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Specification of the Larval Axes in Tunicate Embryos

The axes of the tunicate larva are among its earliest commitments. Indeed, all of its embryonic axes are determined by the cytoplasm of the zygote *prior* to first cleavage (Sardet et al. 2007). The first axis to be determined is the dorsal-ventral axis, which is defined by the cap of cytoplasm at the vegetal pole. This vegetal cap is enriched for mitochondria, endoplasmic reticulum components, and specific maternal mRNAs (such as *macho-1*) that will be involved in cell specification. This vegetal cap prefigures the future dorsal side of the larva and the site where gastrulation is initiated (Bates and Jeffery 1988). When small regions of vegetal pole cytoplasm were removed from zygotes between the first and second waves of zygote cytoplasmic movement, those zygotes neither gastrulated nor formed a dorsal-ventral axis.

The anterior-posterior axis is the second axis to appear and is determined during the migration of the oocyte cytoplasm during fertilization. Microtubules originating from the sperm centrosome, followed by cortical actin microfilaments, cause the vegetal cap to become repositioned to what will be the posterior region of the embryo. This can be followed readily, since the yellow crescent forms in the region of the egg that will become the posterior side of the larva (see textbook Figures 2.3A and 11.18). When roughly 10% of the cytoplasm from this posterior vegetal region of the egg was removed after the second wave of cytoplasmic movement, most of the embryos failed to form an anterior-posterior axis. Rather, these embryos developed into radially symmetrical larvae with anterior fates. This posterior vegetal cytoplasm (PVC) is “dominant” to other cytoplasms in that when it was transplanted into the anterior vegetal region of zygotes that had their own PVC removed, the anterior of the cell became the new posterior, and the axis was reversed (Nishida 1994).

The specification of the left-right axis appears to involve the *Nodal* gene, just as it does in snails, sea urchins, and vertebrates (Morokuma et al. 2002; Yoshida and Saiga 2008). Although the first cleavage divides the tunicate embryo into symmetrical right and left halves, this symmetry is broken before the embryo hatches from its chorion. In the tunicate neurula, the *Nodal* gene is expressed only on the left side, and the brain vesicle and sensory pigment cells are located only on the right side of the brain. The asymmetric expression of *Nodal* appears to result from the rotation of the neurula-stage embryo within its vitelline envelope (Nishide et al. 2012).

When seen from the posterior, the tunicate neurula rotates along the anterior-posterior axis in a counterclockwise direction. When the rotation ceases, the future left portion of the embryo is downward, where the left epidermis touches the vitelline envelope. This contact causes *Nodal* expression in the left (but not the right) epidermis. When neurulae were sandwiched between two pieces of vitelline envelope such that both the right and left sides of the neurula touched the envelope, both sides showed *Nodal* expression and the right-left asymmetry of the brain structures was randomized. It therefore appears that juxtacrine signals from the vitelline envelope induce *Nodal* expression in the neurula, thereby creating left-right asymmetries.

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