Section III

SIGNALS AND SWITCHES IN LINEAGE SPECIFICATION, TISSUE DIFFERENTIATION, AND ORGANOGENESIS

MOLECULAR DRIVERS OF LINEAGE SPECIFICATION

A key milestone of early postimplantation mouse development is the generation of the primary germ layers (ectoderm, mesoderm, and endoderm) from the epiblast during gastrulation. Epiblast cells that are allocated to the germ layers display a progressive restriction in lineage potency, which is accompanied by the dismantling of the pluripotency genetic network and the acquisition of lineage characteristics by the activation of tissue-specific genes. The cells remaining in the epiblast are still multipotent. They display considerable plasticity in lineage fates and are able to generate self-renewing stem cells with full germ layer potency in vitro.

The specification of lineage progenitors is accompanied by switches in the activity of the genome. This is elicited by the action of genetic determinants that activate lineage-specific programs and repress other gene activities that oppose differentiation. For example, the formation of germ cell progenitors (the primordial germ cells) entails the activation of germline-specific genes and concurrent suppression of somatic cell molecular activity (Chapter 15). Superimposed on these transcriptional activities are the epigenetic modulators that activate or silence genome activity through their effects on chromatin conformation and the chemical modification of the DNA. The transcriptional network is further integrated with the activity of the regulatory RNAs, which influence the processing and utilization of RNA transcripts. Progenitors of different cell or tissue lineages vary in the scope of their differentiation potential. Some are restricted in the type of cells they can generate, such as primordial germ cells that produce only one type of cells, the gametes; satellite cells that give rise only to myocytes; mesenchymal progenitors that produce either white or brown adipocytes (Chapter 19); and stem cells residing in different niches in the skin that, under normal circumstances, replenish specific epidermal cell types (Chapter 18). Some progenitors give rise to a few cell types, such as the bipotential cells that can generate hepatocytes and cholangiocytes and the radial glial cells that produce neuronal cells (although there are many subtypes of neurons in the brain) and glial cells (Chapter 17). Interestingly, radial glial cells display different lineage potential during cortex development, generating first the neuronal cells and then switching to the astrocytes. Other progenitors, such as neural crest cells and hematopoietic stem cells, can differentiate into a wider range of cell types and are therefore regarded as multipotent. Neural crest cells can differentiate into many types of cells, including neural ganglionic cells, supporting cells of the neurons, pigment cells, bone cells, and others. Hematopoietic stem cells can generate all types of blood cells. Of note is that both of these cell types shift successively from being multipotent to unipotent as they transit the linear hierarchy of differentiation steps (Chapters 14 and 16).

Analysis of the gene expression profiles of multipotential progenitors has revealed that lineage-specific genes can be expressed simultaneously with genes that maintain the potency. These findings underpin the concept that progenitor cells are molecularly poised to embark upon differentiation but are held back by the activity of potency-regulating genes. Cells may also be maintained in the progenitor stage by the competition among opposing lineage-specific transcription factors for a rate-limiting amount of cofactors or through negative physical interaction. In this model, lineage differentiation is initiated by tipping the balance of competing genetic activity in favor of a particular lineage pathway. In the course of differentiation,

genetic switches operate at every branching point of lineage choices. Such switches may be brought about by the integrated feedback and feed-forward regulatory mechanisms that activate the gatekeeping genes and maintain a stochastic output of dynamic transcriptional activity (Chapters 14, 16, and 17). The activity of signaling molecules mediated by membrane-bound receptors and intracellular transducers intersects with the genetic network and provides an extrinsic force that drives lineage differentiation. During lineage differentiation, the main role of these extrinsic signals is to instruct the execution of the transcriptional program that influences the viability, multiplication, migration, and maturation of the derived cell types. For the germ cell lineage, formation of the primordial germ cells is facilitated by WNT signaling, which may prime the epiblast cells to respond to induction by BMP to kick-start the cascade of transcriptional activity (Chapter 15). During the differentiation of endothelial cells into angioblasts and lymphatic progenitors, the signaling activities of BMP, WNT, Notch, and VEGF regulate the expression cell-type-specific transcription factors to promote cell chemotaxis, choice of tip and stalk cell fates, and differentiation into acinar versus duct cells (Chapter 20). In the cortex, radial glial cells, which are undergoing neurogenesis specified by WNT and Notch signaling, switch to gliogenesis when they are acted on by a combination of FGF, BMP, Notch, and Jak-Stat signals (Chapter 17). In neural crest cells, activation of the neurogenin genes for sensory neuron differentiation and the choice between osteocytes and chondrocytes via differential activation of Sox9 and Runx2 are also influenced by WNT signals (Chapter 16). A complex combination of signaling activity is associated the differentiation of bone, cartilage, and muscle lineages. In chondrocyte and osteocyte differentiation, Sox9-Runx2 and Osterix transcription are coupled with the activity of secreted factors including IHH, PTHrP, BMP, WNT, and FGF (Chapter 22). In myogenesis, the activation of the Six/Pax/Myf/MyoD/Mrf hierarchy of transcription factors is associated with the combinatorial activities of WNT, SHH, Notch, and BMP (Chapter 21). During the differentiation of hematopoietic stem cells, a series of intermediate progenitor cells for different types of blood cells are generated in response to niche-related cytokines and common signals from WNT, Notch, and SHH (Chapter 14). Differentiation of adipocytes, however, is subject to the modulating effect of proadipogenic endocrine factors and inhibitory cytokines that are unique for driving adipogenesis (Chapter 19). The consensus of these studies is that the choice of cell fates is likely to be accomplished by the activation of lineage-specific transcription activity, which is regulated and enhanced by extrinsic signals.

ORGAN FORMATION: MOLECULAR CONTROL AND COMPLEXITY OF MORPHOGENETIC PROCESS

Organogenesis begins with the formation of the organ primordium, which is made up of progenitors of the essential cell types that constitute a functional organ. The complexity of the cellular composition and the germ-layer origin of the progenitors varies among the organ primordia: The thyroid, liver, and pancreatic buds are generated by local proliferation of the endoderm of the embryonic foregut; the eye primordium is formed by the juxtaposition of two ectoderm derivatives, the optic cup and the lens placode; and the ureteric bud and the lung bud also contain two cell types, but they are from different germ layers, an endoderm-derived epithelial outgrowth and the investing mesenchyme from the mesoderm.

During organ formation, cells in the primordium undergo histogenesis to generate the necessary types of tissues and build them into the appropriate architecture for the organ. This may involve epithelium to mesenchyme transition, followed sometimes by condensation of cells into a tissue mass that becomes segregated from other tissues. The transition from mesenchyme to epithelium also occurs to organize cells into an epithelium, which then acquires a more complex morphology through multilayering or folding, or is transformed into luminal, tubular, and branching structures (see also Section II). The mechanistic detail and the role of these morphogenetic processes in organ formation are reviewed in chapters on the development of tooth (Chapter 23), eye (Chapter 24), inner ear (Chapter 25), heart (Chapter 26), lung (Chapter 27), pancreas (Chapter 28), liver (Chapter 29), and kidney (Chapter 30). Among the organs, the heart displays the most complex morphogenetic activities, culminating in asymmetrical looping of the heart tube, chamber formation, septation of cavities, valvular formation, and shaping of the outflow tract (Chapter 26). Next are the kidney and the lung, both of which are noted for the complex branching morphogenesis occurring during organ formation. For example, in the kidney, nephrons are formed in a coordinated process of inductive interaction, tissue condensation, mesenchyme to epithelium transition, and tubulogenesis (Chapter 30). In contrast, there is a stereotypic pattern of asymmetric branching in the lung and the generation of different cell types along the proximal–distal axis of the alveolar pathway (Chapter 27).

The formation of an organ is intimately associated with the establishment of the vascular and lymphatic supply, which is an integral part of the organs throughout life (Chapter 20). The foremost function of the blood and lymphatic system is to provide vital trophic support for

the growth, morphogenesis, and maintenance of organs. The vascular tissue is also a source of signaling activity that maintains stem/progenitor cells (e.g., neural, spermatogonial, and hematopoietic stem cells) and influences the differentiation of cells such as the hypertrophic chondrocytes, acinar and trunk epithelial cells of the pancreas, and the branching process of the respiratory tree (Chapters 22, 27, and 28).

During organ formation, tissue differentiation and patterning are primarily driven by the activity of organ-specific transcription factors, chromatin modifiers, and specific epigenetic factors such as noncoding regulatory RNAs. In the pancreas, bud formation, the transition through morphogenesis and differentiation of the acinar and ductal tissues, and the formation of the islets and the generation of glucagon- versus insulin-producing cells are associated with the transcription of sets of genes (Chapter 28) that are different from those of the liver, another endoderm organ from the foregut (Chapter 29).

Superimposed on the genetic control of organogenesis is the temporal and spatial input of extrinsic signaling activity. For example, the Nkx-Gata-Tbx-Srf transcriptional activity in cardiac cells is regulated by BMP and WNT, and the Pax-Eya-Six-Dach activity in the kidney cells is influenced by GDNF/RET, RTK, and WNT. Although the same repertoire of signaling activity is deployed during the formation of many organs, different outcomes are achieved through the control of time of delivery and the level of signal, the ability of the target cells to respond to the signals, and the influence of concurrent signaling activities (Chapters 21, 23, 27, and 30). Regionalized signaling activity in embryonic structures, such as the optic cup, the early foregut, the pancreatic tubule, and the epithelial tube of the lung bud, predisposes the type of tissue that may be generated in a specific tissue domain (Chapters 24, 27, 28, and 29), whereas in the cochlea, localized Notch and BMP signaling governs the formation of different types of hair cells (Chapter 25). The knowledge of the timing and the tissue-specific intersections of genetic and signaling activities during organogenesis that is gleaned from these studies and the development of the enabling technology for directed cell differentiation are the two key elements of the jigsaw puzzle for devising treatment paradigms of cell-based therapy for tissue and organ repairs.

Patrick P.L. Tam