Evolution of Ascidian Development

Interspecific modifications of the tadpole larva have revealed some of the mechanisms of evolutionary change in development

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Both adulthood and tailless development have evolved multiple times, suggesting that they are mediated by relatively simple control mechanisms

simple differences in ontogeny. By studying closely related species, evolutionary developmental biologists attempt to minimize extraneous differences that have arisen by genetic drift after divergence from a common ancestor. In addition, the embryology of the species should be well known, and the system should be amenable to molecular and genetic analysis. Several systems exhibiting some of these features have recently been introduced to study the evolution of development (Jeffery and Swalla 1992a, Raff 1992, Schneider et al. 1992). Ascidians—marine invertebrates commonly known as sea squirts—have emerged as a leading model system in evolutionary developmental biology. In this article, I review the ascidian system and how it is being used to explore the evolution of development.

Ascidians

Ascidians are sessile filter-feeding animals with a life cycle that includes both larval and adult phases. Some ascidians are solitary and reproduce by sexual processes, whereas others are colonial and exhibit both sexual and asexual (budding) reproduction. The ascidians, along with the larvaceans and salps, are classified in the Subphylum Tunicata (or Urochordata) of the Phylum Chordata. According to recent phylogenetic analyses (Turbeville et al. 1994, Wada and Satoh 1994), the urochordates are the sister group of an assemblage containing the cephalochordates (e.g., amphioxus) and the vertebrates.

The ascidian adult has a saclike body characterized by a filter-feeding apparatus and two muscular siphons (Figure 1d). Except for gill slits, however, there is little in the adult ascidian structure that resembles a chordate. By contrast, the ascidian tadpole larva has a notochord and a dorsal central nervous system (CNS; Figure 2a), the hallmarks of a true chordate. The tadpole larva is often considered a prototypic of the chordate ancestor (Garstang 1928), and studies of ascidians may shed light on the origin and evolution of the chordates (Satoh and Jeffery 1995).

The tadpole larva is composed of the head (or trunk) and the locomotory tail (Figures 1a, 2a, and 5a). The trunk contains the brain and sensory organs (the otolith and, in some species, the ocellus), the endoderm (the precursor of the adult gut and visceral organs), and the mesenchyme cells (the precursors of the adult mesodermal derivatives). The
larva disperses, attaches to a suitable substrate, and undergoes metamorphosis into the sessile adult. During metamorphosis, the tail is retracted and phagocytoposed (Figures 1a–1c), the ampullae (the epidermal structures involved in respiration and in anchoring the juvenile to the substrate) form, and adult tissues and organs differentiate from precursors in the larval trunk.

Ascidians are ideal for studying the evolution of development. The tadpole is a simple organism, consisting of only 2500 cells and six tissues: epidermis, CNS, endoderm, notochord, muscle, and mesenchyme (Figure 2a). Development is rapid: The fertilized egg hatches into a swimming larva in only 12–18 hours. The cell lineages are well known and conserved among species (Satoh 1994), allowing the fate of single blastomeres to be followed throughout embryonic development. The ascidian genome is the smallest of any chordate (1.8 x 10^6 base pairs, approximately the size of the Drosophila genome; Lambert and Laird 1971), simplifying gene cloning and analysis of gene regulatory elements. Most important for evolutionary studies, closely related species can exhibit dramatic differences in development (Jeffery and Swalla 1992a).

Different modes of development

Ascidians are a diverse group of animals that show evolutionary distances as large as those between frogs and humans (Wada et al. 1992). Despite this diversity, the tadpole larva has been conserved in each of the 13 ascidian families. Briefly, ascidian development includes the following steps: the fertilized egg cleaves into a blastula; the blastula undergoes gastrulation; the neural tube is formed during neurulation; the larval tail is extended during the tailbud stage; the tadpole larva hatches, swims, and attaches to a substrate; the larval tail is retracted into the trunk; and the adult is formed during metamorphosis. (For a more detailed account of ascidian development, see Satoh [1994].)

The mode of development in which the adult is formed via a tadpole larva is called tailed development. Two evolutionary modifications in this mode of development, adulation and tailless development, are seen in some species. These alterations have been advantageous in studying the evolution of developmental mechanisms. Adulation is a mode of development in which the adult tissues and organs are formed prior to metamorphosis (Berrill 1935, Jeffery and Swalla 1992a). Adulation can be minimal, with a siphon, a rudimentary circulatory system, and a few gill slits formed in the larval trunk, or it can be more extensive. In the most extreme cases, a miniature adult containing the two siphons, numerous gill slits, a beating heart, a complete digestive tract, and even a precocious sexual bud develops in the larva. In some species with adulation, a more robust tail has evolved to propel the enlarged trunk. The tail is augmented by increasing the number of tail muscle cells. Examples of adulation are especially common in colonial species, but solitary species can also exhibit this mode of development. The existence of adulation in almost every ascidian family suggests that it evolved multiple times, probably to reduce the time between metamorphosis and the initiation of feeding and reproduction.

Tailless development is a mode of development in which the tail and associated brain sensory organs are not formed in the larva (Bates 1994, Berrill 1931, Jeffery and Swalla 1990a, 1992a). Thus, tailless species have lost the ancestral chordate body plan (Figures 2b and 3b). The nonswimming tailless larva hatches, attaches to a substrate, and undergoes metamorphosis near the parent. Tailless species are found in uniform habitats, such as subtidal mud and sand flats, in which dispersal is unnecessary for, or perhaps even detrimental to, survival. Other tailless species inhabit rocky substrates that are exposed to swift currents or extensive wave action.

The tailless mode of development has been reported in fewer than 20 of the approximately 3000 described ascidian species; most tailless species are members of the family Molgulidae (Jeffery and Swalla 1990a). The scarcity of tailless spe-

Figure 1. Ascidian development (Molgula citrina). (a) Midtailbud stage. (b) Larva (retracting its tail). (c) Metamorphosis. (d) Young adult.
cies led de Lacaze-Duthiers, the discoverer of tailless development, to propose a monophyletic origin from a tailed ancestor and to create a single genus *Anurella* for these taxa (de Lacaze-Duthiers 1874, 1877). Based on differences in adult morphology, however, Berrill (1931) and others argued that tailless development evolved multiple times from different tailed ancestors. Modern phylogenies, inferred from 18S and 28S rDNA sequences, support the polyphyletic origin of tailless development (Hadfield et al. 1993). These findings show that the ancestral tailless larva has been converted into a tailed larva at least four separate times in the Molgulidae and once or twice in the Stylocelidae.

In this article, I review recent findings about the mechanisms underlying the evolution of adulation and tailless development in the Roscovita, a clade of molgulid ascidians exhibiting the three contrasting modes of development (Hadfield et al. 1995). The Roscovita clade is named after Roscoff, France, where tailless ascidian species were first discovered (de Lacaze-Duthiers 1874, 1877) and where much of the modern research on tailless development is being conducted. As shown in Figure 3, the Roscovita clade is composed of four closely related solitary species of known phylogeny. These taxa exhibit conventional tailed development (*Molgula oculata*), adulation (*Molgula citrina*), or tailless development (*Molgula bleizi* and *Molgula occulta*). The tadpole larva has been altered to different degrees in the tailless species: *M. occulta* has a completely tailless larva, whereas *M. bleizi* has a larva with a short tail that is nonmotile and retracted into the trunk immediately after hatching. The existence of an intermediate form in *M. bleizi* suggests that tailless development may be a gradual evolutionary process. *M. oculata* and *M. occulta* live partially buried in subtidal sand flats, whereas *M. citrina* and *M. bleizi* live attached to other ascidian species on rocky promontories projecting from these sand flats. Based on biogeography and sequence data, the common ancestor of *M. oculata*, *M. bleizi*, and *M. occulta* is likely to have diverged from *M. citrina* during the past 30–60 million years, and tailless development probably evolved in *M. occulta* and *M. bleizi* within the last 5–15 million years. Therefore, different modes of development have evolved recently in the Roscovita clade.

**Adulation: evolution of novel phenotypes**

The mechanisms of adulation have been studied in *M. citrina* (Grave 1928), which develops ampullae, several gill slits, and the circulatory system and heart prior to metamorphosis. In conventional, tailed developers, the ampullae sprout and mesenchyme cells migrate between the epidermis and endoderm and differentiate into blood cells and the heart during metamorphosis. In *M. citrina*, by contrast, the ampullae are formed and the mesenchyme cells differentiate in the trunk before the larva hatches (Figure 1b).

To investigate whether early differentiation of adult organs in *M. citrina* is controlled by spatial (heterotopic) or temporal (heterochronic) changes, my colleagues and I examined the expression of actin genes in the larva. Ascidians have a family of muscle actin genes (Jeffery 1994a), which are expressed either in the larva, the adult, or both stages of the life cycle. In *M. oculata* and other conventional, tailed species, the larval genes are transcribed in tail muscle cells but not in mesenchyme cells during larval development (Figure 4c). Conversely, the adult genes are silent during larval development and active in differentiating mesenchyme cells during metamorphosis and in adult muscle cells. An adult muscle actin gene has been cloned from *M. citrina* that is expressed either in the larva, the adult, or both stages of the life cycle. In *M. oculata* and other conventional, tailed species, the larval genes are transcribed in tail muscle cells but not in mesenchyme cells during larval development (Figure 4c). Conversely, the adult genes are silent during larval development and active in differentiating mesenchyme cells during metamorphosis and in adult muscle cells. An adult muscle actin gene has been cloned from *M. citrina* that is expressed either in the larva, the adult, or both stages of the life cycle.
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from both M. citrina and M. oculata
(Ma et al. 1996). The function of the
Msx genes is not completely under-
stood, but they code for transcription
factors with possible roles in
expression of genes during organo-
genesis (Davidson 1995). Although
several different Msx genes exist in
vertebrates, only a single Msx gene
has been detected in ascidians (Hol-
land 1991), making the interpretation of
expression patterns straightforward.
In M. oculata, the Msx gene is ex-
pressed in presumptive mesenchyme
and muscle cells and in the neural
plate and folds during gastrulation
and neurulation, and it is then turned
off by a heterochronic process.

The expression patterns of genes
that function early in mesoderm and
muscle cells also indicate that
adultation involves heterochronic
changes. These early genes often
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Msx gene, which encodes a homeo-
domain protein, has been cloned
from both M. citrina and M. oculata
(Ma et al. 1996). The function of the
Msx genes is not completely under-

Figure 4. Larval and adult muscle actin gene expression in ascidians with different
modes of development. (a) and (b) Consecutive serial sections through the trunk
of an M. citrina larva showing the accumulation of adult muscle actin transcripts
(black silver grains) shortly after adultation. h = aggregated mesenchyme cells in
the heart primordium; m = migrating mesenchyme cells. (c)–(e) Expression of larval
muscle actin transcripts shortly after neurulation in (c) M. oculata (tailed species) (d)
M. oculata (tailless species) and (e) hybrid (tailless species' egg fertilized with tailed
species' sperm) embryos. Muscle actin transcripts are present in the muscle cells of
the larval species (c) and hybrids (e) but not of the tailless species (d). mu = muscle
cells; o = oorith. Reprinted from Swalla et al. 1994 and Kusakabe et al.
1996 with permission from John Wiley & Sons and Academic Press, respectively.

not expressed in the larval tail muscle
cells (Swalla et al. 1994). However,
unlike the M. oculata adult muscle
actin gene, the M. citrina gene is
transcribed in mesenchyme cells
during the larval phase (Figures 4a and
4b). Larval muscle actin genes are
not transcribed in the mesenchyme
cells of M. citrina during the period
when the adult actin gene is ex-
pressed. Therefore, adultation in-
volves a temporal change in the ex-
pression of an adult muscle actin
gene, suggesting that it is mediated
by a heterochronic process.

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Transcription of structural genes in the
adult developmental pathway, pre-
sumably as a result of the precocious
activation of regulatory genes.

Tailless development:
regression of chordate features

Tailless development has been studied
in M. oculata, the sister species
of the tailed developer M. oculata
(Figure 3). To avoid confusion raised
by their similar names, I will refer to
these ascidians as the tailed (M.
oculata) and the tailless (M. oculata)
species. Both species have similar
cleavage patterns, cell lineages,
modes of gastrulation, and rates of
development, and both exhibit the
six embryonic tissues that are char-
acteristic of all ascidian larvae (Fig-
ures 2a and 2b; Swalla and Jeffery
1990). Thus, tailless development
does not appear to be controlled by
heterochrony.

The first differences between the
two species are apparent late in gas-
trulation, when the cell movements
that will culminate in tail formation
begin in the tailed species. Tail de-
velopment involves coordinated pos-
terior movements and differentia-
tion of the notochord, tail muscle,
spinal cord, endodermal strand, and
posterior epidermal cells (Figure 2a).
These morphogenetic movements
appear to be driven by notochord
extension (Miyamoto and Crowther
1986). In tailed species, ten pre-
sumptive notochord cells are speci-
fied during the cleavage period by an
inductive signal emanating from
adjacent endoderm cells (Nakatani
and Nishida 1994). The induced cells
develop twice, giving rise to 40 noto-
chord cells, which interdigitate,
converge toward the embryonic mid-
line, and extend to form the
notochord (Figure 2a). Further noto-
chord extension is mediated by the
vacuolation, swelling, and secretion
of an extracellular matrix during
notochord cell differentiation. The
Tailless species also contains approxi-
ately ten presumptive notochord
cells; however, these cells do not
undergo further divisions, morpho-
genetic movements, or differentia-
tion. Instead, the presumptive noto-
chord cells arrest as a clump of
undifferentiated cells in the poste-
rior region of the tailless embryo
(Figure 2b). Thus, the lack of tail formation in the tailless species is mediated in part by abbreviated notochord development.

The tailless species also lacks an otolith, the gravity-sensitive receptor in the tadpole larva (Figures 2a and 2b). (All molgulids lack an ocellus, the larval photoreceptor, and are blind.) In the tailless species, neural cells are induced to develop into the otolith by interactions with presumptive notochord (Reverberi et al. 1960) and/or spinal cord cells (Nishida 1991) during late cleavage. Thus, the missing otolith of the tailless species may be caused by the arrested development of the presumptive notochord cells. Alternatively, the neural cells from which the otolith develops may have lost the competence to respond to the normal inducing signal. Another possibility is that required contacts between the inducing and responding cells may be spatially excluded in the tailless species. The question of what prevents otolith development could be resolved by interspecific cell transplantation experiments, although the small size of Molgula blastomeres during the critical embryonic stages would make such an experiment difficult.

Another hallmark of tailless development is the failure of tail muscle cell differentiation (Whittaker 1979). The ascidian tadpole larva contains approximately 40 muscle cells, with 20 cells flanking each side of the notochord (Figure 2a). The tailless species contains a reduced number of vestigial myoblasts (Figure 2b), but these cells do not differentiate (Swalla and Jeffery 1990). Some larval muscle markers, such as acetylcholinesterase (AChE), are expressed in myoblasts of the tailless species, whereas the expression of others, such as myosin and muscle actin mRNA (Figure 4d), cannot be detected, suggesting that muscle differentiation is initiated but not completed.

The larval muscle cells of ascidians are derived from two sources (Satoh 1994). The primary muscle cells arise from the two vegetal-posterior blastomeres at the 8-cell stage and produce 28 muscle cells in the anterior portion of the tail. Primary muscle cell determination is a cell-autonomous process that is mediated by muscle determinants localized in the egg (Nishida 1994, Swalla 1992). The secondary muscle cells originate from the two vegetal-anterior and the two animal-posterior blastomeres of the 8-cell embryo and produce 10-14 muscle cells in the posterior portion of the tail. Secondary muscle cell determination is a conditional process that requires inductive cell interactions in the zygote (Jeffery 1993a, Nishida 1990). AChE staining patterns indicate that the tailless species develops fewer primary muscle cells than the tailed species and lacks secondary muscle cells (Jeffery and Swalla 1991). These results suggest that both the cell-autonomous and conditional muscle determination processes are altered during development of the tailless species.

The lack of primary muscle cell differentiation in the tailless species can be explained in part by mutations in genes encoding the muscle actin proteins. The tailless species possesses duplicated homologues of a single larval muscle actin gene found in the tailed species (Kusakabe et al. 1996), but both copies have mutated into pseudogenes. The gene duplication event is likely to have occurred while the larval muscle cells were still functional in a direct (perhaps tailed?) ancestor of the tailless species. Remarkably, the promoters of these pseudogenes have retained the ability to drive expression of a reporter gene in muscle cells of the tailless species, suggesting that the relevant transcription factors are present. These transcription factors may be evolutionary relics of a recent tailless ancestry, or they may regulate other, functional muscle genes in the tailless species.

In summary, tailless development involves changes in determinants, inductive signaling events, cell proliferation, morphogenetic movements, and cell differentiation, leading to the loss or modification of ancestral chordate features. These changes have been observed in neural (otolith), muscle, and notochord cells, but similar changes are likely to have occurred in related posterior tissues. Tailless development therefore appears to be mediated by multiple changes in mechanisms controlling development of the larva's posterior region. These alterations could be the result of a single upstream regulatory change, a possibility that is consistent with the central role of the notochord in controlling processes that are modified in the tailless species and with the recurrent evolution of tailless development (Hadfield et al. 1995). Alternatively, tailless development may have evolved by changes in different or redundant regulatory pathways.

**Tailless development: maternal and zygotic control mechanisms**

A drawback of the ascidian system for studies of the evolution of development is the lack of a classical genetic approach. Although genetic analysis of colonial ascidians is to some extent possible, genetic analysis of solitary ascidians is hindered by their long generation times and the difficulty in raising them to sexual maturity in the laboratory. However, the ability to conduct interspecific hybridization experiments (Jeffery and Swalla 1991, 1992b, Swalla and Jeffery 1990) and to use antisense procedures to inhibit gene expression (Swalla and Jeffery 1996) can compensate for the lack of a genetic approach in solitary ascidian species.

Ascidians are well known for their reliance on both maternal and zygotic mechanisms to control cell fate during embryonic development (Satoh 1994), which raises the question of whether the developmental changes in the tailless species are due to alterations in maternal or zygotic processes. Because of their recent divergence, the tailed and tailless species are capable of interspecific hybridization and can be crossed in both directions (Figure 5). Eggs of the tailed species fertilized with sperm of the tailless species develop into conventional tadpole larvae (Figure 5c), implying that tailless development does not result from dominant (e.g., gain-of-function) mutations suppressing tail formation. By contrast, the reciprocal cross (tailless species' eggs fertilized with tailed species' sperm) results in short-tailed hybrids (Figure 5d) displaying an otolith, a notochord, and pri-
The myoplasm: mediator of maternal changes

The knowledge that maternal factors are involved in tailless development, however, as the following observations show. First, as mentioned above, hybrids of the cross between tailless species' eggs and tailed species' sperm (hereafter referred to as hybrids) contain the reduced number of notochord cells that is typical of the tailless species (Figure 2c). Second, the number of myoblasts is increased and muscle actin expression is restored in the hybrid embryos (Figure 4e), but the muscle cells still do not differentiate or move into the tail (Figure 4c). Indeed, the short tail of hybrid larvae is completely immotile. The increase in the number of muscle cells in the hybrid embryos results from the reappearance of the secondary muscle cells (Jeffery and Swalla 1991), presumably because inductive potentials have been restored. These findings suggest that changes in both maternal and zygotic mechanisms are involved in tailless development.

uro genes: regulators of
tailed development

The myoplasm probably exerts its developmental effects by activating...
genes that are involved in axis and muscle development. My colleagues and I developed a strategy to identify these genes that is based on the assumption that they may differ in expression during development of the tailless species. Accordingly, three genes that are differentially expressed in the tailless species were identified by screening cDNA libraries with a subtracted probe (Swalla et al. 1993). The subtracted probe was prepared by hybridizing cDNA prepared from a cDNA library of the tailless species with RNA synthesized from a cDNA library of the tailless species and retaining the noncomplementary cDNA, which corresponds to mRNAs enriched in the tailless species (Swalla 1996).

The three genes that were identified by this strategy were designated uro (for urodele) genes because of their preferential expression in the tailless species (Table 1). The uro-1 (Cymric; the three uro genes are named after tailless cats) gene encodes a nonreceptor protein tyrosine kinase with two SH2 domains and five ankyrin repeats, suggesting multiple options for interactions with other proteins (Swalla et al. 1995). The uro-2 (lynx) gene encodes a leucine zipper protein with several putative phosphorylation sites (Swalla et al. 1993). Leucine zippers are sites of protein–protein interaction in transcription factors and other proteins. Thus, lynx may be a dimer or be present in a complex with another protein containing a leucine zipper. The Cymric and lynx genes are expressed exclusively during oogenesis in the tailless species and are inactive or expressed at low levels in the tailless species. Based on their sequences, the Cymric and lynx proteins are predicted to function in a signal transduction cascade that may be involved in the assembly of the myoplasm during oogenesis or in cell interactions during embryogenesis. The evolutionary modification of such a signaling cascade could be responsible for the lack of myoplasm assembly and changes in inductive potential during tailless development.

The uro-11 (Manx) gene encodes a nuclear protein containing several nucleic acid binding motifs, including a zinc finger (Table 1; Swalla et al. 1993). Zinc fingers usually represent nucleic acid binding domains and are commonly found in transcription factors. Thus, the Manx protein may function as a transcription factor. In contrast to Cymric and lynx, Manx is expressed both maternally and zygotically. Maternal Manx transcripts are restricted to small oocytes, suggesting that the translated protein is functional during oogenesis and/or is stored in the mature egg for use after fertilization. Zygotic Manx transcripts accumulate during gastrulation and neurulation in the presumptive tissues involved in tail morphogenesis (notochord, muscle, CNS, and posterior epidermal cells). Manx transcripts are present at much lower levels in the tailless species than in the tailless species, but they increase to higher levels in hybrids with restored chordate features (Swalla and Jeffery 1996). Manx mRNA is confused to the same tail-forming tissues in these hybrids as in the tailless species. These characteristics suggest that Manx may be a tail-forming regulatory gene whose decreased expression leads to tailless development.

### The Manx gene and beyond

The role of the Manx gene in tail formation has been explored by antisense inhibition of gene expression (Swalla and Jeffery 1996). Antisense methods can be applied to developing embryos only if the protein that is encoded by the gene of interest is not made in advance in the oocyte and stored for later use, but is synthesized after fertilization, when antisense molecules can be introduced into the egg. The presence of Manx protein in the egg, for example, hinders the use of antisense procedures to study the effect of disrupting Manx gene expression in the tailless species. However, hybrid embryos are perfect subjects for antisense inhibition of Manx: They contain zygotic Manx transcripts, but because of their origin from eggs of the tailless species, they are depleted in Manx protein. Treatment of hybrid embryos with Manx antisense deoxyoligonucleotides resulted in the destruction of Manx mRNA and inhibited restoration of the notochord, otolith, and the full number of tail muscle cells. Therefore, zygotically expressed Manx transcripts are required to rescue chordate features in the hybrid embryos. Consequently, loss-of-function mutations in the Manx gene could have resulted in the evolution of tailless development.

The zygotic Manx protein could function by regulating the concerted morphogenetic movements of notochord, epidermal, and muscle cells during tail formation, but the function of the maternal Manx protein is still a mystery. However, it is possible that maternal Manx may be required to organize the myoplasm in the growing oocyte or processes that are necessary for the appearance of inductive potentials in the embryo. Current studies focus on the organization of the Manx gene in the tailless species, the spatial distribution of maternal Manx protein in the egg and embryo, the identity of genes that function upstream and downstream in the Manx pathway, and the status of Manx gene expression in other tailless ascidian species. Finally, it will be interesting to determine whether homologues of the Manx gene arc

### Table 1. Summary of uro and bobcat gene characteristics in the tailed (M. oculata) and tailless (M. occulta) species.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Presence in genome</th>
<th>Expression</th>
<th>Required for chordate features</th>
</tr>
</thead>
<tbody>
<tr>
<td>uro-1 (Cymric)</td>
<td>Tyrosine kinase</td>
<td>Yes</td>
<td>Maternal</td>
<td>No expression regulated</td>
</tr>
<tr>
<td>uro-2 (lynx)</td>
<td>Leucine zipper</td>
<td>Yes</td>
<td>Maternal</td>
<td>Down-regulated</td>
</tr>
<tr>
<td>uro-11 (Manx)</td>
<td>Zinc finger</td>
<td>Yes</td>
<td>Maternal,</td>
<td>Down-regulated</td>
</tr>
<tr>
<td>bobcat</td>
<td>RNA helicase</td>
<td>Yes</td>
<td>Zygotic</td>
<td>Down-regulated</td>
</tr>
</tbody>
</table>
present in other animals, such as the vertebrates, and if so, what their roles are in embryogenesis.

The total number of genes involved in tail development is unknown, but Manx does not appear to be the only zygotically expressed gene required for the restoration of chordate features in hybrid embryos. In both tailed and tailless species, Manx is closely linked to—indeed, it partially overlaps with—another gene (Pederson et al. 1994). This linked gene, which is called bobcat, encodes an ATP-dependent RNA helicase: RNA helicases use energy generated by ATP hydrolysis to unwind double-stranded regions in RNA molecules. The bobcat protein is related to the human RNA helicase, p68 (Iggo and Lane 1989). Although it is conserved from yeast to humans, little is known about p68 function other than that it shuttles between the nucleus and cytoplasm during the cell cycle. The cell cycle-dependent localization implies that p68 may be involved in cell proliferation, perhaps by playing a role in RNA splicing, transport, or decay.

The Manx and bobcat genes show many similar features; they are expressed in the tailed species and downregulated in the tailless species, they are expressed both maternally and zygotically, their zygotic expression is restored in hybrids, and their zygotic transcripts accumulate in presumptive tail-forming tissues in the tailed species and hybrids (Table 1). In addition, antisense experiments indicate that bobcat, like Manx, is required for restoring chordate features to hybrid embryos. As a potential mediator of the cell cycle, bobcat may have a role in the abbreviation of cell proliferation during tailless development, although it could also be involved in morphogenetic movements. It is currently unknown whether bobcat expression depends on Manx or the converse. The Cymric and lynx genes are additional candidates for regulators of tailed development. The requirement of these genes for this process, however, has not been firmly established because their maternal expression precludes the use of antisense methods to investigate their function.

Single copies of the Manx, lynx, and bobcat genes are present per haploid genome of both the tailed and tailless species (Pederson et al. 1994, Swalla et al. 1993, 1995). By contrast, the Cymric gene is a single copy in the tailed species but cannot be detected in the tailless species (Table 1), suggesting that gene loss as well as modulation of gene expression may be involved in tailless development. Based on amplification of specific DNA sequences by the polymerase chain reaction, Ruddle et al. (1994) suggested that the tailless species may lack a gene of the homeobox (Hox) cluster that is expressed in the posterior body region of vertebrate embryos and that is present in the tailed species. This result is interesting because the Hox cluster genes are known to regulate the development and evolution of the chordate body plan (Holland and Garcia-Fernandez 1996). Therefore, determining whether the Hox cluster genes function in concert with the uro genes will be an objective of future studies of tailless development.

Conclusions and prospects

Ascidians with different modes of development provide an excellent opportunity to study the evolution of development in closely related species. Both adulthood and tailless development have evolved multiple times, suggesting that they are mediated by relatively simple control mechanisms. During adulthood, novel phenotypes are generated by heterochronic changes in the expression of regulatory and structural genes. The key question is what factors are responsible for turning on the regulatory genes at inappropriate times. An attractive possibility is that localization in the egg cytoplasm of key maternal factors controlling these genes has been changed during the evolution of adulthood. If this speculation is correct, a heterotopic process may ultimately control the execution of a heterochronic event (Raff 1996).

Heterochrony does not appear to have been a major factor in the evolution of tailless development. Instead, tailless development involves the abbreviation and elimination of developmental programs as a result of changes in both maternal and zygotic gene regulation. The material effects are mediated by gross alterations in myoplasm structure and localization, whereas the zygotic changes are mediated by loss-of-function mutations in regulatory genes. The novel attributes of the ascidian system, including the ability to hybridize species with different modes of development, have allowed some of these genes to be identified. The zinc finger gene Manx and the RNA helicase gene bobcat appear to have important roles in the restoration of chordate features in the tailless larva. A future challenge will be to determine how these and other regulatory genes have resulted in evolution of the tailless phenotype. Ascidians with different modes of development will continue to provide new insights into evolutionary developmental biology.

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