somatic cells. However, more strict criteria and rigorous validation are required to establish tissue stem cell plasticity. For example, observations of transdifferentiation may reflect cell fusion, contamination with progenitor cells from other cell lineages, or persistence of pluripotent embryonic cells in adult organs. Therefore, the assignment of potency to each cultured stem cell in Fig. 88-1 should be considered with caution. Whether transdifferentiation exists and can be used for therapeutic purposes remains to be determined conclusively. A similar, but distinct, concept is the facultative stem cell, which is defined as a unipotent cell or a terminally differentiated cell that can function as a stem cell upon tissue injury. The presence of such cells has been proposed for some organs such as liver, intestine, pancreas, and testis, but is still debated.

Directed Differentiation of Stem Cells Pluripotent stem cells (e.g., ES and iPS cells) can differentiate into multiple cell types, but in culture, they normally differentiate into heterogeneous cell populations in a stochastic manner. However, for therapeutic uses, it is desirable to direct stem cells into specific cell types (e.g., insulin-secreting beta cells). This is an active area of stem cell research, and protocols are being developed to achieve this goal. In any of these directed cell differentiation systems, the cell phenotype must be evaluated critically. Alternatively, the heterogeneity of the cell population derived from pluripotent stem cells can be actively exploited, as different types of cells interact with each other in culture and further enhance their own differentiation. In some instances, e.g., optic cup, self-organizing tissue morphogenesis has been demonstrated in 3D culture.

MOLECULAR CHARACTERIZATION OF STEM CELLS

Genomics and Proteomics In addition to standard molecular biological approaches, high-throughput genomics and proteomics have been extensively applied to the analysis of stem cells. For example, DNA microarray analyses have revealed the expression levels of essentially all genes and identified specific markers for some stem cells. Chromatin immunoprecipitation coupled with next-generation sequencing technologies, capable of producing billions of sequence reads in a single run, has revealed chromatin modifications ("epigenetic marks") relevant to stem cell properties. Similarly, the protein profiles of stem cells have been assessed by using mass spectrometry. These methods are beginning to provide a novel means to characterize and classify various stem cells and the molecular mechanisms that give them their unique characteristics.

ES Cell Regulation It is important to identify genes involved in the regulation of stem cell function and to examine the effects of altered gene expression on ES and other stem cells. For example, core networks of TFs such as *Pou5f1* (*Oct4*), *Nanog*, and *Sox*2, govern key gene regulatory pathways/networks for the maintenance of self-renewal and pluripotency of mouse and human ES cells. These TF networks are modulated by specific external factors through signal transduction pathways, such as leukemia inhibitory factor (*Lif*)/*Stat3*, mitogen-activated protein kinase 1/3 (*Mapk1/3*), the transforming growth factor β (TGFβ) superfamily, and *Wnt*/glycogen synthase kinase 3 beta (*Gsk3b*). Inhibitors of *Mapk1/3* and *Gsk3b* signaling enhance the derivation of ES cells and help maintain ES cells in full pluripotency ("ground" or "naive state"). Recent data also indicate that 20–25 nucleotide RNAs, called microRNAs (miRNAs), play an important role in regulating stem cell function by repressing the translation of their target genes. For example, it has been shown that miR-21 regulates cell cycle progression in ES cells and miR-128 prevents the differentiation of hematopoietic progenitor cells. These types of analyses should provide molecular clues about the function of stem cells and lead to a more effective means to manipulate stem cells for future therapeutic use.