# The Eukaryotic Cell Cycle

The division cycle of most cells consists of four coordinated processes: cell growth, DNA replication, distribution of the duplicated chromosomes to daughter cells, and cell division. In bacteria, cell growth and DNA replication take place throughout most of the cell cycle, and duplicated chromosomes are distributed to daughter cells in association with the plasma membrane. In eukaryotes, however, the cell cycle is more complex and consists of four discrete phases. Although cell growth is usually a continuous process, DNA is synthesized during only one phase of the cell cycle, and the replicated chromosomes are then distributed to daughter nuclei by a complex series of events preceding cell division. Progression between these stages of the cell cycle is controlled by a conserved regulatory apparatus, which not only coordinates the different events of the cell cycle but also links the cell cycle with extracellular signals that control cell proliferation.

**Phases of the Cell Cycle**

A typical eukaryotic cell cycle is illustrated by human cells in culture, which divide approximately every 24 hours. As viewed in the microscope, the cell cycle is divided into two basic parts: mitosis and interphase. Mitosis (nuclear division) is the most dramatic stage of the cell cycle, corresponding to the separation of daughter chromosomes and usually ending with cell division (cytokinesis). However, mitosis and cytokinesis last only about an hour, so approximately 95% of the cell cycle is spent in interphase—the period between mitoses. During interphase, the chromosomes are decondensed and distributed throughout the nucleus, so the nucleus appears morphologically uniform. At the molecular level, however, interphase is the time during which both cell growth and DNA replication occur in an orderly manner in preparation for cell division.

The cell grows at a steady rate throughout interphase, with most dividing cells doubling in size between one mitosis and the next. In contrast, DNA is synthesized during only a portion of interphase. The timing of DNA synthesis thus divides the cycle of eukaryotic cells into four discrete phases (Figure 14.1). The M phase of the cycle corresponds to mitosis, which is usually followed by cytokinesis. This phase is followed by the G1 phase (gap 1), which corresponds to the interval (gap) between mitosis and initiation of DNA replication. During G1, the cell is metabolically active and continuously grows but does not replicate its DNA. G1 is followed by S phase (synthesis), during which DNA replication takes place. The completion of DNA synthesis is followed by the G2 phase (gap 2), during which cell growth continues and proteins are synthesized in preparation for mitosis.

The duration of these cell cycle phases varies considerably in different kinds of cells. For a typical rapidly proliferating human cell with a total cycle time of 24 hours, the G1 phase might last about 11 hours, S phase about 8 hours, G2 about 4 hours, and M about 1 hour. Other types of cells, however, can divide much more rapidly. Budding yeasts, for example, can progress through all four stages of the cell cycle in only about 90 minutes. Even shorter cell cycles (30 minutes or less) occur in early embryo cells shortly after fertilization of the egg (Figure 14.2). In this case, however, cell growth does not take place. Instead, these early embryonic cell cycles rapidly divide the egg cytoplasm into smaller cells. There is no G1 or G2 phase, and DNA replication occurs very rapidly in these early embryonic cell cycles, which therefore consist of very short S phases alternating with M phases.

In contrast to the rapid proliferation of embryonic cells, some cells in adult animals cease division altogether (e.g., nerve cells) and many other cells divide only occasionally, as needed to replace cells that have been lost because of injury or cell death. Cells of the latter type include

skin fibroblasts, as well as the cells of many internal organs, such as the liver, kidney, and lung. As discussed further in the next section, these cells exit G1 to enter a quiescent stage of the cycle called G0, where they remain metabolically active but no longer proliferate unless called on to do so by appropriate extracellular signals.

Analysis of the cell cycle requires identification of cells at the different stages discussed above. Although mitotic cells can be distinguished microscopically, cells in other phases of the cycle (G1, S, and G2) must be identified by biochemical criteria. Cells in S phase can be readily identified because they incorporate radioactive thymidine, which is used exclusively for DNA synthesis (Figure 14.3). For example, if a population of rapidly proliferating human cells in culture is exposed to radioactive thymidine for a short period of time (e.g., 15 minutes) and then analyzed by autoradiography, about a third of the cells will be found to be radioactively labeled, corresponding to the fraction of cells in S phase.

Variations of such cell labeling experiments can also be used to determine the length of different stages of the cell cycle. For example, consider an experiment in which cells are exposed to radioactive thymidine for 15 minutes, after which the radioactive thymidine is removed and the cells are cultured for varying lengths of time prior to autoradiography. Radioactively labeled interphase cells that were in S phase during the time of exposure to radioactive thymidine will be observed for several hours as they progress through the remainder of S and G2. In contrast, radioactively labeled mitotic cells will not be observed until 4 hours after labeling. This 4-hour lag time corresponds to the length of G2—the minimum time required for a cell that incorporated radioactive thymidine at the end of S phase to enter mitosis.

Cells at different stages of the cell cycle can also be distinguished by their DNA content (Figure 14.4). For example, animal cells in G1 are diploid (containing two copies of each chromosome), so their DNA content is referred to as 2n (n designates the haploid DNA content of the genome). During S phase, replication increases the DNA content of the cell from 2n to 4n, so cells in S have DNA contents ranging from 2n to 4n. DNA content then remains at 4n for cells in G2 and M, decreasing to 2n after cytokinesis. Experimentally, cellular DNA content can be determined by incubation of cells with a fluorescent dye that binds to DNA, followed by analysis of the fluorescence intensity of individual cells in a flow cytometer or fluorescence-activated cell sorter, thereby distinguishing cells in the G1, S, and G2/M phases of the cell cycle.

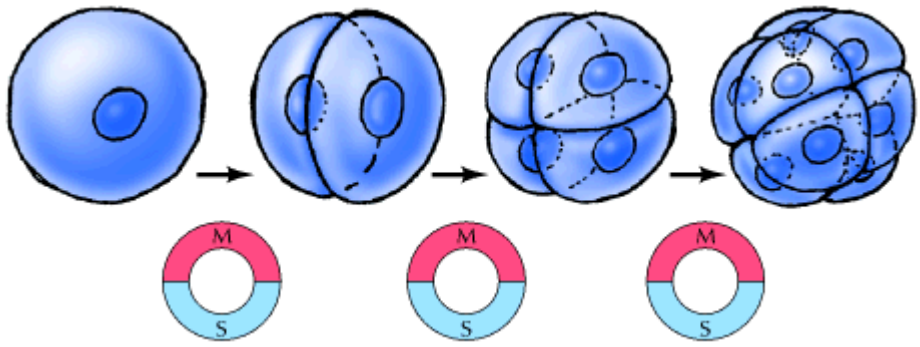


**Figure 14.2. Embryonic cell cycles** Early embryonic cell cycles rapidly divide the cytoplasm of the egg into smaller cells. The cells do not grow during these cycles, which lack G1 and G2 and consist simply of short S phases alternating with M phases.

**Regulation of the Cell Cycle by Cell Growth and Extracellular Signals**

The progression of cells through the division cycle is regulated by extracellular signals from the environment, as well as by internal signals that monitor and coordinate the various processes that

take place during different cell cycle phases. An example of cell cycle regulation by extracellular signals is provided by the effect of growth factors on animal cell proliferation. In addition, different cellular processes, such as cell growth, DNA replication, and mitosis, all must be coordinated during cell cycle progression. This is accomplished by a series of control points that regulate progression through various phases of the cell cycle.

A major cell cycle regulatory point in many types of cells occurs late in G1 and controls progression from G1 to S. This regulatory point was first defined by studies of budding yeast (Saccharomyces cerevisiae), where it is known as START (Figure 14.5). Once cells have passed START, they are committed to entering S phase and undergoing one cell division cycle. However, passage through START is a highly regulated event in the yeast cell cycle, where it is controlled by external signals, such as the availability of nutrients, as well as by cell size. For example, if yeasts are faced with a shortage of nutrients, they arrest their cell cycle at START and enter a resting state rather than proceeding to S phase. Thus, START represents a decision point at which the cell determines whether sufficient nutrients are available to support progression through the rest of the division cycle. Polypeptide factors that signal yeast mating also arrest the cell cycle at START, allowing haploid yeast cells to fuse with one another instead of progressing to S phase.

In addition to serving as a decision point for monitoring extracellular signals, START is the point at which cell growth is coordinated with DNA replication and cell division. The importance of this regulation is particularly evident in budding yeasts, in which cell division produces progeny cells of very different sizes: a large mother cell and a small daughter cell. In order for yeast cells to maintain a constant size, the small daughter cell must grow more than the large mother cell does before they divide again. Thus, cell size must be monitored in order to coordinate cell growth with other cell cycle events. This regulation is accomplished by a control mechanism that requires each cell to reach a minimum size before it can pass START. Consequently, the small daughter cell spends a longer time in G1 and grows more than the mother cell.

The proliferation of most animal cells is similarly regulated in the G1 phase of the cell cycle. In particular, a decision point in late G1, called the restriction point in animal cells, functions analogously to START in yeasts (Figure 14.6). In contrast to yeasts, however, the passage of animal cells through the cell cycle is regulated primarily by the extracellular growth factors that signal cell proliferation, rather than by the availability of nutrients. In the presence of the appropriate growth factors, cells pass the restriction point and enter S phase. Once it has passed through the restriction point, the cell is committed to proceed through S phase and the rest of the cell cycle, even in the absence of further growth factor stimulation. On the other hand, if appropriate growth factors are not available in G1, progression through the cell cycle stops at the restriction point. Such arrested cells then enter a quiescent stage of the cell cycle called G0, in which they can remain for long periods of time without proliferating. G0 cells are metabolically active, although they cease growth and have reduced rates of protein synthesis. As already noted, many cells in animals remain in G0 unless called on to proliferate by appropriate growth factors or other extracellular signals. For example, skin fibroblasts are arrested in G0 until they are stimulated to divide as required to repair damage resulting from a wound. The proliferation of these cells is triggered by platelet-derived growth factor, which is released from blood platelets during clotting and signals the proliferation of fibroblasts in the vicinity of the injured tissue.

Although the proliferation of most cells is regulated primarily in G1, some cell cycles are instead controlled principally in G2. One example is the cell cycle of the fission yeast Schizosaccharomyces pombe (Figure 14.7). In contrast to Saccharomyces cerevisiae, the cell cycle of S. pombe is regulated primarily by control of the transition from G2 to M, which is the

principal point at which cell size and nutrient availability are monitored. In animals, the primary example of cell cycle control in G2 is provided by oocytes. Vertebrate oocytes can remain arrested in G2 for long periods of time (several decades in humans) until their progression to M phase is triggered by hormonal stimulation. Extracellular signals can thus control cell proliferation by regulating progression from the G2 to M as well as the G1 to S phases of the cell cycle.

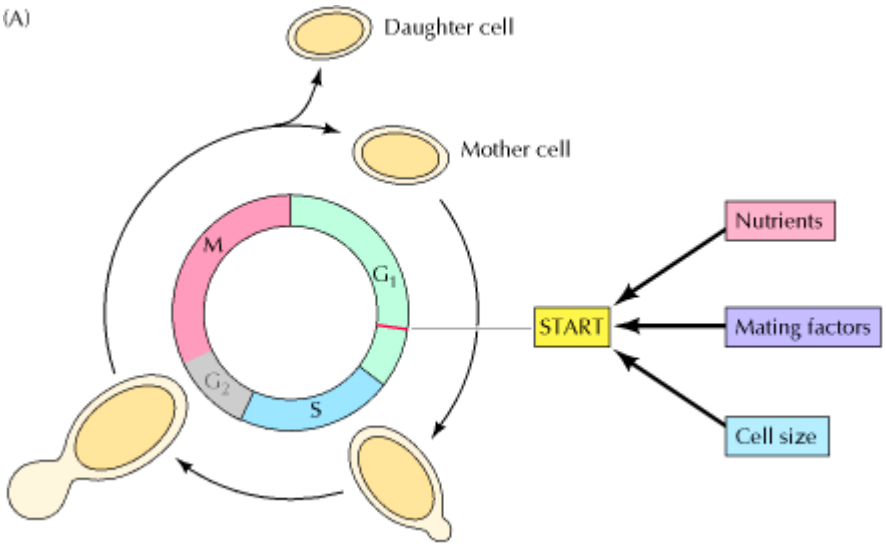




**Figure 14.5. Regulation of the cell cycle of budding yeast** (A) The cell cycle of Saccharomyces cerevisiae is regulated primarily at a point in late G1 called START. Passage through START is controlled by the availability of nutrients, mating factors, and cell size. Note that these yeasts divide by budding. Buds form just after START and continue growing until they separate from the mother cell after mitosis. The daughter cell formed from the bud is smaller than the mother cell and therefore requires more time to grow during the G1 phase of the next cell cycle. Although G1 and S phases occur normally, the mitotic spindle begins to form during S phase, so the cell cycle of budding yeast lacks a distinct G2 phase. (B) Scanning electron micrograph of S.. cerevisiae. The size of the bud reflects the position of the cell in the cycle. (B, David M. Phillips/ Visuals Unlimited.)

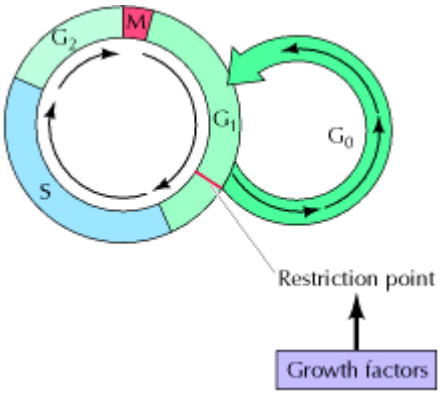

**Figure 14.6. Regulation of animal cell cycles by growth factors** The availability of growth factors controls the animal cell cycle at a point in late G1 called the restriction point. If growth factors are not available during G1, the cells enter a quiescent stage of the cycle called G0.

## Cell Cycle Checkpoints

The controls discussed in the previous section regulate cell cycle progression in response to cell size and extracellular signals, such as nutrients and growth factors. In addition, the events that take place during different stages of the cell cycle must be coordinated with one another so that they occur in the appropriate order. For example, it is critically important that the cell not begin mitosis until replication of the genome has been completed. The alternative would be a catastrophic cell division, in which the daughter cells failed to inherit complete copies of the genetic material. In most cells, this coordination between different phases of the cell cycle is dependent on a system of checkpoints and feedback controls that prevent entry into the next phase of the cell cycle until the events of the preceding phase have been completed.

Several cell cycle checkpoints function to ensure that incomplete or damaged chromosomes are not replicated and passed on to daughter cells (Figure 14.8). One of the most clearly defined of these checkpoints occurs in G2 and prevents the initiation of mitosis until DNA replication is completed. This G2 checkpoint senses unreplicated DNA, which generates a signal that leads to cell cycle arrest. Operation of the G2 checkpoint therefore prevents the initiation of M phase before completion of S phase, so cells remain in G2 until the genome has been completely replicated. Only then is the inhibition of G2 progression relieved, allowing the cell to initiate mitosis and distribute the completely replicated chromosomes to daughter cells.

Progression through the cell cycle is also arrested at the G2 checkpoint in response to DNA damage, such as that resulting from irradiation. This arrest allows time for the damage to be repaired, rather than being passed on to daughter cells. Studies of yeast mutants have shown that the same cell cycle checkpoint is responsible for G2 arrest induced by either unreplicated or damaged DNA, both of which signal cell cycle arrest through related pathways.

DNA damage not only arrests the cell cycle in G2, but also slows the progression of cells through S phase and arrests cell cycle progression at a checkpoint in G1. This G1 arrest may allow repair of the damage to take place before the cell enters S phase, where the damaged DNA would be replicated. In mammalian cells, arrest at the G1 checkpoint is mediated by the action of a protein known as p53, which is rapidly induced in response to damaged DNA (Figure 14.9). Interestingly, the gene encoding p53 is frequently mutated in human cancers. Loss of p53 function as a result of these mutations prevents G1 arrest in response to DNA damage, so the damaged DNA is replicated and passed on to daughter cells instead of being repaired. This inheritance of damaged DNA results in an increased frequency of mutations and general instability of the cellular genome, which contributes to cancer development. Mutations in the p53 gene are the most common genetic alterations in human cancers (see Chapter 15), illustrating the critical importance of cell cycle regulation in the life of multicellular organisms.

Another important cell cycle checkpoint that maintains the integrity of the genome occurs toward the end of mitosis (see Figure 14.8). This checkpoint monitors the alignment of chromosomes on the mitotic spindle, thus ensuring that a complete set of chromosomes is distributed accurately to the daughter cells. For example, the failure of one or more chromosomes to align properly on the spindle causes mitosis to arrest at metaphase, prior to the segregation of the newly replicated chromosomes to daughter nuclei. As a result of this checkpoint, the chromosomes do not separate until a complete complement of chromosomes has been organized for distribution to each daughter cell.

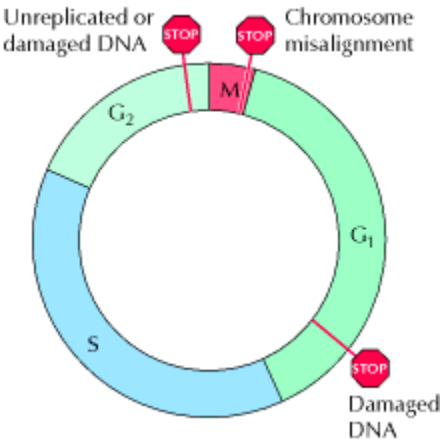


**Figure 14.8. Cell cycle checkpoints** Several checkpoints function to ensure that complete genomes are transmitted to daughter cells. One major checkpoint arrests cells in G2 in response to damaged or unreplicated DNA. The presence of damaged DNA also leads to cell cycle arrest at a checkpoint in G1. Another checkpoint, in M phase, arrests mitosis if the daughter chromosomes are not properly aligned on the mitotic spindle.

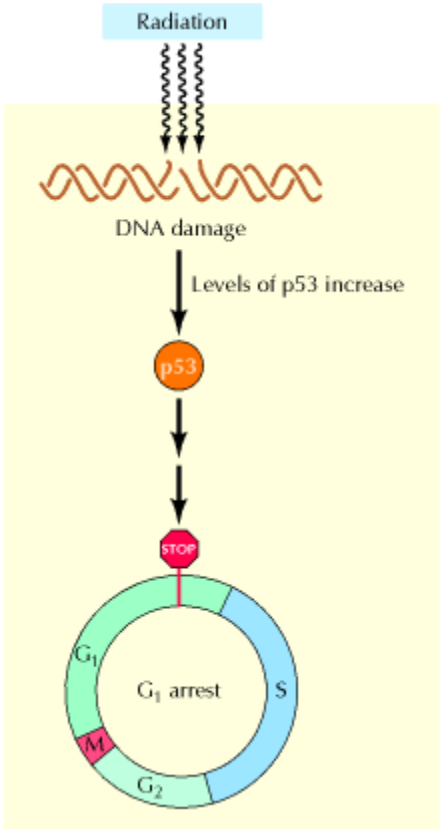


**Figure 14.9. Role of p53 in G1 arrest induced by DNA damage** DNA damage, such as that resulting from irradiation, leads to rapid increases in p53 levels. The protein p53 then signals cell cycle arrest at the G1 checkpoint.

**Coupling of S Phase to M Phase**

The G2 checkpoint prevents the initiation of mitosis prior to the completion of S phase, thereby ensuring that incompletely replicated DNA is not distributed to daughter cells. It is equally important to ensure that the genome is replicated only once per cell cycle. Thus, once DNA has been replicated, control mechanisms must exist to prevent initiation of a new S phase prior to

mitosis. These controls prevent cells in G2 from reentering S phase and block the initiation of another round of DNA replication until after mitosis, at which point the cell has entered the G1 phase of the next cell cycle.

Initial insights into this dependence of S phase on M phase came from cell fusion experiments of Potu Rao and Robert Johnson in 1970 (Figure 14.10). These investigators isolated cells in different phases of the cycle and then fused these cells to each other to form cell hybrids. When G1 cells were fused with S phase cells, the G1 nucleus immediately began to synthesize DNA. Thus, the cytoplasm of S phase cells contained factors that initiated DNA synthesis in the G1 nucleus. Fusing G2 cells with S phase cells, however, yielded a quite different result: The G2 nucleus was unable to initiate DNA synthesis even in the presence of an S phase cytoplasm. It thus appeared that DNA synthesis in the G2 nucleus was prevented by a mechanism that blocked rereplication of the genome until after mitosis had taken place.

The molecular mechanism that restricts DNA replication to once per cell cycle involves the action of a family of proteins (called MCM proteins) that bind to replication origins together with the origin replication complex (ORC) proteins (see Figure 5.17). The MCM proteins act as "licensing factors" that allow replication to initiate (Figure 14.11). Their binding to DNA is regulated during the cell cycle such that the MCM proteins are only able to bind to replication origins during G1, allowing DNA replication to initiate when the cell enters S phase. Once initiation has occurred, however, the MCM proteins are displaced from the origin, so replication cannot initiate again until the cell passes through mitosis and enters G1 phase of the next cell cycle.

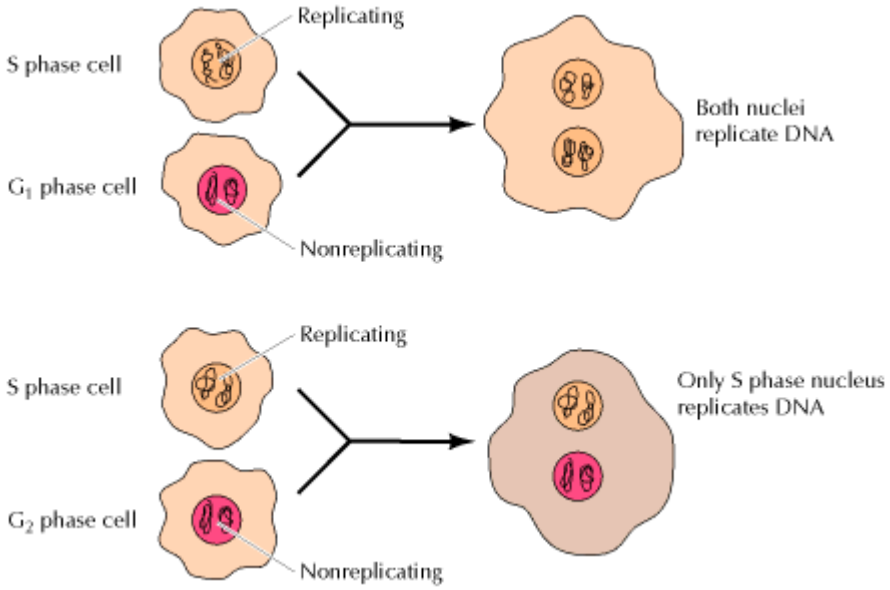


**Figure 14.10. Cell fusion experiments demonstrating the dependence of S phase on M phase**
Cells in S phase were fused either to cells in G1 or to cells in G2. When G1 cells were fused with S phase cells, the G1 nucleus immediately began to replicate DNA. In contrast, when G2 cells were fused with S phase cells, only the S phase nucleus continued DNA replication. It therefore appeared that the G2 nucleus had to pass through M and enter G1 before another round of DNA replication could be initiated.

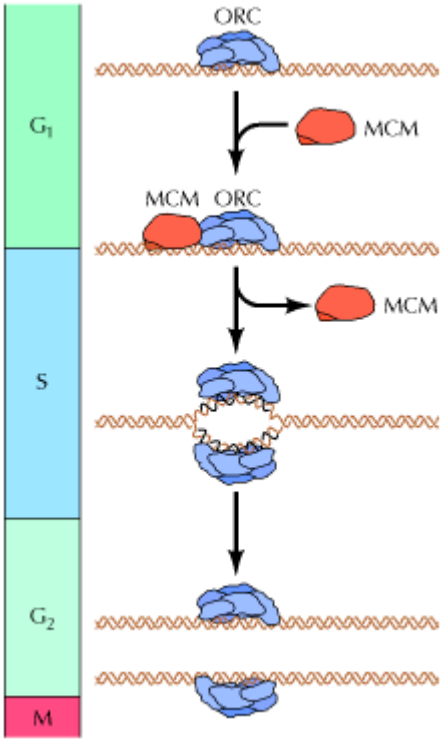


**Figure 14.11. Restriction of DNA replication** DNA replication is restricted to once per cell cycle by MCM proteins that bind to origins of replication together with ORC (origin replication complex) proteins and are required for the initiation of DNA replication. MCM proteins are only able to bind to DNA in G1, allowing DNA replication to initiate in S phase. Once initiation has occurred, the MCM proteins are displaced, so that replication cannot initiate again until after mitosis.

**Regulators of Cell Cycle Progression**

One of the most exciting developments of the last decade has been the elucidation of the molecular mechanisms that control the progression of eukaryotic cells through the division cycle. Our current understanding of cell cycle regulation has emerged from a convergence of results obtained through experiments on organisms as diverse as yeasts, sea urchins, frogs, and mammals. These studies have revealed that the cell cycle of all eukaryotes is controlled by a conserved set of protein kinases, which are responsible for triggering the major cell cycle transitions.

**MPF: A Dimer of Cdc2 and Cyclin**

Three initially distinct experimental approaches contributed to identification of the key molecules responsible for cell cycle regulation. The first of these avenues of investigation originated with studies of frog oocytes (Figure 14.12). These oocytes are arrested in the G2 phase of the cell cycle until hormonal stimulation triggers their entry into the M phase of meiosis (discussed later in this chapter). In 1971, two independent teams of researchers (Yoshio Masui and Clement Markert, as well as Dennis Smith and Robert Ecker) found that oocytes arrested in G2 could be induced to enter M phase by microinjection of cytoplasm from oocytes that had been hormonally stimulated. It thus appeared that a cytoplasmic factor present in hormone-treated oocytes was sufficient to trigger the transition from G2 to M in oocytes that had not been exposed to hormone. Because the entry of oocytes into meiosis is frequently referred to as oocyte maturation, this cytoplasmic factor was called maturation promoting factor (MPF). Further studies showed, however, that the activity of MPF is not restricted to the entry of oocytes into

meiosis. To the contrary, MPF is also present in somatic cells, where it induces entry into M phase of the mitotic cycle. Rather than being specific to oocytes, MPF thus appeared to act as a general regulator of the transition from G2 to M.

The second approach to understanding cell cycle regulation was the genetic analysis of yeasts, pioneered by Lee Hartwell and his colleagues in the early 1970s. Studying the budding yeast Saccharomyces cerevisiae, these investigators identified temperature-sensitive mutants that were defective in cell cycle progression. The key characteristic of these mutants (called cdc for cell division cycle mutants) was that they underwent growth arrest at specific points in the cell cycle. For example, a particularly important mutant designated cdc28 caused the cell cycle to arrest at START, indicating that the Cdc28 protein is required for passage through this critical regulatory point in G1 (Figure 14.13). A similar collection of cell cycle mutants was isolated in the fission yeast Schizosaccharomyces pombe by Paul Nurse and his collaborators. These mutants included cdc2, which arrests the S. pombe cell cycle both in G1 and at the G2 to M transition (the major regulatory point in fission yeast). Further studies showed that S.. cerevisiae cdc28 and S. pombe cdc2 are functionally homologous genes, which are required for passage through START as well as for entry into mitosis in both species of yeasts. To avoid confusion resulting from the difference in genetic nomenclature between S.. cerevisiae and S. pombe, the protein encoded by both genes will be called Cdc2 in this text. Further studies of cdc2 yielded two important insights. First, molecular cloning and nucleotide sequencing revealed that cdc2 encodes a protein kinase—the first indication of the prominent role of protein phosphorylation in regulating the cell cycle. Second, a human gene related to cdc2 was identified and shown to function in yeasts, providing a dramatic demonstration of the conserved activity of this cell cycle regulator.

The third line of investigation that eventually converged with the identification of MPF and yeast genetics emanated from studies of protein synthesis in early sea urchin embryos. Following fertilization, these embryos go through a series of rapid cell divisions. Intriguingly, studies with protein synthesis inhibitors had revealed that entry into M phase of these embryonic cell cycles requires new protein synthesis. In 1983, Tim Hunt and his colleagues identified two proteins that display a periodic pattern of accumulation and degradation in sea urchin and clam embryos. These proteins accumulate throughout interphase and are then rapidly degraded toward the end of each mitosis (Figure 14.14). Hunt called these proteins cyclins (the two proteins were designated cyclin A and cyclin B) and suggested that they might function to induce mitosis, with their periodic accumulation and destruction controlling entry and exit from M phase. Direct support for such a role of cyclins was provided in 1986, when Joan Ruderman and her colleagues showed that microinjection of cyclin A into frog oocytes is sufficient to trigger the G2 to M transition.

These initially independent approaches converged dramatically in 1988, when MPF was purified from frog eggs in the laboratory of James Maller. Molecular characterization of MPF in several laboratories then showed that this conserved regulator of the cell cycle is composed of two key subunits: Cdc2 and cyclin B (Figure 14.15). Cyclin B is a regulatory subunit required for catalytic activity of the Cdc2 protein kinase, consistent with the notion that MPF activity is controlled by the periodic accumulation and degradation of cyclin B during cell cycle progression.

A variety of further studies have confirmed this role of cyclin B, as well as demonstrating the regulation of MPF by phosphorylation and dephosphorylation of Cdc2 (Figure 14.16). In mammalian cells, cyclin B synthesis begins in S phase. Cyclin B then accumulates and forms complexes with Cdc2 throughout S and G2. As these complexes form, Cdc2 is phosphorylated at two critical regulatory positions. One of these phosphorylations occurs on threonine-161 and is required for Cdc2 kinase activity. The second is a phosphorylation of tyrosine-15, and of the

adjacent threonine-14 in vertebrates. Phosphorylation of tyrosine-15, catalyzed by a protein kinase called Wee1, inhibits Cdc2 activity and leads to the accumulation of inactive Cdc2/cyclin B complexes throughout S and G2. The transition from G2 to M is then brought about by activation of the Cdc2/cyclin B complex as a result of dephosphorylation of threonine-14 and tyrosine-15 by a protein phosphatase called Cdc25.

Once activated, the Cdc2 protein kinase phosphorylates a variety of target proteins that initiate the events of M phase, which are discussed later in this chapter. In addition, Cdc2 activity triggers the degradation of cyclin B, which occurs as a result of ubiquitin-mediated proteolysis. This proteolytic destruction of cyclin B then inactivates Cdc2, leading the cell to exit mitosis, undergo cytokinesis, and return to interphase.

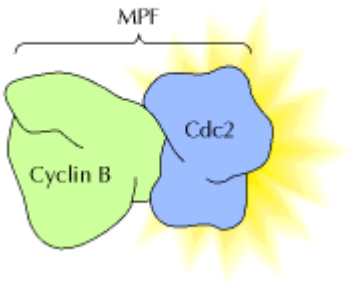


**Figure 14.15. Structure of MPF** MPF is a dimer consisting of cyclin B and the Cdc2 protein kinase.

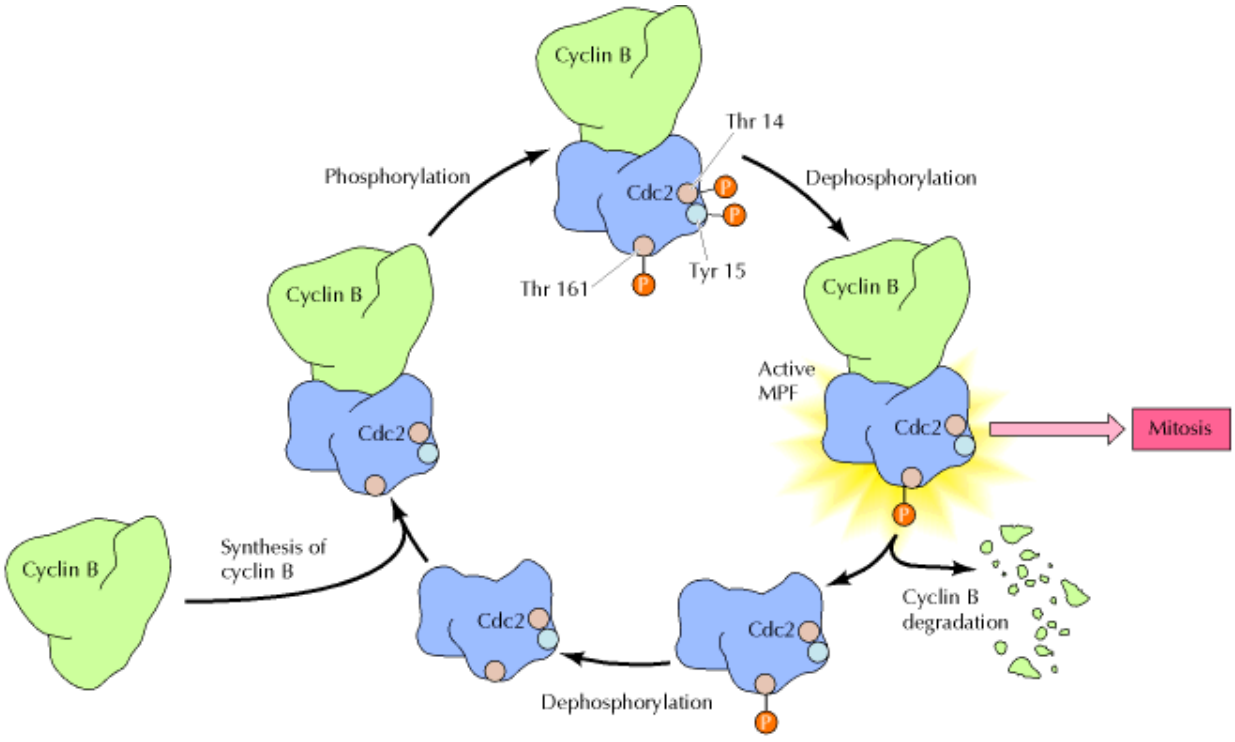


**Figure 14.16. MPF regulation** Cdc2 forms complexes with cyclin B during S and G2. Cdc2 is then phosphorylated on threonine-161, which is required for Cdc2 activity, as well as on tyrosine-15 (and threonine-14 in vertebrate cells), which inhibits Cdc2 activity. Dephosphorylation of Thr14 and Tyr15 activates MPF at the G2 to M transition. MPF activity is then terminated toward the end of mitosis by proteolytic degradation of cyclin B.

**Families of Cyclins and Cyclin-Dependent Kinases**

The structure and function of MPF (Cdc2/cyclin B) provide not only a molecular basis for understanding entry and exit from M phase, but also the foundation for elucidating the regulation of other cell cycle transitions. The insights provided by characterization of the Cdc2/cyclin B complex have thus had a sweeping impact on understanding cell cycle regulation. In particular, further research has established that both Cdc2 and cyclin B are members of large families of related proteins, with different members of these families controlling progression through distinct phases of the cell cycle.

As discussed earlier, Cdc2 controls passage through START as well as entry into mitosis in yeasts. It does so, however, in association with distinct cyclins (Figure 14.17). In particular, the G2 to M transition is driven by Cdc2 in association with the mitotic B-type cyclins (Clb1, Clb2, Clb3, and Clb4). Passage through START, however, is controlled by Cdc2 in association with a distinct class of cyclins called G1cyclins or Cln's. Cdc2 then associates with different B-type cyclins (Clb5 and Clb6), which are required for progression through S phase. These associations of Cdc2 with distinct B-type and G1 cyclins direct Cdc2 to phosphorylate different substrate proteins, as required for progression through specific phases of the cell cycle.

The cell cycles of higher eukaryotes are controlled not only by multiple cyclins, but also by multiple Cdc2-related protein kinases. These Cdc2-related kinases are known as Cdk's (for cyclin-dependent kinases). As the original member of this family, Cdc2 is also known as Cdk1, with other currently identified family members being designated Cdk2 through Cdk8.

These multiple members of the Cdk family associate with specific cyclins to drive progression through the different stages of the cell cycle (see Figure 14.17). For example, progression from G1 to S is regulated principally by Cdk2 and Cdk4 (and in some cells Cdk6) in association with cyclins D and E. Complexes of Cdk4 and Cdk6 with the D-type cyclins (cyclin D1, D2, and D3) play a critical role in progression through the restriction point in G1. Cyclin E is expressed later in G1, and Cdk2/cyclin E complexes are required for the G1 to S transition and initiation of DNA synthesis. Complexes of Cdk2 with cyclin A function in the progression of cells through S phase. As already discussed, the transition from G2 to M is then driven by complexes of Cdc2 with cyclin B.

The activity of Cdk's during cell cycle progression is regulated by at least four molecular mechanisms (Figure 14.18). As already discussed for Cdc2, the first level of regulation involves the association of Cdk's with their cyclin partners. Thus, the formation of specific Cdk/cyclin complexes is controlled by cyclin synthesis and degradation. Second, activation of Cdk/ cyclin complexes requires phosphorylation of a conserved Cdk threonine residue around position 160. This activating phosphorylation of the Cdk's is catalyzed by an enzyme called CAK (for Cdk-activating kinase), which may itself be composed of a Cdk (Cdk7) complexed with cyclin H. Complexes of Cdk7 and cyclin H are also associated with the transcription factor TFIIH, which is required for initiation of transcription by RNA polymerase II (see Chapter 5). It thus appears that this member of the Cdk family may participate in transcription as well as cell cycle regulation.

In contrast to the activating phosphorylation by CAK, the third mechanism of Cdk regulation involves inhibitory phosphorylation of tyrosine residues near the Cdk amino terminus, catalyzed by the Wee1 protein kinase. In particular, both Cdc2 and Cdk2 are inhibited by phosphorylation of tyrosine-15, and the adjacent threonine-14 in vertebrates. These Cdk's are then activated by dephosphorylation of these residues by members of the Cdc25 family of protein phosphatases.

In addition to regulation of the Cdk's by phosphorylation, their activities can also be controlled by the binding of inhibitory proteins (called Cdk inhibitors or CKIs) to Cdk/cyclin complexes. In mammalian cells, two families of Cdk inhibitors are responsible for regulating different Cdk/cyclin complexes (Table 14.1). Members of the Cip/Kip family regulate all stages of progression through G1 and S phase by inhibiting complexes of Cdk2, 4, and 6 with cyclins A, D, and E. In contrast, members of the Ink4 family are specific for complexes of Cdk4 and 6 with cyclin D, so the Ink4 CKIs only regulate progression through the restriction point in G1. In yeast, different CKIs similarly regulate distinct stages of cell cycle progression by inhibiting specific Cdk/cyclin complexes. Control of Cdk inhibitors thus provides an additional mechanism for regulating Cdk activity. The combined effects of these multiple modes of Cdk regulation are responsible for controlling cell cycle progression in response both to checkpoint controls and to the variety of extracellular stimuli that regulate cell proliferation.

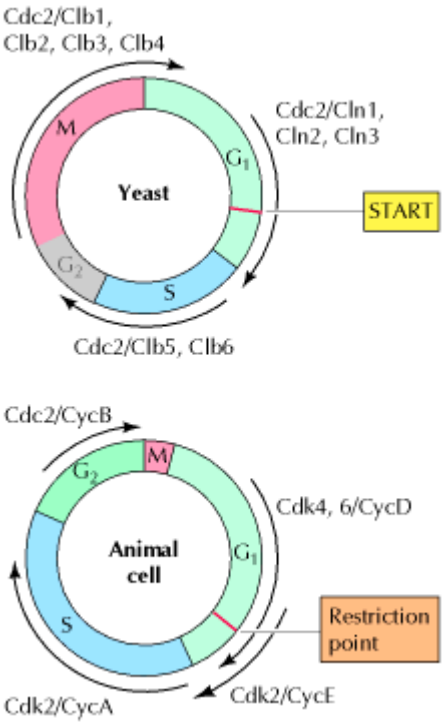


**Figure 14.17. Complexes of cyclins and cyclin-dependent kinases** In yeast, passage through START is controlled by Cdc2 in association with G1 cyclins (Cln1, Cln2, and Cln3). Complexes of Cdc2 with distinct B-type cyclins (Clb's) then regulate progression through S phase and entry into mitosis. In animal cells, progression through the G1 restriction point is controlled by complexes of Cdk4 and Cdk6 with D-type cyclins. Cdk2/cyclin E complexes function later in G1 and are required for the G1 to S transition. Cdk2/cyclin A complexes are

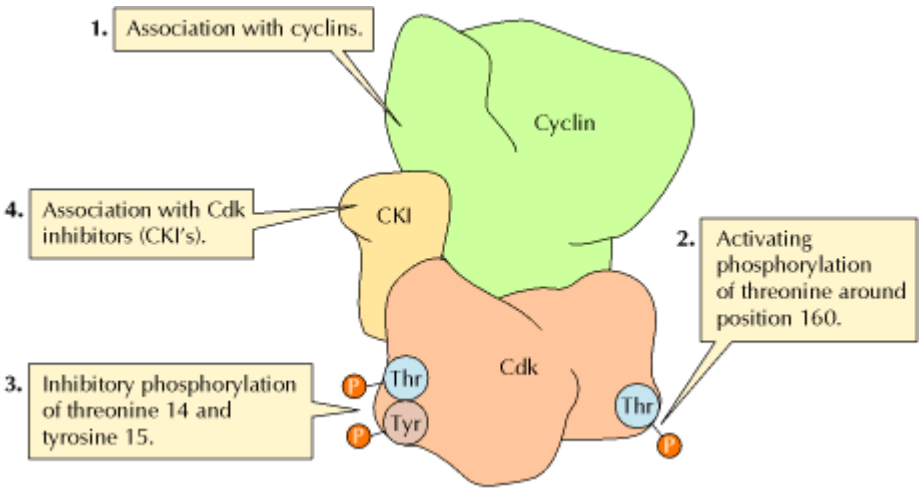


**Figure 14.18. Mechanisms of Cdk regulation** The activities of Cdk's are regulated by four molecular mechanisms.

| Inhibitor | Cdk/cyclin complex | Cell cycle phase affected |
|---|---|---|
| Animal cells | | |
| Cip/Kip family (p21, p27, p57) | Cdk4/cyclin D | G1 |
| | Cdk6/cyclin D | G1 |
| | Cdk2/cyclin E | G1/S |
| | Cdk2/cyclin A | S |
| Ink4 family (p15, p16, p18, p19) | Cdk4/cyclin D | G1 |
| | Cdk6/cyclin D | G1 |
| Yeast (S. cerevisiae) | | |
| Far1 | Cdc2/Cln | G1 |
| Sic1 | Cdc2/Clb5 | S |
| | Cdc2/Clb6 | S |

### Growth Factors and the D-Type Cyclins

As discussed earlier, the proliferation of animal cells is regulated largely by a variety of extracellular growth factors that control the progression of cells through the restriction point in late G1. In the absence of growth factors, cells are unable to pass the restriction point and become quiescent, frequently entering the resting state known as G0, from which they can reenter the cell cycle in response to growth factor stimulation. This control of cell cycle progression by extracellular growth factors implies that the intracellular signaling pathways stimulated downstream of growth factor receptors (discussed in the preceding chapter) ultimately act to regulate components of the cell cycle machinery.

One critical link between growth factor signaling and cell cycle progression is provided by the D-type cyclins (Figure 14.19). Cyclin D synthesis is induced in response to growth factor

stimulation as a result of signaling through the Ras/Raf/ERK pathway, and the D-type cyclins continue to be synthesized as long as growth factors are present. However, the D-type cyclins are also rapidly degraded, so their intracellular concentrations rapidly fall if growth factors are removed. Thus, as long as growth factors are present through G1, complexes of Cdk4, 6/cyclin D drive cells through the restriction point. On the other hand, if growth factors are removed prior to this key regulatory point in the cell cycle, the levels of cyclin D rapidly fall and cells are unable to progress through G1 to S, instead becoming quiescent and entering G0. The inducibility and rapid turnover of D-type cyclins thus integrates growth factor signaling with the cell cycle machinery, allowing the availability of extracellular growth factors to control the progression of cells through G1.

Since cyclin D is a critical target of growth factor signaling, it might be expected that defects in cyclin D regulation could contribute to the loss of growth regulation characteristic of cancer cells. Consistent with this expectation, many human cancers have been found to arise as a result of defects in cell cycle regulation, just as many others result from abnormalities in the intracellular signaling pathways activated by growth factor receptors (see Chapter 13). For example, mutations resulting in continual unregulated expression of cyclin D1 contribute to the development of a variety of human cancers, including lymphomas and breast cancers. Similarly, mutations that inactivate the Ink4 Cdk inhibitors (e.g., p16) that bind to Cdk4, 6/cyclin D complexes are commonly found in human cancer cells.

The connection between cyclin D, growth control, and cancer is further fortified by the fact that a key substrate protein of Cdk4, 6/cyclin D complexes is itself frequently mutated in a wide array of human tumors. This protein, designated Rb, was first identified as the product of a gene responsible for retinoblastoma, a rare inherited childhood eye tumor (see Chapter 15). Further studies then showed that mutations resulting in the absence of functional Rb protein are not restricted to retinoblastoma but also contribute to a variety of common human cancers. Rb is the prototype of a tumor suppressor gene—a gene whose inactivation leads to tumor development. Whereas oncogene proteins such as Ras (see Chapter 13) and cyclin D drive cell proliferation, the proteins encoded by tumor suppressor genes act as brakes that slow down cell cycle progression. Additional examples of cell cycle regulators encoded by tumor suppressor genes include the Ink4 Cdk inhibitors that bind Cdk4, 6/cyclin D complexes and the important growth regulator p53, which was discussed earlier in this chapter.

Further studies of Rb have revealed that it plays a key role in coupling the cell cycle machinery to the expression of genes required for cell cycle progression and DNA synthesis (Figure 14.20). The activity of Rb is regulated by changes in its phosphorylation as cells progress through the cycle. In particular, Rb becomes phosphorylated by Cdk4, 6/cyclin D complexes as cells pass through the restriction point in G1. In its underphosphorylated form (present in G0 or early G1), Rb binds to members of the E2F family of transcription factors, which regulate expression of several genes involved in cell cycle progression, including the gene encoding cyclin E. E2F binds to its target sequences in either the presence or absence of Rb. However, Rb acts as a repressor, so the Rb/E2F complex suppresses transcription of E2F-regulated genes. Phosphorylation of Rb by Cdk4, 6/cyclin D complexes results in its dissociation from E2F, which then activates transcription of its target genes. Rb thus acts as a molecular switch that converts E2F from a repressor to an activator of genes required for cell cycle progression. The control of Rb via Cdk4, 6/cyclin D phosphorylation in turn couples this critical regulation of gene expression to the availability of growth factors in G1.

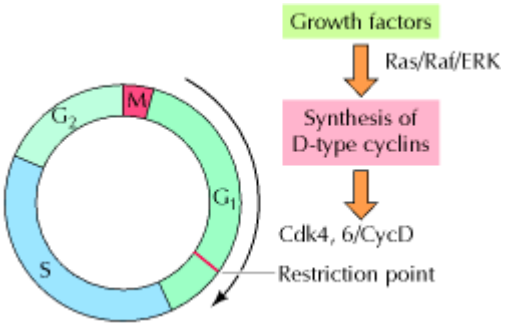


**Figure 14.19. Induction of D-type cyclins** Growth factors regulate cell cycle progression through the G1 restriction point by inducing synthesis of D-type cyclins via the Ras/Raf/ERK signaling pathway.

**Inhibitors of Cell Cycle Progression**

Cell proliferation is regulated not only by growth factors but also by a variety of signals that act to inhibit cell cycle progression. For example, agents that damage DNA result in cell cycle arrest, presumably to allow time for the cell to repair the damage. In addition, cell contacts and a variety of extracellular factors act to inhibit rather than stimulate proliferation of their target cells. The effects of such inhibitory signals are also mediated by regulators of the cell cycle machinery, frequently via the induction of Cdk inhibitors.

A good example of the action of Cdk inhibitors is provided by cell cycle arrest in response to DNA damage, which is mediated by the protein p53 (discussed earlier in this chapter). The p53 protein is a transcriptional regulator that functions, at least in part, to stimulate expression of the Cdk inhibitor p21 (Figure 14.21). The p21 protein inhibits several Cdk/cyclin complexes, and its induction by p53 appears to represent at least one mechanism of p53-dependent cell cycle arrest following DNA damage. In addition to inhibiting cell cycle progression via its interaction with Cdk's, p21 may directly inhibit DNA replication. In particular, p21 binds to proliferating cell nuclear antigen (PCNA), which, as discussed in Chapter 5, is a subunit of DNA polymerase δ. Thus, p21 may play a dual role in cell cycle arrest induced by DNA damage, not only blocking cell cycle progression by inhibiting Cdk's but also directly inhibiting DNA replication in S phase cells.

The best-characterized extracellular inhibitor of animal cell proliferation is TGF-β—a polypeptide factor that inhibits the proliferation of a variety of types of epithelial cells by arresting cell cycle progression in G1. This action of TGF-β appears to be mediated by induction of the Cdk inhibitor p15, which binds to Cdk4, 6/cyclin D complexes. In the resulting absence of Cdk4 activity, Rb phosphorylation is blocked and the cell cycle is arrested in G1.

A different molecular mechanism is used to control cell cycle progression through the G2 checkpoint, which prevents entry into mitosis in the presence of unreplicated or damaged DNA (Figure 14.22). Arrest of the cell cycle at this checkpoint is mediated by a protein kinase called Chk1, which is activated in response to DNA damage or incomplete replication. Chk1 phosphorylates the protein phosphatase Cdc25, thereby preventing Cdc25 from dephosphorylating and activating Cdc2. In the absence of Cdc2 activation, progression to mitosis is blocked and the cell remains arrested in G2.

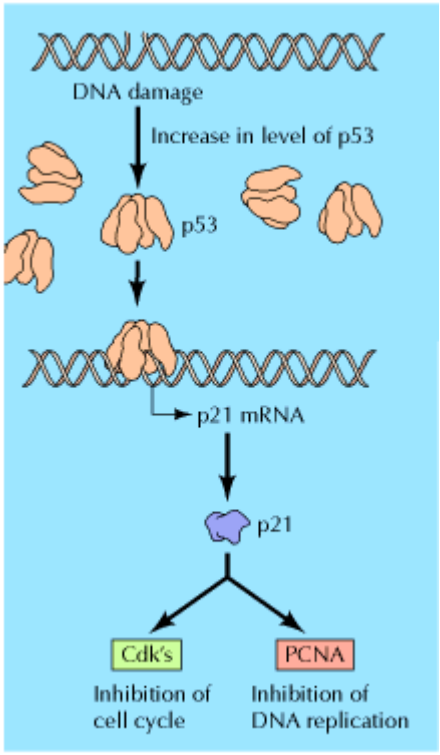


**Figure 14.21. Induction of p21 by DNA damage** DNA damage results in the elevation of intracellular levels of p53, which activates transcription of the gene encoding the Cdk inhibitor p21. In addition to inhibiting cell cycle progression by binding to Cdk/cyclin complexes, p21 may directly inhibit DNA synthesis by interacting with PCNA (a subunit of DNA polymerase δ).

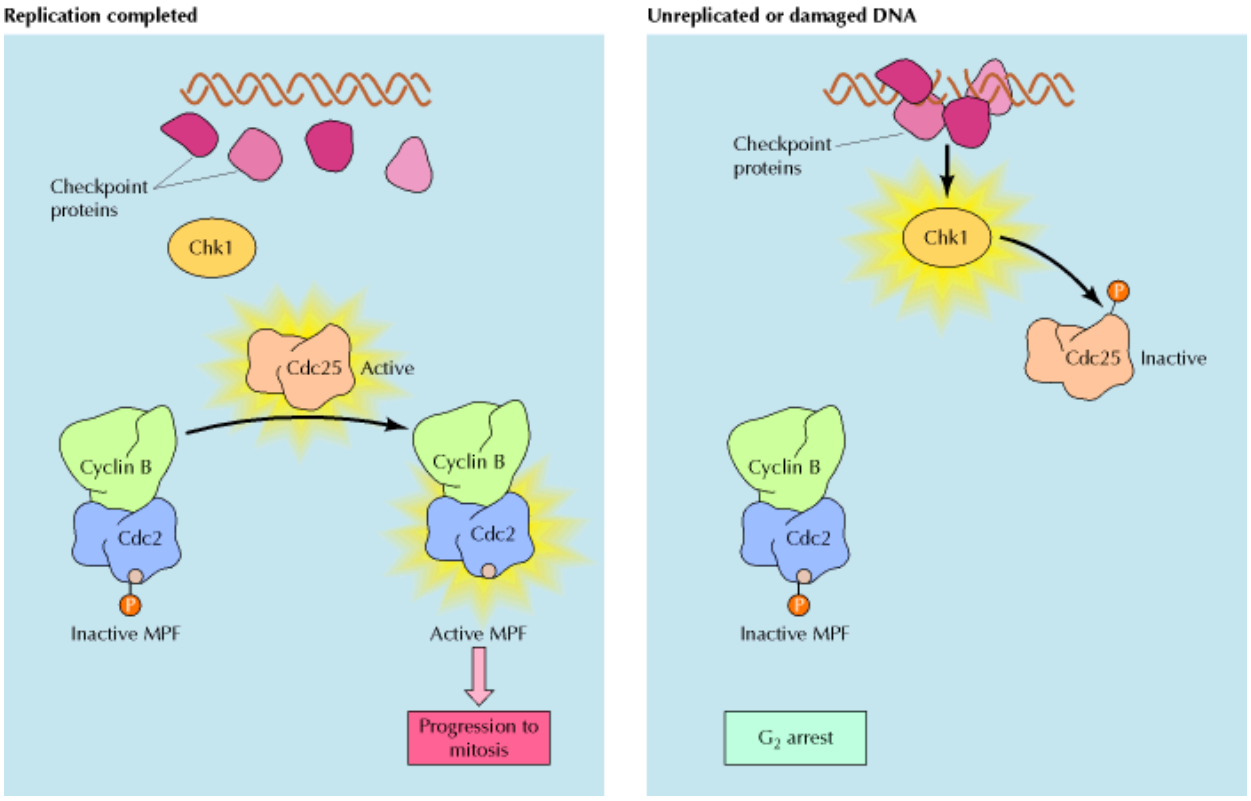


**Figure 14.22. Control of the G2 checkpoint** A complex of checkpoint proteins recognizes unreplicated or damaged DNA and activates the protein kinase Chk1, which phosphorylates and

inhibits the Cdc25 protein phosphatase. Inhibition of Cdc25 prevents dephosphorylation and activation of Cdc2.

14. The Cell Cycle

Self-reproduction is perhaps the most fundamental characteristic of cells—as may be said for all living organisms. All cells reproduce by dividing in two, with each parental cell giving rise to two daughter cells on completion of each cycle of cell division. These newly formed daughter cells can themselves grow and divide, giving rise to a new cell population formed by the growth and division of a single parental cell and its progeny. In the simplest case, such cycles of growth and division allow a single bacterium to form a colony consisting of millions of progeny cells during overnight incubation on a plate of nutrient agar medium. In a more complex case, repeated cycles of cell growth and division result in the development of a single fertilized egg into the more than 1013 cells that make up the human body.

The division of all cells must be carefully regulated and coordinated with both cell growth and DNA replication in order to ensure the formation of progeny cells containing intact genomes. In eukaryotic cells, progression through the cell cycle is controlled by a series of protein kinases that have been conserved from yeasts to mammals. In higher eukaryotes, this cell cycle machinery is itself regulated by the growth factors that control cell proliferation, allowing the division of individual cells to be coordinated with the needs of the organism as a whole. Not surprisingly, defects in cell cycle regulation are a common cause of the abnormal proliferation of cancer cells, so studies of the cell cycle and cancer have become closely interconnected, similar to the relationship between studies of cancer and the cell signaling pathways discussed in Chapter 13.

## Cell Proliferation in Development and Differentiation

Early development is characterized by the rapid proliferation of embryonic cells, which then differentiate to produce the many specialized types of cells that make up the tissues and organs of multicellular animals. As cells differentiate, their rate of proliferation usually decreases, and most cells in adult animals are arrested in the G0 stage of the cell cycle. A few types of differentiated cells never divide again, but most cells are able to resume proliferation as required to replace cells that have been lost as a result of injury or cell death. In addition, some cells divide continuously throughout life to replace cells that have a high rate of turnover in adult animals. Cell proliferation is thus carefully balanced with cell death to maintain a constant number of cells in adult tissues and organs.

### Proliferation of Differentiated Cells

The cells of adult animals can be grouped into three general categories with respect to cell proliferation. A few types of differentiated cells, such as cardiac muscle cells in humans, are no longer capable of cell division. These cells are produced during embryonic development, differentiate, and are then retained throughout the life of the organism. If they are lost because of injury (e.g., the death of cardiac muscle cells during a heart attack), they can never be replaced.

In contrast, most cells in adult animals enter the G0 stage of the cell cycle but resume proliferation as needed to replace cells that have been injured or have died. Cells of this type include skin fibroblasts, smooth muscle cells, the endothelial cells that line blood vessels, and the epithelial cells of most internal organs, such as the liver, pancreas, kidney, lung, prostate, and

breast. One example of the controlled proliferation of these cells, discussed earlier in this chapter, is the rapid proliferation of skin fibroblasts to repair damage resulting from a cut or wound. Another striking example is provided by liver cells, which normally divide only rarely. However, if large numbers of liver cells are lost (e.g., by surgical removal of part of the liver), the remaining cells are stimulated to proliferate to replace the missing tissue. For example, surgical removal of two-thirds of the liver of a rat is followed by rapid cell proliferation, leading to regeneration of the entire liver within a few days. TOP

**Stem Cells**

Other types of differentiated cells, including blood cells, epithelial cells of the skin, and the epithelial cells lining the digestive tract, have short life spans and must be replaced by continual cell proliferation in adult animals. In these cases, the fully differentiated cells do not themselves proliferate. Instead, they are replaced via the proliferation of cells that are less differentiated, called stem cells (Figure 14.43). Stem cells divide to produce daughter cells that can either differentiate or remain as stem cells, thereby serving as a source for the production of differentiated cells throughout life.

A good example of the continual proliferation of stem cells is provided by blood cell differentiation. There are several distinct types of blood cells with specialized functions: Erythrocytes (red blood cells) transport O2 and CO2; granulocytes and macrophages are phagocytic cells; platelets (which are fragments of megakaryocytes) function in blood coagulation; and lymphocytes are responsible for the immune response. All these cells have limited life spans, ranging from less than a day to a few months, and are continually produced by the division of a common stem cell (the pluripotent stem cell) in the bone marrow (Figure 14.44). Descendants of the pluripotent stem cell then become committed to specific differentiation pathways. These cells continue to proliferate and undergo several rounds of division as they differentiate. Once they become fully differentiated, however, they cease proliferation, so the maintenance of differentiated blood cell populations is dependent on continual proliferation of the pluripotent stem cell.

Because stem cells can replicate as well as differentiating to give rise to a variety of cell types, they are of considerable interest with respect to potential medical applications. For example, it may be possible to use stem cells to treat human diseases and repair damaged tissues. The stem cells with the broadest differentiative capacity are the embryonal stem cells (ES cells) that are present in early embryos and can give rise to all of the differentiated cell types of adult organisms. As discussed in Chapter 3, these cells can be cultured from mouse embryos and used to introduce altered genes into mice (see Figure 3.38). In 1998, two groups of researchers reported the isolation of ES cells from human embryos, raising the possibility that these human stem cells could be used for medical applications. Notably, this advance followed the first demonstration, in 1997, that the nucleus of an adult cell could give rise to a viable animal (in this case, a lamb) following transplantation into an oocyte. It is thus theoretically possible that the nucleus of an adult human cell could be used to give rise to ES cells, which could then provide a source of tissue for treatment of that individual. In addition, stem cells have been isolated from adult tissues that give rise not only to blood cells but also to many other cell types, including neurons and cells of connective tissues such as bone, cartilage, fat, and muscle. Therapeutic applications of these adult stem cells may avoid the ethical issues associated with the use of embryos as a source of ES cells. Continuing research on stem cells may thus open new approaches to the treatment of a broad array of human diseases.

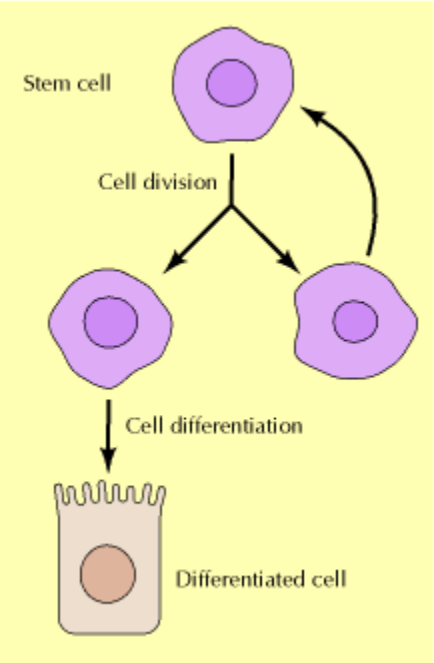


**Figure 14.43. Stem cell proliferation** Stem cells divide to form one daughter cell that remains a stem cell and a second that differentiates (e.g., to an intestinal epithelial cell).

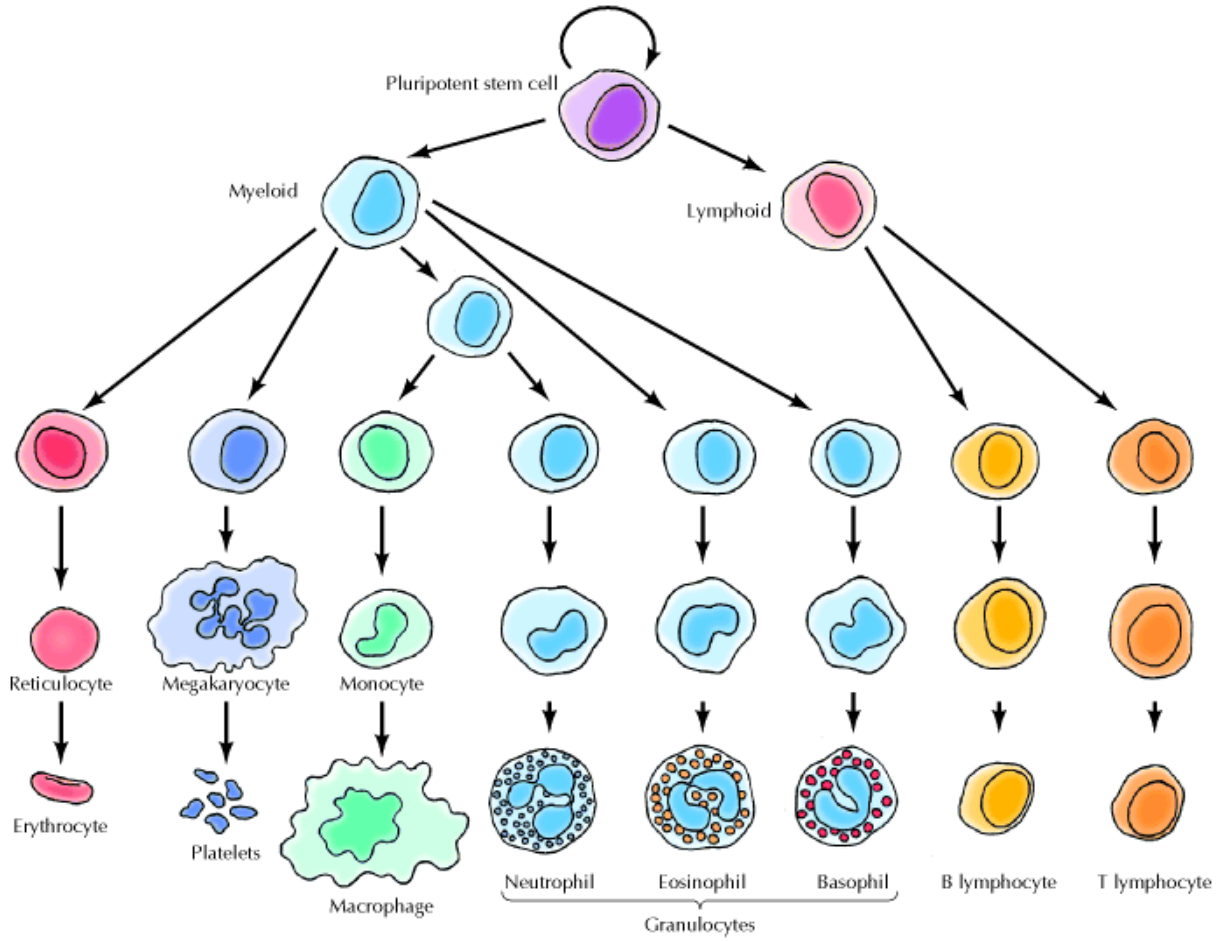


**Figure 14.44. Formation of blood cells** All of the different types of blood cells develop from a pluripotent stem cell in the bone marrow. The precursors of differentiated cells undergo several rounds of cell division as they mature, but cell proliferation ceases at the terminal stages of differentiation.

## The Events of M Phase

M phase is the most dramatic period of the cell cycle, involving a major reorganization of virtually all cell components. During mitosis (nuclear division), the chromosomes condense, the nuclear envelope of most cells breaks down, the cytoskeleton reorganizes to form the mitotic spindle, and the chromosomes move to opposite poles. Chromosome segregation is then usually followed by cell division (cytokinesis). Although many of these events have been discussed in previous chapters with respect to the structure and function of the nucleus and cytoskeleton, they are reviewed here in the context of a coordinated view of M phase and the action of MPF.

### Stages of Mitosis

Although many of the details of mitosis vary among different organisms, the fundamental processes that ensure the faithful segregation of sister chromatids are conserved in all eukaryotes. These basic events of mitosis include chromosome condensation, formation of the mitotic spindle, and attachment of chromosomes to the spindle microtubules. Sister chromatids then separate from each other and move to opposite poles of the spindle, followed by the formation of daughter nuclei.

Mitosis is conventionally divided into four stages—prophase, metaphase, anaphase, and telophase—which are illustrated for an animal cell in Figures 14.23 and 14.24. The beginning of prophase is marked by the appearance of condensed chromosomes, each of which consists of two sister chromatids (the daughter DNA molecules produced in S phase). These newly replicated DNA molecules remain intertwined throughout S and G2, becoming untangled during the process of chromatin condensation. The condensed sister chromatids are then held together at the centromere, which (as discussed in Chapter 4) is a DNA sequence to which proteins bind to form the kinetochore—the site of eventual attachment of the spindle microtubules. In addition to chromosome condensation, cytoplasmic changes leading to the development of the mitotic spindle initiate during prophase. The centrosomes (which had duplicated during interphase) separate and move to opposite sides of the nucleus. There they serve as the two poles of the mitotic spindle, which begins to form during late prophase.

In higher eukaryotes the end of prophase corresponds to the breakdown of the nuclear envelope. As discussed in Chapter 8, however, nuclear envelope breakdown is not a universal feature of mitosis. In particular, yeasts and many other unicellular eukaryotes undergo “closed mitosis,” in which the nuclear envelope remains intact (see Figure 8.30). In these cells the spindle pole bodies are embedded within the nuclear envelope, and the nucleus divides in two following migration of daughter chromosomes to opposite poles of the spindle.

Following completion of prophase, the cell enters prometaphase—a transition period between prophase and metaphase. During prometaphase the microtubules of the mitotic spindle attach to the kinetochores of condensed chromosomes. The kinetochores of sister chromatids are oriented on opposite sides of the chromosome, so they attach to microtubules emanating from opposite poles of the spindle. The chromosomes shuffle back and forth until they eventually align on the metaphase plate in the center of the spindle. At this stage, the cell has reached metaphase.

Most cells remain only briefly at metaphase before proceeding to anaphase. The transition from metaphase to anaphase is triggered by breakage of the link between sister chromatids, which then separate and move to opposite poles of the spindle. Mitosis ends with telophase, during which nuclei re-form and the chromosomes decondense. Cytokinesis usually begins during late anaphase and is almost complete by the end of telophase, resulting in the formation of two interphase daughter cells. TOP

## MPF and Progression to Metaphase

Mitosis involves dramatic changes in multiple cellular components, leading to a major reorganization of the entire structure of the cell. As discussed earlier in this chapter, these events are initiated by activation of the MPF protein kinase (Cdc2/cyclin B). It appears that MPF not only acts as a master regulator of the M phase transition, phosphorylating and activating other downstream protein kinases, but also acts directly by phosphorylating some of the structural proteins involved in this cellular reorganization (Figure 14.25).

The condensation of interphase chromatin to form the compact chromosomes of mitotic cells is a key event in mitosis, critical in enabling the chromosomes to move along the mitotic spindle without becoming broken or tangled with one another. As discussed in Chapter 4, the chromatin in interphase nuclei condenses nearly a thousand fold during the formation of metaphase chromosomes. Such highly condensed chromatin cannot be transcribed, so transcription ceases as chromatin condensation takes place. Despite the fundamental importance of this event, we do not fully understand either the structure of metaphase chromosomes or the molecular mechanism of chromatin condensation. However, protein complexes called condensins have recently been found to drive chromosome condensation by wrapping DNA around itself, compacting chromosomes into the condensed mitotic structure. The condensins are phosphorylated directly by the Cdc2 protein kinase, which drives chromatin condensation by activating condensins as cells enter mitosis. One molecular alteration that generally accompanies chromosome condensation is phosphorylation of histone H1, so it is noteworthy that histone H1 is also a substrate for Cdc2. However, histone H1 phosphorylation is not required for mitotic chromosome condensation, so the significance of H1 phosphorylation by Cdc2 is unclear. In contrast, chromosome condensation has been shown to require phosphorylation of histone H3. Perhaps surprisingly, however, histone H3 is not phosphorylated by Cdc2 and the kinase responsible for H3 phosphorylation in mitotic cells remains to be identified.

Breakdown of the nuclear envelope, which is one of the most dramatic events of mitosis, represents the most clearly defined target for MPF action. As discussed in Chapter 8, Cdc2 phosphorylates the lamins, leading directly to depolymerization of the nuclear lamina (see Figure 8.31). This is followed by fragmentation of the nuclear membrane into small vesicles, which eventually fuse to form new daughter nuclei at telophase. The endoplasmic reticulum and Golgi apparatus similarly fragment into small vesicles, which can then be distributed to daughter cells at cytokinesis. The breakdown of these membranes is also induced by MPF, and may in part be mediated by Cdc2 phosphorylation of the Golgi matrix protein GM130, which is required for the docking of COPI-coated vesicles to the Golgi membrane. Phosphorylation and inactivation of GM130 by Cdc2 inhibits vesicle docking and fusion, leading to fragmentation of the Golgi apparatus. However, additional targets of Cdc2 may also be involved, and the mechanisms by which MPF leads to membrane fragmentation remain to be fully elucidated.

The reorganization of the cytoskeleton that culminates in formation of the mitotic spindle results from the dynamic instability of microtubules (see Chapter 11). At the beginning of prophase, the centrosomes move to opposite sides of the nucleus. The rise in MPF activity then induces a dramatic change in the dynamic behavior of microtubules. First, the rate of microtubule disassembly increases, resulting in depolymerization and shrinkage of the interphase microtubules. This disassembly is thought to result from phosphorylation of microtubule-associated proteins, either by MPF itself or by other MPF-activated protein kinases. In addition, the number of microtubules emanating from the centrosomes increases, so the interphase microtubules are replaced by large numbers of short microtubules radiating from the centrosomes.

The breakdown of the nuclear envelope then allows some of the spindle microtubules to attach to chromosomes at their kinetochores (Figure 14.26), initiating the process of chromosome movement that characterizes prometaphase. The proteins assembled at the kinetochore include microtubule motors that direct the movement of chromosomes toward the minus ends of the spindle microtubules, which are anchored in the centrosome. The action of these proteins, which draw chromosomes toward the centrosome, is opposed by the growth of the spindle microtubules, which pushes the chromosomes away from the spindle poles. Consequently, the chromosomes in prometaphase shuffle back and forth between the centrosomes and the center of the spindle.

Microtubules from opposite poles of the spindle eventually attach to the two kinetochores of sister chromatids (which are located on opposite sides of the chromosome), and the balance of forces acting on the chromosomes leads to their alignment on the metaphase plate in the center of the spindle (Figure 14.27). As discussed in Chapter 11, the spindle consists of both kinetochore microtubules, which are attached to the chromosomes, and polar microtubules, which overlap with one another in the center of the cell. In addition, short astral microtubules radiate outward from the centrosomes toward the cell periphery. TOP

**Proteolysis and the Inactivation of MPF: Anaphase and Telophase**

As discussed earlier in this chapter, an important cell cycle checkpoint monitors the alignment of chromosomes on the metaphase spindle. Once this has been accomplished, the cell proceeds to initiate anaphase and complete mitosis. The progression from metaphase to anaphase results from ubiquitin-mediated proteolysis of key regulatory proteins, triggered by activation of a ubiquitin ligase (see Figure 7.39) called the anaphase-promoting complex. Activation of the anaphase-promoting complex is induced by MPF at the beginning of mitosis, so MPF ultimately triggers its own destruction. The anaphase-promoting complex remains inhibited, however, until the cell passes the metaphase checkpoint, after which activation of the ubiquitin degradation system brings about the transition from metaphase to anaphase and progression through the rest of mitosis.

Activation of the anaphase-promoting complex leads to the degradation of at least two key regulatory proteins (Figure 14.28). The onset of anaphase results from proteolytic degradation of a protein called Scc1, a component of a complex of proteins called cohesins that maintain the connection between sister chromatids while they are aligned on the metaphase plate. Degradation of Scc1 is not catalyzed directly by the anaphase-promoting complex, which instead degrades a regulatory protein called Pds1. Degradation of Pds1 in turn activates another protein, called Esp1, which leads to proteolysis of the cohesin Scc1. Cleavage of Scc1 breaks the linkage between sister chromatids, allowing them to segregate by moving to opposite poles of the spindle (Figure 14.29). The separation of chromosomes during anaphase then proceeds as a result of the action of several types of motor proteins associated with the spindle microtubules (see Figures 11.48 and 11.49).

The other key regulatory protein targeted for ubiquitination and degradation by the anaphase-promoting complex is cyclin B. Degradation of cyclin B leads to inactivation of MPF, which is required for the cell to exit mitosis and return to interphase. Many of the cellular changes involved in these transitions are simply the reversal of the events induced by MPF during entry into mitosis. For example, reassembly of the nuclear envelope, chromatin decondensation, and the return of microtubules to an interphase state probably result directly from loss of MPF activity and dephosphorylation of proteins that had been phosphorylated by MPF at the beginning of mitosis. As discussed next, inactivation of MPF also triggers cytokinesis. TOP

**Cytokinesis**

The completion of mitosis is usually accompanied by cytokinesis, giving rise to two daughter cells. Cytokinesis usually initiates in late anaphase and is triggered by the inactivation of MPF, thereby coordinating nuclear and cytoplasmic division of the cell. As discussed in Chapter 11, cytokinesis of animal cells is mediated by a contractile ring of actin and myosin II filaments that forms beneath the plasma membrane (Figure 14.30). The location of this ring is determined by the position of the mitotic spindle, so the cell is eventually cleaved in a plane that passes through the metaphase plate perpendicular to the spindle. Cleavage proceeds as contraction of the actin-myosin filaments pulls the plasma membrane inward, eventually pinching the cell in half.

The mechanism of cytokinesis is different for higher plant cells, which are surrounded by rigid cell walls. Rather than being pinched in half by a contractile ring, these cells divide by forming new cell walls and plasma membranes inside the cell (Figure 14.31). In early telophase, vesicles carrying cell wall precursors from the Golgi apparatus associate with spindle microtubules and accumulate at the former site of the metaphase plate. These vesicles then fuse to form a large, membrane-enclosed, disclike structure, and their polysaccharide contents assemble to form the matrix of a new cell wall (called a cell plate). The cell plate expands outward, perpendicular to the spindle, until it reaches the plasma membrane. The membrane surrounding the cell plate then fuses with the parental plasma membrane, dividing the cell in two.

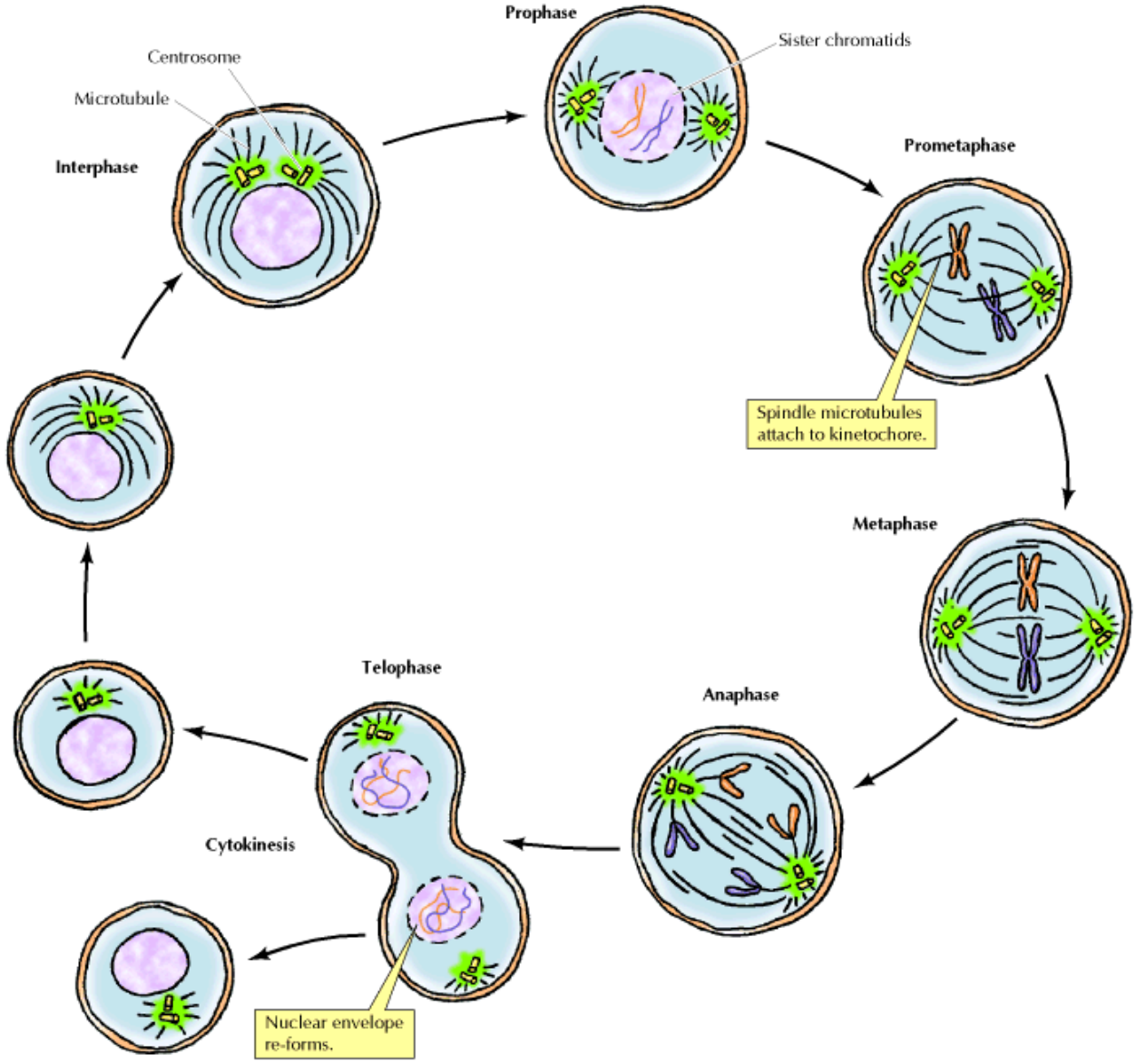


**Figure 14.23. Stages of mitosis in an animal cell** During prophase, the chromosomes condense and centrosomes move to opposite sides of the nucleus, initiating formation of the mitotic spindle. Breakdown of the nuclear envelope then allows spindle microtubules to attach to the kinetochores of chromosomes. During prometaphase, the chromosomes shuffle back and forth between the centrosomes and the center of the cell, eventually aligning in the center of the spindle (metaphase). At anaphase, the sister chromatids separate and move to opposite poles of the spindle. Mitosis then ends with re-formation of nuclear envelopes and chromosome decondensation during telophase, and cytokinesis yields two interphase daughter cells. Note that each daughter cell receives one centrosome, which duplicates prior to the next mitosis.

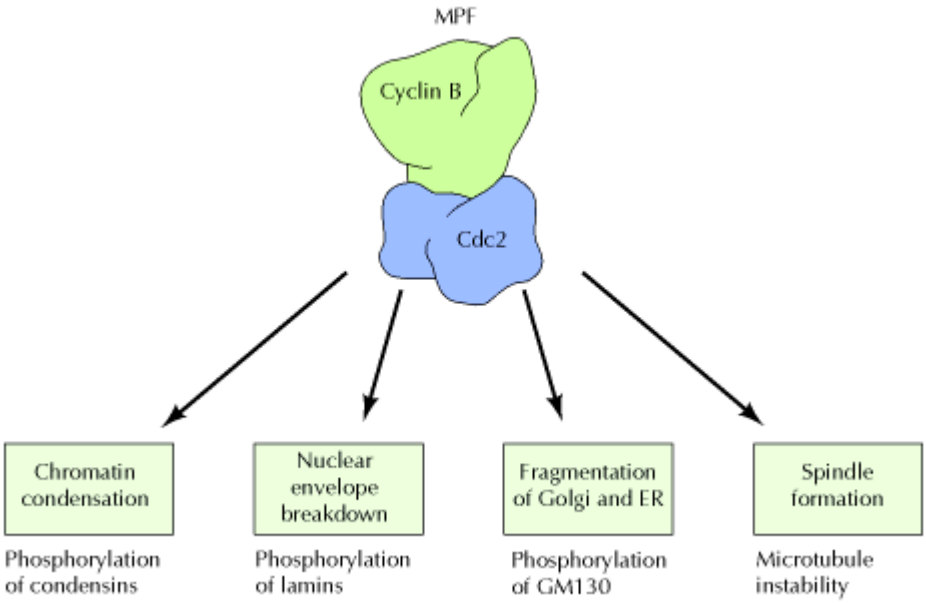


**Figure 14.25. Targets of MPF** MPF induces multiple nuclear and cytoplasmic changes at the onset of M phase, both by activating other protein kinases and by phosphorylating proteins such as condensins and the nuclear lamins.

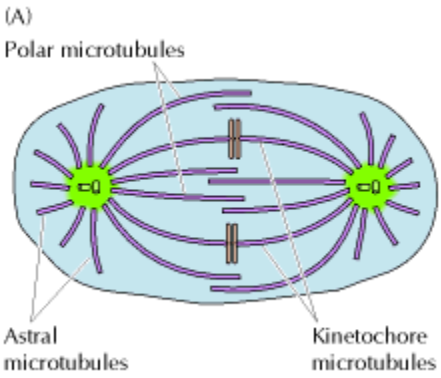




**Figure 14.27. The metaphase spindle** (A) The spindle consists of three kinds of microtubules. Kinetochore microtubules are attached to chromosomes, polar microtubules overlap in the center of the cell, and astral microtubules radiate from the centrosome to the cell periphery. (B) A whitefish cell at metaphase. (B, Michael Abbey/Photo Researchers, Inc.)

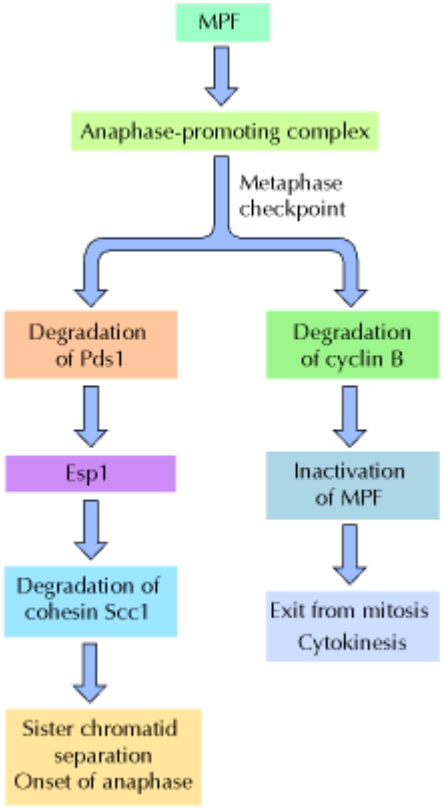


**Figure 14.28. Targets of the cyclin B proteolysis system** The anaphase-promoting complex is a ubiquitin ligase that is activated following passage through the metaphase checkpoint. Its activation brings about the transition from metaphase to anaphase by leading to degradation of the cohesin Scc1, which breaks the link between sister chromatids. The anaphase-promoting complex also targets cyclin B for degradation, leading to inactivation of MPF, exit from mitosis, and cytokinesis.

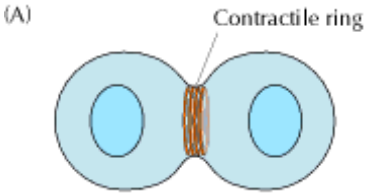


**Figure 14.30. Cytokinesis of animal cells** (A) Cytokinesis results from contraction of a ring of actin and myosin filaments, which pinches the cell in two.