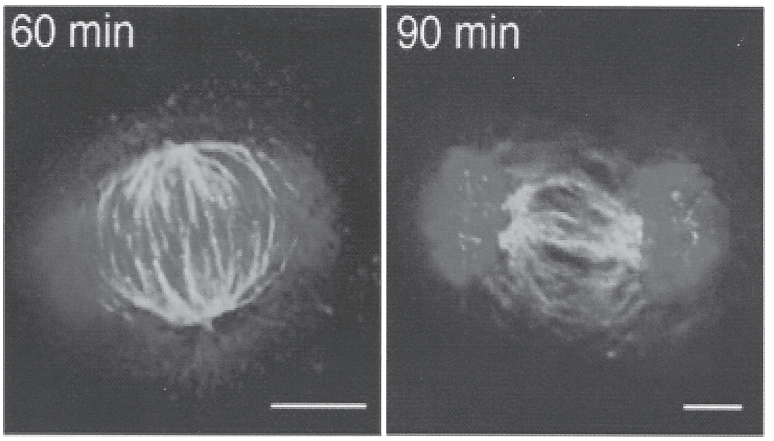
6

FUNDAMENTAL CELLULAR FUNCTIONS



Dynamic rearrangement of chromosomes and microtubules during mitosis. Hela cells are cultured and arrested by treatment with nocodazole, which results in the synchronization of the cells in prometaphase of mitosis. Following the removal of nocodazole, cells start to enter mitosis. Microtubules (green) and chromosomes (blue) undergo dynamic rearrangement during mitosis as shown at 60 and 90 min after the removal of nocodazole. [Reprinted by permission from Macmillan Publishers Ltd: Yasuda S et al: *Nature* 428:767–71, copyright 2004.] See color insert.

Bioregenerative Engineering: Principles and Applications, by Shu Q. Liu

The cell is capable of conducting a number of basic activities, including cell division (cell proliferation and differentiation), migration, adhesion, and apoptosis. These activities play critical roles in the development, morphogenesis, and remodeling of tissues and organs. Cell differentiation, proliferation, and migration are essential processes that contribute to the formation of specialized tissues and organs during development and to the regeneration of malfunctioned and lost cells in injury. Cell-to-cell and cell-to-matrix adhesion are critical processes for the formation of coherent tissue and organ systems. Cell apoptosis is a process that eliminates unnecessary cells, ensuring appropriate morphogenesis and development of tissues and organs. These basic activities are precisely controlled to achieve specialized functions for a living tissue, organ, and system. In a multicellular organism, various cellular activities may occur at different locations and levels. These activities must be precisely coordinated between different systems at the cellular, tissue, and organ levels, which is an essential mechanism for the survival and function of the entire organism. In this chapter, these fundamental cellular activities are briefly reviewed.

CELL DIVISION

Cell division is a process of cell reproduction, which generates progeny with identical genotypes. In mammalian cells, there are two types of division: mitosis and meiosis. *Mitosis* is a cell division process for nongerm cells. This process transmits identical copies of all genes from parent cells to daughter cells. *Meiosis* is a cell division process for germ cells, which produces gametes with half or 23 of the chromosomes.

Mitosis [6.1]

Cell division via mitosis may result in two consequences: cell proliferation and differentiation. *Cell proliferation* is a process of cell division, which results in the reproduction of progeny with identical phenotypes, that is, physical, chemical, and physiological characteristics. *Cell differentiation* is a process of cell division, which results in the production of specialized cells with phenotypes different from the mother cells. These are two fundamental processes, which determine the morphogenesis of tissues and organs during embryonic development and the structure and function of tissues and organs during physiological and pathological remodeling.

Cell proliferation and differentiation may coexist at a given time. The relative activity of cell proliferation and differentiation is dependent on the stage of development. During the embryonic and fetal stages, the two processes occur simultaneously. The differentiation of embryonic stem cells gives rise to various types of specialized cells for various tissue and organ systems, whereas the proliferation of a specialized cell type contributes to cell multiplication and the construction of a specialized tissue and organ. After birth, the activity of cell differentiation is relatively reduced, while cell proliferation remains prominent for tissue and organ growth. After reaching maturation, both cell differentiation and proliferation are reduced significantly. However, a basal level of cell proliferation and differentiation remains for the replacement of malfunctioned, lost, or aging cells. Although cell proliferation and differentiation result in different cell fates, both undergo a common cell division cycle.

Cycle of Mitotic Cell Division

A *cell division cycle* is defined as the period between two mitotic cell divisions. A cell cycle is composed of two major periods: the interphase and mitotic phase (M). The *interphase* is defined as the period from the end of the prior M phase to the beginning of the next M phase and is traditionally divided into the G1 (gap 1), S (synthetic), and G2 (gap 2) phases. The M phase is the period during which cells divide, whereas the interphase is the period during which cells prepare for cell division. These different phases are corresponding to highly ordered and discrete molecular processes that regulate cell division.

The length of a cell cycle is dependent on the stage of development. During the early embryonic stage, the G1 and G2 phases are short or even missing. Embryonic cells primarily undergo alternations of the S and M phases. The duration of a cell cycle is <1 h. The short cell cycle is in coordination with the rapid growth of the early embryo. The length of a cell cycle increases with maturation. In a fully developed mammal, a typical cell cycle may last for 12–24 h with four discrete phases. Each phase of a standard cell cycle is briefly discussed here.

G1 Phase. The G1 phase is the interval during which the cell is prepared for DNA synthesis. It is 6–12 h in length, starting from the end of the M phase to the beginning of the S phase. During this phase, the cell size increases, due to the synthesis of proteins and lipids.

S Phase. The S phase is the period of DNA replication. It lasts for 7–8 hrs, starting from the end of G1 phase to the beginning of the G2 phase. The total DNA content and the number of chromatids are doubled by the end of the S phase. The nucleus increases in size apparently. In addition to DNA synthesis, other cellular components, including RNA and proteins, are also synthesized. However, DNA synthesis occurs only during the S phase, other components are synthesized continuously through the cell cycle. By the beginning of mitosis, the mass of the mother cell is doubled with cellular components equally apportioned for the two daughter cells.

G2 Phase. The G2 phase is the interval of cell preparation for cell mitosis. It is defined as the period (3–4 h) from the end of the S phase to the beginning of the M phase. During this phase, the cell possesses two complete diploid sets of chromosomes and is prepared for entering the mitotic phase. One of the major cellular activities during the G2 phase is the proofreading of the synthesized DNA. The detection of abnormal, damaged, or unreplicated DNA fragments can activate a protein kinase cascade of the G2 DNA damage checkpoint. The consequence of such activity is the inhibition of cyclin-dependent kinases, which are required for the initiation of mitosis, leading to a delay or blockade of entering the M phase.

M Phase. The M phase is the period of chromosome separation (~1 h), staring from the end of the G2 phase to the beginning of the G1 phase. During this phase, the chromosomes are separated into two equal parts for the two daughter cells. The cytoplasm is separated via cytokinesis. Chromosome separation is accomplished through several stages, including the prophase, prometaphase, metaphase, anaphase, and telophase (Fig. 6.1).

During the *prophase*, chromatins, which are composed of DNA and associated proteins and are dispersed in the nucleus during the non-M phases, are condensed into discrete

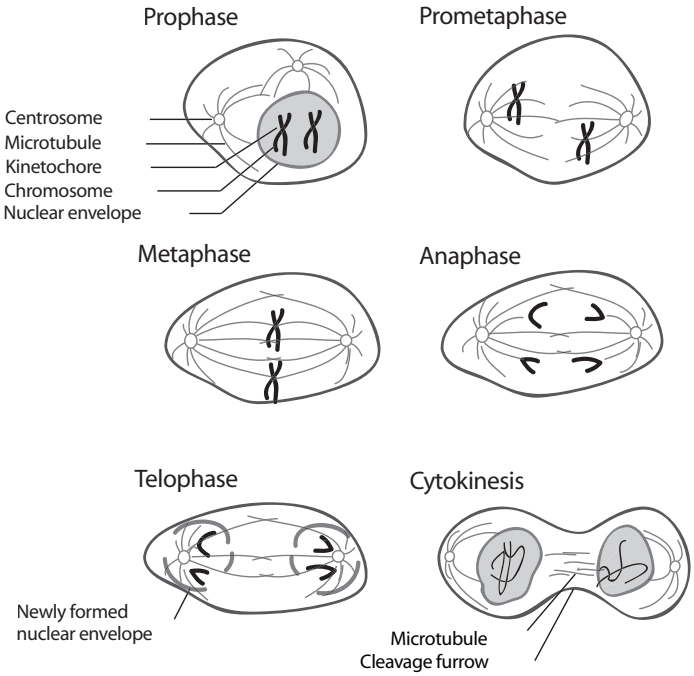


Figure 6.1. Schematic representation of cell mitosis. Mitosis is a process of cell division and consists of several defined phases, including prophase, prometaphase, metaphase, anaphase, telophase, and cytokinesis. Before a cell enters the prophase, the content of DNA is doubled. During the prophase, the chromosomes are organized from dispersed chromatins, the nuclear envelope is disassembled, the two centrosomes are deployed to the two mitotic poles, and microtubules are organized into a spindle-like network. During prometaphase, the chromosomes attach to the spindle microtubules and move toward the cell equator. During metaphase, the chromosomes are aligned along the cell equator and are ready for separation. During anaphase, the two chromatids of each chromosome complex are separated and move toward the opposite mitotic poles. During telophase, the chromosomes approach the mitotic poles, the nuclear envelope of the two daughter nuclei starts to form, and the cell is ready for cytokinesis. Cytokinesis is a process by which the cytoplasm is divided into two equal parts, each with a daughter nucleus. The formation of the daughter cells indicates the end of the cell division cycle. Based on bibliography 6.1.

chromosomes, each containing a pair of chromatids that are connected at the centromere. At the same time, the cytoskeletal microtubules start to reassemble into a mitotic spindle between two centrosomes outside the nucleus.

During the *prometaphase*, the nucleus envelope is disrupted, and the spindle microtubules are redistributed in the nucleus. The kinetochore protein, which forms a complex with the centromere of each chromatid, attaches to a selected spindle microtubule, establishing a kinetochore microtubule. Since each chromosome is composed of a pair of chromatids that are joined by two centromeres, each chromosome contains two kinetochore complexes. The kinetochore-free spindle microtubules are known as *polar microtubules*.

During the *metaphase*, with the help of the kinetochore microtubules, the chromosomes are aligned midway between the two spindle poles on a plane perpendicular to the spindle

microtubules, which are connected to the spindle poles. This chromosome plane is defined as the *metaphase plate*.

During the *anaphase*, the kinetochore–centromere complex is separated and the two chromatids of each chromosome are pulled toward opposite spindle poles at a speed of ~1 μm/min. These activities are induced by the dynamic shortening of the kinetochore microtubules, which connect the chromatids. At the same time, the two spindle poles move away from each other and the polar microtubules, which are not connected to chromosomes, elongate in the spindle direction.

During the *telophase*, separated chromatids, now referred to as *chromosomes*, approach the two spindle poles symmetrically, the kinetochore microtubules gradually disappear, and the nucleus envelope appears around each group of chromosomes surrounding each spindle pole. The chromatin is gradually dispersed in the nucleus and the typical interphase nucleus starts to form, indicating the end of the M phase and beginning of the G1 phase.

Cytokinesis [6.2]

Cytokinesis is the process of cytoplasmic cleavage or segregation, which takes place immediately following chromosomal separation. During the late telophase, the cytoskeletal actin filaments and myosin are deployed along the cell equator, forming a contractile ring or cleavage furrow. The contractile ring gradually constricts the cell until the two daughter nuclei are completely separated with equal cytoplasm contents. This indicates the completion of the cell division cycle.

The formation of the cleavage furrow has always fascinated scientists. This is a complex process, which involves several structures, including the microtubule spindle, actin filaments and myosin, and cell membrane. It is now understood that the assembly of the contractile actin–myosin ring is regulated by several types of molecule. These include microtubule spindle-associated molecules, the RhoA guanosine triphosphatase (GTPase), myosin II, actin and actin-associated factors, and molecules mediating the fusion of membrane structures. The coordinated actions of all these structures and regulatory molecules contribute to the formation and performance of the contractile ring during cytokinesis.

Cell membrane fusion is an important part of cytokinesis. When the cytoplasm is separated by the constriction of the contractile ring, the cytoplasm of each daughter cell must be completely covered with the cell membrane. Two mechanisms are involved in the formation of the membrane of the daughter cells: (1) the daughter cells are able to generate additional cell membrane, which is deployed to the cleavage furrow to cover the open cytoplasm; and (2) membrane vesicles can be produced in the daughter cells. These vesicles can be transported to the cleavage furrow to bridge the open surface of the cytoplasm. Membrane fusion is required for the formation of a complete cell membrane.

Control of Cell Division [6.3]

The mitotic events described above are precisely controlled in a highly coordinated manner. The order and completeness of these events, which are critical to successful cell division, are controlled by two mechanisms: constitutive and extracellular. The constitutive mechanism is intrinsic in nature and ensures the initiation, progression, and completion of cell division. The extracellular mechanism is dependent on the stimulation of extracellular factors and ensures that cell division takes place in coordination with the global function and physiological status of a specialized tissue or organ. In other words,

the extracellular mechanism controls whether, when, and to what degree cell division occurs. Once a cell cycle is triggered by an extracellular cue, the constitutive mechanism controls the progression of cell division.

Both constitutive and extracellular mechanisms are implemented via the mediation of cell cycle regulatory molecules, including cyclins, cyclin-dependent kinases (CDKs), and inhibitors of cyclin-dependent kinases (CKIs). A common feature of cell cycle regulation is the occurrence of oscillatory changes in the activity of regulatory factors, which determine the cyclic events of cell division. In particular, periodic phosphorylation/dephosphorylation of CDKs and cyclic increase and decrease in the level of cyclins play key roles in the initiation, progression, and cessation of cell division.

Constitutive Control of Cell Division. The constitutive control mechanism is dependent on several "checkpoints." These checkpoints inspect and control the progression of cell division and eliminate errors, if any. Checkpoints have been identified in all phases of the cell cycle. Several proteins, including p53, and p21, have been found to play a role in these checkpoints.

In the G1 phase, a major checkpoint is known as the *restriction point*, which controls the initiation of cell division. A cell can pass the G1 phase only if the prior mitosis is complete, DNA is undamaged, and the cell reaches a critical size. DNA damage, if any, must be repaired before a new cell cycle is initiated. Unrepaired DNA may result in the halt of the cell cycle. Also, a critical size of cell mass is required for cell division. The G1 phase is the longest period with the largest variation in length among all cell cycle phases. Thus, the G1 phase is timely flexible to adjust the rate of cell division in response to various stimulations.

The *S-phase checkpoints* assess the integrity of DNA. The checkpoints prevent the cell from synthesizing DNA, if the DNA is damaged or disrupted. All DNA molecules in the genome must be completely replicated, which is a prerequisite for the continuation of cell division to the G2 phase. The cell is arrested in the S phase if damaged DNA is found. Once a DNA replication process is initiated, it must be completed, or the cell cycle is stopped.

During the G2 phase, the newly replicated DNA is examined by the *G2-phase checkpoints* to ensure that the DNA molecules have been correctly duplicated. DNA repair factors can be activated to correct replication errors. These checkpoints determine whether the cell can move to the M phase. If the DNA molecules are not completely replicated, the cell is arrested before the M phase.

The *M-phase checkpoints* are responsible for detecting problems that potentially influence cell division. The checkpoints assess the completeness of proceeding preparatory events as well as the mitotic events. For example, unattached kinetochore can be detected by the checkpoints, leading to the halt of cell cycle progression. The completeness of the entire mitosis is also assessed by the M-phase checkpoints. Any incomplete events may result in cell arrest.

Some cells, especially terminally differentiated cells, are not committed to cell division and enter a phase known as the *G0 phase*, which resembles the G1 phase in certain aspects. These calls cannot pass the restriction points and cannot proceed to the S phase. However, under appropriate extracellular stimulations, some of these cells can be stimulated to pass the restriction points, initiating cell division.

The discovery of the cell cycle checkpoints has led to a significant advance in the understanding of the control mechanisms of cell division. All key processes of cell

division are assessed by the checkpoints. The presence of continuous checkpoints through the cell cycle ensures the initiation, progression, and completion of cell division and the accuracy of cell reproduction.

Extracellular Control of Cell Division. There are a variety of extracellular factors that regulate the initiation and progression of cell division. These include growth factors, nutrient supplies, cell–cell interactions, and mechanical forces. Growth factors, such as platelet-derived growth factor, epidermal growth factor, fibroblast growth factor, activate cyclins and CDKs and promote the cell to enter the S phase and reduce the length of the G1 phase. In contrast, a growth inhibitor, such as transforming growth factor β, activates cell division inhibitors, such as p21, p27, and p57, and induces cell arrest. A reduction or depletion of nutrient supplies may promote the cell to enter the G0 phase. An increase in cell density or cell–cell contact may result in cell arrest. Mechanical forces have also been found to mediate cell mitosis. A decrease in bloodflow or fluid shear stress may activate the division cycle of vascular smooth muscle cells. In contrast, an increase in mechanical stretch or tensile stress in the wall of blood vessels induces mitosis of vascular smooth muscle cells. These mechanical factors may directly regulate cell mitosis or influence cell mitosis via the mediation of mitogenic factors.

Signaling Events of Cell Cycle Control. The progression of the cell cycle is regulated by a cascade of signaling molecules (Fig. 6.2). In the G1 phase, cells either enter the G0 phase or are committed to enter the S phase, initiating the cell division cycle. An increase in growth factors stimulates the cell to enter the S phase by inducing the expression of cyclin D. The level of cyclin D remains high through the G1, S, and G2 phases and rapidly reduces during the M phase via ubiquitination-mediated degradation (see Chapter 5). Increased cyclin D in the G1 phase promotes the formation of the cyclin D-CDK4/6 complex. Activated cyclin D-CDK4/6 complex stimulates cell growth, which is required for the passing of the G1 restriction point.

The cyclin D-CDK4/6 complex can phosphorylate retinoblastoma tumor suppressor (Rb), which contains the transcriptional factor E2F (elongation factor). The phosphorylation of Rb induces the release of E2F, which stimulates the expression of the cyclin E gene, resulting in a transient increase in cyclin E during the transition period from the G1 to S phase. Cyclin E forms a complex with CDK2 and the cyclin E/CDK2 complex is in turn activated by CDC25A-mediated dephosphorylation of CDK2 on the Thr14 and Tyr15 residues. Activated cyclin E-CDK2 complex promotes the cell to pass the G1 restriction point and enter the S phase. (See Table 6.1.)

During the early S phase, the transcriptional factor E2F stimulates the expression of cyclin A, which forms a complex with CDK2. The cyclin A-CDK2 and cyclin E-CDK2 complexes are capable of phosphorylating critical components that initiate and regulate DNA replication. The cyclin E-CDK2 complex is dissociated due to the degradation of cyclin E by the ubiquitin–proteasome system (see Chapter 5) during the early S phase, whereas the cyclin A-CDK2 complex remains active through the S and G2 phases.

During the S phase, another cyclin molecule, cyclin B, is gradually accumulated and forms a complex with cell division cycle protein (CDC)2. The cyclin B/CDC2 complex is known as the *M-phase-promoting factor* (MPF). MPF can be activated by CDC25B/C-mediated dephosphorylation at the G2/M transition. Activated MPF can phosphorylate a number of substrate proteins, including lamin, vimentin, and caldelsom, leading to cell mitosis. The phosphorylation of lamin is thought to induce the disruption of the cell

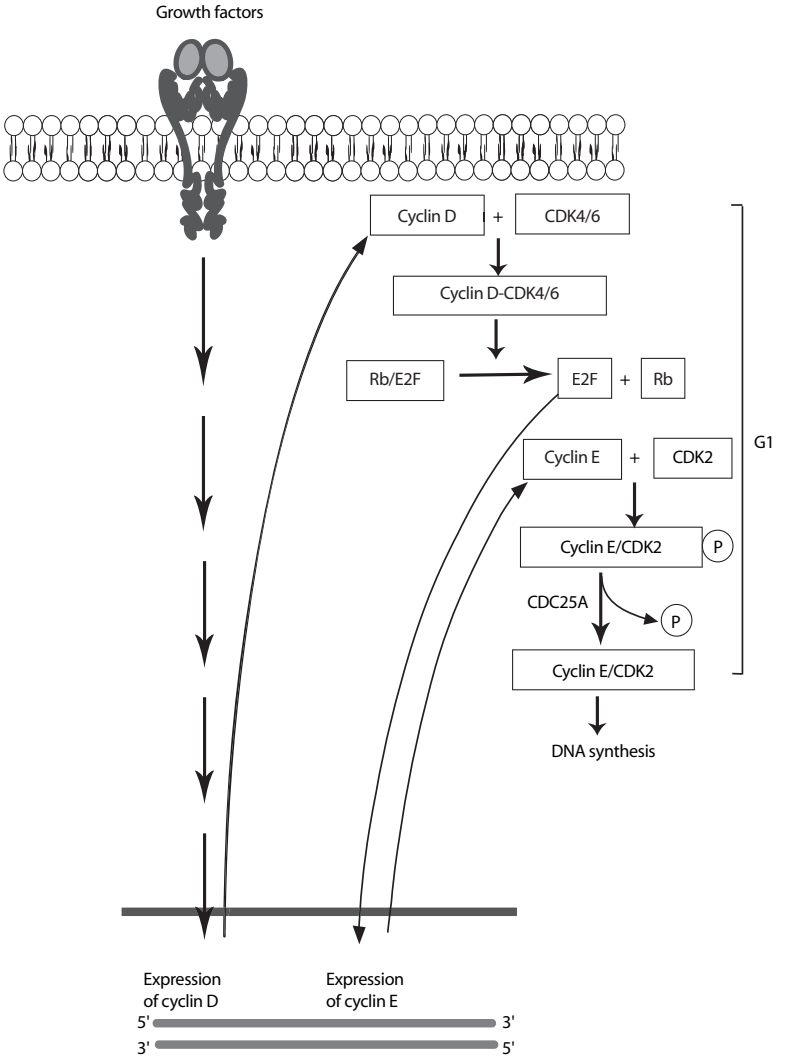


Figure 6.2. Schematic representation of the regulatory mechanisms of cell mitosis. Based on bibliography 6.3.

nucleus. The phosphorylation of vimentin is responsible for dynamic changes in microtubules and the formation of the spindle. The phosphorylation of caldelsom induces the interaction of actin filaments with myosin molecules, leading to the formation of the cleavage furrow and cell cytokinesis. MPF is deactivated by ubi-quitination of cyclin B during the M phase, which indicates the end of cell mitosis. (See Table 6.2.)

Inhibition of Cell Division Cycle. A family of proteins, including p15(INK4B), p16(INK4), p18(INK4C), and p19(INK4D), exerts an inhibitory effect on the activity of the cyclin D/CDK4/CDK 6 complex, and induces cell arrest during the G1 phase. The suppression of these inhibiting molecules leads to uncontrolled cell proliferation.

TABLE 6.1. Characteristics of Selected Cell Cycle Regulatory Molecules*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Cyclin D1	B-cell leukemia 1, BCL1	295	34	Liver, kidney, intestine, uterus, pancreas, placenta	Binding to CDK4 and CDK6, mediating the activity of these CDKs, regulating the transition of the cell division cycle from the G1 to S phase, and promoting cell division and tumorigenesis
Cyclin E1	Cyclin E, G1/S-specific cyclin E1	410	47	Lung	Binding to CDK2, mediating the activity of this CDK, regulating the transition of cell division cycle from the G1 to S phases, and promoting cell division and tumorigenesis
CDK2	Cyclin-dependent kinase 2, cell division kinase 2, p33 protein kinase, cell division protein kinase 2	298	34	Kidney, prostate, lymphocytes, skin	A serine/threonine protein kinase that regulates the transition from G1 to S phases
CDK4	Cyclin-dependent kinase 4, cell division kinase 4	303	34	Ubiquitous	A serine/threonine protein kinase that phosphorylates the retinoblastoma protein and regulates the transition from G1 to S phase
CDK6	Cyclin-dependent kinase 6, cell division protein kinase 6	326	37	Ubiquitous	A serine/threonine protein kinase that forms a complex with CDK4 and regulates the transition from the G1 to S phases
E2F1	E2F transcription factor 1, transcription factor E2F, retinoblastoma associated protein 1, and retinoblastoma-binding protein 3	437	47	Pancreas, connective tissue, skin	Serving as a transcriptional factor, stimulating gene transcription, and regulating cell division, proliferation, differentiation, and apoptosis
CDC25	Cell division cycle 25A, M-phase inducer phosphatase 1, dual-specificity phosphatase Cdc25A	523	59	Brain	A phosphatase that activates CDC2 by dephosphorylation and regulates the progression of cell division cycle from the G1 to S phases

*Based on bibliography 6.3.

The p15 protein may be activated in response to TGF-β, which suppresses the proliferation of several cell types, including epithelial cell and smooth muscle cell. Another group of proteins, including p21, p27, and p57, may inhibit the activity of the cyclin D/CDK4/CDK6 and cyclin A/CDK2 complexes, and induce cell arrest in the G1 phase. The tumor suppressor protein p53 induces activation of p27, leading to cell arrest. (See Table 6.3.)

Meiosis [6.4]

Meiosis is the process of gametogenesis or germ cell division. During such a process, a diploid germ progenitor cell undergoes DNA synthesis and two division events to produce four daughter cells with a haploid set of chromosomes. Each germ progenitor cell contains homologous pairs of chromosomes. Each homologous pair of chromosomes is composed of a maternal chromosome and a paternal chromosome, which can be identical or allelic (not completely identical). In response to the stimulation of signals for gametogenesis, the germ progenitor cell enters the S phase and initiates DNA synthesis, yielding two identical chromatids for each chromosome. The cell then enters two consecutive division processes, designated as meiosis I and meiosis II, to produce daughter germ cells. The meiosis I process is usually divided into several stages, including prophase I, metaphase I, anaphase I, and telophase I. The meiosis II process is divided into metaphase II, anaphase II, and telophase II. By the end of telophase II, four haploid daughter cells are produced from a single diploid germ progenitor cell (Fig. 6.3).

During meiosis prophase I, the nucleus envelope is reorganized, degraded, and disappeared. Centrosomes and microtubule spindles start to form. Scattered chromosomes are organized into apparently double-chromatid structures. The chromosomes can be clearly recognized under an optical microscope. Crossing over of chromatid fragments may occur between the maternal and paternal chromosomes, resulting in homologous recombination (Fig. 6.4). During meiosis metaphase I, a complete spindle and centrosome system is established. The chromosomes are aligned in the equator region. The centromeres of the chromosomes are connected to the spindles. During meiosis anaphase I, the chromosomes remain paired and are pulled toward the poles of the cell. Note that the chromosomal segregation process of meiosis is different from that of mitosis. In mitosis, the pair of chromatids for each chromosome is separated during the anaphase. In meiosis, the paired chromatids of each chromosome are not separated during anaphase I. During meiosis telophase I, the chromosomes are moved to the poles and rearranged. The germ progenitor cell is divided into two cells. However, no nucleus envelope is developed.

Meiosis II is the process by which the two daughter cells are further divided to produce haploid germ cells. The chromosomal separation in meiosis II is similar to that in mitosis. During meiosis metaphase II, the chromosomes in each daughter cell are aligned in the equator region. The centromeres are connected to the microtubule spindles. During meiosis anaphase II, the two identical chromatids of each chromosome are separated and pulled to the cell poles in opposite directions. During meiosis telophase II, each daughter cell is further divided into two granddaughter cells with a haploid set of chromosomes (a single copy of each chromosome from either the mother or the father). The chromosomes are rearranged and enveloped within the nucleus.

Experimental Assessment of Cell Division [6.5]

Cell division can be assessed by detecting DNA synthesis, which occurs only during cell division. There are two basic approaches for the detection of DNA synthesis: measuring

TABLE 6.2. Characteristics of Selected Cell Cycle Regulatory Molecules*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Cyclin A	CCNA1	465	52	Brain, testis, leukocytes	Binding to CDK2 and CDC2 kinases, mediating the activity of these kinases, regulating the transition of cell division cycle from the S to G2 phases
Cyclin B	G2/mitotic specific cyclin B1, CCNB	433	48	Liver, lung, bone marrow	Binding to CDC2 to form the M-phase-promoting factor and regulating the transition of cell cycle from G2 to M phases
CDC2	Cell division cycle 2, cell cycle controller CDC2, p34(CDC2), cyclin-dependent kinase 1 (CDK1), p34 protein kinase, Cdc2 kinase	297	34	Skin	A Ser/Thr protein kinase that serves as the catalytic subunit of the M-phase-promoting factor (MPF); phosphorylates proteins such as lamin, vimentin, and caldelsom; and regulates the transition of cell cycle from G1 to S phases and from G2 to M phases

*Based on bibliography 6.3.

TABLE 6.3. Characteristics of Selected Cell Cycle Inhibitory Molecules*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
p15(INK4B)	p15 inhibitsCDK4, cyclin-dependent kinase inhibitor2B, CDKN2B, p15 INK4b, CDK4B inhibitor, cyclin-dependent kinase 4 inhibitor B, multiple-tumor suppressor 2 (MTS2)	138	15	Skin, placenta	Acting as a cyclin-dependent kinase inhibitor, forming a complex with CDK4 or CDK6, suppressing the activation of these kinases, and inducing the arrest of cell division cycle in G1 phase
p16(INK4A)	p16 inhibits CDK4, CDKN2A, cyclin-dependent kinase inhibitor 2A, CDKN2, CDK4 inhibitor, multiple-tumor suppressor 1 (MTS1)	173	18	Ubiquitous	Serving as an inhibitor for the CDK4 kinase and inducing cell arrest in G1 phase
p18(INK4C)	p18, inhibits CDK4, cyclin-dependent kinase inhibitor 2C, CDKN2C, cyclin-dependent kinase 6 inhibitor, cyclin-dependent kinase 4 inhibitor C, cyclin-dependent inhibitor, CDK6 inhibitor p18, p18-INK6, p18-INK4c, CDKN6	168	18	Brain, liver, testis, thymus	Serving as a cyclin-dependent kinase inhibitor, suppressing the activity of CDK4 and CDK6, and inducing cell arrest in G1 phase
p19(INK4D)	Cyclin-dependent kinase inhibitor 2D, p19 inhibits CDK4, INK4D, p19 INK4D, cyclin-dependent kinase 4 inhibitor D, CDK inhibitor p19 INK4D	166	18	Ubiquitous	Serving as a cyclin-dependent kinase inhibitor, and inducing cell arrest in the G1 phase.
p21	Cyclin-dependent kinase inhibitor 1A, cyclin-dependent kinase inhibitor 1, DNA synthesis inhibitor, CDK-interaction protein 1, wildtype p53-activated fragment 1, melanoma-differentiation-associated protein 6	164	18	Heart, bone	Acting as a cyclin-dependent kinase inhibitor, inhibiting the activity of cyclin-CDK2 and CDK4, and mediating p53-dependent cell arrest in G1 and G2 phases
p27	Cyclin-dependent kinase inhibitor 1B, cyclin-dependent kinase inhibitor p27, p27Kip1, KIP1, CDKN4	198	22	Ubiquitous	Serving as a cyclin-dependent kinase inhibitor, binding to and suppressing the cyclin E-CDK2 and cyclin D-CDK4 complexes, and inducing cell arrest in G1 phase
p57	Cyclin-dependent kinase inhibitor 1C, p57KIP2, KIP2	316	32	Heart, skeletal muscle, B cells, placenta	Acting as an inhibitor for G1 cyclin/CDK complexes and inducing cell arrest in G1 phase

*Based on bibliography 6.3.

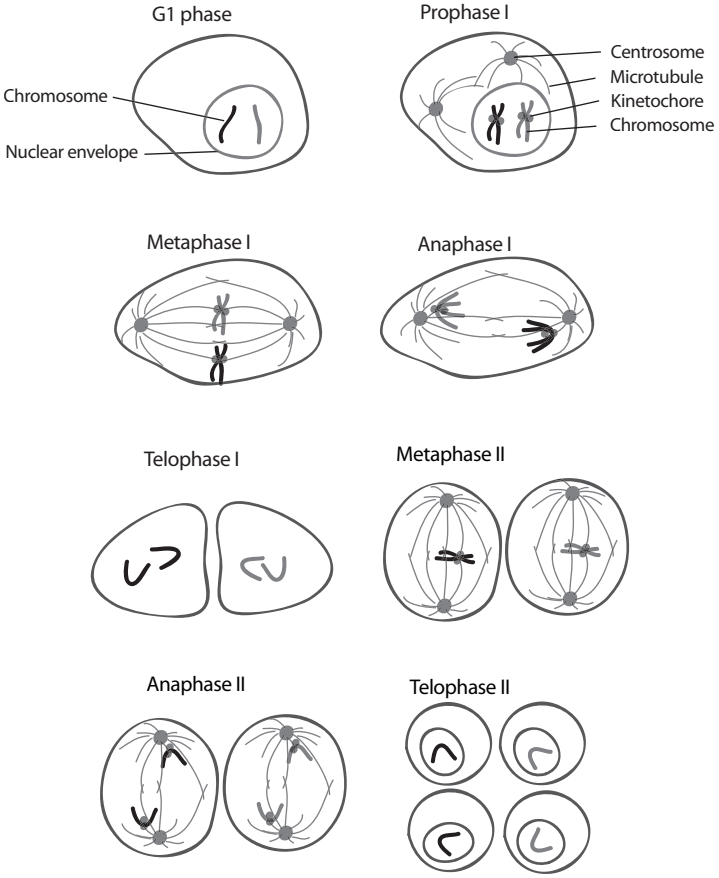


Figure 6.3. Schematic representation of cell meiosis. Meiosis is a process of germ cell division and is composed of two phases, including meiosis I and meiosis II. The phase meiosis I is consists of several stages, including prophase I, metaphase I, anaphase I, and telophase I. The phase meiosis II consists of metaphase II, anaphase II, and telophase II. During prophase I, the nucleus envelope is degraded, centrosomes and microtubule spindles start to form, and scattered chromosomes are organized into apparently double-chromatid structures. During metaphase I, a complete spindle network forms, the two centrosomes are deployed to the mitotic poles, and the chromosomes are aligned in the equator region. During anaphase I, the chromosomes remain paired and are pulled to the mitotic pole of the cell. During telophase I, the chromosomes are completely separated and rearranged near the two poles. The germ progenitor cell is divided into two cells. However, no nucleus envelope is developed. During metaphase II, the chromosomes in each daughter cell are aligned along the equator. The centromeres are connected to the microtubule spindles. During anaphase II, the two identical chromatids for each chromosome are separated and pulled to the mitotic poles. During telophase II, each daughter cell is further divided into two granddaughter cells with a haploid set of chromosomes (a single copy of each chromosome from either the mother or the father). The chromosomes are rearranged and enveloped within the nucleus. Based on bibliography 6.4.

DNA content and measuring the density of cells that undergo DNA synthesis. For DNA content measurement, DNA can be extracted from a given volume of tissue or given area of cultured cells and the DNA content can be measured by DNA extraction and spectrophotometry. Although this method is easy to use, it does not directly give the density of dividing cells.

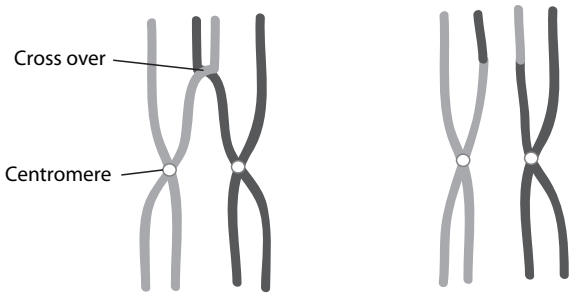


Figure 6.4. Schematic representation of chromosomal cross over. During chromosome segregation, chromosomal segments may exchange location between two chromatids of different chromosome complexes. Based on bibliography 6.4.

To directly measure the density of cells that synthesize DNA, a selected type of deoxynucleotides can be tagged with a marker and delivered to cells. Since only dividing cells take up deoxynucleotides, any cells exhibiting the tagged deoxynucleotides can be considered dividing cells. There are two types of tagging markers—radioactive isotopes and molecules—that can be detected by immunohistochemistry. A common radioactive material used for detecting cell division is [^{3}H]-thymidine. This isotope can be delivered to animal models or cultured cells. Tissue specimens or cultured cells can be collected after 24 h, fixed with 4% formaldehyde in phosphate-buffered saline (PBS), and processed for detecting [^{3}H]-thymidine incorporation. Specimens are exposed to X-ray films and cells with positive [^{3}H]-thymidine signals are considered dividing cells. Such a method is referred to as *autoradiography*. The specimen can be counterstained with hematoxylin and eosin for measuring the total number of cells. The ratio of the number of [^{3}H]-thymidine-labeled cells to that of the total cells can be used as an index for assessing cell division.

Alternatively, 5′-bromodeoxyuridine (BrdU) can be used to detect cell division instead of [^{3}H]-thymidine. BrdU can be directly injected into an animal or delivered to cultured cells. As [^{3}H]-thymidine, BrdU can be taken up only by dividing cells. BrdU can be detected by immunohistochemistry with a BrdU-specific antibody (Fig. 6.5). It is important to address several technical points for the BrdU assay. First, cultured cells or intact tissue specimens without histological sectioning should be treated with a detergent (e.g., 0.5% Triton X-100) to permeabilize cell membrane so that antibody can diffuse through the cell membrane and reach the cell nucleus. For histological tissue sections, since cells are cut open and cell nuclei are exposed, it is not necessary to treat specimens with a detergent. Second, incorporated BrdU is embedded within the cell chromatin, which prevents the anti-BrdU antibody from accessing BrdU. Thus, cultured cells or tissue specimens should be treated with pepsin to digest nucleus proteins and expose DNA, so that the anti-BrdU antibody can access the incorporated BrdU. A DNA-binding fluorochrome, e.g., Hoechst 33258, can be used to counter-staining DNA nonspecifically, allowing the measurement of the total number of cells within a selected specimen. Comparing to the [^{3}H]-thymidine incorporation method, the BrdU method is more advantageous for its simplicity and nonradioactivity.

CELL MIGRATION [6.6]

Cell migration is a fundamental cellular activity observed during development and pathogenic remodeling. During development, cell migration plays a critical role in the initiation

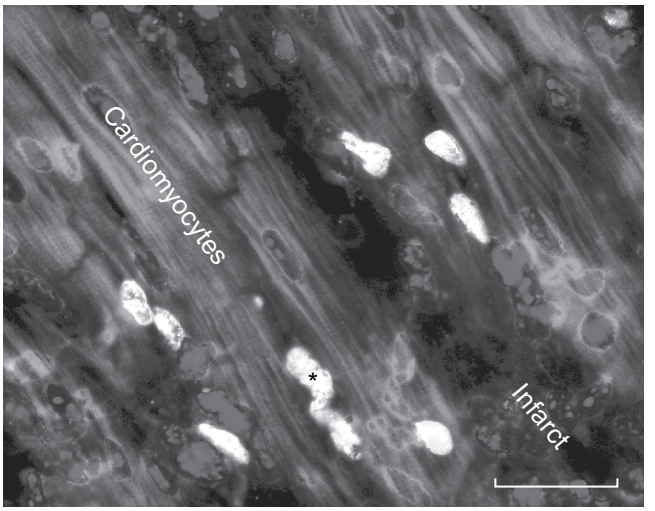


Figure 6.5. 5′-Bromodeoxyuridine (BrdU)-positive cells in injured cardiac tissue at day 5 after ischemic injury. In this preparation, cardiac injury was induced by ligating the left anterior descending coronary artery in a mouse model. BrdU was injected into the skeletal muscle of a mouse 24 hrs before observation. Cardiac specimens were fixed in 4% formaldehyde in phosphate-buffered saline (PBS), cut into cryosections, treated subsequently with 0.5% pepsin and 1.5 N HCl, incubated subsequently with an anti-BrdU antibody and a fluorescein-conjugated secondary antibody, and observed by using a fluorescence microscope. Scale bar: 10 μm.

and formation of tissues and organs, such as the nerve and cardiovascular systems. During pathogenic remodeling, cell migration contributes to the initiation and progression of pathogenic disorders, such as atherogenesis (e.g., smooth muscle cell migration from the arterial media to the arterial intima or arterial substitutes), tumorigenesis (e.g., cancer cell migration and metastasis), and inflammation (leukocyte migration to inflammatory sites). Cell migration is a mechanical event that involves a variety of molecular processes and is controlled by a number of known signaling pathways. In this chapter, the mechanics and regulatory mechanisms of cell migration are briefly reviewed.

Mechanics of Cell Migration

Cell migration is accomplished by a number of mechanical processes at the molecular and subcellular levels. Theses processes include protrusion or extension of cell membrane at the cell leading edge, attachment of protruded cell membrane to a substrate via adhesion receptors, contraction and movement of the cell body, retraction at the cell trailing edge, and recycle of adhesion receptors (Fig. 6.6). A variety of regulatory and contractile proteins are involved in the initiation and progression of cell migration. It is important to note that the five processes outlined above are arbitrarily defined. All these processes take place simultaneously and continuously in a cyclic manner. It is difficult to identify the beginning and end of a migration cycle.

Protrusion of Cell Membrane. When a cell is stimulated by a migration-activating factor, such as a chemoattractant, the cell initiates directed membrane protrusion. The direction of membrane protrusion is often determined by the stimulus. For instance, in the presence

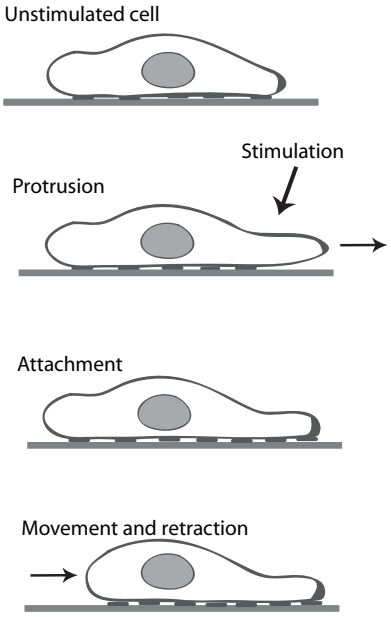


Figure 6.6. Schematic representation of cell migration. Based on bibliography 6.6.

of a chemoattractant, the cell membrane extends toward the chemoattractant. The forces that drive the membrane protrusion are generated by the actin assembly. Although the mechanisms of actin assembly is under debate, it is thought that controlled sequential extension of actin filaments toward the cell leading edge may provide a propelling force for membrane protrusion. In this model, actin subunits are added to the barbed end of the actin filaments, which point at the leading edge of cell migration. Such a process is controlled by regulatory proteins, including the Arp2/3 complex (see Chapter 3).

Attachment of Cell Membrane to Substrate Matrix at the Leading Edge. Following the protrusion of cell membrane due to directed actin assembly, the next step is the attachment of the extended membrane lamellipodia to substrate. Such a process stabilizes the leading edge of the migrating cell and allows the cell to exert forces on the substrate, a necessary condition for cell migration. The attachment of cell leading edge is mediated by integrins. Unstimulated integrins are freely suspended in the cell membrane. In response to the stimulation of extracellular matrix, integrins are activated and bind to the actin cytoskeleton. Cell membrane protrusion enhances the binding of integrins to extracellular matrix in newly formed lamellipodia. Integrin–matrix interaction further stimulates the binding of integrins to the actin cytoskeleton, facilitating the formation of focal adhesion contacts between the actin cytoskeleton and extracellular matrix. Thus, integrins play a critical role for the attachment of cell lamellipodia to substrate. Since focal adhesion contacts link the actin cytoskeleton to extracellular matrix, forces generated by the actin cytoskeleton can be transmitted to the extracellular matrix, which is critical to cell migration.

Cell Traction and Movement. Cell movement is propelled by traction forces generated by the actin cytoskeleton and exerted on the extracellular matrix substrate. In a fibroblast,

for example, the actin cytoskeleton can generate traction forces about 1 nN/μm^2 on a substrate. The generation of traction forces is dependent on the interaction of actin filaments with myosin II in mammalian cells. Actin filaments are distributed around the cell periphery. The barbed ends of the actin filaments are oriented toward the cell periphery in regions near the leading and trailing edges, while those in the middle region are oriented more randomly. The myosin II molecules are distributed more heavily in the middle region than in regions near the leading and trailing edges. With such molecular distributions, the interaction of actin filaments with myosin II results in predominantly peripheral movements. The direction of cell migration may be dependent on the asymmetric distribution of actin filaments and the relationship between actin filaments and the extracellular matrix, which determine the balance of the traction forces between the cell leading and trailing edges. Directed migration can occur only if the traction force at the leading edge exceeds that at the trailing edge.

Retraction of Cell Membrane at the Trailing Edge. To initiate cell migration, the forward movement at the cell leading edge must be accompanied with a retraction at the cell trailing edge. The dynamic interaction of integrins with extracellular matrix may mediate the coordinated leading-edge movement and trailing-edge retraction. The distribution of integrin-containing focal adhesion contacts changes dynamically from the cell leading edge to the trailing edge. The density of focal adhesion contacts is relatively lower at the trailing edge than that at the leading edge. Such a distribution of focal adhesion contacts results in reduced cell membrane adhesion to the extracellular matrix at the cell trailing edge and is in favor of the dissociation of cell membrane from the extracellular matrix. Furthermore, the traction forces generated at the cell leading edge are counterbalanced by those at the trailing edge. A reduction in the density of focal adhesion contacts at the trailing edge likely results in an increase in the traction force per focal adhesion contact, which enhances the disruption of integrin–matrix bonds and thus facilitates the retraction of the cell trailing edge.

In addition to the influence of the physical factors described above, the disruption of the integrin–matrix bonds at the cell trailing edge may be regulated by biochemical processes. For instance, the disruption of $\alpha v\beta 3$–vitronectin interaction in migrating neutrophils requires the presence of calcium. The suppression of the calcium-dependent phosphatase calcineurin prevents the disruption of the $\alpha v\beta 3$–vitronectin interaction. This observation suggests that calcineurin plays a role in regulating the detachment of cell membrane from matrix substrate at the trailing edage. However, the mechanisms of chemically mediated retraction remain to be investigated.

Replenishment of Integrins. Integrins play a critical role in the mediation of membrane attachment, cell traction, and cell retraction during cell migration. The distribution and activity of integrins vary from the cell leading to trailing edges. This suggests that the cell must replenish active integrins at the cell leading edge. There are two possible ways for the replenishment of integrins: integrin synthesis and recycling. New integrins are continuously synthesized and deployed to the cell membrane. Cells are also able to endocytose and reuse the integrin molecules left behind on the substrate during cell trailing-edge retraction. In addition, cells may actively transport membrane integrins from the cell trailing to leading edges. With these approaches, the cell is able to maintain an appropriate distribution of integrins, which is necessary for the conduction of cell migration.

Regulation of Cell Migration

Role of the Rho family of GTPases. As discussed above, cell migration is accomplished by a number of complex molecular processes. These processes are regulated by a variety of signaling molecules. Among the signaling molecules, the Rho family of small GTPases, including Rho, Rac, and Cdc42, plays a critical role in the regulation of cell migration.

The GTPases of the Rho family are GTP-binding proteins with molecular weight of ~21 kDa. These proteins belong to the Ras protein superfamily, which include, in addition to the Rho family GTPases, the Rab, the ADP-ribosylation factor (ARF), and the Ran families. The Rab proteins participate in the regulation of vesicle transport, the ARF proteins mediate signal transduction and vesicle transport, whereas the Ran proteins mediate protein transport to the cell nucleus. All proteins of the Ras superfamily are able to bind GTP.

For the Rho family of small GTPases, 11 different isoforms have been identified in mammalian cells, including RhoA, RhoB, RhoC, RhoD, RhoE, RhoG, Rac1, Rac2, Cdc42, TC10, and TTF. Among these proteins, the role of RhoA (see Table 6.4.), Rac1, and Cdc42 has been extensively studies (see Chapter 3 for characteristics of these molecules). RhoA has been shown to regulate the formation and organization of actin filaments, Rac1 mediates the formation of cell lamellipodia and membrane ruffles, whereas Cdc42 is responsible for the formation of filopodia. All these processes are related to cell migration.

All GTPases of the Rho family can bind GTP or GDP. A GTPase is active when GTP is bound, whereas it is inactive when GDP is bound. Nucleotide exchange factors can stimulate the binding of GTP to GTPases, activating the GTPases. A variety of extracellular signals can activate the nucleotide exchange factors and thus the GTPases. While the exact mechanisms of GTPase activation remains poorly understood, GTPase translocation to the cell membrane or cytoskeleton may play a role. For example, the nucleotide exchange factor for Cdc42 is associated with the cell membrane. Activated nucleotide

TABLE 6.4. Characteristics of RhoA*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
RhoA	Ras homolog gene family member A, ARHA, aplysia ras related homolog1 2 (ARH12), oncogene rho H12, RHOH12, RHO12, RHOA, transforming protein RhoA, Ras homolog gene family member A	193	22	Ubiquitous	Regulating the organization and remodeling of actin cytoskeleton during cell morphogenesis and migration and mediating cell proliferation and differentiation

*Based on bibliography 6.6.

exchange factor can induce translocation of Cdc42 from the cytoplasm to the cell membrane, which facilitates the activation of Cdc42.

Activated RhoA, Rac1, and Cdc42 can interact with and stimulate downstream signaling molecules, including protein kinases, adapter proteins, and phosphoinositide kinases. For instance, Rho can activate Rho-associated kinase, which in turn phosphorylates myosin II light-chain kinase in smooth muscle cells. Myosin II light-chain kinase can activate myosin light chain and facilitate myosin–actin interaction. The Rho family of small GTPases can also interact with molecules that link to the actin cytoskeleton. An example is the interaction of Rho with p140mDia, which induces the activation of p140mDia. Activated p140mDia can interact with profilin, an actin-binding protein.

Rho, Rac1, and Cdc42 are involved in the regulation of actin polymerization and the formation of stress fibers, which are myosin II-associated contractile actin filamentous bundles. In cultured Swiss 3T3 fibroblasts, cell transfection with active Rho and Rac mutants stimulates the formation of stress fibers and lamellipodia, or wide cell membrane protrusions. Cells transfected with a Rho inhibitor C3 transferase or a dominant-negative mutant for Rac exhibit reduced formation of stress fibers. In addition, Rho, Rac, and Cdc42 promote the formation of focal adhesion contacts. Since actin polymerization, cell membrane protrusion (formation of lamellipodia and filopodia), and formation of focal adhesion contacts are essential processes of cell migration, the small GTPases Rho, Rac, and Cdc42 contribute to the regulation of cell migration.

Role of MAPKs. As discussed in Chapter 5, mitogen-activated protein kinases (MAPKs) are key elements for signaling pathways that respond to the stimulation of growth factors, including platelet-derived growth factor, epidermal growth factor, fibroblast growth factor, and vascular endothelial growth factor. The binding of these growth factors to cognate growth factor receptors induces autophosphorylation of the receptor tyrosine kinase located in the cytoplasmic domain of the receptor. Such a process leads to the activation of a cascade of signaling molecules, including the Ras protein, ERK kinase, and ERK1/2, which belongs to the MAPK family. Activated ERK1/2 can directly phosphorylate myosin light-chain kinase, which activates myosin light chain and promotes myosin–actin interaction. These MAPK-involved processes influence cell migration via mediating the contractility of actin filaments.

CELL ADHESION

Cell adhesion is a molecular process that mediates cell–cell (intercell) and cell–matrix interactions and is involved in the regulation of developmental morphogenesis, physiological adaptation, and pathogenic remodeling. Cells can selectively bind to other cells and extracellular matrix by activating cell adhesion mechanisms. Cell adhesion is related to a variety of molecular and cellular processes, such as cytoskeletal reorganization, alterations in cell geometry, signaling activation, gene expression, and cell mitogenic responses. It is now understood that cell–cell adhesion and cell–matrix adhesion are regulated by several classes of cell adhesion molecules. These include immunoglobulin-like cell adhesion molecules, selectins, cadherins, cell surface heparan sulfate proteoglycans, protein tyrosine phosphatases, and integrins. Cell adhesion is a process that requires coordinated interactions between various cell adhesion molecules as well as between adhesion molecules and the actin cytoskeleton. In this section, the structural and functional characteristics of major classes of adhesion molecules are discussed.

Immunoglobulin-Like Domain-Containing Cell Adhesion Molecules [6.7]

Classification and Structure. Immunoglobulin (Ig)-like domain-containing cell adhesion molecules (IgCAMs) (see Table 6.5) are cell surface adhesion molecules that belong to the immunoglobulin superfamily, which contains about 100 members. IgCAMs mediate cell–cell and cell–matrix adhesion and play a role in regulating cell signaling. These molecules contribute to the regulation of embryonic development and pathogenic remodeling. In particular, IgCAMs play a critical role in mediating polarized migration of neurons and the development of the nerve system.

A typical IgCAM is composed of one or more Ig-like domains, which are located in the extracellular region of the molecule (Fig. 6.7). Each Ig-like domain is composed of about 100 amino acids, which constitute two opposed β sheets linked by disulfide bonds between cysteine residues. In addition, a typical IgCAM is composed of fibronectin (FN)-like repeats in the extracellular region. Each repeat consists of two β sheets of about 90 amino acids. Some IgCAMs are widely distributed in almost all tissues and organs, while others are expressed in limited tissues and organs. The expression pattern of IgCAMs is regulated to suit the function of various types of tissues and organs during development and remodeling.

Several types of IgCAMs have been identified and characterized. These include neural cell adhesion molecules (NCAMs), vascular cell adhesion molecules (VCAMs), intercellular adhesion molecules (ICAMs), L1-like IgCAMs, and receptor protein tyrosine phosphatases. A typical NCAM is composed of five Ig-like domains and two FN-like repeats in the extracellular region, a transmembrane domain, and a cytoplasmic domain. NCAMs are primarily expressed in neural cells and are involved in the regulation of neural cell adhesion and development. VCAM is composed of seven extracellular Ig-like domains, a transmem-

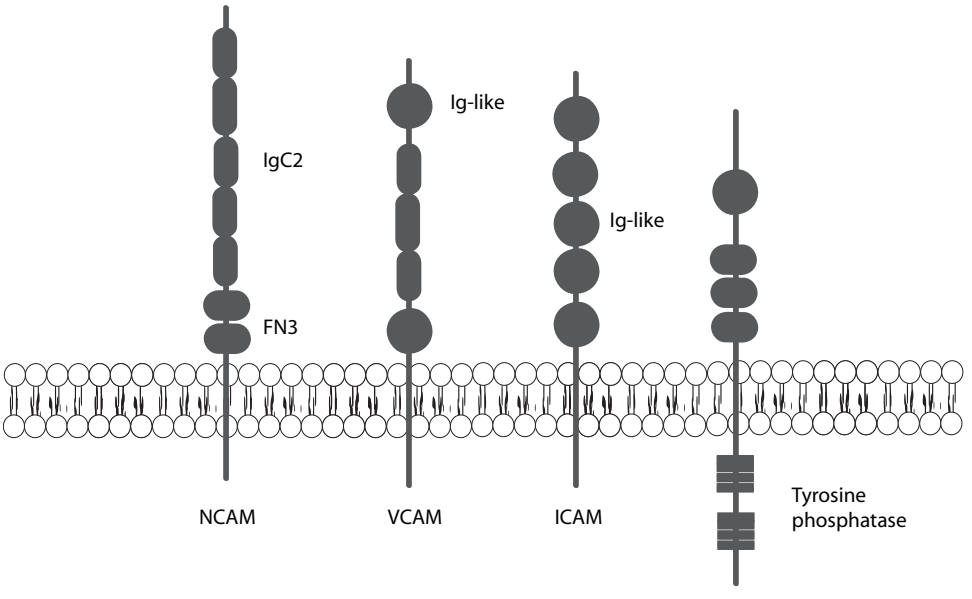


Figure 6.7. Schematic representation of the structure of immunoglobulin (Ig)-like domain-containing cell adhesion molecules. IgC2: Immunoglobulin C2-type domain. FN3: fibronectin type 3 domain. Based on bibliography 6.7.

TABLE 6.5. Characteristics of Selected Immunoglobulin-Like Domain-Containing Cell Adhesion Molecules*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Neural cell adhesion molecule 1	CD56, NCAM, NCAM1, NCA1, NCAM140	848	93	Nervous system	Regulating cell adhesion and signaling
Vascular cell adhesion molecule	VCAM, INCAM100, L1CAM	739	81	Vascular endothelial cells, heart, lung	Mediating the interaction of leukocytes with vascular endothelial cells and regulating cell signal transduction
Intercellular adhesion molecule1	ICAM1, CD54, CD54 antigen, surface antigen of activated B cells BB2	532	58	Leukocytes, vascular endothelial cell, brain, kidney, intestine, skin	Binding to integrins CD11a, CD11b, and CD18, and regulating cell adhesion
Protein tyrosine phosphatase receptor type δ	Phosphotyrosine phosphatase receptor δ, phosphotyrosine phosphatase receptor D, RPTP δ, protein tyrosine phosphatase δ	1912	215	Brain, heart, kidney, placenta	Consisting of three Ig-like and eight fibronectin type III-like domains in extracellular region, promoting neurite growth and axon extension, and mediating cell adhesion and signal transduction
Protein tyrosine phosphatase receptor type κ	Protein tyrosine phosphatase receptor type K, RPTP κ, PTPK, protein tyrosine phosphatase κ	1440	162	Ubiquitous	Regulating intercellular adhesion via interaction with β, γ-catenin at adherens junctions, and inhibiting the proliferation of certain cell types such as keratinocyte
Protein tyrosine phosphatase receptor type μ	Phosphotyrosine phosphatase receptor μ, RPTPμ, protein tyrosine phosphatase receptor-like 1, PTPRL1, protein tyrosine phosphatase μ	1452	164	Blood vessel	Regulating intercellular adhesion

*Based on bibliography 6.7.

brane domain, and a cytoplasmic domain and can be found in vascular endothelial cells. It is involved in the regulation of endothelial cell adhesion. L1-like IgCAMs consists of six Ig-like domains and five FN-like repeats, a transmembrane domain, and a cytoplasmic domain. These IgCAMs can be found in the nerve tissue and play an important role in regulating neuron–neuron and neuron–glial cell interactions. Several receptor protein tyrosine phosphatases (RPTP), including RPTPδ, RPTPκ, and RPTPμ, possess the function of IgCAMs. These molecules are composed of Ig-like domains and FN-like repeats in the extracellular region and two cytoplasmic phosphatase domains. While RPTPs catalyze dephosphorylation of protein tyrosine kinases, they also mediate cell–cell adhesion.

Functions

Role in Mediating Cell–Cell Adhesion. IgCAMs are cell membrane receptors and mediate cell adhesion via interaction between their extracellular domains and target molecules. Various forms of IgCAM interaction have been identified. Under appropriate conditions, some IgCAMs, such as NCAMs and L1-like IgCAMs, can initiate homophilic binding, specifically, interaction between identical IgCAM molecules of different cells. However, most IgCAMs undergo heterophilic interaction between different IgCAMs or between IgCAMs and non-IgCAM molecules, such as laminin and tenascin.

IgCAMs are involved in the mediation of neuron–neuron interaction. For instance, L1-like IgCAMs mediate interaction between different neurons homophilically as well as heterophilically. Such a process is critical to neuron–neuron interaction, which is the basis for neuronal communication. L1-like IgCAM interaction also facilitates neurite extension and outgrowth. In addition to the regulation of neuron–neuron interaction, IgCAMs are also involved in mediating neuron–glial cell interaction. Neural NCAMs are capable of interacting with receptor protein tyrosine phosphatase β in the membrane of glial cells. In this process, receptor protein tyrosine phosphatase β may serve as a substrate for neuron migration and outgrowth. Thus, IgCAM-mediated neuron–neuron and neuron–glial cell interactions contribute to the development of the nerve system.

Role in Mediating Cell–Matrix Adhesion. The interaction of neurons and extracellular matrix is a process that regulates neurite outgrowth and the morphogenesis of the nerve system. IgCAMs are involved in the mediation of neuron–matrix interaction. For instance, L1-like IgCAMs can interact with extracellular matrix-derived tenascin R. Such a process plays an important role in regulating neurite outgrowth. IgCAMs interact not only with extracellular matrix components but also with intracellular actin cytoskeleton. The intracellular interaction is mediated by an actin-binding molecule known as *ankyrin*. L1-like IgCAMs are capable of binding to ankyrin, which links IgCAMs to actin filaments via interaction with spectrin. The IgCAM linkage with actin cytoskeleton may enhance the interaction of the cell with extracellular matrix.

Role in Cell Signaling. IgCAMs are involved in the regulation of cell signal transduction. IgCAMs have been shown to interact with a number of signaling molecules, including the Src family nonreceptor tyrosine kinases, growth factor receptor tyrosine kinases, receptor protein tyrosine phosphatases, and serine/threonine protein kinases. For instance, IgCAM-dependent neurite outgrowth is reduced in the absence of Src and Fyn, suggesting that these nonreceptor tyrosine kinases may relay signals from IgCAMs.

As described above, certain types of receptor protein tyrosine phosphatases (RPTPs) are also IgCAMs. These RPTPs possess dual functions; the extracellular Ig-like region mediates cell adhesion, while the cytoplasmic phosphatase domain transmits adhesion-related signals to intracellular signaling pathways. Although the signaling mechanisms of the cytoplasmic domain remain poorly understood, it is possible that phosphatase-induced dephosphorylation of substrate proteins may play a role.

IgCAMs are also involved in fibroblast growth factor (FGF)-related cell signaling. L1-like IgCAMs and NCAMs can induce phosphorylation of the FGF receptor protein tyrosine kinase, which is independent of FGF ligand stimulation. The phosphorylation of the FGF protein receptor tyrosine kinase induces the activation of a cascade of signaling molecules, leading to mitogenic cellular activities.

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Selectins

Classification and Structure [6.8]. *Selectins* (Table 6.6) are lectin-type adhesion molecules expressed in the membrane of several cell types, including vascular endothelial cells, leukocytes, and platelets. Selectins are classified into several groups: E-selectin, L-selectin, and P-selectin. E-selectin is found in endothelial cells, and its function is to mediate the interaction of endothelial cells with leukocytes via binding to corresponding ligands. L-selectin is expressed in leukocytes and is responsible for binding to ligands on endothelial cells and other leukocytes. P-selectin is expressed in platelets and endothelial cells, and is responsible for binding to ligands on leukocytes and endothelial cells. Selectins are involved in the regulation of several basic leukocyte activities, including leukocyte adhesion to, rolling on, and migration through the endothelium.

A typical selectin is composed of several domains: an *N*-terminal lectin-like domain, an epidermal growth factor (EGF)-like domain, several consensus repeats, a transmembrane domain, and a cytoplasmic domain (Fig. 6.8). The lectin-like and EGF-like domains are similar in amino acid sequence among different selectins, while other domains differ between different selectins. The *N*-terminal lectin domain is responsible for the adhesion properties of selectins in a Ca^{2+}-dependent manner.

Function [6.9]. The primary function of selectins is to mediate interaction between leukocytes, platelets, and endothelial cells. Selectins can selectively bind to the oligosaccharides of glycoproteins in the membrane of a target cell. Endothelial cells express various selectin ligands, including glycosylation cell adhesion molecule-1, CD34, mucosal addressin cell adhesion molecule-1, and podocalyxin. Leukocytes express primarily E-selectin glycoprotein ligand-1 and P-selectin glycoprotein ligand-1. E-selectin and

TABLE 6.6. Characteristics of Selected Selectins*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
E-Selectin	Selectin-E, endothelial leukocyte adhesion molecule 1, ELAM1, ELAM, leukocyte endothelial cell adhesion molecule 2, LECAM2, CD62E	610	67	Vascular endothelial cells	Regulating leukocyte adhesion to endothelial cells and mediating inflammatory reactions
L-Selectin	Lymphocyte adhesion molecule 1, LYAM1, LAM1, CD62 antigen ligand, CD62L, leukocyte adhesion molecule 1, leukocyte endothelial cell adhesion molecule 1, LECAM1, SELL	385	44	Leukocytes, bone marrow cells	Regulating leukocyte adhesion to endothelial cells
P-selectin	Platelet α granule membrane protein, SELP, CD62, granulocyte membrane protein, GRMP, PSGL1	830	91	Platelets, endothelial cells	Regulating platelet adhesion to endothelial cells

*Based on bibliography 6.8.

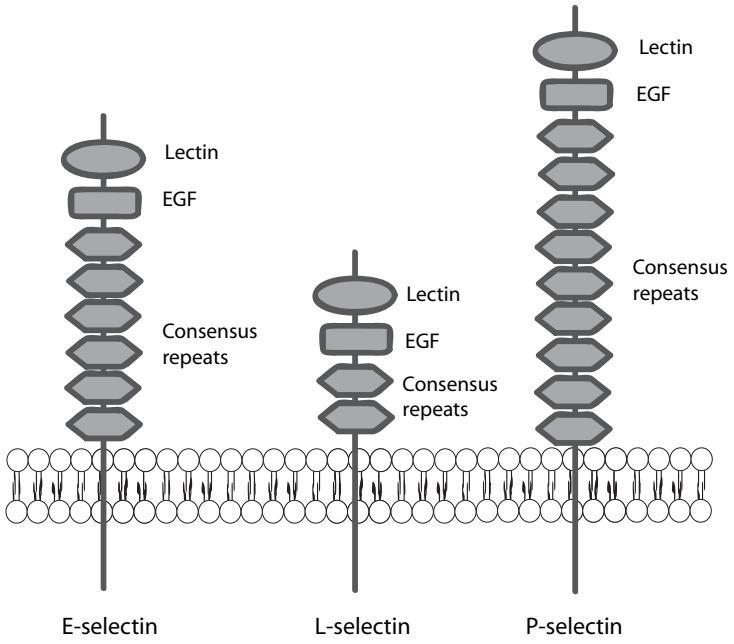

Figure 6.8. Schematic representation of the structure of selectins. Based on bibliography 6.8.

P-selectin of endothelial cells can bind to E-selectin glycoprotein ligand-1 and P-selectin glycoprotein ligand-1 of leukocytes, respectively. L-selectin of leukocytes can bind to glycosylation cell adhesion molecule-1, CD34, mucosal addressin cell adhesion molecule-1, and podocalyxin of endothelial cells. When leukocyte-leukocyte interaction takes place, the L-selectin of one cell can bind to the P-selectin glycoprotein ligand-1 of another cell. Similarly, the P-selectin of platelets can bind to the P-selectin glycoprotein ligand-1 of leukocytes and to the glycosylation cell adhesion molecule-1, CD34, and mucosal addressin cell adhesion molecule-1 of endothelial cells. The binding of selectins with corresponding ligands is the basis for leukocyte adhesion to and rolling on the endothelium.

Under physiological conditions, the constitutive level of selectins in the cell membrane is considerably low, and thus leukocytes and platelets rarely adhere to endothelial cells. Endothelial cells and platelets can synthesize and maintain a constitutive pool of selectins (primarily P-selectin). The synthesized selectin molecules are not deployed to the cell membrane, but stored in the α-granules of platelets and the Weibel–Palade bodies of endothelial cells. These selectin molecules can be redistributed to the cell membrane rapidly in response to inflammatory stimulation. The expression of selectins and ligands are also upregulated in inflammatory reactions. Increased selectin level enhances selectin–ligand interaction and thus facilitates leukocyte and platelet adhesion to the endothelium.

Since leukocytes and endothelial cells are subject to bloodflow, the formation of selectin and ligand bonds must be rapid and the bonds must be sufficiently strong to resist shearing forces imposed by the bloodflow. Under certain shearing conditions, adhered leukocytes may roll on the endothelium. Such a process requires coordination between shear stress and the adhesion bond dynamics, so that the formation of adhesion bonds at the cell leading edge due to selectin–ligand interaction is associated with an equal level

of disruption of adhesion bonds at the cell trailing edge due to shear stress. Leukocyte adhesion to and rolling on the endothelium are critical processes in inflammatory responses. These processes prepare leukocytes for transmigration into the interstitial space, where inflammatory reactions take place.

Selectin–ligand interaction may contribute to signal transduction in leukocytes and endothelial cells. Although a complete mechanism is not yet demonstrated, preliminary studies have shown that leukocyte adhesion to L-selectin ligands induces calcium redistribution and activation of mitogen-activated protein kinases. The level of activation is related to the density of the selectin ligands. There is also evidence that selectin–ligand interaction induces activation of integrins. Further investigations are needed to clarify selectin-related signaling pathways.

Cadherins

Classification and Structure [6.10]. *Cadherins* (Table 6.7) are a family of calcium-dependent cell adhesion molecules, which are characterized by the presence of cadherin-specific repeats in the extracellular region of the molecule. Cadherins are traditionally classified into several subfamilies: classical cadherins, protocadherins, and desmosomal cadherins. Cadherins are usually associated with a class of molecules known as *catenins*. These adhesion molecules are involved in the regulation cell–cell interaction, tissue morphogenesis, as well as mitogenic activities such as cell proliferation and migration.

The classical cadherin subfamily includes E-, P-, and N-cadherins (Fig. 6.9). These molecules are localized to the zonula adherens or adherens junctions, which are intercellular contacts required for cell adhesion, cell–cell communication, and tissue formation and organization. These cadherins share similar amino acid sequences and mediate Ca^{2+}-dependent cell–cell interaction and connection. A typical classical cadherin is composed of an *N*-terminal precursor sequence, which contains a proteolytic processing signal sequence K/RRXKR, four characteristic cadherin repeats immediately following the *N*-terminal precursor sequence, a transmembrane domain, and a well-conserved cytoplasmic domain. Cleavage of the *N*-terminal precursor sequence is required for the activation of cadherin. Each cadherin repeat in the extracellular region contains consensus Ca^{2+}-binding sites. The binding of Ca^{2+} induces dimerization of cadherins and protection of the molecule from degradation. The cytoplasmic domain of cadherins interacts with the actin cytoskeleton via cadherin-associated proteins known as catenins.

Protocadherins constitute another cadherin subfamily. Compared with the classical cadherins, these adhesion molecules are characterized by the lack of the proteolytic precursor sequence and the presence of more than four cadherin repeats in the extracellular region (Fig. 6.10). In addition, unlike the classical cadherins, the cytoplasmic domain of protocadherins is considerably heterogeneous in structure. The structural difference suggests different mechanisms in regulating cell adhesion between protocadherins and classical cadherins. It appears that cell adhesion mediated by protocadherins is not as strong as that mediated by classical cadherins.

The third subfamily of cadherins is found in desmosomes and is defined as desmosomal cadherins. Desmosomes are intercellular structures identified in epithelial and cardiac muscular cells (Fig. 6.11) and are responsible for cell–cell interaction and connection, which play a critical role in regulating the formation and integrity of tissues and organs. A typical desmosome appears under an electron microscope as a complex with two parallel plaques (one from each cell) and a narrow gap (~30 nm in width) between two cell

TABLE 6.7. Characteristics of Selected Cadherins*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
E-Cadherin	Calcium-dependent adhesion protein, cadherin 1, epithelial E-cadherin, ECAD, calcium-dependent adhesion protein, epithelial liver cell adhesion molecule, LCAM	901	100	Ubiquitous	A glycoprotein that regulates cell adhesion, which is a calcium-dependent process
P-Cadherin	Placental P cadherin, cadherin 3, PCAD, placental calcium-dependent adhesion protein, CDHP	829	91	Ubiquitous	A glycoprotein that regulates cell–cell adhesion (note that its action is dependent on calcium)
N-Cadherin	Cadherin 2, neuronal cadherin, N cadherin, NCAD, CDHN.	906	100	Nervous system, ovary, testis	A glycoprotein that regulates calcium-dependent cell–cell adhesion
Protocadherin 1	Protocadherin 42, PCDH42, PC42, cadherin-like protein 1	1237	134	Brain	A membrane protein found at cell–cell junctions, regulating neural cell adhesion and development
Desmoglein 1	Desmosomal glycoprotein 1, DG1, pemphigus foliaceus antigen, desmoglein 1 preproprotein	1049	114	Skin, esophagus	Serving as a calcium-binding glycoprotein component of desmosomes in epithelial cells, and regulating cell–cell adhesion and interaction
Desmocollin	Desmocollin 1A/1B, type I, desmocollins desmosomal glycoprotein 2/3, DG2/DG3	894	100	Skin, thymus	Serving as a calcium-binding glycoprotein component of desmosomes in epithelial cells and regulating cell–cell adhesion and interaction

*Based on bibliography 6.10.

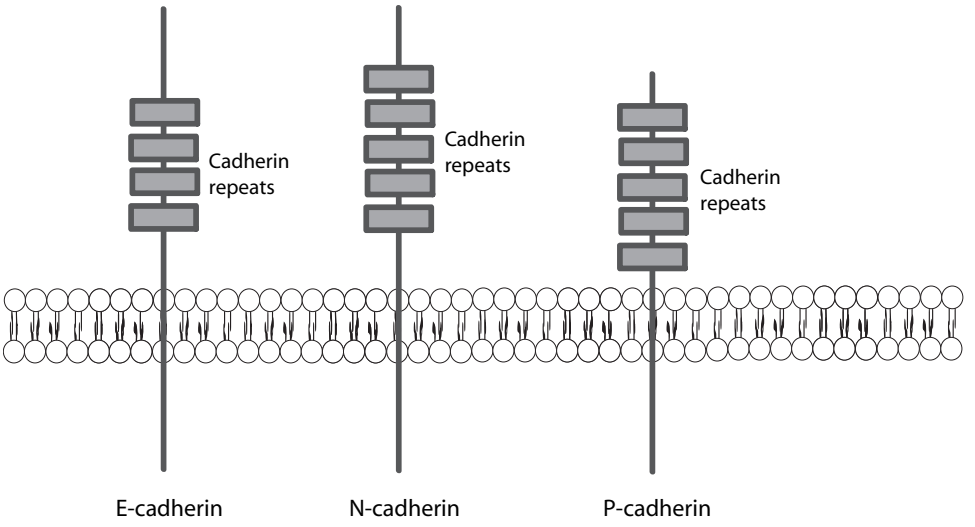

Figure 6.9. Schematic representation of the structure of cadherins. Based on bibliography 6.10.

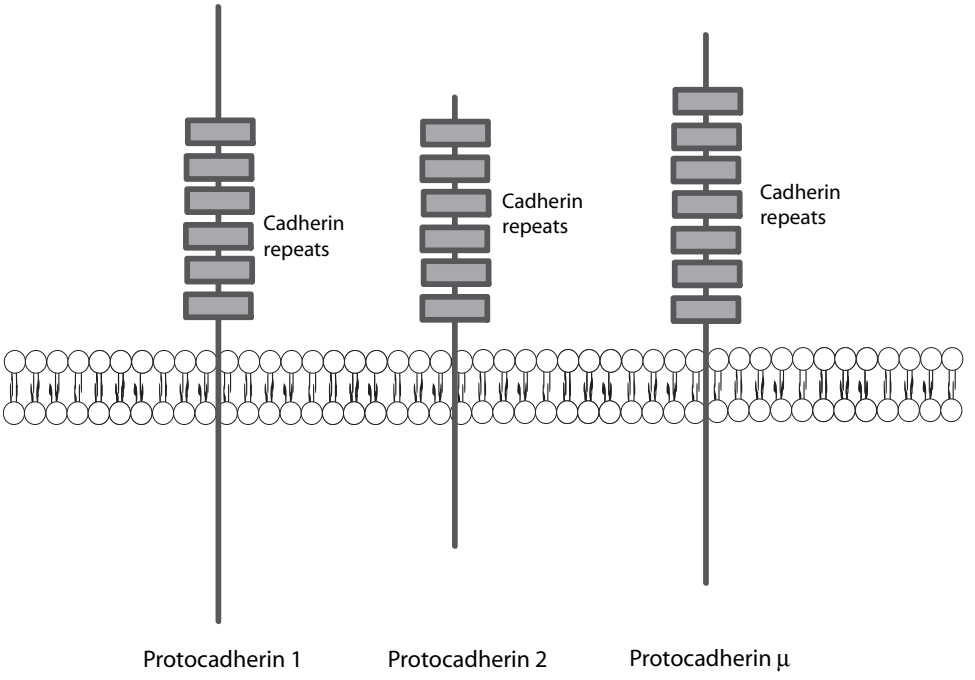

Figure 6.10. Schematic representation of the structure of Protocadherins. Based on bibliography 6.10.

membranes. Such a structure contains several components, including desmosomal cadherins, plakoglobin, plakins, and plakophilins.

There are two types of desmosomal cadherin in desmosomes: desmogleins and desmocollins. These are glycoproteins containing amino acid sequences that are similar to

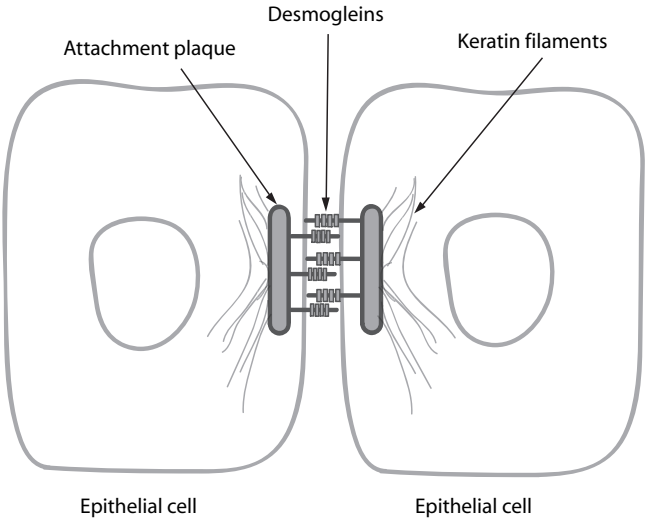

Figure 6.11. Schematic representation of the structure of desmosomes. Based on bibliography 6.10.

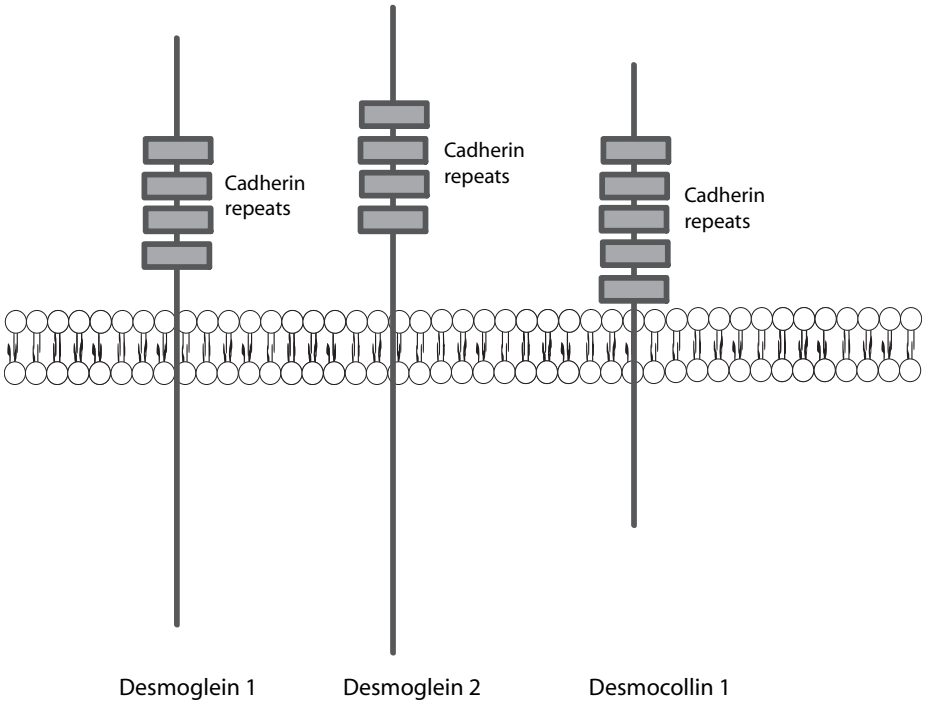

Figure 6.12. Schematic representation of the structure of desmogleins. Based on bibliography 6.10.

the classical cadherins described above. However, desmogleins contain three additional domains in their cytoplasmic tail, including a proline-rich linker, a repeating unit domain, and a terminal domain (Fig. 6.12). Each type of desmosomal cadherin exists in three isoforms. The isoforms for desmogleins are desmoglein-1,2,3, and those for desmocollins are

desmocollin-1,2,3. The distribution of each desmosomal cadherin varies among tissues and organs. For instance, desmoglein-2 and desmocollin-2 are ubiquitously expressed in tissues with desmosomes, whereas desmoglein-3 and desmocollin-3 are found mostly in the basal layer of stratified epithelia.

Function [6.11]. Cadherin-mediated cell adhesion plays an important role in the regulation of tissue and organ morphogenesis and development. A change in the expression pattern of major cadherins is often associated with altered morphogenetic processes. For instance, E-cadherin is highly expressed and activated in the oocyte after fertilization, while N-cadherin is not. At gastrulation, the expression and activation pattern of the E- and N-cadherins is switched; specifically, the E-cadherin is downregulated whereas the N-cadherin is upregulated. Such a switch is associated with epithelial–mesenchymal transition. Furthermore, the overexpression of N-cadherins blocks the segregation of neural crest cells from the neural tube. These observations suggest that the expression and activation of appropriate cadherins are critical to the regulation of tissue and organ morphogenesis during embryonic development.

Cadherins are involved in the regulation of cell differentiation. This function has been demonstrated in E-cadherin-null embryonic stem cells. While wildtype embryonic stem cells can differentiate into well-organized forms of specialized tissues, E-cadherin-null stem cells develop only into tissues without specialized forms. The transfer of E-cadherin gene into E-cadherin-null stem cells restores the differentiation function of the embryonic stem cells. These observations suggest that E-cadherins play a critical role in regulating the differentiation of embryonic stem cells.

Cell adhesion mediated by cadherins plays a role in the regulation of cell survival. This is supported by several lines of evidence. The lack of E-cadherins is associated with the induction of endothelial cell apoptosis. Caspases can cleave cadherins and induce cell dissociation, contributing to cell apoptosis. Thus, the presence of cadherins is essential to cell survival.

Cell Surface Heparan Sulfate Proteoglycans

Classification and Structure [6.12]. Cell surface heparan sulfate proteoglycans are composed of heparan sulfate glycosaminoglycan (GAG) chains, which are attached to core proteins in the extracellular region. These proteoglycans serve as adhesion receptors and participate in the regulation of cell–cell and cell–matrix interactions. Heparan sulfate can form various types of heparan sulfate proteoglycan, including perlecan and agrin in extracellular matrix, serglycin in the cytoplasm, and syndecans and glypicans in the cell membrane. The function of heparan sulfate proteoglycans is determined by the heparan sulfate glycosaminoglycan group.

A heparan sulfate molecule is composed of highly sulfated heparin-like domains and poorly sulfated domains with rich glucuronic acids. These domains alternate with intermediate sulfated domains. The total length and the number of each domain of a heparan sulfate vary considerably between different cell types. The differences between cell types may be due to the existence of multiple isoforms of modification enzymes. A major function of the heparan sulfate chains in a cell surface heparan sulfate proteoglycan is to bind ligands. A large number of proteins can bind to heparan sulfate. Although the amino acid sequences of these binding proteins vary widely, the binding proteins are rich in basic amino acids such as lysine and arginine.

TABLE 6.8. Characteristics of Selected Heparan Sulfate Proteoglycans*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Syndecan 1	Syndecan, SYND1, SDC1, CD138 antigen, CD138.	310	32	Skin, kidney, placenta, lymphocytes	A transmembrane heparan sulfate that mediates cell binding, cell signaling, cytoskeletal organization, cell proliferation, cell differentiation, and HIV transmission to lymphocytes
Glypican 1	GPC1	558	62	Pancreas, intestine, bone marrow, placenta	A heparan sulfate proteoglycan that attaches to external surface of cell membrane and regulates cell–cell interaction

*Based on bibliography 6.12.

Syndecans and glypicans (see Table 6.8) are the most abundant heparan sulfate proteoglycans at the cell surface. Syndecans are transmembrane receptors with heparan sulfate chains attached to the extracellular region at the N-terminus (Fig. 6.13). Syndecans are a family of several heparan sulfate proteoglycans, including syndecan-1,2,3,4 with molecular weights 33, 22, 46, and 22 kDa, respectively. The extracellular domain of the four syndecan molecules differs considerably. Syndecan-2,-3 contain in their extracellular region primarily heparan sulfate chains, whereas syndecan-1,-4 contain chondroitin sulfates in addition to heparan sulfates. The extracellular domain of syndecans is composed of heparan sulfate attachment sites, signal peptide sites, and proteolytic cleavage sites. Syndecans can be shed from the cell membrane via protein cleavage at sites near the cell membrane. Such a process converts receptor-type to soluble syndecans. Both receptor and soluble syndecans can bind to the same type of ligands.

The transmembrane domain of syndecans is well conserved among different types of syndecan. This domain plays a role in mediating the dimerization of the syndecan molecules and localizing syndecans to appropriate membrane compartments. The cytoplasmic tail of all syndecans is highly conserved. This tail is composed of phosphorylation sites and binding sites for cytoskeletal proteins and signaling molecules.

Glypicans are globular molecules with molecular weight ~60 kDa. Six types of glypican have been identified. Each glypican is composed of an *N*-terminal cysteine-rich domain and heparan sulfate attachment motifs. Glypicans are attached to the external surface of the cell membrane via a glycosyl phosphatidylinositol anchor, which is localized to the membrane microdomains with rich glycosphingolipids. The heparan sulfate chains are attached to the *C*-terminus of the core proteins near the cell membrane. Glypicans do not pass through the cell membrane.

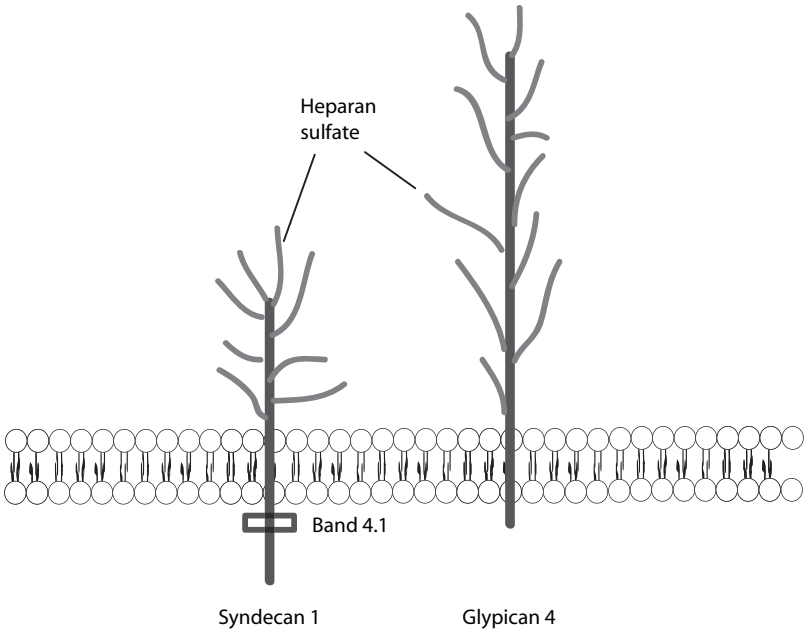

Figure 6.13. Schematic representation of the structure of syndecans. Based on bibliography 6.12.

Function [6.13]. Cell surface heparan sulfate proteoglycans participate in the regulation of cell–cell adhesion and interaction. Syndecans are localized to adherens junctions and can interact with several adhesion molecules, such as L-selectin, N-CAM, and PE-CAM. The lack of syndecan-1 is associated with reduced cell aggregation, and the transfection of the syndecan-1 gene restores cell aggregation. These observations suggest that the presence of syndecans is essential to cell–cell adhesion. However, the exact mechanisms remain to be investigated.

Cell surface heparan sulfate proteoglycans play a role in the regulation of cell–matrix adhesion. Extracellular matrix contains a number of components, including collagen, elastin, fibronectin, laminin, tenascin, vitronectin, and thrombospondin, which are capable of interacting with syndecans. During development, syndecans are colocalized with extracellular matrix components. Certain types of syndecans, such as syndecan-1 and -4 are localized to the focal adhesion contacts. In heparan sulfate-deficient cells, the formation of focal adhesion contacts is impaired. These observations demonstrate the importance of cell surface heparan sulfate proteoglycans in the control of cell–matrix interaction. The role of glypicans in regulating cell–matrix adhesion remains to be determined, although glypicans have been found to bind to collagen and fibronectin.

Integrins

Classification and Structure [6.14]. Integrins are transmembrane glycoproteins that mediate cell–matrix adhesion, providing a linkage between the cytoskeleton and extracellular matrix (Fig. 6.14). Integrins also mediate cell–cell interactions. Each integrin is a heterodimer composed of a variable α subunit and a relatively conserved β subunit. To date, at least 18 α subunits and 8 β subunits have been identified. The binding ligands of

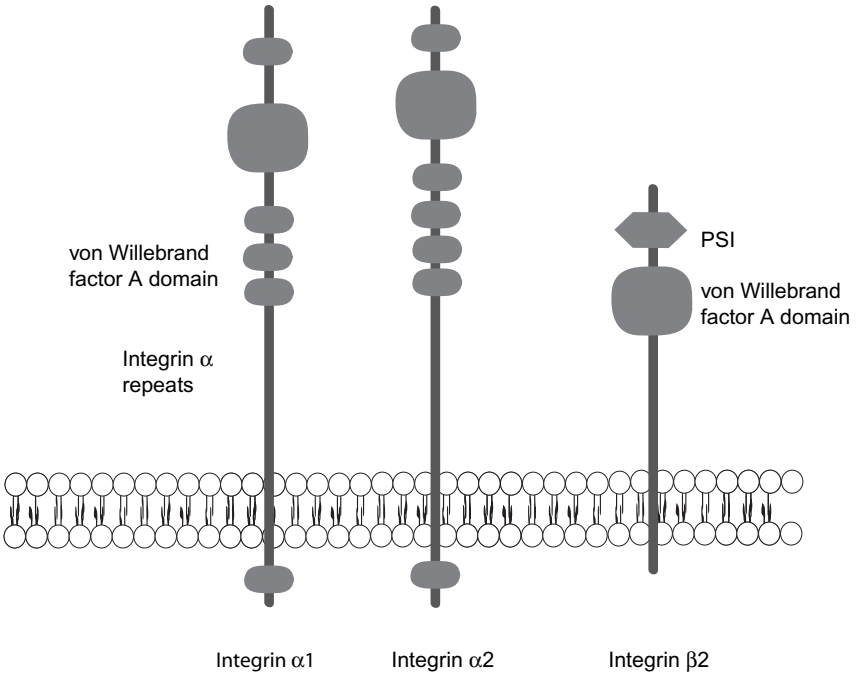

Figure 6.14. Schematic representation of the structure of integrins. PSI: domain found in plexins, semaphorins, and integrins. Based on bibliography 6.14.

integrins are determined by the combination of specific α and β subunits. Several α integrins and a common β integrin are presented in Table 6.9; some of these molecules are used for treatment of muscular dystrophy.

Both integrin α and β subunits contain an extracellular domain, a transmembrane domain, and a cytoplasmic tail. The α and β subunits contain distinct amino acid sequences. The β subunit is about 760–790 amino acids in length, whereas the α subunit is about 1000–1200 amino acids. The majority of amino acids are distributed in the extracellular region for both α and β subunits. The extracellular region of the β subunit is composed of an I-domain, which is responsible for ligand binding, and several cysteine-rich repeats. The extracellular region of the α subunit is composed of an I-domain (in at least 9 α subunits) and several repeats with a consensus sequence DxDxDGxxD (x = any amino acid). These repeats are responsible for integrin binding.

Although the cytoplasmic tail of integrins is considerably short, this region plays an important role in mediating interactions between integrins and intracellular signaling molecules, between integrins and cytoskeletal components, and between the integrin α and β subunits. The cytoplasmic tail is composed of a large number of binding sites for these interactions. The binding of integrins to the actin cytoskeleton is the basis for the formation of cell focal adhesion contacts, essential structures for the control of cell attachment to extracellular matrix and cell migration.

Each cell contains a number of different types of integrins. The β1 subunit forms a dominant subfamily of integrins with the α subunits. The β1-containing integrins are defined as β1 integrins. The ligands of β1 integrins are determined primarily by the specificities of the α subunits. For example, α1β1 and α2β1 bind to collagen and laminin, α4β1 and α5β1 bind to fibronectin, whereas α6β1 binds to laminin.

TABLE 6.9. Characteristics of Selected Integrins*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Integrin α1	Laminin and collagen receptor, very late activation protein 1, VLA1, CD49a	1179	131	Ubiquitous	Forming a heterodimer with integrin β1, serving as a receptor for collagen and laminin, regulating cell–matrix interaction, and mediating cell survival, migration, and proliferation
Integrin α2	ITGA2, very late activation protein 2 receptor α2 subunit, VLA2 receptor α2 subunit, VLA2 α chain, VLAA2, CD49B, platelet glycoprotein Ia/IIa, platelet membrane glycoprotein Ia, GPIa, collagen receptor	1181	129	Ubiquitous	Forming a heterodimer with integrin β1 and serving as a receptor for collagen
Integrin α3	ITGA3, CD49C, very late activation antigen 3 (VLA-3), very common antigen 2 (VCA-2), extracellular matrix receptor 1 (ECMR1), and galactoprotein b3 (GAPB3), VLA3 α chain	1066	119	Ubiquitous	Forming a heterodimer with integrin β1 and and mediating cell interaction with extracellular matrix components, such as collagen, fibronectin, and laminin
Integrin α4	ITGA4, CD49D, very late activation protein 4 receptor α4 subunit, VLA4 receptor α4 subunit	1038	115	Blood vessels, blood cells, skin.	Forming a heterodimer with integrin β1 regulating cell interaction with fibronectin, and mediating vascular cell–cell interaction

Integrin α5	ITGA5, CD49e, fibronectin receptor α subunit, very late activation protein 5α subunit	1049	115	Ubiquitous	Forming a heterodimer with integrin β1, regulating cell interaction with fibronectin
Integrin α6	ITGA6, CD49f, very late activation protein 6α subunit, VLA-6	1073	120	Skin, lung, intestine, stomach, pancreas.	Forming a heterodimer with integrin β1, regulating cell interaction with laminin
Integrin α7	ITGA7	1137	124	Heart, skeletal muscle, nervous system, lung, intestine, overy, prostate gland	Joining with integrin β1 to form an integrin complex, which is a major integrin complex expressed in differentiated muscle cells, binding to the extracellular matrix protein laminin-1, and regulating cell attachment to extracellular matrix
Integrin αv	ITGAV, vitrionectin receptor α polypeptide, CD51	1048	116	Ubiquitous	Forming heterodimers with several β integrins, such as integrin β1, 3, and 5; mediating cell adhesion to extracellular matrix (note that the integrin complex αvβ3 regulates cell adhesion to vitronectin), and regulating TGFβ-related signal transduction
Integrin β1	ITGB1, CD29, very late activation protein, β polypeptide, VLAβ	825	92	Ubiquitous	Joining with an integrin α subunit to form integrin complexes, regulating cell adhesion to extracellular matrix, regulating various cellular activities, including embryogenesis, cell proliferation and migration, immune response, and metastasis of tumor cells

*Based on bibliography 6.14.

Function [6.15]. Integrins play a critical role in the regulation of cell migration. As discussed on page 270 of this chapter, in order to induce migration, a cell must attach to a matrix substrate at the leading edge while detaching from the substrate at the trailing edge at a given time. Although the exact mechanisms are poorly understood, integrins are likely involved in the regulation of these processes. During cell migration, integrins at the leading edge must be engaged to form strong adhesion bonds between focal adhesion contacts and the matrix substrate (Fig. 6.15), whereas integrins at the trailing edge must dissociate from the matrix substrate. The location-dependent integrin activation and deactivation within a single cell remains a subject of research.

In addition to the regulation of cell membrane attachment to matrix substrate, integrins are involved in regulating the contractile activity of the actin cytoskeleton. Integrins can be activated by exposure to extracellular matrix. Activated integrins can lead to phosphorylation of the mitogen-activated protein kinases (MAPKs) via the mediation of focal adhesion kinase and adaptor proteins. MAPKs can directly phosphorylate the myosin light-chain kinase (MLCK), which in turn activates myosin light chain, inducing and enhancing actin–myosin interaction. Actin–myosin interaction generates forces necessary for cell protrusion and traction during migration.

Integrins are involved in regulating the assembly of extracellular matrix. One example is the control of basement membrane formation by β1 integrins in epithelial tissues. Embryonic stem cells derived from β1 integrin-null mice are not able to form a basement membrane in epidermal tissue. Another example is integrin-related fibronectin fibrillogenesis. The assembly of fibronectin fibrils can be initiated on the binding of fibronectin to the α5β1 integrin. The loss of this type of integrin is associated with impairment of fibronectin fibrillogenesis. Other types of integrin, such as α4β1 and αvβ3, also contribute to the regulation of fibronectin fibrillogenesis.

Integrins play a critical role in the regulation of cell differentiation and proliferation. In several experimental models, integrins have been shown to mediate the pattern of gene expression and cell differentiation. For instance, salivary gland cells can differentiate into duct and acinar epithelial cells in response to the interaction of integrins and extracellular matrix, whereas these cells cannot differentiate in the absence of extracellular matrix. Furthermore, a treatment with antibodies specific to collagen IV and integrin α6 and β1

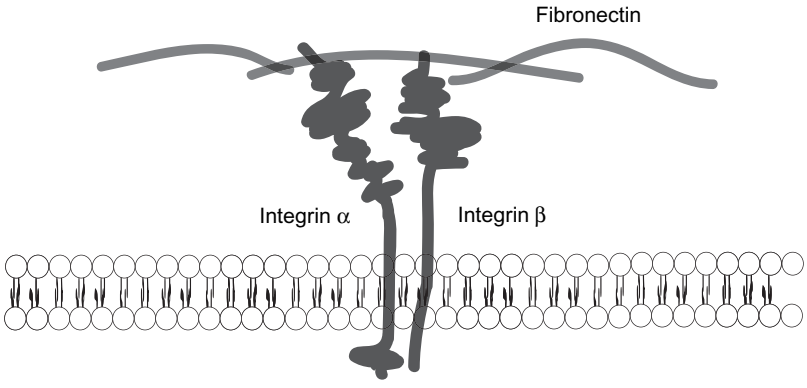

Figure 6.15. Schematic demonstration of interaction of integrins with fibronectin. Based on bibliography 6.15.

reduces the capability of cell differentiation. The attachment of cells to fibronectin- and collagen-containing matrix is often associated with extensive changes in gene expression. Altered gene activities may likely contribute to integrin-initiated cell differentiation and proliferation. The loss of β1 integrins in keratinocytes is associated with a reduction in cell differentiation and proliferation. These observations suggest a critical role for integrin-matrix interaction in the regulation of cell differentiation and proliferation.

Mechanisms of Integrin-Related Activities [6.15]. The primary function of integrins is to regulate the adhesion of cells to extracellular matrix. Integrins can be activated by exposure to extracellular matrix, which induces conformational changes and clustering of integrins. The activation of integrins is associated with an increase in integrin binding affinity. Integrins are major constituents of focal adhesion contacts, structures regulating cell adhesion to extracellular matrix. Focal adhesion contacts were original identified in cultured fibroblasts by electron microscopy, which demonstrates the presence of electron dense plaques with filamentous structures. These plaques were later found to contain a number of molecules, including integrins, actin filaments, talin, filamin, α-actinin, vinculin, profilin, paxillin, tensin, focal adhesion kinase, the Src family kinases, protein tyrosine phosphatases, the Grb2 adaptor protein, and phosphoinositide 3-kinase. Integrins serve as links between the intracellular actin cytoskeleton and extracellular matrix, and transmit signals from the extracellular matrix to the actin cytoskeleton and intracellular signaling pathways.

Integrins do not possess intrinsic catalytic activity. However, the β subunit of integrins can transmit a variety of extracellular signals via their connection with intracellular signaling molecules and actin cytoskeleton-associated molecules as described above. The importance of the β subunit can be tested by selected sequence deletion and restoration. The deletion of the β subunit is associated with diminished interaction of integrins with intracellular signaling molecules. Compared with the β subunit, the α subunit binds to fewer molecules. Identified molecules that bind to the α subunit include calreticulin, guanine nucleotide exchange factor Mss4, and calcium-binding protein. The physiological function of the α subunit-binding proteins remains to be determined.

The interaction of extracellular matrix with integrins often initiates intracellular signaling events, leading to molecular activities such as phosphorylation of protein tyrosine kinases, changes in the level of cAMP and calcium, and expression of mitogenic genes. In particular, integrins can transmit signals to two nonreceptor protein tyrosine kinases: focal adhesion kinase (FAK) and Src protein tyrosine kinase. These protein tyrosine kinases are localized to focal adhesion contacts. FAK can be phosphorylated in response to interaction of integrins with fibronectin, although the mechanisms of FAK activation remain poorly understood. Phosphorylated FAK induces the recruitment of Src to FAK. Recruited Src in turn phosphorylates FAK at various sites, enhancing the activity of FAK. These activities lead to the recruitment of adaptor proteins, including Grb2 and $pp130^{Cas}$, to the focal adhesion contacts. These adapter proteins link the integrin-FAK pathway to other signaling pathways, including the PI3-kinase, Ras, and MAPK pathways, which play critical roles in regulating cell proliferation and migration.

Integrin-related signaling molecules can communicate with the Rho family of small GTPases, including Rho, Cdc42, and Rac, which regulate the assembly and function of the actin cytoskeleton. The interaction of integrins with extracellular matrix can induce activation of Rho, Cdc42, and Rac. Activated Rho, Cdc42, and Rac enhance the assembly of focal adhesion contacts and the activity of related signaling molecules, including FAK.

However, the exact regulatory mechanisms remain to be investigated. These investigations suggest that integrin-dependent signaling pathways can "crosstalk" to other signaling pathways. Such interaction provides a synergistic mechanism for the regulation of cell adhesion, migration, proliferation, and differentiation.

APOPTOSIS [6.16]

Apoptosis is a process of naturally occurring cell death, which eliminates malfunctioned and undesired cells during development and remodeling. During development, apoptosis and cell division together contribute to the morphogenesis of tissues and organs. While cell division contributes to the growth of cell and tissue mass, apoptosis contributes to the removal of excessive cells. These processes are unnecessary for the formation of tissues and organs. After reaching maturity, apoptosis continues to play an important role in the maintenance of the homeostasis. Cells with damaged or mutant DNA are eliminated by apoptosis. Without apoptotic elimination, these cells may develop into tumor cells. Under a physiological condition, the cell density is kept at a relatively constant level through coordinated cell proliferation and apoptosis. An increase in apoptotic activity results in tissue degeneration, whereas a decrease in apoptotic activity results in hyperplasia, both of which contribute to pathogenic disorders of tissues and organs. Thus, it is important to maintain a physiological level of apoptotic activity.

Morphological Characteristics of Apoptosis [6.16]

In biological research, it is important to identify apoptotic cells, which helps to understand the mechanisms of apoptosis. Apoptotic cells undergo several stages of morphological change. On the stimulation of apoptotic signals, a cell usually starts to round up and becomes spherical in shape. These morphological changes are usually associated with cell membrane budding. The next noticeable change is DNA condensation, resulting in an increase in the nucleus density and reduction in the nucleus size, which can be seen under an optical and electron microscope (Fig. 6.16). DNA condensation is followed by DNA fragmentation and nucleus disruption. The entire cell eventually disintegrates into small pieces, which are phagocytosed by macrophages or neighboring cells. These morphological features can be used to identify apoptotic cells.

Apoptosis-Inducing Factors [6.16]. Apoptosis can be induced by a variety of extracellular factors, such as depletion of growth factors and nutrients, hypoxia, UV irradiation, mechanical stress, and binding of apoptotic ligands. Intrinsic changes, such as DNA damage and disruption, and immunoreactions, such as T-lymphocyte activation, can also trigger apoptosis. In addition, cancer cells can initiate apoptosis, an important mechanism for the elimination of cancer cells. Suppression of the apoptotic function increases the possibility of tumorigenesis.

Regulation of Apoptosis [6.16]. Apoptosis can be induced and regulated by two known signaling pathways: Fas ligand (FasL)- and tumor necrosis factor (TNF)-activated pathways. FasL and TNF are apoptosis-inducing proteins. These proteins are generated in the ER, deployed to the cell membrane, and cleaved from the cell membrane to form soluble ligands. The forms of the ligands determine the effectiveness of the ligands. For the Fas ligand, the membrane-bound form is more effective than the soluble form. In

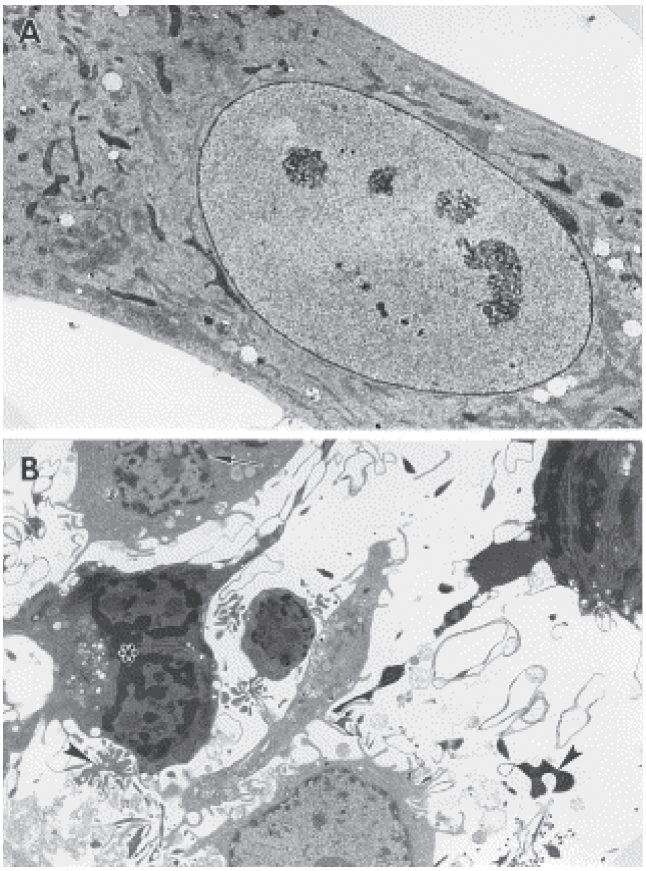

Figure 6.16. Electron micrographs of apoptotic human fibroblasts. (A) A control cell and (B) cells exposed to 0.5-μM naphthazarin (5,8-dihydroxy-1,4-naphthoquinone), an apoptosis inducer, for 8 h are shown. Different stages of apoptosis can be discerned in the treated cells: reduced cell size, condensed chromatin (stars), fragmented nuclei (arrow), and apoptotic bodies (arrow heads). Reprinted from Roberg K et al., Lysosomal release of cathepsin D precedes relocation of cytochrome c and loss of mitochondrial transmembrane potential during apoptosis induced by oxidative stress, *Free Radical Biol Med* 27:1228–37, 1999, with permission from Elsevier.

contrast, soluble TNF is more active than the membrane-bound form of TNF. The mechanism of Fas ligand-induced apoptosis is similar to that induced by TNF. Here, the FasL signaling pathway is used as an example to demonstrate the mechanisms of apoptosis (Fig. 6.17).

A Fas ligand can interact with the Fas ligand receptor, resulting in oligomerization (often trimerization) and activation of the receptor. Activated Fas ligand receptor in turn stimulates the Fas ligand-associated death domain or FADD (note that TNF can interact with and activate TNF receptor, which stimulates the TNFR-associated death domain or TRADD). Activated FADD (or TRADD) binds to a downstream protein known as *caspase 8* (cysteine aspartate protease 8), which belongs to the caspase family and possesses protease catalytic activity. In unstimulated cells, caspase 8 exists in an inactive form known as *procaspase 8*. In response to the stimulation of FADD (or TRADD), caspase 8 can be

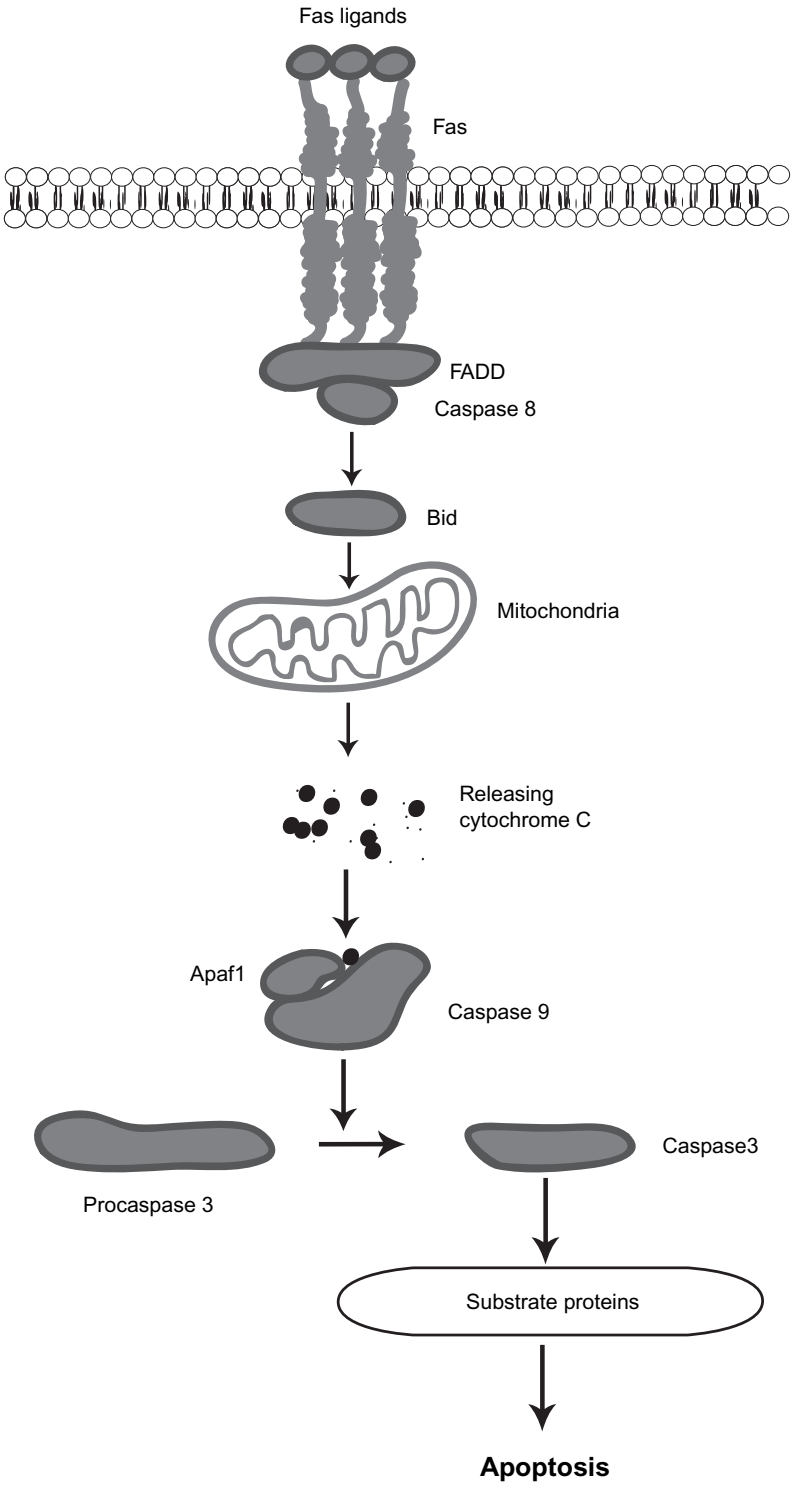

Figure 6.17. Schematic demonstration of the mechanisms of Fas-induced apoptosis. Based on bibliography 6.16.

activated via autocatalytic cleavage. Caspase 8 can further activate a downstream protein known as *Bid*, a member of the Bcl2 family, by cleaving the C-terminal domain of the substrate. Following the cleavage by caspase 8, Bid can be translocated to the mitochondrial membrane. A major action of Bid is to release mitochondrial cytochrome *c* into the cytoplasm. Cytochrome *c* in turn activates a downstream protein named apoptosis *protein-activating factor-1* (Apaf-1), which binds ATP and interacts with procaspase 9, resulting in the formation of the active form of caspase 9 via autocatalytic cleavage. Caspase 9 can cleave procaspase 3, releasing the active form caspase 3. Caspase 3 is a terminal-stage protease, which cleaves and degrades a variety of signaling and structural proteins, including protein kinases, poly[A]polymerse, and actin filaments. Caspase 3 can also cleave DNA fragmentation factor (DFF), releasing a DFF subunit. This subunit can activate nucleases, which induces DNA degradation. These activities eventually lead to DNA fragmentation and cell degeneration. (See Table 6.10.)

Assessment of Cell Apoptosis [6.17]

There are several methods that can be used for assessing cell apoptosis. These methods have been developed on the basis of cell morphological and molecular changes in apoptotic cells and are classified into several groups: (1) methods based on changes in the structure of cell membrane, (2) methods based on changes in cell morphology, (3) methods based on DNA fragmentation, (4) methods based on cytochrome *c* translocation, and (5) methods based on caspase activities. These methods are briefly discussed here.

Assessing Changes in Cell Membrane Structure. A cell membrane contains asymmetrically distributed phospholipid species in the membrane bilayer. The cytoplasmic layer of the cell membrane is composed of phosphatidylserine, phosphatidylinositol, phosphatidylethanolamine, and phosphatidylcholine. The extracellular layer is composed of sphingomyelin, phosphatidylcholine, and phosphatidylethanolamine, but not phosphatidylserine. The asymmetrical distribution of phosphatidylserine is created and maintained by the activity of aminophospholipid translocase, which transports phosphatidylserine from the extracellular layer to the cytoplasmic layer of the cell membrane. In apoptosis, the activity of the aminophospholipid translocase is inhibited, and the content of phosphatidylserine in the extracellular layer of the cell membrane increases, even though the integrity of the cell membrane is uncompromised. Thus, the appearance of phosphatidylserine in the extracellular layer of the cell membrane is indicative of early cell apoptosis. Phosphatidylserine in the extracellular layer can be detected by using an assay for Annexin A5, which is a phosphatidylserine-binding protein. To detect phosphatidylserine, Annexin A5 can be tagged with a marker (e.g., biotin or fluorochrome) and incubated with cell samples. Positive labeling of cells with Annexin A5 suggests the translocation of phosphatidylserine to the extracellular layer of the cell membrane, which indicates the occurrence of apoptosis in the labeled cells.

In addition, the permeability of cell membrane is often increased in cell apoptosis because of the disorganization of phospholipids. In such a case, the cell membrane is permeable to certain types of fluorescent dyes, such as merocyanine (MC) 540 and 7-aminoactinomycin D (7-AAD), which can not pass through the plasma membrane of normal cells. These dyes can be incubated with cell samples and detected by fluorescence microscopy. The appearance of the dye within the cell suggests the occurrence of cell apoptosis. However, these fluorescent dyes are not specific to cell apoptosis. Any factors that cause an increase in cell membrane permeability can induce positive cell labeling.

TABLE 6.10. Characteristics of Selected Apoptosis Regulatory Proteins*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Fas ligand	CD95 ligand, CD178, FAS, tumor necrosis factor ligand superfamily member 6, TNFSF6, FASL, apoptosis antigen ligand 1, apoptosis (APO 1) antigen ligand 1	281	31	Lymphocyte, testis	A protein that interacts with the Fas receptor and induces apoptosis
Fas receptor	Tumor necrosis factor receptor superfamily member 6, CD95, FAS1, apoptosis-mediating surface antigen FAS, apoptosis antigen 1	335	38	Ubiquitous	A transmembrane receptor that interacts with the Fas ligand and induces apoptosis
FADD	FAS-associated protein with death domain	208	23	Ubiquitous	An adaptor protein that interacts with the Fas receptor and TNF receptor; also mediates cell apoptosis
TNFα	Tumor necrosis factor, tumor necrosis factor α, TNFA, cachectin	233	26	Monocyte, macrophage	Interacting with TNF receptor and regulating cell apoptosis, proliferation, differentiation, and inflammatory reactions
TNF receptor	TNFR1α, TNFR1, tumor necrosis factor receptor superfamily member 1A, p55 TNFR	455	50	Heart, blood vessel, leukocytes	Interacting with TNFα, and mediating apoptosis and inflammatory reactions
TRADD	TNFR1-associated death-domain protein, tumor necrosisfactor receptor 1-associated death-domain protein, tumor necrosis factor receptor 1-associated protein	312	34	Ubiquitous	Interacting with TNF receptor 1 and regulating apoptosis and inflammation

Caspase 8	FADD homologous ICE/CED-3-ICE/CED-3-like protease, FADD-like ICE, MACH, MCH5, FLICE	496	58	Ubiquitous	A cysteine–aspartic acid protease that interacts with FADD and mediates the transduction of apoptotic signals
Bid	BH3 interacting death-domain agonist, BID	195	22	Ubiquitous	An apoptotic agonist activated by caspase 8, stimulating the release of cytochrome *c*, and regulating cell apoptosis
Cytochrome *c*	CYC	105	12	Ubiquitous	A mitochondrial electron transport chain component that mediates electron transfer and regulates apoptosis
Apaf-1	Apoptotic protease-activating factor 1	1248	142	Ubiquitous	Interacting with cytochrome *c* and forming an apoptosome, a structure that cleaves the preproprotein of caspase 9 and generates active caspase 9, resulting in apoptosis
Caspase 9	CASP9, apoptotic protease MCH6, MCH6, ICE-like apoptotic protease 6, apoptotic protease-activating factor 3, APAF3	416	46	Ubiquitous	A cysteine–aspartic acid protease that cleaves procaspase 3, inducing the formation of caspase 3 and apoptosis
Caspase 3	CASP3, cysteine protease CPP32, CPP32, apoptosis-related cysteine protease, APOPAIN	277	32	Ubiquitous	A downstream cysteine–aspartic acid protease that cleaves a variety of cytoplasmic proteins, including protein kinases, nuclear proteins, and cytoskeletal proteins, and induces apoptosis

*Based on bibliography 6.16.

Assessing Changes in Cell Morphology. Apoptotic cells are associated with morphological changes, including cell membrane blebbing, DNA condensation with increased nucleus density, and nucleus disruption. The entire cell is eventually disintegrated into small pieces. Optical and electron microscopic approaches can be used to examine these morphological features. At the optical level, hematoxylin can be used to examine the morphology of cell nuclei. In addition, cell nucleus-binding fluorescent dyes, such as DAPI and Hoechst 33258, can be used for the same purpose. Usually, the fluorescent approach provides better images. At the electron microscopic level, cell membrane blebbing and DNA condensation (Fig. 6.16) can be observed with a much better resolution compared with the optical approach. Morphological examination is a key method for the identification of cell apoptosis and is often used as a standard for the confirmation of cell apoptosis detected by using other methods.

Assessing DNA Fragmentation. DNA fragmentation is a hallmark of cell apoptosis. Thus, apoptotic cells can be identified by assessing DNA fragmentation. Two approaches can be used for such purpose: DNA electrophoresis and terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL). For the DNA electrophoresis method, the pattern of DNA bands can be analyzed by comparing to DNA samples from normal control cells. An increase in the number of DNA bands in a large range of molecular size suggests the occurrence of cell apoptosis.

TUNEL is a method used for visualizing DNA fragments in situ. A key enzyme used for this assay is the terminal deoxynucleotidyl transferase, which catalyzes DNA synthesis at the ends of DNA fragments in the presence of deoxynucleotides (dNUPs). When a dNTP is tagged with a marker, such as a fluorescent molecule, the DNA fragments with the added dNTP can be visualized by fluorescence microscopy. This method can be used at the single cell level. However, the method is not apoptosis-specific. DNA fragmentation induced by other factors, such as cell injury, can be detected. Thus, the identification of morphological changes in apoptotic cells is often conducted together with the TUNEL method to confirm the results by TUNEL.

Assessing the Translocation of Cytochrome c. Cytochrome *c* is a protein component of the respiratory chain in the mitochondria. It is localized to the surface of the internal membrane of the mitochondria. Cytochrome *c* can be translocated from the mitochondria to the cytoplasm and contributes to the activation of apoptotic signaling pathways. The translocation of cytochrome *c* is a critical step in apoptosis. Thus, the detection of cytochrome *c* translocation from the mitochondria to the cytoplasm is indicative of apoptosis. An antibody can be used for examining the distribution of cytochrome *c*. Typical images of cytochrome *c* translocation are shown in Fig. 6.18.

Assessing the Activity of Caspases. Caspases are a group of proteinases that degrade proteins, ranging from signaling protein kinases to structural proteins, and play critical roles for the induction of cell apoptosis. The activation of caspases indicates the occurrence of apoptosis. Caspases are expressed in the form of inactive precursors, which can be activated by proteolytic cleavage at specific sites induced by proteinases. Thus, caspases cleavage is a sign of caspase activation. Immunoblotting is an effective method for the detection of caspase cleavage. The presence of reduced caspase subunits is indicative of caspase activation and the occurrence of apoptosis.

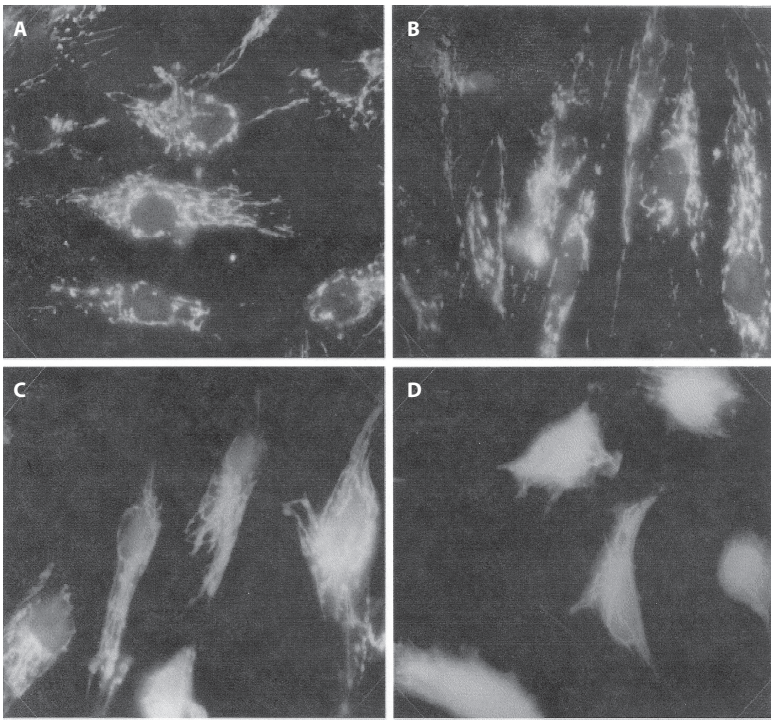

Figure 6.18. Immunofluorescence detection of cytochrome *c* in fibroblasts. The micrographs show: (A) control cells; (B–D) cells exposed to 0.5 µM naphthazarin (5,8-dihydroxy-1,4-naphthoquinone, an apoptosis inducer) for 1, 2, and 3 h, respectively. Note the translocation of cytochrome c from the mitochondria to the cytoplasm in panels B, C, and D. (Reprinted from Roberg K et al: Lysosomal release of cathepsin D precedes relocation of cytochrome c and loss of mitochondrial transmembrane potential during apoptosis induced by oxidative stress, *Free Radical Biol Med* 27:1228–37, 1999, with permission from Elsevier.)

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