Mayo KE. 2006. Switching of NR5A proteins associated with the inhibin alpha subunit gene promoter after activation of the gene in granulosa cells. *Mol Endocrinol*, 20:1090-1103.

White R, Morganstein D, Christian M, Seth A, Herzog B, Parker MG. 2008. Role of RIP140 in metabolic tissues: connections to disease. *FEBS Lett*, 582:39-45.

Xu PL, Liu YQ, Shan SF, Kong YY, Zhou Q, Li M, Ding JP, Xie YH, Wang Y. 2004. Molecular mechanism for the potentiation of the transcriptional activity of human liver receptor homolog 1 by steroid receptor coactivator-1. *Mol Endocrinol*, 18:1887-1905.

Yang FM, Pan CT, Tsai HM, Chiu TW, Wu ML, Hu MC. 2009. Liver receptor homolog-1 localization in the nuclear body is regulated by sumoylation and cAMP signaling in rat granulosa cells. *FEBS J*, 276:425-436.

Yazawa T, Inaoka Y, Okada R, Mizutani T, Yamazaki Y, Usami Y, Kuribayashi M, Orisaka M, Umezawa A, Miyamoto K. 2010. PPAR-gamma coactivator-1 alpha regulates progesterone production in ovarian granulosa cells with SF-1 and LRH-1. *Mol Endocrinol*, 24:485-496.

Zetterstrom RH, Solomin L, Jansson L, Hoffer BJ, Olson L, Perlmann T. 1997. Dopamine neuron agenesis in Nurr1-deficient mice. *Science*, 276:248-250.

Zhao H, Li Z, Cooney AJ, Lan ZJ. 2007. Orphan nuclear receptor function in the ovary. *Front Biosci*, 12:3398-3405.

Zhao L, Bakke M, Krimkevich Y, Cushman LJ, Parlow AF, Camper SA, Parker KL. 2001. Steroidogenic factor 1 (SF1) is essential for pituitary gonadotrope function. *Development*, 128:147-154.

Zheng W, Yang J, Jiang Q, He Z, Halvorson LM. 2007. Liver receptor homologue-1 regulates gonadotrope function. *J Mol Endocrinol*, 38:207-219.