21 Cell Birth, Lineage, and Death

Review the Concepts

1. By definition, a stem cell divides to give rise to a copy of itself and to a differentiated cell or a cell capable of differentiating into multiple cell types, such as a mutipotential progenitor cell. Totipotential stem cells can give rise to every tissue in an organism. Pluripotent stem cells give rise to multiple, but not necessarily all, cell types. Progenitor cells give rise to more than one cell type but, unlike stem cells, do not self-renew.

2. In plants, stem cells are located in meristems, such as shoot apical meristems (SAMs) and floral meristems. In adult animal cells, stem-cell populations are thought to exist in low numbers in many organs including skin, intestine, and bone marrow. SAMs in plants are embryo-like in their concentration of totipotent stem cells. However, stem cells are difficult to purify from adult animals, and the only totipotent stem cells found in animals are in very early stage embryos.

3. Because Dolly was derived from an egg containing a nucleus from an adult, differentiated cell, we know that differentiated nuclei (at least adult mammary cells) have the potential to dedifferentiate and become totipotent. Since Dolly was derived from a differentiated nucleus placed into an egg and not from an intact, differentiated cell, we can conclude nothing about the ability of a differentiated cell to become totipotent. Other than the nucleus, the organelles, including mitochondria, which also contain genetic material, were derived from a germ cell. Differentiation of cells is maintained by cytoskeletal structures, organelles that confer cell properties, particular modifications of key regulatory proteins, and accessibility of regulatory genes in the chromatin. The Dolly experiment best indicates that the chromatin of differentiated nuclei can be remodeled from a differentiated to a totipotent state.

4. Because *C. elegans* consists of a small, invariant number of cells, it has been possible to generate a fate map of every cell from the fertilized egg to adulthood. *C. elegans* is also very amenable to genetic manipulation. Therefore, it is possible to alter the expression of specific genes and then determine the effect of this manipulation on cell division, cell differentiation, and cell death. Because many differentiation pathways are highly conserved between *C. elegans* and mammals (e.g., the apoptotic pathway) much of the information derived from studies in *C. elegans* can be applied by analogy to mammalian systems and homologous genes can be discovered.

5. To use retrovirus infection for tracing cell lineage, the viral genome is first engineered to produce a library of viruses that are defective so they can infect only once. Each virus in the library contains a unique DNA sequence. Any host cell infected by a single virus gives rise to a clone of cells that each carry that particular virus' unique DNA sequence. By using probes for that particular sequence, researchers can identify all of the cells that derived from the original infected cell.

6a. MCM1 binds efficiently to the P site of **a**-specific genes in **a** cells to promote transcription.

6b. MCM1 alone does not bind efficiently to the P site of α-specific genes in **a** cells.

6c. MCM1 binds efficiently to the P site of α-specific genes when α1 is bound to the adjacent Q site in cells.

6d. MCM1 bound to the P site complexed with α2 dimers bound to the adjacent α2 sites inhibits transcription of **a**-specific genes in cells.

7. **a** cells secrete only **a**-type mating pheromone and express only **α**-type pheromone receptor. α cells

secrete only α-type mating pheromone and express only **a**-type pheromone receptor. Therefore, each haploid cell is able to attract and respond only to cells of the opposite mating type.

8. Exposure of C3H 10T½ cells to 5-azacytidine is thought to induce muscle differentiation by incorporation of this compound, which cannot be methylated, into DNA. As a result, genes that previously had been inactivated by methylation are re-activated, and a different phenotype is expressed. The first step in isolating the genes involved in muscle differentiation was to demonstrate that DNA isolated from cells treated with 5-azacytidine, called azamyoblasts, could transform untreated C3H 10T½ cells into muscle. The mRNAs isolated from azamyoblasts were then converted to cDNAs and subjected to subtractive hybridization with mRNAs extracted from untreated cells. The azamyoblast-specific cDNAs then were used as probes to screen an azamyoblast cDNA library. The genes isolated by this procedure were tested for their ability to promote muscle differentiation by transfecting them into C3H 10T½ cells and assaying for the muscle protein myosin with an immunofluorescence assay.

9. MyoD is a member of the helix-loop-helix family of transcription factors.

9a. MyoD binds the E-box of target genes with tenfold higher affinity when dimerized with E2A.

9b. MEF homodimers synergize with MyoD-E2A dimers to induce transcription of target genes.

9c. Id contains a dimerization region but lacks a DNA-binding region. Id forms heterodimers with MyoD, preventing its association with DNA and thereby blocking its activity as a transcription factor.

10. MyoD = Achaete and Scute; myogenin = Asense; Id = Emc; E2A = Da. Injection of *MyoD* mRNA into *Xenopus* embryos should result in an increase in the amount of muscle tissue as measured by the expression of muscle-specific genes.

11a. Since HO endonuclease is required to catalyze allele switching, neither cell should be able to undergo mating-type switching.

11b. Constitutive expression of HO endonuclease should render both mother and daughter cells capable of mating-type switching.

11c. Since SWI/SWF is the transcription factor that induces expression of the HO gene, both mother and daughter cells should be capable of mating-type switching.

12. In *S. cerevisiae*, the myosin motor protein, Myo4p, localizes Ash1 mRNA to the bud that will form the daughter cell. In *Drosophila* neuroblasts, microtubules are required for assembly of the Baz/Par6/PKC3 protein complex at the apical end.

13. In mutant mice in which either neurotrophins or their receptors are knocked out, specific classes of neurons die by apoptosis. These results indicate that apoptosis occurs by default unless a specific extracellular signal is transduced to block the apoptotic program.

14. Apoptosis is characterized by cell shrinkage and DNA fragmentation. During necrosis, cells swell and lyse, inducing damage and inflammation in surrounding tissue. Although external signals such as TNF and Fas ligand induce apoptosis, the responding cell must still transduce the death signal through an intracellular pathway and induce its own death by activation of the caspase enzymes. The morphologic events of this death are indistinguishable from apoptosis triggered by an intrinsic pathway.

15a. The cell should undergo apoptosis even in the presence of trophic factors.

15b. The cell should not undergo apoptosis even in the absence of trophic factors.

15c. The cell should not undergo apoptosis even in the absence of trophic factors.

The mutations named in the question in both (b) and (c) could be found in cancer cells since either would block apoptosis even in the absence of trophic factors.

16. IAPs have zinc-binding domains that can bind directly to caspases and inhibit their protease activity, thus preventing apoptosis. SMAC/DIABLOs, a family of mitochondrial proteins, can bind to the zinc-binding domains in IAPs and prevent them from binding to caspases.

Analyze the Data

a. The lower panel in the text figure on page 946 shows a cell that appears to have differentially segregated most of its BrdU labeled DNA strands to only one of its two daughter cells. Because the older DNA strands would contain BrdU and the newer strands, synthesized during the 18-hour period in the absence of BrdU (chase), would not contain BrdU, this observation suggests that older (labeled) strands have been co-segregated to the lower daughter while the upper daughter received few or no detectable BrdU-labeled strands. In contrast, both daughter cells in the upper panel contain BrdU-labeled strands. The asymmetric partitioning of 40 labeled chromosomes, observed here to occur with a frequency of about 1.5 in 10^2 after only two divisions in the absence of BrdU, would be expected to occur by chance at a frequency of about $(1/2)^{39}$ or about 1.8 in 10^{12} (i.e., the loss of label from one of the two daughter cells after two divisions is occurring about 10 billion times more frequently than expected).

b. The cell that acquires most of the Numb also acquires the older (BrdU-labeled) DNA strands and thus may become the new stem cell. To determine if Numb is required for co-segregation of older DNA strands, one could deplete satellite cells of Numb by using a siRNA directed against Numb mRNA and then examining the progeny to determine if any cells exhibit co-segregation of older DNA strands.

c. The established cell line has none of the properties of stem cells and apparently has lost the ability to mark or detect the older DNA strands at each division and thereby co-segregate them preferentially to one of the two daughter cells. Only symmetric cell divisions, with random segregation of the DNA strands, would be expected.