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More similar than you think: Frog metamorphosis as a model of human perinatal endocrinology

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ABSTRACT

Hormonal control of development during the human perinatal period is critically important and complex with multiple hormones regulating fetal growth, brain development, and organ maturation in preparation for birth. Genetic and environmental perturbations of such hormonal control may cause irreversible morphological and physiological impairments and may also predispose individuals to diseases of adulthood, including diabetes and cardiovascular disease. Endocrine and molecular mechanisms that regulate perinatal development and that underlie the connections between early life events and adult diseases are not well elucidated. Such mechanisms are difficult to study in uterus-enclosed mammalian embryos because of confounding maternal effects. To elucidate mechanisms of developmental endocrinology in the perinatal period, *Xenopus laevis* the African clawed frog is a valuable vertebrate model. Frogs and humans have identical hormones which peak at birth and metamorphosis, have conserved hormone receptors and mechanisms of gene regulation, and have comparable roles for hormones in many target organs. Study of molecular and endocrine mechanisms of hormone-dependent development in frogs is advantageous because an extended free-living larval period followed by metamorphosis (1) is independent of maternal endocrine influence, (2) exhibits dramatic yet conserved developmental effects induced by thyroid and glucocorticoid hormones, and (3) begins at a developmental stage with naturally undetectable hormone levels, thereby facilitating endocrine manipulation and interpretation of results. This review highlights the utility of frog metamorphosis to elucidate molecular and endocrine actions, hormone interactions, and endocrine disruption, especially with respect to thyroid hormone. Knowledge from the frog model is expected to provide fundamental insights to aid medical understanding of endocrine disease, stress, and endocrine disruption affecting the perinatal period in humans.

Hormonal control of human perinatal development

Maturational changes in vital organs such as the lungs, liver, kidneys, and gut occur several weeks before birth in humans in preparation for extra-uterine life (Liggins, 1994). These changes ensure activation of physiological processes essential for survival immediately at birth, such as pulmonary gas exchange, adaptations in cardiac function, hepatic gluconeogenesis, and thermogenesis. The fetus also increases in size dramatically in the weeks before birth, and development of the brain and nervous system continue apace throughout the perinatal period (Patel et al., 2011; Sferruzzi-Perri et al., 2013). Regulation and coordination of these post-embryonic developmental events, as well as the timing of birth itself, are accomplished by many hormones, where thyroid hormone (TH) and glucocorticoids (GCs) in particular play prominent and widespread roles (Fowden and Forhead, 2013).

Deviations from normal TH or GC signaling, arising from congenital endocrine diseases in the mother or fetus or from environmental insults, for example nutrition stress or medical interventions, can cause deleterious alterations in organ maturation and timing of birth. Similarly, endocrine disrupting chemicals, which alter TH or GC action, synthesis, or degradation during critical developmental windows, can also dramatically perturb normal development (Fudvoye et al., 2014; Préau et al., 2015).

Low TH availability to the fetus or newborn causes cretinism characterized by mental retardation, short stature, and impaired development of the neuromotor and auditory systems (Delange, 2005). Also, mutant TH receptors, plasma membrane TH transporters, and cytosolic TH binding proteins cause various forms of resistance to TH and hypothyroidism (Abe et al., 2003; Refetoff, 2005; Visser et al., 2010). Even mild reductions in maternal TH signaling in early pregnancy are associated with reduced IQ in offspring (Biondi and Cooper, 2008). Such critical dependence on

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appropriate TH levels for proper brain development highlights the importance of careful control of timing and dose of TH treatments to minimize negative effects on nervous system development (Nunez et al., 2008).

GCs are essential for survival at birth, most critically for inducing lung surfactant, but also for inducing organ maturation in the brain, intestine, liver, and kidney (Fowden et al., 1998; Fowden and Forhead, 2009; Grier and Halliday, 2004; Liggins, 1994). Consequently, synthetic GCs are routinely used to induce organ maturation and avoid neonate suffocation by lung atelectasis in cases of preterm delivery. On the other hand, fetal stress and elevated GCs experienced early in life are associated with lifelong negative health consequences, including cardiovascular disease, diabetes, and obesity (Harris and Seckl, 2011).

These significant health consequences from genetic or environmental changes in the hormonal regulation of perinatal development motivate efforts to look for treatment options to regulate organ maturation and the timing of birth while mitigating long-term side effects. Unfortunately, many mechanisms pertinent to perinatal endocrinology are not well understood. For example, the extent to which TH alters development of the tissues, such as the autonomic nervous system, either prenatally or in the long term and the developmental and epigenetic mechanisms by which hormones act and interact at the cellular and molecular levels *in utero* still remain largely unknown. Such knowledge is required to better diagnose and treat or prevent medical issues from maternal endocrine disease, stress, or endocrine disruption that may affect perinatal development.

In order to elucidate mechanisms in developmental endocrinology and generate preventative and therapeutic strategies, animal models are required. Also, the fact that the rate of preterm delivery is increasing despite extensive efforts to stop it points to the importance of broadening the net of basic research to develop treatment options (Goldenberg et al., 2008; Wang et al., 2014). Studying the endocrine control of frog metamorphosis has contributed to understanding hormonal control of post-embryonic developmental events, particularly with respect to the roles of TH and GCs (Denver et al., 2009; Shi, 1999). Historically, key insights into hypothalamic and pituitary control of TH and GC secretion (Allen, 1938; Dodd and Dodd, 1976), the effects of TH on mRNA and protein synthesis (Tata, 1965; Tata, 1966), morphological and biochemical changes induced by TH during development (Fox, 1983; Frieden and Just, 1970), and identification of TH response genes (Brown et al., 1995) have stemmed from metamorphosis

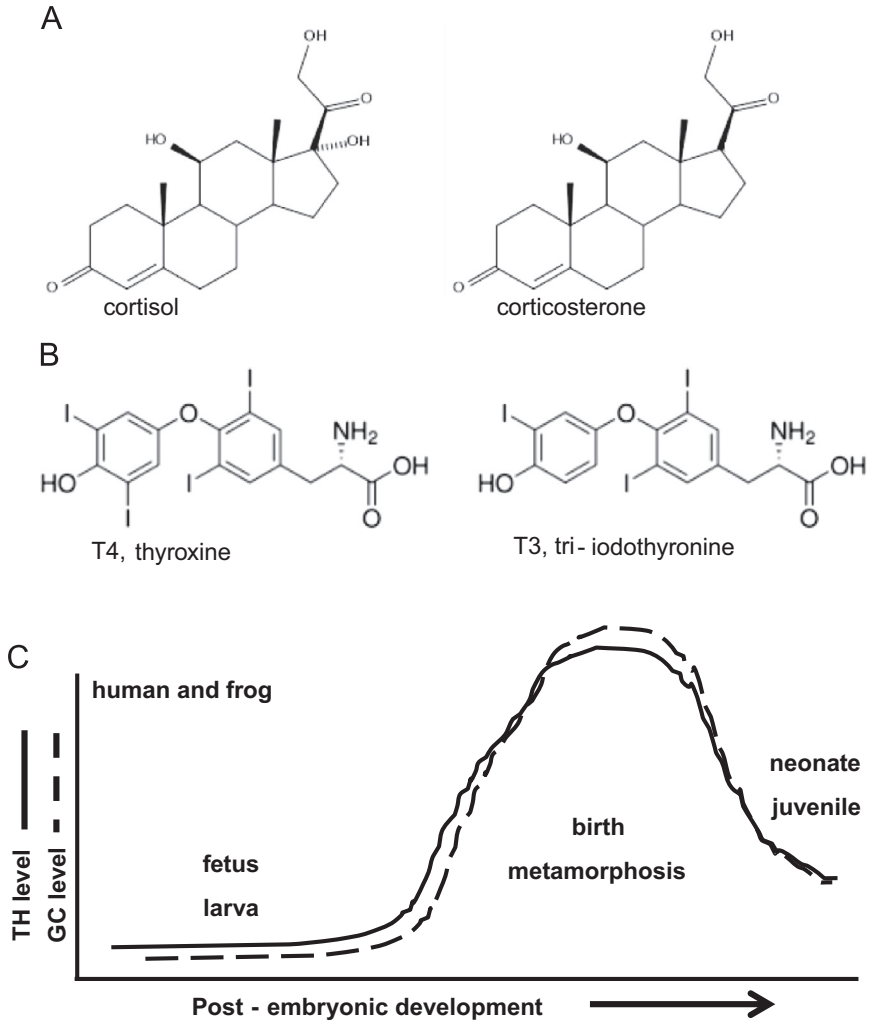


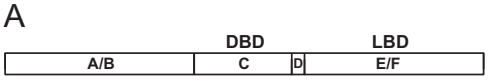
Fig. 1. Hormone structures and developmental profiles. (A) Cortisol is released from the adrenal gland in humans and fish, whereas corticosterone is released in mice and frogs. Both cortisol and corticosterone bind the two GC receptors, Type I and Type II with high affinity. (B) Thyroxine (T4) is the prohormone form of TH, and tri-iodothyronine (T3) is the active form, and the structure of these hormones is identical among all vertebrates. Two TH receptors exist in all vertebrates, TH receptor alpha and TH receptor beta (TRα and TRβ), and both receptors bind T3 with 10-fold higher affinity than T4. (C) Diagram of developmental profiles of hormone levels in the blood in humans and frogs. The plasma levels of TH and GCs peak in the blood at birth in humans and metamorphosis in frogs. Profile curves were extracted from (Carr et al., 1981; Hume et al., 2004;Jolivet Jaudet and Leloup Hatey, 1984;Leloup and Buscaglia, 1977;Tata, 1993).

research. Recent reviews on amphibian metamorphosis detail the molecular and developmental mechanisms of hormone action in frogs (Brown and Cai, 2007; Denver et al., 2009; Grimaldi et al., 2013; Ishizuya-Oka, 2011). To a large degree, the extensive research on hormonal control of development in frogs and mammals is not directly comparable in detail because of the different perspectives. Frog researchers rarely treated tadpoles with hormones to better understand endocrine diseases found in humans, and research on the endocrinology of birth has not had dramatic hormone-induced morphology to elucidate. This review attempts to bring these two paradigms closer together by focusing on the current need and utility of the frog model to elucidate developmental actions of TH and GCs, what the model has accomplished, and future promises of the model with respect to understanding the hormone regulation of human perinatal development.

Birth and metamorphosis

Significant parallels can be drawn between frog metamorphosis and mammalian birth (Tata, 1993; Wada, 2008). Both frogs and mammals undergo a life history transition from aquatic (amniotic fluid or water) to terrestrial habitat with air-breathing and lung maturation, during which they (1) transition to a new food source accompanied by the maturation of the intestine to the adult form, (2) switch from the fetal or larval to adult type of hemoglobin, (3) increase production of albumin and other plasma proteins, (4) induce urea cycle enzymes in the liver, (5) undergo skin keratinization, (6) have limb elongation, and (8) experience developmental progression and restructuring of the central and peripheral nervous systems.

Underlying these conserved developmental events are conserved endocrine components. The same hormones, GCs and TH (Fig. 1A and B), experience peak plasma levels at birth in humans (Carr et al., 1981; Hume et al., 2004; Kawahara and Yokoya, 2002) and at metamorphosis in frogs (Jolivet Jaudet and Leloup Hatey, 1984; Leloup and Buscaglia, 1977) (Fig. 1C). In rodents, GCs peak at birth and at weaning 15 days post-partum, and TH peaks only at weaning (Hadj-Sahraoui et al., 2000; Lamers et al., 1986). Conservation is also observed in hormone receptors (Fig. 2A and B). Furthermore, gene regulation by TH involves structurally well-conserved TH receptor isoforms (alpha and beta), heterodimer partners (retinoic acid receptors alpha, beta, gamma), and receptor-associated co-repressors and co-activators that bind canonical TH response elements in enhancer or promoter regions of TH target genes (Buchholz et al., 2006). Similarly, GCs bind conserved Type I and Type II GC receptors, which are cytoplasm-



B

	Type I	Type II	TRα	TRβ
Human v Mouse	88	90	98	79
Human v Frog	62	68	85	75
Human v Fish	46	50	78	73
Frog v Fish	46	49	81	87

Fig. 2. Structure and conservation of TH and GC nuclear receptors. (A) The receptors for GCs and TH belong to the family of ligand-activated nuclear receptors and share a common structure with a variable A/B transactivation domain, the conserved C domain or DNA binding domain (DBD), the hinge region (D domain), and the E/F ligand binding domain (LBD) where co-repressors and co-activators also bind. (B) The percent similarity comparisons in amino acid sequence between human, mouse, frog (*Xenopus laevis*), and zebrafish (*Danio rerio*) were done using full-length protein sequences. For each comparison, the sequence divergences are predominantly due to disparity in the A/B domains rather than the conserved DBD or LBD.

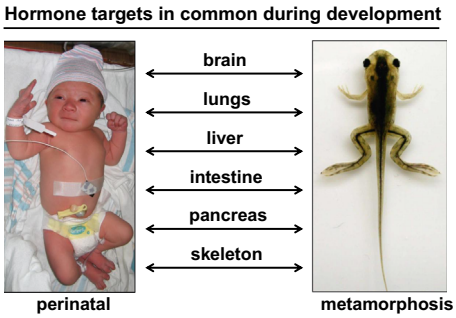


Fig. 3. Hormone target organs in common at birth and metamorphosis. Many organs require GC and TH hormone signaling for proper development in humans and frogs (Brown and Cai, 2007;Dodd and Dodd, 1976;Forhead and Fowden, 2014; Fowden and Forhead, 2013;Liggins, 1994).

resident nuclear receptors that travel to the nucleus upon hormone binding to alter gene expression (Kulkarni and Buchholz, 2014; Ratman et al., 2013).

Like the hormones and their receptors, many known developmental roles of TH and GCs are comparable in frogs and humans. During the perinatal period and metamorphosis, numerous organs require the action of hormones for proper development (Fig. 3). Intrauterine exposure to GCs in humans and other mammals and treatment of tadpoles with GCs (1) reduces overall growth rate (Dodd and Dodd, 1976; Fowden, 1995; Reinisch et al., 1978) (2) causes a marked increase in the accumulation of liver glycogen (Forhead et al., 2009; Hanke and Leist, 1971) (3) causes regression of lymphoid tissue in the spleen and thymus regresses late in fetal life (Liggins, 1994; Rollins-Smith et al., 1997) (4) can permanently alter responsiveness of the stress hormone axis through to adulthood (Braun et al., 2013; Hu et al., 2008; Reynolds, 2013). For TH, neuronal proliferation in the central nervous system begins very early in human fetal development and is one of the earliest TH-dependent events in tadpoles as well (Kollros, 1981; Patel et al., 2011). TH is also required early in metamorphosis for skeletal growth, and short stature is a hallmark of cretinism caused by perinatal and neonatal TH deficiency in humans (Dodd and Dodd, 1976; Van Vliet, 2005). In several organs, TH and GC appear to have extensive interactions in common between frogs and humans, such as in lung structural maturation (Buchholz, unpub. data) (Forhead and Fowden, 2014), intestinal structural development (Ishizuya-Oka and Shimozawa, 1991; Ishizuya-Oka, 1996; Sirakov and Plateroti, 2011; Trahair and Sangild, 1997), and intestinal digestive enzyme expression (El Maraghi-Ater et al., 1986; Henning et al., 1994). In conclusion, TH-dependent development in frogs can serve as a model for perinatal events in humans because the hormones, their receptors, molecular mechanisms, and developmental roles of TH and GC signaling are conserved to a high degree.

Advantages of the frog model for hormonal control of development

The study of perinatal endocrinology in mammals is complicated by several issues. First, fetal tissues are constantly exposed to maternal hormones through the placenta (Forhead and Fowden, 2014). Consequently, endocrine regulation of fetal development is not independent of influence by the mother, and manipulation of fetal endocrine signaling is difficult to achieve without pathologically disrupting maternal endocrine physiology. Using free-living tadpoles, study of endocrine regulation on a developing organism isolated from maternal influence is possible. Second, receptor function cannot be studied in plus or minus hormonal states

under normal developmental conditions in mammals. In contrast, undetectable hormones levels occur naturally during the frog larval period prior to metamorphosis (Leloup and Buscaglia, 1977). Precise timing of change from the unliganded to the liganded state is readily achieved by addition of hormones to the rearing water mimicking natural metamorphosis (Shi, 1999). Third, the lower cost by at least a factor of 100 of husbandry in frogs versus mice should not be overlooked in the light of tight federal budgets. Fourth, the frog model provides direct observation and manipulation at the developmental stages that are comparable to fetal stages in mammals. Tadpoles are easily accessible throughout their development and take up TH and GCs from the aqueous rearing medium, where consequent metamorphic changes in gene expression and morphology occur at a rate and degree unequaled among terrestrial vertebrates.

Accomplishments of the model

In-vivo molecular mechanisms of TH action during development

In-vitro cell culture studies showed that TH receptors repress gene expression in the absence of TH and activate those same genes when TH is added (Damm et al., 1989; Sap et al., 1989). However, a mechanistic connection between the dual action of TH receptors on gene regulation in vitro and a developmental role for TH receptors was in need of elucidation. To that end, TH receptor knockout mice and dominant negative TH receptors in knock-in mutant mice that mimic human diseases clearly showed developmental defects in nervous system and skeletal growth among other defects due to lack of TH gene induction (Flamant and Samarut, 2003). On the other hand, few developmental roles for TH receptors in the absence of TH in mice and none in humans have been identified (Bernal and Morte, 2013). Direct evidence in mice for unliganded TH receptors was shown in TH receptor alpha knockout mice in cerebellum (Morte et al., 2002), heart (Mai et al., 2004), and cochlea (Winter et al., 2007). A mutant phenotype was seen in cochlea only in hypothyroid but not euthyroid conditions. Mutant receptor-related phenotypes exposed by hypothyroid conditions, which are not uncommon in women of reproductive age and occur naturally in tadpole development, points to frog metamorphosis as an important and convenient model to study developmental roles of unliganded TH receptors.

A dual function model (Fig. 4) for the role of TH receptors in development was proposed upon cloning of the *Xenopus* receptors (Yaoita et al., 1990) and elaborated later (Buchholz et al., 2006; Sachs et al., 2000; Shi, 2009). The dual function model states that: (1) TH receptors act to recruit co-repressors and reduce expression of genes involved in developmental progression in the absence of TH to allow continued growth, and (2) upon TH release into circulation, liganded TH receptors bind co-activators and induce the previously repressed genes to initiate TH-dependent events. Strong evidence exists for all aspects of this model, derived comprehensively from the frog system where precise control and assessment of TH action is most feasible.

Pioneering in-vivo application of the chromatin immunoprecipitation assay, which quantifies DNA binding by proteins using specific antibodies, used chromatin isolated from tadpoles treated with and without TH (Sachs and Shi, 2000). In the absence of TH, TH receptors recruit co-repressors, which deacetylate histones and repress gene expression and, in the presence of TH, TH receptors favor binding to co-activators to acetylate and methylate histones promoting gene induction, as observed in vitro (Fig. 4) (Shi, 2009). The developmental consequences of this TH-dependent chromatin remodeling and gene regulation were then functionally validated using transgenic frogs overexpressing TH-interacting cofactors and their various dominant negative forms. Blocking co-repressor activity by a dominant negative NCoR (Sato et al., 2007) or TALEN-mediated knockout of TH receptor alpha (Choi et al., 2015; Wen and Shi, 2015) resulted in de-repression of TH response genes important for metamorphosis causing precocious initiation of metamorphic events. Blocking co-activator activity using dominant negative TH receptor, SRC3, or p300 inhibited histone acetylation, gene induction, and developmental progression (Buchholz et al., 2003; Paul et al., 2005; Paul et al., 2007). These critical in-vivo validation steps in the molecular mechanisms of TH and TH receptors established the dual function model for the role of TR in development applicable across vertebrates.

Endocrine disruption

Endocrine disruption by man-made small molecules alters hormone synthesis, metabolism, or action and poses a great concern for human health (de Cock et al., 2014; DiVall, 2013; Schug et al., 2011). The ease of hormone or chemical treatment of free-living tadpoles makes them amenable to be an intact, vertebrate model for the biological activity of TH receptor agonists and antagonists, TH receptor isoform-specific actions, and endocrine disrupting effects of environmental contaminants on early development in general. The in-vivo effectiveness of the first TH receptor antagonist, NH3, was initially shown in tadpoles (Lim et al., 2002), where it blocked natural and TH-induced metamorphic events. Likewise, the isoform-selective agonist, GC-1, which preferentially binds and transactivates mammalian TH receptor beta compared to alpha, induced metamorphic changes in tadpole tissues known to induce high levels of TH receptor beta compared to tissues that express high levels of TH receptor alpha (Furlow et al., 2004).

Many chemicals and chemical mixtures have been tested for their effects on tadpole development by simply adding chemicals to the tadpole rearing water and analyzing effects of gene expression and morphology (Hayes et al., 2006; Heimeier and Shi, 2010; Säfholm et al., 2014; Searcy et al., 2012). By virtue of the small size of early tadpoles, medium-throughput assays have been developed to measure the effect of chemicals on TH-dependent gene expression. One week-old free-living, post-embryonic tadpoles are small enough to fit into a 96-well plate for assay using a fluorescence plate reader. A transgenic frog line was produced that express green fluorescent protein (GFP) under control of promoter sequences from the highly sensitive and ubiquitously expressed TH-response gene, TH/bZIP (Fini et al., 2007). Upon treatment with TH or chemicals that affect TH signaling, the translucent tadpoles express GFP quantitated using a plate reader. This transgenic frog line is currently being marketed to assess endocrine disruption capability of test samples from homes and hospitals and is being used to monitor water quality on-site before and after wastewater treatment (Gies, 2013).

Bisphenol A (BPA) is a well-known xenoestrogen that affects sexual development in mouse and humans (Mileva et al., 2014), but BPA had effects on brain development not known to be regulated by estrogens (Heimeier and Shi, 2010). Insight came when BPA was discovered to inhibit frog metamorphosis via antagonizing TH-dependent development and gene regulation (Goto et al., 2006; Iwamuro et al., 2003; Iwamuro et al., 2006). The ability of BPA to bind to both estrogen and TH receptors to elicit disruption makes it very difficult to study the actions of BPA during mammalian development because of estrogenic actions in the fetus. Due to limited effects of estrogens on metamorphic events, frog metamorphosis is a suitable in-vivo model to evaluate the effects of BPA on TH function during development, where global analysis of gene expression has been done (Heimeier and Shi, 2010; Heimeier et al., 2009).

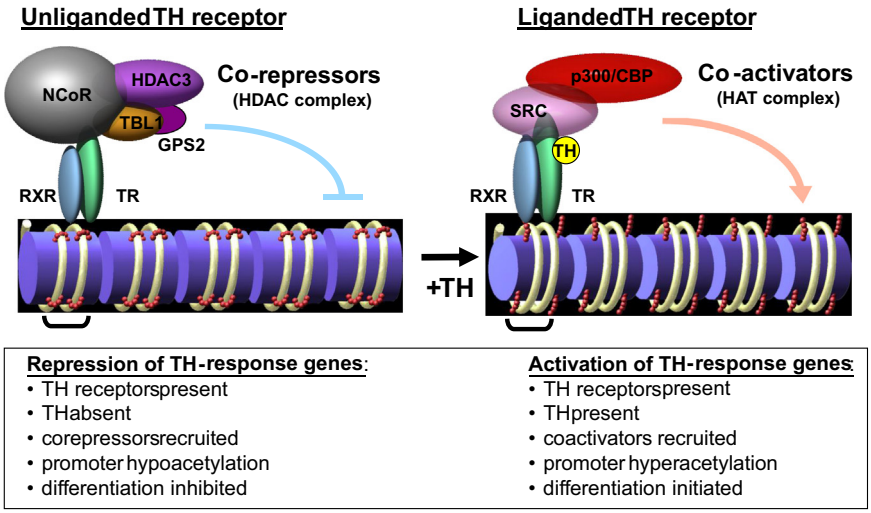


Fig. 4. Dual function model for the role of TH receptors in gene regulation and development. Unliganded TH receptor: in the absence of TH, TH receptors inhibit TH-dependent differentiation by forming heterodimers with RXR (retinoid X receptor), binding promoter or enhancer regions of TH-response genes, recruiting co-repressors to deacetylate histones, and repressing gene expression. NCoR-nuclear co-repressor, HDAC3-histone deacetylase 3, TBL1- transducin beta-like protein 1, GPS2-G protein pathway suppressor 2. Liganded TH receptor: In the presence of TH, TH receptors initiate TH-dependent development by forming heterodimers with RXR (retinoid X receptor), binding promoter or enhancer regions of TH-response genes, recruiting co-activators to acetylate histones, and inducing gene expression. SRC-steroid receptor co-activator, p300/CBP-cAMP response element binding protein, HAT-histone acetytransferase.

Hormone interactions

Synergism between TH and GCs has been observed in a number of prepartum maturational processes, especially in lung, liver, and brain (Barker et al., 1991; Forhead and Fowden, 2014; Fowden et al., 1998; Fowden et al., 2001; Hillman et al., 2012; Mendelson and Boggaram, 1991). Such hormone synergy is due, in part, to GC-induced ontogenic changes in tissue deiodinase activities that increase circulating concentration of T3 (tri-iodothyronine, the active form of TH) in the fetus toward term (Forhead et al., 2006; Fowden and Forhead, 2009). In turn, the prepartum increase in T3 bioavailability in the fetus may mediate, at least in part, the maturational effects of both endogenous GCs and exogenous GCs given as a clinical treatment to improve neonatal viability in threatened or actual preterm delivery (Forhead and Fowden, 2014). As in mammals, GCs synergize with TH to increase the rate of developmental progression in many tissues, and this synergy works in part by GC-regulated deiodinase expression that increases T3 bioavailability as well as increases TH receptor expression (Bonett et al., 2010; Denver et al., 2009; Denver, 2013; Galton, 1990; Kulkarni and Buchholz, 2014).

In contrast to synergism between these two hormones during development on morphology, their synergy at the level of gene regulation is largely unknown. The best-studied example comes from frogs, where TH and GCs synergize to induce KLF9 expression in all tissues examined, and the molecular mechanism of this synergy has been localized to a "synergy module", i.e., a genomic enhancer region with TH and GC receptor binding sites (Bagamasbad, 2012; Bagamasbad et al., 2008). Similar synergy was later identified at the *KLF9* locus in mouse brain (Denver and Williamson, 2009). Taking advantage of negligible levels of GC and TH in premetamorphic tadpoles, a systematic study of TH and GC interaction in gene regulation was conducted using microarray analysis of hormone-treated tadpoles (Kulkarni and Buchholz, 2012). The diversity of TH/GC interactions identified on gene regulation was surprising given that synergy was the only previously known interaction effect between TH and GC. As expected, genes regulated by only TH or only GC were identified, as well as genes that required both hormones for regulation. However, nearly 20% of the more than 5000 genes regulated by one or both hormones had novel, antagonistic interactions between the two hormones. These data greatly expanded the understanding of the hormonal cross-talk underlying TH and GC control of development in a way not predicted based on previously known effects of these two hormones. Further analysis is required to elucidate the significance of this diversity of interaction effects for development. The ease of the frog system to identify previously unknown diversity of interactions between these two hormones can be applied to additional hormone interactions in development.

Developmental origin of intestinal stem cells

Many developmental events dependent on TH in frogs do not appear to require TH for normal morphological development in humans throughout fetal development, including limbs, intestine, gene switching in liver, and skin development (Wasan et al., 2005). Nevertheless, the frog model for perinatal development can be used to understand mechanisms of developmental events by virtue of the dramatic requirement for TH, which provides a unique "hook" or "tool" to probe development mechanisms common to all vertebrates (Tata, 1993). The most thorough example is in intestinal remodeling, which transitions from the long, simple hollow tube with thin connective tissue and muscle in tadpoles to the short, muscular tube with numerous in-foldings of epithelium into the gut lumen in adults (McAvoy and Dixon, 1977; Shi and Ishizuya-Oka, 1996; Shi and Ishizuya-Oka, 2001). The human intestine goes through a period of remodeling in the 6–8 week human fetus reminiscent of the larval to adult transition during intestine metamorphosis, though without known involvement of TH or GC (Montgomery et al., 1999; Wasan et al., 2005). All intestinal epithelial cells are capable of proliferation in fetal mammals and tadpoles, whereas the transition to a niche/stem cell structure is triggered by TH only in frogs (Ishizuya-Oka and Shi, 2011). In rodents, villus and crypt formation, presumably with stem cells, occurs before and after birth, respectively, but before TH-dependent remodeling at weaning (Sirakov and Plateroti, 2011). Signaling through TH receptors is required for proper morphological development in mice (Plateroti et al., 1999), but the ability to induce stem cell formation with exogenous TH in frogs provides a unique window and level of control into the developmental mechanisms of intestinal development not readily available in other models.

While most intestinal epithelial cells undergo apoptosis at metamorphosis, a subpopulation of proliferative epithelial cells lining the immature intestinal lumen undergo dedifferentiation

and redifferentiation upon receiving the TH signal (Hasebe et al., 2011; Ishizuya-Oka et al., 2009; Ishizuya-Oka et al., 2014; Ishizuya-Oka, 2005). Epithelial and mesenchymal interactions involving, hedgehog, bone morphogenic protein, and non-canonical wnt signaling are required to establish adult intestinal stem cell in their niche during development. Understanding of the origin of intestinal stem cells in frogs will help elucidate developmental mechanisms in humans and may provide avenues or methods to direct differentiation pathways towards intestinal stem cells from iPS cells.

Future prospects

Molecular mechanisms of gene regulation and developmental roles of hormones are complex involving transcriptional and epigenetic machinery that vary among cell types, hormone interactions at multiple levels, and cell–cell interactions (Fowden and Forhead, 2009; Fowden and Forhead, 2013; Hillman et al., 2012). Study of frog metamorphosis has contributed greatly towards understand this complexity (Brown and Cai, 2007; Gilbert et al., 1996; Grimaldi et al., 2013) and continued development of the frog tool kit, including transgenic animals and gene knockout technology, will promote future insights. Open questions include studying the role of non-genomic actions of TH and GCs in development, identifying how other hormone systems influence TH action in development, and determining the roles of epigenetic modifiers. These questions are currently being addressed by transgenic overexpression or nuclease-mediated knockout using gene knockout technology to target hormone production, hormone receptors, and epigenetic modifiers.

The roles of epigenetic modifications are of particular interest regarding the developmental origin of adult disease (Braun et al., 2013; Moisiadis and Matthews, 2014; Vickers, 2014). Environment-dependent hormone action, mediated especially by GCs, is likely to underlie the environmental influence on the epigenome responsible for developmental programming. The complex interactions between GCs and TH during development and the actions of these hormones on epigenetic modifications point to a value for frog metamorphosis. Indeed, developmental programming of GC physiology has been determined in *Xenopus* and the TH gene regulation cascade includes many epigenetic modifiers (Grimaldi et al., 2013; Hu et al., 2008). Further study of the endocrine basis of epigenetic signatures laid down during development is critical to understand the mechanisms of developmental programming.

Frogs are the most closely related animals to humans with aquatic, free-living embryos and larva. As such, frogs have been a useful testing ground for small molecule therapeutics and EDCs as an in-vivo developmental model (Fini et al., 2007; Préau et al., 2015; Wheeler and Liu, 2012). Humans are exposed to thousands of chemicals, not all of which have been tested for teratogenic or endocrine disruption potential (Colborn et al., 1993). A major arm of ensuring continued healthy human development is to derive basic knowledge concerning the effects of chemicals that we are exposed to during critical periods of human development. High and medium-throughput screening efforts will continue to identify EDCs and additional studies will elucidate their modes of action on vertebrate development (Hayes et al., 2010; Helbing et al., 2010; Turque et al., 2005).

Unlike mammals, frogs have the amazing ability to regenerate tail tips, hind limbs, and even transected spinal cords as tadpoles (Gibbs et al., 2011; King et al., 2012; Lee-Liu et al., 2014; Slack et al., 2008). Significantly, this regenerative ability is lost during metamorphosis. Loss of the ability to regenerate can be induced by treatment with TH, providing a model to pin point mechanisms associated with regeneration that may be applicable to mammals. Identifying the mechanistic basis of the TH-initiated blockade to regeneration is a fascinating future goal with implications for understanding the mammalian inability to regenerate body parts and for potential therapies in human spinal cord injury and limb amputation.

Conclusions

Frogs, fish, and mice all have unique features with respect to humans, and some of these unique features provide valuable advantages for use in understanding the human condition. As presented in this review, the molecular, endocrine, and developmental mechanisms of TH actions and hormone interactions during metamorphosis are unique strengths of the frog model to understand human perinatal development. Taking advantage of the frog model is valuable because of the vital and numerous developmental roles of TH and GCs for proper development in humans and because of the potential impact of endocrine disrupting chemicals on development. A pragmatic approach to elucidating the role of hormone action and interactions during the human perinatal period is to conduct exploratory studies in frogs. Then, the high degree of conservation in molecular and endocrine mechanisms during development makes it likely that many discoveries in frogs can be translated to mice and humans. In addition, developmental events, such as red blood cell switching, gene switching in liver, and skin development, are not hormone dependent in humans but common developmental mechanisms can be analyzed using TH-dependent development in frogs.

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