PART I
CHAPTER 1

CELL FATES

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Cells develop phenotypes that are determined by organized and regulated molecular processes. Then diverse fates include proliferation, differentiation, and apoptosis. They proceed along several pathways of molecular signaling that are initiated by external factors, which activate cascades of kinases that bring these signals to the nucleus where they initiate transcriptions. These processes require an organized series of cellular and events in which numerous catalytic and regulatory proteins are involved, which in this book are discussed in detail.

PURPOSE AND ORGANIZATION OF THIS BOOK

A living cell can proceed along alternative pathways to a variety of destinations. These include proliferation to form two daughter cells, irreversible or reversible growth arrest, differentiation to a new type of cell as in development or metamorphosis, and death by necrosis or by programmed cell death (apoptosis). At any time the net number of cells is the result of a balance between proliferation and death. These cell fates may be changed in diseases such as the increased growth and decreased apoptotic death of cancer cells. And also they can be modified by drugs and other extracellular agents.

The purpose of this book is to summarize what has been learned about structural, biochemical, and molecular biological events that are the basis for these cell biological processes and their regulations. The emphasis is on vertebrate cells. Thousands of molecules and reactions have already been reported and organized into functional patterns that connect molecules with phenotypes (Fig. 1.1). In this and the next chapter are provided general concepts and underlying principles of regulation and structure. In the other chapters of this book are presented the mass of information, with references and illustrative examples.

CELL CYCLE BIOLOGY

As an example of a cell fate pathway we outline the general organization of the cell cycle and its biology and biochemistry. By this orderly process one cell grows into two. It is fundamental for the organism’s growth and for replacement of cells lost during normal wear and tear (Murray, 1993). Cells from a mature eukaryotic organism can require an interval of a day or more between successive divisions in tissue culture. During this time duplications of all of the myriad molecules that comprise each cell are required, at different times throughout the cycle. The most evident is duplication of deoxyribonucleic acid (DNA), the heredity-carrying material in chromosomes. DNA does not duplicate continuously, but only during several hours in midcycle, a period named the S phase for (DNA) synthesis. The cycle is organized, for simplicity, into a sequence of only four major biological and biochemical events which are grouped as gap 1 (G1 phase) during which a cell prepares for DNA synthesis, DNA synthesis (S phase), preparation for mitosis (G2 phase), and mitosis (M phase), after which the cell divides and the cycles of the two new cells can commence. For a historical summary, see Baserga (1985).

Figure 1.1. Cell molecular and information transfer. The central path of information flow from DNA to cell functions is regulated by feedbacks, indicated on the left. Syntheses from precursors are counterbalanced by degradations, as indicated on the right.
Quiescence

Most cells in vivo are performing their specialized functions in support of the whole organism. They are quiescent (in G0 phase), not usually progressing through the cycle, and divide very infrequently. Some cells can remain quiescent for a limited time, an example being fibroblasts whose proliferation resumes after wound upon stimulation by platelet growth factors. Others such as nerve and muscle cells have become permanently quiescent. Quiescent cells have left the cycle during G1, and so they contain the unduplicated quantity of DNA, as do G1 cells. But they differ from G1 cells in many other properties; in particular, they lack the regulatory molecules required for growth. KI-67 protein is a marker for distinguishing proliferating G1 from G0 cells.

G1 Phase

Quiescent cells are activated to proliferate by providing suitable conditions. Nutrients including sugars, salts, vitamins, and essential amino acids are needed for their growth (Baserga, 1985). Normal (nontumor) cells also require epidermal growth factor (EGF), insulin-like growth factor (IGF-1), and transferrin. In an organism growth factors and nutrients must be supplied from blood. For cells to grow in tissue culture, a nutrient medium is required that supplies growth factors usually from added serum. Cells again become quiescent if growth factors are removed. These proteins are required to overcome inhibitions created by contacts between receptors on the cell surface with proteins present in the medium such as growth-negative factor TGF-β, in the extracellular matrix, and on other cells with which a cell is in contact at high density.

Cells increase in size in G1 phase, but they do not exhibit dramatic changes in morphology. But many molecules are synthesized, and molecular processes take place successively during this interval (see below). The time that cells in culture spend traversing this phase is highly variable, for example, from 6 to 24 hours, unlike the rather uniform times they spend in each of the other phases. G1 culminates in initiation of DNA synthesis. Growth factors initiate a multiple-step cascade of signals that ultimately activate genes to produce messenger ribonucleic acids (mRNA) and proteins.

S Phase

The requirements of growth factors for passage through G1 phase are lost at the restriction point (R), located shortly before cells start to synthesize DNA (Pardee, 1989). Progression through later phases of the cell cycle depends on internally generated signals. During the 6 to 8 hours of S phase the nuclear DNA comprising possibly 50,000 genes that are located on 23 pairs of chromosomes is replicated. Each gene is duplicated at a definite time. For example, the dihydrofolate reductase gene that is required for synthesis of DNA is replicated in very early S phase.
CELL FATES

G2 Phase, M Phase, and Cell Division (Cytokinesis)

Cells pass through G2 phase for a few hours after DNA synthesis is completed and before mitosis commences, an interval presumed to be needed to produce the machinery required for mitosis. The complex processes of mitosis then require less than an hour, during which the nuclear membrane breaks down, duplicated chromosomes condense, are paired, and microtubule proteins segregate them equally between the two daughter cells. These daughter cells then divide, separate, and each can reinitiate its cycle.

BIOCHEMISTRY AND MOLECULAR BIOLOGY OF CYCLE PHASES

Growth Stimulation

The pathways to cell fates are activated by various extracellular and internal molecular signals. Each pathway has its distinctive molecular basis, a complex set of interactions between very numerous molecules that carry these signals from cell surface into nucleus (Murray and Hunt, 1993; Andreef, 2003). Alternative pathways and their enzymes often perform the same function, a redundancy that provides fail-safe mechanisms. Details of these complex pathways are presented in other chapters this volume.

We illustrate this complexity with as an example an overview of only a part of one pathway. Activation of proliferation by EGF commences when this growth factor binds to its receptor, on the part located on the cell surface. This external stimulus causes the receptor proteins to form a dimer. The entire receptor extends into the cell, and dimerization activates its protein tyrosine kinase portion inside the cell. This in turn initiates signal transduction, a phosphorylation cascade that begins on the membrane’s internal surface and ends in the nucleus. Located on the inner surface of the membrane are enzymes and their regulatory noncovalent binding effectors, such as the GTP-binding Ras protein. From there, a cascade of downstream enzymes including kinases B and C carry the signal on to the nucleus, where transcription factors are phosphorylated and form large complexes with accessory proteins that bind to specific promoter and enhancer sequences in DNA of target genes (Naar, 2001).

Steroid hormones also activate transcriptions and initiate growth. These molecules move directly into the nucleus where they activate genes, unlike growth factors that initiate cytoplasmic signaling pathways from the membrane. For example, estrogen activates hormone responsive breast cells by ligating to specific receptor proteins in the nucleus that bind to DNA sequences in promoters and activate growth-stimulating target genes.
Signal Transduction

Numerous genes that are activated during the cycle were discovered by researches with yeast mutants having modified cycle-controls (Hartwell and Kastan, 1994). Activation of G₁ phase in mammalian cells results in expression of at least 100 genes. Biochemistry and molecular biology has identified new key enzymes and regulatory proteins, especially cycle-dependent kinases (cdks) that phosphorylate proteins required for cell cycle progression (Nurse, 2000). A series of regulatory proteins regulate transition through the cycle (Roberts, 1999) by binding to and activating these kinases (Murray, 1993). As a cell proceeds through its cycle, four major cyclins (D, E, A, and B) are produced sequentially, and they activate several cyclin dependent kinases. These complexes catalyze successive stages of cell cycle progression. Cyclin D increases in early to mid G₁ phase and regulates cyclin dependent kinases cdk4 and cdk6 (Sherr, 1996). Cyclin D/cdk4 and cdk6 trigger synthesis of cyclin E in late G₁ phase, which in turn activates cdk2/cyclin A and DNA synthesis. Cyclins rise and fall during the cycle because of periodic changes in both their synthesis and destruction (Minshull, 1989).

Families of other proteins bind to and block activities of cyclin/cdk complexes. Some named inhibitors of kinases (INK) counterbalance the cyclin’s activation of cdks, thereby affecting cycling, development, and tumorigenesis (Sherr, 1996). p27 blocks progression; its level is high in quiescent cells and decreases during late G₁ to release cdk/cyclin activities. Inhibition of cyclins by the cdk inhibitor p21 has been demonstrated to be induced under many conditions that arrest growth.

In addition to the synthesis of cyclins, phosphorylations of these complexes are regulatory. Another kinase, CAK, activates the cyclin-dependent kinases by phosphorylation, and also inhibitory phosphates are removed by phosphatases. Furthermore a major regulatory role during the cell cycle is played by relocalization of cyclin/cdks to the nuclear compartment within a cell. Importantly, proteolytic destruction of these regulatory proteins is vital after a cell passes each phase in the cycle (Koepp, 1999). Proteins targeted for removal, including cyclins, are first specifically labeled with the small ubiquitin protein, and then the proteosome, a biochemical machine composed of many enzymatic subunits, chews them up (Benaroudj, 2001).

Downstream Events

Activated cdks phosphorylate proteins that are essential for progression through the cell cycle. When they phosphorylate the retinoblastoma tumor-suppressor protein pRb, which is absent in retinoblastomas, it releases the E2F1 protein to which it was bound. E2F1 then activates transcriptions of many genes that are necessary for initiating S phase, including those coding for enzymes of DNA synthesis. An example is DNA polymerase-α whose transcription is thereby up regulated at G₁/S phase. These enzymes increase at the beginning of S phase, and also
they move from the cytoplasm into the nucleus where they duplicate DNA.

The DNA replication process is initiated at numerous origins of replication, which are sequences in DNA, and is catalyzed by a complex of proteins that includes DNA polymerases. It is closely controlled. In the early S phase cyclins D and E must be degraded by proteasomes. Progression through S phase depends on cyclin A-cdk2 kinase.

After completion of S phase, events in G2 phase are preparatory for entry into mitosis (M). The maturation-promoting factor (MPF) obtained from mitotic cells was early shown to activate mitosis when introduced into another cell. The cyclin-dependent kinase cdk1 is by itself inactive but has been demonstrated to be essential. It must be activated by binding cyclin B, newly produced in late S and G2, which forms MPF. It phosphorylates the nuclear membrane protein laminin, which causes breakdown of the nuclear membrane. At the beginning of M phase, after the nuclear membrane is degraded, cyclins A and E2F are removed by proteosome-catalyzed degradation, a process necessary to prevent apoptosis—see below (Lees, 1999). These events are basic to the complex molecular mechanism enabling progression into M phase. To again briefly illustrate the complexity of regulatory mechanisms, this G2/M checkpoint mechanism is a complex molecular network of phosphorylations and dephosphorylations, catalyzed by several enzymes and proteins. MPF activity is regulated by a variety of proteins that include not only cyclin B but phosphatases, kinases, and also its subcellular localization; cyclin B/cdk1 is rapidly relocated from the cytoplasm to the nucleus at the G2/M transition.

Thereafter the processes of chromosome condensation, pairing, and segregation in mitosis proceed. The destruction of cyclin B, involving a specialized multiple-subunit anaphase promoting complex, is essential for completion of the cycle. These many phosphorylations are important for the massive morphological changes that are necessary for a cell to divide. Cell separation (cytokinesis) soon follows, but it is not necessary for progression through the next cycle because this is accomplished normally by binucleate cells that can be produced after daughter cell separation is blocked by cytochalasin B.

The cell must prepare for DNA synthesis in its next cycle. Normally only one DNA replication can occur per cycle; DNA synthesis cannot be reinitiated until after mitosis is complete. The retinoblastoma protein pRb is a critical determinant in preventing DNA reduplication. Perhaps related is the breakdown during mitosis of the membrane around the nucleus, which permits interactions between molecules from the nucleus and cytoplasm. Degradation of cyclin B by proteasomes is also necessary to start S phase in the following cycle. This “licensing” of DNA synthesis can be disrupted: cells that have lost the cdk inhibitor p21 undergo multiple rounds of DNA synthesis without mitosis, and this process is also activated by the anticancer agent staurosporin, which eliminates the dependence of DNA synthesis on the prior M phase (Nurse, 2000).
GROWTH DISREGULATION

Proliferative Regulation

The cycle of a normal cell is very closely regulated. Proliferation is determined in G1 phase by the presence of suitable growth conditions. These controls ensure that a phase of the cell cycle does not begin until the preceding phase has been completed with high fidelity. If a regulation control fails, programmed cell death (apoptosis) or genomic instability can result. In mammalian nontumor cells a surveillance system in G1 phase is engaged to throw the switch between cell growth and quiescence (Pardee, 1974). A similar regulation point in yeast named START was discovered by Hartwell. These cells cannot pass beyond a specific point in late G1 phase, named the restriction point (R), if the stimulation by growth factors or nutrients is inadequate, and they remain in or revert to quiescence. The final steps that are needed to pass R require synthesis of an unstable protein, later proposed to be cyclin E. Under inadequate conditions this protein’s synthesis does not keep up with its loss, and so it cannot be accumulated to be in excess of the cdk inhibitor p21 and so is insufficient to move the cell into S phase. This G1 regulatory mechanism is defective in cancer cells, which therefore readily pass through R, and so they proliferate excessively (Pardee, 1974).

DNA Damage-Induced Checkpoints

Uncorrected failures of DNA repair are important in the progression from normal to cancerous mammalian cells. DNA damage results in blocked proliferation. The name checkpoint was proposed for this set of cell cycle controls that are activated after DNA is damaged (Hartwell, 1994). A checkpoint delays entry into the next phase of the cell cycle. A major checkpoint acts upon the G1 to S transition, and prevents damaged G1 cells from beginning DNA synthesis until DNA has been repaired, and another is especially evident at the G2/M interface (Fingert, 1988). Several proteins have been implicated in this checkpoint mechanism, in particular, p53, a tumor suppressor called “the guardian of the genome.” It is inactivated by mutation in more than 50% of cancers (Levine, 1997). After DNA is damaged, p53 increases owing to its greater stability; it induces protein p21, which blocks proliferation by inhibiting cyclin/cdk. The ataxia telangectasia protein (ATM) phosphorylates and increases p53. The gene coding for ATM is mutated in individuals that are very sensitive to X rays and that have a high incidence of tumors.

Mammalian cells in S phase exhibit a dose-dependent reduction in DNA synthesis within several minutes of exposure to DNA damaging agents such as X rays. Less is known about the mechanism of this S phase checkpoint than about those in G1 or G2. As little as one double-strand break in DNA activates a G2/M phase checkpoint control and stops cells at the G2/M boundary. This is important because it provides time for DNA repair before a cell goes through mitosis. If this interval is short-
ened by a drug treatment, the cells progress into mitosis without repairing all the damage, which results in death (Fingert, 1988).

Mitosis segregates the duplicated chromosomes between the daughter cells. Accurate segregation depends on proper chromosome alignments on, and attachment to, the mitotic spindle, which is composed of microtubule proteins. A mitotic checkpoint ensures that segregation process occurs correctly by delaying completion of mitosis until all chromosomes are properly attached to the mitotic spindle. This mechanism blocks progression through mitosis if chromosomes are misaligned.

**Programmed Cell Death (Apoptosis)**

Apoptosis is a terminal cell fate, a highly regulated “suicide” process that eliminates physiologically unneeded or dangerous cells. It may prevent mutations that cause cancer (Sellers, 1999). After a cell is severely damaged the time of checkpoint arrest may be too brief to permit complete repair, and such cells are eliminated by apoptosis. As an example, the cyclin A-kinase complex necessary for S phase progression is inhibited in cells treated with X rays, which can result in apoptosis because of inability of this complex to remove the apoptotic factor E2F (Lees, 1999). Checkpoint genes including p53 are involved in activating apoptosis, and other proteins including NF-κB can prevent apoptosis. Apoptosis is performed by proteases named caspases and by nucleases, activated by a family that includes positively acting Bax and negatively acting Bcl-2 proteins. Various cells have different responses to damage or drugs partly because they express various members of the Bcl-2 family and the modulating proteins.

**Tumor Progression**

A cancerous cell’s regulatory balance is perturbed by additional mutations, which arise though defects of checkpoint regulation and DNA repair. These lead to further errors in repair, replication, and chromosome segregation. The mutations cause further losses of proliferation control, and they block apoptosis, differentiation, and related growth arrest, and limited life span (immortalization). Metastasis follows, the ability of cancer cells to move about in the body and proliferate in unusual environments, and so on (Onn, 2002). Various molecular mechanisms that control cancer cell growth and apoptosis are now being discovered. These differences between cancer and normal cells can provide novel targets for therapy.

**MAJOR REGULATORY MECHANISMS**

Throughout this book there are detailed discussions and explications of the major molecular pathways that determine cell fates. This chapter concludes with a brief listing of general regulatory mechanisms that apply
to cell cycle control, apoptosis, and the other cell fates described in this book. For example, differentiation pathways are activated and regulated by extracellular factors including hormones, retinoic acids, and drugs. These alter expressions of genes that determine the properties of their target cells.

1. Transcription is activated when complexes of proteins bind to specific DNA sequences in a gene’s promoter and enhancer regions. An example is binding to DNA of p53, which turns on transcription of many genes, among them ones involved in growth arrest (p21) and then apoptosis. In some cases this functioning depends on covalent bond formation such as protein phosphorylation, or on noncovalent attachment of a small molecule as by retinoic acid’s attachment to its receptor proteins.

2. Chromatin structure also regulates transcription. Methylation of the cytosines in CPG islands of DNA favors local histone deacetylations, which is reversed by acetylation. This changes chromatin structure and inactivates transcriptions. Processes of this general kind may be responsible for long term silencing of long DNA regions, as of the entire one of the two X-chromosomes in each female cell.

3. Pre-RNA processing, splicing and export from the nucleus determine the quantity of mRNA available in the cytoplasm to be translated by ribosomes. Of great current interest are the mechanisms by which different splicing of a pre-mRNA produce several mRNAs, and the regulation of these events.

4. Degradation by nucleases limits mRNAs life times, and together with synthesis, rates determine their steady state concentrations.

5. Translation control is an important element in establishing the amount of protein produced from an mRNA. Inequality between a mRNA and its protein has often been observed.

6. Degradation of a protein counteracts its synthesis, and this too can alter the ratio of a protein to its mRNA. The ultimate example of protein degradation is by proteosome action. This process is initiated by a series of three enzymes that specifically identify the proteins to be removed by covalently tagging them with ubiquitins. As an important example, cyclins are degraded by proteosomes after they have served their transient functions in a phase of the cycle.

7. Covalent modifications are among the best known mechanisms of regulation of activities of a protein such as catalysis, ligand binding, and stability. Cleavage by a protease can either activate or inactivate a protein. Protein phosphorylations are frequently identified modifiers of activities. In signal transduction are sequential activations by cascades of kinases, such as MAP kinase kinase kinase, MAP kinase kinase, and MAP kinase. These activities may be positively or negatively modified.

8. The major components of metabolic machinery are catalytic proteins (enzymes) and noncatalytic binding proteins (which might be named “enphores”). A functional molecule’s activity is altered by its spe-
cific noncovalent bindings. Regulatory “allosteric” sites of a protein bind small molecules that modify activities of the primary sites on the same or on an associated protein. For example, activation by GTP binding to accessory Ras proteins is involved in signal transduction. Many biosynthetic pathways that produce essential metabolites are closely regulated by feedback mechanisms; an initial enzyme in the pathway is inhibited by its noncovalent binding of the end product metabolite.

9. An example of noncovalent regulation by a large molecule is binding of a growth factor to its receptor on the cell surface, which activates the latter’s internal kinase.

10. Control can depend on intracellular localization. As one example, NF-κB activates transcriptions when it is moved from cytoplasm to nucleus.

SUMMARY

The several fates of a cell are produced by organized and regulated processes. Recurring principles of regulation are evident. Their molecular mechanisms are similar in diverse organisms. External factors initiate pathways of signaling to create these cell fates. Very many proteins are involved, both catalytic and regulatory. They function in large complexes. Cascades of kinases bring the message to the nucleus, where it initiates transcriptions. The mRNAs produced are translated by cytoplasmic ribosomes to make the cell’s machinery. This leads to an organized series of cellular and molecular processes, of which DNA duplication near the middle of the cycle and mitosis at the end stand out.

The ying-yang principle of regulation by opposing dynamic actions is observed throughout biology. Both positively and negatively acting molecules are involved at every level. This is illustrated by proliferation versus apoptosis with cells, by activating cyclin proteins versus inhibitory regulators of cdc kinases in proliferation, by apoptosis action of Bax versus inhibitory Bcl-2, by histone acetylation versus deacetylation, by macromolecules synthesis versus degradation, by enzyme phosphorylation catalyzed by kinases balanced by phosphatases.

The cell cycle must be closely regulated if life is to remain in balance. Problems arise, especially serious being errors in DNA replication and mitosis that can cause mutational insertion of incorrect bases and chromosome rearrangements, respectively. Important safeguards are DNA repair mechanisms, redundant pathways to produce an end result, checkpoints that provide time for repair, and elimination of defective proteins by proteasomes. As the final safeguard, there is apoptosis, causing death of defective and dangerous cells.
REFERENCES


