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Review

Thyroid hormones in male reproductive development: Evidence for direct crosstalk between the androgen and thyroid hormone axes

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ABSTRACT

Thyroid hormones (THs) exert a broad range of effects on development in vertebrate species, demonstrating connections in nearly every biological endocrine system. In particular, studies have shown that THs play a role in sexual differentiation and gonadal development in mammalian and non-mammalian species. There is considerable evidence that the effects of THs on reproductive development are mediated through the female hormonal axis; however, recent findings suggest a more direct crosstalk between THs and the androgen axis. These findings demonstrate that THs have considerable influence in the sexual ontogeny of male vertebrates, through direct interactions with select sex-determining-genes and regulation of gonadotropin production in the hypothalamus–pituitary–gonad axis. THs also regulate androgen biosynthesis and signaling through direct and indirect regulation of steroidogenic enzyme expression and activity. Novel promoter analysis presented in this work demonstrates the potential for direct and vertebrate wide crosstalk at the transcriptional level in mice (*Mus musculus*), Western clawed frogs (*Silurana tropicalis*) and medaka (*Oryzias latipes*). Cumulative evidence from previous studies; coupled with novel promoter analysis suggests mechanisms for a more direct crosstalk between the TH and male reproductive axes across vertebrate species.

1. Introduction

Thyroid hormones (THs) influence many developmental processes, including reproduction. However, the specific mechanisms underlying this hormonal interaction are largely debated. Initially, it was believed that the female axis solely mediated the effects of THs on the reproductive development of either sex. Consequently, the interrelationships between the estrogen and THs axes are well established and have been previously reviewed (teleost fish (Habibi et al., 2012; Liu et al., 2011); mammals (Doufas and Mastorakos, 2000; Vasudevan et al., 2002)). Over the last two decades, a more direct crosstalk between the androgen and TH axes has also been suggested (Wagner et al., 2008, 2009). However, the molecular basis for cross-regulation between these two hormonal pathways is still largely unexplored and has not been extensively reviewed. In this comparative review, we provide further evidence for direct crosstalk between the androgen and TH axes throughout male reproductive development, weakening the proposal that the female reproductive axis solely mediates TH effects.

TH regulation has been implicated in the reproductive development of many different vertebrate species (rainbow trout, *Oncorhynchus mykiss* (Holloway et al., 1999); zebrafish, *Danio rerio* (Filby et al., 2007); African clawed frog, *Xenopus laevis* (Goleman et al., 2002); Western clawed frog, *Silurana tropicalis* (Duarte-Guterman et al., 2010; Duarte-Guterman and Trudeau, 2011; Langlois et al., 2010a, 2011); Bocage's Wall Lizard, *Podarcis bocagei* (Bicho et al., 2013); Indian garden lizard, *Calotes versicolor* (Haldarmisra and Thapliyal, 1981); American tree sparrows, *Spizella arborea* (Reinert and Wilson, 1996); Indian finch, Lal munia, *Estrilda amandava* (Thapliyal and Pandha, 1967); red munia, *E. amandava* (Saxena et al., 2011); sheep, *Ovis aries* (Karch et al., 1995); rat, *Rattus norvegicus* (Tamura et al., 1998)). This cross regulation has been studied extensively on the physiological level, with many studies examining the role of the TH axis in testes function and development (*X. laevis* (Goleman et al., 2002); Hokkaido salamander, *Hynobius retardatus* (Kanki and Wakahara, 1999); *P. bocagei* (Bicho et al., 2013); *C. versicolor* (Haldarmisra and Thapliyal, 1981); *Lonchura punctulata* (Gupta and Thapliyal, 1984), *E. amandava* (Saxena et al., 2011; Thapliyal and Pandha, 1967); Chicken, *Gallus gallus* (Akhlaghi and Zamiri, 2007); *O. aries* (Parkinson et al., 1995); *R. norvegicus* (Anbalagan et al., 2010; Cristovao et al., 2002;

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Holsberger and Cooke, 2005; Jannini et al., 1995; Lagu et al., 2005; Marchlewska et al., 2011; Wagner et al., 2008, 2009; Wajner et al., 2009); *Homo sapiens* (Maran, 2003)). Therefore, we focus primarily on transcriptional, hormonal and cellular responses within the male reproductive-axis to elucidate the molecular controls behind this hormonal interaction. We reviewed information concerning both mammalian and non-mammalian species to stress the conserved nature of TH regulation in male reproductive development. We acknowledge that species-specific variation in the interaction between THs and male reproduction does exist; however, crosstalk between the androgen and TH axes appear to be maintained in some degree across vertebrates. We believe our findings effectively demonstrate that THs have considerable influence in the sexual ontogeny of male vertebrates and strengthen the proposal of cross-regulation between the androgen- and TH-axes.

2. Androgenic involvement in TH biosynthesis

An understanding of the TH hierarchy enables us to better identify and elucidate points where crosstalk is possible. The hypothalamus–pituitary–thyroid axis (HPT) has been extensively reviewed in vertebrate species (teleosts and amphibians (Brown and Cai, 2007; Carr and Patino, 2011); mammals, (Wagner et al., 2008, 2009)). Previous reviews solely examined the regulatory role of THs in reproductive development. Here we focus on the potential for cross-regulation between androgenic factors and THs throughout TH biosynthesis in a brief outline of the HPT structure.

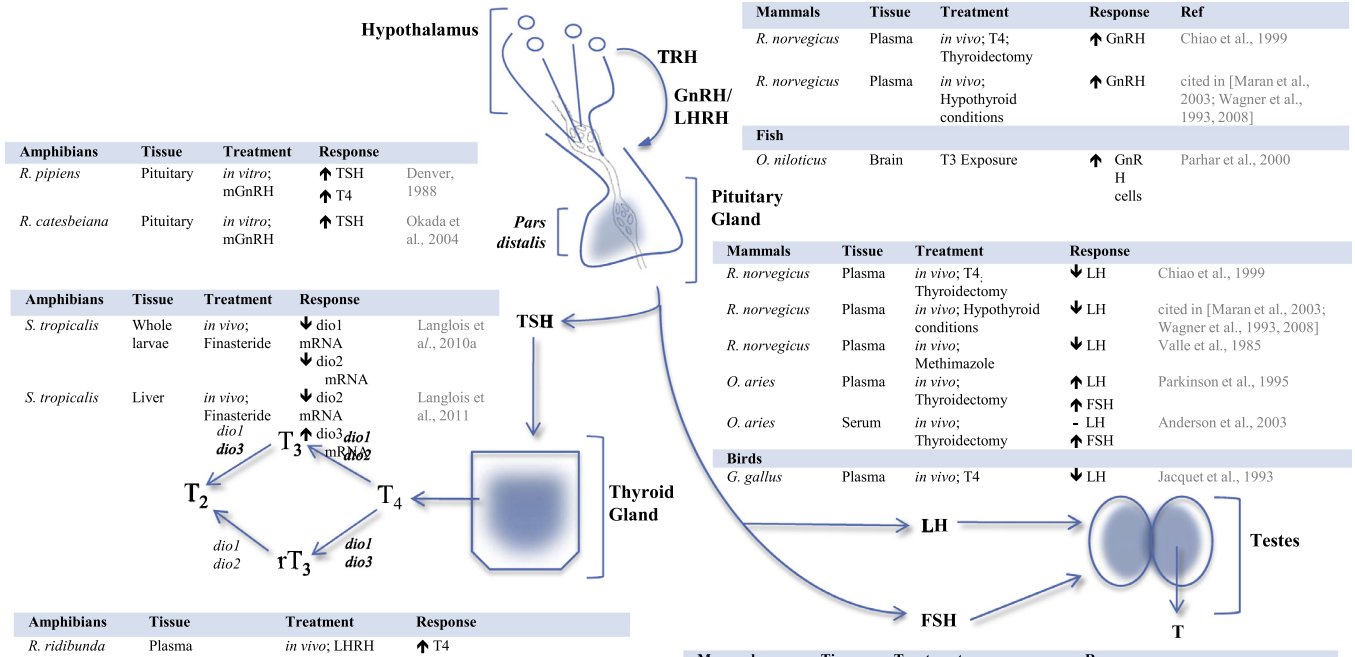
Considering the extent of the proposed regulatory role of THs in the androgen axis, an understanding of TH biosynthesis could assist in elucidating where potential crosstalk between androgens and THs can occur. Throughout normal thyroid gland functioning, the thyroid-releasing hormone (TRH) is released from the hypothalamus. TRH then triggers the release of thyroid-stimulating hormone (TSH) or thyrotropin, from thyrotrope cells in the *pars distalis* of the adenohypophysis (Fig. 1). Studies show that TRHs and TSHs are susceptible to regulation by other endocrine systems at the hypothalamic and pituitary levels. Gonadotropin-releasing hormones (GnRHs), from the hypothalamus–pituitary–gonad axis (HPG) interfere with the TH axis, increasing TSH secretion (Northern leopard frog, *Rana pipiens* (Denver, 1988); American bullfrog, *Rana catesbeina* (Okada, 2004)). An increase in TSH production would lead to a subsequent increase in TH synthesis. TSH binds to receptors on the thyroid follicle cell membrane, stimulating the biosynthesis of the iodine-containing THs, tetraiodothyronine or thyroxine (T4), and triiodothyronine (T3). T4 is the principle form of TH secreted from the thyroid gland; however, it is quickly metabolized into T3, the more potent form of TH. In addition to increased TSH concentrations, a number of studies have demonstrated that exposure to GnRH induces T4 secretion, increasing T4 production and serum concentrations in fish (Barfin flounder, *Verasper moseri* (Chiba et al., 2004); masu salmon, *Oncorhynchus masou* (Chiba et al., 2004); goldfish, *Carassius auratus* (Chiba et al., 2004) and amphibians (*R. pipiens* Denver, 1988; *A. mexicanum* (Jacobs and Kuhn, 1987);; Marsh frog, *Rana ridibunda* (Jacobs et al., 1988); Common frog, *Rana temporaria* (Jacobs et al., 1988); European frog, *Rana esculenta* (Jacobs et al., 1988)). However, no changes in circulating T3 concentrations were observed in fish in response to GnRH increases (*C. auratus* (Mackenzie et al., 1987)). Discrepancies between T4 and T3 level fluctuations suggest that gonadotropins can increase the baseline circulating TH concentration, but the appropriate deiodinase activity would have to be stimulated in order to increase the concentration of the active TH.

Deiodinases activate and deactivate THs via iodination and deiodination of their phenolic rings (Fig. 1; reviewed in Kohrle (1996) and Visser and Schoenmakers, 1992). Thus, the coordination of the expression and activity of the deiodinase enzymes in individual tissues regulates the concentration of active THs, according to the specific needs of the tissue. The spatiotemporal distribution and expression of deiodinases have been shown to respond to androgen signaling. For example, exposure to finasteride, a known disruptor of androgen biosynthesis, increases type II deiodinase (*dio2*) and decreases *dio3* mRNA levels in brain and liver tissues of pre-metamorphic tadpoles (Fig. 1; *S. tropicalis* (Langlois et al., 2011)), suggesting that TH axis is responsive to circulating androgen concentrations, and increases T3 concentrations accordingly. In support of observed deiodinases activity, T4 and T3 ratios and concentrations fluctuate with androgen levels. Testosterone (T) treatment had no effect on the plasma concentrations of T4, but reduced the T3 concentrations in rainbow trout (*Salmo-gairdneri Richardson* (Leatherland, 1985). Moreover, studies have identified androgen receptors (ar) in the thyroid gland of different vertebrate species (American alligator, *Alligator mississippiensis* (Bermudez et al., 2011); mammals, reviewed in Pelletier (2000)), suggesting that the androgen axis directly regulates TH synthesis. Androgenic regulation of both the synthesis and the peripheral metabolism of THs alone, demonstrates considerable crosstalk between the androgen and TH axes. Further research on these potential regulatory and feedback mechanisms will further strengthen the proposed mechanism.

3. TH-related machinery within gonadal tissues

Deiodinases are responsible for TH peripheral metabolism and thyroid receptors (trs) mediate TH activity at sites of action, both are present within gonadal tissues. Moreover, it has become clear that the distribution of TH-related machinery in gonadal tissues is highly sex-specific. Studies have identified deiodinases in the testes of vertebrate species, and the role of deiodinases within testicular functioning in mammalian species has been reviewed in detail (see reference Wagner et al. (2009)). In developing rats (*R. norvegicus*), dio1 and dio2 activity are higher in the testes compared to ovaries, whereas dio3 activities is higher in ovary tissue (Bates et al., 1999). Recently, similar observations have been confirmed in non-mammalian species. Testes of striped parrotfish (*Scarus iseri*) are characterized by higher *dio2* and *dio3* mRNA levels than ovaries (Johnson and Lema, 2011). Also, testes of *O. mykiss* are characterized by higher transcripts of *dio2*, and *dio2* expression is dependent on spermatogenic stages, increasing at the beginning of spermatogenesis (Sambroni et al., 2001). Moreover, Duarte-Guterman and Trudeau (2011) demonstrate that *dio1*, *dio2* and *dio3* mRNAs are significantly higher in testes compared to ovaries in the frog *S. tropicalis*. Altogether, this demonstrates that maintenance of a baseline level of active THs by deiodinases could be necessary to vertebrate testes development.

TRs mediate TH signaling and are crucial for testes development and function. The expression of *trs* in testicular tissues and their physiological implications in mammalian species have been reviewed thoroughly (Bagamasbad and Denver, 2011; Tsai-Morris et al., 1993; Valadares et al., 2008). *trα* and *trβ* genes code for a number of tr-isoforms including: *trα1*, *trα2*, *trα3*, *trβ1*, *trβ2*, and *trβ3*. These various tr-isoforms are expressed in a range of tissue types including testes (fish (Johnson and Lema, 2011; Sambroni et al., 2001); reptiles (Cardone et al., 2000) mammals (Buzzard et al., 2000; Holsberger and Cooke, 2005; Jannini et al., 1990, 1999; Williams, 2000, 2011). Apriletti et al. (1998) reviewed in detail the various modes of action of tr isoforms in mammals. The expression of *trs* in testes is dependent on circulating TH concentrations. Recent studies demonstrate that *tr* mRNA within gonadal tissues fluctuate with TH production, reinforcing auto-regulation by THs (De Paul et al., 2008; Wagner et al., 2008, 2009). Similar



Amphibians	Tissue	Treatment	Response	
R. pipiens	Pituitary	*in vitro*; mGnRH	↑ TSH ↑ T4	Denver, 1988
R. catesbeiana	Pituitary	*in vitro*; mGnRH	↑ TSH	Okada et al., 2004

Amphibians	Tissue	Treatment	Response	
S. tropicalis	Whole larvae	*in vivo*; Finasteride	↓ dio1 mRNA ↓ dio2 mRNA	Langlois et al., 2010a
S. tropicalis	Liver	*in vivo*; Finasteride	↓ dio2 mRNA ↑ dio3 mRNA	Langlois et al., 2011

Amphibians	Tissue	Treatment	Response	
R. ridibunda	Plasma	*in vivo*; LHRH	↑ T4	
R. temporaria	Plasma	*in vivo*; LHRH	↑ T4	Jacobs et al., 1988
R. esculenta	Plasma	*in vivo*; LHRH	↑ T4	
A. mexicanum	Plasma	*in vivo*; LHRH	↑ T4	Jacobs and Kuhn, 1987
Fish				
V. moseri	Plasma; Thyroid follicles	*in vivo, in vitro*; sbGnRH	↑ T4	
O. *masou*	Plasma; Thyroid follicles	*in vivo, in vitro*; sGnRH	↑ T4	Chiba et al., 2004
C. auratus	Plasma; Thyroid follicles	*in vivo, in vitro*; sGnRH	↑ T4	
C. auratus	Plasma	*in vivo*; GnRH	↑ T4 – T3	Mackenzie et al., 1987
S. gairdneri Richardson	Plasma	*in vivo*; Testosterone	– T4 ↓ T3	Leatherland and Barkataki, 2010

Mammals	Tissue	Treatment	Response	Ref
R. norvegicus	Plasma	*in vivo*; T4; Thyroidectomy	↑ GnRH	Chiao et al., 1999
R. norvegicus	Plasma	*in vivo*; Hypothyroid conditions	↑ GnRH	cited in [Maran et al., 2003; Wagner et al., 1993, 2008]
Fish				
O. niloticus	Brain	T3 Exposure	↑ GnRH cells	Parhar et al., 2000

Mammals	Tissue	Treatment	Response	
R. norvegicus	Plasma	*in vivo*; T4, Thyroidectomy	↓ LH	Chiao et al., 1999
R. norvegicus	Plasma	*in vivo*; Hypothyroid conditions	↓ LH	cited in [Maran et al., 2003; Wagner et al., 1993, 2008]
R. norvegicus	Plasma	*in vivo*; Methimazole	↓ LH	Valle et al., 1985
O. aries	Plasma	*in vivo*; Thyroidectomy	↑ LH ↑ FSH	Parkinson et al., 1995
O. aries	Serum	*in vivo*; Thyroidectomy	- LH ↑ FSH	Anderson et al., 2003
Birds				
G. gallus	Plasma	*in vivo*; T4	↓ LH	Jacquet et al., 1993

Mammals	Tissue	Treatment	Response	
R. norvegicus	Plasma	*in vivo*; T4, Thyroidectomy	↑ T	Chiao et al., 1999
R. norvegicus	Leydig cells	Propylthiouracil	↓ T	Chiao et al., 2002
R. norvegicus	Plasma	*in vivo*; Hypothyroid conditions	↓ T	cited in [Maran et al., 2003; Wagner et al., 1993, 2008]
R. norvegicus	Plasma	*in vivo*; 2,8-Dimercapto-6-hydroxypurine	↓ T	Jahan et al., 2012
Birds				
G. gallus	Serum	*in vivo*; Propylthiouracil	↑ T	Akhlaghi and Zamiri, 2007
C. japonica	Plasma	*in vivo*; Methimazole	↓ T	Weng et al., 2007
Fish				
C. gariepinus	Plasma	*in vivo*; Thiourea	↓ T	Swapna et al., 2006

Fig. 1. Schematic representation of hypothalamus–pituitary TH/gonadal axis interactions. Chart includes a list of endpoints compiled from gonadotropin and TH related studies. An upward pointing arrow indicates an increase in hormone concentration, gene expression or enzyme activity, whereas a downward pointing arrow indicates a decrease in hormone concentration, gene expression or enzymatic activity and a flat line indicates no observable change in endpoints. This figure incorporates both the hypothalamic regulation of androgen and TH synthesis and effectively brings together aspects of crosstalk from numerous functional levels. Results highlight possible evolutionary differences between species as well as demonstrating vertebrate wide patterns in hormone response. Moreover, considerable redundancy in possible mechanism of action exists. Abbreviations: TRH, thyroid releasing hormone; TSH, thyroid stimulating hormone; GnRH, gonadotropin releasing hormone; LHRH, Luteinizing releasing hormone; dio1, deiodinase type 1; dio2, deiodinase type 2; dio3, deiodinase 3; T4, thyroxine; T3, triiodothyronine;rT3, reverse triiodothyronine; T2, 3,5-diiodo-L-thyronine; LH, luteinizing hormone; FSH, follicle stimulating hormone; T, testosterone. (See above-mentioned references for further information.)

to deiodinases, significant differences in *tr* expression exist between sexes. In fish and amphibians, testes are characterized by greater *tr* mRNA levels than ovaries (*S. iseri* (Johnson and Lema, 2011); *S. tropicalis* (Duarte-Guterman and Trudeau, 2011)). Sex-specific ratios of deiodinases and *trs* suggest that the TH-axis is involved in gonadal differentiation. Indeed, An et al. (2010) observed a significant decrease in *trα* expression in gonadal tissues following male-to-female sex changes in the protandrous black porgy (*Acanthopagrus schlegeli*). However, no changes are observed in *trβ* transcripts between the male and female gonadal tissue (An et al., 2010). In the testes, *trs* have distinct patterns of spatiotemporal expression dependent on stage of development. The expression of *trs* decreases with sexual maturation, suggesting that THs play a crucial role in early testes development and cessation of signaling is responsible for testes maturation (*R. norvegicus* (Buzzard et al., 2000; Canale et al., 2001; Jannini et al., 1990, 1995, 1999)). In addition, extra-thyroidal expression of TSH-receptors and TRH-receptors has been identified in the testes (European sea bass, *Dicentrarchus labrax* (Rocha et al., 2007); fathead minnow, *Pimephale promelas* (Lema et al., 2009); Japanese quail, *Coturnix japonica* (Catena et al., 2003); mouse, *Mus musculus*; *R. norvegicus*; Guinea pig, *Cavia porcellus*; *O. aries; H. sapiens* [reviewed in Williams (2011)]). However, the regulatory role of these receptors in male gonads remains unclear.

In summary, the transcriptional profiles of *tr* in testicular tissues suggests a direct regulatory role for THs in male gonadal development. The concentration and distribution of trs in the gonadal tissues change in response to thyroidal state, implying a functional role for THs within the testicular development. Moreover, TH-related gene expression and enzyme activity appear to be sexually dimorphic in vertebrates, with enhanced gene expression and enzymatic activity within the testes. Lastly, the distribution and abundance of TH-related machinery is dependent on the stage of testicular development. However, further research on TH transcriptional regulation in gonadal tissues is necessary to fully understand the role of THs in testicular development, especially in non-mammalian species.

4. TH transcriptional regulation

TH actions within the testes can be performed through rapid membrane and cytoplasm-initiated actions (Zamoner et al.,

2011), as well as through more classical and well-known nuclear receptor activation. Non-genomic regulation is a relatively unexplored mechanism of potential androgen and TH crosstalk. However, here we focus here on tr-mediated regulation of androgen-related gene expression. Transcriptional regulation of target genes has been established as the primary regulatory mechanisms of THs. At the genomic level, the effects of THs are mediated through nuclear trs (amphibians (Shi et al., 1996); mammals (Glass, 1994)). Nuclear receptors are intimately associated with chromatin and bind respective hormones with high affinity and specificity (Lazar, 2003). T3 is the main biological TH and readily binds to tr isoforms over T4 (Apriletti et al., 1998). Hormone binding is associated with the conformational change of the receptor that causes it to function as a transcriptional activator. Transcription of TH-inducible genes is initiated by trs recruiting co-activators and binding to specific nucleotide sequences in the promoter region. Common TH-related co-activators include retinoid X receptor (RXR) and retinoic acid receptor (RAR) (Yu et al., 1991; Zhang et al., 1992). The specific DNA sequences that mediate transcriptional activation or repression in response to T3 are referred to as thyroid response elements (TREs). trs bind to the TREs with high affinity as monomers, homodimers, and even heterodimers in the promoter region of the genes (Apriletti et al., 1998). In addition, trs can bind TREs constitutively independent of ligand occupancy; unliganded trs repress the transcription of TH-inducible genes by recruiting co-repressors when binding to TREs in promoters (Glass, 1994; Ribeiro et al., 1995; Zhang and Lazar, 2000). The presence of T3 in target tissues, such as testes, and TREs in target genes thereby dictates when and where trs can activate or repress gene expression. To understand potential cross-regulation between the androgen- and TH-axes, it is necessary to review tr mechanisms of action and TH auto-regulation.

One of the primary target genes for tr transcriptional activation is the *tr* gene itself (Apriletti et al., 1998). Thus, auto-regulation may be a means to amplify the effects of THs in responsive tissues by increasing the expression of *trs*. Using *in silico* modeling, we predict potential auto-regulation in TH-related genes of mammals, amphibians, and fish (Fig. 2). TREs (*i.e.*, TGACCT, TGTCCT, TTGAGCTA, TGTCCT/nnnnn/TGTCCT, TGACCT/nnnnn/TGTCCT) are observed in the promoter regions of *dio1*, *dio2*, *dio3*, *trα*, and *trβ* of *M. musculus*, *S. tropicalis*, and medaka (*Oryzias latipes*). In TH-related genes, more TREs are found in promoters of mammals ($n = 15$) compared to amphibians ($n = 9$) and fish ($n = 9$). These findings highlight potential evolutionary differences in TR auto-regulation and may explain differences in species-specific responses to disruptions in TH-biosynthesis. Moreover, species-specific patterns of auto-regulation may reveal differences in the extent of genome mediated regulation between species. For instance, we observed fewer TREs in *trα* ($n = 1$) and *trβ* ($n = 0$) gene promoter regions of *S. tropicalis*, compared to both *O. latipes* (*trα*, $n = 3$; *trβ*, $n = 3$) and *M. musculus* (*trα*, $n = 5$; *trβ*, $n = 2$). Interestingly, even though no TREs are found in *trβ* of *S. tropicalis*, Duarte-Guterman and Trudeau (2011) observed dramatic increases in *trβ* in the gonad–mesonephros-complex of *S. tropicalis* larvae treated with T3 suggesting possible non-genomic regulation by THs in frogs. Non-genomic mechanisms (e.g., ion fluxes at the plasma membrane) can be connected via signal transduction pathways to nuclear events, meaning hormonal signals that begin in the plasma membrane can still modulate gene transcription (reviewed in Zhou et al. (2002)). This offers a possible explanation regarding the sensitivity of *trβ* to T3 despite the apparent lack of TREs in the promoter region of *S. tropicalis* analyzed in the present revision, however further investigation into non-genomic regulation of TH-related genes in vertebrate species is required.

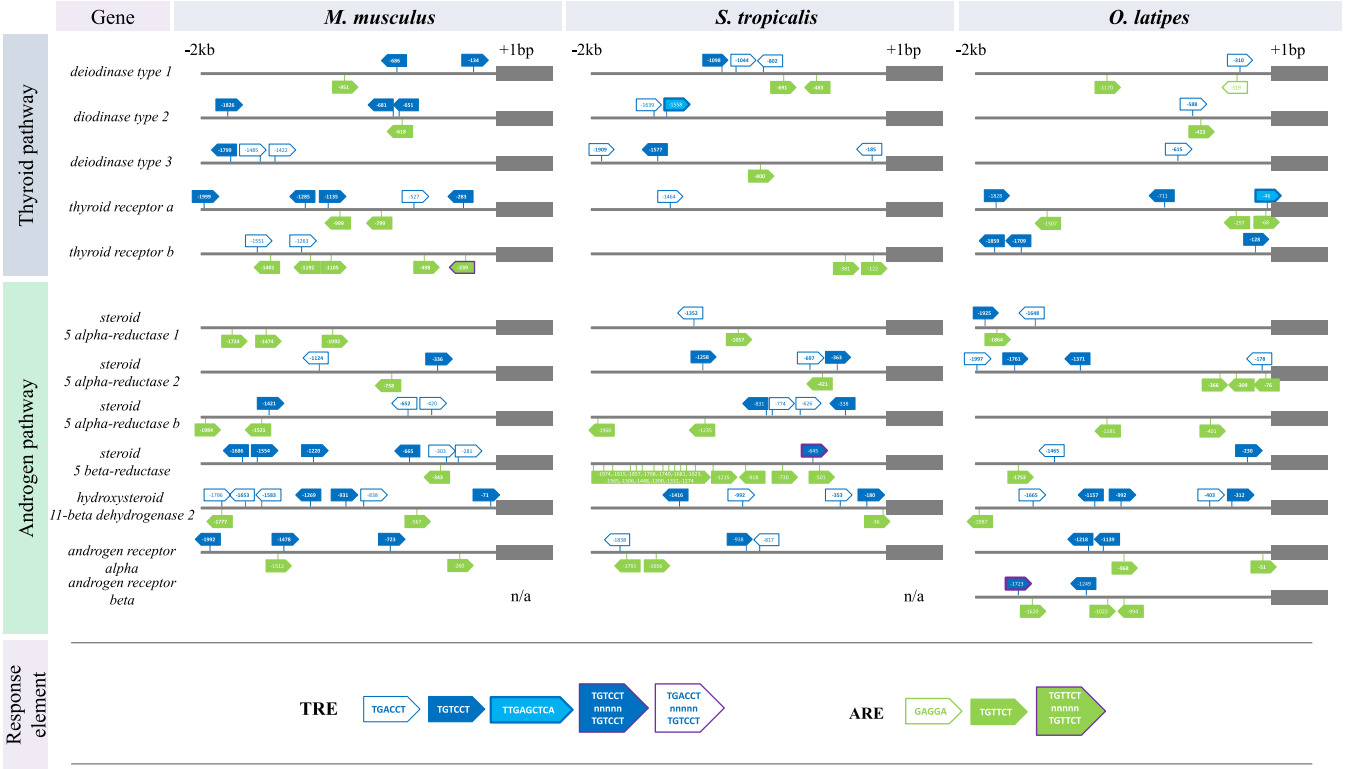


Fig. 2. Promoter analysis of *M. musculus*, *S. tropicalis* and *O. latipes)* TH- (*dio1*, *dio2*, *dio3*, *tr1*, *tra* and *trb*) and androgen- (*srd5a1*, *srd5a2*, *srd5a3*, *srd5b*, *hsd11b2* and *ar*) related genes. TREs are shown in blue and AREs are represented by green arrows. All sequences used for analysis were collected from the Ensembl Project (http://www.ensembl.org) and the GenBank/EMBLDatabase of the National Center of Biotechnology Information (NCBI). Putative transcription factor binding sites (TFBSs) within the putative promoter (−2000 to +1) were identified by Transcription Element Search Software (TESS) Schug, 2008 and MULAN software (Ovcharenko et al., 2005). The nucleotide sequence homology was analyzed using the software BLASTN at http://www.ncbi.nlm.nih.gov/BLAST. Legend: Blue, TREs and Green, AREs.

Moreover, seeing as deiodinases are largely responsible for concentrations of active circulating THs, it is imperative that we consider the genomic regulation of TH peripheral metabolism. This form of autoregulation may amplify the effects of THs in responsive tissues by raising the circulating concentrations of active THs. The TRE profile for the deiodinase promoter regions (*dio1*, *dio2*, and *dio3*) reveals potential evolutionary trends. The deiodinase promoters of *M. musculus* (2–3 per gene; $n = 8$) and *S. tropicalis* (2–3 per gene; $n = 8$) has the same cummulative number of TREs, whereas the promoter regions of *O. latipes* ($n = 3$) has fewer (1 per gene; Fig. 2). Nelson and Habibi (2009) suggest that *dio3* transcription is primarily regulated by trs in fish. However, our findings may weaken the proposal that trs solely regulate deiodinases in fish, as only one TRE is observed in *dio3* of *O. latipes*. Similarly, only one TRE is observed in the promoter regions of *dio1* and *dio2* in *O. latipes*, suggesting possible species-specific differences in genomic and non-genomic regulation of deiodinases. However, further experimental confirmation of promoter binding in different species is needed to elucidate possible species-specific differences.

4.1. The influence of THs on androgen-related receptors and enzymes

The presence of TH machinery in testicular tissues implies that the TH axis must regulate aspects of testicular functioning. Indeed, hypothyroid males exhibit testes and sperm dysfunction. Sertoli and Leydig cells are responsible for androgen biosynthesis and spermatogenesis in vertebrates (Cristovao et al., 2002; Lema et al., 2009; Nagendra Prasad et al., 1999; Panno et al., 1996; Santos et al., 2007). The proliferation and functioning of Sertoli and Leydig cells are co-regulated by ar. Consequently, we can hypothesize that THs may interact with the ar promoter region, which in turn mediates the effects of THs. We now recognize that THs can influence androgen responsiveness in testicular tissues, with previous studies demonstrating that THs are involved in the direct regulation of the ar. THs can influence androgen gene expression by directly interacting with components of ar transcriptional apparatus. *In silico* modeling of TREs in promoter regions of the androgen-related genes in mammals and tetrapods provides evidence for a direct and vertebrate-wide crosstalk (Fig. 2). TREs were identified in the *ar* promoter region in *M. musculus* ($n = 3$), *S. tropicalis* ($n = 3$),and *O. latipes* ($n = 2$). The *arβ* gene is only expressed in *O. latipes* and only two TREs were identified in the promoter region of this gene. Studies show that THs directly regulate *ar* expression by binding to ligand binding domains (LBD) within the *ar* promoter. Varriale and Esposito (2005) previously identified a putative TH response element in the hamster AR promoter. In additon, Estebanez-Perpina et al. (2007) demonstrated that the analog 3,3′,5-triiodothyloacetic acid can bind to a second LBD in *ar* in mammals. Accordingly, THs can induce changes in *ar* expression supporting of the presences of TREs in the *ar* promoter. Studies demonstrate that T3 treatment increases *ar* mRNA in vertebrate testes (*S. tropicalis* (Duarte-Guterman and Trudeau, 2011); Tungara frog, *Physalaemus pustulosus* (Duarte-Guterman et al., 2012); Italian wall lizard, *Podarcis sicula* (Cardone et al., 2000); *M. musculus* (Wagner et al., 2008, 2009); *R. norvegicus* (Arambepola et al., 1998; Panno et al., 1996; Sisci et al., 1997)). Based on similar findings, Cardone et al., (2000) also concluded that THs must directly modulate *ar* mRNA levels in Italian wall lizard, *P. sicula*. Moreover, T3 enhances *ar* expression in whole embryo and whole larvae brain and liver tissues in *S. tropicalis* (Langlois et al., 2011), demonstrating crosstalk across tissues. Conversely, reduced T4 levels decrease *ar* expression within the testicular tissues of *R. norvegicus* (Xiao et al., 2010). Similarly, Anbalagan et al. (2010) demonstrated that transient gestational-onset hypothyroidism affects male fertility by altering *ar* expression in different testicular tissues, further confirming the direct relationship between these two axes. Overall, the regulation of *ar* by THs appears to be consistent with the presence of TREs in fish, mice and frog *ar* promoter regions (Fig. 2).

In addition to ar, TH may also regulate other genes and enzymes involved in androgen biosynthesis and signaling. The enzyme 5α-reductases (srd5α) is essential as it converts T into the more potent androgen, 5α dihydrotestosterone (5α-DHT). TH treatment enhances 5α-reductase expression and activity within the testes (Duarte-Guterman et al., 2010, 2012; Duarte-Guterman and Trudeau, 2011; Kala et al., 2002; Ram and Waxman, 1990), increasing circulating 5α-DHT concentrations. Kala et al. (2002) demonstrated that persistent hypothyroidism in rats exposure significantly decreased 5α-reductase activity in testicular tissues. TREs are present in the promoter regions every reductase-isoform (2–6 pergene; $n = 10$) except *srd5α1* in *M. musculus*. TREs are present in every reductase-isoform in *S. tropicalis* (1–4 per gene $n = 9$). Whereas, TREs are observed however only in *srd5α, srd5α1,* and *srd5α2* in *O. latipes* (2–4 per gene $n = 8$). Further experimental confirmation of promoter binding in different species is needed to distinguish between isoform preferences between species. We also identified TREs in the promoter region of 11-β hydroxysteroid dehydrogenase 2 (*11β-hsd2*). In vertebrates, *11β-hsd2* converts the active ligand cortisol to cortisone, an inactive form unable to bind to glucocorticoid receptors; in fish however, it presents an additional function as a key step in the biosynthesis of the major fish androgen 11-ketotestosterone (11-KT). In fish, *11β-hsd2* is important for the masculinization of the gonad (Nagendra Prasad et al., 1999). Interestingly, mammals had the greatest number of TREs present in the *11β-hsd* gene ($n = 7$), compared to *S. tropicalis* ($n = 4$) and *O. latipes* ($n = 4$). Seeing as THs can directly regulate these steroidogenic genes as well as indirectly via AR, there is a calculated level of redundancy suggesting that TH signaling plays a crucial role in androgen signaling. These findings in mammalian and non-mammalian species propose a direct role for THs in steroidogenesis, which will be examined further in later sections.

4.2. Androgenic regulation of TH-related receptors and enzymes

Recent studies have demonstrated that *tr* transcript levels and distributions within testes are responsive in turn to androgen fluctuations. Filby et al. (2007) demonstrate that flutamide, an anti-androgenic compound increases *trβ* expression in male *P. promelas* liver. Similarly, intersex individuals are characterized by different *tr* gene profiles than classic male and female phenotypes in *S. tropicalis* (Langlois et al., 2011). These findings demonstrate the potential for ar to directly regulate the TH axis, a possibility that has been largely overlooked by previous studies. Using *in silico* modeling, we confirmed that androgen response elements (AREs; *i.e.*, GAGGA, TGTTCT, TGTTCT/nnnnn/TGTTCT) are present in the promoter regions of deiodinase and tr isoforms of *M. musculus* ($n = 9$), *S. tropicalis* ($n = 5$) and *O. latipes* ($n = 6$) (Fig. 2). AREs are present in deiodinases in the three species ($n = 2$–3 per promoter). However, AREs are absent in the *dio3* promoter region of *M. musculus* and *O. latipes*, and *dio2* in *S. tropicalis*. In addition, more AREs are observed in the promoter regions of *tr*-isoforms in *M. musculus* ($n = 7$) compared to *S. tropicalis* ($n = 2$) and *O. latipes* ($n = 3$). No AREs were identified in the promoter of *trα* in *S. tropicalis* and *trβ* of *O. latipes*. These differences in response element distribution suggest varying degrees of crosstalk between genes as well as a potential mechanism to preserve TH signaling without input from other endocrine axes. Androgen antagonists can modify the activation state of ar. Thus, if AREs are present in the promoter region of TH-related genes, we can expect disrupted androgen signaling to result in induced changes in the expression of that gene. Langlois et al. (2011) observed no changes in *trα* mRNA brain and liver tissues of chemically induced intersexed *S. tropicalis*, consistent with the absence of AREs in the promoter region of this receptor.

Similarly, Nelson and Habibi (2009) demonstrated that *trβ* mRNA did not increase with T and 11-KT treatment in *C. auratus*. These findings further demonstrate androgens can directly regulate *tr* expression; thus it is therefore necessary to examine ar auto-regulation to better understand cross-regulation between the androgen and TH axes.

In silico modeling also demonstrates that androgens regulate ar isoforms and have considerable transcriptional influence on other genes involved in androgen biosynthesis. AREs were identified in the *ar* promoter region in fish and tetrapods ($n = 2$ per species). In addition, AREs are present in *arβ* in fish ($n = 3$). Androgens also regulate genes involved in steroidogenesis. The promoter region of *srd5β*, the enzyme responsible for the production of 5β-DHT (Langlois et al., 2010b), is characterized by higher abundance of AREs in *S. tropicalis* ($n = 17$) compared to *M. musculus* ($n = 1$) and *O. latipes* ($n = 1$), highlighting an important regulatory role for *srd5β* in androge biosynthesis in frogs.

In summary, *in silico* analysis provides novel insight into mechanisms of direct crosstalk between the androgen and TH axes. The presence of AREs and TREs in promoter regions of *ar* and *trs* makes it possible for androgen- and TH-related genes to be directly auto-regulated by their own nuclear receptors as well as indirectly by the other endocrine receptors. Seeing as THs can manipulate *ar* expression, the identification of AREs in promoter regions of androgen-related genes also reveals genes susceptible to TH level changes. Consequently, fluctuations in circulating androgens or THs have the ability to disrupt each transcriptional regulation of either hormonal axis. These findings strengthen the proposal of crosstalk between the androgen and TH axes and highlight that THs can have considerable influence in the sexual ontogeny of male vertebrates. Promoter analysis reveals potential evolutionary trends and species-specific differences in TRE and ARE profiles. Further experimental confirmation of promoter binding is needed to distinguish between species-specific differences, as well as between genomic and non-genomic regulation. In particular, transcriptional evidence between androgen and TH axes is lacking in avian and reptilian species.

5. Putative role of THs in the gonadal fate

Sex-determining-genes are epitomized by linkage to a specific sex chromosome or by having considerable influence in sexual differentiation and gonadal formation (Morais da Silva et al., 1996; Nakamura, 2009, 2010). Given that the expression of the sex-determining-genes occurs primarily during a relatively brief and sensitive period of development, the susceptibility of these genes for transcriptional interference by exogenous hormones or endocrine disrupting chemicals (EDCs) represents an important area of future study.

Responsible for male gonadal differentiation, the sex-determining region Y gene (*sry*) activates male-specific transcription factor sex-determining region Y box 9 (*sox9*), which induces the bipotential cells of the testes to differentiate into testicular Sertoli cells (Kent et al., 1996; Morais da Silva et al., 1996). Conversely, inhibition of *sox9* expression results in differentiation into ovarian granulosa cells in mammals (Kent et al., 1996; Kobayashi et al., 2005; Piprek, 2009). In amphibians (Dumond et al., 2011), reptiles (reviewed in Peter (2011) and Rhen and Schroeder (2010)), birds (Reinert and Wilson, 1996; Smith and Sinclair, 2001) and mammals (Kent et al., 1996; Kobayashi et al., 2005; Piprek, 2009) the expression of *sox9* is sexually dimorphic, with higher mRNA levels observed in males. Fish and amphibians present a more interesting case. *sox9* is expressed in both male and female Japanese wrinkled frogs (*Rana rugosa;* Suda et al., 2011). Similarly, in fish, *sox9* isoforms are expressed in both testes and the ovaries (Kluver et al., 2005; Liu et al., 2007; Santos et al., 2007). However, Kluver et al. (2005) show that *sox9a* is ovary-specific while *sox9b* is exclusively expressed in the testes, demonstrating significant differences in the gonadal expression of sox9 isoforms. Despite differences in *sox9* expression between species, studies suggest *sox9* may interact with the TH axis (Fig. 3).

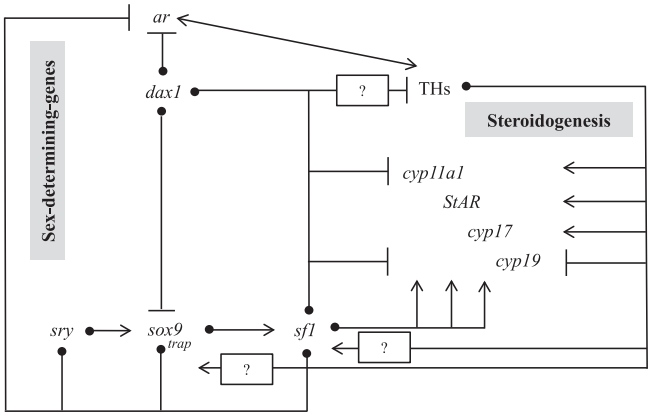


Fig. 3. Proposed mechanisms of TH-regulation in molecular genetic events surrounding the initiation of sexual determination and differentiation in males. Arrows indicate activation, whereas solid lines indicate inhibition. Boxes with question marks signify potential connections that require further investigation. In the male gonad, *sry* activation is followed by *sox9* activation by processes that possibly involving nuclear receptors. Interactions between, *sox9* and TRAP suggest TRAP may be a part of a coactivator complex, regulating its expression. In turn, *sox9* activates *sf1*, which activates steroidogenic enzymes. It has been suggested that *dax1* could act at multiple steps inhibiting and activating gene expression. Similarly, TH could act in multiple steps and levels. *sry*, *sox9*, and *sf1* have all been shown to independently repress *ar* expression. Abbreviations: AR, androgen receptor; *cyp11a1*, P450 cholesterol side-chain cleavage enzyme; *cyp17*, Cytochrome P450 17α Hydroxylase; *cyp19*, aromatase; *dax1*, DSS–AHC on X chromosome gene 1; *sf1*, steroidogenic factor-1 gene; *sox9*, SRY box-9 gene; *sry*, sex-determining region Y chromosome gene; *StAR*, steroidogenic acute regulatory protein; THs, thyroid hormones; TRAP, thyroid receptor associated protein.

TH-related transcription factors may influence *sox9* expression in mammalian testes, as *sox9* has been shown to interact with TRAP230, a component of the TH receptor associated protein (TRAP) to govern developmental processes (*D. rerio* (Rau et al., 2006); *H. sapiens* (Zhou et al., 2002)). Moreover, this member of the mediator complex expressed in both testes and ovaries mediates effects on either sex axis (Treuter et al., 1999). Indeed, it has been implied that TRAP proteins are required for testicular differentiation (wang et al., 2002; Zhou et al., 2002). Further evaluation of potential sex-differences in this transcription factor is required. The possibility of shared co-activating factors between the two axes suggests possible cross-regulation between sex differentiating genes and trs. Outside the reproductive axes, studies show that THs influence *sox9* expression (Okubo and Reddi, 2003). Okubo and Reddi (2003) demonstrated that *sox9* expression in *M. musculus* chondrocytes significantly decreases with T4 exposure. Thus, THs may also have the potential to negatively regulate *sox9* in vertebrate gonadal structures; however, further investigation into the co-regulation by THs and *sox9* in non-mammalian species is required.

Furthermore, *sox9* stimulates the nuclear receptor steroidogenic factor 1 (*sf1*), which in turn activates a suite of genes required for gland functioning and hormone biosynthesis. Encoded by the NR5A1 gene, *sf1* has an important role in sexual differentiation because it is expressed in primordial organ cell clusters fated to differentiate into mammalian adrenal glands, testes and ovaries (reviewed in Vilain and McCabe (1998) and Zhao et al. (2001)). This transcription factor is primarily expressed in the Leydig cells; however, Parker and Schimmer (2002) provided a list of putative

targets for *sf1* in the mammalian system, which include both Leydig and Sertoli cells, and their associated transcriptional factors. Expression of *sf1* is associated with the onset of steroidogenesis and is thought to be responsible for the regulation of cholesterol side-chain cleavage cytochrome P450 (P450scc; *cyp11a1*), steroidogenic acute regulatory protein (*StAR*), 17α-hydroxylase (*cyp17*) and 3β-hydroxysteroid dehydrogenase (*3β-hsd*) genes (Parker and Schimmer, 2002). It has been demonstrated that sf1 binds to AGGTCA-like half sites found in all steroidogeneic *cyp*-promoters (reviewed in Honkakoski and Negishi (2000)). NF5A1 recognition sequences have been identified in mammalian *StAR* promoter regions (Hiroi et al., 2004a,b; Sugawara et al., 1997a,b). Furthermore, Manna et al. (2001a,b) demonstrate that T3 exposure increases *StAR* mRNA levels, and moreover, that T3-mediated *StAR* responses are dependent on *sf1* expression, as inhibition of the latter by dosage sensitive sex reversal (DSS), adrenal hypoplasia congenita (AHC) critical region on the X chromosome, gene 1 (*dax1*) considerably diminishes T3 mediated regulation. Furthermore, TH treatment decreases aromatase activity within Sertoli cells of *R. norvegicus*, whereas in a cell line with mutations in *sf1* promoters results in no changes, indicating that the *sf1* response element must be present with its integrity preserved for T3 to have any effect (Catalano et al., 2003). Therefore, it establishes *sf1* as a mediator of T3 during male sexual differentiation. It has been noted that there is a lack of TRE in the *sf1* gene in mammalian species (cited in Manna et al., 2001a,b) suggesting other cofactors are required for THs to influence *sf1* expression or THs may even act in a species-specific manner. Using plasma resonance techniques, Valadares et al. (2008) demonstrated that *trβ* is capable of binding to *sf1* regulatory activator peptides. Coinciding with higher *trβ* concentrations within the testes, it appears that THs can regulate *sf1* through regulatory complexes. However, it is possible that trs may regulate *sf1* expression by binding to different promoter regions. These studies emphasize secondary control by THs, where one gene component upstream mediates a multitude of effects further downstream, efficiently and effectively enabling THs to regulate a number of processes and gain access to other endocrine axes.

In mammalian species, *dax1* expression decreases with testes differentiation (Iyer and McCabe, 2004; Parker and Schimmer, 2002; Vilain and McCabe, 1998). In contrast, *dax1* mRNA levels do not decrease during testes differentiation in reptiles (reviewed in Rhen and Schroeder (2010)) and birds (Smith and Sinclair, 2001). Exhibiting a greater degree of conservation across vertebrate species, *dax1* works in parallel with the *sry* network to regulate testicular cell differentiation. Studies have shown *dax1* expression negatively regulates *sf1* expression in vertebrates (Iyer and McCabe, 2004; Park et al., 2005). Similarly, studies show that *dax1* expression inhibits *sox9* transcription in *M. musculus* (Ludbrook et al., 2012). dax1 has considerable influence over gonadal development, as the receptor interacts with other nuclear receptors within gonadal tissues.

Studies demonstrate that *dax1* can regulate TH-related gene expression. *In vitro* studies indicate that the protein *dax1* can bind to and negatively regulate the expression of *trβ* in vertebrates (Moore et al., 2004; Sugawara et al., 1997b; Valadares et al., 2008). Therefore, dax1 has the potential to limit the masculinizing activities of TRs. Studies show that the two nuclear receptors work in an antagonistic manner on different regulatory levels with one another to govern male sexual differentiation. Expression of *dax1* also regulates factors involved with gonadal maintenance as response elements for *dax1* are found in *ar* (mammalian cell line (Holter et al., 2002). Indeed, the dax1 receptor binds to the promoter region of *ar* and represses gene expression in mammals (Holter et al., 2002; Zamoner et al., 2011). Conversely, studies show that TH treatment induces corresponding increases in *ar* expression (*P. sicula* (Cardone et al., 2000); *S. tropicalis* (Duarte-Guterman and Trudeau, 2011; Langlois et al., 2011); *P. pustulosus* (Duarte-Guterman et al., 2012); *M. musculus* (Wagner et al., 2008, 2009); *R. norvegicus* (Arambepola et al., 1998; Panno et al., 1996; Sisci et al., 1997)). Moreover, we have confirmed the presence of TREs in the *ar* promoter region as well as in other androgen related genes (Fig. 2). Thus, it appears that *dax1* and THs have antagonistic roles in regulating *ar* gene expression.

In addition, dax1 and trs interfere with androgen biosynthesis by interacting directly with steroidogeneic genes (Fig. 3). Studies show that dax1 represses expression of *cyp11a1* and *StAR* in fish during steroidogenesis (Zhao et al., 2006). dax1 binds directly to the promoter transcription factor recognition elements of *StAR* in mammals, negatively regulating expression (reviewed in Lalli and Sassone-Corsi (2003), Manna et al. (2001a,b) and Park et al. (2005)). THs on the other hand positively regulate *StAR* mRNA expression and activity. Antagonistic crosstalk between these two nuclear receptors highlights potential time sensitive regulatory mechanisms. Critically lacking is data on the effects of hyper- or hypothyroid conditions on various gonadal differentiation genes' mRNA levels (e.g., *sry*, *sox9*, *dax1*, *sf1*, *dmrt1*) as well as evidence of direct interactions, such as analysis of promoter regions of these genes in non-mammalian species (*i.e.*, fish, amphibians, reptiles and birds). The lack of research on non-mammalian species may be a result of greater research focus placed on external environmental factors (*i.e.*, temperature, photoperiod, etc.) regulating sex determination in these species or simply that sex determining genes have not yet been identified in those select species.

6. TH regulation of gonadotropins

Fluctuations in circulating TH concentrations induce subsequent responses in the synthesis, secretion, circulation, metabolism and the physiological action of androgen hormones. The HPG-axis ultimately regulates androgen signaling and biosynthesis. Originating from the hypothalamus, GnRH regulates the biosynthesis and secretion of the gonadotropins: luteinizing hormone (LH) and follicle stimulating hormone (FSH), which are largely responsible for gonadal formation and maintenance of the gonadal structures. Circulating gonadotropins and their physiological effects are typically governed by hypothalamic-hormones and feedback from down-stream products; however gonadotropins are also subject to regulation by other endocrine systems (Fig. 1). Both induced hyper- and hypo-thyroidic conditions alter circulating gonadotropin concentrations, as well as related-gene expression in vertebrates (mammals (Chiao et al., 1999, 2002; Wagner et al., 2008, 2009)). Studies have identified TREs in the GnRH promoter region (Kakar, 1997). In addition, studies demonstrate that decreased TH levels result in an increase in GnRH cell proliferation and (Parhar et al., 2000) and circulating GnRH levels (Chiao et al., 1999; Kent et al., 1996; Wagner et al., 2008, 2009). Moreover, we previously showed that this regulation is bidirectional with gonadotropins altering TH synthesis. Thus, crosstalk at the level of hormone synthesis will have down stream consequences in androgen and TH signaling. Here we will review the interactions of THs with gonadotropins in relation to the male reproductive axis.

Studies show that LH biosynthesis is subject to the influence of THs (Fig. 1), with THs sharing an inverse relationship with the gonadotropin across different regulatory levels. LH induces steroidogenesis in the Leydig cells, which are responsible for the production of potent androgens, such as T. Mendis-Handagama and Ariyara demonstrated that TH exposure stimulates Leydig cell testosterone production (2004). Hypothyroid conditions decrease circulating LH concentrations in vertebrates (*G. gallus* (Jacquet et al., 1993); *R. norvegicus* (Chiao et al., 1999; Maran, 2003; Valle

et al., 1985; Wagner et al., 2008, 2009)). Similarly, Cristovao et al. (2002) demonstrated that severe hypothyroidism induces decreased proliferation of leydig cells. For mammalian species, TREs are present within the promoter of the LH receptor (LHR) gene (Tsai-Morris et al., 1993) and increases in LHR expression are observed in *M. musculus* Leydig cells exposed to T3 (Manna et al., 2001a). This demonstrates that THs can directly regulate LH actions and provides an indirect mechanism of action in which THs can impact androgen biosynthesis. Interactions between this gonadotropin and THs suggest the existence of vertebrate wide crosstalk between the two axes. However, further investigation into mechanisms of direct similarly, cross-regulation studies in demonstrate non-mammalian species is required.

Similarly, studies demonstrate that THs interfere with the FSH pathway, which is responsible for the proliferation and functioning of Sertoli cells. Found within the semiferous tubules, Sertoli cells are responsible for spermatogenesis. The mechanistic influence of FSH on Sertoli cells in mammals has been well reviewed by Holsberger and Cooke (2005). The gonadotropin shares an inverse relationship with THs (Fig. 1), with FSH concentrations increasing in hypothyroid conditions. Studies reveal that serum concentrations of FSH increase in thyroidectomized male *O. aerius* compared to control individuals (Anderson et al., 2003; Parkinson et al., 1995). Accordingly, studies have demonstrated that TH fluctuations influence the rate and cessation of Sertoli cell proliferation (Cristovao et al., 2002; Marchlewska et al., 2011). TSH has been shown to positively bind to the promoter region of the FSH receptor in birds (Dobozy et al., 1982) ensuring the initiation of spermatogenesis and Sertoli cell proliferation. Thus, it has been proposed that the FSH partially mediates the effects of TSH in male sexual development. A number of studies attribute subsequent changes in steroid- and androgen-related gene expression to fluctuations in FSH and LH induced by TH interference. Given that THs induce responses in LH and FSH production and secretion, THs have the potential to indirectly impact steroid biosynthesis.

7. Regulatory role of THs in steroidogenesis

Steroidogenesis is a complex pathway in which hormones involved in the male and female reproductive-axes, as well as from the stress-axis are produced. Many enzymes are involved in cholesterol degradation which leads to androgen production. Interference in the functioning of one of these enzymes could ultimately lead to changes in T production. Studies have identified interaction between THs and the steroidogenic enzymes responsible for androgen biosynthesis. Fig. 4 illustrates androgen biosynthesis, a section of the steroidogenesis pathway and includes a list of studies that investigate the effects of TH regulation on the steroidogenic enzymes.

Cholesterol serves as the precursor of androgens and is metabolized into intermediate forms in a series of steps to ultimately produce testosterone. StAR mediates transport of cholesterol to the P450scc, which converts cholesterol to pregnenolone, both respond to changes in TH concentrations (Chiao et al., 2002; Kim et al., 2011; Manna et al., 2001a). *In vitro* T3 exposure increase mRNA levels of *cyp11a1* and *StAR* (*M. musculus* (Kim et al., 2011; Manna et al., 2001a)), conversely however, exposure to the goitrogen propylthiouracil decreases mRNAs and enzymatic activity (Cooke et al., 2004). Thus, THs have considerable influence over the initiation of steroidogenesis, as StAR and P450scc are limiting factors in androgen production (Miller and Bose, 2011).

3β-hsd is responsible for catalyzing the synthesis of intermediate progesterone and androgen in the steroidogenesis pathway. *In vitro* TH treatments increase in 3β-hsd activity in fish (Nagendra Prasad et al., 1999) and mammals (Antony et al., 1995; Kim et al., 2011). *In vivo* studies show that induced hypothyroid conditions decrease *3β-hsd* activity and mRNA levels in mammals (Antony et al., 1995). Whereas, Rasheeda et al. (2005) conversely highlight no changes in gene expression of *3β-hsd* under hypothyroidic conditions in testes in the air-breathing catfish (*Clarias gariepinus*). The enzyme *cyp17* is responsible for the conversion of pregnenolone and progesterone intermediates to 17α-hydroxypregnenolone and 17α-progesterone, respectively, and it also converts androstenedione from the previous precursors. Acute T3 treatments result in an increase in *cyp17* expression in Leydig cells of *R. norvegicus* (Manna et al., 2001a). Fish with hypothyroidism demonstrate no changes in expression of *cyp17* in testes were observed (*C. gariepinus* (Rasheeda et al., 2005)). T3 exposure enhances *3β-hsd* and *cyp17* transcript levels in accordance with the presence of presumptive TREs in the promoter. Suda et al. (2011) identified AREs in the promoter of *cyp17a1* in *R. rugosa* tadpoles. THs are a putative regulator of *ar* expression (Fig. 2), consequently ar could serve as an additional transcriptional mediator for THs in androgen biosynthesis. Further experimental confirmation of promoter binding in steroidogenesis is needed to distinguish between direct and indirect mechanisms of TH regulation.

The final steps of androgen biosynthesis are catalyzed by 17β-hydroxysteroid dehydrogenase (*17β-hsd*), the enzyme responsible for producing androstenediol and T. Consequently, 17β-hsd has high protein levels in testes and seminal vesicles. *In vivo* and *in vitro* studies demonstrate that *17β-hsd* mRNAs decrease following diminished circulating TH concentrations in testes of *R. norvegicus* (Biswas et al., 1994). After androstenedione is reduced to T by 17β-hsd in Leydig cells, Sertoli cells are then responsible for the reduction of T into the potent androgen 5α-DHT by 5α-reductases. In fig. 2, we show that TREs are present in the promoter region of 5α-reductases. TH treatment enhances 5α-reductase expression and activity within the testes (Fig. 4; *P. pustulosus* (Duarte-Guterman et al., 2012); *S. tropicalis* (Duarte-Guterman et al., 2010; Duarte-Guterman and Trudeau, 2011); *R. norvegicus* (Kala et al., 2002; Ram and Waxman, 1990)), increasing 5α-DHT concentrations. Conversely, goitrogen exposure inhibits 5α-reductase in *R. norvegicus* (Chiao et al., 2002; Kala et al., 2002; Ram and Waxman, 1990). To attest that THs influence 5α-reductase expression, studies have demonstrated that induced hypothyroid conditions decrease T concentrations (fish (Swapna et al., 2006), birds (Akhlaghi and Zamiri, 2007; Weng et al., 2007), and mammals (Chiao et al., 2002, 1999; Maran, 2003; Wagner et al., 2008, 2009)). This indicates that high TH concentrations favor the formation of androgens via the increasing of androgen-related enzyme activity. Therefore, THs represent potentially a local regulatory mechanism in the testes.

In fish, unlike mammals and other tetrapods, 11-KT is the most important physiological androgen in males (Borg and Mayer, 1995). Recently, the androgenic potency of 5α-DHT and 11-KT was compared in juvenile *P. promelas*, revealing that both androgens have comparable power in their ability to activate somatic growth, the expression of secondary sexual characteristics and spermatogenesis in males (Margiotta-Casaluci and Sumpter, 2011). Thiourea treatment results in a reduction of 11-KT in *C. gariepinus* serum and tissue levels (Swapna et al., 2006), thus providing evidence that THs regulation of androgen production is conserved across vertebrates. All together, these findings demonstrate that steroidogenesis is responsive to TH-level fluctuations, and each intermediate enzymatic step of androgen biosynthesis may interact with THs (Fig. 4). In vertebrates, androgen and TH responses appear to be fixed across *in vivo* and *in vitro* studies, highlighting the extent to which transcriptional crosstalk is conserved.

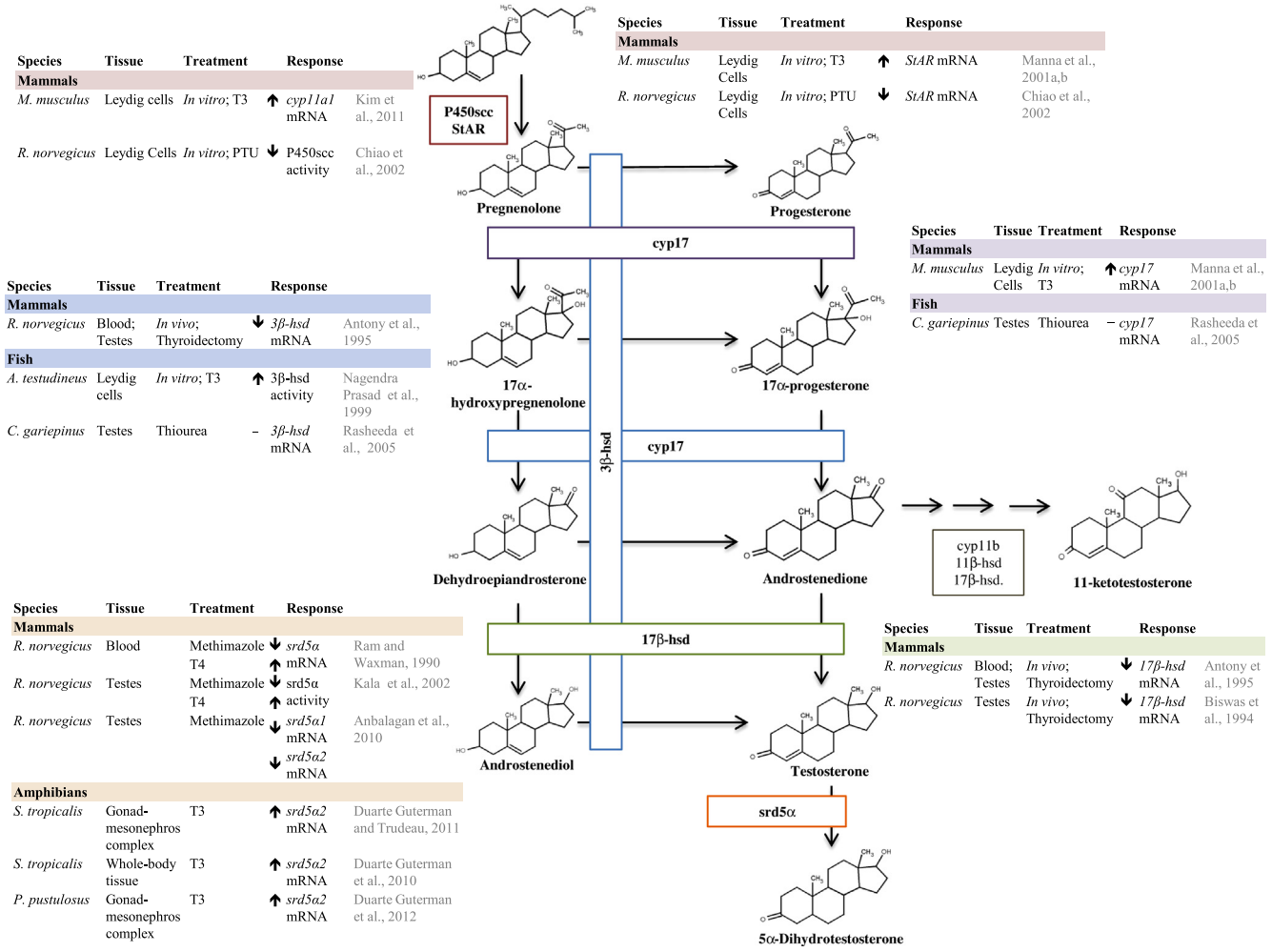


Fig. 4. Schematic representation of the effects of THs on the steroidogeneic enzymes involved in androgen biosynthesis within the testes. Chart includes a list of endpoints compiled from TH and goitrogen experiments investigating TH regulation on steroidogenic enzymes; they are provided beside each respective enzymatic steps. An upward pointing arrow indicates an increase in gene expression or enzyme activity, whereas a downward pointing arrow indicates a decrease in gene expression or enzymatic activity and a flat line indicates no observable change in endpoints. Results highlight possible evolutionary differences between species as well as demonstrating vertebrate wide patterns in enzyme response. Interestingly, THs demonstrate direct interactions and regulation of steroidogenic mRNA in testes. Moreover, considerable redundancy in possible sites of action exists. Abbreviations: StAR, steroidogenic acute regulatory protein; cyp11a1, P450 cholesterol side-chain cleavage enzyme; cyp17, 17a hydroxylase/17,20 lyase; 3b-HSD, 3-beta-hydroxysteroid dehydrogenase; 17b-HSD 17-beta-hydroxysteroid dehydrogenase.

8. Significance

Researchers acknowledge that the various endocrine axes work in an integrative fashion yet we continue to view and examine them as separate entities. We believe this has contributed to an oversight in the degree of crosstalk between the androgen and TH-axes as well as between other hormone pathways. A number of review papers have examined the complex interactions of THs with the androgen ($n = 4$), estrogen ($n = 5$), and stress ($n = 2$) pathways (Bagamasbad and Denver, 2011; Cooke et al., 2004; Denver, 2009; Doufas and Mastorakos, 2000; Habibi et al., 2012; Jannini et al., 1999; Leatherland et al., 2010; Maran, 2003; Peter, 2011; Vasudevan et al., 2002; Wagner et al., 2008, 2009; Wajner et al., 2009; Zamoner et al., 2011). Based on the degree of crosstalk between these systems and the influence of THs in each, we propose that the TH-axis orchestrates and responds to feedback from other hormone pathways rather than working in parallel to them (Fig. 5). Other studies have also suggested that THs play a central role in hormonal signaling, for example Hayes (1997) placed THs as the center of a regulatory network of other hormones (e.g., estrogen, T and growth hormones, etc.) that interact to induce developmental changes in anurans.

Many examples of the versatile regulatory role of the TH axis have been highlighted in this review: (i) THs play a crucial role in early development influencing many developmental programs (e.g., sexual differentiation); (ii) THs are responsible for seasonal changes in various species (e.g., gonadal recrudescence), thus demonstrating a continuous active role throughout the life span of an organism; (iii) the regulatory roles of THs are tissue specific, however THs, TR and deiodinases are universal to every tissue; and (iv) TH-related machinery is characterized by a sex bias, with greater TH expression associated with the male phenotype. These conclusions along with the findings of previous studies show that the TH axis is highly integrated with other endocrine networks and through crosstalk influences an organism's development on many spatiotemporal scales. Therefore, we propose greater emphasis be placed on the regulatory potential of the TH axis and crosstalk with other endocrine axes in vertebrate species.

9. Conclusion

In summary, this review illustrates transcriptional, cellular and hormonal responses to TH treatment within the male

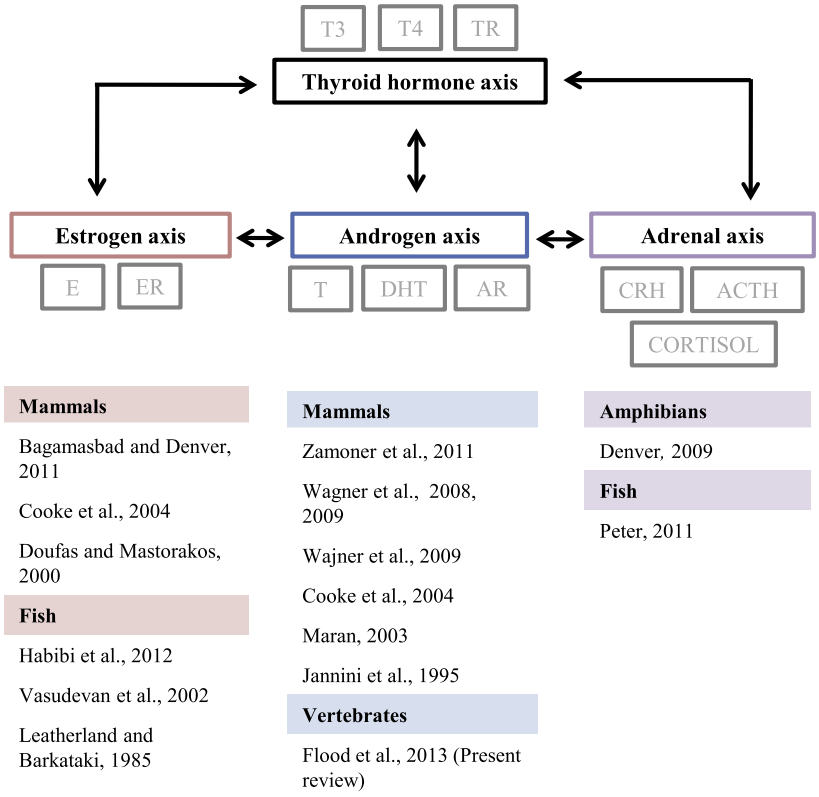


Fig. 5. Proposed mechanisms of TH-regulation of estrogen, androgen, and adrenal axes. Arrows indicate endocrine interactions. Review papers that have examined the complex interactions of THs with the androgen ($n = 4$), estrogen ($n = 5$), and stress ($n = 2$) pathways are shown. Based on the degree of crosstalk between these systems and the influence of THs in each, we propose that the TH-axis orchestrates and responds to feed back from other hormone pathways rather than working in parallel to them. Abbreviations: E, estrogen; ER, estrogen receptor; T, testosterone; DHT, 5α-dihydrotestosterone; AR, androgen receptor; CRH, corticotropin-releasing hormone; ACTH, adrenocorticotropic hormone; T3, triiodothyronine; T4, thyroxine; TR, thyroid receptor.

reproductive-axis. Interestingly, within the various processes governing male sexual development considerable redundancy exists in androgen and TH-mediated actions to ensure normal testicular development. Moreover, an androgen and TH crosstalk seems to be conserved across vertebrate species. Overall, the findings provide support for the hypothesis that THs are important contributing factors in male sexual development and weaken the proposal that the female reproductive axis solely mediates TH effects. In summary, there is strong evidence that THs directly regulate male reproductive development by (1) coordinating sex-determining-genes that set the reproductive ontogeny in favor of males; (2) enhancing steroidal gene expression, which contributes to stimulated androgen synthesis; (3) enhancing androgen responsiveness by increasing *ar* expression; and (4) directly binding to response elements within select androgen-related genes. Moreover, we identified AREs in the promoter regions of TH-related genes which suggest potential for the androgen axis to regulate components of TH biosynthesis. The consequences of androgen-mediated regulation of the TH axis in vertebrate development should be investigated further in future studies.

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