21 Endocrinology of Complex Life Cycles: Amphibians

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Glossary

adrenocorticotropic hormone (ACTH) Small polypeptide hormone derived from a larger precursor (proopiomelanocortin) produced by the anterior pituitary gland that stimulates the adrenal cortex (interrenal glands in nonmammalian species) to produce corticosteroids (primarily glucocorticoids).

corticotropin-releasing factor (CRF) A 41-amino-acid polypeptide produced in the hypothalamus and extrahypothalamic sites that stimulates the release of ACTH (all vertebrates studied) and TSH.
(nonmammalian vertebrates) by the anterior pituitary gland. CRF-like peptides play central roles in developmental, behavioral, and physiological responses to stressors.

growth hormone (GH) Simple polypeptide hormone produced by the anterior pituitary gland that stimulates growth in all vertebrate species studied.

metamorphic climax The final and most rapid phase of morphological change when thyroid activity is at its peak.

pedomorphosis Reproductive maturity is attained while in a larval or branchiate form.

premetamorphosis Stage of amphibian larval development when the animal grows but little or no morphological change occurs; plasma thyroid hormone concentrations are low.

prolactin (PRL) Simple polypeptide hormone produced by the anterior pituitary gland that stimulates lactation in mammals and has anti-metamorphic effects in amphibians.

prometamorphosis Stage of amphibian larval development when metamorphosis begins. Hindlimb growth and development is evident externally. The thyroid gland becomes active and secretes thyroid hormone in response to increasing plasma concentrations of pituitary thyroid-stimulating hormone (TSH).

thyroid-stimulating hormone (TSH) Glycoprotein hormone comprised of two subunits produced by the anterior pituitary gland that stimulates the production of thyroid hormone by the thyroid gland.

thyrotropin-releasing hormone (TRH) Tripeptide produced in the hypothalamus and extrahypothalamic sites that stimulates the release of TSH by the anterior pituitary gland.

21.1 Most Amphibian Species Have Complex Life Cycles

Amphibians exhibit considerable diversity in behavioral, physiological, and life history strategies. They are geographically widespread, occupying a diverse range of habitats. The life history strategies of amphibian species include complex life cycles (e.g., metamorphosis) and direct development (Callery et al., 2001). The majority of amphibian species exhibits complex life cycles and, thus, have two very different life stages that are affected differently by environmental factors. Most anuran (frog) larvae are aquatic, and tadpoles are found in a wide variety of habitats, ranging from water-filled crevices in rocks, logs, or leaves to larger ponds or streams. Most then undergo morphological, biochemical, and physiological transformation into adults, which are sensitive to different environmental variables than larvae, due to this shift in habitat (Duellman and Trueb, 1994). Some amphibians have lost the larval form and develop directly into the adult morphology (direct development); others do not metamorphose but reproduce in the aquatic habitat while retaining the larval morphology (pedomorphosis).

Amphibians that undergo a metamorphosis exhibit strong variation, both between and within species, in the duration of the larval period (Werner, 1986; Wilbur and Collins, 1973). Larvae encounter diverse ecological conditions during development. Variation in abiotic factors (e.g., water availability, temperature, and photoperiod) as well as biotic factors (e.g., intra- and interspecific competition and predation) can interact in complex ways to influence larval growth and development (Alvarez and Nicieza, 2002; Downie et al., 2004; Relyea, 2002, 2007; Rowe and Dunson, 1995; Semlitsch, 1987; Sredl and Collins, 1992; Taylor and Scott, 1997). The timing of metamorphosis is a central amphibian life history trait that likely reflects the quality and relative permanence of the larval habitat. Species that breed in predictable habitats (i.e., permanent or semi-permanent lakes and ponds) tend to have longer larval periods. Species that breed in unpredictable habitats (i.e., ephemeral pools) generally have much shorter larval periods (Denver, 1997b).

Wilbur and Collins (1973) suggested that there is a threshold of minimum body size that must be reached before metamorphosis is possible and that larval growth rates determine the timing of metamorphosis after this minimum size has been attained. Werner (1986) added mortality risk in the larval and adult habitat to the list of factors that ultimately influence metamorphosis. The effects of environmental factors may differ depending on the animal's stage of development. These observations led Day and Rowe (2002) to incorporate developmental thresholds into the Wilbur–Collins model. Environmental factors that influence growth rate or mortality risk therefore should alter the timing of metamorphosis, and the effects of the environment may be influenced by the stage of development that has been achieved.
For example, the same factor may be inhibitory to growth if present early in the larval phase or stimulatory to development if present during metamorphosis (e.g., population density, food availability, pond drying, or predation – reviewed by Denver (1997b)). Thus, body size and stage of development may interact in complex ways to determine the phenotypic response to specific environmental variables.

**21.1.1 Plasticity in Amphibian Life Cycles**

Amphibian larvae exhibit plasticity in the timing of metamorphosis and can capitalize on favorable conditions for growth as long as such conditions last (up until a genetically determined upper limit to the length of the larval period; Newman, 1992; Rudolf and Rodel, 2007). Such plasticity may permit amphibian larvae to match their phenotype (morphology, physiology, and metamorphic timing) to prevailing environmental conditions. Animals capable of phenotypic plasticity may have a higher probability of surviving in unpredictable habitats compared with those with a genetically fixed, or canalized phenotype (Gomez-Mestre and Buchholz, 2006; Newman, 1992; Stearns, 1989; Van Buskirk, 2002).

The upper and lower limits to the length of the larval period are determined by genetic factors that are subject to natural selection. The plasticity of larval period length within these limits is also subject to natural selection and is influenced at both proximate and ultimate levels by the environment. While metamorphic timing is determined by both genetic and environmental factors, its expression depends on the development and activity of endocrine glands and the actions of the hormones that these glands produce.

Among the most extreme evolutionary modifications of the ancestral, complex life history is pedomorphosis. Most amphibian larvae undergo a metamorphosis to an adult form before becoming sexually mature. Some species of urodele amphibians (e.g., salamanders and newts) exhibit pedomorphosis, where reproductive maturity is attained while in a larval or branchiate form. Pedomorphosis refers to the retention of juvenile characteristics in sexually mature adults (Gould, 1977). Pedomorphosis can be either obligate or facultative depending on the species. Obligate pedomorphs never undergo metamorphosis and remain in an aquatic habitat their entire lives (e.g., *Necturus*, *Proteus*, *Amphiuma*, *Ambystoma mexicanum*). Facultatively pedomorphic species can either become pedomorphic and remain in the aquatic habitat, or metamorphose and move into the terrestrial environment where they become sexually mature (e.g., *Ambystoma tigrinum*, *A. talpoidum*, *A. gracile*, *Notophthalmus viridescens*; Duellman and Trueb, 1994). The developmental decision to become pedomorphic or to metamorphose in facultative species depends on the prevailing environmental conditions rather than the animal’s genotype (Harris, 1987; Licht, 1992; Semlitsch, 1987; Denoel and Poncin, 2001; Jackson and Semlitsch, 1993) and may be controlled by the interplay of antagonistic hormonal pathways (Rosenkilde and Ussing, 1996; Wakahara, 1994, 1996).

**21.2 Endocrinology of Metamorphosis**

**21.2.1 Overview**

Hormones orchestrate the diverse morphological and physiological changes that occur during metamorphosis and also function as mediators of environmental effects on development. Gudernatsch (1912) first showed that the vertebrate thyroid gland contained a factor that could induce precocious metamorphosis if fed to tadpoles. This compound, later identified as 3,5,3'-tetraiodothyronine (thyroxine T₄; Harrington, 1926, 1927; Kendall, 1915) and referred to as TH, is now known to be the primary hormone controlling amphibian metamorphosis. While hormones produced by the anterior pituitary gland and the interrenal glands (amphibian homologs of the mammalian adrenal cortex) influence the rate of metamorphosis, exogenous TH alone can induce the entire suite of tissue transformations (Kikuyama et al., 1993; Shi, 1996). Furthermore, chemical or surgical thyroidectomy results in metamorphic stasis (Dodd and Dodd, 1976; Kikuyama et al., 1993).

The work of William Etkin laid much of the foundation for our current understanding of the endocrine control of metamorphosis. Etkin (1968) proposed a model for the hormonal changes that occur during amphibian metamorphosis. He also coined the terms in common use today among amphibian endocrinologists for describing three stages of anuran development: (1) premetamorphosis, when the larvae grow but little or no morphological change occurs and plasma TH concentrations are low; (2) prometamorphosis, when hindlimb growth accelerates and plasma TH concentration rises; and (3) metamorphic climax, the final and most rapid phase of morphological change when thyroid activity is at its peak (Dodd and Dodd, 1976; White and Nicoll, 1981; Table 1).
The objective of the following section is to describe the cast of endocrine characters that interact to control metamorphosis. Each endocrine axis involved in metamorphosis will first be presented in terms of its developmental schedule. This should allow predictions of when the endocrine system is sufficiently developed to allow the animal to become competent to respond to the external environment. The multiple levels at which the activity and functioning of each endocrine axis can be regulated will then be considered. The goal, which is addressed in Section 21.3, is to understand how the endocrine system determines the timing of metamorphosis and mediates environmental effects on amphibian development.

### 21.2.2 Thyroid Hormone

#### 21.2.2.1 Role of thyroid hormone in amphibian development

Perhaps, the most striking characteristic of amphibian metamorphosis, from the perspective of hormonal control, is that a single signaling molecule, produced by a highly restricted group of cells (the thyroid epithelial cells), can orchestrate the entire suite of molecular, biochemical, and morphological changes. Depending on the tissue, TH can induce cell proliferation, cell death, differentiation, or migration. Target cells for TH are now known to activate both similar and different sets of genes according to the concentration of this single signaling molecule.

### Table 1

A comparison of three of the most widely cited staging tables for postembryonic, feeding stages of anuran larvae

<table>
<thead>
<tr>
<th>N–F&lt;sup&gt;a&lt;/sup&gt; staging for X. laevis</th>
<th>Major, common diagnostic features/ morphological changes</th>
<th>T–K staging&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Gosner staging&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Terminology of Etkind&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–45</td>
<td>Nonfeeding stages (comparable to Shumway stages&lt;sup&gt;e&lt;/sup&gt; 1–24)</td>
<td>I</td>
<td>1–25</td>
<td></td>
</tr>
<tr>
<td>46</td>
<td></td>
<td></td>
<td>26</td>
<td>Premetamorphosis</td>
</tr>
<tr>
<td>47–48</td>
<td>Feeding begins</td>
<td>II</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>49–50</td>
<td></td>
<td>III</td>
<td>28</td>
<td></td>
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<tr>
<td>51</td>
<td></td>
<td>IV</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>52</td>
<td>Foot paddle stages</td>
<td>V</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>53</td>
<td></td>
<td>VI</td>
<td>31</td>
<td></td>
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<tr>
<td>54</td>
<td></td>
<td>VII</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>55</td>
<td>Hindlimb stages</td>
<td>VIII</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>56</td>
<td></td>
<td>IX</td>
<td>34</td>
<td>Prometamorphosis</td>
</tr>
<tr>
<td>57–58</td>
<td></td>
<td>X</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>59</td>
<td></td>
<td>XI</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>Tadpole reaches maximum length</td>
<td>XII</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>61</td>
<td></td>
<td>XIII</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>62</td>
<td>Rapid tail resorption begins, front limbs erupt&lt;sup&gt;f&lt;/sup&gt;</td>
<td>XIV–XVI</td>
<td>39–40</td>
<td></td>
</tr>
<tr>
<td>63</td>
<td></td>
<td>XVII</td>
<td>40</td>
<td></td>
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<tr>
<td>64</td>
<td></td>
<td>XVIII</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>65</td>
<td></td>
<td>XIX</td>
<td>42</td>
<td>Climax</td>
</tr>
<tr>
<td>66</td>
<td>Stump of tail remains</td>
<td>XX</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>66</td>
<td>Tail completely resorbed, juvenile frog</td>
<td>XXI</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>66</td>
<td></td>
<td>XXII</td>
<td>45</td>
<td></td>
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<tr>
<td>66</td>
<td></td>
<td>XXIV</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>66</td>
<td></td>
<td>XXV</td>
<td>47</td>
<td></td>
</tr>
</tbody>
</table>

Note: Table is derived from similar tables published by Nieuwkoop and Faber (1956), Dodd and Dodd (1976), and Kikuyama et al. (1993), with the addition of Gosner staging. Note that the table is modified somewhat with respect to the table published by Kikuyama et al. (1993) with deference to the comparison between X. laevis and the staging of R. pipiens (Taylor and Kollros, 1946) made by Nieuwkoop and Faber (1956). Comparison of Taylor and Kollros (1946) with Gosner (1960) staging tables is based on that of Gosner (1960).

<sup>a</sup>Stages of Nieuwkoop and Faber (1956).

<sup>b</sup>Stages of Taylor and Kollros (1946).

<sup>c</sup>Stages of Gosner (1960).

<sup>d</sup>Terminology of Etkin (1968).

<sup>e</sup>Stages of Shumway (1940).

<sup>f</sup>Note that the front limbs erupt in X. laevis at stage 58 and continue to grow and develop through metamorphic climax. In other amphibians such as ranids or pelobatids (e.g., Scaphiopus), front limbs develop internally and then erupt at metamorphic climax.
Specific tissues exhibit different dose sensitivities to TH, and the challenge for investigators studying the molecular basis of TH action during metamorphosis is to determine how and why individual tissues respond differently to the hormone and exhibit differential dose responses (see Brown and Cai (2007)).

21.2.2.2 Thyroid gland development and hormone production

The thyroid gland develops early in the amphibian embryo when the anlage consists of a thickening of the pharyngeal epithelium; these cells are capable of synthesizing small iodoproteins (reviewed by Dodd and Dodd (1976) and Regard et al. (1978)). The gland matures functionally at the time of hatching when it separates into two distinct lobes and is essentially completely developed. The thyroid is thought to be functional at metamorphosis (Dodd and Dodd, 1976; Regard et al., 1978; Dodd and Dodd, 1976; Kaye, 1959, 1961; Nieuwkoop and Faber, 1956; Regard et al., 1978; Saxen et al., 1957a,b). Multiple measures of thyroid activity, including radioiodine uptake, gland ultrastructure, and plasma concentration or tissue content of THs, show that thyroid activity increases markedly during metamorphosis (Table 1 and Figure 1), peaks at metamorphic climax, and declines thereafter to reach an adult level of activity (Dodd and Dodd, 1976; Kaye, 1959, 1960; Kikuyama et al., 1993; Regard et al., 1978). Ultrastructural analyses show a dramatic increase in thyroid follicular cell height during metamorphosis with a peak at metamorphic climax that corresponds to the peak in plasma concentrations (and tissue content) of THs (Dodd and Dodd, 1976; Regard et al., 1978).

When Etkin proposed his endocrine-based model for metamorphosis, investigators at the time did not have sensitive and quantitative methods for determining plasma TH concentrations. Early methods relied on the determination of protein-bound iodide to estimate plasma TH titers (Just, 1972). Subsequently, sensitive and specific radioimmunoassays (RIAs) were developed that allowed determination of plasma thyroxine (the primary product of the thyroid gland) and 3,5,3′-triiodothyronine (T3; derived from T4 by monodeiodination in target tissues) concentrations during metamorphosis. These studies confirmed earlier studies and the predictions of Etkin by demonstrating low-to-nondetectable plasma TH concentrations during premetamorphosis, increasing concentrations during prometamorphosis, and a dramatic peak at metamorphic climax (Figure 1(b); Leloup and Buscaglia, 1977; Miyauchi et al., 1977; Mondou and Kaltenbach, 1979; Weil, 1986; Niinuma et al., 1991a; Regard et al., 1978; Suzuki and Suzuki, 1981; Weber et al., 1994; Denver, 1998a; Krain and Denver, 2004).

Because of the difficulty of obtaining blood from small tadpoles for analysis by RIA, until recently only those species with tadpoles large enough to obtain a serum sample were analyzed. Thus, most blood measurements have been done on ranid species (e.g., Rana catesbeiana; Rana clamitans; however, Leloup and Buscaglia (1977) and Tata et al. (1993) measured THs in plasma pools of Xenopus laevis; see also Buscaglia et al. (1985) for measures of plasma T3 and T4 in other Xenopus spp.). In species with small tadpoles, developmental changes in TH content of whole bodies and individual tissues have been determined. These analyses have shown that changes in whole-body TH content in the smaller species essentially parallel changes observed in the plasma of tadpoles of the larger species (Bufo japonicus (Niinuma et al., 1991a); Spea hammondii (Denver, 1993, 1998a); X. laevis (Krain and Denver, 2004); Bufo marinus (Weber et al., 1994)). The peak in whole-body T3 and T4 coincides with peak uptake of 131I in B. japonicus (Niinuma et al., 1991a). Thus, it is likely that determination of whole-body hormone content provides a reasonable estimate of physiological changes in TH production in species for which blood samples are unobtainable.

21.2.2.3 Thyroid hormone metabolism

The major product of the amphibian thyroid gland is T4 with minor amounts of T3 produced (Buscaglia et al., 1985; Rosenkilde, 1978). The result is that plasma T4 concentration tends to be an order of magnitude greater than T3 (Larras-Regard et al., 1981; Regard et al., 1978). The only case where this relationship may not hold is for X. laevis where the reported plasma T3:T4 ratio is very similar and may even exceed 1 at metamorphic climax (Buscaglia, 1985; Leloup and Buscaglia, 1977). Measures of tissue content of T4 and T3 in various species show that the two hormones are present in roughly similar amounts (Niinuma et al., 1991a; Weber et al., 1994; Denver, 1997a, 1998a; Krain and Denver, 2004). Although a comprehensive analysis of both blood concentrations and tissue contents of THs has not been done for any species, it is likely that the higher T3:T4 ratio in tissues compared with T3:T4 ratios in blood reflects high tissue T3′-monodeiodinase activity.

Tissue monodeiodinases convert the product of the thyroid gland, T4, to T3 by removing one iodine atom at the T3′ position. T3′ is often referred to as the biologically active form of TH since the TH receptors (TRs) possess 10 times greater affinity for T3 than
for T₄ (Leonard and Visser, 1986; Oppenheimer et al., 1995). Similarly, T₃ exhibits 3–10 times greater biological activity than T₄ in amphibia as in other vertebrates (Lindsay et al., 1967; Rosenkilde, 1978; Wahlborg et al., 1964; Frieden, 1981; White and Nicoll, 1981). Thus, current data support the view that while T₄ is the primary product of the thyroid gland, T₃, derived from conversion within the target tissues, is the biologically active form of the hormone. T₄ can also be inactivated by conversion to reverse T₃ (3,3',5'-triiodothyronine; rT₃) and diiodothyronine (T₂); neither compound binds to the TRs. Similarly, T₃ can be inactivated by deiodination.

The tissue deiodinases catalyze two basic reactions: a 5' monodeiodination (outer ring) that results in bioactivation and a 5' monodeiodination (inner ring)
that results in bioinactivation of the substrate, T₄ or T₃. Three types of vertebrate deiodinases have been described that differ in their substrate specificity, kinetics, and sensitivity to inhibitors. Thus, the isozymes were originally identified by operational definitions based on their biochemical and pharmacological characteristics and not as specific polypeptides. However, recent cloning of complementary DNAs (cDNAs) for subunits of each of these enzymes now allows assignment of biochemical attributes to specific proteins (see Brown (2005) and Galton (2005)).

Tadpoles possess both 5' and 5'-deiodinase activities. Early biochemical studies identified enzymes with primarily type 2 and type 3 activities, but no evidence for an enzyme with type 1 characteristics (Becker et al., 1997). cDNAs for two enzymes, corresponding to these two different activities, have been cloned in R. catesbeiana and in X. laevis (see Brown (2005)). In both species these two enzymes exhibit tissue-specific and developmental stage-specific expression patterns (Becker et al., 1997; Brown, 2005; Cai and Brown, 2004). Recently, a cDNA homologous to the mammalian type 1 deiodinase was isolated from X. laevis and its expression patterns, and the biochemical properties of its protein product were characterized (Dubois et al., 2006; Kuiper et al., 2006). For simplicity, in the following discussion the type 1 enzyme is abbreviated as D1, the type 2 enzyme as D2, and the type 3 enzyme as D3.

During metamorphosis, coincident with rising plasma titers of T₃ and T₄, there is an increase in both D2 and D3 activities in target tissues (Becker et al., 1997; Brown, 2005; Brown et al., 1996; Buscaglia, 1985; Galton, 1988; Kawahara et al., 1999). In bullfrog tadpoles the D2 and D3 enzymes exhibit differential tissue expression. For example, D2 enzyme activity (and mRNA) is expressed in tail, intestine, hindlimb, forelimb, eye, and skin, but no D2 could be detected in the liver or kidney of bullfrog tadpoles at any stage of development (Galton, 1988; Galton and Hiebert, 1988; Becker et al., 1997). This finding contrasts sharply with many other vertebrates where both the liver and kidney possess high 5' deiodinase activities, and both organs are thought to be the primary sources of circulating T₃ (St. Germain and Galton, 1997). By contrast with D2, D3 enzyme activity (and mRNA) is expressed in liver and kidney, as well as tail, intestine, hindlimb, forelimb, eye, skin (R. catesbeiana: Becker et al., 1997; X. laevis: Brown et al., 1996; Wang and Brown, 1993), and brain (X. laevis: head: Brown et al., 1996; brain: Denver et al., 1997). Little is known about the expression or potential roles in metamorphosis of the D1 enzyme. The expression of D1 mRNA has been investigated during early embryogenesis and in juveniles of X. laevis, but little is known about its expression patterns in the tadpole except that it may be expressed in brain ventricular zones of climax-stage tadpoles (Dubois et al., 2006; Kuiper et al., 2006).

In tissues where both D2 and D3 are expressed, they exhibit comparable ontogenetic expression profiles (Becker et al., 1997; Brown, 2005). In the tadpole, the expression patterns of each of these genes correlate well with the schedule of metamorphic changes in particular organs (Brown, 2005). The constitutive expression of D2 is correlated with early events in metamorphosis, while TH-induced expression correlates with late events. For example, D2 activity is highest in the eyes and hindlimbs during prometamorphosis, at which time the major part of the retina and the limbs are differentiating, and declines at metamorphic climax. In the tail, which is the last organ to undergo metamorphic transformation (resorption), D2 activity is very low until metamorphic climax; a similar pattern is seen in the intestine (Becker et al., 1997). The importance of D2 activity for hindlimb development is supported by findings that T₄ has no effect on the hindlimb in the presence of the deiodinase inhibitor, iopanoic acid (IOP). By and large, the ontogenetic expression of D2 in X. laevis was found to be similar to R. catesbeiana, with the notable exception of the eyes where in R. catesbeiana D2 enzymatic activity and mRNA were detected from early in development, but not in X. laevis (Becker et al., 1997; Brown, 2005).

The expression of D3 exhibits similar ontogenetic profiles to D2 in R. catesbeiana (Becker et al., 1997). These findings led Becker et al. (1997) to hypothesize that the co-expression of the two enzymes during metamorphosis generates a push/pull mechanism, thereby providing for tight control of intracellular T₃ concentrations in tissues at times of maximum metamorphic changes. However, while these findings in the bullfrog were partially corroborated in X. laevis for D3 mRNA expression, species differences were also evident (Kawahara et al., 1999). D3 mRNA in X. laevis showed similar ontogenetic profiles to R. catesbeiana in tail, intestine, and liver, but expression in the hindlimb and kidney showed patterns of expression that were directly opposite. In Rana pipiens, brain D2 mRNA expression showed a progressive decrease through metamorphosis, while brain D3 mRNA was dramatically upregulated at metamorphic
climax similar to *X. laevis* (Hogan et al., 2007). The meaning of such species differences in expression patterns is unknown, but must be understood in order to derive general principles regarding the roles that the deiodinases play in regulating tissue responsiveness to TH during metamorphosis.

The regulation of deiodinase gene expression is poorly understood. Conflicting results for the regulation of D2 activity have been published. Buscaglia et al. (1985) reported that in *X. laevis* treated with the goitrogen perchlorate, D2 activity remained at low, premetamorphic levels. Replacement with T3 or T4 in these animals induced D2 activity, suggesting that TH positively regulates 5’ deiodination. By contrast, Becker et al. (1997) reported that in bullfrog tadpoles treated with the goitrogen methimazole D2 activity was elevated, and replacement with T4 but not T3 downregulated this activity. The expression of D2 mRNA is upregulated in *X. laevis* tail by TH treatment, but this is a delayed response requiring days of TH administration (Brown, 2005). In tadpoles of *R. pipiens*, D2 mRNA was induced in brain by a 48-h treatment with T3 (Hogan et al., 2007). By contrast to D2, D3 enzyme activity and mRNA are clearly upregulated by T3. The cDNA for the *X. laevis* D3 gene was twice isolated as a T3-regulated gene in differential screens of tail and brain (Denver et al., 1997; Wang and Brown, 1993). Response kinetics and the resistance of upregulation of the mRNA to protein synthesis inhibition suggest that D3 is a direct T3 response gene in *X. laevis* and *R. catesbeiana* (Becker et al., 1995; St. Germain et al., 1994); however, a thyroid hormone response element has not yet been identified in the D3 gene. This gene is upregulated in tail, brain, intestine, and hindlimb but is downregulated in liver (Denver et al., 1997; Hogan et al., 2007; Kawahara et al., 1999; Wang and Brown, 1993). This pattern of T3 responsiveness fits the ontogenetic expression profiles for the gene where it is upregulated during late metamorphosis/m metamorphic climax in each of the tissues in which it responds positively to the hormone but downregulated in the liver (Kawahara et al., 1999). Clearly, the roles of THs and other physiological and environmental factors in the regulation of deiodinase gene expression and enzyme activity require further study.

What is the evidence for a physiological role for tissue deiodinases in the control of metamorphosis? Several investigators have treated tadpoles with IOP, which blocks D2 and D3 activities in tadpoles (Becker et al., 1997; Buscaglia, 1985; Cai and Brown, 2004; Galton, 1989; Huang et al., 2001). The hypothesis tested was: if conversion of T4 to T3 is important for the metamorphic process then IOP should block metamorphosis. As predicted, treatment with IOP inhibited metamorphosis, and this blockade could be overcome by replacement with T3 but not with T4 (Becker et al., 1997; Cai and Brown, 2004; Galton, 1989). These findings support the view that T3 is the biologically active hormone and its generation from T4 is essential to metamorphosis. Similarly, the importance of the degradation of THs to the coordination of metamorphic transformations is supported by studies with transgenic frogs. Overexpression of a D3-green fluorescent protein (GFP) fusion protein in transgenic *X. laevis* resulted in metamorphic stasis and resistance to exogenous TH (Huang et al., 1999). At a finer level, type 3 deiodinase has been implicated in the modulation of T3-dependent development of the visual system in tadpoles (Marsh-Armstrong et al., 1999) and the development of motor connections between the spinal cord and hindlimbs (Marsh-Armstrong et al., 2004). Taken together, the current data point to a central role for tissue deiodinases in modulating tissue responsiveness to T3 through their exertion of tight control over intracellular concentrations of the hormone.

### 21.2.2.4 Thyroid hormone transport in blood

Once synthesized, T4 diffuses out of thyroid follicular cells and into the bloodstream where it becomes reversibly bound to plasma proteins. The plasma proteins serve to transport the hormone from the site of production to its target tissues. Several vertebrate plasma-binding proteins that bind T4 and T3 with varying affinities have been identified. Throxine-binding globulin (TBG) is found only in large, eutherian mammals, and it binds T4 with high affinity and low capacity (Power et al., 2000). Transthyretin (TTR; also known as prealbumin) is found in all vertebrates and it binds T4 with moderate affinity and intermediate capacity. Both TBG and TTRs can also bind T3, although in most cases with 10 times lower affinity than T4 (Power et al., 2000; although the situation in amphibia is the reverse – see below). The two primary sites for TTR expression in vertebrates are the liver and the choroid plexus (although it is expressed at other sites; Power et al., 2000). In most mammals TTR is expressed in both tissues, in reptiles it appears to be expressed only in the choroid plexus, and in teleosts and amphibians it is expressed primarily in the liver (Power et al., 2000; although see Funkenstein et al. (1999) for TTR expression in the...
skin and other tissues of the teleost fish, *Sparus aurata*). An essential function of TTR is its interaction with retinol-binding protein, which acts as a carrier for all-*trans*-retinol in the blood. The functional significance of this interaction is not known, but it is intriguing that T3 and 9-*cis*-retinoic acid (which is a metabolite of all-*trans*-retinol) serve as ligands for the TR-retinoid X receptor (RXR) heterocomplex. Current evidence supports the hypothesis that the TR-RXR heterodimer is the active complex, which binds to promoters of TH target genes and activates transcription in the presence of TH. Serum albumin also binds T3 and T4 in many species with low affinity and high capacity. Power et al. (2000) suggest that albumin might be the principal T4-binding protein in amphibians.

By contrast with other tetrapods, but similar to teleost fish, amphibian TTRs exhibit much greater affinity for T3 than for T4 (Yamauchi et al., 1993, 1998, 1999, 2000). The functional significance of the apparent evolutionary transformation of TTR from a T3-binding to a T4-binding protein is not known (Power et al., 2000). In *R. catesbeiana*, the binding affinity of TTR for T3 is much lower than the bullfrog (affinity for T4: 100–360 nM; Yamauchi et al., 1993; whole plasma or recombinant TTR: 8–9 nM; Yamauchi et al., 2000). By contrast, the affinity of recombinant *X. laevis* TTR for T3 is much lower than the bullfrog protein (550 nM; Yamauchi et al., 2000). However, a similar relationship between the affinities of TTR for T3 and T4 exists in *X. laevis* (affinity for T4: 13 μM; Yamauchi et al., 2000). Circulating TTR protein is present in bullfrog and *X. laevis* tadpoles during premetamorphosis and prometamorphosis but declines at metamorphic climax (Prapunpooj et al., 2000; Yamauchi et al., 1998, 2000).

What might be the functional significance of the developmental expression pattern of TTR in tadpoles? TTR expression is high during metamorphosis when thyroid activity is increasing (see above) and plasma T4 and T3 concentrations are rising. Based on the free hormone hypothesis (Ekins, 1990; Mendel, 1989), one would predict that TTR at this stage of development would reduce the free fraction of hormone in the blood and, thus, limit the availability of the hormone to target tissues. On the other hand, TTR would serve as a sink for the hormone in the blood, thus maintaining increasing plasma concentrations of THs before thyroid gland activity accelerates in response to rising titers of plasma TSH. At metamorphic climax, when plasma T3 and T4 concentrations are maximal, TTR concentration in the blood declines. The continued rise in plasma TH concentrations (without a high-affinity plasma protein binder to slow hormone clearance) would likely result in an increased free hormone fraction (at least for T3) in the blood. At the same time the rate of clearance of T3 from the circulation would likely increase. However, because the thyroid synthetic rate is so high at metamorphic climax, total T3 concentrations continue to rise. Thus, one would predict that not only does the hormone production rate increase at metamorphic climax, but so does the proportional availability of T3 to the target tissues. To my knowledge, T3 or T4 clearance rates have not been calculated in tadpoles at different stages of development. Based on TTR expression profiles one would predict that clearance rates would be lower during prometamorphosis compared with premetamorphosis or metamorphic climax. Furthermore, given the lower affinity of TTR for T4 compared with T3, one would predict that the clearance rate for T4 would be higher than T3.

### 21.2.2.5 Cellular uptake of thyroid hormone

Saturable, carrier-mediated uptake of THs has been demonstrated in tadpole RBCs (Galton et al., 1986; Murata and Yamauchi, 2005; Yamauchi et al., 1989), but little is known about TH transporters in amphiabians. Krain and Denver (2004) found that tadpoles take up and concentrate T3 from their environment to 4–6 times than the beginning environmental concentration. This finding suggests that tadpole cells have the capacity to actively take up TH from the circulation, and this activity could be regulated during metamorphosis. The genes that encode TH transport proteins could be important loci for the regulation of metamorphosis.

Until recently, it was thought that because of their lipophilicity, THs entered cells by simple diffusion across plasma membranes. However, the highly polar nature of the alanine side chain precludes free membrane passage of the iodothyronines (Friesema et al., 1999). It is now clear that THs can be actively taken up by cells via plasma membrane transporters (Friesema et al., 2005; Jansen et al., 2005; Visser et al., 2008). These transporters belong to two classes of proteins, the organic anion transporters and the amino acid transporters. The monocarboxylate transporters MCT8 and MCT10, and the organic anion-transporting polypeptide OATP1C1 demonstrate the highest degree of specificity for TH transport.
Amino acid permeases have been implicated in the uptake of THs by cells (see Ritchie et al. (1999, 2003)). The T3-inducible gene IU12 from *X. laevis* intestine (Liang et al., 1997; Shi and Brown, 1993) encodes a subunit of a heterodimeric amino acid permease complex (System L; Ritchie et al., 2003; Torrents et al., 1998). This permease complex efficiently transports T3 and T4 when expressed in the *Xenopus* oocyte expression system, but is inhibited by reverse T3 (Ritchie et al., 1999). Overexpression of System L in *Xenopus* oocytes increased cytoplasmic and nuclear delivery of THs from the external medium and enhanced transcriptional activation by TRs (Ritchie et al., 2003). By contrast, blocking endogenous System L activity in mammalian cells reduced both TH uptake and TR function (Ritchie et al., 2003). The fact that IU12 is a T3-inducible gene suggests that it could play a role in mediating T3 uptake by cells during tadpole metamorphosis (Liang et al., 1997). The possibility of specific receptors for TTR also has been demonstrated, although this means of hormone uptake requires further investigation (Divino and Schussler, 1990; Schussler, 2000).

Upon entering cells, and before binding to nuclear receptors (see below), THs encounter a series of intracellular-binding proteins. These cytoplasmic TH-binding proteins (CTHBPs) are represented by several classes of multifunctional proteins. These proteins have a variety of enzymatic activities within the cell. For example, two genes were cloned in *X. laevis* that are CTHBPs: one is a cytosolic aldehyde dehydrogenase which catalyzes the formation of retinoic acid (an important developmental signaling molecule that signals via nuclear receptors (Yamauchi and Tata, 1994) and the other is homologous to mammalian M2 pyruvate kinase (Shi et al., 1996a). Protein disulfide isomerase (PDI) and related proteins catalyze the formation of disulfide bonds within and between proteins and human PDI possesses a high-affinity binding site for TH (Cheng et al., 1987; Yamauchi et al., 1987). A cDNA encoding a PDI-like protein was isolated as a T3-responsive gene from *X. laevis* tadpole brain (Denver et al., 1997).

It has been suggested that the functional significance of hormone binding to these CTHBPs is to serve to transport THs within the cytoplasm to the nucleus where the TRs are located. Alternatively, they could serve as chelators to limit the cellular free TH concentration or act as buffer proteins in the maintenance of intracellular levels of TH (Shi, 2000a). However, in considering a role for these proteins in TH transport, the possibility that TH might serve a regulatory role for the enzymatic activities of these proteins should not be overlooked. As an example, the human M2 pyruvate kinase functions as a kinase in its tetrameric form, but only binds TH in its monomeric form. The binding of TH results in a shift toward the monomeric form and, thus, the inhibition of the kinase activity (Ashizawa and Cheng, 1992). Thus, one would predict that TH would serve to inhibit this enzymatic pathway.

### 21.2.2.6 Mechanisms of thyroid hormone action

Tadpoles become competent to respond to exogenous TH at the time of hatching (Tata, 1968). This establishment of competence to respond to the hormone likely depends on the expression of TH receptors (TRs; Shi et al., 1996b). Thyroid hormone receptors are ligand-activated transcription factors that belong to the steroid hormone receptor superfamily (Mangelsdorf et al., 1995). There are two TR genes, termed α and β, in all vertebrates studied to date; owing to its pseudotetraploidy, *X. laevis* possesses four TR genes, two α and two β (Buchholz et al., 2006). The two *X. laevis* TRα genes, each appear to give rise to single, unique proteins; whereas, alternative mRNA splicing of TRβ transcripts can give rise to two different receptor isoforms for each TRβ gene (Buchholz et al., 2006).

The TRα genes are first expressed shortly after hatching in *X. laevis* and their expression rises during premetamorphosis and remains high throughout metamorphosis (Baker and Tata, 1990; Banker et al., 1991; Kawahara et al., 1991; Yaoita and Brown, 1990). It has been hypothesized that the early expression of TRα establishes hormone responsiveness of tadpole tissues (Baker and Tata, 1990; Shi et al., 1996a). TRβ mRNA is not detected until early prometamorphosis, but its expression increases during prometamorphosis in parallel with TH synthesis (Baker and Tata, 1992; Kamamori and Brown, 1992; Kawahara et al., 1991; Yaoita and Brown, 1990). Several studies have shown that the TR genes are upregulated by T3 in *X. laevis* and *Rana catesbeiana* (Helbling et al., 1992; Kawahara et al., 1991; Schneider and Galton, 1991; Yaoita and Brown, 1990; a phenomenon termed autoinduction; see Tata et al. (1993)). A thyroid hormone response element to
which TRs can bind and regulate transcription has been identified in the X. laevis TRβA gene (Machuca et al., 1995; Ranjan et al., 1994). Autoinduction may require the upregulation of accessory transcription factors such as the immediate early, TH-inducible gene basic transcription element binding protein 1 (BTEB1) (Bagamasbad et al., 2008).

A central role for TRs in metamorphosis is supported by transgenic studies in X. laevis. For example, transgenic expression of a dominant negative TR blocks metamorphosis (Buchholz et al., 2003; Schreiber et al., 2001), while expression of a dominant positive TR promotes metamorphic changes in the absence of TH (Buchholz et al., 2004). Specific functions for the different receptors in amphibia are poorly understood. Results of gene targeting experiments in mice point to a network of specific and common TR pathways, but have as yet failed to provide a complete picture of the roles for these different receptors (Flamant and Samarut, 2003; Forrest and Vennstrom, 2000). There is evidence in mammals that the TRs possess different functional characteristics (Zhu et al., 1999) and can mediate different cellular responses to T3; (Lebel et al., 1994), presumably by regulating different sets of genes (Denver et al., 1999; Guissouma et al., 1998; Sandhofer et al., 1998; Dupre et al., 2004; Guissouma et al., 2005; Flamant and Samarut, 2003). Functional studies addressing specific functions for the different TRs in amphibians have been limited by the inability to specifically delete or knockdown the TRs. Expression studies suggest differential roles, as do recent pharmacological studies with TR subtype selective agonists. For example, studies with the TRα selective agonist CO23 support that this receptor subtype is involved in cell proliferation (RJ Denver, F Hu, TS Scanlan, and JD Furlow, unpublished results; Ocasio and Scanlan, 2006), while studies with the TRβ selective agonists GC1 and GC24 support that this subtype is primarily involved with tissue resorption (apoptosis) and cell differentiation (RJ Denver, F Hu, TS Scanlan, and JD Furlow, unpublished results; Furlow et al., 2004; Ocasio and Scanlan, 2006). The low basal expression level and the failure of TH to upregulate TRβ expression in the obligate pedomorphic salamander Necturus maculosus supports the view that this receptor subtype is necessary for promoting tissue resorption, as the gills of this species fail to resorb in response to TH treatment unlike metamorphosing or facultatively pedomorphic salamanders (Safi et al., 1997, 2004, 2006; Vlaeminck-Guillem et al., 2006).

TRs function as dimers, that is, the DNA consensus sequences that TRs bind to are six nucleotides in length and are referred to as half sites. Two of these half sites comprise a TH response element (TRE) (Williams and Brent, 1995). These TREs can be located within the promoter, within the structural part of the gene, or upstream of the transcription start site. Homodimers of TRα or TRβ can form on most TREs, but the preferred configuration appears to be as a heterodimer with RXR (Puzianowska-Kuznicka et al., 1997; Wong and Shi, 1995). TR–RXR heterodimers bind DNA and transactivate TRE-containing genes much more effectively than TR homodimers. In the unliganded form, the TR–RXR complex functions as a transcriptional repressor (Wong and Shi, 1995). The TR–RXR heterocomplex recruits cofactor proteins that mediate the repressive or activation actions of the complex (McKenna and O'Malley, 2002; Shi, 2000b). The unliganded receptor may have important developmental functions in the premetamorphic tadpole (discussed below). The TR and RXR genes exhibit more or less coordinate regulation during metamorphosis and this coordination may be essential to the timing of tissue-specific changes (Wong and Shi, 1995).

Hormone binding to the TR–RXR receptor complex induces gene expression in target tissues. The TRs cause modifications of chromatin through recruitment of coactivator and corepressor proteins. In the unliganded state, TRs function primarily as transcriptional repressors, recruiting corepressors such as nuclear receptor corepressor (NcoR) and silencing mediator of retinoid and thyroid hormone receptor (SMRT) which then recruit histone deacetylases (Tomita et al., 2004). This leads to a compact, repressive chromatin structure (Jones and Shi, 2003; Sachs et al., 2001). When TH binds to TRs, corepressors are exchanged for coactivators, leading to transcriptional derepression and transactivation. Many of the coactivators have intrinsic histone acetyl transferase activity, leading to the addition of acetyl groups to lysine residues on histone tails. Coactivator expression and recruitment to target genes is correlated with tissue transformation and gene activation (Paul et al., 2005a; Paul and Shi, 2003), and transgenic analysis suggests that the recruitment of coactivators by TRs is essential for metamorphosis to proceed (Paul et al., 2005b, 2007). A role for unliganded TR in tadpole development, whereby it represses the expression of adult genes (Sachs et al., 2002; Sato et al., 2007), has led to the development of the dual function model for TR action during metamorphosis.
This model proposes that unliganded TRs play an important role in development by repressing adult genes in the tadpole prior to the onset of thyroid activity, but activate expression of metamorphosis-associated genes when ligand is present (reviewed by Buchholz et al. (2006)). Discussion of the characteristics of the gene regulation cascades and the functions of the gene products induced by TH in different tissues during metamorphosis is beyond the scope of this chapter. The reader is referred to reviews by Furlow and Neff (2006), Buchholz et al. (2006), and Brown and Cai (2007) for detailed discussions of these topics.

21.2.3 Corticosteroids

Corticosteroids are the primary vertebrate stress hormones and are produced in response to a variety of environmental signals (Selye, 1976). The production of corticosteroids changes with development and likely reflects the functional maturation of the hypothalamic–hypophyseal–interrenal axis.

21.2.3.1 Hormones produced by amphibian interrenal glands

Corticosterone and aldosterone appear to be the major corticosteroids produced by the amphibian interrenal glands (Carstensen et al., 1961; Macchi and Phillips, 1966). In many species there is an elevation in plasma concentrations of these hormones during metamorphic climax that is more or less synchronous with increases in plasma TH.

The interrenal gland is generally less active in early, premetamorphic developmental stages and more active during prometamorphosis and metamorphic climax (Dodd and Dodd, 1976). The ultrastructural appearance of X. laevis interrenal cells indicates relative inactivity in midprometamorphs, increasing to peak activity at metamorphic climax (reviewed by Dodd and Dodd (1976); however, see below for contrary evidence). Activity of the interrenal enzyme, $\Delta^3$-3$\beta$-hydroxysteroid dehydrogenase (HSD), is present throughout development in R. catesbeiana and X. laevis but increases at metamorphic climax in R. catesbeiana (Hsu et al., 1980; Kang et al., 1995). Carr and Norris (1988) found a similar pattern for plasma corticosterone and interrenal HSD activity in the tiger salamander, A. tigrinum.

RIAs for corticosteroids have been done on plasma samples collected throughout the metamorphic period for several amphibian species (R. catesbeiana: Jaffe, 1981; Kikuyama et al., 1986; Krug et al., 1983; B. japonicus: Niinuma et al., 1989, X. laevis: Jolivet-Jaudet and Leloup-Hatey, 1984; A. tigrinum: Carr and Norris, 1988). Whole-body measures of corticosteroid content have also been determined throughout development (S. hammondii: Denver, 1998a, X. laevis: Kloas et al., 1997; Krain and Denver, 2004; Glennemeier and Denver, 2002a, R. pipiens: Glennemeier and Denver, 2002a). The majority of these studies showed low-to-nondetectable corticosteroids during premetamorphosis, and a marked increase at metamorphic climax, more or less in parallel with the rise in THs. The only exception to this rule is whole-body corticosteroid content in X. laevis. Kloas et al. (1997) reported that whole-body corticosterone content in X. laevis increases during prometamorphosis to reach a peak at NK stage 48 and then declines during metamorphosis and is low at metamorphic climax. Kloas et al. (1997) also measured whole-body aldosterone and found a similar increase during prometamorphosis but the peak production was during early metamorphosis (NK stage 54) and it declined thereafter. Glennemeier and Denver (2002a) obtained similar results with corticosterone, although in X. laevis there was a small increase at metamorphic climax. Whether these findings in X. laevis represent species differences, or whether changes in whole-body corticosteroid content are not representative of changes in plasma concentrations is currently unknown.

Corticosteroids, being lipophilic, are transported in blood bound to plasma proteins. Corticosteroid-binding globulin (CBG) is the primary plasma protein to which corticosteroids bind in mammals, although albumin also plays a transport role (Hammond, 1990; Rosner, 1990). Recently, binding properties of a putative CBG present in amphibian serum (A. tigrinum) were reported by Orchinik et al. (2000). However, the expression of CBG has not been studied in amphibians nor is there anything known of the role that such a protein might play in maintaining corticosteroid balance in frogs and tadpoles.

21.2.3.2 Roles of corticosteroids in amphibian growth and development

Corticosteroids may influence growth and development of larval anurans, but their influence is more complex than that of TH. Exogenous corticosteroids can either accelerate or decelerate metamorphosis, depending on the animal’s developmental stage and TH status. Studies using relatively large doses of exogenous corticosteroids have shown that these hormones inhibit forelimb emergence when administered during premetamorphosis (Darras et al., 2002; Frieden and Naile, 1955; Gray and Janssens, 1990; Hayes et al., 1993; Hayes, 1995; Kobayashi, 1958; Wright et al., 1994).
The effects of exogenous corticosteroids on tadpole growth are more straightforward than their developmental effects. Administration of various corticosteroid doses to both pre- and prometamorphic tadpoles inhibits their growth (Belden et al., 2005; Glennemeier and Denver, 2002c; Hayes et al., 1993; Hayes, 1995; Hu et al., 2008; Wright et al., 1994). A physiological role for elevated corticosteroids in growth inhibition is supported by the finding that blockade of corticosteroid synthesis reversed the growth suppressive effects of crowding in tadpoles (Glennemeier and Denver, 2002b).

While exogenous corticosteroids, when administered alone during premetamorphosis, can inhibit growth and development, the hormones accelerate TH-induced metamorphosis in most species (Frieden and Naile, 1955; Kikuyama et al., 1983; Gray and Janssens, 1990; Wright et al., 1994; Hayes, 1995; Kikuyama et al., 1993; Darras et al., 2002; Kuhn et al., 2004; or CRF plus T4; Kuhn et al., 2005). Prometamorphic Bufo boreas (Hayes et al., 1993), or axolotl (Darras et al., 2002), exposed to exogenous corticosteroids alone showed accelerated metamorphosis, due likely to synergy of the corticosterone with rising endogenous TH levels.

While studies in which tadpoles were treated with exogenous corticosteroids with or without TH suggest a role for corticosteroids in the regulation of tadpole metamorphosis, they do not address whether endogenous corticosteroids play a physiological role in this process. Inhibitors of corticosteroid synthesis have been used to address the role of endogenous corticosteroids. Hayes and Wu (1995a) found that a 33% reduction in whole-body corticosterone content by treatment with metyrapone (an inhibitor of corticosteroid biosynthesis) slowed TH-induced acceleration of hindlimb development but did not affect the rate of tail resorption (Hayes and Wu, 1995a,b). Glennemeier and Denver (2002b) found that a 50% reduction in whole-body corticosterone by treatment with metyrapone throughout prometamorphosis, increased size at metamorphosis by more than 10% but did not affect the rate of metamorphosis in R. pipiens tadpoles. More work is required to determine a potential role for endogenous corticosteroids in tadpole growth and development (Belden et al., 2007).

In summary, the dose of corticosteroid administered, the stage at which the hormone is given, and whether it is administered with TH determines the developmental effects of the steroid. Whether these effects represent physiological actions remains to be determined. If these actions turn out to be physiologically relevant, then one would predict that increased corticosteroid biosynthesis (perhaps in response to a stressor) in prometamorphic tadpoles might retard growth and delay metamorphosis. Conversely, increased corticosteroids in prometamorphic tadpoles might retard growth but accelerate metamorphosis.

21.2.3.3 Mechanisms of corticosteroid action
Corticosteroids, like all steroid hormones, act primarily through binding to receptors that function as ligand-dependent transcription factors. These receptors are members of the same superfamily of receptor proteins that include the TH receptors (see above). Corticosteroid receptors are found primarily in the cytosol in the absence of ligand where they are complexed with a series of heat shock proteins and immunophilins (a foldosome) that serve to maintain the receptors in a conformation that favors ligand binding (Pratt and Toft, 1997). Binding of hormone results in dissociation of the foldosome complex and translocation of the receptor to the nucleus (Pratt and Toft, 1997). Vertebrates possess two distinct corticosteroid receptors that were originally identified in mammals based on their differential binding affinities: the high-affinity type 1 receptor (also called the mineralocorticoid receptor, MR) and the lower-affinity type 2 receptor (also called the glucocorticoid receptor, GR). The GR and MR belong to the nuclear hormone receptor superfamily, and phylogenetic analysis suggests that these two receptors arose by a gene duplication event in the gnathostome lineage (Bridgham et al., 2006; Thornton, 2001). Homologous genes to mammals for both receptor types have been isolated in X. laevis (Gao et al., 1994a,b; Csikos et al., 1995). The distribution in the brain and the regulation of expression by corticosteroids of the GR were recently reported in X. laevis (Yao et al., 2008).

The mechanisms of corticosteroid inhibition of growth in tadpoles have not been investigated. In mammals, it is known that corticosteroids produce growth inhibition through actions at multiple levels. At the organismal physiological level, corticosteroids mobilize stored fuels during increased metabolic demand (Sapolsky et al., 2000). Chronic elevation in plasma corticosteroid concentrations promotes protein catabolism and muscle wasting. Corticosteroids are known to downregulate growth hormone (GH) biosynthesis in the anterior pituitary gland of mammals (Harvey et al., 1995).

The molecular mechanisms by which corticosteroids promote metamorphosis involve, at least in part, the enhancement of TH bioactivity through increased TR and monodeiodinase expression. Corticosteroids
were shown to increase maximal nuclear-binding capacity for T3 in a dose-dependent manner and, thus, alter tissue responsiveness (Kikuyama et al., 1993; Niki et al., 1981; Suzuki and Kikuyama, 1983). This increase in nuclear-binding capacity for T3 is paralleled by the upregulation of TRα and TRβ mRNAs in X. laevis tail and in frog cell lines; this occurs in a synergistic manner, with low or subthreshold doses of TH plus corticosterone causing superinduction of TRs (RM Bonett, ED Hoopfer, and RJ Denver, unpublished results). Corticosterone has also been shown to increase 5′-deiodinase activity in bullfrog tadpoles, thereby increasing availability of T3 at peripheral tissues (RM Bonett, ED Hoopfer, and RJ Denver, unpublished results; Galton, 1990).

Darras et al. (2002) showed that treatment with dexamethasone increased hepatic D3 and brain D2 activities, plasma T3, and induced metamorphic changes (without concommittant TH treatment) in the axolotl. Kuhn et al. (2005) showed that treatment with CRF plus T4 caused a strong synergistic activation of brain D2 activity in the axolotl.

Thyroid hormone target genes may also be synergistically regulated by T3 and corticosteroids through mechanisms that are not directly, or immediately dependent on increased TRs or deiodinases (i.e., direct synergy between TRs and GR or MR at the target gene). BTEB1, a T3 target gene, is also induced by corticosterone (RM Bonett, F Hu, M Yao, and RJ Denver, unpublished results), and is superinduced with rapid kinetics by combined treatment with T3 and corticosterone, both in vivo in X. laevis, and in frog tissue culture cells (RM Bonett, ED Hoopfer, and RJ Denver, unpublished results). Similar synergistic regulation of BTEB1 by T3 and corticosteroids was found in the mammalian hippocampal cell line HT-22 (Bagamasbad and Denver, 2008). These findings suggest that synergistic gene regulation by TH and corticosteroids may be a general and important phenomenon in animal development.

21.2.4 Neuroendocrine Control of Metamorphosis

The vertebrate neuroendocrine system comprises the hypothalamus and the pituitary gland. The secretion of these pituitary hormones and, subsequently, the production of hormones by peripheral endocrine glands (e.g., thyroid and interrenals) are controlled by hypothalamic neurohormones. These neurohormones, termed releasing and release-inhibiting factors, are released from modified nerve terminals in the median eminence into capillaries that drain into the hypophysial portal vessels that deliver blood to the anterior pituitary gland. The importance of hypothalamic control of metamorphosis has long been recognized (reviewed by Kikuyama et al. (1993) and Denver (1996)). Early studies suggested that the pituitary hormones TSH and ACTH are primarily under stimulatory hypothalamic control in amphibians (reviewed by Denver (1996)). The anterior pituitary gland controls both the thyroid gland and the interrenal glands by production of TSH and ACTH, respectively.

While environmental influences on the timing of metamorphosis can occur at the level of peripheral tissues (e.g., direct thermal effects and osmotic effects), much environmental information is gathered by neural sensory systems and integrated within the hypothalamus to alter the secretion of pituitary hormones and consequently the activity of peripheral endocrine glands. The neuroendocrine system serves as an interface between the central nervous system (CNS) and the endocrine system, and transduces signals obtained through a variety of sensory inputs into appropriate physiological responses (Figure 2).

21.2.4.1 Developmental expression and regulation of thyroid-stimulating hormone

The increase in thyroid gland growth and biosynthetic activity during prometamorphosis is dependent upon the pituitary hormone thyrotropin (thyroid-stimulating hormone, TSH). The development of the thyroid gland is arrested in hypophysectomized tadpoles, resulting in the failure to metamorphose (Dodd and Dodd, 1976; Regard and Mauchamp, 1971, 1973). This condition can be reversed by injecting TSH (Regard and Mauchamp, 1971, 1973). It is likely that the early development of the thyroid gland does not depend on TSH since its development occurs before immunoreactive TSH cells are present in the anterior pituitary (which occurs at NK stage 42 in X. laevis and at similar stages in ranid frogs; Moriceau-Hay et al., 1982; Tanaka et al., 1991; Gracia-Navarro et al., 1992). However, it cannot be ruled out that small amounts of TSH sufficient to support thyroid development are produced earlier than these stages but cannot be detected due to limitations in the sensitivity of the immunohistochemical detection methods.

While functional thyroid follicles are present at stages that precede the prometamorphic rise in TH production, the rate of hormone synthesis is coordinate with the development of the pituitary gland and the production of TSH (Kaye, 1961;
Dodd and Dodd, 1976; Buckbinder and Brown, 1993; Denver, 1996; Manzon and Denver, 2004). The amphibian thyroid gland develops sensitivity to TSH during late embryogenesis (just prior to hatching) as demonstrated by increased radioiodine uptake by thyroids following TSH injection (Kaye, 1961). Immunoreactive TSH was detected in the circulation by RIA in adult and larval bullfrogs (Okada et al., 2004), but there have been no measurements of changes in plasma TSH by RIA during metamorphosis. Evidence for an increase in circulating TSH at the early limb bud stage (TK stage 3) in R. pipiens tadpoles was provided by Kaye (1961) using indirect measures of $[^{131}I]$ uptake.

Thyrotropin is comprised of two subunits, $\alpha$ and $\beta$, that are derived from two separate genes. The $\alpha$ subunit (alpha glycoprotein hormone subunit; $\alpha$-GSU) is common among the glycoprotein hormones (i.e., the gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), and TSH; whereas, the $\beta$ subunit confers hormonal specificity on the molecule; Pierce and Parsons, 1981). The cDNAs for TSH$\beta$ subunit have now been isolated from four amphibian species (three anurans: X. laevis – Buckbinder and Brown, 1993; R. catesbeiana – Okada et al., 2000; Bufo japonicus – Komoike and Ishii, 2003; one urodele – Hyobius retardatus – Kanki and Wakahara, 2000 – partial cDNA). Deduced amino acid sequences of the amphibian TSH$\beta$ subunits show that they have between 40% and 73% sequence similarity to known vertebrate TSH$\beta$ proteins.

Pituitary expression of TSH$\beta$ mRNA has been studied throughout metamorphosis in tadpoles of X. laevis (Buckbinder and Brown, 1993; Manzon and Denver, 2004) and R. catesbeiana (Okada et al., 2000; Figures 1(c) and 1(d)). In X. laevis TSH$\beta$ mRNA levels rise from NF stage 52 to peak values at stage 59 just prior to metamorphic climax, then drop thereafter to levels comparable to those of late prometamorphosis (e.g., NF stage 57; Figures 1(c) and 1(d)). Changes in $\alpha$-GSU mRNA parallel TSH$\beta$ (Buckbinder and Brown, 1993; Manzon and Denver, 2004). A similar expression pattern for TSH$\beta$ mRNA was found in R. catesbeiana, although expression remained at a high level throughout metamorphic climax and declined thereafter (Okada et al., 2000). Okada et al. (2000) speculated that species differences in TSH$\beta$ expression are related to differences in the time required to complete metamorphosis, with X. laevis proceeding more rapidly than R. catesbeiana. Thus, TSH biosynthesis is coordinate with thyroid gland development and hormone secretion, and the stimulatory action of pituitary TSH is necessary for thyroid gland growth and hormone biosynthesis.

21.2.4.2 Neurohormones regulating TSH: Thyrotropin-releasing hormone

The tripeptide pyro-glutamyl-histidyl-proline-amide was the first hypophysiotropic peptide isolated and its structure determined (Reichlin, 1989). It was named thyrotropin-releasing hormone (TRH) for
its ability to stimulate the release of TSH in mammals where it appears to be the principal stimulator of TSH secretion (Morley, 1981). However, its role as a TSH-releasing factor (TRF) in nonmammalian vertebrates is less certain. While TRH is expressed in the brain of larval and adult amphibia, injections of TRH are without effect on the thyroid axis or in altering the timing of tadpole metamorphosis (Denver, 1996; Kikuyama et al., 1993; Norris and Dent, 1989). This is explained by the lack of effect of TRH on the release of TSH by the tadpole pituitary gland (Denver and Licht, 1989; Okada et al., 2004). By contrast, TRH can elevate plasma TH concentrations when injected into adult frogs (Darras and Kuhn, 1982) and can stimulate the release of TSH by adult frog pituitaries \textit{in vitro} (Denver, 1988; Jacobs and Kuhn, 1992; Okada et al., 2004). However, the magnitude of the TSH response to TRH \textit{in vitro} is far lower than that achieved with similar doses of CRF (Denver, 1988; Okada et al., 2004). These findings support that pituitary TSH cell responsiveness to TRH is regulated in a developmental stage-specific manner. Expression of the type 2 TRH receptor appears during late prometamorphosis in the \textit{X. laevis} tadpole pituitary; whereas, the type 1 TRH receptor is expressed during pre- and prometamorphosis and is downregulated during metamorphic climax (Figure 3; Manzon and Denver, 2004). In chicken and mammals, the type 1 TRH receptor is expressed in thyrotropes and somatotropes (De Groef et al., 2003a; Yu et al., 1998), and TRH receptors are negatively regulated by TH (Hinkle and Goh, 1982; Harvey and Baidwan, 1990; Schomburg and Bauer, 1995). This downregulation of the receptor during metamorphosis, perhaps in response to rising plasma TH concentrations, and perhaps leading to decreased thyrotrope responsiveness to TRH, could account for the failure of this neuropeptide to stimulate TSH release in the tadpole. The type 2 TRH receptor does not appear to be expressed in the rodent pituitary gland (Cao et al., 1998). At present, the possibility that TRH plays a hypophysiotropic role in larval amphibians is uncertain. It could regulate prolactin (PRL), perhaps via the type 2 TRH receptor, both of which increase at metamorphic climax (Buckbinder and Brown, 1993; Kikuyama et al., 1993; Manzon and Denver, 2004). Further research is needed to clarify the role of TRH in the tadpole.

\subsection{21.2.4.3 Corticotropin-releasing factor and related peptides}

Corticotropin-releasing factor is a 41-amino-acid polypeptide that was first isolated based on its ability to stimulate ACTH secretion in mammals (Turnbull and Rivier, 1997; Vale et al., 1981). Members of the CRF family of peptides play central roles in the regulation of neuroendocrine, autonomic, and behavioral responses to physical and emotional stress (Aguilera, 1998; Yao and Denver, 2007). Members of the CRF family of peptides play central roles in the regulation of neuroendocrine, autonomic, and behavioral responses to physical and emotional stress (Aguilera, 1998; Yao and Denver, 2007). Corticotropin-releasing factor is a member of a family of related peptides in vertebrates that includes the fish urotenins-I, frog sauvgaine, and the urocortin peptides (urocortins 1–3; Dautzenberg and Hauger, 2002; Boorse et al., 2005; Boorse and Denver, 2006). 

\begin{figure}
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\includegraphics[width=\textwidth]{figure3.png}
\end{figure}

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Phylogenetic analysis has shown that tetrapod vertebrates possess four paralogous lineages of CRF-like peptides that likely arose prior to the divergence of the actinopterygian and sarcopterygian fishes (Lovejoy and Balment, 1999; Lovejoy and Jahan, 2006; Boorse et al., 2005). These vertebrate peptides share an evolutionary relationship with diuretic peptides present in invertebrates, thus emphasizing the phylogenetically ancient origin of these important hormonal signaling molecules (reviewed by Lovejoy and Balment (1999) and Lovejoy and Jahan (2006)). The regulation of ACTH secretion by CRF in mammals is considered to be its primary hypophysiotropic role (Vale et al., 1997).

21.2.4.4 Corticotropin-releasing factor is a TSH-releasing factor

Recent studies have shown that the stress neurohormone CRF is a potent stimulator of the thyroid axis in larval amphibians and other nonmammalian vertebrates (reviewed by Denver (1999) and De Groef et al. (2006)). Injections of CRF peptides elevate whole-body TH content in tadpoles of several species (Figure 4; Gancedo et al., 1992; Denver, 1993, 1997a). Using bioassays or RIA, CRF-like peptides have been shown to stimulate TSH secretion by acting directly on the pituitary gland in several vertebrate species, including amphibians (Figure 5; reviewed by De Groef et al. (2006)). Interestingly, while CRF is stimulatory to TSH secretion by cultured salmon pituitaries, TRH lacks activity in this regard (Larsen et al., 1998).

Taken together, the findings point to a central, and perhaps primitive role for CRF-like peptides in the regulation of both the thyroid and the interrenal (adrenal) axes. A role for CRF-like peptides in influencing thyroid activity in tadpoles and thus regulating metamorphosis comes from studies in several species, which showed that injections of CRF-like peptides accelerate metamorphosis (Figure 6). Injections of CRF and related peptides accelerated metamorphosis in the anurans *Rana perezi* (Gancedo et al., 1992), *R. catesbeiana*, *Spea (Scaphiopus) hammondii* (Denver, 1993, 1997a), and *Bufo arenarum* (Miranda et al., 2000), and in the salamander *A. tigrinum* (Boorse and Denver, 2002). CRF injections elevated whole-body TH content of *R. perezi* and *S. hammondii* tadpoles (Denver, 1993, 1997a; Gancedo et al., 1992; Boorse and Denver, 2004). In *S. hammondii*, injections of synthetic *X. laevis* CRF (which is identical in primary structure to *S. hammondii* CRF; Boorse and Denver, 2004) produced a dose-dependent increase in whole-body T3, T4, and corticosterone when measured 4 h after injection (Denver, 1997a).

Passive immunization with CRF antiserum slowed spontaneous metamorphosis in *R. catesbeiana* tadpoles (Denver, 1993). Also, injections of the CRF receptor antagonist α helical CRF(9–41) blocked simulated pond drying–induced metamorphosis in *S. hammondii* (Denver, 1997a). Furthermore, hypothalamic CRF peptide content was increased in spadefoot toad tadpoles that accelerated metamorphosis in response to simulated pond drying (Denver, 1997a). Taken together, these findings support a physiological role for CRF in controlling metamorphosis. Because CRF is a stress neurohormone, endogenous CRF may participate in environmentally induced (stress-induced) metamorphosis (Boorse and Denver, 2004; Denver, 1997a).

![Figure 4](image_url)

**Figure 4** Intraperitoneal injections of CRF-like peptides elevate plasma T4 in tadpoles of *S. hammondii*. Gosner stage 37–39 tadpoles were given i.p. injections of either saline vehicle, *X. laevis* CRF (× CRF; 0.5 µg), or Urocortin 1 (× UCN1; 0.5 µg) or Urocortin 3 (× UCN3; 2 µg) and plasma T4 was measured 2 h later by RIA. Means with the same letter are not significantly different. Reproduced from Okada R, Miller MF, Yamamoto K, De Groef B, Denver RJ, and Kikuyama S (2007a). Involvement of the corticotropin releasing factor (CRF) type 2 receptor in CRF-induced thyrotropin release by the amphibian pituitary gland. *General and Comparative Endocrinology* 150: 437–444, with permission from Elsevier.

21.2.4.5 Modulation of CRF actions – receptors and binding protein

The actions of CRF are mediated by two, G-protein-coupled receptors (CRF₁ and CRF₂; Dautzenberg and Hauger, 2002) and are modulated by a secreted binding protein (CRF-BP; Seasholtz et al., 2002). Genes for the two receptors have been isolated from *X. laevis* (Dautzenberg et al., 1997) and *R. catesbeiana* (Ito et al., 2006), and the CRF-BP from *X. laevis* (Brown et al., 1996). The receptors exhibit different rank order affinities for CRF peptides and tissue-specific patterns of expression. Structure–function
relationships among CRF ligands and binders have been established for several mammalian species (reviewed by Dautzenberg and Hauger (2002) and Seasholtz et al. (2002)) and for the frog, *X. laevis* (Valverde et al., 2001; Seasholtz et al., 2002; Boorse et al., 2005; Boorse and Denver, 2006) and are evolutionarily conserved. CRF and urocortin 1 bind to and activate CRF1, and CRF2, although CRF has greater affinity for CRF1, and urocortin has greater affinity for CRF2 (Boorse et al., 2005; Dautzenberg and Hauger, 2002); whereas urocortins 2 and 3 are selective ligands for CRF2 (Boorse et al., 2005; Dautzenberg and Hauger, 2002). In frogs and mammals, the receptors are expressed throughout the brain, in the pituitary, and in peripheral tissues (Boorse and Denver, 2006; Ito et al., 2006).

Current evidence suggests that the modulation of pituitary ACTH and TSH release by CRF-like peptides is mediated by different receptors expressed on corticotropes and thyrotropes. The CRF1 is expressed in the pituitary glands of mammals and chicken and appears to be predominantly in corticotropes (Van Pett et al., 2000; De Groef et al., 2003a,b). Although CRF1 is also expressed in frog pituitary, it is not yet known in which cell types (Manzon and Denver, 2004; Boorse and Denver, 2006; Ito et al., 2006). The expression of the CRF2 in mammalian pituitary is controversial, but this subtype is clearly expressed in pituitary of chicken (De Groef et al., 2003b) and frog (Boorse and Denver, 2006; Ito et al., 2006; Manzon and Denver, 2004). There is now evidence that TSH release by thyrotropes is mediated by the CRF2 (Figure 7). De Groef et al. (2003b) found that chick thyrotropes express CRF2 and that TSH release is mediated by this receptor. Recently, Okada et al. (2007a) found a similar relationship in amphibians. Treatment with CRF2-selective ligands (urocortins 2 and 3, and sauvagine, which binds to

Figure 5 CRF-like peptides that preferentially (urocortin 1, sauvagine) or selectively (urocortin 2, urocortin 3) bind to the CRF2 receptor stimulate TSH secretion by dispersed adult bullfrog pituitary cells *in vitro* in a dose-dependent manner. Means with the same letter are not significantly different. Reproduced from Okada R, Miller MF, Yamamoto K, De Groef B, Denver RJ, and Kikuyama S (2007a) Involvement of the corticotropin releasing factor (CRF) type 2 receptor in CRF-induced thyrotropin release by the amphibian pituitary gland. *General and Comparative Endocrinology* 150: 437–444, with permission from Elsevier.
CRF$_2$ with 40 times greater affinity than CRF$_1$ increased plasma T$_4$, accelerated metamorphosis, and stimulated in vitro TSH release by dispersed frog pituitary cells (Figures 5 and 6; Okada et al., 2007a). The CRF$_2$-selective antagonist anti-sauvagine 30, but not the CRF$_1$-selective antagonist antalarmin, blocked CRF-dependent TSH release by frog pituitary cells in vitro (Figure 7).

The expression of CRF$_1$ and CRF$_2$ mRNAs in the pituitary gland of *X. laevis* tadpoles was analyzed throughout metamorphosis using reverse transcriptase polymerase chain reaction (RT-PCR) (Figure 8; Manzon and Denver, 2004). The CRF$_1$ was expressed during premetamorphosis and its level increased during premetamorphosis, reaching a plateau through metamorphic climax. The expression of CRF$_1$ during premetamorphosis is consistent with findings that premetamorphic tadpoles are capable of increasing corticosterone following exposure to a physical stressor (Glennemeier and Denver, 2002a), and in response to environmental stressors (Denver, 1998a; Glennemeier and Denver, 2002b). By contrast, CRF$_2$ mRNA is barely detectable during pre- and early metamorphosis, but exhibits a dramatic increase during late metamorphosis and metamorphic climax. The expression of the CRF$_2$ parallels the increase in sensitivity of the pituitary to CRF-like peptides that occurs during metamorphosis (Kaneko et al., 2005). The upregulation of CRF$_2$ expression during late metamorphosis may play a central role in the regulation of TSH secretion.
role in the timing of metamorphosis by mediating the actions of CRF-like peptides on pituitary TSH release.

The CRF-BP has high-affinity binding for CRF peptides (in the range of the receptors) and may play an important role in modulating CRF bioavailability (see Behan et al. (1996) and Seasholtz et al. (2002)). Analyses of the primary structures of the vertebrate CRF-BPs reveal a protein with high evolutionary conservation, which suggests strong selective pressure to maintain its structure and function (Seasholtz, 2002; see also Huising and Flick (2005)). The *X. laevis* CRF-BP was originally isolated from a subtractive tadpole tail cDNA library as a T3-regulated gene (Brown et al., 1996). Its expression in the tadpole tail influences CRF bioavailability, which in turn affects tail muscle cell survival (Boorse et al., 2006). In frogs and mammals, CRF-BP is expressed in diverse tissues, with high expression in the brain and pituitary gland (Boorse and Denver, 2006). CRF-BP circulates in the blood.

**Figure 7** The general CRF receptor antagonist astressin, and the CRF2-specific antagonist antisauvagine-30, but not the CRF1-specific antagonist antalarmin block CRF-induced TSH release by dispersed adult bullfrog pituitary cells *in vitro*. Means with the same letter are not significantly different. Reproduced from Okada R, Miller MF, Yamamoto K, De Groef B, Denver RJ, and Kikuyama S (2007a) Involvement of the corticotropin releasing factor (CRF) type 2 receptor in CRF-induced thyrotropin release by the amphibian pituitary gland. *General and Comparative Endocrinology* 150: 437–444, with permission from Elsevier.
in humans but not in rats, which may be explained by the lack of expression in rat liver; it is not yet known whether the CRF-BP is present in the circulation of frogs.

Currently, the role that this protein plays in modulating CRF action in any species is poorly understood, and few comparative studies in nonmammalian species have been done (Huising et al., 2004; Seasholtz, 2002). The protein could modulate CRF action by binding it and thus blocking its availability to receptors, or by targeting the peptide for clearance (Behan et al., 1996; Boorse et al., 2006; Seasholtz, 2002). Alternatively, the CRF-BP might serve to maintain high concentrations of CRF within tissues or in tissue fluids and, perhaps, facilitate CRF action. Brown et al. (1996) suggested that the upregulation of this protein during metamorphic climax might serve a negative feedback function by sequestering CRF and thus modulating its bioavailability. However, the expression of CRF-BP in the tadpole pituitary gland suggests the opposite, that is, it increases during prometamorphosis, but is strongly downregulated at metamorphic climax, which may lead to increased bioavailability of CRF within the pituitary gland (Manzon and Denver, 2004; see Figure 8).

21.2.4.6 Other neurohormones that stimulate TSH secretion

According to studies from Sakae Kikuyama’s laboratory, approximately 50% of the TSH-releasing activity of hypothalamic extracts (derived from both tadpoles and adult frogs) can be blocked by coincubation of adult bullfrog pituitary cells with 10⁻⁴ M CRF receptor antagonist α-helical CRF₁(9–41) (Ito et al., 2004). These findings suggest that a significant proportion of TSH-releasing activity in the amphibian hypothalamus is contributed by CRF-like peptides. They also suggest that other factors may be involved in the regulation of TSH, or that α-helical CRF₁(9–41) may not fully antagonize CRF-like peptide activity in hypothalamic extracts (Rivier et al., 1984).

Relatively few neuropeptides have been found to be stimulatory to TSH release in amphibians. Gonadotropin-releasing hormone (GnRH) stimulated the thyroid axis in axolotl and Rana ridibunda (Jacobs et al., 1988; Jacobs and Kuhn, 1988), and this action of GnRH is direct on the pituitary gland (Denver, 1988; Okada et al., 2004). The physiological significance of this finding is currently unknown, since the magnitude of the response is far lower than that achieved with similar doses of CRF.

![Figure 8](image-url)

(Denver, 1988; Okada et al., 2004). GnRH did not increase TSH release by tadpole pituitary cells 

in vitro (Okada et al., 2004). Other potential stimulators of TSH by the amphibian pituitary gland include vasoactive intestinal polypeptide (VIP) and pituitary adenylate cyclase activating polypeptide (PACAP), which have been shown to stimulate TSH secretion by primary pituitary cells from adult bullfrogs (Okada et al., 2007b). The physiological significance of these actions is unknown, as are whether such actions occur in the tadpole.

21.2.4.7 Negative feedback regulation of TSH

As discussed above, the rise in TH production during metamorphosis is driven by pituitary TSH. The synthesis and secretion of TSH are controlled by neuropeptides produced by the hypothalamus, and by maturational effects, and negative feedback actions of TSH on the hypothalamus and the anterior pituitary gland. The challenge has been to decipher which of these processes account for the sustained rise in TSH (and, thus, thyroid activity) that occurs during metamorphosis, and the subsequent decline in thyroid activity after metamorphic climax.

As in mammals, TH has been shown to reduce basal and stimulated TSH release 

in vitro by pituitary glands of adult frogs (Jacobs and Kuhn, 1992; Kaneko et al., 2005). In the tadpole, Etkin (1968) hypothesized that negative feedback on pituitary TSH does not develop until metamorphic climax, and that this lack of feedback is responsible, at least in part, for the sustained rise in TSH production and thyroid activity during metamorphosis. However, Kaye (1961) showed that premetamorphic R. pipiens tadpoles treated with TH at Taylor Kollros stage 3 exhibited depressed thyroidal [131]I uptake, suggesting a suppression of TSH at this stage of development. Also, treatment of premetamorphic tadpoles with goitrogens caused enlargement of the thyroid gland and degranulation of pituitary thyrotropes (Goos, 1968, 1978; Goos et al., 1968b; Dodd and Dodd, 1976). Conversely, replacement with T4 reversed the effects of the goitrogen on the thyrotropes, suggesting that negative feedback on TSH was operative in the premetamorphic tadpole (Goos et al., 1968a). TSHb mRNA in the pituitary of premetamorphic tadpoles is dramatically elevated by treatment with the goitrogen methimazole (Buckbinder and Brown, 1993; Huang et al., 2001). Manzon and Denver (2004) used 

in vitro pituitary explant cultures to show that physiological concentrations of T4 or T3 can act directly on the pituitary glands of X. laevis tadpoles throughout metamorphosis to suppress TSHb mRNA expression. They observed no significant differences between stages in the effects of TH treatment on TSH mRNA levels, although pituitaries derived from early prometamorphic tadpoles tended to show the greatest sensitivity to thyroid negative feedback. Kaneko et al. (2005) showed that CRF-induced TSH release by bullfrog primary pituitary cells was suppressed by T3 throughout metamorphosis. Taken together, these findings support that negative feedback at the level of the pituitary is active from premetamorphosis/early metamorphosis.

Despite evidence for negative feedback on TSH being present from early in metamorphosis, Huang et al. (2001) embraced Etkin’s hypothesis that it is absent until metamorphic climax (NF stage 62), and they proposed that feedback is established at this time by the upregulation of pituitary D2 which converts T4 to T3. They based their conclusion in part on evidence for the presence of D2 mRNA at stage 62 but not at stage 56 as measured by Northern blotting (Huang et al., 2001; but note that only these two stages were analyzed). However, Manzon and Denver (2004), using a more sensitive technique (RT-PCR), found that D2 mRNA is present in pituitaries from early prometamorphic X. laevis tadpoles and increases during metamorphosis, reaching maximal expression by NF stage 59, earlier than stage 62 proposed by Huang and colleagues. Like other pituitary genes, the expression of both D2 and D3, and TRβ (which is known to be essential for transcriptional repression of the TSHb and TRH genes in mammals; Flamant and Samarut, 2003; Guissouma et al., 2005) increase in parallel with TH levels and TSHb mRNA throughout metamorphosis (Figure 9; Manzon and Denver, 2004). Huang and colleagues provided no evidence for the absence of negative feedback on TSH before climax in their experiments, and, as discussed above, a body of evidence suggests that negative feedback is functional in the premetamorphic/early metamorphic tadpole.

Deiodinase type 2 is known to play an important role in negative feedback on TSH in mammals (Schneider et al., 2001; St. Germain et al., 2005) and could have a similar role in frogs (Brown, 2005). The expression of D2 throughout metamorphosis (Manzon and Denver, 2004) supports the findings discussed above that T4 (perhaps through its conversion to T3) can exert negative feedback on TSH during metamorphosis/early metamorphosis (Figure 9). If the hypothesis that the upregulation of D2 at metamorphic climax is responsible for the establishment of negative feedback were correct (Huang et al., 2001), one
would predict that the late prometamorphic and climax stages (i.e., stages 59–62) would be most sensitive to TH negative feedback, particularly for T₄. However, Manzon and Denver (2004) observed the opposite trend in pituitary explant culture studies, with stage 56 pituitaries being more sensitive to T₃ and, perhaps more importantly, to T₄ than either stage 59 or 62 pituitaries. The downregulation of TSH expression by T₄ suggests that D2 is either active in the pituitary throughout metamorphosis, or the conversion of T₄ to T₃ via D2 is not required for negative feedback. The data do not support that a switch, in the form of D2 expression, is turned on at metamorphic climax.

The rise in TSH during metamorphosis occurs in the face of elevated TH and functional thyroid negative feedback on the pituitary gland. Etkin (1968) proposed that TH exerts a maturational effect on the hypothalamus, median eminence, and pituitary gland, and this is responsible for the sustained rise in plasma TH concentrations that drive metamorphosis (and that negative feedback is established at climax, which is likely incorrect—see above). Thus, there may be strong hypothalamic drive for TSH production that overcomes the negative feedback exerted by the elevated plasma TH concentrations during metamorphosis. Evidence for this is that (1) the neurosecretory neurons and median eminence, the structure necessary for the delivery of neurohormones to the pituitary, develop during prometamorphosis under the influence of TH (reviewed by Denver (1998b)) and (2) the expression of neuropeptide receptors by anterior pituitary cells, and the responsiveness of these cells to secretagogues increases during metamorphosis (Kaneko et al., 2005; Manzon and Denver, 2004; see Figure 9).

The set point of TSH expression after metamorphosis is comparable to the expression level achieved during late prometamorphosis (Manzon and Denver, 2004; see Figures 1(c) and 1(d)). Therefore, negative feedback may be physiologically important for limiting TSH secretion once the system has matured, and perhaps during maturation of the neuroendocrine system; that is, the coordination of morphogenesis may require the temperance of TSH expression by TH throughout metamorphosis. However, the sustained rise in thyroid activity during metamorphosis is primarily due to the maturational effects of TH on the CNS (and perhaps the pituitary) rather than the absence of negative feedback.

**Figure 9** Changes in mRNAs for thyroid hormone receptor beta A (TRβA), deiodinase type 2 (D2) and deiodinase type 3 (D3) in the pituitary gland of *X. laevis* throughout metamorphosis as determined by semi-quantitative RT-PCR. Means with the same letter are not significantly different. Modified from Manzon RG and Denver RJ (2004) Regulation of pituitary thyrotropin gene expression during *Xenopus* metamorphosis: Negative feedback is functional throughout metamorphosis. *Journal of Endocrinology* 182(2): 273–285. © Society for Endocrinology (2004). Reproduced by permission.
feedback. The relatively lower levels of pituitary D2 expression during early prometamorphosis might be permissive for the sustained rise in TSH during prometamorphosis.

Pituitary TSH is regulated by predominantly stimulatory input from the hypothalamus and, as discussed above, by strong negative feedback by TH. Some have argued that the dominant regulation of pituitary TSH is by negative feedback (St. Germain et al., 1995). However, Nikrothandan et al. (2006) showed that mice in which both the TRH and TRβ genes had been knocked out were unable to mount a rise in plasma TSH when made hypothyroid. The authors concluded that TRH is necessary for the rise in TSH that occurs upon a drop in plasma TH. The findings suggest that hypothalamic drive for TSH secretion, rather than simply the release from negative feedback at the level of the pituitary gland, is essential for the regulation of TSH.

21.2.5 Developmental Expression and Regulation of ACTH

There have been far fewer studies conducted on the hypothalamic control of ACTH than on TSH in amphibians. There are relatively few reports analyzing the activity of the hypothalamic–pituitary–interrenal axis throughout metamorphosis at levels other than the interrenal gland. Carr and Norris (1990) reported low immunoreactive CRF in the median eminence and arginine vasotocin (AVT) in the preoptic nucleus of premetamorphic R. catesbeiana tadpoles, which increased dramatically by late prometamorphosis. Both CRF and arginine vasopressin (AVP; AVT is the amphibian hormone) are potent stimulators of ACTH secretion by cultured adult frog pituitaries (Tonon et al., 1986). To my knowledge, no direct measures of ACTH production over development have been reported in amphibians. However, the expression of the mRNA for the precursor of ACTH, proopiomelanocortin (POMC), in the anterior pituitary of bullfrog tadpoles is low during prometamorphosis, increases during metamorphosis, and remains high during metamorphic climax (Aida et al., 1999).

Whether this mRNA expression pattern reflects production and secretion of ACTH peptide is unknown. The tadpole hypothalamic–hypophyseal–interrenal axis becomes functional during prometamorphosis. For example, the interrenal glands of prometamorphic tadpoles of R. pipiens and X. laevis respond to ACTH injections in vivo by increasing whole-body corticosterone content (Glennemeier and Denver, 2002a). These experiments show that functional ACTH receptors are expressed before metamorphosis. The functionality of higher levels of the hypothalamic–hypophyseal–interrenal axis in prometamorphic animals is shown by their ability to mount a corticosterone response (increased whole-body corticosterone content) following exposure to a physical stressor (shaking/confinement stressor; Glennemeier and Denver, 2002a). Thus, there is the potential for environmental stressors to cause elevations in endogenous corticosteroid biosynthesis during prometamorphosis. Such early activation of the hypothalamic–hypophyseal–interrenal axis could result in growth retardation and metamorphic inhibition, as described above.

21.2.6 Prolactin and Growth Hormone

The pituitary hormones GH (also called somatotropin) and PRL (also called lactotropin) are simple polypeptides at 200 amino acids in length and are paralogous members of a multigene family. A key component of the Etkin (1968) model was that the stimulatory actions of TH on metamorphosis were counterbalanced by the inhibitory effects of the pituitary hormone PRL. Etkin proposed that PRL production would be high during larval life and then decline at metamorphic climax. This prediction was based largely on the inhibitory effects that preparations of mammalian PRLs had on metamorphosis when injected into tadpoles (White and Nicoll, 1981). Based on the antimetamorphic actions of these mammalian PRL preparations, several investigators suggested that PRL exerted a juvenilizing action in amphibian larvae akin to that of juvenile hormone in insects (Bern et al., 1967; Etkin and Gona, 1967).

The early studies that led to the development of the Etkin model have been extensively reviewed (White and Nicoll, 1981; Dodd and Dodd, 1976; Kikuyama et al., 1993; Denver, 1996; Kaltenbach, 1996). Studies using primarily mammalian preparations of GH or PRL suggested different roles for these hormones, with PRL enhancing larval growth and blocking the actions of TH on metamorphosis, and GH primarily stimulating postmetamorphic growth as it does in other vertebrates (Denver, 1996; see also Takada and Kasai (2003)). A role for GH in regulating body growth in amphibia as it does in other vertebrates (Harvey, 1995) has been borne out by numerous studies in which GH was injected into tadpoles or frogs (Denver, 1996; Kikuyama et al., 1993; White and Nicoll, 1981) and more recently
through the use of transgenic techniques in *X. laevis* (Huang and Brown, 2000a). A role for PRL in the stimulation of tadpole growth and the inhibition of metamorphosis has been questioned by Huang and Brown (2000b).

The early studies supported the view that treatment of tadpoles with PRL can inhibit metamorphosis and stimulate larval growth. Most of these studies, done with mammalian PRL (and GH) preparations, showed that tadpole tissues have the capacity to respond to PRL/GH-like molecules; that is, functional receptors are expressed in amphibian tissues which can transmit a signal that can both promote tadpole growth and block T3-induced metamorphosis, likely by preventing the autoinduction of the TRs (Tata et al., 1993). Furthermore, studies with amphibian PRL preparations show that the homologous PRL has similar effects to the mammalian hormones (Kikuyama et al., 1993). Passive immunization studies with prolactin antisera suggested a physiological role for endogenous PRL (Denver, 1996; Kikuyama et al., 1993).

But do these effects represent a physiological role for endogenous GH and PRL, or pharmacological actions of the exogenous hormones? The strongest argument against a role for PRL as a juvenilizing hormone in amphibians comes from expression analyses. As mentioned above, Etkin (1968) proposed that larval growth and metamorphosis is controlled by a balance between TH and PRL, and that the two should show an inverse relationship in their blood concentrations at metamorphic climax. The rise in circulating concentrations of TH during prometamorphosis and climax has been confirmed (see above). However, circulating concentrations of PRL and levels of pituitary PRL mRNA are low during prometamorphosis and also rise, more or less in parallel with TH, during late prometamorphosis and climax (Clemons and Nicoll, 1977; Yamamoto and Kikuyama, 1982; Takahashi et al., 1990; Niinuma et al., 1991b; Buckbinder and Brown, 1993), thus contradicting the earlier hypothesis of an inverse relationship of the two hormones (Etkin, 1968). The rise in PRL production tends to occur slightly later than the rise in TSH expression and circulating TH (Buckbinder and Brown, 1993). Similarly, [125I]-PRL binding to kidney membrane fractions was low in premetamorphic bullfrog tadpoles and increased during metamorphic climax (White and Nicoll, 1979). Huang and Brown (2000b) measured PRL receptor mRNA by Northern blotting in whole *X. laevis* tadpole and tail tissue and found increased expression at metamorphic climax. Hasunuma et al. (2004) reported the cloning of the PRL receptor gene in the bullfrog and showed that the mRNA increases in the tail fin and kidney during metamorphic climax. Taken together, these PRL and PRL receptor (PRL-R) expression analyses argue against the hypothesis that PRL plays a juvenilizing role in amphibian metamorphosis (Buckbinder and Brown, 1993; Huang and Brown, 2000b). However, Kikuyama et al. (1993) have argued, based on their experiments with passive immunization with antisera to bullfrog PRL, that low levels of PRL during the premetamorphic/early metamorphic period might be sufficient to support larval growth and inhibit TH action.

Huang and Brown (2000a,b) used a germ line transgenesis approach to address the question of the roles of GH and PRL in amphibian development. They created transgenic tadpoles of *X. laevis* that overexpressed either *X. laevis* GH, *X. laevis* PRL, or ovine PRL. The expression of the transgenes was driven by the simian cytomegalovirus (sCMV) promoter; thus, all tissues expressed the transgenes (i.e., expression was not restricted to the pituitary gland where the hormones are normally expressed). They found that overexpression of GH had no effect on the timing of metamorphosis but resulted in larger tadpoles and larger juvenile frogs, a finding that confirms earlier studies in frogs and studies in other vertebrates that show that GH promotes growth (hence, its name; Harvey et al., 1995). Overexpression of *X. laevis* (xPRL) or ovine PRL (oPRL) did not alter the timing of metamorphosis, but blocked tail resorption in some tadpoles. The overexpression of the mRNAs was confirmed by Northern blotting; however, they were unable to detect the xPRL in serum of transgenic frogs by Western blotting but apparently were able to detect the oPRL. The authors concluded that their results disprove the hypothesis that PRL is a juvenile hormone in *X. laevis*. One caution in this interpretation is that the PRL was overexpressed in all tissues throughout the entire developmental period. Such stage-inappropriate overexpression of a hormone might result in compensatory changes in physiological systems; alternatively, the PRL-responsive cells could become desensitized by receptor internalization following chronic exposure to very high concentrations of the hormone, which is a common phenomenon in endocrine systems.

Whether PRL plays any role in larval growth or development, the rise in PRL biosynthesis at metamorphic climax suggests that the hormone might either modulate the rapid tissue transformations.
that occur at climax (e.g., provide a brake on TH action in concert with the upregulation of the 5 monodeiodinase; see Denver (1996) or perhaps play an important physiological role in the postmetamorphic frog (Huang and Brown, 2000b). Shintani et al. (2002) reported that PRL and GH upregulated D3 mRNA expression in X. laevis tadpole tail. These authors suggested that the effects of PRL and GH on metamorphosis may be mediated in part by the tissue-specific regulation of D3 expression. It is currently unknown what causes the surge in PRL that occurs at metamorphic climax. Buckbinder and Brown (1993) found that PRL increases after 3 days of TH treatment of premetamorphic tadpoles, thus showing that it is a late-responding TH inducible gene. Thus, other factors that may be upregulated at the time of metamorphic climax are likely responsible for PRL expression/secretion at this time. It is interesting to note that in mammals, PRL secretion is induced by stressors (Cooke et al., 2004; Soares et al., 2006). This raises the possibility that the activation of neuroendocrine stress pathways that occurs during metamorphosis may participate in the late rise in PRL secretion.

21.3 Integrating Endocrinology with the Ecology of Metamorphosis

The activity of the thyroid axis in tadpoles can be regulated at multiple levels, and this activity ultimately determines when larvae enter metamorphosis and the rate at which metamorphosis progresses. Because the stress hormonal axis is closely linked to the thyroid axis, central nervous stress pathways may play a critical role in transducing environmental information and regulating metamorphic timing. From a developmental/physiological perspective, the upper and lower limits to the larval period in different species are established genetically through programming the developmental schedules for each of the components of the endocrine system (the establishment of functional endocrine cells and tissue competence to respond to thyroid and steroid hormones) and epigenetically through the regulated secretion, metabolism, and action of hormones. The environment could impact the developmental schedules and most certainly impacts the production and perhaps the action of hormones. Also, antagonism between growth-promoting hormones and morphogenetic hormones might underlie the trade-offs between growth rate and development rate.

At present, few studies have addressed these issues from an integrative perspective (physiology and ecology). The following sections discuss these issues and develop several hypotheses to explain how the limits to the larval period are established (in a physiological/developmental sense) and how plasticity in metamorphic timing within those limits is controlled.

21.3.1 Limits to the Length of the Larval Period

Why do amphibians differ in the lower and upper limits to the lengths of their larval periods? What determines tadpole growth and development rates and size at transformation? How does the timing of metamorphosis evolve? Few studies have attempted to address natural selection for the timing of metamorphosis; however, there is strong correlative evidence for the hypothesis that the length of the larval period is a reflection of the characteristics of the ancestral habitat (permanence and predictability, resource availability and competition, thermal environment, predation, etc.) The most important variable in this equation is habitat permanence, since amphibian larvae depend on an aquatic environment for growth and development. It is also important to consider how factors operating in both life history stages (larval and adult) influence selection for the timing of metamorphosis (Werner, 1986). Here the author considers the question: What specific physiological regulatory systems in amphibian larvae might be targets for selection?

21.3.1.1 The lower limit

The earliest time at which tadpoles initiate metamorphosis in nature is likely influenced by the animal’s size and the environmental conditions. But what determines the earliest possible time that a tadpole can enter metamorphosis and why does this timing differ among species? The Wilbur–Collins model (Wilbur and Collins, 1973) proposes that tadpoles must reach a minimum body size before metamorphosis; however, there is strong correlative evidence for the hypothesis that the length of the larval period is a reflection of the characteristics of the ancestral habitat (permanence and predictability, resource availability and competition, thermal environment, predation, etc.) The most important variable in this equation is habitat permanence, since amphibian larvae depend on an aquatic environment for growth and development. It is also important to consider how factors operating in both life history stages (larval and adult) influence selection for the timing of metamorphosis (Werner, 1986). Here the author considers the question: What specific physiological regulatory systems in amphibian larvae might be targets for selection?
size (7–9 mm snout–vent length), while others exhibit considerable growth and metamorphose at a larger size (e.g., R. catesbeiana, 20–60 mm snout–vent length; reviewed by Werner (1986)).

Is the minimum, taxon-specific size for metamorphosis correlated with the establishment of competence to respond to metamorphic hormones? In X. laevis the capacity to respond to TH (i.e., increased RNA and protein synthesis) is established early, just after hatching (Tata, 1968). Thus, competence to respond to TH is established well before the minimum size for normal metamorphosis is reached. Is the minimum size correlated with the establishment of competence to produce metamorphic hormones in sufficient quantities to drive morphogenesis? The capacity to upregulate hormone production takes longer to develop and depends on the maturation of the neuroendocrine system (reviewed by Denver (1996)).

There is considerable variation among species in the time it takes to proceed from hatching to the first appearance of limb buds (premetamorphosis), then from limb bud appearance to late prometamorphosis. These two periods are likely to be independent targets for selection. During the premetamorphic period, selection for growth rate may be most important. Plasticity in the length of the premetamorphic period depends primarily on growth opportunities, and tadpoles have no choice but to make a living in the larval habitat and attain the minimum size for metamorphosis. During the prometamorphic period, selection for development of the endocrine system is likely the more important factor. During this period, a tadpole’s endocrine system is sufficiently developed to allow it to make developmental decisions. That is, if conditions are favorable, the rate of TH production should remain low and tadpoles should continue to capitalize on favorable growth conditions. If conditions deteriorate, tadpoles have the capacity to activate endocrine systems and transition from the aquatic to the terrestrial habitat.

Where is the metamorphic clock/environmental sensor located? Etkin (1968) argued that the clock is located in the hypothalamus. For example, autotransplantation of the pituitary primordium to the tail of the frog embryo (separation from stimulatory control by the hypothalamus) results in a failure to metamorphose (Etkin, 1968). Similarly, destruction of the preoptic nucleus or surgical removal of the primordium of the posterior hypothalamus (and thus isolation of the pituitary from the brain) prevents metamorphosis (reviewed by Denver (1996)). Studies of the normal development of the neurosecretory centers of the hypothalamus and the median eminence further support this hypothesis (Etkin, 1968).

While the neuroendocrine system is central to the control of metamorphic timing, the rate of metamorphic transformation may be influenced at other levels. For example, hormone transport in the blood by TTR, hormone conversion by monodeiodinases, hormone uptake and intracellular transport by membrane TH transporters and CTH-BPs, and cellular responsiveness determined by the expression of TRs or nuclear receptor coregulators could each influence metamorphic timing. Buchholz and Hayes (2005) showed that closely related species of spadefoot toads that differ in the duration of their larval periods show differences in the tissue content of T3 and T4, and the sensitivity of their tissues to thyroid hormone. They speculated that these differences might be due to differences in TH uptake into cells and/or TH metabolism, although they did not identify the underlying mechanisms.

21.3.1.2 The upper limit
An environment with good growth conditions and low predation favors a longer larval period in most species; that is, under such circumstances tadpoles would be expected to push the upper limit. But even if one maintains tadpoles in the laboratory under constant, favorable conditions they will ultimately metamorphose; that is, they will not grow indefinitely. What physiological/developmental mechanism is responsible for the spontaneous activation of the endocrine system controlling metamorphosis? Perhaps the slow increase in thyroid activity eventually reaches a threshold such that the system is pushed into metamorphic climax. The better the conditions, the lower the thyroid activity, but it eventually reaches a level where positive feedback is initiated. Or, perhaps the activation follows from the animal reaching some upper size limit, and the subsequent decline in growth promoting hormones removes antagonism on the thyroid system. Because anurans are not pedomorphic, the costs of remaining in the larval habitat longer should eventually outweigh the benefits of larger size at metamorphosis.

21.3.2 Plasticity in the Timing of Metamorphosis
Within the lower and upper limits to the larval period, tadpoles exhibit considerable plasticity in their timing of metamorphosis. This phenotypic plasticity depends on environmental factors; that is,
the quality and suitability of the larval habitat for
growth and survival. Majority of amphibians that
have been studied exhibit phenotypic plasticity
within the limits of the length of the larval period,
rather than exhibiting a fixed rate of development.
The following section addresses the question: What
physiological systems enable plasticity in the timing
of development and are thus targets for selection?

21.3.2.1 The integrated endocrine system
controlling metamorphosis and potential
loci for environmental modification of
endocrine activity

Points of regulation by the environment might
include the neuroendocrine system, peripheral endo-
crine organs, hormone transport and metabolism, and
hormone action (Figure 10). But how are environ-
mental factors sensed: Thermal, osmotic, and effects
related to the gaseous environment could be sensed
directly by most or all tissues. The influence of other
factors, such as photoperiod, resource availability,
predator presence, and crowding, is likely integrated
by the neuroendocrine system, and transduced by the
hypothalamus into changes in peripheral endocrine
gland activity.

Availability of biologically active thyroid hormone
is regulated within tissues by the monodeiodinases
and the expression of these enzymes could be mod-
ified either directly or indirectly by environmental
factors (Figure 10). An example of indirect regulation
of monodeiodinases by environmental factors is by
corticosteroids, which have been shown to increase
3′D activity, with the result that more of the active
hormone T3 is generated. This regulatory relation-
ship suggests that stress and stress hormones can
accelerate metamorphosis by upregulating 3′D. Simi-
larly, TR synthesis might be regulated directly or
indirectly by environmental factors which would
then influence metamorphic timing. Currently, there
is little known about what factors, either physiological
or environmental, regulate nuclear receptor expres-
sion in any species. As for monodeiodinase, evidence
suggests that corticosteroids can enhance TH action
by upregulating TR expression, and so TR biosyn-
thesis is an additional site where stress and stress
hormones may modulate timing of metamorphosis.

21.3.2.2 Plasticity mediated by the
neuroendocrine system

As described above, the neuroendocrine system is
likely to be the clock regulating spontaneous metamor-
phic timing. Furthermore, the external and internal
environments can modify the activity of the neuroen-
docrine system. Many biotic and abiotic environmental
factors are detected by animal sensory systems,
integrated in higher brain centers, and then informa-
tion is transduced via the neuroendocrine system.

The most important environmental variable for a
tadpole is water availability, and duration of the
aquatic habitat can profoundly influence the rate of
metamorphosis in many species. This is especially
ture for desert amphibians that tend to breed in
ephemeral habitats. As discussed earlier, CRF–like
peptides are implicated in the control of TSH secre-
tion, acting via the CRF2 receptor. Because the secre-
tion of CRF is activated by stressors, CRF may play a
central role in mediating a tadpole’s developmental
response to a deteriorating larval habitat (e.g., in the
case of the Western spadefoot toad; Denver, 1997a,
1998a; Denver et al., 1998; Boorse and Denver, 2004).
Furthermore, CRF may represent a phylogenetically
ancient developmental cue that vertebrates use to
assess changes in their habitat and to mount an
appropriate developmental/physiological response.
Recent findings in mammals show that CRF of fetal
and/or placental origin controls the timing of the
length of gestation and may shorten the gestational
period under conditions of fetal stress (Challis et al.,
2005; Smith et al., 2002).

Do other environmental factors, that are known to
alter the timing of metamorphosis, also act through
the neuroendocrine stress axis? Whole-body cortico-
sterone content was elevated in *R. pipiens* tadpoles that
were food restricted or subjected to high conspecific
density, compared to their high-resource, low-density
counterparts (Glennemeier and Denver, 2002b). Both
low food and increased density resulted in slowed
growth and development in premetamorphic tad-
poles, which agrees with other studies showing
growth- and development-inhibiting effects of these
factors in premetamorphs (but contrast this with pro-
metamorphic animals which accelerate development
in response to food restriction or crowding). This
slowed growth caused by crowding stress was reversed
by treatment of tadpoles with the corticosterone
synthesis inhibitor metyrapone, again suggesting a
functional role for the hypothalamic–hypophyseal–
interrenal axis in mediating the larval developmental
response to environmental conditions (Glennemeier
and Denver, 2002b). Hayes (1997) also reported an
elevation in whole-body corticosterone content in
*B. boreas* tadpoles caused by crowding. By contrast,
Belden et al. (2007) did not find such a relationship
in a mesocosm study. Predation, temperature,
photoperiod, or other environmental factors could conceivably work through similar neuroendocrine pathways to exert their effects on larval development. If larvae have a means of detecting the state of environmental conditions, through visual, chemical, or other sensory systems, then the neuroendocrine system is a likely pathway through which developmental responses to the environment can operate.
Acknowledgment

The preparation of this chapter and the published and unpublished work reported herein was supported by NSF grant IBN 0235401 to R.J.D.

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Further Reading


Biographical Sketch

Dr. Robert J. Denver is professor of molecular, cellular, and developmental biology (MCDB) and professor of ecology and evolutionary biology (EEB) at the University of Michigan, Ann Arbor. He earned the PhD in 1989 from the University of California at Berkeley and was appointed to the UM faculty in 1994. Professor Denver's expertise is in hormone action in brain development, development and evolution of the neuroendocrine stress axis; mechanisms of developmental plasticity; and endocrinology, ecology, and molecular biology of amphibian metamorphosis. He has published over 80 research articles on these topics. He is currently a member of the NIH Integrative and Clinical Endocrinology and Reproduction (ICER) Study Section, he has served on several NSF grant review panels and on several EPA scientific advisory panels. He served as Chair of the Division of Comparative Endocrinology, Society for Integrative and Comparative Biology; Secretary-Treasurer and Member-at-Large, Intl. Federation of Comparative Endocrine Societies; Associate Editor: General and Comparative Endocrinology, and is on the editorial board of the Journal of Experimental Zoology Part A: Comparative Experimental Biology and Integrative and Comparative Biology.