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MINI-REVIEW

Direct Development: An Alternative Way to Make a Frog

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Mammals are direct developers. They lack a morphologically distinct larval form like the butterfly's caterpillar or the frog's tadpole, and they lack the corresponding metamorphosis from the larva to the adult. It is a surprise to realize that many frogs lack a tadpole and are direct developers. An extreme example is the Puerto Rican tree frog, *Eleutherodactylus coqui.* Anyone who has been to Puerto Rico has heard their beautiful singing in the evening, but few are aware that *E. coqui* never goes in the water. It lays its eggs on land, where they are protected by the father until the embryos hatch from their jelly capsules as tiny frogs (Fig. 1). *E. coqui* is but one of 500 species in this genus, all of which are direct developers, and this mode of development is found in multiple frog lineages (Duellman and Trueb, 1986; Hanken, 1999).

Direct development in *E. coqui* is derived, which means that its ancestor had a tadpole. The tadpole was eliminated in evolution, although traces remain. The embryo has a tail, obviously not used for swimming but co-opted to provide a surface for gas exchange. Rudimentary gills form, as does an operculum, the flap of skin that covers the developing forelimbs in a tadpole (Callery and Elinson, 2000a). Despite these tadpole remnants, *E. coqui* embryos begin to look like frogs early in development (Fig. 2). The jaws and eyes appear adultlike from inception, and prominent limb buds arise shortly after neurulation.

E. coqui provides an opportunity to investigate direct development. Adults can be kept as a reproductive colony in the laboratory, and a good mating pair will produce a clutch of 30–40 embryos monthly (Elinson *et al.,* 1990). The fact that *E. coqui* lives in cities as well as the countryside probably accounts for its continued reproductive activity despite the noise and irregular lighting schedule in a lab. Using *E. coqui,* we can ask what developmental changes underlie the loss of the tadpole morphology.

Tadpole Morphology

The tadpole looks like no other vertebrate, either larva or adult, with its bulbous head and body attached to a tail (Fig. 3). The tadpole's jaws are unique, with keratinous teeth rather than neural crest-derived ones and two tadpole-specific jaw cartilages supporting the keratinous beak and teeth (Altig and McDiarmid, 1999). Meckel's cartilage, which elongates to form the basis for the lower jaw in frogs and other terrestrial vertebrates, remains short in tadpoles (Wassersug and Hoff, 1982; Cannatella, 1999). The tadpole is herbivorous, and most of the body is filled with a long coiled intestine. The stomach is not prominent (Viertel and Richter, 1999; Smith *et al.,* 2000), and the continuous feeding by the tadpole pushes the wastes through to the anus (Wassersug, 1997). Limbs are initiated late and grow slowly. The hind limbs are tucked behind the bulbous body, while the forelimbs are hidden from view by the operculum. The tadpole relies on its tail for swimming, since it lacks fins or functional limbs, and its short trunk allows little flexure, unlike the case in many fish, newts, snakes, otters, and other swimming vertebrates. Unlike tails in other vertebrates, the muscular tail of the tadpole lacks vertebral cartilages (Wassersug, 1989), and the notochord persists in both the trunk and tail.

Loss of Tadpole-Specific Structures

The removal of the tadpole from the life history of *E. coqui* involved many morphological changes (Fig. 3). These changes can be classified into two groups: loss of tadpole-specific structures and early development of adult structures. With respect to the former, *E. coqui* fails to form many tadpole-specific structures, including cement and hatching glands, lateral line organs, a long coiled intestine, suprarostral cartilages of the jaw, and tadpole teeth and scraping beak (Elinson, 1990). In tadpoles, the cement and hatching glands are transient and degenerate before feeding begins, while most of the intestine, the suprarostral cartilage, and the rest of the tadpole mouth degenerate in response to thyroid hormone. The lateral line also degenerates upon metamorphosis to a terrestrial frog but is retained in aquatic frogs, such as *Xenopus.*

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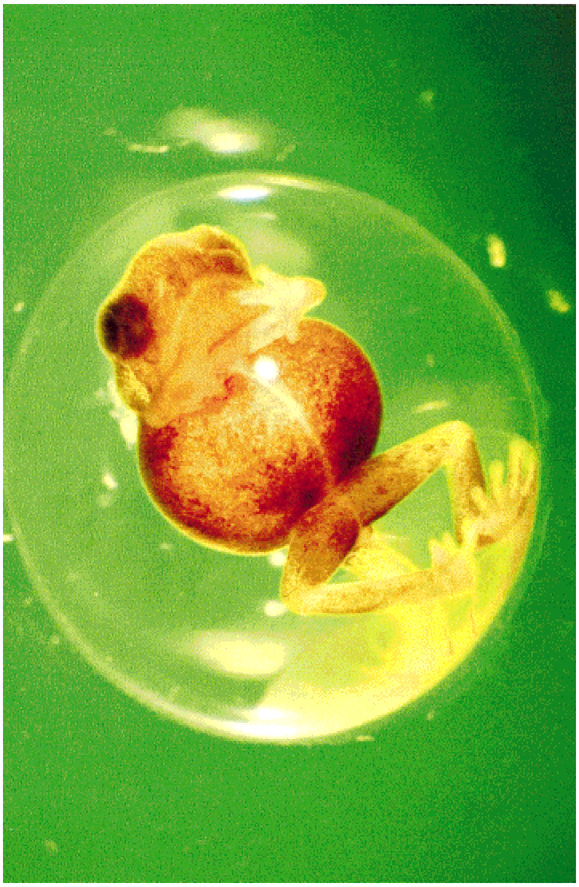

FIG. 1. *E. coqui* embryo developing within its fertilization membrane. The embryo (TS stage 13; Townsend and Stewart, 1985) is about 20 days old and measures 5 mm from snout to vent. It is clearly a frog.

The failure of the cement gland and the lateral line to form in *E. coqui* is due to a lack of response of the ectoderm to inducing signals. When prospective ectoderm from *Xenopus laevis* was transplanted to the prospective neural-epidermal boundary in *E. coqui* gastrulae, the signals in the *E. coqui* embryo were sufficient to cause cement gland development in the *X. laevis* transplant (Fang and Elinson, 1996). Transplantation of *E. coqui* ectoderm into the cement gland-forming region of *Rana pipiens* embryos failed to cause cement gland development in the *E. coqui* tissue. Similar results were obtained with reciprocal transplants between *E. coqui* and the *Ambystoma mexicanum* with respect to lateral line formation (Schlosser *et al.,* 1999). In addition to these embryological experiments, domains of *distalless* gene expression associated with the cement gland and of *NeuroD* expression associated with the lateral line do not appear in *E. coqui* (Fang and Elinson, 1996; Schlosser *et al.,* 1999). It is clear that the *E. coqui* prospective ectoderm differs in its response to some inducers, but the molecular nature of this difference is unknown.

Similarly, nothing is known about the developmental basis for the absence in *E. coqui* of the tadpole feeding apparatus—the specialized mouth, jaw, and intestine. The tadpole has a long gut for herbivory, the frog has a short gut for carnivory, and *E. coqui* has a short, yolk-filled gut. At metamorphosis, up to 90% of the length of the tadpole gut is lost, and a new adult gut epithelium forms (reviewed in Shi, 2000; Smith *et al.,* 2000). Whether the tadpole and frog epithelia are generated from the same cells or from different ones is not well resolved. Molecular differences between tadpole and adult guts likely exist, since their physiology is different. The discovery of gut molecular markers would be very useful both to follow the cellular changes of the gut at

FIG. 2. Comparison between embryos of *E. coqui,* a rat, and a chicken. The *E. coqui* embryo (center) has been removed from the large yolk mass and is about 14 days old (TS stage 9). It has large eyes, a froglike head, and developing limbs. The rat embryo (left) was removed from the amnion and the rest of the placenta and is at embryonic day 13. The chicken embryo (right) has been removed from its amnion and yolk and is four days old. The size and body proportions are clearly different between these three direct developers.

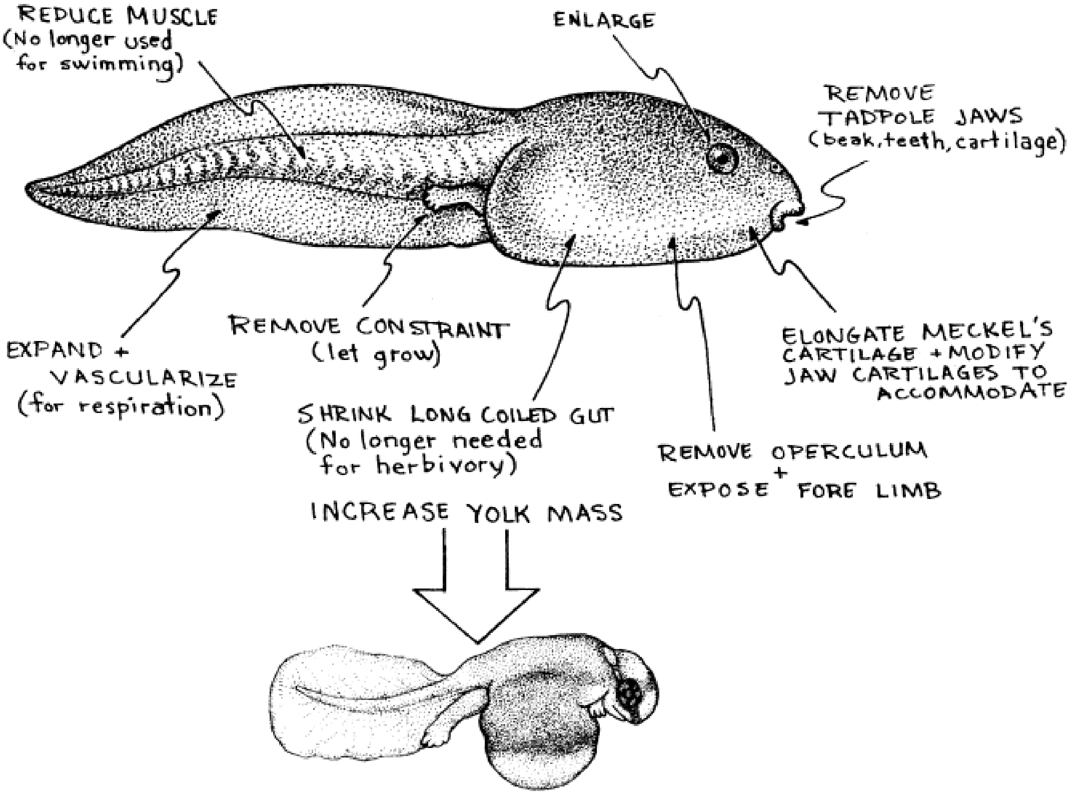

FIG. 3. Tadpole renovation. The major structural changes required to derive the *E. coqui* embryo from a tadpole are summarized in this architect's plan. Both the tadpole and the *E. coqui* TS10 embryo are prior to metamorphosis. Drawing by Mark A. Klingler.

metamorphosis and to determine the type of gut in *E. coqui*.

Early Development of Adult Morphology in *E. coqui*

Many adult structures form relatively late in frogs, as they depend on stimulation by thyroid hormone. For instance, Meckel's cartilage does not elongate in the tadpole, and another jaw cartilage, the palatoquadrate, assumes an unusual acute angle relative to the skull. At metamorphosis, thyroid hormone stimulates elongation and a restructuring of the jaw (Hanken and Summers, 1988; Huang *et al.,* 1999). In *E. coqui,* Meckel's and other jaw cartilages first appear around TS stage 8 (Hanken *et al.,* 1992), a few days before TS stage10 when thyroid hormone starts acting (Jennings and Hanken, 1998; Callery and Elinson, 2000b). Thus, the initial formation of the jaw cartilages joins directly with the thyroid hormone-dependent elongation and restructuring in *E. coqui*.

A second example of this joining of embryogenesis with thyroid-dependent metamorphosis is the fusion at the ventral midline of the rectus abdominis muscle, derived from the somites on the right and left sides. This fusion is delayed until metamorphosis, so tadpoles lack muscle on the ventral midline. In *E. coqui,* the gradual movement of the prospective rectus abdominis over the yolk mass joins seamlessly in time with its thyroid hormone-dependent fusion at the ventral midline (Elinson and Fang, 1998; Callery and Elinson, 2000b). The ventral fusion line appears as a thin, white stripe on the belly (Fig. 1). The relationship between events occurring during embryogenesis, tadpole life, and metamorphosis are best described by a heterochrony plot, and this plot indicates that there is a smooth joining of embryonic and metamorphic trajectories in *E. coqui* (Schlosser and Roth, 1997; Callery and Elinson, 2000a).

While this joining accounts for much of the early appearance of the frog morphology, there are other obvious accelerations, most notably the limbs (Figs. 2, 3). Large limb buds form shortly after neurulation in *E. coqui,* much earlier than in tadpole species. The subsequent growth and development of cartilage in *E. coqui* is not that different from metamorphic species, whose limbs elongate and have cartilages before the massive stimulation by thyroid hormone at metamorphosis (Elinson, 1994). The difference between *E. coqui* and a tadpole lies more in the initial recruitment of cells into the limb bud or their proliferation. In effect, the *E. coqui* pattern is more like a bird's or a mammal's (Fig. 2), with the tadpole having some kind of suppression of early limb development. The molecular or cellular basis of this suppression is unknown. Similarly the reversion of *E. coqui* to a pattern seen in amniotes could be due to the elimination of the tadpole suppressive mechanism or to the establishment of a pathway, circumventing the suppression. This is an open question.

Was There a Larva in the Tetrapod Lineage Leading to Mammals?

Since the tadpole has been deleted in direct development in frogs, we can ask whether a tetrapod ancestor once had a larval form that was deleted in the lineage leading to mammals to give direct development. This is a difficult question for which the fossil record provides no clue. It is very unlikely that there was a tadpole per se in the lineage leading to mammals. Frogs and mammals last shared a common ancestor about 350 million years (MY) ago, and frogs do not appear in the fossil record until about 210 MY ago. While tadpoles are primitive within the frog lineage, the tadpole could have evolved in the 140 MY between the last common ancestor of mammals and frogs and the origin of frogs. This is likely, since neither urodeles nor caecilians, the other extant orders of amphibians, have a tadpole.

Although the tadpole appears unique to anuran amphibians, there still could have been another larval form in the mammalian lineage, which was deleted. A potential source of data for the existence of a metamorphosing larva in the mammalian lineage is the utilization of thyroid hormone. Thyroid hormone is the key stimulator of metamorphosis of the tadpole to the frog. It is also important for the less extreme metamorphosis in urodele amphibians (Rose, 1999) and for some changes in teleost fish (Brown, 1997; de Jesus *et al.,* 1998). The movement of the eye and other morphological changes during metamorphosis of the flounder depends on thyroid hormone (Inui and Miwa, 1985; Miwa *et al.,* 1988). Paradoxically, metamorphosis of lamprey, a jawless fish, involves the depletion of thyroid hormone, opposite to the situation in teleosts and amphibians (Youson, 1997).

Although *E. coqui* no longer has a tadpole, there are several lines of evidence for a cryptic thyroid hormone-dependent metamorphosis. As described earlier, developmental events, which are widely separated in metamorphosing species, are juxtaposed in *E. coqui* (Schlosser and Roth, 1997; Callery and Elinson, 2000a,b; Callery *et al.,* 2001). The point of juxtaposition is around two-thirds of the way through development, the same time that the thyroid gland appears to become functional, based on its histology (Jennings and Hanken, 1998). The gene for thyroid hormone receptor β (TRβ) is up-regulated around this time (Callery and Elinson, 2000b), similar to the up-regulation of TRβ at the onset of metamorphosis of the *X. laevis* tadpole. Inhibition of thyroid hormone synthesis results in a developmental arrest, which can be reversed by exogenous thyroid hormone (Callery and Elinson, 2000b). These results support the idea that a tadpole cassette was removed from the life history of *E. coqui* (Elinson, 1990), with the point of removal marked by thyroid hormone-dependent events.

Since thyroid hormone events indicate where the tadpole was removed from *E. coqui* ontogeny, the question can be asked whether a similar mark is found in mammalian or amniote development. Thyroid hormone receptors (TRs) are expressed in several tissues of mouse fetuses and chicken embryos (Nagasawa *et al.,* 1997; Forrest *et al.,* 1990; Sjoberg *et al.,* 1992). Functional analysis of the TRs has been recently achieved by producing targeted mutations in mice, including null alleles of TRα and TRβ and an abnormal TRβ allele (Göthe *et al.,* 1999; Gauthier *et al.,* 1999; Kaneshige *et al.,* 2000). Growth and bone and intestinal development were retarded, and the pituitary-thyroid axis was abnormal. The mice were viable, however, with no obvious peculiarities of specific organs. Coupled with observations of mammalian development with reduced thyroid hormone levels (hypothyroidism) (see Göthe *et al.,* 1999), thyroid hormone is not as important for the completion of development in a mammal as it is for *E. coqui*.

The relative lack of importance of thyroid hormone for mammalian development may suggest that there never was a larval form with thyroid-dependent metamorphosis in the lineage leading to mammals. On the other hand, such a thyroid dependency may have dissipated over millions of years. The question of this larval form may not be answerable at present; nevertheless, the existence of direct development in frogs alerts us to developmental and evolutionary histories that we may not otherwise have considered.

LITERATURE CITED

Altig R, McDiarmid RW. 1999. Body plan, development and morphology. In: McDiarmid RW, Altig R, editors. Tadpoles, the biology of anuran larvae. Chicago: The University of Chicago Press. p 24–51.

Brown DD. 1997. The role of thyroid hormone in zebrafish and axolotl development. Proc Natl Acad Sci USA 94:13011–13016.

Callery EM, Elinson RP. 2000a. Opercular development and ontogenetic re-organization in a direct developing frog. Dev Genes Evol 210:377–381.

Callery EM, Elinson RP. 2000b. Thyroid hormone-dependent metamorphosis in a direct developing frog. Proc Natl Acad Sci USA 97: 2615–2620.

Callery EM, Fang H, Elinson RP. 2001. Frogs without polliwogs: Evolution of anuran direct development. BioEssays (in press).

Cannatella D. 1999. Architecture, cranial and axial musculoskeleton. In: McDiarmid RW, Altig R, editors. Tadpoles, the biology of anuran larvae. Chicago: The University of Chicago Press. p 52–91.

de Jesus EG, Toledo JD, Simpas MS. 1998. Thyroid hormones promote early metamorphosis in grouper *(Epinephelus coioides)* larvae. Gen Comp Endocrinol 112:10–16.

Duellman WE, Trueb L. 1986. Biology of amphibians. New York: McGraw-Hill Book Co.

Elinson RP. 1990. Direct development in frogs: Wiping the recapitulationist slate clean. Sem Dev Biol 1:263–270.

Elinson RP. 1994. Leg development in a frog without a tadpole *(Eleutherodactylus coqui).* J Exp Zool 270:202–210.

Elinson RP, Fang H. 1998. Secondary coverage of the yolk sac by the body wall in the direct developing frog, *Eleutherodactylus coqui:* An unusual process for amphibian embryos. Dev Genes Evol 208:457–466.

Elinson RP, del Pino EM, Townsend DS, Cuesta FC, Eichhorn P. 1990.

A practical guide to the developmental biology of terrestrial-breeding frogs. Biol Bull 179:163–177.

Fang H, Elinson RP. 1996. Patterns of distal-less gene expression and inductive interactions in the head of the direct developing frog *Eleutherodactylus coqui.* Dev Biol 179:160–172.

Forrest D, Sjoberg M, Vennström B. 1990. Contrasting developmental and tissue-specific expression of alpha and beta thyroid hormone receptor genes. EMBO J 9:1519–1528.

Gauthier K, Chassande O, Plateroti M, Roux J-P, Legrand C, Pain B, Rousset B, Weiss R, Trouillas J, Samarut J. 1999. Different functions for the thyroid hormone receptors TRα and TRβ in the control of thyroid hormone production and post-natal development. EMBO J 18:623–631.

Göthe S, Wang Z, Ng L, Kindblom JM, Campos Barros A, Ohlsson C, Vennström B, Forrest D. 1999. Mice devoid of all known thyroid hormone receptors are viable but exhibit disorders of the pituitary-thyroid axis, growth, and bone maturation. Genes Dev 13: 1329–1341.

Hanken J. 1999. Larvae in amphibian development and evolution. In: Hall BK, Wake MH, editors. The origin and evolution of larval forms. New York: Academic Press. p 61–108.

Hanken J, Summers CH. 1988. Skull development during anuran metamorphosis: III. Role of thyroid hormone in chondrogenesis. J Exp Zool 246:156–170.

Hanken J, Klymkowsky MW, Summers CH, Seufert DW, Ingebrigtsen N. 1992. Cranial ontogeny in the direct-developing frog, *Eleutherodactylus coqui* (Anura: Leptodactylidae), analyzed using whole-mount immunohistochemistry. J Morph 211:95–118.

Huang H, Marsh-Armstrong N, Brown DD. 1999. Metamorphosis is inhibited in transgenic *Xenopus laevis* tadpoles that overexpress type III deiodinase. Proc Natl Acad Sci USA 96:962–967.

Inui Y, Miwa S. 1985. Thyroid hormone induces metamorphosis of flounder larvae. Gen Comp Endocrinol 60:450–454.

Jennings DH, Hanken J. 1998. Mechanistic basis of life history evolution in anuran amphibians: thyroid gland development in the direct-developing frog, *Eleutherodactylus coqui.* Gen Comp Endocrinol 111:225–232.

Kaneshige M, Kaneshige K, Zhu X, Dace A, Garrett L, Carter TA, Kazlauskaite R, Pankratz DG, Wynshaw-Boris A, Refetoff S, Weintrub B, Willingham MC, Barlow C, Cheng S. 2000. Mice with a targeted mutation in the thyroid hormone beta receptor gene exhibit impaired growth and resistance to thyroid hormone. Proc Natl Acad Sci USA 97:13209–13214.

Nagasawa T, Suzuki S, Takeda T, DeGroot LJ. 1997. Thyroid hormone receptor β1 expression in developing mouse limbs and face. Endocrinology 138:1276–1281.

Rose CS. 1999. Hormonal control in larval development and evolution—Amphibians. In: Hall BK, Wake MH, editors. The origin and evolution of larval forms. New York: Academic Press. p 167–216.

Schlosser G, Roth G. 1997. Evolution of nerve development in frogs. II. Modified development of the peripheral nervous system in the direct-developing frog *Eleutherodactylus coqui* (Leptodactylidae). Brain Behav Evol 50:94–128.

Schlosser G, Kintner C, Northcutt RG. 1999. Loss of ectodermal competence for lateral line placode formation in the direct developing frog *Eleutherodactylus coqui.* Dev Biol 213:354–369.

Shi YB. 2000. Amphibian metamorphosis. New York: Wiley-Liss.

Sjoberg M, Vennström B, Forrest D. 1992. Thyroid hormone receptors in chick retinal development: differential expression of mRNA for alpha and N-terminal variant beta receptors. Development 114: 39–47.

Smith DM, Grasty RC, Theodosiou NA, Tabin CJ, Nascone-Yoder NM. 2000. Evolutionary relationships between amphibian, avian, and mammalian stomachs. Evol Dev 2:348–359.

Townsend DS, Stewart MM. 1985. Direct development in Eleutherodactylus coqui (Anura: Leptodactylidae): A staging table. Copeia 1985:423–436.

Viertel B, Richter S. 1999. Anatomy, viscera and endocrines. In: McDiarmid RW, Altig R, editors. Tadpoles, the biology of anuran larvae. Chicago: The University of Chicago Press. p 92–148.

Wassersug RJ. 1989. Locomotion in amphibian larvae. Amer Zool 29:65–84.

Wassersug RJ. 1997. Where the tadpole meets the world. Amer Zool 37:124–136.

Wassersug RJ, Hoff K. 1982. Developmental changes in the orientation of the anuran jaw suspension. Evol Biol 15:223–246.

Youson JH. 1997. Is lamprey metamorphosis regulated by thyroid hormones? Amer Zool 37:439–460.