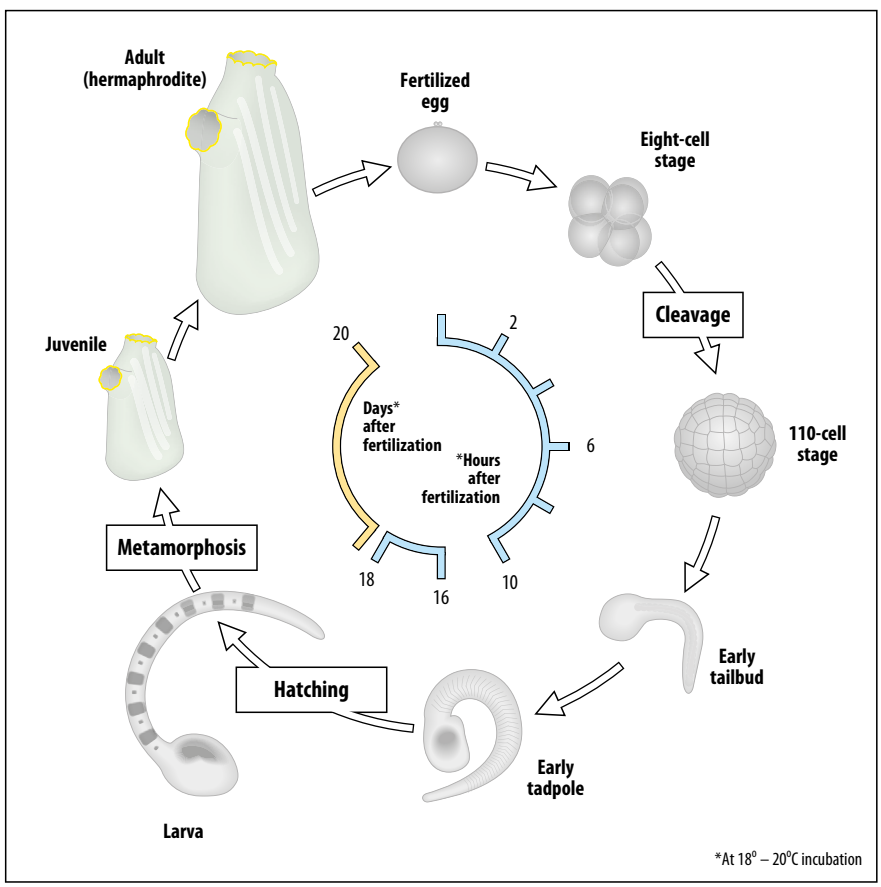
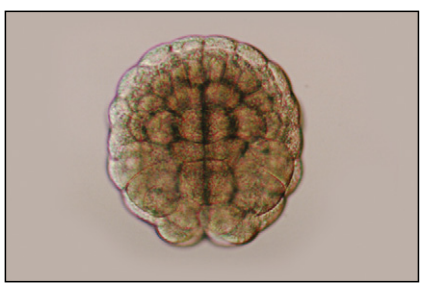
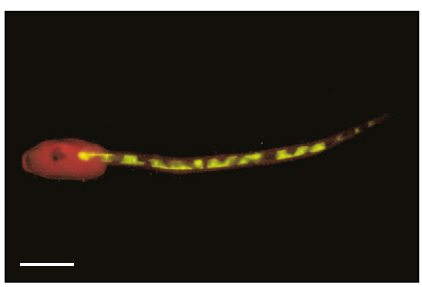
Ascidians

Adult ascidians, also known as sea squirts, are sessile marine animals. They are urochordates, which are included in the same phylum—the Chordata—as the vertebrates because their free-living, tadpole-like larvae possess a notochord, neural tube, and muscles, and are rather similar to the tailbud stage of a vertebrate embryo. The larva, which has about 2600 cells, undergoes metamorphosis into a sac-like sessile adult (Fig. S5.1). Although they are chordates, some aspects of ascidian development are very different







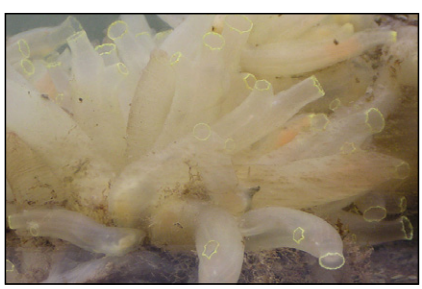


Fig. S5.1 **Life-cycle diagram of the ascidian *Ciona intestinalis*.** *Ciona* is hermaphrodite and eggs are fertilized externally. The fertilized egg takes about 18 hours to hatch into a larva, depending on the temperature of the water. The free-swimming larva undergoes metamorphosis into the sessile juvenile (about 2 cm tall) in around 20 days. Development of the juvenile into a sexually mature adult (which is about 5–8 cm tall) takes a further 2 months. The photographs show: (top) a 110-cell embryo; (middle) a larva (with some notochord cells labeled green; scale bar = 0.1 mm); (bottom) adult C. intestinalis. *Top photograph courtesy of Shigeki Fujiwara and Naoki Shimozono; middle photograph reproduced with permission from Corbo, J.C., et al.:* ***Characterization of a notochord-specific enhancer from the Brachyury promoter region of the ascidian,* Ciona intestinalis**. *Development 1997,* ***124****: 589-602. Published by permission of The Company of Biologists Ltd Lower photograph © Perezoso, reproduced under the Creative Commons Attribution-Share Alike 3.0 Unported license.*

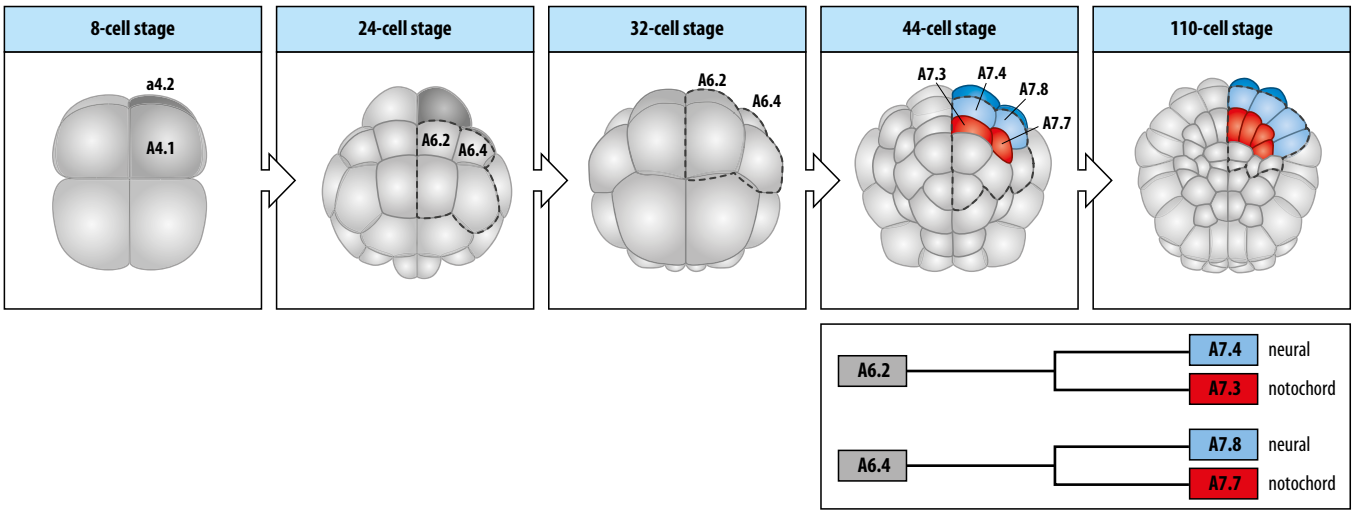


Fig. S5.2 Cleavages in the egg of the ascidian *Halocynthia roretzi*. The first cleavage divides the fertilized egg along the dorso-ventral plane into left and right halves. From this stage onwards the development of the embryo is bilaterally symmetrical. Corresponding right- and left-half blastomeres are given the same designation, but left-half blastomeres and their descendants are conventionally underlined. The second cleavage divides the embryo along the antero-posterior axis. Note that the view of the embryo has been rotated by 90° towards the viewer, between the first and second cleavages, so that the antero-posterior axis can be shown clearly.

from those of vertebrates. Ascidian embryos have an invariant cleavage pattern (Fig. S5.2), and localized cytoplasmic factors appear to have a much more important role in specifying cell fate. The genomes of the sea squirts *Ciona intestinalis* and *C. savigynyi* have been sequenced, and *C. intestinalis* has an estimated 15,800 genes, which is comparable to the other model invertebrates. Knowing the genome sequences will enable the full panoply of genomic techniques to be applied to the study of ascidian development, and enable its similarities to, and differences from, the development of vertebrates and other chordates to be investigated at the genetic and molecular level.

Knowledge of the *C. intestinalis* genome has already been exploited in a large-scale screen for *cis*-regulatory control elements controlling Hox gene expression. Nine Hox genes have been identified in *Ciona*, seven of which are dispersed on the same chromosome, with the rest on another chromosome. Hox genes are expressed along the neural tube during development, and some are expressed in the endoderm that gives rise to the intestine. But, unlike flies and vertebrates, the timing of expression of the various Hox genes in *Ciona* seems not to be coordinated, and several are missing. Some 670 transcription factors have already been identified from the genome sequence, along with 119 major signal transduction proteins. All the major vertebrate developmental intercellular signaling pathways are present in ascidians, including the Hedgehog, Wnt, TGF-β, and Notch pathways.

Ascidian embryogenesis has long been regarded as a typical example of mosaic development, with cytoplasmic factors specifying cell fate during cleavage, and cell interactions playing only a relatively minor part. It is now clear, however, that cell interactions are more important than was previously thought.

S5.1 Animal-vegetal and antero-posterior axes in the ascidian embryo are defined before first cleavage

The developmental axes in an ascidian embryo are related to the early pattern of cleavage. Like amphibian and sea-urchin eggs, the unfertilized ascidian egg is polarized along an animal–vegetal axis, with the prospective territories of ectoderm, mesoderm, and endoderm lying along this axis from animal to vegetal. Another axis, which

is conventionally called the antero-posterior axis, is established perpendicular to this. Note that this naming convention differs from that of the axes in amphibian embryos. This difference is largely semantic, and is due to the way in which ascidian embryos gastrulate and to a difference in the position of the developing embryo used as a reference point by ascidian researchers. The animal–vegetal axis of the egg is conventionally considered as the future ventro-dorsal axis of the tadpole, as the vegetal pole becomes covered with dorsal tissues at gastrulation.

Important maternal factors are localized in the vegetal region of the fertilized egg, among them components of the Dishevelled–β-catenin signaling pathway. As in the sea urchin, localized activation of this pathway stabilizes maternal β-catenin and induces its preferential entry into nuclei in vegetal cells, where it is required to specify endoderm in the vegetal region. In contrast, the development of an epidermal fate in animal blastomeres requires suppression of β-catenin function.

The animal–vegetal and antero-posterior axes of the ascidian embryo are specified in the fertilized egg before first cleavage by movement of the cortical cytoplasm. The unfertilized egg is radially symmetrical along its animal–vegetal axis. As in *Xenopus*, sperm entry at fertilization triggers a rotation of the egg cortex that breaks this symmetry (see Section 4.2). In the ascidian, part of the cortical cytoplasm moves towards the vegetal pole, generating a characteristic bulge at the pole. This movement is associated with the cytoskeleton, principally cortical actin filaments and a deeper network of intermediate filaments. The cortical region that relocates is rich in mitochondria and is called the **myoplasm**. It contains maternal cytoplasmic determinants, such as the mRNA *macho-1*, that specify muscle, and it will eventually give rise to the muscle of the ascidian tadpole's tail. Its position after cortical movement specifies the posterior end of the antero-posterior axis, which is also where gastrulation will start.

The first cleavage is in the plane of the animal–vegetal axis and gives rise to two cells with similar developmental potential, each of which can regulate to grow into a half-size tadpole if separated. The second cleavage is in the same plane but at right angles to the first and delimits the anterior and posterior halves of the embryo. The third cleavage demarcates the animal and vegetal halves (see Fig. S5.2). The planes of further cleavages are strictly controlled; this stereotyped pattern of cleavage determines what cytoplasmic maternal determinants the resulting cells contain and thus how they will develop. An important influence on further unequal cleavages in the posterior region is the so-called chromosome-attracting body (CAB) in the myoplasm. The CAB contains PAR proteins, which, as in nematodes (see Section 6.1), help position the mitotic spindle so that the cell divides asymmetrically.

By the time the embryo reaches the 110-cell stage, at which gastrulation begins, the fate of each blastomere has become restricted by a combination of the maternal cytoplasmic determinants it contains and intercellular signaling, such that most of the cells give rise to a single cell type in the larva (Fig. S5.3). When individual blastomeres from this stage are isolated and cultured they develop into the cell types specified by the fate map.

Despite the prominent role of cytoplasmic determinants in specifying cell fate in ascidians, the development of some types of mesodermal tissue at least depends on inductive signals, as in vertebrates and echinoderms. Three distinct types of mesodermal tissue are formed in ascidian embryos—the muscles of the larval tail, the mesenchymal tissue that gives rise to internal tissues, and the notochord. Here we shall look at the ways in which the fate of these three tissues is determined.

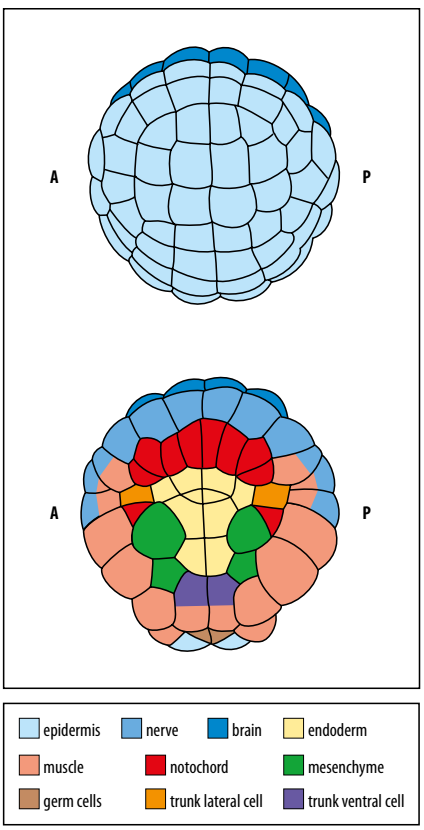


Fig. S5.3 Fate map of the 110-cell ascidian embryo. Top panel: view looking down on the animal pole. Almost all the cells of the animal half become ectoderm. Bottom panel: view from the vegetal pole. *Illustration from Nishida, H.:* ***Specification of embryonic axis and mosaic development in ascidians****. Dev. Dyn. 2005,* ***233****: 1177-1193.*

S5.2 In ascidians, muscle is specified by localized cytoplasmic factors

The fate of the myoplasm in the ascidian *Styela* is particularly easy to follow as it contains yellow pigment granules that are visible in the embryo, and this was the first indication, discovered at the beginning of the twentieth century, that a particular

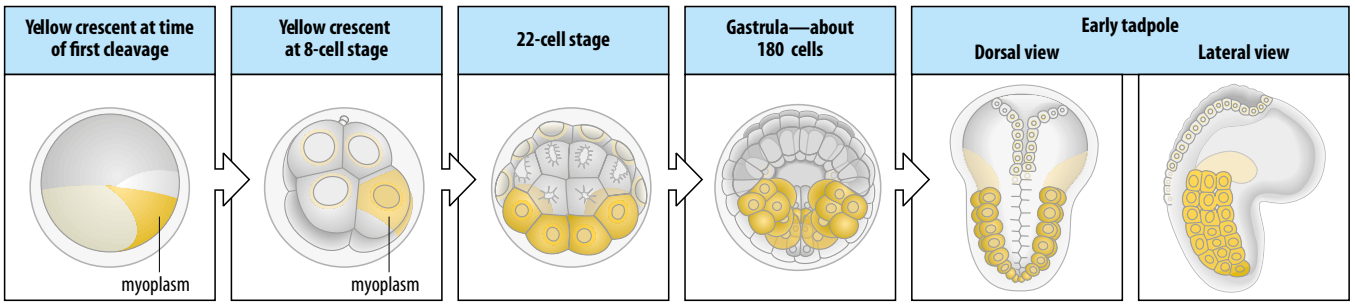


Fig. S5.4 Muscle development and cytoplasmic determinants in the ascidian *Styela*. Following fertilization, the myoplasm, which is colored with yellow granules, moves laterally and toward the equator. This movement forms a yellow crescent at the future posterior end of the embryo. Gastrulation starts at this site. The muscle of the ascidian tadpole's tail comes both from cells that contain the yellow myoplasm and the cells adjacent to them. *After Conklin, E.G.:* ***Mosaic development in ascidian eggs****. Journal of Experimental Zoology 1905,* ***2****:2 145-223.*

region of the ascidian egg cytoplasm could give rise to a particular tissue. A hundred years later we are at last beginning to find out how. The cells that acquire the yellow myoplasm during cleavage give rise to the muscle cells of the larval tail (Fig. S5.4). Before fertilization, the yellow granules are more or less uniformly distributed throughout the egg; following fertilization and cortical rotation, there is a dramatic rearrangement of the myoplasm to form the yellow crescent at the equator.

By the eight-cell stage, the myoplasm is largely confined to the two vegetal posterior cells, with a small amount in adjacent cells (see Fig. S5.4). In the species *Halocynthia roretzi*, the cell lineage has been worked out in detail by the injection of a tracer into the early cells. The two posterior B4.1 cells (see Fig. S5.2) that contain myoplasm contribute to the primary muscle cells, which are 28 of the 42 muscle cells that lie on each side of the tail. The secondary muscle cells are derived from blastomeres adjacent to B4.1 at the eight-cell stage and end up at the top of the tail. The lineage is complex; for example, at the 128-cell stage one of the descendants of B4.1 is still an endomesodermal cell that will give rise to both muscle and endoderm. So, while there is a good correlation between muscle development and myoplasm, these observations on their own did not establish that it is something in the myoplasm that is causing differentiation into muscle.

Experiments that altered the distribution of the myoplasm also provided suggestive, but not conclusive, evidence that the myoplasm on its own can specify muscle cells. But the most persuasive evidence that the myoplasm specifies muscle is provided by more recent experiments that deplete it of a key maternal mRNA—*macho-1* mRNA. This is localized in the myoplasm in the egg and its depletion results in loss of the primary muscle cells in the tail. If the posterior-vegetal cytoplasm containing the *macho-1* mRNA is removed from the fertilized egg, the blastomeres that would normally develop as muscle develop as nerve cord. Conversely, injection of *macho-1* mRNA into non-muscle cell lineages causes ectopic muscle differentiation.

S5.3 Notochord, neural precursors, and mesenchyme in ascidians require inducing signals from neighboring cells

When first visible in the early tadpole, the notochord of the *Ciona* larva consists of a single row of 40 cells aligned along the center of the tail, which undergo shape changes to eventually form a hollow tube. The notochord derives mainly from A lineage cells with a small contribution from the B lineage and requires induction by adjacent vegetal cells for its formation. Blastomeres that normally give rise to notochord do not do so if they are isolated at the 32-cell stage, unless they are combined with vegetal blastomeres. However, prospective notochord cells isolated at the 110-cell stage do develop into notochord.

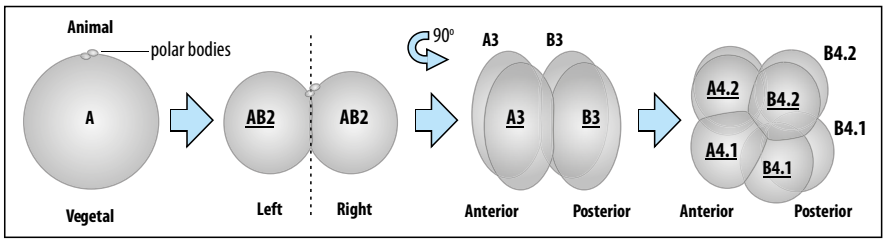


Fig. S5.5 A binary choice between notchord and neural fates in ascidian embryos. The embryo is viewed from the vegetal pole, in the same orientation as shown in the lower panel in Fig. S5.3. Anterior is up. All notochord cells are derived from blastomeres A4.1 and B4.1, formed at the eight-cell stage. In the A lineage, the notochord derives from an asymmetric division of the A6.2 and A6.4 mother cells at the late 32-cell stage. These cells were formed from A4.1 by two successive rounds of cell division. At the 44-cell stage, A6.2 and A6.4 divide along their antero-posterior axis to give rise to one (posterior) notochord precursor each (red, A7.3 and A7.7) and one (anterior) neural precursor each (blue, A7.4 and A7.8). Each precursor cell then divides in the medio-lateral plane to generate four notochord precursors and four neural precursors in the 110-cell embryo. *After Picco, V., et al.:* ***Ephrin-Eph signalling drives the asymmetric division of notochord/neural precursors in Ciona embryos.*** *Development 2007.* ***134****: 1491–1497.*

The specification of the notochord precursor cells of the A lineage provides a good example of how a pattern of asymmetric cell division, together with signals from neighboring cells, can allocate daughter cells to different germ layers. Blastomeres A7.3 and A7.7 in the 64-cell embryo give rise to notochord only, whereas their sister cells, A7.4 and A7.8, respectively, give rise to spinal cord. (This applies also to the corresponding cells on the right-hand side of the embryo. We will follow events on just one side for simplicity.) The division that creates these cells with very different fates occurs between the 32-cell and 44-cell stage (Fig. S5.5). When A6.2 and A6.4 in the 32-cell embryo divide, the posterior daughter of each division, the one adjacent to the vegetal blastomeres contributes to the notochord. The anterior daughter, adjacent to animal cap cells, gives rise to neural tissue (spinal cord).

As we saw with the asymmetric division of the *Caenorhabditis* EMS cell into an MS cell and an E cell (see Section 6.3), the assignment of notchord and neural fates to the daughters of A6.2 and A6.4 is the result of a polarization of the mother cells by intercellular signals followed by a functionally asymmetric division. The growth factor FGF can induce notochord *in vitro* and is probably the inducer of a notochord fate *in vivo*, as preventing FGF signaling in the early embryo prevents notochord formation. If the FGF signaling pathway is blocked, both the daughter cells of A6.2 and A6.4 are neural precursors. The acquisition of a neural fate requires contact with anterior animal cap cells and Eph–ephrin signaling, which suppresses the notochord fate. The involvement of Eph receptors and their ephrin ligands as signals that determine cell fate in the early embryo came as something of a surprise. In other chordates they are usually involved in adhesive cell–cell interactions, such as those that delimit rhombomere boundaries in the chick brain (see Section 11.4), and in the selective adhesive interactions that guide growing axons to their targets during nervous system development (see Section 11.16).

Although the early development of ascidians is very different from that of vertebrates, the presence of a notochord and its induction by vegetal cells is a striking parallel between the two groups. Moreover, the same genes are involved in notochord specification in both ascidians and vertebrates. The gene *Brachyury* is expressed in early mesoderm in vertebrates and then becomes confined to the notochord (see Section 4.12). Expression of the ascidian homolog of *Brachyury* is first detected at the 64-cell stage in the A-lineage precursors of the notochord, and this stage appears to

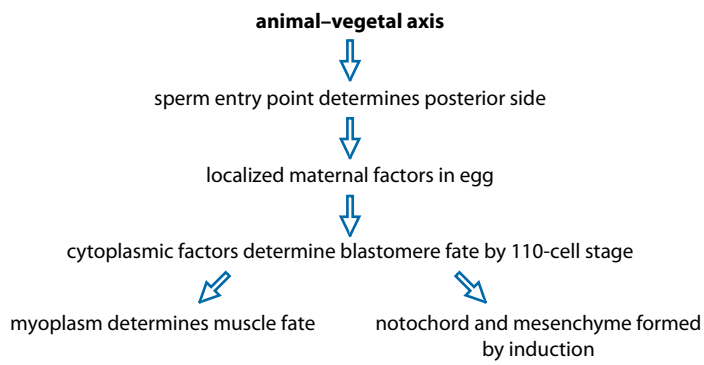
correspond with the time at which induction is complete. Ectopic expression of the ascidian *Brachyury* gene can transform endoderm to notochord. Thus, there appear to be similar mechanisms involved in notochord formation in all chordates.

Specification of mesenchymal precursors (which will give rise to internal tissues) from a mesodermal precursor also requires signals from adjacent cells. The mesenchyme precursors are located adjacent to the endodermal cells at the vegetal pole of the 110-cell embryo (see Fig. S5.4). When blastomeres are isolated at the 32-cell stage, all those blastomeres whose normal fate is mesenchyme develop into muscle. It appears that a signal from the endoderm, probably FGF, normally suppresses muscle formation in these blastomeres. In the absence of such signals they develop into muscle, as a result of the presence of maternal muscle determinants, such as Macho-1, within them. The interplay between intercellular signals and intracellular determinants in setting cell fate is complicated. Embryos lacking Macho-1 form notochord in place of mesenchyme, whereas the overexpression of *macho-1* causes the usual notochord precursors to develop as mesenchyme.

SUMMARY

Ascidians are members of the chordates, the same phylum as vertebrates, and show a mixture of mosaic development and cell-cell interactions. In the ascidians, there is evidence that localized cytoplasmic factors are involved in specifying cell fate, particularly of muscle, but that cell interactions are also involved. The notochord develops through a well-defined cell lineage but also requires induction. The ascidian homolog of the vertebrate gene *Brachyury*, which is involved in specifying the notochord in vertebrates, is expressed in the presumptive ascidian notochord after induction. The third type of mesodermal tissue in ascidian embryos, the mesenchyme, also requires inductive signals from neighboring cells.

SUMMARY: Ascidian early development



End of chapter questions

Long answer (concept questions)

1. What is the myoplasm of ascidian embryos? How is the myoplasm related to cortical rotation, and what tissue will it form?

2. Why are ascidians chordates, but not vertebrates? What similarities and differences exist between the formation of the notochord of ascidians and the notochord of amphibian or chick embryos?

Multiple choice (factual recall questions)

NB There is only one right answer to each question.

1. In the ascidian *Styela* the yellow-pigmented myoplasm marks cells fated to become muscle cells in the tail; however, at the molecular level, the key determinant of tail muscle seems to be:

a) expression of the *Brachyury* gene
b) induction of muscle by FGF
c) localization of β-catenin to the nuclei in the vegetal portion of the embryo
d) the gene product of the *macho-1* gene

2. The role of the *Brachyury* gene in ascidians is similar to that in *Xenopus* in that

a) *Brachyury* is the master-switch gene for muscle development on both organisms
b) *Brachyury* sets up the animal–vegetal axis
c) signaling that leads to induction of the mesoderm results in expression of the *Brachyury* gene
d) the *Brachyury* gene is expressed specifically in those cells that will form somites, and hence muscle

Answers: 1: d, 2: c

Further reading

Ascidians

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