22 The Molecular Cell Biology of Development

Review the Concepts

1. Two examples are given in this chapter. Nuclear-transfer cloning is one example. In this procedure, the nucleus of a differentiated adult somatic cell is transferred into an enucleated egg. Occasionally, the egg develops to form an embryo that can be implanted into a foster mother. The cloned sheep, Dolly, is an example. This procedure shows that, in at least some cases, differentiated adult somatic cells have all the genetic information needed to develop into a fully functional adult. (There are, however, frequent failures in this procedure.) In the other example, post mitotic olfactory sensory neurons were genetically marked with green fluorescence protein (GFP) and then used as donor of nuclei implanted into enucleated mouse oocytes. Some of the oocytes developed into blastocysts that produced GFP. Cells from the blastocysts were used to derive embryonic stem cell lines, which were then used to generate mouse embryos and eventually healthy mice. This experiment demonstrated that the genome of a differentiated cell can be reprogrammed completely to form all the tissues of a mouse.

2. In *Drosophila,* both Spatzle and Dpp proteins play critical roles in determining the dorsal/ventral axis. Spatzle sets off a signal-transduction pathway that eventually leads to Dorsal protein accumulation in the nuclei of ventral cells. In ventral nuclei, Dorsal turns on expression of genes that induce ventral cell fates. Dorsal also represses Dpp. In the dorsal side of the embryo, no Dorsal protein is present, so Dpp is expressed and dorsal cell fates are induced. In *Xenopus*, the TGF family members BMP2 and BMP4 have similar effects to those of the *Drosophila* Dpp protein. One difference in *Xenopus* dorsal/ventral axis specification is that the axis is flipped in vertebrates as compared with invertebrates.

3. *Dorsal* mRNA would be expressed in all areas of the syncytial embryo. Dorsal protein is also expressed in all regions of the embryo, but accumulates only in the ventral side nuclei, where this protein acts to repress genes whose expression is needed for dorsal specification (i.e., *dpp*).

4. Microarray analysis reveals patterns of gene expression for many genes. If wild-type and *dorsal* mutant embryos were compared, the results would yield a wild-type gene-expression pattern that could be compared to the pattern when the Dorsal protein is not expressed. The only difference in the types of embryos is the presence/absence of Dorsal, so differences in the two expression patterns would reveal genes regulated by Dorsal, either directly or indirectly. The genes we would expect to see increased when *dorsal* gene function is lost are *dpp, tolloid, short gastrulation,* and *zerknullt*, since Dorsal normally represses these genes. The genes we would expect to see decreased when dorsal gene function is lost are *twist, snail,* and *rhomboid*, since Dorsal normally activates expression of these genes.

5. *Bicoid* RNA localization at the anterior end produces a Bicoid protein gradient. This localization is dependent on the *bicoid* 3′ UTR. If this 3′ UTR regulatory sequence were removed, *bicoid* RNA and protein would NOT be localized at the anterior end, so anterior structures would not be exclusive to this region. Instead, formation of anterior structures would be expanded to occupy a greater proportion of the embryo.

6. The five gap genes are expressed in specific spatial domains (i.e., broad overlapping stripes) and function to regulate one another. This complexity creates combinations of transcription factors that can lead to the development of more than five cell types.

7. Somites form after the embryo's three axes have been established. Four signaling systems are involved: an FGF signal, retinoic acid, and Wnt and Notch signaling. Somite development depends on concentration gradients and interactions between the signals.

8. Homeosis is the development of an organ or body part that has the characteristics of another organ or body part. There are three main flower homeotic mutants. Mutants in A function contain carpels instead of sepals, and stamens in place of petals. A genes thus function to specify sepals and part of petal identity. Mutants in B function contain sepals instead of petals, and carpels in place of stamens. B genes thus function to specify part of petal identity and part of stamen identity. Mutants in C function contain petals instead of stamens, and sepals in place of carpels. C genes thus function to specify part of stamen identity and all of carpel identity.

9. Regulatory regions of some of the Hox genes contain binding sites for their corresponding proteins. In this way, Hox proteins can maintain their own expression through an autoregulatory loop. Another mechanism for maintaining Hox gene expression involves proteins such as Trithorax proteins and Polycomb proteins, which modulate chromatin structure. (Polycomb complexes act to repress Hox gene expression; Trithorax proteins maintain Hox gene expression.)

10. Neuron formation was inhibited when Notch mRNA was injected into *Xenopus* embryos during primary neurogenesis. Researchers also injected mRNA for an altered form of Delta that prevents Notch activation. In this case, too many neurons were formed.

11. Antibodies to Sonic hedgehog (Shh) protein block the formation of different ventral neural-tube cells in the chick. This shows that Shh is required for ventral neural-tube cell development. In addition, different concentrations of Shh were added to chick neural-tube explants. These different concentrations induced development of different types of cells.

12. Implantation of a bead soaked in FGF10 in a developing embryo can cause an extra limb to grow even where limbs do not normally form. Mouse mutants that lack FGF10 develop without limbs.

13. Synpolydactyly is caused by a Hox gene mutation, specifically Hox D13. This gene normally becomes active as limb buds grow during the period 9–10.5 days after birth and remains active during limb development.

Analyze the Data

a. The insertion of a transposable element appears to affect production of *oskar* mRNA; either the mRNA is not made or it is unstable and degrades. The mRNA is present in the nonsense mutant. The absence of an *oskar* band in lane 3 is not the result of lack of mRNA loaded on the gel because the rp49 probe gives a strong signal.

b. Oskar function appears to be required twice; early, to complete oogenesis and then again later, to specify posterior parts. Since the early function does not occur in the *oskX* mutant females that lack *oskar* mRNA but does occur in the *oskNS* mutants that make *oskar* mRNA but no Oskar protein, oogenesis may require *oskar* mRNA rather than Oskar protein.

c. Expression of the *oskar* transgene in *Drosophila* females lacking a functional copy of the *oskar* gene results in rescue of *Drosophila* oocyte development, as long as the transgene encodes the 3′ UTR of the *oskar* mRNA. Accordingly, these finding suggest that there is a function for *oskar* mRNA in oocyte development that is separate from the function of the Oskar protein and that the former function localizes to the 3′ untranslated domain of the message. Development of the posterior parts of the embryo requires Oskar protein, which is made in constructs 1 and 2 but not 4.