Chapter 9
Signal Transduction and Cell Growth

One characteristic of organisms is that they exhibit various behaviors in response to changes in their environment (i.e., the outside world). Cells are equipped with numerous receptor proteins in order to detect changes that occur on an extracellular level. When bound with signaling molecules, receptors change their structure, thereby greatly altering the structure and functions of cells via intracellular signal transduction pathways.

In unicellular organisms, information related to chemicals (such as nutrients and oxygen) and physical stimuli (such as temperature and light) are primarily communicated, whereas in multicellular organisms, signal transduction between cells also takes place. Transduced signals regulate cell functions through protein activation in the short term and through genetic regulation in the long term. The series of events through which one parent cell replicates its genetic information and is divided into two daughter cells is called the cell cycle. This cycle is arrested in many of the cells of multicellular organisms, and is resumed in response to cell growth signals. In cancer cells, this regulation system is mutated, causing uncontrolled cell growth.

I. Signal Transduction

Organisms exhibit a range of adaptive behaviors in response to changes in the environment. Even unicellular organisms respond to various physical and chemical stimuli. Multicellular organisms, including animals and plants, have a well-developed intercellular signal transduction system, and networks of cells come together to establish these organisms. Intracellular signal transduction is activated when intercellular signals reach cells.

Figure 9-1A shows the basic mechanism of intracellular signal transduction. Cells have many receptor proteins on their plasma membrane surface, and molecules that bind specifically to these receptors are called signaling molecules. When bound with signaling molecules, receptors go through structural changes (such as becoming dimers) and are activated, which then changes the shape, movement
Chapter 9  Signal Transduction and Cell Growth

and functions of the cell by activating intracellular signal transduction proteins, and regulates gene expression through the relocation of intracellular signaling molecules to the nucleus.

While a large number of physical and chemical stimuli exist in the environment, organisms respond only to particular stimuli. This is due to the signal detection and communication mechanisms of their cells, and in particular due to the limited number of receptor proteins. Analyzing the genes and proteins involved in signal transduction will therefore enable a better understanding of the basic mechanisms of cellular regulation.

In multicellular organisms, intercellular exchanges of signals are performed in various ways, as shown in Figure 9-1B. Intercellular signaling molecules include hormones, growth factors and cytokines. Hormones are generally signaling molecules that act on distant target cells. Endocrine disrupters, a.k.a. environmental hormones, are exogenous chemicals that exhibit hormone-like effects by acting on receptors. The molecules involved in signal transduction between cells are called first messengers, while the diffusible molecules engaged in intracellular signal transduction in response to first messengers are known as second messengers (e.g., Ca²⁺ and cAMP).

As exceptional signalers, gaseous molecules (such as nitric oxide) and fat-soluble molecules (such as steroid hormones) enter the cytoplasm by crossing the plasma membrane; the receptors are therefore located in the cell or the nucleus. Gaseous molecular ethylene (a plant hormone) reacts with the receptors on the plasma membrane. Light penetrates the cytoplasm and is detected by specific pigment-carrying receptors.

Figure 9-1
Basic mechanism of signal transduction
(A) Basic mechanism of intracellular signal transduction, (B) Mode of intercellular signal transduction: (a) autocrine mode: cells are activated by the signaling molecules they secrete, (b) paracrine mode: cells activate surrounding cells, (c) endocrine mode: signaling molecules activate distant cells by traveling through blood vessels and other pathways, (d) cell-contact mode: cells are activated through contact with their membrane proteins, and (e) nerve mode: part of a cell protrudes and forms a synapse.
II. Intracellular Signal Transduction

Intracellular signal transduction is a system in which signaling molecules bind to the receptors on the cell surface, triggering signaling within a cell. In intracellular signal transduction, information is transduced mainly by protein phosphorylation, G proteins and second messengers. This section discusses three basic mechanisms of signal transduction that are common to many organisms.

Phosphorylation and Dephosphorylation of Proteins

The most important among the intracellular signal transduction mechanisms is the phosphorylation of the side chains of tyrosine, serine and threonine in proteins. As shown in Figure 9-2A, phosphorylation is one of the most effective ways of changing the structure of proteins due to the large size and negative charge of the phosphate group; for the same reason, it is also effective as a recognition marker for other proteins. The enzymes that perform phosphorylation are called protein kinases (referred to simply as “kinases” in this chapter), and many types exist. In signal transduction, a reaction that removes the phosphate group takes place. This reaction is called dephosphorylation, and the enzymes that perform it are called protein phosphatases (referred to as “phosphatases” in this chapter) (see Chapter 7).

For signal transduction by phosphorylation to take place, kinases need to be activated, as shown in Figure 9-2B. First, receptors themselves may have kinase activity. In such cases, receptors on the plasma membrane surface become bound with a signaling molecule (i.e., a first messenger) and form dimers, thereby activating the kinase domain of the receptor proteins within the cell (Fig. 9-2B(a)). A kinase may also bind with a second messenger located in the cell and become activated (Fig. 9-2B(b)).

Kinases in the cytoplasm may also be activated. A kinase, which is activated by cAMP (a second messenger), is normally deactivated when it binds to an A-kinase inhibitory protein. When cAMP binds to this kind of protein, the kinase and the inhibitor are separated from each other, thereby activating A-kinase and thus phosphorylating the target proteins.

Kinases activated in the cell may induce chain reactions (Fig. 9-2B(c)). This phenomenon is called a kinase cascade, since a series of reactions occur in the same way as a waterfall flowing over a cliff. A well-known example is that of MAPK (mitogen-activated protein kinase), which is activated by extracellular...
stimuli. MAP kinase kinase kinase (or MAPKKK), when activated by stimuli, phosphorylates MAP kinase kinase (MAPKK), which activates MAP kinase by phosphorylation. The activated MAP kinase activates genes by phosphorylating various transcription factors.

Figure 9-2
Signal transduction by protein phosphorylation
(A) Phosphorylation of amino acids – serine, threonine and tyrosine. (B) The three activation patterns of kinase: (a) activation of receptor kinases by first messengers (growth factors, hormones, etc.), (b) activation of kinases by (intracellular) second messengers, and (c) chain reactions of kinases (the Figure shows the MAPK cascade)
G Proteins

G proteins are a family of intracellular proteins bound with GDP or GTP (see Fig. 2-5 in Chapter 2). They function by alternating between an inactive GDP-bound state and an active GTP-bound state.

There are two G protein groups. One is low-molecular-weight G proteins, which act as monomers (molecular weight: 20,000 – 30,000). As shown in Figure 9-3A, low-molecular-weight G proteins exist normally as an inactive GDP-bound form, are activated by a factor that exchanges GDP with GTP in response to signal transduction from receptors, and on completion of their roles are inactivated by RGS and GAP – GTPase activating proteins. Low-molecular-weight G proteins are used to transfer information from one place to another within a cell.

The other G protein type is trimetric G proteins, which consist of three subunits – Gα, Gβ and Gγ (Fig. 9-3B). A trimetric G protein binds to a G protein-coupled receptor – a characteristic receptor that penetrates the plasma membrane seven times – in which GDP binds to Gα, thereby inactivating the protein. When a signaling molecule binds to the receptor protein, the Gα subunit releases GDP and is bound instead with GTP, thereby becoming activated. This activated Gα transduces signals to target factors through protein-protein interaction. This active state is brought back to an inactive state through the hydrolysis of GTP by RGS and GAP activity. Gβ and Gγ transfer signals in some cases.

Figure 9-3
G protein functioning cycle
(A) G proteins alternate between an active GTP-bound state and an inactive GDP-bound state. There are activating factors that release GDP and exchange it with GTP located in the cytoplasm, and inactivating factors (RGS and GAP) that promote GTPase activity in Gα subunits and shift the protein to an inactive state.
(B) Binding of a signaling molecule to the receptor transforms the Gα subunit to a GTP-bound form, which releases the subunit as well as Gβ and Gγ from the receptor. When the GTP of the Gα subunit is hydrolyzed into GDP, the subunits are returned to their original position.
Low-molecular-weight Second Messengers

As discussed in the section on phosphorylation, in intracellular signal transduction, not only proteins but also low-molecular substances and ions (such as cAMP, inositol trisphosphate (IP3) and Ca^{2+}) are second messengers that play important roles. These low-molecular substances transduce information by diffusing within the cell. Figure 9-4 shows how the concentration of Ca^{2+}, a second messenger, increases. The Ca^{2+} level inside a cell is normally significantly lower than that outside it. As shown in Figure 9-4B, the Ca^{2+} level is high in an endoplasmic reticulum; if a channel that releases Ca^{2+} from the endoplasmic reticulum opens in response to a signal from the receptor, the intracellular Ca^{2+} level increases.

\[ H + R \leftrightarrow HR \]

When hormone-receptor binding is in equilibrium, the rates of the normal and reverse reactions are the same.
where \( k_{on} \) is the binding rate constant, \( k_{off} \) is the dissociation rate constant, \([H]\) is the concentration of free hormones that are not bound with receptors, \([R]\) is the concentration of free receptors, and \([HR]\) is the concentration of hormones bound with receptors.

This can be changed to:

**Equation 9-1**

\[
K_d = \frac{[H][R]}{[HR]} = \frac{k_{off}}{k_{on}}
\]

where \( K_d \) is the dissociation constant.

For a simple system in which one receptor has a single binding site, the total concentration of receptors in a cell, \( R \), can be expressed as follows:

\[
[R] = R_{tot} - [HR]
\]

By assigning this to Equation 9-1:

\[
[HR] = \frac{R_{tot} [H] / (K_d + [H])}{[H][R]}
\]

In an actual experiment, hormones labeled by a radioisotope are added to a cell culture, and after equilibrium is reached, the cells are washed to separate bound hormones from free hormones. \( K_d \) is determined by measuring the concentration of hormones bound to 50% of the receptors, as illustrated in Column Figure 9-1A. Column Figure 9-1B uses a semi-logarithmic scale as the concentration range extends over multiple digits. Using this, the total concentration of receptors (i.e., the total number of receptors to which hormones can bind) and \( K_d \) (the concentration at which 50% of receptors are occupied) are calculated.

**Column Figure 9-1**

Saturation binding

Direct binding curves that use (A) the linear scale and (B) the semi-logarithmic scale.
III. Intracellular Signal Transduction Pathways Mediated by Receptors

Actual intracellular signal transduction is performed through a combination of the aforementioned mechanisms. The intracellular signal transduction pathways, mediated by four main receptors – enzyme-linked receptors, G protein-coupled receptors, channel receptors and transcription factor receptors, which respond to extracellular signaling molecules – are shown in Figure 9-5 for discussion on the signal transduction pathways through which a stimulus applied to the cell surface activates nuclear genes.

Enzyme-linked Receptors

Many of the enzyme-linked receptors on the plasma membrane have the kinase domain inside the cell. These receptors form dimers when bound with a signaling molecule, and the pair receptors perform mutual tyrosine phosphorylation. In this way, the binding of a signaling molecule to a receptor transduces information to the interior of the cell.

Figure 9-5(1) shows the signal transduction of a growth factor called epidermal growth factor (EGF). Binding with EGF phosphorylates the tyrosine of the receptor protein, and a protein with the SH2 region that recognizes phosphorylated tyrosine binds to it to form an activated complex. This complex activates Ras – a small G protein – by transforming it to the GTP-bound form. The activated Ras regulates nuclear gene expression through a MAPK chain reaction.

Some enzyme-linked receptors have, in addition to tyrosine kinase, a domain that phosphorylates serine or threonine in a protein. Conversely, some proteins have a domain for phosphatase, an enzyme that removes a phosphate group. These proteins also play an important role in signal transduction.
As a means of signal transduction, a mechanism is used in which a target protein is cleaved through the activation of a protein cleavage enzyme. The cleaved protein may be activated and used for signal transduction, or may be fully degraded.

As examples of cleavage through activation, membrane-bound proteins may be cleaved and move from the plasma membrane to the nucleus, thereby activating genes, or signals may be transduced through a chain reaction involving protease, as in the cell death discussed later (Fig. 9-11).

There is another mechanism in which particular proteins are selectively degraded using ATP as energy. Ubiquitin, a small protein, is bound to a protein as a marker for degradation. This ubiquitinated protein is promptly degraded in a proteasome – a complex consisting of many proteins. Since ubiquitination acts on particular proteins, a complex mechanism regulates this process. The action of the plant hormone auxin, which causes the bending of plant stems, is such an example.

**G Protein-coupled Receptors**

This is a family of receptors that are encoded by more genes in humans than other proteins are (see the Column on p.179). Approximately on thousand types of G protein-coupled receptors are encoded by the human genome, and are involved in the reception of various kinds of information, from stimuli such as light and odor to the actions of blood pressure regulation hormones. The proteins are given this name because, as shown in Figure 9-5(2), they penetrate the plasma membrane seven times and are bound with a trimeric G protein in the cytoplasm. The trimeric G protein bound with the receptor consists of three subunits – α, β and γ. When the receptor is activated and the Gα subunit is transformed from the GDP-bound form to the GTP-bound form, Gα is released from the receptor and binds to adenylate cyclase, thereby activating this enzyme. Subsequently, the GTP loses the phosphate group by cleavage and becomes GDP, causing the inactivation of the G protein. cAMP, which is generated by adenylate cyclase, activates a kinase called cAMP-dependent kinase and regulates gene expression.
In addition, Gα released by the receptor, together with a Gβ-Gγ complex, generates inositol trisphosphate by activating an enzyme called phospholipase C. This inositol trisphosphate increases the Ca^{2+} concentration in the cell by opening the Ca^{2+} channel on an endoplasmic reticulum. As a result, Ca^{2+}-dependent kinase is activated, and gene expression is regulated.

Figure 9-5
Signal transduction pathways mediated by four main receptors

1. Enzyme-linked receptors: The signal transduction pathway of a growth factor receptor is shown. When bound with EGF, the two receptors form a dimer and phosphorylates each other. A protein that recognizes and binds to this phosphorylated site activates Ras, a low-molecular-weight G protein, thereby initiating the kinase cascade reaction.

2. G protein-coupled receptor: When a signaling molecule binds to the G protein trimer, the GDP-GTP exchange reaction occurs in the Gα of the G protein trimer, and the G protein trimer released from the receptor activates target enzymes such as adenylate cyclase and phospholipase C. As a result, the level of cAMP and Ca^{2+} increase, thereby transducing signals.

3. Channel receptor: Binding with a signaling molecule regulates the opening and closing of a channel, thereby transducing signals.

4. Transcription factor receptor: since receptors for fat-soluble signaling molecules are located in the cytoplasm and the nucleus, the signaling molecules cross the plasma membrane into these areas, where they bind to the receptors and transduce signals.
Channel Receptors

Channel receptors on the plasma membrane and the endoplasmic reticulum membrane are opened when they are bound with signaling molecules. The resultant changes in intracellular ion concentration transduce signals (Fig. 9-5(3), see Chapter 5).

Transcription Factor Receptors

Fat-soluble steroid hormones such as adrenocortical hormones and male and female hormones (and many other fat-soluble signaling molecules) cross the plasma membrane into cells. Transcription factor receptors that specifically bind to these signaling molecules exist in cells and nuclei (Fig. 9-5(4)). These receptors are also called nuclear receptors, and are a type of transcription factor equipped with a special zinc finger structure for binding to DNA. In the case of steroid hormones, for example, the receptor binds to a regulatory protein in the cytoplasm. However, if a steroid hormone binds to the receptor, the latter changes its structure and dissociates itself from the regulatory protein. The free receptor then moves to the nucleus and regulates gene expression there.

IV. Cell Cycle

One parent cell divides into two daughter cells (the word “daughter” is used customarily and does not implicate the gender of cells). In humans, one fertilized egg keeps dividing, finally forming an adult human with 60 trillion cells. After this, cell multiplication continues since the body needs to keep replenishing cells for metabolism and regeneration.

What is the Cell Cycle?

For cells to grow, a process is repeated in which cellular components, including genetic information, are doubled and distributed equally to two cells. This repetition is called the cell cycle (Fig. 9-6A). The cycle consists of the mitotic (M) phase (during which the cell is divided into two units), the synthetic (S) phase (during which DNA is synthesized), the G1 (gap 1) phase from the M phase to the S phase, and the G2 phase (gap 2) from the S phase to the M phase. The
phase is a period of preparation for DNA synthesis, while the G2 phase involves preparation for cell division. In multicellular organisms, including humans, many cells stop dividing despite their ability to do so; such cells are considered to have moved out of the cell cycle and entered the G0 phase. One cell cycle in humans lasts about a day, of which the S phase takes six to eight hours to complete and the M phase takes one hour.

In eukaryotic cells, the most obvious change under the microscope is the M phase. As shown in Figure 9-6B, nuclear division occurs following DNA synthesis, during which the nuclear envelope disappears, DNA-containing chromosomes become clearly visible and are pulled by microtubules to the two opposite ends of the cell (see Fig. 6-5 in Chapter 6). In animal cells, the plasma membrane then pinches inward to form two daughter cells, and in plant cells a dividing wall is constructed within the cell, thus forming two daughter cells.

**Figure 9-6  Cell cycle**

(A) The cell cycle consists of four phases – the S phase (during which DNA is synthesized), the M phase (during which the nucleus and cytoplasm are divided), and two interphases between the S and M phases. These interphases are the G1 phase from the end of the M phase to the S phase, and the G2 phase from the end of the S phase to the M phase. Many cells arrest the cell cycle during the G1 phase and enter the G0 phase.

(B) In the cell cycle, only the M phase can be seen under a microscope. The remaining three phases are collectively referred to as interkinesis.
Classifying the estimated 26,000 genes in the human genome by the protein families they encode, the most abundant is G protein-coupled receptors, including odorant receptors, totaling approximately 1,000 types. The second most abundant is a nuclear protein family called zinc finger proteins, totaling approximately 900 types, of which 48 are nuclear receptors. In humans, the number of receptors involved in the detection of extracellular information is estimated to be between 2,000 and 3,000, and these are the targets of drugs. Since drugs that are effective in humans bind to receptors, these 2,000 – 3,000 receptors are basic candidate targets for drugs. Over half the superior drugs that have entered widespread use in the world target G protein-coupled receptors or nuclear receptors, and receptor-binding drugs are used to combat various diseases such as high blood pressure, stomach ulcers, lifestyle-related diseases, allergies and cancer. However, since the human proteins that have so far been targeted by drugs number 500 types at most, there are 1,500 – 2,500 drug targets left. Receptors whose signaling molecules have not been identified are known as orphan receptors; the identification of signaling molecules for such receptors leads to the development of new medicines, prompting drug companies to scramble to find these signaling molecules.

To locate such molecules, a receptor gene whose functions are unknown is expressed in a cultured cell, and a range of chemicals is added to the culture to observe signal transduction from the receptors. In the case of G protein-coupled receptors, for example, increased Ca^{2+} and cAMP levels are observed as a result of the activation of G proteins. Drugs with new effects for cancer and diabetes are now being developed.
Symmetric and Asymmetric Cell Divisions

Two daughter cells produced by a cell division have the same characteristics (symmetric cell division) in many cases, but cells with different characteristics may also be formed (asymmetric cell division). As an example, one stem cell divides into two cells: one is a stem cell and the other is differentiated into a leukocyte. In this way, red and white blood cells are produced, while stem cells are preserved for later use. In the developmental process (the process during which a fertilized egg grows to become an individual), regulation by asymmetric cell division is the key to the formation of complex bodies. In this kind of cell division, the asymmetric distribution of organelles and intracellular proteins is often formed in advance (see Chapter 10).

While two daughter cells produced by asymmetric cell division have the same genes (genome), they are maintained as cells with different characteristics due to the difference in the types of genes expressed. As mentioned in Chapter 4, this difference is maintained through modification by DNA methylation, acetylation and methylation of the histone proteins constituting the chromatin structure, and other mechanisms.

V. Regulation of Cell Growth

Roughly speaking, unicellular organisms keep multiplying as long as their living environment permits. On the other hand, the cell growth of eukaryotic multicellular organisms is regulated to avoid uncontrolled growth despite the well-maintained internal environment the cells are in (e.g., nutrients and oxygen are supplied, waste materials and carbon dioxide are eliminated, and temperature and pH are appropriately maintained [internal homeostasis]). Tissues consisting of somatic cells contain many cells that do not grow by default (i.e., they are in the G0 phase) but do so as necessary.

Positive and Negative Regulation

The cell growth of eukaryotic multicellular organisms is strictly regulated, which is achieved by balancing anti-cell-growth signals (negative growth signals) and pro-cell-growth signals (positive growth signals). As an example, cells are firmly attached to each other in the epithelial tissue of mammals including humans (see
Chapter 11), thereby negatively regulating cell growth. When a cell is detached from the adjacent cell (e.g., by injury), the negative regulation is lost and cell growth is enabled. This, however, is still not sufficient for the initiation of cell growth, which begins only when positive growth signals are transduced to cells by growth factors (many of which are proteins) precommitted to particular cell types. If cells adhere to each other, the presence of growth factors does not induce cell growth. In this way, cell growth is regulated both positively and negatively.

VI. Signal Transduction for the Initiation of Cell Growth

Signal Transduction leading up to the Initiation of Cell Growth

Figure 9-7 shows a schematic diagram of the intracellular signal transduction that acts from the moment of a growth induction signal reaching a receptor on the plasma membrane to the initiation of cell growth. A transcription factor generated by a newly expressed early gene induces the expression of various genes. Among the genes expressed as a result, a group of protein kinases called cyclin-dependent kinases (CDKs) and a group of proteins required for their
activation called **cyclins** are important. These two are synthesized, accumulated and form a complex; when activated, they phosphorylate other proteins (e.g., Rb) and consequently activate the transcription factors (e.g., E2F) of the genes necessary for DNA synthesis. This then activates gene-encoding enzymes that synthesize the DNA materials necessary in the S phase and DNA polymerase, thereby allowing the cell to enter the S phase.

**Positive and Negative Regulation**

The process of cell growth initiation also has both positive and negative regulation. Cyclins and CDKs are important as positive regulatory factors with a central role in the progression of cells through the cell cycle. On the other hand, a protein group called cyclin-dependent kinase inhibitors (**CKIs**) negatively regulates the cell cycle. These are synthesized by various signals that suppress cell growth in situations where, for example, cells adhere to each other or growth factors are absent, and suppress the activity of protein kinase by binding to CDKs [Fig. 9-7]. CKIs degrade in environments where cells can grow. Another group of proteins that negatively regulate the progression of cells through the cell cycle is produced by genes collectively called tumor suppressor genes, which include Rb and p53 as shown in Figure 9-7. In quiescent cells, these proteins suppress the genes necessary in the S phase, but the cells lose antiproliferative activity once Rb has been phosphorylated through the activation of CDKs by growth factors. In this way, both positive and negative systems are often involved in many intracellular reactions.

**Cyclins and CDKs – Involved in Every Stage of the Cell Cycle**

As outlined in Figure 9-7, the G1 cyclins and G1 CDKs act at the onset of the S phase. Cyclins and CDKs, each consisting of a group (family) of several similar proteins, also play an important role in helping cells progress through the cell cycle. As shown in Figure 9-8, the G2 cyclins and G2 CDKs (cyclin B-Cdc2 complex) act at the onset of the M phase; once activated, they induce the breakdown of the nuclear envelope and the formation of chromosomes. There are several types of G1 cyclin and G1 CDK, including the cyclin D-CDK4/6 complex and the cyclin E-CDK2 complex, but these are not discussed here.

Cyclins are rapidly synthesized at certain stages of the cell cycle. Once their work is complete, they become ubiquitinated and rapidly degraded by protease...
elements called proteasomes. Degradation takes place not only because the cyclins are no longer needed but also because the process is often essential for cells to move on to the next stage of the cell cycle. Although CDKs, on the other hand, need to be synthesized for cells to exit the G0 phase and initiate growth, some types are degraded once they enter the cell cycle while others are not. The overall picture of CDK activity regulation is very complex, and is governed by at least four mechanisms (Fig. 9-9). These are: synthesis and binding of cyclins; activation by phosphorylation; activity suppression by phosphorylation of the ATP binding site and activation by dephosphorylation; and activity suppression through the binding of CKIs. Although the overall picture is still not clearly understood, it is considered to be an intricate mechanism that causes DNA replication and cell division to progress relentlessly by performing molecular-level reactions (such as the synthesis and phosphorylation of proteins), using many types of factors.

VII. Checkpoint Mechanism of the Cell Cycle

Cells grow through repetition of the cell cycle. One of the most important points in cell growth is that genetic information (the genome) is accurately replicated.
and distributed to the two resulting cells. To make sure that these processes take place without fail, checkpoints located at each phase of the cell cycle verify whether the cell is ready to progress to the next phase.

The G1 checkpoint determines whether the cell should initiate DNA synthesis. DNA is routinely damaged in various ways; if DNA synthesis is initiated before the damage is repaired, correct replication does not take place, likely resulting in mutation or cell death. Cells therefore have a mechanism that checks whether the DNA sequence or structure is correct, and when errors (or damage) are detected, p53 is activated by acetylation or phosphorylation, and the activated p53 in turn activates (or suppresses, depending on the gene) many genes.

One of the roles of p53 is to activate the gene encoding p21 (a CKI) to produce many molecules of p21, thereby suppressing the action of the G1 cyclin and the G1 CDK. As a result, the cell does not progress into the S phase, and DNA damage is repaired during this delay time. Once the repair is complete, p53 is inactivated and p21 is degraded, allowing the cell to progress into the S phase. If the DNA damage is too severe to be repaired, p53, after being modified, activates the gene that induces apoptosis (discussed later), leading to the death of the cell.

To prevent defective DNA from being distributed to the daughter cells, the G2 checkpoint suppresses the activity of the G2 cyclin-CDK complex (thus keeping the cell from entering the M phase) until it confirms that the DNA is not damaged and its synthesis is complete.

To ensure that the genome is accurately distributed to the two daughter cells, the M checkpoint keeps the cell from progressing to the next phase until microtubules (see Fig. 6-5 in Chapter 6) correctly bind to all chromosomes. These “check and go” systems are located at key points of the cell cycle, making sure that the genome is distributed to the two daughter cells without fail. In terms of the growth regulation mechanism for the somatic cells of eukaryotic multicellular animals (particularly that of mammals), a condition in which the uncontrolled growth of cells is suppressed is set as the default, and a positive growth regulation mechanism is temporarily activated to initiate the cell cycle only when cell growth is necessary. However, even then, a mechanism is at work to ensure that the genome is accurately replicated and distributed to daughter cells through careful operation of the “check and go” system. In unicellular organisms, on the other hand, a mechanism is in place to increase the number of individuals (cells) as much as the living environment will permit.
VIII. Cancer and Cancer Genes

Cancer cells are generally considered to grow rapidly. However, their cell cycle turns no more quickly than that of normal cells. They grow fast because in cancer tissues, many cells exist within the cell cycle, whereas in normal tissues, a higher proportion of cells are in the G0 phase. Cancer cells overgrow because their cell growth does not arrest in the G0 phase – in other words, their growth regulation mechanism is defective.

Cancer Genes

As shown in Figure 9-10A, it has been demonstrated that normal cells can be transformed into cancer cells if DNA extracted from the latter is fragmented and incorporated into the DNA of the former. The incorporated genetic information caused malignant transformation, and the genes identified were named oncogenes. The oncogene first discovered in humans was ras*1. As shown in Figure 9-5, Ras*1 (a G protein) acts at the early stage of the signal transduction pathway for cell growth, and if mutation occurs in this gene (thus triggering the production of the activated Ras protein without the presence of growth factors), the signal transduction pathway starts acting continuously. The difference between normal

![Diagram](image)

**Figure. 9-10**

Discovery of cancer genes and tumor suppressor genes

(A) Cancer genes were discovered by fragmenting DNA extracted from cancer cells and identifying the DNA fragments that transform cultured cells into cancer cells. (B) In a family line characterized by a high rate of hereditary retinal cancer, one of the two copies of the tumor-suppressing Rb gene is mutated, and the cancer develops when the other copy is mutated.

*Genes are basically expressed by three italic letters, while the proteins encoded by the genes are expressed by three regular letters with the first letter capitalized. However, there are many exceptions.*
Ras and Ras that turns cells cancerous is one amino acid caused by a single base mutation. The cancer-causing Ras, bound with GTP, is constantly activated, and maintains activation without the presence of growth factors, thus causing uncontrolled cell growth. As exemplified by the constant activation of MAP kinase kinase kinase (Fig. 9-2B (c)) and early genes produced in large amounts (Fig. 9-7), it has been shown that many of the oncogenes known today have a mutation that causes the runaway of the signal transduction pathway for cell growth.

**Tumor Suppressor Genes**

The negative regulation of cell growth stops functioning if genes, such as Rb and p53, are damaged or mutated, thereby halting the functions of the proteins encoded by the genes. Cell growth is initiated not only in this way, it also occurs as a result of slight stimuli. Such genes in the normal state suppress cancer, and are called tumor suppressor genes. The first tumor suppressor gene discovered in humans was identified in a study of a family with frequent occurrence of hereditary retinal cancer in childhood (Fig. 9-10B), which found a mutation in the Rb gene. It was shown that one copy of the Rb gene in this family line is inherently defective, and that the cancer occurs if there is a mutation in the other normal Rb gene. On the other hand, in nonhereditary retinal cancer that occurs in adulthood, the possibility of mutation occurring in both copies of the Rb gene is extremely low. In the case of the p53 gene, mutation in one gene copy is often enough for cancer to develop (even if the other copy is normal), and the defective p53 gene has been identified in over fifty percent of human cancer cases. Other examples of tumor suppressor genes can also be found in various proteins that form part of the signal transduction pathway (e.g., signals from cell adhesion) that negatively regulates cell growth.

**Independent Growth of Cancer**

It has been shown that in many cancer cells in humans, both cancer genes and tumor suppressor genes are mutated, resulting in the abnormal growth regulation of cells. This is the cause of the independent growth of cancer cells. The term “independent” here means that cells grow in an uncontrolled manner without being subjected to the homeostasis of the number of cells – an important cellular regulation mechanism for individuals. Cancer cells also have the ability to translocate and escape from immune surveillance mechanisms.
IX. Signals of Cell Death

There are signals designed to lead cells to death. In nematodes, some cells are genetically destined to die during the developmental process (see the Column on p.194 in Chapter 10). In such cells, the nucleus is condensed following the disappearance of the nuclear envelope, blebs are formed in the cytoplasm, and the cell shrinks and eventually dies — a characteristic process from which the phenomenon derives its name, apoptosis (apo means “away” and ptosis means “falling” in Greek). The signal transduction in apoptosis is transduced by a chain reaction involving the protease known as caspase. As shown in Figure 9-11, the caspase chain reaction may be initiated by the release of the protein known as cytochrome c from mitochondria to the cytoplasm or by the activation of the Fas receptor on the plasma membrane by extracellular signaling molecules mediating apoptosis. The latter mechanism is effective for killing cells infected with viruses. The dysfunction of apoptosis reactions, which allows cells that are supposed to die to remain alive, is one of the causes of cancer, and is also known to exacerbate immune disorders and other diseases.

Figure 9-11
Apoptotic signals leading to cell death
Apoptosis takes place as a result of a chain reaction involving caspase (a protease). It may occur as a result of the release of a protein known as cytochrome c from mitochondria in the cell or the activation of a receptor known as Fas on the cell surface.
• Cells perform various reactions to detect and adapt to extracellular chemicals and physical stimuli.
• In multicellular organisms, many signaling molecules are exchanged between cells.
• Signals are detected by receptor proteins located on the cell surface. Some receptors are located in the cell.
• Signaling molecules capable of binding to receptors are efficiently detected.
• Receptors change their structure when bound with signaling molecules and transduce signals into the cell.
• Intracellular signal transduction takes place by the four main methods of phosphorylation, G proteins, second messengers such as cAMP and Ca^{2+}, and protein degradation.
• There are four main receptor types: enzyme-linked receptors with kinase or other enzymes, G protein-coupled receptors, channel receptors and transcription factor receptors.
• In the short term, transduced signals change the functions of proteins, thereby altering the structure and functions of the cell. In the long term, the signals change gene transcription, thereby affecting cell growth and differentiation as well as various other functions.
• One cell replicates its genetic information and divides into two cells.
• There are two types of cell division: symmetric cell division (by which two cells with the same characteristics are produced) and asymmetric cell division (by which two cells with different characteristics are produced).
• Cell division progresses in a cyclic reaction known as the cell cycle, consisting of the G1, S, G2 and M phases.
• The cell cycle of many cells in multicellular organisms is arrested in the G1 phase.
• Growth signals cause the cell cycle to resume.
• The cell cycle is regulated by the continuous activation and inactivation of the cyclin-CDK complex, centering around the cyclic expression of cyclins.
• Cells progress to the next stage after verification that the previous stage is complete (checkpoints).
• Cancer cells start to grow independently after the mutation of genes that encode proteins involved in the signal transduction mechanism of cell growth into cancer genes, resulting in the loss of the functions of protein that suppress cell division (tumor suppressor genes).
• Cells have signal transduction pathways that induce their own death as an active process (apoptosis).
Problems

[1] Depending on intercellular soluble molecules, signal transduction can be roughly classified into endocrine, paracrine, autocrine, cell contact and nerve modes. Explain the difference between these types. Insulin produced in the pancreas is secreted, and acts on receptors located across the body. Indicate which of the above signal transduction types is applied to this mechanisms, and explain the reasons for your choice.

[2] Compare signal transduction by neurons (nerve type), which secrete neurotransmitters from the synapse, with signal transduction by endocrine cells, which secrete hormones into the blood (endocrine type). Outline the advantages of both types.

[3] Cells may be programmed to actively die even with a sufficient supply of nutrients. Provide the name of this mechanism and outline the circumstances in which it is used.

[4] Using specific names, outline a typical mechanism in which a ligand binds to a G protein-coupled receptor, which then activates a G protein trimer, which in turn increases the intracellular Ca\(^{2+}\) level to transduce signals.

[5] 1) List the names of the cell cycle stages. 2) Explain the molecular mechanisms necessary for the cell cycle to progress irreversibly in one direction.

[6] In a normal cell culture, the cell cycle varies among cells, and those in the M and S phases are mixed together. Explain the method by which the cell cycle of all cultured cells is synchronized (known as synchronized culture).

(Answers on p.257)