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Review

Stress hormones mediate environment-genotype interactions during amphibian development

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ABSTRACT

Environments experienced by organisms during early development shape the character and timing of developmental processes, leading to different probabilities of survival in the developmental habitat, and often profound effects on phenotypic expression later in life. Amphibian larvae have immense capacity for plasticity in behavior, morphology, growth and development rate. This creates the potential for extreme variation in the timing of, and size at metamorphosis, and subsequent phenotype in the juvenile and adult stage. Hormones of the neuroendocrine stress axis play pivotal roles in mediating environmental effects on animal development. Corticotropin-releasing factor, whose secretion by hypothalamic neurons is induced by environmental stress, influences the timing of amphibian metamorphosis by controlling the activity of the thyroid and interrenal (adrenal; corticosteroids) glands. At target tissues, corticosteroids synergize with thyroid hormone to promote metamorphosis. Thus, environmental stress acts centrally to increase the activity of the two principle endocrine axes controlling metamorphosis, and the effectors of these axes synergize at the level of target tissues to promote morphogenesis. While stress hormones can promote survival in a deteriorating larval habitat, costs may be incurred such as reduced tadpole growth and size at metamorphosis. Furthermore, exposure to elevated corticosteroids early in life can cause permanent changes in the expression of genes of the neuroendocrine stress axis, leading to altered physiology and behavior in the juvenile/adult stage. Persistent effects of stress hormone actions early in life may have important fitness consequences.

1. Introduction

Although the genotype provides the template for organismal form and function, the environment experienced by a developing organism often has profound effects on phenotypic expression, a phenomenon generally known as phenotypic plasticity (Pigliucci, 2001; DeWitt and Scheiner, 2004). Phenotypic plasticity is the process by which organisms modify their behavior, morphology, or physiology in response to changing environments, and has been described in almost every group of plant and animal (Via et al., 1995). The term 'developmental plasticity' is often used in place of phenotypic plasticity to recognize that plasticity is often (but not exclusively) a developmental phenomenon (West-Eberhard, 2003). Other terms used are 'prenatal programming' or 'fetal programming', which refer to the effects of early life experience on later life phenotypic expression. Studies of plasticity have expanded in recent years to encompass most biological disciplines including human biology, where the impact of the environment experienced by the developing embryo, fetus and neonate on later life health and disease has come to the forefront of biomedical research (the developmental origins of disease hypothesis; Ozanne and Costancia, 2007). The science of *ecological developmental biology* has developed recently as a distinct discipline, which, in the words of Gilbert and Epel (2008), is the study of how "Development weaves genotype and environment into phenotype".

Organisms respond to their environment by altering development, either by changing the timing of developmental events or by modifying their morphology, physiology or behavior. Plastic responses to the environment expressed during early development can have particularly important fitness consequences (Gilbert, 2003; Nijhout, 2003; Frankino and Raff, 2004; Gilbert and Epel, 2008). Phenotypic plasticity can be adaptive if it increases survival during the embryonic/larval life stage (e.g., accelerated tadpole metamorphosis in response to pond drying; Newman, 1992; Denver et al., 1998) or generates a juvenile/adult phenotype that may be better adapted to the prevailing environmental conditions (West-Eberhard, 2003; Gilbert and Epel, 2008). However, there are trade-offs associated with such plasticity, which can affect traits expressed later in life, resulting in either positive or negative fitness consequences. Thus, phenotypic plasticity can be neutral, adaptive or maladaptive depending on the trait or traits affected,

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and the nature of the environment in which the traits are expressed.

Virtually all organisms exhibit some form of phenotypic plasticity. However, the proximate mechanisms that underlie variable phenotypic responses to the environment are poorly understood. Hormones mediate many environmental effects on organismal development (Gilbert and Epel, 2008). They influence the timing and level of gene expression, and therefore can alter the timing of development, modify the character of morphological development, or 'program' the behavioral and physiological phenotype expressed in a subsequent life history stage. Hormones of the neuroendocrine stress axis (the hypothalamo–pituitary–adrenal, or HPA axis) are principal mediators of physiological and behavioral responses to environmental change (Denver, 2009). The term 'stress hormone' is used here to refer to hormones that are produced and act within the HPA axis, and include neurohormones such as corticotropin-releasing factor (CRF) and related peptides, pituitary adrenocorticotropic hormone (ACTH; also known as corticotropin) and glucocorticoids (GCs) produced by adrenal cortical cells (e.g., cortisol and corticosterone). Hormone action often leads to epigenetic changes at gene regulatory regions, involving modifications to chromatin structure (e.g., histone methylation, acetylation, phosphorylation, ubiquitination) and possibly DNA methylation (Gilbert and Epel, 2008). Epigenetic changes in chromatin or DNA lead to altered gene expression, which drives phenotypic expression; such changes may be passed on to subsequent generations (i.e., transgenerational effects; Anway et al., 2005; Crews, 2008; Gore, 2008; Morgan and Whitelaw, 2008).

Because of their complex life cycles, amphibians are ideal for investigating environmental effects on early development, and their impact on future phenotypic expression and fitness. Studies by amphibian ecologists have shown that environmental conditions experienced during the larval stage, such as conspecific density, food availability, habitat desiccation, and exposure to predators, have significant effects on metamorphic timing, body size and morphology of the tadpole and the adult (Wilbur and Collins, 1973; Werner, 1986; Newman, 1992; Goater, 1994; Denver et al., 1998; Relyea, 2007). Growth is reduced when tadpoles face deteriorating environmental conditions, but developmental responses (i.e., acceleration of metamorphosis or polyphenism) depend on the stage and body size of the tadpole. In premetamorphosis, before hindlimb and thyroid gland development (Etkin, 1968), tadpoles slow development in response to adverse environmental conditions (Glennemeier and Denver, 2002b). After a minimum body size is reached and the thyroid gland develops, during prometamorphosis, tadpoles respond to adverse conditions by accelerating metamorphosis (Wilbur and Collins, 1973; Newman, 1992; Denver et al., 1998, 2002).

Developmental plasticity is adaptive for amphibian species that live in arid environments since it increases the probability of survival (Newman, 1992; Denver et al., 1998). However, in such circumstances metamorphosis occurs at a smaller body size, which may be associated with future fitness costs. Tadpoles reared in suboptimal environments metamorphose at a smaller body size, and the juveniles are thus more likely to exhibit slower growth rates, inferior locomotor abilities, greater susceptibility to starvation and higher mortality when reared under conditions where resources are limited (Semlitsch et al., 1988; Berven, 1990; Goater, 1994; Scott, 1994; Beck and Congdon, 1999, 2000; Van Buskirk and Saxer, 2001; Alvarez and Nicieza, 2002; Altwegg and Reyer, 2003; Relyea and Hoverman, 2003). In most species, this body size disadvantage at metamorphosis is retained through the age at first reproduction, thus compromising reproductive fitness (Semlitsch et al., 1988; Berven, 1990; Goater, 1994; Scott, 1994; Altwegg and Reyer, 2003). However, this is in contrast to the growth potential that newly metamorphosed frogs have when provided with abundant resources (i.e., they exhibit catch-up growth; Hu et al., 2008).

The effects of environmental stress on tadpole growth and development in many ways parallel those of intrauterine stress on fetal growth and development in mammals. Maternal malnutrition or repeated acute stress (e.g., shock, restraint) cause intrauterine growth retardation and pre-term birth (Weinstock et al., 1992, 1998; Challis et al., 2001; Bloomfield et al., 2003), and both of these factors have been associated with reproductive dysfunction and increased susceptibility to disease later in life (Barker, 1997; Weinstock, 2001). These later-life effects of the *in utero* environment are associated with activation of the neuroendocrine stress axis in both mothers and fetuses (Weinstock, 2001; Welberg and Seckl, 2001; Matthews, 2002). This activation, which causes an elevation in plasma GCs during critical windows of brain development, has been shown to permanently alter the functioning of the stress axis and the expression of behaviors throughout the life of the animal.

2. Hormones of the neuroendocrine stress axis

Hormones of the neuroendocrine stress axis (HPA axis) play key roles in phenotypic plasticity, and may mediate the long term effects of early life experience on later life phenotypic expression. Below I first describe the major components of the HPA axis (Fig. 1; for a more detailed description see Boorse and Denver, 2006; Yao and Denver, 2007; Denver, 2009). I then discuss the roles of stress hormones in mediating environmental effects on amphibian development, and in 'programming' the phenotype of the juvenile/adult.

2.1. Corticotropin-releasing factor and related peptides

Corticotropin-releasing factor and related peptides play central roles in developmental plasticity in vertebrates. The presence of CRF activity in the hypothalamus was originally described by Harris in the 1940s and was the first neurohormonal activity to be discovered (reviewed by Harris, 1955). Working in the late 1970s and early 1980s, three groups isolated peptides from different species that had CRF activity. Vale and colleagues (1981) reported the isolation of a 41 amino acid peptide from ovine hypothalamus that stimulated the release of ACTH and β-endorphin by the rat anterior pituitary gland *in vitro* and *in vivo*. In the same year, Montecucchi and Henschen (Montecucchi and Henschen, 1981; earlier working with Vittorio Erspamer; Montecucchi et al., 1979) reported the isolation of a peptide from the skin of the monkey frog, *Phyllomedusa sauvageii*, that they named sauvagine (Montecucchi and Henschen, 1981). Sauvagine had earlier been found to cause the release of ACTH and β-endorphin *in vivo* and *in vitro* (reported in abstract form: Montecucchi et al., 1979). One year later, Lederis and colleagues (1982) reported the isolation of a peptide from the caudal neurosecretory organ (urophysis) of the white suckerfish (*Catostomus commersoni*) that had potent hypotensive activity in mammals and birds, and ACTH releasing activity in fish and mammals. In the 1981 paper by Vale and colleagues (1981), referencing the paper by Montecucchi and Henschen (1981), the authors discuss the sequence similarity of ovine CRF and sauvagine, and, citing a personal communication with Karl Lederis, they pointed out that "Another nonmammalian hypotensive peptide, urotensin I, isolated from teleost urohypophysis is closely related structurally to sauvagine and thus to CRF." Prior to the publication of the ovine CRF peptide sequence, Vittorio Erspamer predicted that "the long-searched hypothalamic CRF is sauvagine-like" (Erspamer et al., 1981; see Lederis, 1987). Thus began three decades of intensive work on the structure, function and evolution of CRF and related peptides.

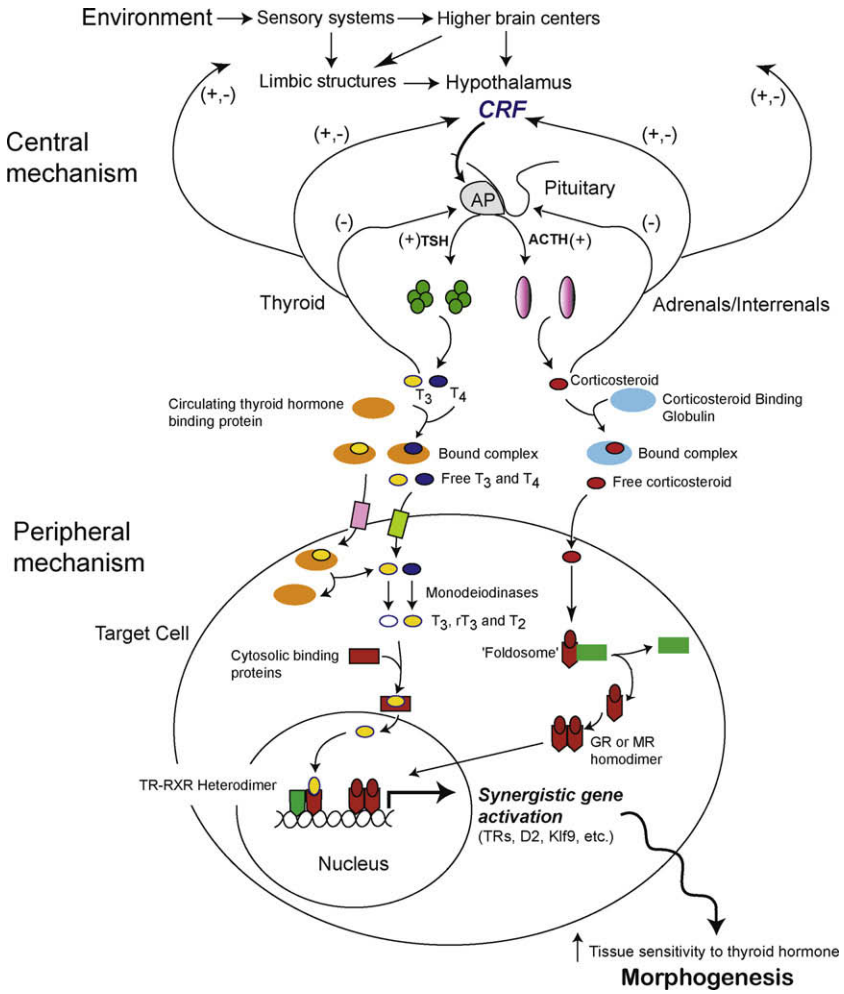

Fig. 1. Central and peripheral integration of the stress and thyroid endocrine axes in the control of amphibian metamorphosis. Shown is a schematic representation of the organization of the hypothalamo–pituitary–adrenal (HPA; stress) and hypothalamo–pituitary–thyroid (HPT) axes in amphibian tadpoles, their regulation by input from the external environment, transduction of this input by neural and neuroendocrine pathways, and synergistic interactions among thyroid hormones and corticosteroids in target cells leading to the promotion of morphogenesis. The two endocrine axes are controlled centrally by corticotropin-releasing factor (CRF; and possibly CRF-related peptides) which acts on the anterior pituitary gland (AP) to stimulate the release of thyrotropin (TSH) and corticotropin (ACTH). TSH acts on the thyroid gland to stimulate release of thyroxine (T_4) and to a lesser extent 3,5,3′-triiodothyronine (T_3). Thyroid hormones are transported in the blood by serum binding proteins (transthyretin, thyroxine binding globulin and albumin). ACTH acts on adrenal cortical cells (interrenal glands) to stimulate biosynthesis and release of glucocorticoids which are transported in the blood bound to corticosteroid binding globulin. Cellular uptake of T_3 and T_4 is achieved by amino acid and organic ion transporters; there is also evidence that thyroid hormones may enter cells bound to transthyretin via a receptor-mediated process. Glucocorticoids are thought to enter cells primarily by passive diffusion across the plasma membrane. Upon entering the cell thyroid hormone is bound to cytosolic binding proteins, some of which (the monodeiodinases) serve to convert the hormone to either active (T_3; deiodinases types 1 and 2) or inactive forms (reverse T_3 [rT_3], diiodothyronine [T_2]; deiodinase type 3). Thyroid hormone receptors (TR) form heterodimers with retinoid X receptors (RXR) and are bound to DNA in the unliganded form where they actively repress gene transcription. Upon thyroid hormone binding to TR gene transcription is derepressed and activated. Upon entering the cell glucocorticoids bind to corticosteroid receptors (glucocorticoid receptor [GR or mineralocorticoid receptor [MR]) that are located in the cytosol bound to heat shock proteins and immunphilins (the 'foldosome'). Hormone binding causes a conformational change in the receptor, the release of proteins that comprise the foldosome, and dimerization and translocation of receptors to the nucleus where they bind DNA to activate or repress target genes. When cells are exposed to low concentrations of thyroid hormone plus glucocorticoids genes such as the TRs, deiodinase type 2, and the immediate early thyroid hormone inducible transcription factor *Klf9* are activated in a synergistic manner. This leads to enhanced sensitivity of cells to the actions of thyroid hormone, which serves to accelerate morphogenesis.

At the time of their isolation, sauvagine and urotensin I were thought to represent orthologs of mammalian CRF, but subsequent analysis showed that fishes and frogs have orthologous CRF genes that are distinct from urotensin I and sauvagine (Okawara et al., 1988; Stenzel-Poore et al., 1992). Subsequently, Vaughan and colleagues (1995), using antibodies to fish urotensin I, found urotensin I-like immunoreactivity in rat brain. They then used a urotensin I cDNA probe to screen a rat brain cDNA library and isolated a mammalian gene that codes for a 122 amino acid precursor that is processed to a 40 amino acid mature peptide with 63% sequence similarity to suckerfish urotensin I, and 45% sequence similarity to rat CRF (Vaughan et al., 1995). They named this peptide urocortin to recognize its similarity to CRF and fish urotensin I (now urocortin 1; Vaughan et al., 1995; Donaldson et al., 1996). We recently isolated a gene orthologous to mammalian urocortin 1 from the frog *Xenopus laevis* (Boorse et al., 2005).

Following the discovery of mammalian urocortin, two other CRF/urocortin paralogs were isolated by genomic analysis and molecular cloning from mouse and human and named urocortin 2 and urocortin 3 (Hsu and Hsueh, 2001; Reyes et al., 2001; Lewis et al., 2001; Hauger et al., 2003). Orthologous genes for mammalian urocortins 2 and 3 were subsequently identified in pufferfish (Hsu and Hsueh, 2001; Lewis et al., 2001; Boorse et al., 2005), urocortin 2 in the chicken and urocortin 3 in the frog *X. laevis* (Boorse et al., 2005). Current evidence supports the existence of at least four paralogous CRF/urocortin genes in vertebrates (Denver,

2009). Sauvagine remains somewhat of an enigma, since it shares only 50% sequence similarity with *X. laevis* urocortin 1 (Boorse et al., 2005). A full length sauvagine cDNA sequence isolated from the skin of *P. sauvageii* has been deposited in Genbank (Accession # AY943910). Analysis of this sequence suggests that sauvagine may be a highly divergent urocortin 1 that could be specific to the Phyllomedusidae, but more study is needed.

The actions of CRF-like peptides are mediated by at least two G protein-coupled receptors and a secreted binding protein (CRF-BP). The major function of the CRF-BP appears to be to modulate access of CRF and related peptides to CRF receptors (Seasholtz et al., 2001). The first CRF receptor was isolated from human by Vale's group using an expression cloning strategy (Chen et al., 1993). Subsequently, two CRF receptor genes were identified and named CRF_1 and CRF_2 (Dautzenberg and Hauger, 2002). Orthologs of the mammalian CRF_1 and CRF_2 receptors have been isolated by molecular cloning from other vertebrates including frogs (Dautzenberg et al., 1997; Ito et al., 2006). Both receptors have distinct tissue distributions in mammals and frogs, and they mediate the actions of CRF peptides in the central nervous system and in peripheral tissues (reviewed by Boorse and Denver, 2006). Within the hypothalamo–pituitary system, CRF binds to and activates CRF_1 receptors expressed on pituitary corticotropes to induce secretion of ACTH (reviewed by Yao and Denver, 2007). ACTH stimulates corticosteroid biosynthesis by the adrenal cortex (mammals) or interrenal glands (amphibians). Corticotropin-releasing factor and urocortin 1 bind to and activate both CRF_1 and CRF_2, but CRF has higher affinity for CRF_1, and urocortin 1 (and sauvagine) has higher affinity for CRF_2 (Dautzenberg et al., 1997; Dautzenberg and Hauger, 2002; Boorse et al., 2005). Urocortins 2 and 3 are selective for CRF_2 (Hsu and Hsueh, 2001; Reyes et al., 2001; Lewis et al., 2001; Hauger et al., 2003). These findings have led to the hypothesis that the CRF_2 functions primarily as a receptor for urocortins (Dautzenberg and Hauger, 2002).

In addition to their critical roles as hypophysiotropins, CRF-like peptides are widely expressed in the central nervous system (CNS) of vertebrates where they function as neurotransmitters or neuromodulators to coordinate behavioral and autonomic responses to stress (Lovejoy and Balment, 1999; Boorse and Denver, 2006). Corticotropin-releasing factor and related peptides play central roles in the regulation of food intake (Crespi and Denver, 2005; Mastorakos and Zapanti, 2004), behavioral responses to stress (Sapolsky et al., 2000; Bale et al., 2002; Bale and Vale, 2004; Flik et al., 2006), and learning and memory consolidation (Gulpinar and Yegen, 2004; Fenoglio et al., 2006; Roozendaal et al., 2008; Todorovic et al., 2007).

Peptides of the CRF family, their receptors and binding protein are expressed in many peripheral tissues where they may influence diverse physiological functions (reviewed by Boorse and Denver, 2006). Corticotropin-releasing factor and urocortins have some overlapping, but many distinct roles in physiology and behavior (Fekete and Zorrilla, 2007), and these differences may in part reflect the differential expression and functions of the CRF_1 and CRF_2 (Dautzenberg and Hauger, 2002; Bale et al., 2002; Bale and Vale, 2004; Boorse and Denver, 2006; Rissman et al., 2007).

Corticotropin-releasing factor-like peptides function as cytoprotective factors, protecting neuronal and cardiac cells from apoptosis (Fox et al., 1993; Brar et al., 1999, 2000, 2002; Pedersen et al., 2001; Radulovic et al., 2003; Linden et al., 2005; Martin et al., 2005; Tao et al., 2006) and inducing proliferation in cultured mammalian cells (Jessop et al., 1997; Mitsuma et al., 2001; Ikeda et al., 2002). We recently discovered a novel, cytoprotective role for CRF in the *X. laevis* tadpole tail (Boorse et al., 2005). Corticotropin-releasing factor, expressed by tail muscle cells, functions as an autocrine cytoprotective factor for tail muscle cell survival. Treatment of tadpole tail explants with CRF slowed tail regression *in vitro*, and reduced caspase 3/7 activity. The expression of CRF-BP mRNA in tadpole tail increased during spontaneous metamorphosis and was induced precociously by treatment with thyroid hormone (Brown et al., 1996; Valverde et al., 2001; Boorse et al., 2006). Increased CRF-BP at metamorphic climax reduces the bioavailability of CRF to its receptors on tail muscle cells, thus promoting tail regression by neutralizing the cytoprotective actions of CRF (Boorse et al., 2006).

The adaptive significance of CRF's cytoprotective role may be to maintain the viability of the tadpole tail, an essential locomotory organ required for feeding and escape from predators. Environmental insults such as thermal and osmotic stress, hypoxia, hypercapnia, and tissue damage caused by predatory attack could negatively impact tail cell survival. The expression of CRF and urocortin 1 mRNAs in tail explants cultures was increased, but CRF-BP mRNA was decreased by different environmental stressors (Boorse et al., 2006). The upregulation of CRF and urocortin 1, and the downregulation of CRF-BP by environmental stressors suggest that the production and bioavailability of these peptides, and thus their cytoprotective actions, can be modulated by direct environmental effects on the tail.

2.2. Corticosteroids and their receptors

The corticosteroids, produced by adrenal cortical cells, are the primary effectors of the HPA axis of vertebrates, and have been classified into two groups, the glucocorticoids (GCs) and the mineralocorticoids, owing to their often distinct physiological functions. Corticosteroids act primarily through binding to intracellular receptors that function as ligand-activated transcription factors. Vertebrates possess two distinct corticosteroid receptors that were originally identified in mammals based on their differential binding affinities: the high affinity type I receptor (also called the mineralocorticoid receptor; MR) and the lower affinity type II 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 (Thornton, 2001; Bridgham et al., 2006). Homologous genes to mammals for both receptor types have been isolated in the frog *X. laevis* (Gao et al., 1994a,b; Csikos et al., 1995), and we recently reported the distribution in the brain, and the regulation of expression by corticosteroids of the GR in *X. laevis* (Yao et al., 2008a). There is also evidence for corticosteroid receptors located in the plasma membrane that mediate rapid actions of these hormones (Tasker et al., 2006).

The corticosteroids have diverse actions in animal development, physiology and behavior, although the molecular bases for these actions are poorly understood. They influence development of the brain, lungs and other organ systems, mobilize stored energy and stimulate feeding to replenish depleted energy stores following a stress response, and have important effects on the brain where they influence learning and memory consolidation. Corticosteroids exert negative feedback at the level of the brain and pituitary gland to inhibit the activity of the HPA axis, thus returning the system to basal following a stress response (Yao and Denver, 2007; Yao et al., 2008b).

3. Roles of stress hormones in amphibian development and phenotypic plasticity

Stress hormones play important and diverse roles in animal development. In many animals the neuroendocrine stress system is sufficiently developed to respond to physical stressors by upregulating corticosteroid secretion during early postembryonic stages (Feist and Schreck, 2001; Glennemeier and Denver, 2002a; Wada,

2008). Corticosteroids have widespread effects on growth and development (Welberg and Seckl, 2001; Denver et al., 2002; Radulovic et al., 2003), altering the timing of life history transitions (Wada, 2008), and leading to permanent alterations in physiology, morphology and behavior (Barker, 1992, 1997; Brunson et al., 2001; Matthews, 2002; Hu et al., 2008; Korosi and Baram, 2008). Elevations in corticosteroids may be causally linked to the timing of smoltification in salmonids, metamorphosis of flatfish and amphibians, hatching in reptiles and birds, and parturition in mammals (Wada, 2008).

3.1. Stress hormones and larval growth, behavior and morphology

Corticosteroids have complex influence on growth and metamorphosis of larval amphibians. The effects of corticosteroids on metamorphosis (i.e., whether they accelerate or decelerate metamorphosis) depend on the animal's developmental stage and thyroid hormone status (discussed below). By contrast, elevations in corticosteroids in tadpoles, either by administration of exogenous hormone or through increased endogenous hormone production reduce tadpole growth at all developmental stages (see Hu et al., 2008; Denver, 2009). A physiological role for corticosteroids in growth inhibition in tadpoles is supported by the finding that inhibition of corticosteroid synthesis using the drug metyrapone reversed the growth suppressive effects caused by crowding in *Rana pipiens* tadpoles (Glennemeier and Denver, 2002b).

Recent findings support a role for corticosteroids in the behavioral and morphological responses of tadpoles to predation. When confronted with a predator, or a predator chemical cue, tadpoles display rapid behavioral inhibition, whereby they settle to the bottom of the pond and remain still. This is presumed to facilitate avoidance of detection by the predator (Fraker, 2008). Some chemical cues may be derived from the predator (i.e., kairomones), but tadpoles actively secrete an alarm pheromone under predatory attack that is detected by conspecifics (Fraker et al., 2009). Unlike the neuroendocrine response to predator cues in mammals where the HPA axis becomes activated (Figueiredo et al., 2003; Apfelbach et al., 2005; Roseboom et al., 2007), exposure of tadpoles to alarm pheromone caused a rapid, dose-dependent suppression of whole body corticosterone content (Fraker et al., 2009). The suppression of the HPA axis was permissive for expression of behavioral inhibition, since reversing the decline in corticosterone by the addition of a low dose of hormone to the aquarium water in which the tadpoles were reared partially blocked the behavioral response.

While predation causes rapid (minutes to hours) behavioral and physiological responses in tadpoles, predation risk extending over days to weeks leads to distinct morphological changes which can have indirect effects on fitness (Benard, 2004; Relyea, 2007). For example, nonlethal predator presence (predators housed in cages and fed conspecifics) over several weeks results in a relatively smaller body and larger tail (Benard, 2004; Relyea, 2007). The larger tail may serve as a lure to distract predator strikes from the more vulnerable body, or may confer enhanced burst locomotion for escape (Benard, 2004; Johnson et al., 2008). We recently found that 3 week exposure of wood frog tadpoles, *R. sylvatica* to the nonlethal presence of a predator (larvae of the dragonfly *Anax junius* fed conspecific tadpoles) elevated whole body corticosterone content (J. Middlemis-Maher, E.E. Werner and R.J. Denver, unpublished data; this is in contrast to the acute suppression of the HPA axis discussed above). Furthermore, elevation of tissue corticosterone content within the physiological range by addition of the hormone to the aquarium water, or exposure to tadpole alarm pheromone caused wood frog tadpoles to develop larger tails relative to their body size; the effect of the alarm pheromone on tail morphology could be blocked by treatment with metyrapone (J. Middlemis-Maher, unpublished data). *R. pipiens* tadpoles treated with corticosterone (62 or 125 nM in the aquarium water for 18 days) had deeper tails (increased tail muscle depth:tail length; Glennemeier and Denver, 2002c). Tail explants from *X. laevis* tadpoles cultured for 7 days in the presence of different doses of corticosterone were larger (greater dry mass) than controls at the end of the culture period (E.D. Hoopfer and R.J. Denver, unpublished data). Taken together, the findings suggest that adaptive changes in tadpole tail morphology in response to predation, that can have indirect effects on fitness, are mediated by corticosteroids. While the development of defensive morphologies can reduce predation risk, such phenotypic plasticity can incur costs such as reduced growth rates (Benard, 2004). As discussed above, corticosteroids reduce tadpole growth, and therefore may mediate the cost/benefit trade-off in predator-induced morphological changes and slowed growth rate.

3.2. Stress hormones and amphibian metamorphosis

Thyroid hormone controls amphibian metamorphosis and corticosteroids synergize with thyroid hormone to accelerate metamorphosis (Denver, 2009). Thus, if a tadpole experiences environmental stress during prometamorphosis it may accelerate metamorphosis. For example, exposure to habitat desiccation, crowding or food restriction during mid to late prometamorphosis shortens the time to metamorphosis in different amphibian species (Denver et al., 2002). There are two physiological/molecular mechanisms, one central nervous, the other via actions in peripheral tissues, that likely account for these effects (Fig. 1).

3.2.1. Central nervous system mechanism

A central mechanism involves the dual role that CRF plays in amphibian larvae to regulate the HPA and the hypothalamo–pituitary–thyroid axes (Fig. 1). In all vertebrates studied, within the hypothalamo–pituitary system, a major action of CRF is to stimulate ACTH secretion, and thus to serve as the central regulator of the HPA axis (Yao and Denver, 2007; Denver, 2009). However, CRF-like peptides are also potent thyrotropin (TSH)-releasing factors in nonmammalian species, particularly during early developmental stages (Denver, 1999, 2009; De Groef et al., 2006). This suggested the hypothesis that CRF plays a key role in controlling life history transitions such as amphibian metamorphosis, which is dependent on thyroid hormone (see De Groef et al., 2006; Denver, 2009 for reviews). There is now strong evidence to support this hypothesis. For example, injection of CRF-like peptides into amphibian tadpoles increased whole body thyroid hormone and corticosterone content, and accelerated metamorphosis (Gancedo et al., 1992; Denver, 1993, 1997; Okada et al., 2007). Blockade of endogenous CRF availability or action through passive immunization, or administration of the general CRF receptor antagonist alpha helical $CRF_{(9-41)}$ slowed spontaneous metamorphosis (Denver, 1993) or blocked accelerated metamorphosis caused by water volume reduction to mimic pond drying (Denver, 1997).

Stimulation of TSH release by CRF-like peptides is mediated by the CRF_2 expressed on pituitary thyrotrope cells (shown in chick and frog; De Groef et al., 2003, 2006; Okada et al., 2007; Okada et al., 2009). The CRF_2 selective ligands, urocortins 2 and 3 accelerated tadpole metamorphosis when injected into tadpoles, and they stimulated TSH release by frog pituitary cells *in vitro* (Okada et al., 2007). The actions of CRF on TSH release *in vitro* could be blocked by the CRF_2 receptor antagonist anti-sauvagine 30, but not by the CRF_1 receptor antagonist antalarmin (Okada et al., 2007). Sauvagine, which binds to the frog CRF_2 with 40 times greater affinity than to the frog CRF_1 (Boorse et al., 2005), is a potent stimulator of tadpole metamorphosis (Denver, 1997) and *in vitro* TSH release (Okada et al., 2007), but does not stimulate ACTH release by frog pituitaries *in vitro* (while CRF stimulated ACTH release in the same experiment; Tonon et al., 1986). Lastly, using histochemistry, CRF_2

has been shown to be expressed on thyrotropes but not corticotropes in chick (De Groef et al., 2003) and frog (Okada et al., 2009) pituitaries. Taken together, the findings support that stimulation of TSH release from the amphibian pituitary gland by CRF-like peptides is mediated by CRF_2 expressed on thyrotropes; whereas, stimulation of ACTH release is mediated by CRF_1 expressed on corticotropes.

In the pituitary gland of *X. laevis* tadpoles, CRF_1 mRNA is expressed during premetamorphosis and increases throughout metamorphosis, while CRF_2 mRNA expression can be detected by RT-PCR at the start of prometamorphosis and shows a distinct increase in expression in late prometamorphosis leading up to metamorphic climax (Manzon and Denver, 2004). This expression pattern suggests that the CRF_1 expressed on corticotropes provides for responsiveness of the tadpole HPA axis to environmental stress from early in development; premetamorphic tadpoles are capable of mounting robust HPA responses following exposure to a physical stressor (Glennemeier and Denver, 2002a). Whereas, upregulation of CRF_2 during prometamorphosis establishes competence of the pituitary thyrotropes to respond to hypothalamic signals, thus driving the prometamorphic rise in plasma TSH, and allowing for modulation of TSH secretion (and thus the timing of metamorphosis) in response to a changing environment (mediated by CRF neurons located in the anterior preoptic area; Yao et al., 2004).

3.2.2. Peripheral mechanisms

Although exogenous corticosteroids administered alone during premetamorphosis inhibit tadpole growth and development, they can accelerate thyroid hormone-induced metamorphosis (Denver, 2009). In some species, such as *Bufo boreas* (Hayes et al., 1993) or the Mexican axolotl (Darras et al., 2002), exposure to exogenous corticosterone alone (without exogenous thyroid hormone) resulted in accelerated metamorphosis, which may have been due to synergy of the corticosterone with rising endogenous levels of thyroid hormone.

The molecular mechanisms for the synergistic actions of corticosteroids and thyroid hormone on tissue transformation likely involve the enhancement of thyroid hormone bioactivity in cells by the upregulation of thyroid hormone receptors (TRs) and thyroid hormone converting enzymes (monodeiodinases), and of immediate early thyroid hormone target genes (Fig. 1; Denver, 2009; R.M. Bonett, E.D. Hoopfer and R.J. Denver, unpublished data). For example, corticosteroids increased maximal nuclear binding capacity for 3,5,3′-triiodothyronine (T_3; Niki et al., 1981; Suzuki and Kikuyama, 1983; Kikuyama et al., 1993). Combined treatment of *X. laevis* tail explants or frog cell lines with corticosterone and T_3 caused synergistic upregulation of TRα and TRβ mRNAs (R.M. Bonett, E.D. Hoopfer and R.J. Denver, unpublished data). Corticosterone also increased 5′-deiodinase activity in bullfrog tadpoles, thereby increasing bioavailability of T_3 at peripheral tissues (Galton, 1990; and see Darras et al., 2002; Kuhn et al., 2005).

In addition to TRs and monodeiodinases, some thyroid hormone target genes are synergistically upregulated by thyroid hormone plus corticosterone through mechanisms that may not be directly, or immediately dependent on an increase in TRs or deiodinases. That is, the target genes may be direct targets for TRs and for corticosteroid receptors (GR, MR) which then function in a synergistic manner to activate gene transcription. For example, Krüppel-like factor 9 (*Klf9*; also known as basic transcription element binding protein 1; *bteb1*) is a T_3 target gene that is also induced by corticosterone acting via the GR (Bonett et al., 2009), and is superinduced with rapid kinetics by combined treatment with T_3 plus corticosterone, both *in vivo* in *X. laevis*, and in frog tissue culture cells (R.M. Bonett, E.D. Hoopfer and R.J. Denver, unpublished data). We found similar synergistic upregulation of the *Klf9* gene by T_3 and corticosteroids in a mouse hippocampal cell line (HT-22; P. Bagamasbad and R.J. Denver, unpublished data). We have identified regions of the *Klf9* gene in frog and mouse that support synergistic gene activation in transfection assays, and that exhibit hyperacetylation of histones upon hormone treatment (P. Bagamasbad and R.J. Denver, unpublished data). These findings suggest that synergistic gene regulation by thyroid hormone and corticosteroids may be a general, and important phenomenon in animal development.

The common regulation of the HPT and the HPA axes by CRF-like peptides, and the sensitization of target tissues to low concentrations of thyroid hormone by corticosteroids may provide a mechanism by which a tadpole can modulate its rate of development in response to a changing environment. Similar to the role that stress hormones play in timing amphibian metamorphosis, in mammals, CRF and corticosteroids have been shown to play critical roles in the timing of birth. In humans, the increase in CRF of fetal and placental origin, and adrenal steroids of fetal origin have been implicated in controlling the timing of parturition (McLean and Smith, 2001; Hillhouse and Grammatopoulos, 2002). The HPA axis of mammals matures during mid- to late-gestation, and as birth approaches concentrations of CRF and corticosteroids in the maternal circulation increase exponentially, and this is driven by a positive feedback loop between the placenta and the fetus (McLean and Smith, 2001; Hillhouse and Grammatopoulos, 2002). Studies in sheep have shown that CRF derived from the fetal paraventricular nucleus (PVN; located in the hypothalamus where it houses CRF neurosecretory neurons; homologous to the frog anterior preoptic area—POA) plays a critical role in timing gestation (Brooks and Challis, 1988; Matthews and Challis, 1996; Challis et al., 2000, 2005). Unlike the ovine placenta, the human placenta synthesizes CRF (Torricelli et al., 2007) which, together with fetal CRF is implicated in timing parturition (i.e., the 'CRF placental clock'; McLean et al., 1995). Early elevations in maternal circulating CRF driven by placental CRF secretion is associated with higher probabilities of pre-term birth in humans (McLean et al., 1995). In addition to CRF, the CRF-BP is expressed in the human placenta and is secreted into the maternal circulation (Linton et al., 1993; Behan et al., 1996). The expression of CRF-BP declines during late-gestation, at which time placental CRF expression increases. Together, this leads to an increase in free CRF in the circulation, which is hypothesized to play an important role in the timing of birth (McLean and Smith, 2001; Hillhouse and Grammatopoulos, 2002).

4. Effects of early life experience on later life phenotypic expression

In addition to modifying the timing and size at life history transitions such as metamorphosis or birth, the environment experienced by developing organisms can have profound effects on phenotypic expression later in life, and thus affect individual fitness (often referred to as phenotypic 'carry-over', or developmental 'programming'). Exposure to stressors during early development can result in higher probabilities of reproductive dysfunction and adult-onset diseases in humans. Studies in frogs and mammals point to elevated GCs, caused by exposure to stressors during early development, as the proximate mechanism for effects on the function of physiological and behavioral systems later in life. The molecular mechanism of GC action during early development may involve the promotion of epigenetic changes such as DNA methylation and chromatin modifications, which leads to the reprogramming of neuroendocrine stress axis genes.

In mammals, maternal malnutrition or repeated acute stress cause intrauterine growth retardation and pre-term birth (Weinstock et al., 1992, 1998; Challis et al., 2001; Bloomfield et al., 2003), and are associated with reproductive dysfunction and increased susceptibility to disease later in life (Barker, 1997; Wein-

stock, 2001; Matthews, 2002; Sloboda et al., 2006). These later-life effects of the *in utero* environment are associated with elevated neuroendocrine stress axis activity in mothers and fetuses (Weinstock, 2001; Welberg and Seckl, 2001; Matthews, 2002). Elevations in plasma GCs at critical windows of brain development may permanently alter the functioning of the stress axis and the expression of behaviors throughout life. Similarly, in amphibian tadpoles, exposure to environmental stressors (high conspecific density, decreased food availability, habitat desiccation, predators), or to a physical stressor all elevate corticosterone (Denver, 1997; Hayes, 1997; Glennemeier and Denver, 2002b; Denver et al., 2002). Activation of the tadpole HPA axis is linked to decreased growth and thus smaller size at metamorphosis (Hayes, 1997; Denver et al., 2002; Glennemeier and Denver, 2002b,c). Previously, it was thought that the complex life cycle (larval stage followed by a metamorphosis to the juvenile/adult form) was a means to dissociate phenotypic correlations between stages, thus allowing each life history stage to evolve independently (Ebenman, 1992; Moran, 1994; Pechenik et al., 1998). However, a growing body of evidence now shows that phenotypic carry-over occurs between different stages of the amphibian life cycle and may have wide-ranging effects on individual fitness (Goater, 1994; Scott, 1994; Van Buskirk and Saxer, 2001; Alvarez and Nicieza, 2002; Altwegg and Reyer, 2003).

Exposure to stress early in life is typically associated with a "hyper-responsive" neuroendocrine stress axis, with elevated basal expression of hypothalamic CRF and plasma GCs (McCormick et al., 1995; Weinstock, 2001; Meaney, 2001), fearful behaviors and anxiety (Smythe et al., 1996; Meaney, 2001), magnified or prolonged responses of CRF and GCs to acute stressors (Meaney, 2001; Lesage et al., 2004), and increased food intake associated with higher probabilities of obesity and metabolic dysfunction (Barker, 1997; Breier et al., 2001). These responses are complex, as they often depend on gender, the duration of exposure to stress, and the developmental stage when the stress was experienced (Meaney, 2001; Matthews, 2002). The hyper-reactivity of the HPA axis may result from reduced GC negative feedback, as shown by the simultaneous elevation in basal plasma GCs and CRF expression in the PVN, prolonged elevations in plasma GCs after a stress response, and reduced GR expression in the hippocampus (Meaney, 2001; Welberg and Seckl, 2001; Weaver et al., 2004).

Recent work from my laboratory showed that food restriction during the tadpole stage altered post-metamorphic growth and HPA axis activity in *X. laevis* (Hu et al., 2008). We manipulated food availability of tadpoles from Nieuwkoop Faber stage 56–57 (Nieuwkoop and Faber, 1994) to the completion of metamorphosis. Food restriction increases whole body corticosterone content in tadpoles (Glennemeier and Denver, 2002b; Crespi and Denver, 2005). Tadpoles that were food restricted had reduced body weight at metamorphosis, but juvenile frogs showed catch-up growth, and reached similar body size to controls by 21 days after metamorphosis. Food restricted animals had greater food intake, size-specific growth rates, and whole body corticosterone content (Hu et al., 2008). Our findings in the frog compare with those from mammals that show that exposure to GCs during the fetal/perinatal period leads to elevated basal plasma GC concentrations during later life stages (Meaney et al., 2007). The elevated corticosterone may be causally related to the increased food intake seen in juvenile frogs, since corticosterone facilitates feeding in juvenile *X. laevis* (Crespi and Denver, 2004).

We also tested for a causal relationship between elevated corticosterone and later life phenotypic expression by exposing early prometamorphic tadpoles to 100 nM corticosterone in their aquarium water for 5 or 10 days, and then allowing them to develop and grow until 2 months after metamorphosis (Hu et al., 2008). This treatment increased whole body corticosterone content by ~3-fold, which is within the physiological range achieved in tadpoles exposed to shaking/confinement stressor (Glennemeier and Denver, 2002a). Also, the level reached, and duration of corticosterone elevation is comparable to that seen with pond drying (Denver, 1997, 1998) or intraspecific competition (Denver et al., 1998; Glennemeier and Denver, 2002b; Boorse and Denver, 2004). Corticosterone treatment as tadpoles reduced body weight at metamorphosis (growth inhibition discussed above), but juvenile frogs showed catch-up growth, reaching similar body size as controls 2 months after metamorphosis (Hu et al., 2008). Juvenile frogs that had been treated with corticosterone as tadpoles had increased HPA activity as evidenced by higher basal plasma corticosterone concentration. Also, treatment with corticosterone as a tadpole decreased the number of GR immunoreactive (GR-ir) cells throughout the brain (POA, amygdala, bed nucleus of the stria terminalis [BNST], medial pallium [homolog of the mammalian hippocampus]; Fig. 2—only the POA shown) and in the anterior pituitary gland of juvenile frogs (Hu et al., 2008). Corticosterone treatment as a tadpole also lead to the sensitization of CRF neurons in the POA/hypothalamus to a physical stressor (shaking/confinement stressor; Yao et al., 2004) as shown by measures of CRF mRNA on microdissected brain sections (F. Hu and R.J. Denver, unpublished data). Our findings show that elevations in corticosterone during the tadpole stage alters neuroendocrine gene expression, leading to altered feedback relationships and activity of the HPA axis, which could have long term fitness consequences.

In rodents, neonatal stress alters CRF neuronal morphology in the PVN and other areas of the brain involved in the stress response; e.g., the amygdala, BNST, hippocampus, and locus coeruleus (Meaney, 2001). The amygdala and BNST play central roles in the expression of fear and anxiety-related behaviors (Charney et al., 1998; Herman et al., 2005; Schafe et al., 2005; Schulkin et al., 2005; Morgane et al., 2005). These limbic structures have extensive connections with the telencephalon, hypothalamus, thalamus and brainstem, and are known to influence neuroendocrine and autonomic functions (Gray, 1991; Ongur and Price, 2000; Herman et al., 2005; Morgane et al., 2005). It is well established in rodents that CRF neurons in the amygdala and BNST are activated in response to fear/anxiety-provoking stressors (Casada and Dafny, 1991; Gray, 1993; Merali et al., 1998; Makino et al., 1999; Bruijnzeel et al., 2001; Becker et al., 2007; Rotllant et al., 2007). The BNST composes the main relay between the amygdala and the hypothalamus, and is the major direct non-hypothalamic input to the parvocellular PVN (Sawchenko and Swanson, 1983; Cunningham et al., 1990). The CRF-expressing pathways in the amygdala and BNST may be involved in relaying stress input to the hypothalamus and facilitating CRF release from the PVN (Gray, 1993).

Our recent work in the frog *X. laevis* suggests that the basic functions of limbic structures in the stress response, and the nature of the feedback regulation by GCs likely arose before the divergence of the amphibian and amniote lineages, and may be common features in tetrapods (Yao et al., 2004, 2008a,b; Yao and Denver, 2007). Alterations in CRF neuronal physiology in frogs by exposure to stressors early in life could lead to behavioral modifications with long term fitness consequences. For example, individuals that are less active in response to larval predators (i.e., more fearful) may also show reduced activity in the post-metamorphic environment, and thus be less effective at obtaining food or mates.

4.1. Effect of early life stress on later life food intake

Hormones of the neuroendocrine stress axis (CRF, corticosterone) influence food intake, with CRF potently anorexigenic, while corticosterone is orexigenic (Heinrichs and Richard, 1999; Carr, 2002; Crespi et al., 2004; Crespi and Denver, 2004, 2005). The temporal relationship between these opposing actions modulates

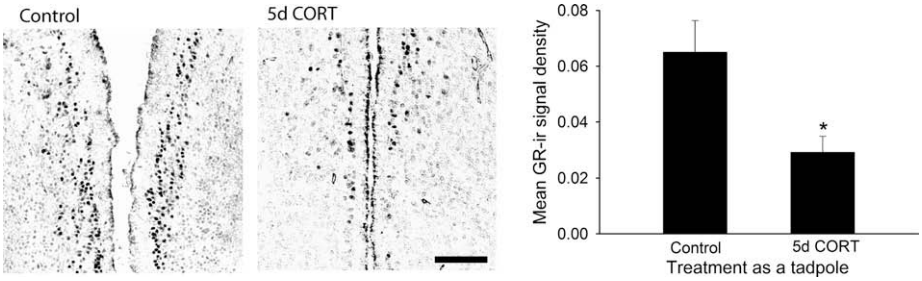

Fig. 2. Treatment of *X. laevis* tadpoles with corticosterone leads to decreased glucocorticoid receptor (GR) immunoreactivity (-ir) in the anterior preoptic area (POA) of 2-month-old juvenile frogs. Early prometamorphic tadpoles (Nieuwkoop Faber stage 52–54) were treated with 100 nM corticosterone added to the aquarium water for 5 days then reared to 2 months post-metamorphosis. (Left) Photomicrographs of representative transverse sections through the POA of juvenile frogs. (Right) Quantitative morphometric analysis showing the mean GR-ir signal density in the POA of juvenile frogs. The asterisk indicates a significant difference based on *t*-test ($P < 0.05$; $n = 5$/treatment; scale bar = 100 μm). Modified from Hu et al. (2008) with permission.

appetite and feeding during the stress response; e.g., CRF acting rapidly to suppress appetite, corticosterone acting later to stimulate appetite and replenish energy reserves (Heinrichs and Richard, 1999; Crespi and Denver, 2004). Diurnal changes in plasma corticosterone influence appetite through actions on the hypothalamus (Dallman et al., 1993, 1995).

A striking effect of exposure to stressors during early mammalian development is the programming of the appetite and metabolic phenotype (Sloboda et al., 2006). Maternal nutritional stress during pregnancy is often associated with hyperphagia and "catch-up growth" in offspring (Breier et al., 2001). Neonates from undernourished mothers have higher basal PVN CRF content and plasma corticosterone concentration (Breier et al., 2001), which may be causally related to the increased food intake. Programming of the metabolic phenotype is reflected in changed gene expression in different organs through epigenetic modifications at gene promoters, including the GR and peroxisome proliferator activated receptors (PPARs; Burdge et al., 2007). These changes are thought to be due, at least in part, to increased exposure to GCs early in life (Burdge et al., 2007). As discussed above, food restriction, or exposure to corticosterone during the tadpole stage leads to increased food intake and compensatory growth in juvenile frogs when food is abundant (Hu et al., 2008; Morey and Reznick, 2001). This is associated with elevated plasma corticosterone concentration, which may be causally related to the hyperphagia (Hu et al., 2008). Compensatory growth may be a general phenomenon in tetrapod vertebrates, and thus could have similar mechanistic underpinnings.

Compensatory growth could quickly reverse any competitive disadvantage that smaller individuals have at metamorphosis (or birth). However, there is evidence that such compensation comes at a variety of costs later in life, and although an organism might appear to recover through catch-up growth, early life nutritional deficits result in profound and permanent changes in adult physiology and behavior (Metcalfe and Monaghan, 2001). This is true for many organisms (Metcalfe and Monaghan, 2001), and perhaps best illustrated in humans, where despite compensation in body size through catch-up growth, prenatally stressed individuals have higher probabilities of hypertension, obesity, and type II diabetes, among other diseases during later life (Barker, 1997; Breier et al., 2001).

4.2. Epigenetic programming of gene expression by early life experience

There is a growing body of evidence that epigenetic mechanisms of gene regulation, such as DNA methylation and chromatin modifications, play important roles in mediating the relationship between the early environment and later life phenotypic expression (Jaenisch and Bird, 2003; Junien et al., 2005; Vickaryous and Whitelaw, 2005; Meaney et al., 2007; Waterland and Michels, 2007; Mathers, 2007). Early development is an active period of DNA methylation mediated by DNA methyltransferases (DNMTs) and leading to gene repression/silencing. Methylation of DNA occurs in genomic regions known as CpG islands, and nuclear proteins that bind to methyl-CpG mediate gene repression. Two families of DNA binding proteins that recognize and bind to methyl-CpG dinucleotides have been identified. There are five methylated CpG-binding domain (MBD) protein genes in mammals (MeCP2, MBD1–4; Clouaire and Stancheva, 2008). Chromatin immunoprecipitation (ChIP) experiments have localized MBD proteins to methylated and silenced genes (Gregory et al., 2001; Ballestar et al., 2003; Lopez-Serra and Esteller, 2008). A second family of methyl-CpG-binding proteins is the BTB/POX zinc finger domain proteins of which the protein *Kaiso* is the founding member (also ZBTB4 and ZBTB38; Lopez-Serra and Esteller, 2008; Clouaire and Stancheva, 2008). We recently identified *Kaiso* as a GC target gene in *X. laevis* brain (F. Hu and R.J. Denver, unpublished data).

Early life experience can influence the degree of DNA methylation at CpG islands in gene promoters (Szyf et al., 2005, 2007a,b). Whether these changes are mediated by GCs has yet to be determined. The promoter region of the rat GR gene (exon 17) has a CpG island that is differentially methylated depending on early life experience, and the methylation state is thought to determine the level of GR expression (Weaver et al., 2004). The frog (*X. tropicalis*) GR gene has the same number of exons as the rodent genes and conserved CpG islands located in the first two noncoding exons (Y. Kyono and R. Denver, unpublished), suggesting that its expression may also be modified by DNA methylation and may account for the decreased GR-ir that we observed in juvenile frogs following exposure to corticosterone as a tadpole (Hu et al., 2008). Several transcription factor binding sites have been identified in this region of the rat GR gene, and the methylation state of DNA may be influenced by, or may influence the binding of transcriptional activators such as nerve growth factor I-A (NGFI-A; Weaver et al., 2007). NGFI-A may serve to reverse epigenetic marks at the GR promoter, and may thus mediate maternal effects (Weaver et al., 2007). Recent findings show that the CRF gene also has CpG islands and is thus a target for epigenetic regulation by DNA methylation (McGill et al., 2006; Murani et al., 2006). Mice harboring a truncated allele of MeCP2 had increased CRF mRNA in the PVN, central amygdala and BNST, increased anxiety-like behavior, and elevated serum corticosterone concentration (McGill et al., 2006). These authors also showed using ChIP assay that MeCP2 associates with the CRF promoter.

Another important means for epigenetic modulation that may be influenced by early life experience, and by GCs is the modification of chromatin structure by histone acetylation, methylation,

ubiquitination, phosphorylation and ADP-ribosylation (Lachner and Jenuwein, 2002; Lachner et al., 2003; Cheung and Lau, 2005; Caiafa and Zampieri, 2005). Methylation of DNA is accompanied by post-translational modification of histones that modulate chromatin structure and transcriptional activity. The histones in chromatin of methylated, silenced genes are hypoacetylated and methylated (H3 methylated on lysine 9), both of which are indicative of a repressive chromatin structure (Gregory et al., 2001; Ballestar et al., 2003; Clouaire and Stancheva, 2008). Acetylation of histone tails (e.g., H3 and H4) is often, but not always, associated with transcriptional activation. Methylation of H3 at lysine 9 is associated with heterochromatin and gene silencing, while methylation of histone H3 at lysine 4 or 27 is associated with transcriptional activation (Lachner and Jenuwein, 2002; Lachner et al., 2003). Each lysine residue may become mono-, di-, or tri-methylated; the consequences of this diversity of histone modification is poorly understood.

5. Summary

Hormones mediate many effects of the environment on amphibian development, and consequently on phenotypic expression in the juvenile and adult. Hormones of the neuroendocrine stress axis have especially important roles in mediating environmental effects on developmental plasticity. Since the stress axis plays key roles in behavior and growth, early environmental effects, mediated in part by GCs, on the development of the neuroendocrine system can have profound fitness consequences. The actions of GCs in programming long term changes in gene expression likely result from epigenetic changes such as DNA methylation and histone modifications. Amphibians and other nonmammalian species can serve as important model organisms for elucidating the molecular mechanisms of stress hormone actions in early development, and the consequences of these actions for later life phenotypic expression.

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