<Further Development 10.12>

Effects of the Dorsal Protein Gradient

What does the Dorsal protein do once it is located in the nuclei of the ventral cells? A look at the fate map of a cross section through the *Drosophila* embryo at the division cycle 14 shows that the 16 cells with the highest concentration of Dorsal are those that generate the mesoderm (textbook Figure 10.27). The next cell up from this region generates the specialized glial and neural cells of the midline. The next two cells give rise to the ventrolateral epidermis and ventral nerve cord, and the nine cells above them produce the dorsal epidermis. The most dorsal group of six cells generates the amnioserosal covering of the embryo (Ferguson and Anderson 1991). This fate map is generated by the gradient of Dorsal protein in the nuclei. Large amounts of Dorsal instruct the cells to become mesoderm, whereas lesser amounts instruct the cells to become glial or ectodermal tissue (Jiang and Levine 1993; Hong et al. 2008).

The first morphogenetic event of *Drosophila* gastrulation is the invagination of the 16 ventralmost cells of the embryo to create the ventral furrow. All of the body muscles, fat bodies, and gonads derive from these mesodermal cells (Foe 1989). Dorsal protein specifies these cells to become mesoderm in two ways. First, the protein activates specific genes that create the mesodermal phenotype. Five of the target genes for the Dorsal protein are *twist*, *snail*, *fgf8, fgf8 receptor*, and *rhomboid*. These genes are transcribed only in nuclei that have received high concentrations of Dorsal, since their enhancers do not bind Dorsal with a very high affinity (Thisse et al. 1988, 1991; Jiang et al. 1991; Pan et al. 1991). Both Snail and Twist are also needed for the complete mesodermal phenotype and proper gastrulation (Leptin et al. 1991b). The Twist protein activates mesodermal genes, whereas the Snail protein represses particular non-mesodermal genes that might otherwise be active. The combination of Snail and Twist transcription factors in the future mesoderm cells also induces myosin contractile proteins to accumulate at the apical ends of the mesoderm cells. This localization enables the future mesoderm to respond to random wavelike contraction events by invaginating into the embryo (Pouille et al. 2009).

The *rhomboid* and *fgf8* genes are interesting because they are activated by Dorsal but repressed by Snail. Thus, *rhomboid* and *fgf8* are not expressed in the most ventral cells (i.e., the mesodermal precursors) but are expressed in the cells adjacent to the mesoderm. These *rhomboid*- and *fgf8*-expressing cells will become the mesectoderm. The mesectoderm tissue is fated to become the ventral midline, once the mesoderm invaginates and brings these ventrolateral regions together. This mesectoderm gives rise to glial cells and to the midline structures of the central nervous system. Unlike the neurogenic ectoderm adjacent to it, the mesectoderm cells never form typical neuroblasts, never form epidermis, and are not a stem cell population.

The high concentration of Twist protein in the nuclei of the ventralmost cells activates the gene for the Fgf8 receptor (the product of the *heartless* gene) in the presumptive mesoderm (Jiang and Levine 1993; Gryzik and Müller 2004; Strathopoulos et al. 2004). The expression and secretion of Fgf8 by the presumptive neural ectoderm is received by its receptor on the mesoderm cells, causing these mesoderm cells to invaginate into the embryo and flatten against the ectoderm.

Meanwhile, *intermediate* levels of nuclear Dorsal activate transcription of the *Short gastrulation* (*Sog*) gene in two lateral stripes that flank the ventral *twist* expression domain, each 12–14 cells wide (François et al. 1994; Srinivasan et al. 2002). *Sog* encodes a protein that prevents the ectoderm in this region from becoming epidermis and begins the processes of neural differentiation. At gastrulation, when the mesoderm (at the most ventral region) invaginates into the embryo, the *Sog*-expressing cells become the most ventral cells.

Dorsal protein also determines the mesoderm indirectly. In addition to activating the mesoderm-stimulating genes (*twist* and *snail*), it directly inhibits the dorsalizing genes *zerknüllt* (*zen*) and *decapentaplegic* (*dpp*). Thus, in the same cells Dorsal can act as an activator of some genes and a repressor of others. Whether Dorsal activates or represses a given gene depends on the structure of the gene's enhancers. The *zen* enhancer has a silencer region that contains a binding site for Dorsal as well as a second binding site for two other DNA-binding proteins. These two other proteins enable Dorsal to bind a transcriptional repressor protein (Groucho) and bring it to the DNA (Valentine et al. 1998). Mutants of *dorsal* express *dpp* and *zen* genes throughout the embryo (Rushlow et al. 1987), and embryos deficient in *dpp* and *zen* fail to form dorsal structures (Irish and Gelbart 1987). Thus, in wild-type embryos, the mesodermal precursors express *twist* and *snail* (but not *zen* or *dpp*); precursors of the dorsal epidermis and amnioserosa express *zen* and *dpp* (but not *twist* or *snail*). Glial (mesectoderm) precursors express *twist* and *rhomboid*, while the lateral neural ectodermal precursors do not express any of these five genes (Kosman et al. 1991; Ray and Schüpbach 1996). By the cellular responses to the Dorsal protein gradient, the embryo becomes subdivided from the ventral to dorsal regions into mesoderm, neurogenic ectoderm, epidermis (from the lateral and dorsal ectoderm), and amnioserosa.

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