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REVIEWS

Evolutionary Reorganizations of Ontogenesis in Sea Urchins

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Abstract—The data published during recent 15–20 years on comparative, experimental and molecular embryology of unusually developing sea urchins have been reviewed. These animals are characterized by large lipid-rich eggs, highly modified embryogenesis, and the absence of a planktotrophic larva. Such a type of development is evolutionary advanced and arose independently in various phylogenetic lineages of the sea urchins.

Key words: sea urchins, evolution of development, *Heliocidaris, Holopneustes, Strongylocentrotus*.

Class Echinoidea includes about 1000 extant species of sea urchins. Some of them, above all *Stronglyocentrotus purpuratus*, as well as *S. droebachiensis, Arbacia punctulata, Lytechinus pictus, Paracentrotus lividus, Psammechinus miliaris* and others, are classical species for morphological and experimental studies of early ontogenesis of multicellular animals (Buznikov and Podmarev, 1975, 1990; Ivanova-Kazas, 1978; Davidson *et al.,* 1982, 1998; Arenas-Mena *et al.,* 1998, 2000; Biermann *et al.,* 2003). These sea urchins, as well as most other Echinoidea, have eggs with a diameter of up to 100–150 μm and comparatively poor in yolk. Soon after gastrulation, the planktotrophic larva pluteus is formed, which has, in most cases, up to four–six pairs of long processes (arms). Pluteus lives in plankton for several weeks and then undergoes metamorphosis, as a result of which a juvenile sea urchin is formed. This type of development is considered to be primitive for the class Echinoidea (Strathmann, 1978, 2000; Kas'yanov, 1989; Ivanova-Kazas, 1992).

However, in about 20% of sea urchins, which belong to several different orders, development proceeds in a different way: the development of pluteus is fully or partially suppressed and an adult organism is formed at a higher rate. These animals occur predominantly in sea waters of the Southern hemisphere (Hyman, 1955; Kas'yanov *et al.,* 1983; Raff, 1987) and they have large eggs, 300 to 2000 μm in diameter, rich in stored nutrients. Modifications of development include the formation of facultatively planktotrophic plutei (Strathmann, 1979; Hart, 1996) and nonplanktotrophic (lecithotrophic) plutei (Okazaki and Dan, 1954; Olson *et al.,* 1993; Amemiya and Arakawa, 1996) or armless lecithotrophic larvae (Parks *et al.,* 1989; Amemiya and Emlet, 1992; Morris, 1995). Finally, the pelagic larval stage can be absent and brooding is observed (Poulin and Feral, 1996; Schatt and Feral, 1996), when the embryo develops at a slower rate.

The authors of reviews on evolutionary embryology of sea urchins (see, for example, Raff, 1987; Emlet, 1995a) proposed that reorganizations of ontogenesis, including the formation of lecithotrophic larvae or brooding, proceeded independently in different phylogenetic lineages. The types of development were described for more than 200 sea urchins (see table). The discussion of controversial problems of taxonomy of the Echinoidea, such as possible joining of orders Echinoida and Temnopleura in the order Camarodonta, is beyond the scope of this paper. The results of novel studies (Jeffery and Emlet, 2003; Jeffery *et al.,* 2003) suggest that evolutionary transitions from an ancestral planktotrophic modus of larval development to the lecithotrophic one appear to be irreversible.

Among sea urchins with lecithotrophic larvae, the endemic Australian species *Heliocidaris erythrogramma* (order Echinoida, family Echinometridae)

Different types of development in different orders of sea urchins

Subclasses, orders*	Types of development within the order in different species		
Cidaroidea			
Cidaroida	P	L	B
Euechinoidea			
Echinothurioida		L	
Diadematoida	P		
Cassiduloida	P		B
Clypeasteroida	P	L	B
Spatangoida	P	L	B
Arbacoida	P		
Temnopleuroida	P	L	B
Echinoida	P	L	B

* Types of development within the order in different species

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became the most extensively studied. Its development is characterized by the full absence of pluteus stage and was first described by Mortensen (1915) and later by Williams and Anderson (1975). Note that the studies of embryogenesis of *H. erythrogramma* were stimulated by the review paper of Raff (1987), in which the attention was attracted to the importance of using model systems comprising two (or more) closely related echinoderm species characterized by different types (modes) of development. The genus *Heliocidaris* consists only of two species whose ranges are overlapped on eastern coast of Australia in the region of Sydney and the second species *H. tuberculata* is characterized by the usual type of development with planktotrophic larva. The molecular phylogeny data (analysis of 18S rRNA and mitochondrial DNAs) suggest that these two representatives of the genus *Heliocidaris* diverged from each other 4 to 10 million years ago (Smith *et al.,* 1990; McMillan *et al.,* 1992) and from *Strongylocentrotus purpuratus* (order Echinoida, family Strongylocentrotidae) 30 to 40 million years ago (Smith, 1988; Littlewood and Smith,1995). Therefore specific features of development of the latter species are often compared to those of *H. erythrogramma* (Parks *et al.,* 1988; Kissinger and Raff, 1998; Raff, 1999). Note also that the *Strongylocentrotus* species are sometimes (Littlewood and Smith, 1995) included in the family Echinometridae, but the discussion of this problem is beyond the task of our paper.

Mature eggs of *H. erythrogramma*, 400 to 450 μm in diameter, are opaque (the nucleus, up to 100 μm in diameter, is located in the center of the egg) and float on the water surface (Williams and Anderson, 1975). The volume of mature eggs exceeds those of *H. tuberculata* and *S. puprpuratus* approximately 100- to 150-fold (the egg diameters in the latter are 90–95 and 80 μm, respectively).

Studies of oogenesis have shown similar patterns and levels of vitellogenic gene expression in *H. erythrogramma* and *H. tuberculata* (Byrne *et al.,* 1999). Moreover, vitellogenesis proceeds in both species in a similar way: yolk stores are rather small. However, *H. erythrogramma* has an additional, terminal phase of oogenesis which is characterized by mass storage of nonvitellogenic material, including accessory maternal protein and lipid uniformly distributed over the oocyte. Hypertrophy of egg size is achieved at the expense of this comparatively recent (no more than a few million years ago) evolutionary change.

Analysis of the composition of lipids in mature eggs of various sea urchins (Villinski *et al.,* 2002) has shown the predominance of triglycerides in the eggs of *H. tuberculata* and *S. purpuratus* and two other species with planktotrophic larvae (*A. punctulata* and *Eucidaris tribuloides*) (55–95% of total mass), while wax esters predominate (74–78% of total mass) in the eggs of *H. erythrogramma* and another species with lecithotrophic armless larva *Holopneustes purpurescens* (order Temnopleuroida, family Temnolpeuridae). However, the results of experiments on removal of lipids (up to 50% of egg dry mass) from the early embryos of *H. erythrogramma* (Emlet and Hoegh-Guldberg, 1997; Hoegh-Guldberg and Emlet, 1997) suggest that the major part of stored organic substances is not required for larval development, but is used for feeding of juvenile sea urchin after larval settlement onto the bottom.

The results of experiments on analysis of differentiation of the blastomeres isolated from the early embryos of *H. erythrogramma*, on tracing the fate of cell lineages after microinjection of fluorescent dyes, and on fertilization of deformed eggs elongated in narrow silicon tubes suggest that in this sea urchin, the dorsoventral axis is specified before the 1st cleavage division (Henry and Raff, 1990; Henry *et al.,* 1990). Of course, similar experiments and experiments with centrifugation and treatment by various poisons were carried out on eggs and early embryos of Echinoidea with the usual planktotrophic type of development (for reviews see Lindahl, 1942; Davidson *et al.,* 1998). Note, however, that the term "oral-aboral embryonic axis" has been recently used more frequently with reference to these sea urchins than the term "dorsoventral axis" (Davidson *et al.,* 1998; Coffman and Davidson, 2001; Gross *et al.,* 2003), since the former, unlike in sea urchins that lost the pluteus stage, does not coincide with the adult dorsoventral axis. Representatives of the orders Echinoida (Hörstadius, 1938; Kominami, 1988; Kominami and Takata, 2003), Arbacoida (Lindahl, 1932a, 1932b), and Clypeasteroida (Pease, 1939, 1941) were earlier studied. As a rule, the oral-aboral embryonic axis is specified in sea urchins with planktotrophic larvae at the 4–32- cell stage and certain variations in the stage of axis specification (from 4- to 32-cell stage) are possible even in different species of the order Echinoida. Note that in *Peronella japonica* (order Clypeasteroida) with a lecithotrophic pluteus, the oral-aboral axis appears to be specified also only after the 4-cell stage (Okazaki and Dan, 1954).

Henry and Raff (1990) explain the earlier specification of the dorsoventral axis in development of *H. erythrogramma*, as compared to other sea urchins, by evolutionary changes in localization of certain cytoplasmic determinants in very large eggs of this species, which has also an additional phase of oogenesis. It has been proposed that in *P. japonica*, the pattern of development underwent changes independently of that *H. erythrogramma* and these changes are less pronounced (Amemiya and Arakawa, 1996). Unfortunately, there are no experimental data on specification of the dorsoventral axis in other Echinoidea species with lecithotrophic type of development.

The cleavage of *H. erythrogramma* is complete, equal, radial (Wray and Raff, 1989, 1990) and differs morphologically from that in *S. purpuratus, H. tuberculata* and other sea urchins with planktotrophic type of development. For example, four small micromeres are

formed at the vegetal pole of 16-cell embryo in these representatives of Echinoidea, which are characterized by a slower division rate and give rise to the lineage of primary mesenchymal cells migrating in the blastocoel shortly before the beginning of gastrulation. The primary mesenchymal cells form syncytium and secrete calcareous larval skeleton. The micromeres give also rise to another cell lineage that forms coelomic sacs lying on the sides of larval intestine (Pehrson and Cohen, 1986; Davidson, 1989; Ransick *et al.,* 1996). However, micromeres are not formed in the early embryos of *H. erythrogramma*. Skeletogenic mesenchymal cells and cells of coelomic mesoderm develop predominantly from the ventral vegetal blastomeres and, to a lesser extent, from the dorsal vegetal blastomeres (Wray and Raff, 1989, 1990; Raff, 1999). Thus, reorganizations of the early embryogenesis in this sea urchin include evolutionary modifications of cell lineages.

The period of synchronous cleavage in *H. erythrogramma* comprises seven equal divisions with the minimal cell cycle duration of about 15–30 min at 22–25°C (Williams and Anderson, 1975). The midblastula stage is achieved in this species, like in *H. tuberculata*, approximately within 8 h after fertilization (Parks *et al.,* 1988). However, the embryo of the former species at this stage consists of approximately 3500 cells (11–12 divisions after fertilization), while the embryo of the latter species has only 500 cells (9 divisions). There is a small blastocoel in the midblastula of *H. erythrogramma*, but by the late blastula stage (13–15 h after fertilization) the embryo is segregated into a transparent peripheral blastoderm, ca. 50 μm thick, and opaque central lipid mass, 300 to 350 μm in diameter (Williams and Anderson, 1975).

The stage of early/middle gastrula is achieved in both *Heliocidaris* species within approximately 18 h after fertilization (Parks *et al.,* 1988). In *H. erythrogramma* and *H. tuberculata*, the embryo consists of 9000–13000 cells (13–14 divisions) and 1000 cells (10 divisions), respectively. Thus, it can be calculated that the cell cycle length in the interval between midblastula and midgastrula amounts to about 10 and 5 h in *H. tuberculata* and *H. erythrogramma*, respectively. Hence, under the same laboratory conditions, cell proliferation in embryogenesis of *H. erythrogramma* proceeds at a higher rate than in *H. tuberculata*. It would be interesting to compare changes in cell cycle during early development of both species using autoradiography and flow cytophotometry and determination of mitotic index, which were successfully used in embryological studies of sea urchins with the usual type of development (Agrell, 1954; Andreeva, 1970; Andreeva *et al.,* 1989).

It is interesting that the gastrula of *H. erythrogramma* contains 1700–2200 mesenchymal cells or approximately 17–19% of the total number of cells in the embryo, which actively proliferate and penetrate in the inner lipid mass, while only 30–60 primary mesenchymal cells (3–6% of the total number of cells), which have already ceased to divide, are located in the blastocoel of *H. tuberculata* blastula and gastrula (Parks *et al.,* 1988). The mesenchymal cells of these two species differ also in the expression the gene *msp130* specific for this cell type (Klueg *et al.,* 1997). Its expression in the primary mesenchyme of *H. tuberculata*, just as in *S. purpuratus*, begins at the late blastula stage and larval skeleton is actively formed at the early stages of gastrulation, while in the mesenchyme of *H. erythrogramma*, its expression is first detected only after gastrulation, which is followed by predominant secretion of skeletal material of the juvenile sea urchin, rather than of larval skeleton.

Gastrulation in *H. erythrogramma* is also characterized by the formation of a very short archenteron via invagination at the vegetal pole and this process comprises unusual helical movements of cells (Wray and Raff, 1991). Hatching from the embryonic membranes and transition to a very short larval period (3 to 3.5 days, i.e., approximately 10 to 15 times less than in *H. tuberculata* and *S. purpuratus*) take place at the early gastrula stage (Williams and Anderson, 1975). Two coelomic sacs are "pinched" from the archenteron at a high rate and the left (larger) coelom gives rise to the hydrocoel. No mouth and functioning intestine are formed in the larva; the oral and aboral ectoderm and larval arms are fully absent. At this stage, the larva is characterized by bilateral symmetry, a band of cilia, and strongly reduced larval skeleton (Emlet, 1995b). Thus, the lecithotrophic larva of *H. erythrogramma* is significantly simplified as compared to the usual planktotrophic plutei but it should be considered a strongly modified evolutionarily new form, rather than a degenerative armless pluteus (Wray and Raff, 1990).

This was confirmed by molecular-genetic studies of the larval ectoderm in the *Heliocidaris* species (Haag and Raff, 1998; Haag *et al.,* 1999). Expression of the gene *HeET-1* was detected only in *H. erythrogramma* and not in *H. tuberculata*. This gene encodes an evolutionarily new extracellular protein apextrin, which is in tight association with the plasma membrane and appears to be essential for adhesion of the apical ectodermal cells. The appearance of this protein may be considered a peculiar adaptation related to comparatively large embryos and larvae of *H. erythrogramma*. Expression of the *Wnt* genes (encoding proteins of intercellular signaling) was rather similar in both *H. erythrogramma* and *H. tuberculata* (Ferkowicz *et al.,* 1998; Ferkowicz and Raff, 2001). On the contrary, significant qualitative differences were described in expression of the actin genes in the embryos and larvae of these two species (Kissinger and Raff, 1998). In both *Heliocidaris* species, the number of actin genes expressing during early ontogenesis was reduced, as compared to *S. purpuratus*. In *H. erythrogramma*, the *CyIII* expression is lost, which correlates with the loss

of aboral ectoderm, while in *H. tuberculata*, the *CyII* expression is lost.

Hence, I would agree with Emlet (1995b) that the term "direct development" with reference to *H. erythrogramma* used by Raff and his coauthors (Raff, 1987, 1992; Parks *et al.,* 1988) is misleading, since direct development implies the full loss of larval features. Nevertheless, this term is still used in descriptions of the development of sea urchins with lecithotrophic larvae (Hano *et al.,* 2001; Raff *et al.,* 2003).

Let us remember the classical experiments with the use of the vegetalizing agent LiCl, which induced exogastrulation and enhanced differentiation of mesenchymal cells in sea urchins with the standard type of development (Runnström, 1928; Brachet, 1961). It has recently been shown that LiCl exerted a similar effect on the early embryos of *H. erythrogramma* (Kauffman and Raff, 2003).

The development of juvenile *H. erythrogramma* (Williams and Anderson, 1975; Parks *et al.,* 1988; Minsuk and Raff, 2002) is morphologically similar to that in Echinoidea with the standard type of ontogenesis, but it is not preceded by long-term larval period and proceeds at very high rate. This process begins directly after the completion of gastrulation (24–25 h after fertilization) as a result of interaction of the left coelom and vestibular ectoderm and lasts approximately three days. By this time (approximately within four days after fertilization), an armless lecithotrophic larva settles onto the bottom. The juvenile sea urchin is also a lecithotroph for at least three weeks after settlement and begins active feeding only after this period.

Some specific features of the juvenile sea urchin development have been recently described in species with different modes of development using molecular-genetic methods (Nielsen *et al.,* 2003; Morris *et al.,* 2004). Expression of the gene *Orthodenticle (Otx)*, which plays an important role in neurogenesis of insects and chordates, is quite distinct in differentiating rudiments of sea urchins *H. erythrogramma, Holopneustes purpurescens*, and two other species with lecithotrophic type of development, *Asthenosoma ijimai* (order Echinothurioida) and *Phyllacanthus parvispinus* (order Cidaroida). Expression of this gene is absent in developing rudiments of sea urchins *H. tuberculata, S. purpuratus*, and other species with the usual type of development (Lowe and Wray, 1997; Nielsen *et al.,* 2003).

Let us consider the data on development of the hybrids between sea urchins with different modes of development (Raff *et al.,* 1999, 2003). When the *H. tuberculata* eggs are fertilized by *H. erythrogramma* sperm, lethal hybrids are formed, whose development is arrested at the gastrula stage. The experiments with reciprocal hybridization proved to be more interesting. The development of *H. erythrogramma* eggs fertilized by *H. tuberculata* sperm proceeds according to the maternal type until the end of gastrulation. However, further development leading to the formation of a juvenile sea urchin within approximately seven days after fertilization differs from those in both parental species. As a result of the paternal genome expression, some larval structures are restored that were lost in the course of evolution of the maternal species. Specifically, the hybrid has a mouth, larval intestine (possibly, functioning), and anus, although no return to the pluteus takes place. Raff *et al.* (1999) believe that this hybrid larva somewhat resembles the bilateral symmetrical dipleurula, which is considered to be an ancestral larval form in the type Echinodermata.

Similar hybrid larvae were obtained when the *H. erythrogramma* eggs were fertilized by the sperm of *Pseudoboletia maculata* (order Echinoida, family Toxopneustidae) (Raff *et al.,* 2003), the sea urchin with planktotrophic type of development. Note that these two species diverged ca. 40 million years ago, rather than 4–10 million years, like *H. erythrogramma* and *H. tuberculata*. Hence, a conclusion was drawn on significant conservatism of the molecular mechanisms of ontogenesis in sea urchins with the usual mode of development. As a results of hybridization of *H. erythrogramma* and *Holopneustes purpurescens* (two species with lecithotrophic type of development that diverged approximately 70 million years ago), characteristic armless lecithotrophic larvae were obtained and no restoration of any usual planktotrophic larva features was observed. These data support the hypothesis on parallel evolution of lecithotrophic development in two phylogenetic lineages. This hypothesis is also supported by another group of authors (Jeffery *et al.,* 2003), who proposed that *Holopneustes purpurescens* diverged from the related sea urchins with planktotrophic type of development approximately 4 to 7 million years ago. It is desirable that so far rare morphological and molecular studies of development of the Australian species *Holopneustes purpurescens* be continued (Morris, 1995; Morris *et al.,* 1997, 2002, 2004).

Finally, Raff *et al.* (2003) support the concept of "punctuated evolution" of embryos elaborated by this group of researchers (Wray and Raff, 1991; Wray, 1995). Indeed, the main features of early ontogenesis of sea urchins with the usual type of development were formed no less than 250 million years ago, i.e., before the divergence of the subclasses Cidaroidea and Euechinoidea. Since that time, relatively fast reorganizations of development could take place in different phylogenetic lineages after long-term (tens or more million years) periods of stable existence. The evolution of ontogenesis within the genus *Heliocidaris*, which was completed within several million years, appears to be a typical example. It was proposed that the complexes of genes controlling the development of Echinoidea are also evolved in a punctuated way; they are sharply changed during short periods of fast morphological evolution, but are relatively stable during the long-term periods of slow morphological evolution

(Raff *et al.,* 2003). Since the evolutionary concept of "punctuated equilibrium" is not generally accepted (see, for example, Grant, 1985), the corresponding reasoning of Raff and coauthors is better be regarded as open to discussion. It would be desirable to obtain detailed embryological and molecular data not only for *H. erythrogramma* and *Holopneustes purpurescens*, but also for at least a few other sea urchins with lecithotrophic armless larvae.

It would be of great interest to compare the above concepts of patterns of evolutionary reorganizations of early ontogenesis in sea urchins with similar theories on embryology of other animals. Certain advances in studying evolutionary reorganization of the mode of development (revealed in studies of related species) were achieved for ascidians (Jeffery, 1997; Jeffery *et al.,* 1999), starfishes (Hart *et al.,* 1997; Cerra and Byrne, 2004), anuran amphibians (Callery *et al.,* 2001; Desnitskiy, 2004), insects (Tikhomirova, 1991; Ivanova-Kazas, 1997), etc. The protistan genus *Volvox* is also a promising model in this respect (Desnitskiy, 1991, 1995).There are grounds to believe that each of these groups has specific features of evolutionary reorganizations of ontogenesis. It would also be interesting to reveal the general patterns of ontogenetic evolution of multicellular organisms and, in particular, test the concept of "punctuated evolution" of embryos. Such an analysis could have been done in a separate paper.

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