
REVIEWS

Evolutionary Reorganizations of Ontogenesis in Ascidians of the Genus *Molgula*

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Abstract—The data on comparative, experimental, and molecular embryology of ascidians (genus *Molgula*) published during the last 15 years have been reviewed. Some representatives of this genus evolved from development with a tailed larva (tadpole) to direct development associated with the loss of larval structures, such as tail, notochord, sensory organs, and differentiated muscles. The data on evolutionary reorganizations of ontogenesis in ascidians of the genus *Molgula* have been compared with those in sea urchins, anuran amphibians, and some other organisms.

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The class Ascidiacea, one of the most primitive in the phylum Chordata, includes more than 2000 species. These marine animals are widely used in embryological studies during the entire century and their development has been studied well enough in both morphological and experimental aspects (see, for example, Conklin, 1905; Jeffery *et al.*, 1984; Satoh, 1987, 1999; Whittaker, 1987; Dehal *et al.*, 2002; Lemaire *et al.*, 2002). Ascidiaceans have also been recently used for molecular-phylogenetic studies (Wada *et al.*, 1992; Wada, 1998; Swalla *et al.*, 2000; Yokobori *et al.*, 2003). Studies of the mechanisms underlying the evolution of ontogenesis in ascidians may shed light on the origin of more complex developmental patterns in vertebrates.

Ciona intestinalis and *C. savignyi* (order Enterogona, family Cionidae), *Halocynthia roretzi* (order Pleurogona, family Pyrosidae), *Styela partita*, and *S. plicata* (order Pleurogona, family Styelidae) are among the most characteristic representatives of the class Ascidiacea. In ontogenesis of these and most other species from both orders of ascidians, the larval tadpole stage proceeds soon after the completion of gastrulation. At this stage, epidermal, nervous, endodermal, notochordal, muscle, and mesenchymal cells are distinguished. Note that the corresponding cell lineages are segregated already at the early cleavage stages (Ivanova-Kazas, 1978; Nishida and Satoh, 1983; Swalla, 1992; Satoh, 1999). The total number of larval cells amounts to about 2500. The larva is differentiated into the head (trunk) region containing the brain rudiment with sensory organs and tail containing notochord (ca. 40 cells), neural tube (ca. 380 cells), and muscles (ca. 40 cells). The tadpole hatches, swims, enhancing the species distribution (but not feeds), and then fixes on the substrate. Thereafter, an adult sedentary ascidian

is formed as a result of regressive metamorphosis including tail resorption.

However, in a few ascidians, such as some species from the families Molgulidae (order Pleurogona) and Styelidae, ontogenesis proceeds in a different way (Berrill, 1931; Ivanova-Kazas, 1978; Whittaker, 1979). In ontogenesis of these animals, comprising less than 1% of the total number of species in the class Ascidiacea, the tadpole stage is replaced by an immobile tailless stage, which is called tailless embryo or tailless larva and is characterized by the absence of not only tail structures, but also larval sensory organs. The tailless larva hatches, fixes to the substrate in the direct vicinity of the parental individual, and metamorphoses into an adult animal. Hence, in this case we can speak about direct development. The species with different types of ontogenesis are often called tailed and tailless, respectively, which is in a way incorrect, since the stage of tailed larva in ascidians is very short-term: from several hours to several days. The results of experimental studies (Hadfield *et al.*, 1995; Jeffery *et al.*, 1999) confirm the suggestion (Berrill, 1931) that the tailless development of the Ascidiacea evolved independently several times from ancestors characterized by tailed development and stage of swimming tadpole.

TWO CLOSELY RELATED SPECIES OF ASCIDIANS WITH DIFFERENT TYPES OF DEVELOPMENT

Studies of embryology of the ascidians with altered type of development began in the early 1990s (Swalla and Jeffery, 1990; Jeffery and Swalla, 1991, 1992). In order to study the evolutionary transition from one type of development to another, those authors used a model

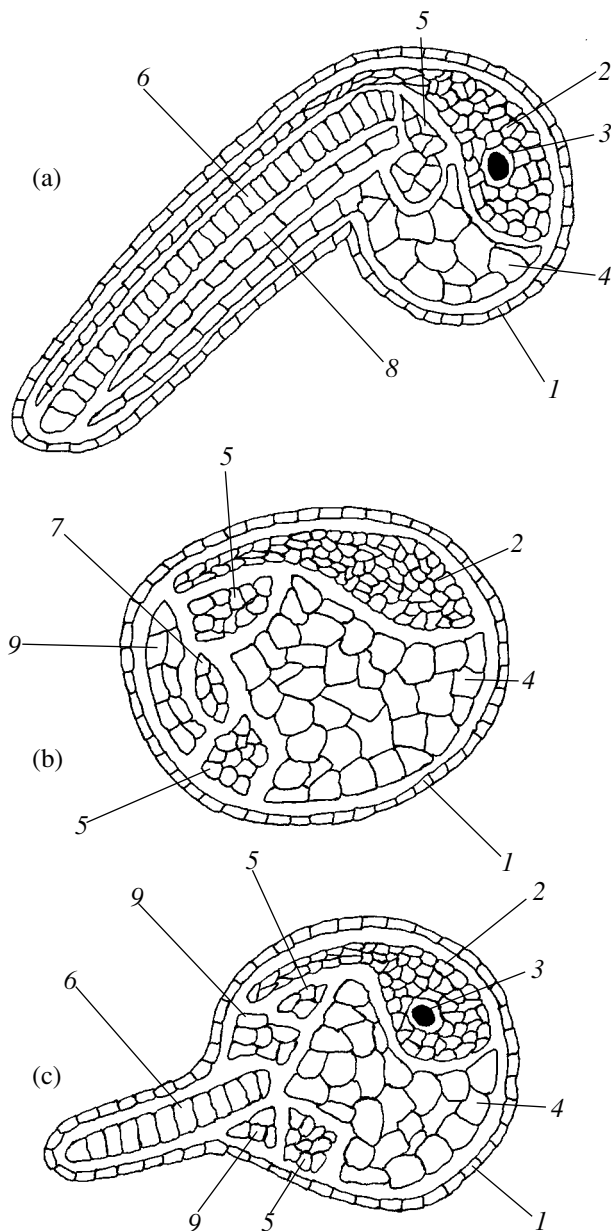


Fig. 1. Larvae of ascidians of the genus *Molgula*: (a) a tadpole of *M. oculata* (species with tailed development); (b) a larva of *M. occulta* (species with tailless development); (c) a hybrid larva with a short tail obtained as a result of fertilization of *M. occulta* egg by *M. oculata* spermatozoon. 1, epidermis; 2, central nervous system; 3, otolith; 4, endoderm; 5, mesenchyme; 6, notochord; 7, degenerating notochordal cells; 8, muscles; 9, undifferentiated muscle cells.

system including the species with tailed and tailless development (*Molgula oculata* and *M. occulta*, respectively) (Figs. 1a and 1b). These two closely related species occur on a sandy bottom at depths down to 100–120 m (more frequently 20–40 m) off the north-western coast of Europe. The developing *M. occulta* individuals lack larval structures necessary for swimming, such as tail, notochord, and otolith (unicellular

pigmented organ of equilibrium), while muscle cells remain in the rudimentary undifferentiated state. Note that all species of the family Molgulidae, including those with tailed larvae, are characterized by the absence of ocellus, the main component of larval photosensory organ, which is present in tadpoles from other ascidian families (Berrill, 1931; Jeffery, 2004).

These species practically do not differ from each other by the size of mature eggs and rate of embryogenesis (Swalla and Jeffery, 1990), which can be explained by their lecithotrophic development. The *M. oculata* eggs are weakly pigmented and have a diameter of 80 μm ; the larva hatches at 18°C within 11–12 h after fertilization. The *M. occulta* eggs are dark pigmented and have a diameter of 80–100 μm ; the larva hatches also within 12 h. Cleavage and gastrulation proceed similarly in both species. However, subsequent development of *M. occulta* sharply differs from that of *M. oculata* and other ascidians with usual (tailed) type of ontogenesis. Specifically, presumptive notochord cells do not migrate to the posterior end of the *M. occulta* embryo. These cells cease to proliferate prematurely and their differentiation is blocked (Takada *et al.*, 2002). The neural tube is formed, but muscles and unicellular pigmented otolith do not differentiate. The interval between hatching and beginning of metamorphosis in the swimming *M. oculata* tadpole and immobile slug-like *M. occulta* larva is 3–4 and 1 h, respectively (Swalla and Jeffery, 1990).

These two species were hybridized. When *M. oculata* eggs were fertilized by *M. occulta* spermatozoa, typical tadpoles were formed. In a reciprocal experiment, hybrids with otolith and markedly shortened tail containing notochord were formed, but muscle cells did not differentiate (Swalla and Jeffery, 1990), i.e. the larval features lost during evolution were only partially restored (Fig. 1c). But when the *M. occulta* eggs were fertilized by UV-irradiated *M. oculata* spermatozoa, no ancestral larval features were restored in the resulting gynogenetic hybrids (Jeffery and Swalla, 1992). Hence, the results of experiments on interspecies hybridization suggest that two independent processes are responsible for the loss of larval features. In other words, both maternal and zygotic changes, i.e. changes occurring during oogenesis and early development, are involved in the appearance and evolution of direct development, characteristic of some *Molgula* species.

A critical role of maternal changes for suppression of muscle cell differentiation in *M. occulta* was demonstrated by studies of the localization of cytoskeletal protein p58 (Swalla *et al.*, 1991). In ascidians with tailed development, this protein is localized in the egg myoplasm, the zone of prospective tail larval muscle. At the tadpole stage, protein p58 is specifically localized in the tail muscle cells and its content is constant throughout the larval development. On the contrary, in *M. occulta* and some other ascidians with tailless development, the egg content of protein p58 is very low or it

is altogether absent; it was also absent in presumptive muscle cells of tailless embryos. The hypothesis of the role of protein p58 in muscle determination in ascidians with tailed development may be tested experimentally by microinjection of this protein in the eggs of ascidians with tailless development.

Note also that synthesis of cytoskeletal (Jeffery *et al.*, 1998) and muscle (Kusakabe *et al.*, 1996) actin mRNAs is suppressed during development of *M. occulta* and there are certain structural differences between muscle actin genes of *M. occulta* and *M. oculata* (Kusakabe *et al.*, 1996). The genes *Uro-2* and *Uro-11* related to the development of tail structures are present in the genomes of both species but the corresponding mRNAs are expressed predominantly during oogenesis or early embryogenesis in *M. oculata*, rather than in *M. occulta* (Swalla *et al.*, 1993). Genetic mechanisms underlying some zygotic changes essential for restoration of the tail, notochord, and some other larval structures were studied in detail and the data were obtained that explain why these structures are absent in the species with tailless development (Swalla and Jeffery, 1996; Jeffery, 1997; Swalla *et al.*, 1999). It was shown that genes *Manx* and *bobcat* were expressed in embryogenesis of *M. oculata*, but their expression in *M. occulta* was suppressed. Active expression of these genes in hybrid embryos suggests their significant role in tail restoration and notochord rudiment development after fertilization of the eggs of a tailless species by spermatozoa of a tailed species. Interactions and cell functions of these “tail-forming” genes were also studied. They are expressed cooperatively during oogenesis and embryogenesis, but during spermatogenesis the corresponding mRNAs are accumulated independently (Swalla *et al.*, 1999).

In addition, the role of gene *FHI* encoding protein HNF-3 β (Olsen and Jeffery, 1997; Olsen *et al.*, 1999) was studied, which was later (Jeffery, 2002) called *FoxA5*. This gene is expressed during gastrulation and neurulation in the endodermal and notochordal cells of both *Molgula* species, as well as in hybrids. However, its expression in *M. occulta* is arrested after the completion of neurulation, while in *M. oculata* and hybrids, it is active also at later developmental stages. Antisense oligodeoxynucleotides suppress the expression of *FoxA5* and development of larval structures in *M. oculata* and hybrids. On the other hand, a similar treatment of *M. occulta* embryos did not prevent the formation of characteristic tailless larvae. Hence, in addition to genes *Manx* and *bobcat*, gene *FoxA5* is involved in evolutionary reorganization of the larva in ascidians of the genus *Molgula*, which also enhances the appearance of tailless development (Olsen *et al.*, 1999).

Jeffery (2002) has shown that the absence of otolith in the *M. occulta* tailless larva is due to apoptosis. He also described a massive death of presumptive notochord and muscle cells in *M. occulta* embryos and dem-

onstrated that the normal expression of genes *Manx* and *FoxA5* prevented extensive apoptosis not only in *M. oculata* embryos, but also in hybrids. Note also that inductive signals from the endodermal cells to the presumptive notochord cells appear to be markedly weakened in the early embryogenesis of *M. occulta*.

It can be proposed that in *M. occulta*, the transition to direct development is due to the loss or inactivation of muscle determinants and modification of inductive influences from axial determinants.

SPECIFIC DEVELOPMENTAL FEATURES OF OTHER *MOLGULA* SPECIES

M. bleizi was referred for a long time to the group of species with typical tailless development (Berrill, 1931). However, it was found later (Jeffery, 1997; Jeffery *et al.*, 1999) that a very short tail with notochord rudiment was formed in this species during late embryogenesis, although cleavage and gastrulation are similar to those in *M. oculata* and *M. occulta*. Just after hatching, this rudimentary tail is drawn into the trunk of larva, which remains immobile. No otolith is present. Thus, reorganizations of ontogenesis are expressed in *M. bleizi* to a somewhat lesser extent than in the closely related species *M. occulta*. The larva of *M. bleizi* is an intermediate form between the ancestral tailed and evolutionarily advanced tailless larvae, but the results of molecular-phylogenetic analysis suggest (Jeffery *et al.*, 1999) that the reorganization of development in *M. bleizi* occurred independently on that in *M. occulta* and, apparently, later.

On the contrary, in another species, *M. pacifica*, deviations from the usual tailed development are expressed to even greater extent than in *M. occulta*, and, hence, in this case, we can speak about a “maximally direct development” (Bates, 2004). The cleavage is similar to that in most ascidians by morphology, but gastrulation is markedly modified; specifically, endodermal cells do not invaginate and archenteron is not formed. It has been proposed that changes in ooplasmic segregation and egg cortex contribute to modification of tailless development in *M. pacifica*.

Finally, note that during ontogenesis of *M. citrina*, species with tailed development, adult tissues and organs develop during the larval phase, before metamorphosis (Swalla *et al.*, 1994; Jeffery, 1997).

Berrill (1931) summarized the available data on embryology and ecology of ascidians of the family Molgulidae and stressed the role of ecological factors that enhanced the appearance of tailless development. He emphasized the circumstance that the species with direct development might be well adapted to the life on a homogenous substrate, for example, on the sandy or muddy ground of the upper sublittoral zone, where the swimming larva does not seem to provide any selective advantage. However, it turned out later that some species with direct development, such as Atlantic *M. retor-*

tiformis (Bates, 1995) and *M. bleizi* (Jeffery *et al.*, 1999) and Pacific *M. pacifica* (Young *et al.*, 1988) and *M. tectiformis* (Tagawa *et al.*, 1997) occur on rock ground, where most species with tailed larvae also occur. Hence, this hypothesis has been discredited and appears to be invalid (Bates, 1995; Jeffery *et al.*, 1999).

CONCLUSION

Ascidians of the family Molgulidae have already been studied at a level equal to representatives of other families of the class Ascidiacea, which were traditionally used for developmental biology research. Moreover, the results of investigations on embryos and larvae of ascidians with tailless development helped to understand better how the mechanisms of ontogenesis are realized in ascidians with usual tailed development. The genus *Molgula*, like the genus *Heliocidaris* (phylum Echinodermata, class Echinoidea) (Raff, 1987, 1999), is now among the main model systems for studying evolutionary reorganizations of ontogenesis in closely related species of marine invertebrates.

The idea that reorganization of ontogenesis is a factor of evolution of closely related species was put forward in the area of comparative and evolutionary embryology of insects (Tikhomirova, 1991) and starfishes (Byrne and Cerra, 1996; Hart *et al.*, 1997). We believe that it should be true also for other organisms. However, some specific features can be observed. For example, in *M. occulta* embryo, suppression of proliferation and enhanced cell mortality are observed as a result of evolutionary reorganization of ontogenesis, as distinct from reorganizations of ontogenesis in some other animals, which lead to direct development. For example, enhanced cell proliferation leading to an increased cell number in the embryo is observed at the blastula and gastrula stages in the sea urchin *Heliocidaris erythrogramma* (Parks *et al.*, 1988) and during spinal cord formation in the anuran *Eleutherodactylus coqui* (Schlosser, 2003). On the contrary, in the colonial green flagellates of the genus *Volvox* (Chlorophyta, Volvocales), the rates of cell division are slowed down during evolutionary reorganizations of ontogenesis (exemplified by species *V. aureus*, *V. globator*, and *V. tertius*) (Desnitskiy, 1995).

Note that the evolution of tailless ontogenesis in ascidians lacks the transition from planktotrophic larval development to the lecithotrophic one. In all ascidians with the tadpole stage, this tailed larva is already lecithotrophic. Thus, the larval development of ascidians differs in the physiological respect from that of echinoids, since most sea urchin species have planktotrophic plutei, while only few of them have lecithotrophic plutei (for review see Raff, 1987; Desnitskiy, 2005). Among the anuran amphibians with the tadpole stage, only few have lecithotrophic larvae (Callery *et al.*, 2001).

There are also some general laws of the evolution of development within different groups of multicellular organisms, such as parallel directed evolutionary reorganizations in different representatives of the same group and variations (intermediate or transitional forms) in reorganization of ontogenesis within the same group. This is characteristic not only for ascidians, but also for sea urchins (Raff, 1987; Desnitskiy, 2005), anuran amphibians (Callery *et al.*, 2001; Desnitskiy, 2004), and colonial Volvocales (Desnitskiy, 1995). Finally, there are molecular-phylogenetic data for ascidians of the genus *Molgula* (Hadfield *et al.*, 1995; Jeffery, 1997), sea urchins of the genus *Heliocidaris* (McMillan *et al.*, 1992), and green flagellates of the genus *Volvox* (Liss *et al.*, 1997), which distinctly indicate that reorganizations of ontogenesis in species of these three genera are relatively recent evolutionary events, within the limits of several last million of years.

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