CHAPTER 10

1) In *Drosophila*, the identity of each segment and the structures that it will form are determined by the Hox genes which are expressed in overlapping patterns along the anteroposterior axis. The embryo is a collection of segments which would be patterned in the same manner were it not for the Hox genes. Mutant analysis suggests that the second thoracic segment is the baseline, the segment which will develop if no Hox genes are expressed. Then, for example, each pair of legs is different and results from the expression of a different Hox gene. The second pair of legs is under the control of Antennapedia and misexpression of Antennapedia in the head leads to the appearance of a leg with the characteristics of the second thoracic segment.
2) The most important signal downstream of Engrailed, associated with pattern formation, is the signalling molecule Hedgehog in both the embryo and the wing imaginal disc. In both instances Hedgehog signals to adjacent cells and affects their patterns of expression and the patterning they produce. Important effects on pattern are mediated by the maintenance of the expression of morphogens in adjacent cells. In the embryo, this is Wingless and in the wing disc, it is Dpp.
3) The product of *wingless* is a secreted signal, whereas the products of *apterous* and *vestigial* are transcription factors. Loss of function of any of these genes results in the absence of a wing but their molecular activities and patterns of expression are different which means that the way they influence the development of the wing is different. Wingless acts on adjacent cells to define a primordium from which the wing will develop. Apterous and Vestigial act on the cells they are expressed in i.e. they are cell autonomous. Vestigial is expressed throughout the wing primordium and is under the control of Wingless; so, it could be said that Vestigial executes the activity of Wingless. Apterous is different as its expression is restricted to the dorsal cells in the wing disc and the phenotype, the same as wingless and vestigial mutants suggests that the development of the wing requires interactions between dorsal and ventral cells, specifically at their boundary.
4) Implantation of a bead soaked in FGF protein to the interlimb region of the flank of an early chick embryo. Include the transcription factors Tbx4/Tbx5 and Pitx1 in the pathway and FGF10 as the ligand that normally initiates limb development.
5) The apical ectodermal ridge rims the distal tip of the developing limb bud extending from anterior to posterior and lies at the boundary between dorsal and ventral ectoderm. See Fig. 10.20 and 10.21for different views of the apical ectodermal ridge which will help you to appreciate its disposition in three-dimensions.
6) Removal of the apical ridge leads to limb truncations. The extent of truncation depends on the time at which ridge is removed, with more severe truncations resulting when the ridge is removed earlier in development. These results suggest that the structures of the limb are laid down in a proximo-distal sequence.
7) Models are shown in Fig.10.30. FGF signaling from the apical ectodermal ridge acts either to maintain a region of undifferentiated cells at the tip of the limb bud in which a timing mechanism operates or to specify distal positional values depending on the model.
8) The polarizing region is at the posterior margin of the limb bud and its properties were revealed by grafting experiments in chick wing buds. Sonic hedgehog is the signaling molecule. Evidence includes embryological experiments in chick embryos, and genetic experiments in mice. See also additional material in the on-line *in silico* practical on chick wing development.
9) Gli3 is one of the three Gli transcription factors in the intracellular pathway that transduces the Sonic Hedgehog signal. It can function as either a transcriptional activator or a transcriptional repressor with processing to a repressor taking place in absence of Shh. Include gradient of Gli3A/Gli3R across the

antero-posterior axis of the limb bud and specification of digit identity, gene targets identified by chromatin immunoprecipitation- Hox genes, *Bmp2*- , also *Tbx3*.

10) Dorso-ventral pattern of structures developing from the limb bud mesoderm is reversed suggesting that signals from the ectoderm control pattern formation across this axis of the limb. Include Wnt signaling by dorsal ectoderm and function of the transcription factor, Engrailed, expressed in ventral ectoderm. Discuss evidence from genetic experiments in mice. Could also include function of Lmx1 in the discussion of specification of dorsal pattern.
11) Wnt signaling from dorsal ectoderm and FGF signaling from apical ectodermal ridge. The positive feedback loop in which Shh maintains expression of *Fgf* genes in the ridge includes BMP antagonist Gremlin.
12) Cells that will form muscle migrate into the limb bud from the adjacent somites. Experiments in which future neck somites are grafted opposite the position in which the limb buds will develop show that muscle cells are equivalent.
13) Include expression domains of homeobox genes in the jaw, FGF and BMP signaling, the effects of experimentally manipulating BMP signaling and the results of genetic experiments in mice.
14) Interactions involve retinoic acid, hedgehog, Wnt and BMP signaling (see Fig. 10.46). In a protocol to generate lung cells, the iPS cells would first have to be specified as endoderm (see Chapter 4) followed by sequential treatment with retinoic acid and then Wnts.
15) The branching pattern of the mouse lung is shown in Fig. 10.45. Answer should include discussion of the roles of FGF and Shh signaling in lung branching and mechanisms to prevent excessive branching. Could mention parallels with early branching of the *Drosophila* larval respiratory system (see Chapter 7, Section 7.17).
16) Include description of development of all three layers as indicated in question. Epicardium covers the outside of the heart and gives rise to the connective tissues supporting the myocardial cells and the endothelial cells of the coronary arteries. See further discussion of importance of epicardial-myocardial interactions in heart regeneration and the signals involved in Chapter 12.
17) Review the transcription factors involved in specifying development of the heart and their functions. Have any of the genes encoding these transcription factors been shown to be mutated in human patients with congenital heart defects? What does the fact that these conditions are syndromes signify?
18) *Pax6* encodes a transcription factor and is the 'master' gene for eye development. Include discussion of function of *Drosophila eyeless* gene and its relationship to *Pax6*. What are the effects of expressing mouse *Pax6* in a *Drosophila* antennal disc? Patients heterozygous for *Pax6* have aniridia – see definition in glossary.