Chapter 5

Thyroid Hormones and Reproduction in Fishes

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SUMMARY
Thyroid hormones (THs) appear to be involved in various aspects of growth and development in fishes, but their potential role in reproduction is not clear. A large number of published studies have attempted to show a relationship between THs and the reproductive cycle in fishes, but differences in species and their reproductive strategies, and methods used to analyze changes in TH regulation and signaling, have likely contributed to conflicting results. There is evidence to suggest that changes in thyroid function do take place during the reproductive cycle, but whether these changes reflect a direct role in reproductive events or merely the corresponding changes in energy utilization, decreased growth, and other factors accompanying the onset of the reproductive cycle is not apparent. This chapter reviews the current understanding of the regulation and signaling pathways of THs and their potential involvement in fish reproduction.

1. INTRODUCTION

Thyroid hormones (THs), the iodinated thyronines triiodothyronine (T_3) and thyroxine (T_4), are thought to be involved in a large number of biological processes in fishes, including metabolism, growth, development, and reproduction. However, very few definitive roles have actually been attributed to THs. In many cases, the literature is rife with experimental evidence both substantiating and challenging various proposed functions of THs. In mammals, maintenance of metabolic homeostasis could be considered the major function of THs, and likely the best known. Thyroid hormones are also involved in a number of physiological and developmental processes, and the involvement of T_3 in many aspects of mammalian metabolism and temperature regulation is well established. The role of THs in thermogenesis likely evolved in concert with endothermy, and it is evident that thermogenesis is closely tied to metabolic regulation. This suggests that THs were involved in metabolic regulation prior to the evolution of endothermy, and that the role of THs changed with the development of endothermy. Thus, there is a possibility that metabolic processes in poikilotherms involve THs, even though metabolic rates in these animals are primarily driven by ambient temperature. Although a number of studies have described effects of THs on metabolism in fishes, the evidence is generally conflicting, and the doses of T_4 or T_3 administered in many of these studies are in the pharmacological range (see Leatherland, 1994, for review). Nevertheless, the possibility exists that T_3 plays multiple permissive roles in metabolic regulation in fishes, as it does in mammals.

Thyroid hormones appear to play a major role in vertebrate development. In particular, a large body of evidence suggests that THs are strongly associated with neural development and maturation. In mammals, T_3 is essential for neuronal proliferation, migration, synaptogenesis, and myelination during brain development (Howdeshell, 2002; Horn & Heuer, 2010). Developmental abnormalities such as mental retardation, deaf-mutism, and motor disorders can result from hypothyroidism in the developing human fetus, but these problems can be partially reversed by TH replacement after birth (Hulbert, 2000). Further, in cases where the mother or developing fetus are hypothyroid, there is neurological impairment of the fetus (congenital cretinism), and the degree of impairment is correlated with the level of hormone insufficiency (LaFranchi, 1999). In fishes, neurological effects of THs also occur. Hindbrain formation in zebrafish (*Danio rerio*) embryos is impaired when the TH receptor (TR) isoform, TRα1, is overexpressed (Essner, Johnson, & Hackett, 1999). Moreover, THs induce programmed cell death (apoptosis) of ultraviolet-sensitive (UVS) cone cells and corresponding loss of sensitivity to ultraviolet (UV) light over most of the neural retina of rainbow trout (*Oncorhynchus mykiss*) during development (Browman & Hawryshyn, 1992; 1994; Kunz, Wildenburg, Goodrich, & Callaghan, 1994; Deutschlander, Greaves, Haimberger, & Hawryshyn, 2001; Allison et al., 2003;

Hawryshyn, Martens, Allison, & Anholt, 2003; Allison, Dann, Veldhoen, & Hawryshyn, 2006; Kunz, 2006; Veldhoen et al., 2006; Raine & Hawryshyn, 2009; Raine, Coffin & Hawryshyn, 2010).

One well-studied aspect of TH functions involves the events surrounding organ restructuring in poikilothermic vertebrates, either during embryonic development or during metamorphosis. Thyroid hormones control the initiation of metamorphosis of tadpoles to adult amphibians, and are directly associated with many tissue-specific changes that take place during this metamorphic process (see Buchholz, Paul, Fu, & Shi, 2006; Brown & Cai, 2007, for review). Similarly, flatfishes of the order Pleuronectiformes undergo a metamorphic transformation during development that is directly regulated by THs. These flatfishes transform from a bilaterally symmetrical, pelagic larval stage into a benthic, asymmetrical juvenile. Major behavioral, physiological, and morphological changes take place in these fish during metamorphosis and this process can be stimulated with the addition of exogenous T_4, or inhibited with the addition of goitrogens, drugs that inhibit production of endogenous THs (Miwa & Inui, 1987; Power et al., 2001). Thyroid hormones also may play a role in other developmental stages of fishes that are not considered metamorphic, but do involve substantial changes in morphology, behavior, and physiology (Hoar, 1988; Lam, 1994; Leatherland, 1994; Power et al., 2001; Campinho, Silva, Sweeney, & Power, 2007). These include the transition of embryonic/larval fish stages that feed exclusively from yolk reserves containing THs of maternal origin, to the free-feeding juvenile stage as well as smoltification, the transformation of salmonid fishes (trout and salmon) during migration from shallow, freshwater streams to the ocean.

In all vertebrates, a role for THs in reproduction has been investigated, yet the function of THs, if any, in this process is elusive. In mammals and birds, THs have been linked to changes in day length and the initiation of seasonal reproduction, although the mechanism by which THs act is unknown (Nakao, Ono, & Yoshimura, 2008). In fishes, a role for THs in reproduction has been sought for more than 50 years, and there is still no clear answer. Many fish studies have examined the reproductive cycle with a dominant regulatory function for THs in mind, and evidence that THs could be involved in the process of gonadal maturation in some species has emerged. It may be that, in searching for a major controlling role of THs in reproduction, more specific and understated roles of THs have been overlooked. Further, our increasing knowledge of TH regulation and action suggests that classical means of analyzing TH action and involvement in reproduction may need to be reassessed. This chapter reviews the current understanding of TH physiology and the relationship between THs and reproduction in fishes.

2. THYROID HORMONE DELIVERY

2.1. Regulation of Circulating Thyroid Hormone (TH) Levels

The concentration of THs in the blood is generally maintained within a relatively narrow range, especially in mammals where THs are tied to metabolic rate and thermogenesis. The regulatory pathway responsible for maintaining stable levels of circulating THs is referred to as the hypothalamic–pituitary–thyroid (HPT) axis (Figure 5.1). The model for TH regulation is similar for all vertebrates, but in mammals it begins with the release of thyrotropin-releasing hormone (TRH) from the hypothalamus. Thyrotropin-releasing hormone is a tripeptide that is highly conserved across all vertebrate groups, although its functions at least with respect to TH homeostasis seem to be dissimilar (Zoeller, Tan, & Tyl, 2007). In mammals, TRH stimulates the release of thyrotropin (TSH) from thyrotropes in the anterior pituitary gland (Zoeller, Tan, & Tyl, 2007). Thyrotropin-releasing hormone is transported directly from the hypothalamus to the adenohypophysis region of the pituitary gland through the hypophysial portal blood system, which is found in mammals (Zoeller et al., 2007). Teleost fish lack this portal blood system and instead the adenohypophysis is directly innervated by hypothalamic neurosecretory fibers (Peter, Yu, Marchant, & Rosenblum, 1990; Leatherland, 1994). In mammals, TRH controls blood levels of TSH through binding to a TRH receptor on a thyrotrope, which results in activation of the phosphatidyl inositol-protein kinase C pathway, and initiates the synthesis and release of TSH (Rondeel et al., 1988; Bogazzi et al., 1997). Also in

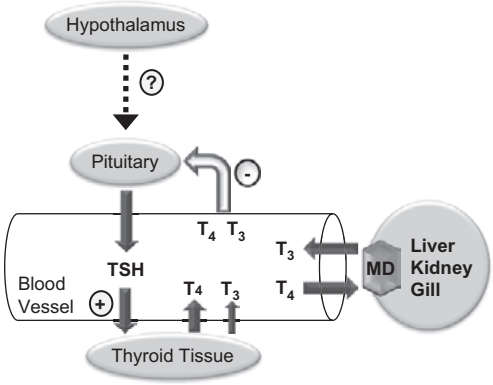

FIGURE 5.1 Simplified diagram demonstrating the regulation of plasma thyroid hormones (THs) in fishes through the hypothalamic–pituitary–thyroid (HPT) axis. Release of thyrotropin (TSH) from the anterior pituitary stimulates thyroxine (T_4) and lesser amounts of triiodothyronine (T_3) to be synthesized and released from the thyroid tissue. Most of the T_3 circulating in the bloodstream comes from deiodination of T_4 by monodeiodination enzymes (MD) in peripheral tissues. High plasma TH levels decrease TSH release through negative feedback. The regulation of TSH release may involve an unidentified hypothalamic factor.

mammals, somatostatin (SS) inhibits TSH secretion in a similar manner and acts as a negative control of TH levels in the blood (Vtiger, 1987). Although several studies have attempted to show the effects of TRH on either TSH secretion or T_4 and T_3 levels in fishes, the results regarding the possible involvement of SS and TRH in thyroidal control have been ambiguous (see Leatherland, 1994, for review). Recently, isolated pituitary cells from bighead carp (*Austichthys nobilis*) have been shown to increase TSH mRNA levels after exposure to TRH (Chatterjee, Hsieh, & Yu, 2001). Additionally, cell-surface TRH receptors have been identified in a fish pituitary and they are structurally similar to those of mammals (Harder et al., 2001). In anurans, corticotropin-releasing factor (CRF), not TRH, appears to stimulate TSH release, and there is some evidence that CRF could play a similar role in fishes (Larsen, Swanson, Dickey, Rivier, & Dickhoff, 1998; Okada et al., 2007). However, there is also evidence that the release of TSH from the thyrotropes of the pituitary gland of some teleost fishes may be under inhibitory hypothalamic control, and this has been demonstrated by hypothalamic lesioning and pituitary transplantation studies (see Leatherland, 1994, for review). However, the potential hypothalamic factors responsible for this inhibitory action have still not been fully identified in fishes.

In teleost fishes, as in mammals, TSH appears to be the main factor regulating TH release from the thyroid follicles (Figure 5.1). Thyrotropin is one of three glycoprotein hormones in the pituitary gland that share a common alpha subunit (glycoprotein hormone subunit α (GPHα)) together with a beta subunit that determines the hormone's biological activity; i.e., the TSHβ subunit for TSH (Zoeller, Tan, & Tyl, 2007). Sequencing of the beta subunit of the TSH gene in several fish species has demonstrated a highly conserved DNA sequence (Ito, Koide, Takamatsu, Kawauchi, & Shiba, 1992; Pradet-Balade, Schmitz, Salmon, Dufour, & Quérat, 1997; 1998; Martin, Wallner, Youngson, & Smith, 1999; Yoshiura, Sohn, Munakata, Kobayashi, & Aida, 1999; Chatterjee, Hsieh, & Yu, 2001; Wang, Zhou, Yao, Li, & Gui, 2004; Lema, Dickey, Schultz, & Swanson, 2009). In several of these species, TSHβ expression was detected in the pituitary and a number of other tissues including the liver, kidney, testis, and ovary (Wang et al., 2004; Lema et al., 2009). Extra-pituitary expression of TSHβ was unexpected and current research is aimed at understanding the functional significance of this finding.

In the last several years, TSH receptor (TSH-R) cDNAs have been cloned from tissues of the striped bass (*Morone saxatilis*) (Kumar et al., 2000), amago salmon (*Oncorhynchus rhodurus*) (Oba, Hirai, Yoshiura, Kobayashi, & Nagahama, 2000; 2001), sunrise sculpin (*Pseudoblennius cottoides*) (Kumar & Trant, 2001), African catfish (*Clarias gariepinus*) (Vischer & Bogerd, 2003), European sea bass (*Dicentrarchus labrax*) (Rocha et al., 2007), and channel catfish (*Ictalurus punctatus*) (Goto-Kazeto, Kazeto, & Trant, 2009). Thyrotropin receptors are 30 kDa dimeric proteins transmembrane G-protein-coupled receptors belonging to the glycoprotein hormone receptor family, which includes the luteinizing hormone (LH) receptor (LH-R) and follicle-stimulating hormone (FSH) receptor (FSH-R) (Vassart, Pardo, & Costagliola, 2004). Thyrotropin receptors are expressed solely in thyrocytes of amago salmon, whereas TSH-R expression has been found to be abundant not only in thyroid tissue but also in gonads of the striped bass, European sea bass, sunrise sculpin, African catfish, and channel catfish (Kumar et al., 2000; Kumar & Trant, 2001; Vischer & Bogerd, 2003; Rocha et al., 2007; Goto-Kazeto et al., 2009). Thyrotropin receptors transcripts have also been detected in various other tissues of these fishes, including skeletal muscle, heart, brain, liver, and kidney (Kumar et al., 2000; Vischer & Bogerd, 2003; Rocha et al., 2007; Goto-Kazeto et al., 2009). The presence of TSH-R in thyroid tissue provides further support for the regulatory role of TSH in thyroid function, whereas the presence of TSHβ-subunit and TSH-R in extra-thyroidal tissues suggests that TSH possibly has some as yet unknown functions.

Administration of TSH increases plasma T_4 concentrations and increases thyroid tissue mass and activity in several species of teleost fishes (see Smith & Grau, 1986; Leatherland, 1994; for review see Raine, Takemura, & Leatherland, 2001). Thyrotropin increases iodide uptake by thyrocytes and iodination of tyrosyl residues (Zoeller et al., 2007). Thyrotropin also increases releases of T_4 and T_3 from thyrocytes in both rainbow trout and medaka (*Oryzias latipes*) (Raine et al., 2001) by upregulating the expression of genes involved in TH synthesis and release (Vassart & Dumont, 1992; De Felice, Postiglione, & Di Lauro, 2004).

In the majority of *in-vivo* studies carried out in fishes, TSH does not directly alter plasma T_3 levels, even though plasma T_4 levels are elevated (Leatherland, 1994). Similarly, a corresponding decrease in conversion of T_4 to T_3 commonly occurs when plasma T_3 levels are elevated following the administration of exogenous T_3 (Eales et al., 1990; Eales & Finnson, 1991). This provides strong evidence for the independent regulation of plasma T_4 and T_3 levels (Leatherland, 1994; Leiner & MacKenzie, 2001).

In all vertebrates, relatively high levels of circulating T_4 and T_3 initiate negative feedback mechanisms that result in decreased T_4 and T_3 release from the thyroid tissue and reduced circulating T_4 and T_3 levels (Figure 5.1). In this way, organismal plasma T_4 and T_3 levels are maintained within a reasonably narrow range. In mammals, in addition to stimulating TSH release, TRH is thought to modulate the sensitivity of the pituitary to negative feedback by THs (Greer, Sato, Wang, Greer, & McAdams, 1993). Both T_4 and T_3 negatively regulate the synthesis and release of TSH in the mammalian pituitary and indirectly affect TSH synthesis through changes in TRH synthesis (Bogazzi et al.,

1997). In both mammals and teleosts, THs decrease TSH synthesis and release by binding to nuclear TRs in the thyrotropes. Triiodothyronine is thought to bind to thyrotrope TRs whereas T_4 acts indirectly through intra-pituitary and intra-hypothalamic T_4 to T_3 conversion by monodeiodinating enzymes (Bogazzi et al., 1997). Nevertheless, both T_4 and T_3 directly decrease transcription of the TSHβ-subunit gene in both the mammalian and teleost pituitary (Bogazzi et al., 1997; Pradet-Balade, Schmitz, Salmon, Dufour, & Quérat, 1997; 1998). Recently, human pituitary thyrotropes have been shown to contain TSH receptor transcripts, suggesting the existence of a negative feedback loop enabling downregulation of TSH transcription in the presence of high TSH levels (Prummel et al., 2000; Theodoropoulou et al., 2000; Zoeller et al., 2007).

Three main forms of iodinated thyronines are found in the blood of fish: T_4, T_3, and the inert metabolite of T_4, reverse-triiodothyronine (rT_3) (Figure 5.2). Thyroxine is the main hormone released from the thyroid tissue of teleost fishes, while T_3 is released in lesser amounts (Chan & Eales, 1975; Eales, 1979; Grau et al., 1986; Byamungu, Mol, & Kuhn, 1992; Raine & Leatherland, 1999; 2000; Raine et al., 2001). Traditionally, T_4 was considered a prohormone for T_3, which was thought to be the biologically active form of TH, due to the much higher affinity of nuclear TRs for T_3. However, the recent discovery of a cell surface TR specific for T_4 in mammals suggests that this paradigm may need to be re-evaluated (Bergh et al., 2005). Deiodinating enzymes (deiodinases) located primarily in the liver as well as in TH target tissues are important regulators of vertebrate TH concentrations in the blood and provide either an increased peripheral supply of T_3 or the clearance of both T_4 and T_3 from the circulation (Eales & Brown, 1993; Leatherland, 1994; Köhrle, 1999; Bianco, Salvatore, Gereben, Berry, & Larsen, 2002; Hernandez & St. Germain, 2003; Brown et al., 2005; Nunez, Celi, Ng, & Forrest, 2008) (Figure 5.1). Local tissue deiodination is considered an important mechanism for supplying T_3 in the nervous system, and T_3 levels in a target cell can be increased or decreased by deiodination (Darras, Mol, Van der Geyten, & Kuhn, 1998; Nunez, Celi, Ng, & Forrest, 2008). Enzymatic monodeiodination of T_4 to T_3 involves the removal of one iodine atom from the outer ring of the molecule (Leatherland, 1994; Mol et al., 1998). Reverse T_3 is generated by the removal of one iodine atom from the inner ring of the T_4 molecule (Figure 5.2). The liver, gill, and kidney are responsible for the majority of the deiodination (Figure 5.1), although many other tissues have deiodinase activity (Byamungu et al., 1992; MacLatchy & Eales, 1992; Morin, Hara, & Eales, 1993; Mol et al., 1998).

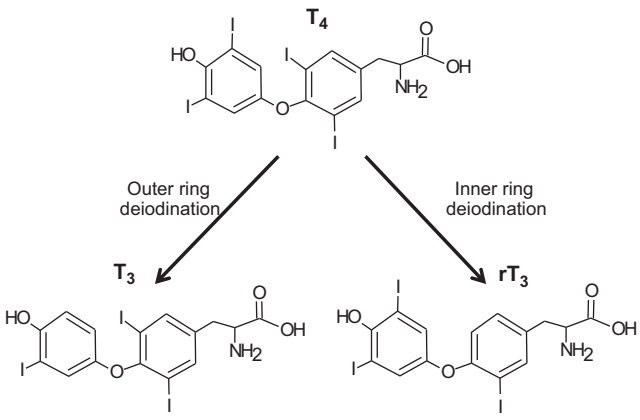

FIGURE 5.2 The chemical structure of the main thyroid hormones in vertebrates showing the position of iodine atoms on the phenolic (tyrosine) rings. Removal of an iodine atom from the outer ring of thyroxine (T_4) by deiodination enzymes creates triiodothyronine (T_3), while removal of an iodine atom from the inner ring results in reverse T_3 (rT_3).

Three types of deiodinase enzyme have been identified thus far in mammals, birds, amphibians, and fishes, namely type I, type II, and type III. In mammals, type I deiodinase is thought to be found in all tissues, but it has the highest activity in the liver, the kidney, thyroid tissue, and the central nervous system (CNS) (Hulbert, 2000). Type I deiodinase can perform both inner- and outer-ring deiodination, is inhibited by propylthiouracil (PTU), and is responsible for generating the majority of the circulating T_3 via conversion of T_4 into T_3. It also generates rT_3 from T_4 (Yen, 2001). Type II deiodinase performs outer-ring deiodination only, and is not inhibited by PTU. It has been identified in the CNS, brown adipose tissue, anterior pituitary gland, and placenta (Hulbert, 2000). Type II deiodinase functions predominately to convert T_4 into T_3 to supply the local intracellular needs for T_3 (Yen, 2001). Type III deiodinase performs only inner-ring deiodination (converting T_4 into rT_3 and T_3 into the inactive diiodothyronine, T_2). It is found in the CNS, placenta, and skin and also is not inhibited by PTU (Hulbert, 2000; Yen, 2001). Less is known about the monodeiodinases of fishes relative to mammals, but deiodinase types I, II, and III have been found in a number of fish species (see, e.g., Mol et al., 1998), and cDNAs for all three deiodinase enzyme types have been isolated from killifish, (*Fundulus heteroclitus*) and tilapia (*Oreochromis niloticus*) (Valverde, Croteau, Lafleur, Orozco, & St. Germain, 1997; Sanders et al., 1999; Orozco, Villalobos, & Valverde, 2002), which suggests that the deiodinases have been highly conserved among vertebrate classes. However, the type I deiodinase in teleosts appears to possess a number of functional characteristics, including an insensitivity to PTU and altered responsiveness to increased T_3 levels, that suggest that this enzyme is not as similar to its mammalian counterpart as once thought (Van der Geyten, Byamungu, Reyns, Kuhn, & Darras, 2005). Several studies have shown that teleost type I deiodinase activity is not affected and gene expression is downregulated during hyperthyroidism, in contrast to the

response of this enzyme observed in mammals (Berry, Kates, & Larsen, 1990; Mol et al., 1998; Plohman et al. 2002; García, Lopez-Bojorquez, Nunez, Valverde, & Orozco, 2004). In addition, hepatic type I deiodinase activity and expression is upregulated in hypothyroid tilapia, again disparate to the response of this enzyme seen in mammals (Berry et al., 1990; Van der Geyton, 2001).

2.2. Thyroid Hormone (TH) Transporters

Thyroid hormones exist in two forms in the blood: free and bound to transport proteins (Figure 5.3). Less than 1% of the T_4 and T_3 present in the blood of teleosts is estimated to be present in the free form, and thus readily accessible to target cells (Eales & Shostak, 1985; Weirich, Schwartz, & Oppenheimer, 1987). The remainder of the hormone is reversibly bound to transport proteins, of which albumin was thought to be the primary form in teleosts until transthyretin (TTR) cDNA was identified in the gilthead sea bream (*Sparus auratus*) (Santos & Power, 1999). The T_4-binding globulin (TBG) present in mammals has not been detected in fishes. Both TTR and albumin in mammals have a higher affinity for T_4 than T_3, whereas in fish, birds, and amphibians TTR has a higher affinity for T_3 than for T_4 (Richardson, 2009). In addition to the liver, TTR transcripts have been detected in the heart, skeletal muscle, kidney, testis, gills, and pituitary of adult gilthead sea bream, and in the liver of several salmonids during smoltification (see Richardson, 2009).

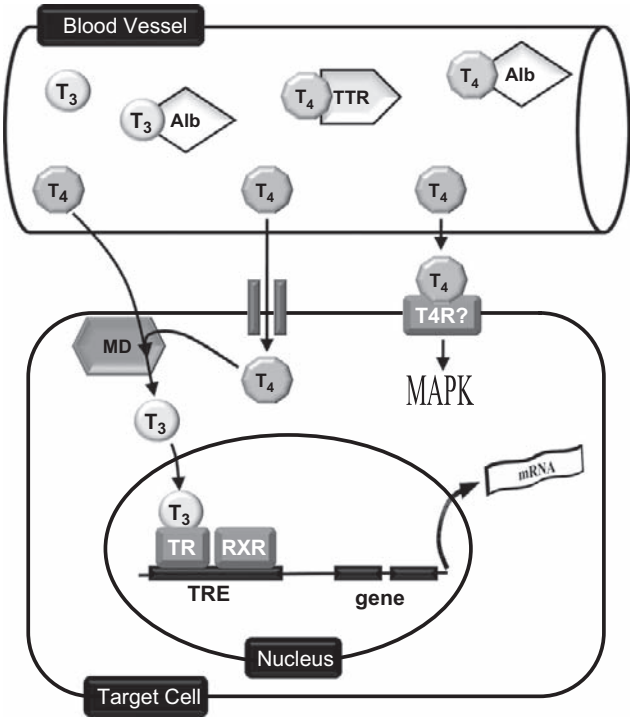

FIGURE 5.3 Thyroid hormone action at the cellular level in teleost fishes. Thyroid hormones are present in the blood bound to the plasma binding proteins, albumin (Alb) and transthyretin (TTR), or in free form. Bound thyroxine (T_4) and triiodothyronine (T_3) are inaccessible to target tissues and provide a circulating store of hormone, while the free form is available to enter target cells via a specific cellular transporter. Alternatively, T_4 may bind to a specific receptor on the cell membrane as in mammals. Triiodothyronine may be transported directly into the cell, but it is postulated that mainly T_4 in the cell provides the substrate for T_3 generation through deiodination enzymes (MD). Triiodothyronine enters the nucleus where it binds to its receptor already associated with a thyroid response element (TRE) on the target gene. Thyroid hormone receptor (TR) usually binds as a heterodimer with a retinoid X receptor (RXR).The TR alone represses transcription, while T_3 binding to the receptor initiates transcriptional machinery to generate messenger RNA. MAPK, mitogen-activated protein kinase.

Recent studies in mammals suggest that there are specific transporters responsible for transfer of THs into target cells. These transmembrane transporters appear to be Na^+-independent organic anion transport systems, which transport THs across the membranes of target cells in the CNS, liver, kidney, and retina (Heuer & Visser, 2009). They may also transport conjugated and unconjugated steroid hormones from the bloodstream into hepatocytes (Abe, Suzuki, Unno, Tokui, & Ito, 2002). Although there is no direct evidence for similar TH transmembrane transporters in fishes, indirect evidence suggests that T_4 and T_3 transporters are present in teleosts. Thyroxine and T_3 entry into red blood cells occurs by simple diffusion, as well as by pH- and temperature-sensitive saturable transport mechanisms (McLeese & Eales, 1996a; 1996b). There also is evidence for separate T_4 and T_3 transporters. Transport of T_3 into red blood cells is Na^+-independent and relatively rapid, whereas T_4 uptake is slower, very specific, and appears to depend on binding to red blood cell protein (McLeese & Eales, 1996b).

2.3. Thyroid Hormone (TH) Clearance

In mammals, THs are conjugated in the liver through sulfation/sulfonation by sulfotransferases, or glucuronidation by UDP-glucuronosyl transferase prior to elimination through the bile (Hood & Klaassen, 2000a; 2000b). These enzymes alter the solubility of THs and prevent deiodination (Zoeller et al., 2007). In teleosts, a similar clearance mechanism appears to exist. All of the principle thyronines (T_4, T_3, and rT_3) undergo glucuronidation and sulfation in rainbow trout *in vivo*, and by hepatocytes *in vitro* (Finnson & Eales, 1996; Finnson, McLeese, & Eales, 1999). Glucuronides of T_3 occur in the blood of male tilapia (DiStefano, Ron, Nguyen, Weber, & Grau, 1998). Deiodination also serves to clear T_4 and T_3 from the blood and tissues of teleosts (DiStefano et al., 1998).

2.4. Thyroid Hormone (TH) Receptors

Thyroid hormones produce their effects primarily after binding to two classes of TR: a nuclear receptor with

a higher affinity for T_3 than for T_4 and a plasma membrane receptor that has an affinity primarily for T_4. Thyroid nuclear receptors have a genomic mechanism of action whereas the plasma membrane receptor works mainly through kinase cascades.

2.4.1. Nuclear triiodothyronine (T_3) receptors

In mammals, THs readily enter target cells and exert most of their effects at the genomic level via their interaction with the TR in the nucleus of target cells (Figure 5.3). Intracellular type I or II deiodinases can convert T_4 into T_3, which then binds to the TR. The occupied TR may bind to the thyroid response element (TRE) on DNA as a homodimer but binds most strongly as a heterodimer with the retinoid X receptor (RXR) (Hulbert, 2000; Yen et al., 2006; Mengeling et al., 2008; Koury et al., 2009).

In the absence of ligand, TRs are thought to bind to DNA as either a homodimer or a heterodimer with RXR, and stimulate the binding of a corepressor complex that actively represses basal transcription of the target gene (Hulbert 2000; Harvey & Williams, 2002). Two key corepressor proteins that frequently make up the corepressor complex with TRs are the silencing mediator of retinoid and TH receptor (SMRT) and the nuclear receptor corepressor (NCoR), found in both mammalian and amphibian systems (Tomita, Buchholz, & Shi, 2004; Choi et al., 2008). This model is the generally accepted method of T_3-dependent gene activation.

In vertebrates, two highly conserved forms of TR, TRα and TRβ, have been identified, each encoded by a separate gene (Harvey & Williams, 2002). Two additional TR transcripts have been identified for each of the TRα and TRβ proteins in mammals: TRα-1 and TRα-2, and TRβ-1 and TRβ-2. TRα-1 and TRα-2 arise from alternate splicing of the initial RNA transcript, while TRβ-1 and TRβ-2 are generated through the use of one of the two promoters located on the TRβ gene (Yen, 2001). TRα-2 is the only isoform that does not bind T_3. A number of other TRα and TRβ subtypes have also been identified in mammals and fishes, although their functional significance is still being investigated (Zoeller et al., 2007; Nelson & Habibi, 2009). TRα-1 and TRβ-1 are expressed in most tissues, but their relative abundance varies (Harvey & Williams, 2002).

In fishes, TRα and TRβ mRNA have been identified in a number of species, including the Japanese flounder (*Paralichthys olivaceus*) (Yamano, Araki, Sekikawa, & Inui, 1994; Yamano & Inui, 1995), halibut (*Hippoglossus hippoglossus*) (Llewellyn et al., 1999), gilthead sea bream (Nowell, Power, Canario, Llewellyn, & Sweeny, 2001; Power et al., 2001), rainbow trout (Jones, Rogers, Kille, & Sweeney, 2002; Raine, Cameron, Vijayan, Lamarre, & Leatherland, 2004), zebrafish (J. Essner, Breuer, R. Essner, Fahrenkrug, Hackett, 1997; Liu, Lo, & Chan, 2000), Atlantic salmon (*Salmo salar*) and tilapia (Marchand et al., 2001), conger eel (*Conger myriaster*) (Kawakami, Tanda, Adachi, & Yamauchi, 2003a; 2003b), turbot (*Scophtalmus maximus*) (Marchand, Duffraisse, Triqueneaux, Safi, & Laudet, 2004), goldfish (*Carassius auratus*) (Nelson & Habibi, 2006), and Pacific bluefin tuna (*Thunnus orientalis*) (Kawakami, Nozaki, Seoka, Kumai, & Ohta, 2008). TRα and TRβ mRNA has been detected in most tissues of the Japanese flounder, zebrafish, and other species, although the isoforms are generally differentially expressed in different species (Yamano & Miwa, 1998; Liu et al., 2000; Nelson & Habibi, 2009). Nuclear TRs also are present in oocytes and early embryos of zebrafish and rainbow trout, fertilized eggs of Japanese flounder, and blastula-stage embryos of gilthead sea bream (Essner et al., 1997; Yamano & Miwa, 1998; Liu et al., 2000; Power et al., 2001; Raine et al., 2004; Li, Raine, & Leatherland, 2007). Receptor binding and signaling by T_3 in fishes is highly conserved relative to that found in mammals and other vertebrates (e.g., see Nunez et al., 2008; Nelson & Habibi, 2009).

Thyroid hormones upregulate their own nuclear TRs in mammals, amphibians, and fishes, as shown in a number of studies using adult, juvenile, and embryonic developmental stages. Fathead minnows (*Pimephales promelas*) fed a T_3-enhanced diet increased both TRα and TRβ transcripts in the liver and brain (Lema et al., 2009). Similarly, exogenous T_4 exposure through immersion or body cavity implant consistently upregulated retinal TRα and TRβ gene expression in rainbow trout parr during the TH-dependent developmental process of UVS cone degeneration (Raine & Hawryshyn, 2009; Raine et al., 2010). In amphibians and several fish species, including the Japanese flounder, Senegalese sole (*Solea senegalensis*), and conger eel, that undergo TH-dependent metamorphosis, increases in whole body TH levels precede metamorphic climax and correspond to increased accumulation of TR transcripts (Yamano & Miwa, 1998; Kawakami et al., 2003a; 2003b; Tata, 2006; Manchado, Infante, Rebordinos, & Canavate, 2009). Both TRα and TRβ gene expression were upregulated in response to exogenous T_4 and T_3 treatment in conger eel hepatocytes cultured *in vitro* (Kawakami et al., 2006), whereas T_4 treatment upregulated TRβ expression in larval Senegalese sole and treatment with a goitrogen (thiourea) decreased TRβ transcripts (Manchado et al., 2009). The findings of these studies suggest that TH upregulation of TRs may be a necessary phenomenon that exists to sensitize target tissues/cells to T_3 in response to temporal tissue-specific requirements.

2.4.2. Plasma membrane thyroxine (T_4) receptor

It has been recognized for some time that not all actions initiated by THs are mediated through nuclear TRs, and the existence of a nongenomic TH signaling pathway was

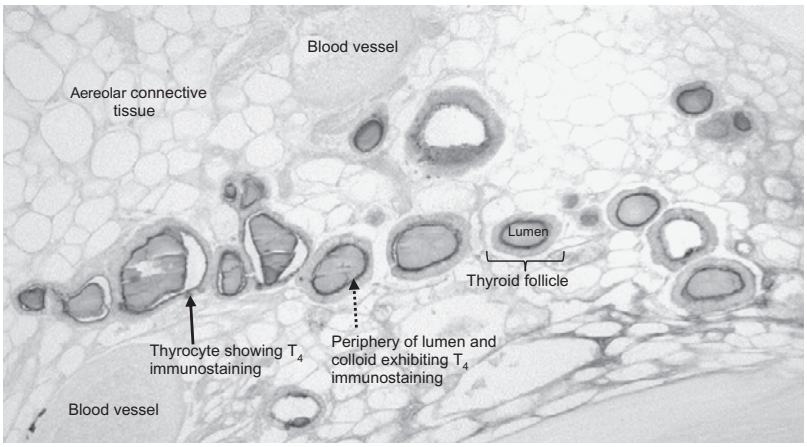

FIGURE 5.4 Thyroid tissue of juvenile rainbow trout immunostained for thyroxine (T_4). This histological image shows the general appearance of the thyroid tissue in many species of teleost fish. Thyroid follicles are scattered throughout the areolar connective tissue of the lower jaw in close association with the blood supply. Thyroxine immunostaining demonstrates the presence of T_4 in some of the thyroid epithelium cells (thyrocytes), the periphery of the colloid, and the lumen. This staining technique can be used to help assess the activity level of the thyroid tissue. An increase in the number of T_4-immunostained thyrocytes, and increased intensity of staining in and around the colloid, indicate increased activity or storage. Processing of the tissue for histology can result in shrinkage or loss of the colloid from the lumen of the thyroid follicles, as seen in this image.

suspected. In fishes, TH-induced rapid stimulation of succinate dehydrogenase in hepatic mitochondria of carp (*Cyprinus carpio*) and the potential enhancement of gonadotropin-induced estradiol (E_2) secretion in trout oocytes was thought to take place too quickly for T_3 to bind to its nuclear receptor and initiate transcription (Cyr & Eales, 1989; Peter & Oommen, 1989). Many more examples of nongenomic actions of THs can be found in mammals, including effects on mitochondria, glucose uptake, and actin polymerization, the latter of which promotes neural outgrowth and may be a key factor in TH effects on brain development (Shih et al., 2004; Farwell & Leonard., 2005; Moeller, Cao, Dumitrescu, Seo, & Refetoff, 2006; Mousa, O'Connor, F. Davis, & P. Davis, 2006; Zoeller et al., 2007; Casas et al., 2008; Leonard, 2008; Lombardi et al., 2009). A plasma membrane receptor for T_4, the integrin vβ3 receptor, was recently identified in mammals and it appears to be the source of these nongenomic effects of THs (see P. Davis, Leonard, & F. Davis, 2008, for review). This membrane receptor has a higher affinity for T_4 than T_3, and binding of T_4 to the receptor activates a mitogen-activated protein kinase (MAPK) signal transduction pathway in angiogenesis studies (Davis et al., 2009). Although a cell membrane-associated T_4 receptor has yet to be identified in any fish, it may be that this is also the source of a number of nongenomic effects in this vertebrate group too. It is clear that a re-evaluation of the classical model of TH action, where T_4 is considered to be only a prohormone for T_3, is in order.

3. THE THYROID TISSUE OF FISHES

The thyroid follicle is considered to be the functional unit of the thyroid gland and is highly conserved in all vertebrate species. Thyroid follicles are made up of a single layer of epithelial cells (thyrocytes) creating an extracellular compartment or lumen containing colloid (Figure 5.4). The formation of an extracellular lumen is highly conserved among all vertebrates and is thought to be a required adaptation for TH synthesis, which involves a highly reactive oxidative step, and extracellular storage (Leatherland, 1994). It may be that, by sequestering this oxidative reaction in an extracellular compartment, the thyroid follicle provides protection for other tissues and cells.

Mammals, birds, amphibians, and reptiles possess a discrete glandular thyroid surrounded by a connective tissue covering. Similarly, cartilaginous fishes also possess an encapsulated thyroid gland, but, in most species of bony fish, the thyroid tissue does not form a discrete gland but consists of isolated thyroid follicles scattered throughout the well-vascularized areolar connective tissue of the lower jaw, in the vicinity of the ventral aorta (Leatherland, 1994) (Figure 5.5).

In teleost fishes, although seemingly active thyroid follicles are detected prior to hatch in a number of freshwater species, activity of the tissue is based solely upon histological criteria and corresponding increases in whole body TH levels have not been observed to corroborate this finding (Atlantic salmon (Hoar, 1939), fathead minnows (Wabuke-Bunoti & Firling, 1983) chinook salmon (*Oncorhynchus tschawytscha*), and coho salmon (*Oncorhynchus kisutch*) (Leatherland & Lin, 1975; Greenblatt, Brown, Lee, Dauder, & Bern, 1989)). Further, the pattern of active thyroid follicle appearance in marine species differs from that of freshwater fish, and active follicles are not found until after yolk absorption, again using only histological characteristics (Nacario, 1983; Brown et al., 1988). Immunohistochemical detection of T_4 and T_3 in the developing thyroid tissue of rainbow trout embryos demonstrated that, in these fish, synthesis of the THs begins prior to hatch and release of THs from the thyroid tissue does not occur until much later after hatch but before the onset of exogenous feeding (Raine & Leatherland, 1999; 2000). An example of T_4-immunostained thyroid tissue from a juvenile rainbow trout can be seen in Figure 5.5.

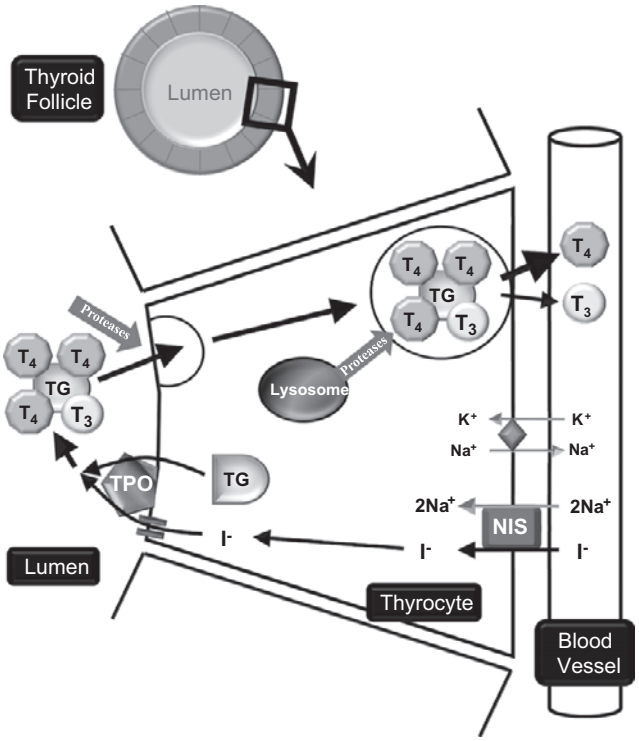

FIGURE 5.5 Synthesis and release of thyroid hormone from thyroid tissue. Iodide (I^-) is taken up by the thyroid epithelial cell (thyrocyte) by sodium iodide symporters (NIS), which rely on a sodium (Na^+) gradient established by sodium potassium ATPases (diamond). I^- travels through the cell and likely enters the lumen of the follicle through a channel protein. The protein that makes up thyroglobulin (TG) is manufactured in the thyrocyte and is iodinated upon entering the thyroid follicle lumen by a thyroid-specific thyroid peroxidase (TPO) to form the insoluble thyroid hormone-associated TG that makes up the colloid. The stored TG is solubilized by proteases and taken up by the thyrocyte by endocytosis. Lysosomal proteases release the thyroid hormones from the TG molecule and mainly thyroxine (T_4), with some triiodothyronine (T_3), is released from the cell into the bloodstream.

Similarly to mammalian thyroid gland development, TSH does not appear to play a role in early thyroid tissue development of teleost fishes, although TSH RNA has been detected in unfertilized oocytes of channel catfish (*I. punctatus*) and could potentially be involved (Raine & Leatherland, 2000; Alt et al., 2006, Goto-Kazeto et al., 2009). Thyrotropin immunoreactive cells have been found prior to the detection of TH synthesis in the developing thyroid tissue of rainbow trout embryos, and may be involved in stimulating the onset of TH synthesis (Raine & Leatherland, 1999; 2000).

3.1. Thyroid Hormone (TH) Synthesis and Release

Most of the information on TSH stimulation of TH synthesis and release can be found in studies of the mammalian thyroid gland. However, the highly conserved morphology of thyroid follicles, the equivalent chemical structure of THs, and the similarities in regulation and signaling of TH suggest that synthesis and release of THs by thyroid follicles would be similar among all vertebrates, and studies in teleosts appear to corroborate this assumption (Eales & Brown, 1993; Leatherland, 1994; Cyr & Eales, 1996; Raine & Leatherland, 2000; Raine et al., 2001; Blanton & Specker, 2007; Nelson & Habibi, 2009). Details of the process of TH synthesis that applies to all vertebrates are provided in Figure 5.4.

4. THYROID HORMONE (TH) AND REPRODUCTION IN FISHES

A possible role for THs in reproduction in fishes has been sought for several decades, but, despite the large body of literature published in this area, a clear function for THs in this process has not been revealed, and a role in reproduction is still unclear. There are many excellent reviews dealing with TH and reproduction in fishes that compile, synthesize, and discuss the main studies in this area prior to the 21st century, and these should be consulted for a comprehensive review of the earlier literature (see Eales, 1979; Leatherland, 1982; 1987; 1988; 1994; Cyr & Eales, 1996). The general consensus of these prior reviews exploring the role of THs in fish reproduction appears to be that, although there is evidence to suggest involvement of THs in reproduction, there is also a wealth of evidence suggesting that THs are not directly involved in the reproductive process, which is a pervasive theme when reviewing the literature and attempting to define a role for THs in fish. As has been discussed in other reviews, the basis of the conflicting results attained with much of this research may be attributed to the diverse methods used to investigate TH action, the variety of species examined, and the many different reproductive strategies that they employ. A great deal of the early studies on TH and reproduction involved the correlation of plasma T_4 and T_3 levels with the reproductive cycle of various species of fish. However, as discussed earlier in this review, it is now evident that TH levels are highly regulated both in the blood and at the tissue/cellular level. Moreover, it has become quite apparent that detection of a change in one measurement does not automatically infer a functional change in TH action. Changes in the regulation of THs can generally compensate and overcome disturbances to individual components of this regulatory system (Zoeller et al., 2007). Thus, there is a need to ensure that evaluation of TH physiology and action involves the measurement of multiple factors involved in TH regulation and action. Just as critical is the need to develop TH-specific biomarkers with clear functional endpoints that can be used to gauge the severity of perturbations to general TH physiology and

regulation, and to determine whether this interference results in a real impact at the organismal level. A number of reviews have addressed this growing concern of how to accurately assess a change in thyroid function and this will not be directly addressed in this chapter (see Eales & Brown, 1993; Brown, Adams, Cyr, & Eales, 2004; Blanton & Specker, 2007).

The more informative published studies that address the involvement of TH in fish reproduction fall into one of the following categories: (1) correlative studies examining changes in TH function during reproductive maturation; (2) identification of TH regulatory elements in gonadal tissue; (3) assessment of thyroid function following sex steroid treatment; or (4) manipulation of thyroid function to induce changes in reproductive maturation. These categories are employed in subsequent sections to aid in the review of TH in fish reproduction.

4.1. Correlative Studies Examining Changes in Thyroid Hormone (TH) Function During Reproductive Maturation

A great many early studies have investigated the possibility that THs play a major role in controlling reproductive events. These investigations generally employed measurement of plasma TH levels and/or thyroid tissue histology to correlate changes in reproductive events that take place during the breeding cycle. A thorough review of this early body of work can be found in Cyr & Eales (1996). In general, there is a positive correlation between the activity level of the thyroid tissue and the reproductive stage of seasonally breeding, non-salmonid teleost fishes, despite variations in life history and habitat (Cyr & Eales, 1996). Thyroid tissue activity tends to increase during the early stages of gonadal development, and these levels are maintained or increased during reproduction, and decrease after spawning (Cyr & Eales, 1996). This pattern of thyroid activity also is seen in fish species with short breeding cycles where thyroidal status increases during the early stages of each cycle. Further, reproductive status and thyroid tissue activity appear to possess a clear temporal relationship in salmonids, based on thyroid histology and elevated plasma TH levels (Cyr & Eales, 1996). These fishes exhibit an increase in thyroid activity with the onset of gonadal development and a general decrease in plasma TH levels with the upstream spawning migration in both males and females. In non-anadromous species there is often an increase in plasma TH levels at spawning, especially in males, followed by an increase in thyroid activity with the continuation of growth (Cyr & Eales, 1996). Similarly, in another seasonal spawner, the burbot (*Lota lota*), elevated blood T_3 has been observed prior to spawning and decreased blood T_3 at spawning, although no change was seen in T_4 (Mustonen, Nieminen, & Hyvrinen, 2002). An earlier study did find increases in blood T_4 at spawning, especially in females (Hornsey, 1977). However, despite the large amount of correlative evidence to bolster this interpretation, changes in water temperature, day length, and other seasonal parameters greatly complicate the matter (Leatherland, 1994; Cyr & Eales, 1996). This can be seen in the brown bullhead (*Ictalurus nebulosus*) and channel catfish (*I. punctatus*), which do not exhibit any relationship between plasma TH and gonadal development, but in which plasma TH levels correlate with ambient temperature (Burke & Leatherland, 1983; MacKenzie, Thomas, & Farrar, 1989; Leatherland, 1994).

In oviparous elasmobranchs, similar positive correlations of thyroid activity with reproductive stage can be found, with minimal thyroid function observed in immature females and maximal thyroid activity seen at the peak of egg development and vitellogenesis, although the elevation in male thyroid activity is less pronounced (Cyr & Eales, 1996). In the stellate sturgeon (*Acipenser stellatus*), high thyroid activity occurs along with gonadal maturation during the prespawning migration as well as at spawning and even shortly after spawning has concluded (Pickford & Atz, 1957; Cyr & Eales, 1996). The age at which sturgeon achieve reproductive maturity varies from approximately seven to twenty-five years, and they generally have four- to seven-year intervals between successive spawning cycles, depending on the species and sex of the fish (Sulak & Randall, 2002). Moreover, it has been suggested that female sturgeon do not commonly reach as great an age as previously thought, and thus may not spawn many times during their average lifespan (Sulak & Randall, 2002).

The effects of environmental factors on TH and reproductive events have been examined in trout of similar age exposed to altered photoperiod and constant water temperature and ration, in an effort to separate these parameters from reproductive processes (Cyr, MacLatchy, & Eales, 1988). This experimental regimen generated plasma TH levels that were highest during early ovarian development, as found previously, and it was found that plasma THs decreased as E_2 increased. This pattern was present irrespective of photoperiod. In a comparable study, Pavlidis, Dessypris, and Christofidis (1991) found low THs in female rainbow trout during spawning compared to nonspawning females.

Triploid stinging catfish (*Heteropneustes fossilis*) possess an uneven number of chromosomes and exhibit irregular meiotic division resulting in reduced gonadal development, as compared with diploid individuals (Biswas et al., 2006). Thyroid hormone levels were similar in diploid and triploid fish until spawning when diploid females exhibited significantly reduced plasma total T_4 and T_3 levels relative to the triploid females. No differences were seen in plasma TH levels in diploid and triploid males

with spawning (Biswas et al., 2006). During the spawning period, female diploid catfish exhibited an increase in thyroid gland activity assessed by histology, and a significantly higher oocyte content of T_4 and T_3 than triploid females. It was suggested that oocyte TH uptake is responsible for the decrease in plasma TH profiles during spawning in diploid females and thyroid gland activity is increased to compensate for the low plasma TH levels at this time (Biswas et al., 2006).

Interestingly, a change in the visual system of anadromous salmonids accompanies sexual maturation and upstream spawning migration of these fishes. The UVS cones and sensitivity to UV wavelengths of light reappear in maturing salmonids, and are thought to be required for navigation to natal streams (Browman & Hawryshyn, 1994; Beaudet, Novales-Flamarique, & Hayashi, 1997; Hawryshyn, 2000; Novales-Flamarique, 2000; Hawryshyn et al., 2003). The regeneration of UVS cones accompanies the surge in plasma T_4 seen during the reproductive cycle in these fishes, and T_4 treatment precociously induces UVS cone reappearance and sensitivity to light in the UV spectra (Browman & Hawryshyn, 1994; Hawryshyn et al., 2003). Although this retinal change is not generally considered a reproductive event, it does consistently accompany the spawning migration in salmonids and is an event that during reproduction can be tied to THs.

Although TH levels, and in some cases thyroid histology, often correlate quite well with the reproductive cycle in many fish species, particularly during early gonadal maturation, there are other physiological changes, such as changes in energy partitioning, that accompany this stage that should be taken into account (see Cyr & Eales, 1996). The general inhibition of thyroid function as the reproductive cycle progresses is considered to be reflective of a shift in energy partitioning, where somatic growth is decreased to allow available energy to be used for gonadal growth (Leatherland, 1994; Cyr & Eales, 1996). However, even if the primary role of THs during the reproductive cycle is metabolic, this function still contributes to successful reproduction and thus could still be considered a reproductive role.

4.2. Identification of Thyroid Hormone (TH) Regulatory Elements in Gonadal Tissue

In the year 2000, the TSH-R gene was sequenced and its expression detected in the thyroid tissue of amago salmon (Oba et al., 2000). Interestingly, at the same time, TSH-R expression was also found in the gonadal tissue of striped bass (Kumar et al., 2000). A short time later, gonadal TSH-R expression was confirmed in the testis of the African catfish, the ovaries of the channel catfish, and the gonads of European sea bass, with additional expression observed in several other tissues (Vischer & Bogerd, 2003; Goto-Kazeto, Kazeto, & Trant, 2003; Rocha et al., 2007). In both the European sea bass (*D. labrax*) and the channel catfish, TSH-R expression levels increased with ovarian maturation, but differed in the onset of TSH-R downregulation either before or after spawning (Rocha et al., 2007; Goto-Kazeto et al., 2009). Similarly, TSH-R expression increased to a maximal level during early testes development in these fishes (Rocha et al., 2007; Goto-Kazeto et al., 2009). Surprisingly, TSHβ subunit transcripts were also detected for gonadal tissues of the orange-spotted grouper (*Epinephelus coioides*) and the red-spotted grouper (*Epinephelus akaara*)—in the ooplasm of maturing oocytes and in spermatogenetic cysts, but not in the ovarian follicular cells (Wang et al., 2004). The TSHβ expression pattern in these groupers complements that of the TSH-R expression pattern found in the other fish species (Kumar et al., 2000; Goto-Kazeto et al., 2003; Vischer & Bogerd, 2003; Rocha et al., 2007). Extra-thyroidal activity of TSH has been suggested previously in mammalian systems. In various mammalian tissues, TSH-R has been found in ovaries, lymphocytes, thymus, pituitary, testes, kidneys, brain, adipose/fibroblast, heart, and bone (reviewed by Davies, Marians, & Latif, 2002; Abe et al., 2003; Aghajanova et al., 2009). Recently, granulosa cells have been shown to have TSH-R, and TSH has been shown to increase cAMP concentration in these cells in culture (Aghajanova et al., 2009). These studies suggest that TSH may be directly involved in some aspects of gonadal physiology, and that this hormone may do more than just stimulate TH release from the thyroid.

Receptors for THs have been detected in all stages of rat and human testicular development (Jannini et al., 1990; Jannini, Ulisse, & D'Armiento, 1995; Cooke, Zhao, & Bunick, 1994; Wagner, Wajner, & Maia, 2008; 2009). Further, T_3 regulates the maturation and growth of testes, and controls Sertoli cell and Leydig cell proliferation and differentiation during testicular development (Wagner et al., 2008). Human ovarian granulosa cells have TRs, and T_4 treatment of granulosa cells in culture initiates MAPK activation within 10 minutes, suggesting activation of the membrane T_4 receptor (Rae et al., 2007; Aghajanova et al., 2009). In fishes, TRs have been reported in Leydig cells of freshwater climbing perch (*Anabas testudineus*), gonadal tissues of the hermaphroditic black porgy (*Acanthopagrus schlegeli*), and the gonads of the gonochoristic goldfish and fathead minnow (Jana & Bhattacharya, 1993; Nelson & Habibi, 2006; Lema et al., 2009; K. An, M. An, Nelson, Habibi, & Choi, 2010).

In addition to TRs, deiodination enzymes are present in the gonads of several vertebrate species. Boar seminal plasma contains type II deiodinase activity, and this enzyme in the testes is postulated to act as a local regulator of TH levels and to provide the predominant source of T_3

for the testes (Brzezinska-Slebodzinska, Slebodzinski, & Kowalska, 2000). The activity level of this deiodination enzyme increases in piglet testicular homogenates from one to four weeks after birth (Brzezinska-Slebodzinska et al., 2000). Moreover, deiodinase II and III expression is present in luteinized human granulosa cells and human ovarian thecal cells (Rae et al., 2007; Aghajanova et al., 2009). In fishes, type II deiodinase expression has been detected in the testes and ovaries of the rainbow trout, and high plasma T_4 levels correspond to the maximal number of type II deiodinase transcripts detected in the testes (Sambroni et al., 2001). Additionally, the increase in type II deiodinase expression correlates with the onset of spermatogenesis and increases in plasma T_4, which suggests local abundance of T_3 (Sambroni et al., 2001).

The detection of TRs and deiodinases in gonadal tissues is not surprising, as TRs are present in most vertebrate tissues. Their presence does suggest a function in transcriptional regulation of some genes, but, without further research, a specific function cannot be ascribed. However, the additional presence of TSH in reproductive and other tissues is unexpected and warrants further investigation.

4.3. Assessment of Thyroid Function Using Sex Steroid Treatment

Treatment with sex hormones has generated little supporting evidence for the general trends found in earlier studies examining correlative changes in the HPT and the hypothalamic−pituitary−gonadal (HPG) axes during reproductive maturation. There is a general trend in a number of fish species for E_2 to suppress thyroid function, while THs decrease production of E_2. Treatment with E_2 decreases total and free plasma T_3 and sometimes plasma T_4 levels in a number of fish species, including freshwater European eels (*Anguilla anguilla*) (M. Olivereau & J. Oliverau, 1979; M. Olivereau, Leloup, De Luze, & J. Olivereau, 1981), mollies (*Poecilia* sp.) (Sage & J. Bromage, 1970), rainbow trout (Cyr et al., 1988; Flett & Leatherland, 1989a; 1989b), and masu salmon (*Oncorhynchus masou*) (Yamada, Hiriuchi, Gen, & Yamauchi, 1993). Further, E_2 suppresses hepatic T_3 production and plasma T_3 levels in salmonids (Cyr et al., 1988; Flett & Leatherland, 1989a; 1989b; Mercure et al., 2001). Moreover, decreased thyroid epithelial cell heights, reflecting decreased thyroid activity, have been found in European eel and rainbow trout following E_2 treatment, even when no changes in circulating T_4 levels were observed, suggesting a decrease in T_4 clearance (Olivereau et al., 1981; Leatherland, 1985). Similarly, E_2 treatment decreases T_4 clearance and secretion rates via decreased T_4 to T_3 conversion in rainbow trout and the Southern lamprey (*Geotria australis*) (Cyr et al., 1988; Flett & Leatherland, 1989b; Leatherland et al., 1990; Mercure et al., 2001) and decreases free plasma T_3 and total T_4 levels, perhaps due to some degree of reduction in T_4 binding to plasma carrier proteins (Cyr & Eales, 1989; 1992) or possibly by a decrease in the availability of TR for T_3 binding (Bres, Cyr, & Eales, 1990). This inhibitory role of estrogens was demonstrated recently, when treatment with a synthetic estrogen (ethinylestradiol) resulted in TRα and TRβ downregulation in fathead minnow liver, although TRβ was simultaneously upregulated in the ovary (Filby, Thorpe, Maack, & Tyler, 2007).

Alternatively, E_2 enhances thyroidal status in the climbing perch (*A. testudineus*), dwarf snakehead (*Channa gachua*), and striped dwarf catfish (*Mystus vittatus*) (Singh, 1968; 1969; Chakraborti, Rakshit, & Bhattacharya, 1983; Chakraborti & Bhattacharya, 1984; Bandyopadhyay, Banerjee, & Bhattacharya, 1991). Further, removal of the ovaries from climbing perch results in a depression of thyroid function and plasma T_4 levels that can be reinstated with E_2 treatment (Chakraborti et al., 1983; Chakraborti & Bhattacharya, 1984). In contrast, thyroid function in sockeye salmon (*Oncorhynchus nerka*) and rainbow trout does not respond to E_2 treatment (Van Overbeeke & McBride, 1971; Milne & Leatherland, 1978), although perhaps the thyroid axis was already depressed due to fasting (Milne & Leatherland, 1980). Clearly, there is no uniform pattern, but these differences in the effects of E_2 may be species-specific or may be due to variations in experimental design, such as dose used, nutritional status of subjects, developmental stage, water temperature, and the method used to assess thyroid function.

Interestingly, androgen treatment generally produces results opposite to those of E_2 treatment, and enhances thyroidal function in most teleosts examined (Cyr & Eales, 1996). Testosterone (T) or methyltestosterone (MT) have been found to increase plasma TH levels in dwarf snakeheads (Singh, 1968; 1969), rainbow trout (Hunt & Eales, 1979; Leatherland & Sonstegard, 1980; Leatherland, 1985; MacLatchy & Eales, 1988; Shelbourn, Clarke, McBride, Fagerlund, & Donaldson, 1992), coho salmon (*O. kisutch*) (Fagerlund, Hijman, McBride, Plotnikoff, & Dosanjh, 1980), masu salmon (Ikuta, Aida, Okumoto, & Hanyu, 1985; Yamada et al., 1993), and the guppy (*Poecilia reticulata*) (Schwerdtfeger, 1979). Exposure to MT also stimulates increased activity of thyroid tissue in medaka (Nishikawa, 1976). Testosterone treatment of both rainbow trout and Arctic char (*Salvelinus alpines*) resulted in increased T_4 to T_3 conversion, further supporting the positive correlation between T and thyroid function (Hunt & Eales, 1979; MacLatchy & Eales, 1988). However, no effect of androgen treatment on plasma TH levels or conversion of T_4 to T_3 has been reported in other studies (Milne & Leatherland, 1980; Leatherland, 1985; Yamada et al., 1993). Nevertheless, as with E_2 treatment, differences in experimental design, dosing, water temperature, sex, and

the nutritional status confound the interpretation of these experiments.

The use of hypothalamic gonadotropin-releasing hormone (GnRH) and pituitary gonadotropin (GTH) to study the role of THs in reproduction has generated mixed outcomes. Some of the varied results may stem from the fact that only one GTH was identified in fish prior to 1989, and many studies employed injection of isolated fish pituitary GTH that could potentially have included one or both forms of GTH. The fish GTHs are designated GTH-I, considered homologous to mammalian FSH, and GTH-II, homologous to mammalian LH (Kawauchi & Sower, 2005). Thyroid epithelial cell height increases following treatment of gonadectomized sockeye salmon with salmon GTH (Donaldson & McBride, 1974) and both GnRH analogs and salmon GTH increase plasma T_4 in female sea lampreys (Sower, Plisetskaya, & Gorbman, 1985). Both salmon GTH and ovine LH markedly increase plasma T_4 levels in climbing perch, while ovine FSH has no effect (Chakraborti & Bhattacharya, 1984). Further, treatment with a GnRH analog increases plasma T_3 levels but not T_4 in immature rainbow trout (Plate et al., 2002). However, plasma TH levels are unchanged with salmon GnRH treatment of goldfish, and salmon GTH does not alter secretion of T_4 in medaka (Cyr & Eales, 1996). Further, suppression of thyroid function resulting from removal of the ovaries in climbing perch is not reinstated with subsequent salmon GTH treatment (Chakraborti et al., 1983; Chakraborti & Bhattacharya, 1984).

4.4. Manipulation of Thyroid Function to Induce Changes in the Reproductive System

A number of fish studies have approached elucidation of the relationship between reproduction and TH in another way. These studies employed altered thyroidal status to examine the reproductive system for correlative changes. Using the advantage of an encapsulated thyroid tissue in cartilaginous fishes, thyroidectomy of female dogfish sharks (*Scyliorhinus canicula*) has been shown to prevent development of vitellogenic ovarian follicles (Lewis & Dodd, 1974). Since most teleosts possess diffuse thyroid tissue that cannot be completely removed from the living fish, chemical goitrogens have been widely used to reduce the synthesis and release of THs from the thyroid tissue. To this end, the use of chemically induced hypothyroidism in a number of teleost fishes has generally resulted in impaired gonadal function (Leatherland, 1987; 1994; Cyr & Eales, 1996; Swapna & Senthilkumaran, 2007). There is evidence that some goitrogens, such as thiourea, are considered to have toxic effects on fishes, and this could interfere with the interpretation of many of the studies using these and other potentially toxic chemicals (Leatherland, 1994). However, the relatively consistent trend of decreased GSI and gonadal development/maturation accompanying decreased thyroid function reported in many studies is difficult to ignore. Decreased TH production in male African catfish treated with thiourea prior to spawning results in decreased spermatozoa in the testicular lumen (Swapna et al., 2006). Further, lowered circulating THs in a number of Asian teleosts interfere with a variety of testicular processes during maturation of the testes, which resume with a return of the fish to a euthyroid state (Swapna & Senthilkumaran, 2007). Although there are other studies that have found no effect or a detrimental effect of THs on several testes-related processes, reproductive stage and treatment dose are likely factors in the range of outcomes reported (Swapna & Senthilkumaran, 2007). Nevertheless, these studies do indicate that THs can affect maturation of the testes in teleost fishes and suggest that THs may be involved in milt production, and sperm viability and maintenance.

Decreased thyroidal activity has been shown to accompany reduced ovarian function in rainbow trout and fathead minnows exposed to sublethal concentrations of thiocyanate (Ruby, Idler, & So, 1993; Lanno & Dixon, 1994). Similarly, inhibition of thyroid function using sodium ipodate injections decreases the gonadosomatic index (GSI) in rainbow trout, whereas treatment with T_3-supplemented food increases GSI (Cyr & Eales, 1988a). Ovarian follicle development is impaired in African catfish following thiourea treatment, and T_4 treatment increases the growth rate of ovarian follicles and the number of mature follicles present in the ovarian tissue (Supriya et al., 2005). In zebrafish, PTU administration increases the number of oocytes produced, but decreases the size of the mature oocytes (Van der Ven, Van den Brandhof, Vos, Power, & Wester, 2006). Thyroxine also stimulates ovarian maturation in immature goldfish, but has no effect on ovarian development in hypophysectomized adults, suggesting a pituitary factor is also involved (Hurlburt, 1977). In the guppy, T_4 treatment decreases spawning interval and brood time (Lam & Loy, 1985). Further, T_3 restores the responsiveness of oocytes to GTH in the female stellate sturgeon, with delayed sexual development resulting from decreased water temperature (Detlaf & Davydova, 1979). Treatment with T_4 increases the action of salmon GTH and ovine LH *in-vitro* on the ovary of the climbing perch (Chakraborti & Bhattacharya, 1984). However, T_4 only had this effect two hours prior to salmon GTH treatment. In rainbow trout, T_3 and/or salmon GTH increase GSI after 21 days, and this effect is greater in trout treated with both T_3 and GTH than with GTH treatment alone (Cyr & Eales, 1988a). Moreover, T_3 consistently potentiates salmon GTH stimulation of gonadal steroid secretion *in vitro* (Hurlburt, 1977; Cyr & Eales, 1988b; Cyr et al., 1988; Soyano, Saito, Nagae, & Yamauchi, 1993). In rainbow trout, this effect has been found to be bimodal, with low T_3 treatment

stimulating E_2 production and higher T_3 concentrations suppressing E_2 production (Cyr et al., 1988). In medaka, a similar effect has only been seen 32 hours prior to ovulation, even though a surge of T_4 and T_3 release took place at 12 hours preovulation (Soyano et al., 1993). Further, synthesis of GnRH is decreased with T_3 treatment in tilapia (Parhar et al., 2000).

Recently, fathead minnows fed a T_3-elevated diet have exhibited significantly elevated TSHβ transcripts in the testes, and TRβ and GPHα transcripts have been shown to be significantly elevated in both the ovaries and the testes (Lema et al., 2009). Gonadal TRα expression in the fathead minnow gonads, however, did not change following T_3 treatment. In mice, TRα1 and TRβ appear to have opposite roles in the control of reproductive behavior (Forrest, Reh, & Rüsch, 2002). Estrogens stimulate female receptivity to mating, and THs interfere. Further, TRα1-deficient mice exhibit decreased mating behavior, whereas TRβ-deficient mice show increased mating behavior (Forrest et al., 2002).

Clearly, changes in thyroid function can affect reproductive events, but this, in and of itself, does not prove a direct role for TH in reproduction. It may be that effects on reproduction are indirect through permissive effects of TH on related components of the reproductive system, or as a result of an interrelationship between physiological changes incurred with altered TH availability and reproductive events.

5. CONCLUSIONS

Although there is still no clearly defined role for THs in reproduction, there is evidence to suggest that THs are involved in the reproductive cycles of fishes. The strong correlation between THs and nutritional status suggests that energy partitioning is a key role for THs during the reproductive cycle, shifting the mobilization of energy reserves from growth to reproductive maturation. There is also evidence that THs play major roles in testicular and ovarian development. However, teleosts are an incredibly large and diverse group encompassing many very different life histories and reproductive strategies, which can make interpretation and comparison between species exceedingly difficult and perhaps unrealistic. Further, much of the research in this area has employed single-parameter assessment of thyroid function/physiology, namely plasma TH levels, and searched for an overarching regulatory role for TH during the reproductive cycle. The delivery of THs to their target cells is highly regulated through redundant and compensatory mechanisms to maintain TH signaling despite perturbations resulting in altered plasma TH (Zoeller et al., 2007). This new appreciation for preservation of TH action irrespective of plasma TH levels requires re-evaluation of previous dogma, and revision of experimental assessment of TH physiology and action in many vertebrates, especially in fishes. This includes a more thorough investigative method for experiments required to interpret functional changes in TH physiology, where analysis of multiple players involved in TH signaling and regulation is essential. Further, it is highly probable that the control of reproductive events involves the interaction of multiple hormones and that TH is only one of many components in this process. The success of future research in this area would appear to benefit from an emphasis on the potential involvement of THs in the various tissue-/cell-specific changes that take place during the reproductive cycles of fishes, rather than the overall regulation of this event. Evaluation of tissue/cellular regulation of THs, standardization of methods of analysis, and an understanding of the reproductive strategies of the species of fishes under investigation may be key to evaluating and understanding other possible roles of THs in the reproductive process.

ABBREVIATIONS

CNS	Central nervous system
CRF	Corticotropin-releasing factor
E_2	Estradiol
FSH	Follicle-stimulating hormone
FSH-R	Follicle-stimulating hormone receptor
GnRH	Gonadotropin-releasing hormone
GPHα	Glycoprotein hormone subunit α
GSI	Gonadosomatic index
GTH	Gonadotropin
HPG	Hypothalamic−pituitary−gonadal
HPT	Hypothalamic−pituitary−thyroid
LH	Luteinizing hormone
LH-R	Luteinizing hormone receptor
MAPK	Mitogen-activated protein kinase
MT	Methyltestosterone
NCoR	Nuclear receptor corepressor
PTU	Propylthiouracil
rT_3	Reverse-triiodothyronine
RXR	Retinoid X receptor
SMRT	Silencing mediator of retinoid and thyroid hormone receptor
SS	Somatostatin
T	Testosterone
T_3	Triiodothyronine
T_4	Thyroxine
TBG	Thyroxine-binding globulin
TH	Thyroid hormone
TR	Thyroid hormone receptor
TRα1	Thyroid hormone receptor isoform
TRE	Thyroid response element
TRH	Thyrotropin-releasing hormone
TSH	Thyrotropin
TSH-R	Thyrotropin receptor
TTR	Transthyretin
UV	Ultraviolet
UVS	Ultraviolet-sensitive

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