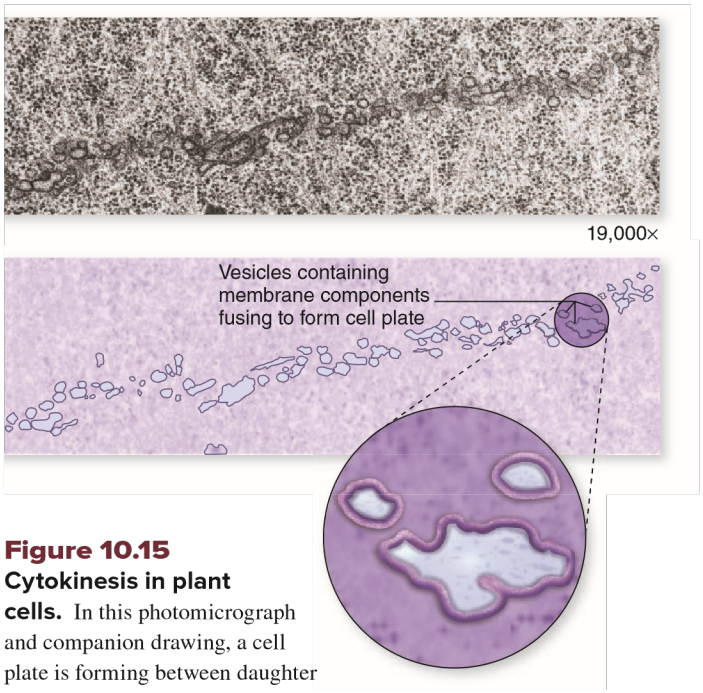


Figure 10.15
Cytokinesis in plant cells. In this photomicrograph and companion drawing, a cell plate is forming between daughter nuclei. The cell plate forms from the fusion of Golgi-derived vesicles. Once the plate is complete, there will be two cells.

organisms does the nucleus divide into two daughter nuclei; then, during cytokinesis, one nucleus goes to each daughter cell. This separate nuclear division phase of the cell cycle does not occur in plants, animals, or most protists.

After cytokinesis in any eukaryotic cell, the two daughter cells contain all the components of a complete cell. Whereas mitosis ensures that both daughter cells contain a full complement of chromosomes, no similar mechanism ensures that organelles such as mitochondria and chloroplasts are distributed equally between the daughter cells. But as long as at least one of each organelle is present in each cell, the organelles can replicate to reach the number appropriate for that cell.

Learning Outcomes Review 10.5

Mitosis is divided into phases: prophase, prometaphase, metaphase, anaphase, and telophase. The early phases involve restructuring the cell to create the microtubule spindle that pulls chromosomes to the equator of the cell in metaphase. Chromatids for each chromosome remain attached at the centromere by cohesin proteins. Chromatids are then pulled to opposite poles during anaphase when cohesin proteins are destroyed. The nucleus is re-formed in telophase, and cytokinesis then divides the cell cytoplasm and organelles. In animal cells, actin pinches the cell in two; in plant cells, a cell plate forms in the middle of the dividing cell.

- ***What would happen to a chromosome that loses cohesin protein between sister chromatids before metaphase?***

10.6 Control of the Cell Cycle

Learning Outcomes

1. *Distinguish the role of checkpoints in the control of the cell cycle.*
2. *Characterize the role of the anaphase-promoting complex/cyclosome in mitosis.*
3. *Describe cancer in terms of cell-cycle control.*

Our knowledge of how the cell cycle is controlled, although still incomplete, has grown enormously in the past 45 years. Our current view integrates two basic concepts. First, the cell cycle has two irreversible points: the replication of genetic material and the separation of the sister chromatids. Second, the cell cycle can be put on hold at specific points called *checkpoints.* At any of these checkpoints, the process is checked for accuracy and can be halted if there are errors. This leads to extremely high fidelity overall for the entire process. The checkpoint organization also allows the cell cycle to respond to both the internal state of the cell, including nutritional state and integrity of genetic material, and to signals from the environment, which are integrated at major checkpoints.

Research uncovered cell cycle control factors

The history of investigation into control of the cell cycle is instructive in two ways. First, it allows us to place modern observations into context; second, we can see how biologists using very different approaches often end up at the same place. The following brief history introduces three observations and then shows how they can be integrated into a single mechanism.

Discovery of MPF

Research on the activation of frog oocytes led to the discovery of a substance that was first called *maturation-promoting factor (MPF).* Frog oocytes, which go on to become egg cells, become arrested near the end of their development at the G_2 stage before meiosis I, which is the division leading to the production of gametes (see chapter 11). They remain in this arrested state and await hormonal signaling to complete this division process.

Cytoplasm taken from a variety of actively dividing cells could prematurely induce cell division when injected into oocytes (figure 10.16). These experiments indicated the presence of a positive regulator of cell-cycle progression in the cytoplasm of dividing cells: MPF. These experiments also fit well with cell fusion experiments done with mitotic and interphase cells that also indicated a cytoplasmic positive regulator that could induce mitosis (figure 10.16).

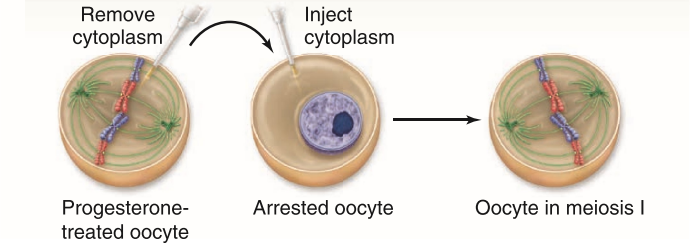
Further studies highlighted two key aspects of MPF. First, MPF activity varied during the cell cycle: low in early G_2, rising throughout this phase, and then peaking in mitosis (figure 10.17). Second, the enzymatic activity of MPF involved the phosphorylation of proteins. This second point is not surprising given the importance of phosphorylation as a reversible switch on

SCIENTIFIC THINKING

Hypothesis: *There are positive regulators of cell division.*

Prediction: *Frog oocytes are arrested in G_2 of meiosis I. They can be induced to mature (undergo meiosis) by progesterone treatment. If maturing oocytes contain a positive regulator of cell division, injection of cytoplasm should induce an immature oocyte to undergo meiosis.*

Test: *Oocytes are induced with progesterone, then cytoplasm from these maturing cells is injected into immature oocytes.*

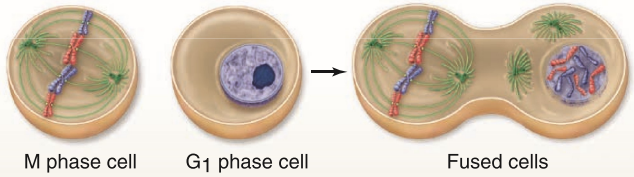


Result: *Injected oocytes progress from G_2 into meiosis I.*

Conclusion: *The progesterone treatment causes production of a positive regulator of maturation: maturation promoting factor (MPF).*

Prediction: *If mitosis is driven by positive regulators, then cytoplasm from a mitotic cell should cause a G_1 cell to enter mitosis.*

Test: *M phase cells are fused with G_1 phase cells, then the nucleus from the G_1 phase cell is monitored microscopically.*



Conclusion: *Cytoplasm from M phase cells contains a positive regulator that causes a cell to enter mitosis.*

Further Experiments: *How can both of these experiments be rationalized? What would be the next step in characterizing these factors?*

Figure 10.16 Discovery of positive regulator of cell division.

the activity of proteins (see chapter 9). The first observation indicated that MPF itself was not always active, but rather was being regulated with the cell cycle, and the second showed the possible enzymatic activity of MPF.

Discovery of cyclins

Other researchers examined proteins produced during the early divisions in sea urchin embryos. They identified proteins that were produced in synchrony with the cell cycle, and named them **cyclins** (figure 10.17). These observations were extended in another marine invertebrate, the surf clam. Two forms of cyclin were found that cycled at slightly different times, reaching peaks at the G_1/S and G_2/M boundaries. Despite much effort, no identified enzymatic activity was associated with these proteins. Their hallmark was the timing of their production and not any intrinsic activity.

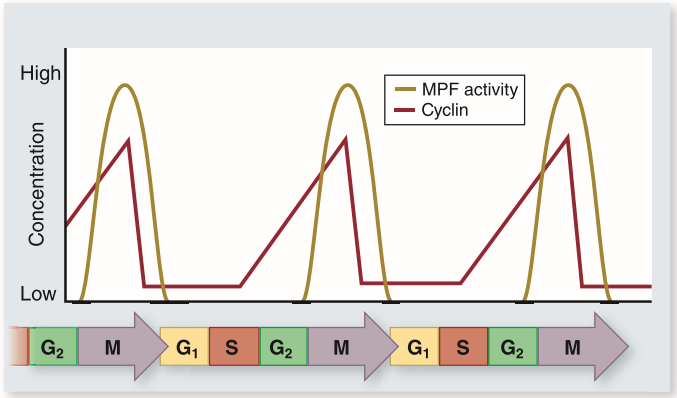


Figure 10.17 Correlation of MPF activity, amount of cyclin protein, and stages of the cell cycle. Cyclin concentration and MPF activity are shown plotted versus stage of the cell cycle. MPF activity changes in a repeating pattern through the cell cycle. This also correlates with the level of mitotic cyclin in the cell, which shows a similar pattern. The reason for this correlation is that cyclin is actually one component of MPF, the other being a cyclin-dependent kinase (Cdk). Together these act as a positive regulator of cell division.

Genetic analysis of the cell cycle

Geneticists using two different yeasts, budding yeast and fission yeast, as model systems set out to determine the genes necessary for control of the cell cycle. By isolating mutants that were halted during division, they identified genes that were necessary for cell cycle progression. These studies indicated that in yeast, there were two critical control points: the commitment to DNA synthesis, called START, as it meant committing to divide, and the commitment to mitosis. One particular gene, named *cdc2,* from fission yeast, was shown to be critical for passing both of these boundaries.

MPF is cyclin plus cdc2

All of these findings came together in an elegant fashion with the following observations. First, the protein encoded by the *cdc2* gene was shown to be a protein kinase. Second, the purification and identification of MPF showed that it was composed of both a cyclin component and a kinase component. Last, the kinase itself was the cdc2 protein!

The cdc2 protein was the first identified **cyclin-dependent kinase (Cdk)**—that is, a protein kinase enzyme that is only active when complexed with cyclin. This finding led to the renaming of MPF as *mitosis*-promoting factor, as its role was clearly more general than simply promoting the maturation of frog oocytes.

These Cdk enzymes are the key positive drivers of the cell division cycle. They are often called the engine that drives cell division. The control of the cell cycle in higher eukaryotes is much more complex than the simple single-engine cycle of yeast, but the yeast model remains a useful framework for understanding more complex regulation. The discovery of Cdks and their role in the cell cycle is an excellent example of the progressive nature of science.

The cell cycle is controlled at three checkpoints

Although we divide the cell cycle into phases and further subdivide mitosis, cells actively control only three points in the cycle we call checkpoints: G_1/S, G_2/M, and late metaphase (the spindle checkpoint). These checkpoints allow the cycle to be delayed or halted when necessary. The cell uses these checkpoints to both assess its internal state and integrate external signals (figure 10.18). Passage through these checkpoints is controlled by the Cdk enzymes described in this chapter.

G_1/S checkpoint

The **G_1/S checkpoint** is the primary point at which the cell "decides" whether or not to divide. This checkpoint is therefore the primary point at which external signals can influence events of the cycle. It is the phase during which growth factors (discussed later in this section) affect the cycle and also the phase that links cell division to cell growth and nutrition.

In yeast systems, where the majority of the genetic analysis of the cell cycle has been performed, this checkpoint is called START. In animals, it is called the restriction point (R point). In all systems, once a cell has made this irreversible commitment to replicate its genome, it has committed to divide. Damage to DNA can halt the cycle at this point, as can starvation conditions or lack of growth factors.

G_2/M checkpoint

The **G_2/M checkpoint** has received a large amount of attention because of its complexity and its importance as the stimulus for the events of mitosis. Historically, Cdks active at this checkpoint were first identified as MPFs, a term that has now evolved into **M phase-promoting factor (MPF).**

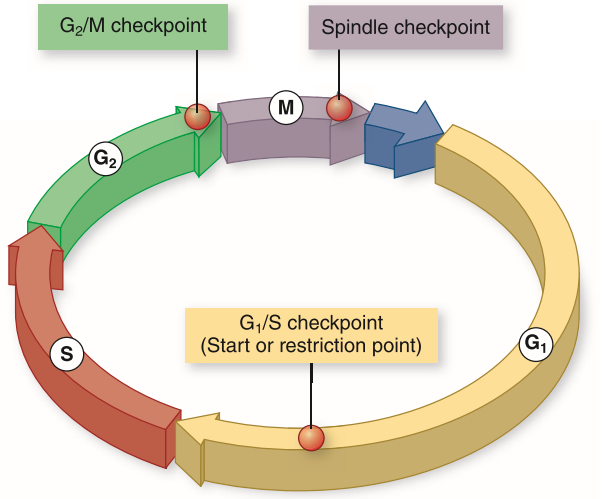


Figure 10.18 Control of the cell cycle. Cells use a centralized control system to check whether proper conditions have been achieved before passing three key checkpoints in the cell cycle.

Passage through this checkpoint represents the commitment to mitosis. This checkpoint assesses the success of DNA replication and can stall the cycle if DNA has not been accurately replicated. DNA-damaging agents result in arrest at this checkpoint as well as at the G_1/S checkpoint.

Spindle checkpoint

The **spindle checkpoint** ensures that all of the chromosomes are attached to the spindle in preparation for anaphase. The second irreversible step in the cycle is the separation of chromosomes during anaphase, and therefore it is critical that they are properly arrayed at the metaphase plate.

Cyclin-dependent kinases drive the cell cycle

The primary molecular mechanism of cell cycle control is phosphorylation, which you may recall is the addition of a phosphate group to the amino acids serine, threonine, and tyrosine in proteins (see chapter 9). The enzymes that accomplish this phosphorylation are the Cdks (figure 10.19).

The action of Cdks

The first important cell cycle kinase was identified in fission yeast and named Cdc2 (now also called Cdk1). In yeast, this Cdk can partner with different cyclins at different points in the cell cycle (figure 10.20).

Even in the simplified cycle of the yeasts, we are left with the important question of what controls the activity of the Cdks during the cycle. For many years, a common view was that cyclins drove the cell cycle—that is, the periodic synthesis and destruction of cyclins acted as a clock. It later became clear that the Cdc2 kinase is also itself controlled by phosphorylation: Phosphorylation at one site activates Cdc2, and phosphorylation at another site inactivates it (see figure 10.19). Full activation of the Cdc2 kinase requires complexing with a cyclin and the appropriate pattern of phosphorylation.

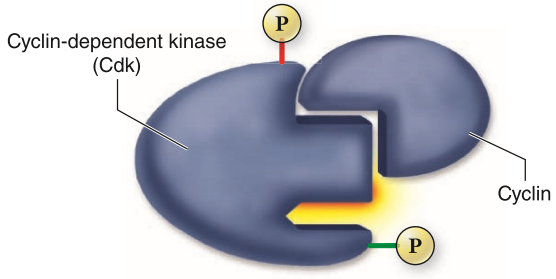


Figure 10.19 Cdk enzyme forms a complex with cyclin. Cdk is a protein kinase that activates numerous cell proteins by phosphorylating them. Cyclin is a regulatory protein required to activate Cdk. This complex is also called mitosis-promoting factor (MPF). The activity of Cdk is also controlled by the pattern of phosphorylation: Phosphorylation at one site (represented by the *red* site) inactivates the Cdk, and phosphorylation at another site (represented by the *green* site) activates the Cdk.

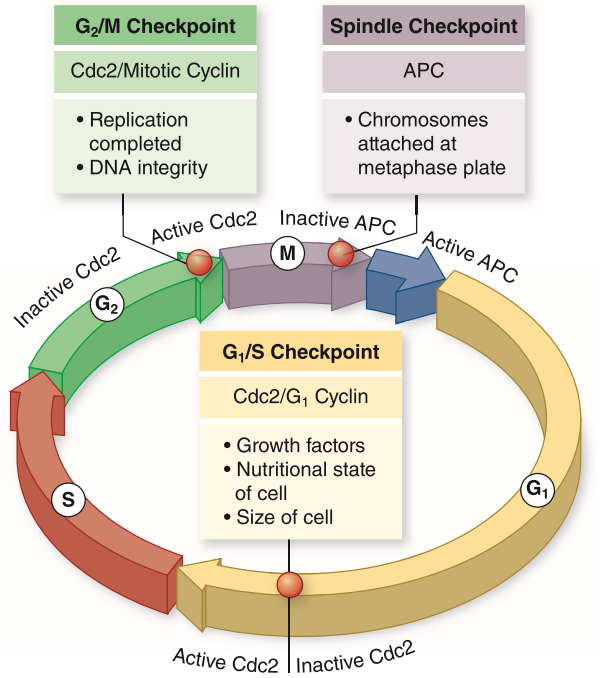


Figure 10.20 Checkpoints of the yeast cell cycle.
The simplest cell cycle that has been studied in detail is the fission yeast. This is controlled by three main checkpoints and a single Cdk enzyme, called Cdc2. The Cdc2 enzyme partners with different cyclins to control the G_1/S and G_2/M checkpoints. The spindle checkpoint is controlled by the anaphase-promoting complex (APC).

As the G_1/S checkpoint is approached, the triggering signal in yeast appears to be the accumulation of G_1 cyclins. These form a complex with Cdc2 to create the active G_1/S Cdk, which phosphorylates a number of targets that bring about the increased enzyme activity for DNA replication.

The action of MPF

MPF and its role at the G_2/M checkpoint has been extensively analyzed in a number of different experimental systems. The control of MPF is sensitive to agents that disrupt or delay replication and to agents that damage DNA. It was once thought that MPF was controlled solely by the level of the M phase-specific cyclins, but it has now become clear that this is not the case.

Although M phase cyclin is necessary for MPF function, activity is controlled by inhibitory phosphorylation of the kinase component, Cdc2. The critical signal in this process is the removal of the inhibitory phosphates by a protein, phosphatase. This action forms a molecular switch based on positive feedback because the active MPF further activates its own activating phosphatase.

The checkpoint assesses the balance of the kinase that adds inhibitory phosphates with the phosphatase that removes them. Damage to DNA acts through a complex pathway that includes damage sensing and a response to tip the balance toward the inhibitory phosphorylation of MPF. Later in this section, we describe how some cancers overcome this inhibition.

The anaphase-promoting complex

The molecular details of the sensing system at the spindle checkpoint are not clear. The presence of all chromosomes at the metaphase plate and the tension on the microtubules between opposite poles are both important. The signal is transmitted through the **anaphase-promoting complex,** also called the *cyclosome (APC/C).*

The function of the APC/C is to trigger anaphase itself. As described in section 10.5, the sister chromatids at metaphase are still held together by the protein complex cohesin. The APC does not act directly on cohesin, but rather acts by marking a protein called *securin* for destruction. The securin protein acts as an inhibitor of another protease called *separase* that is specific for one component of the cohesin complex. Once inhibition is lifted, separase destroys cohesin.

This process has been analyzed in detail in budding yeast, where it has been shown that the separase enzyme specifically degrades a component of cohesin called Scc1. This leads to the release of the sister chromatids and results in their sudden movement toward opposite poles during anaphase.

In vertebrates, most cohesin is removed from the sister chromatids during chromosome condensation, possibly with cohesin being replaced by condensin. At metaphase, the majority of the cohesin that remains on vertebrate chromatids is concentrated at the centromere (see figure 10.10). The destruction of this cohesin explains the anaphase movement of chromosomes and the apparent "division" of the centromeres.

The APC/C has two main roles in mitosis: it activates the protease that removes the cohesins holding sister chromatids together, and it is necessary for the destruction of mitotic cyclins to drive the cell out of mitosis. The APC/C complex marks proteins for destruction by the proteosome, the organelle responsible for the controlled degradation of proteins (see chapter 16). The signal to degrade a protein is the addition of a molecule called *ubiquitin,* and the APC/C acts as a ubiquitin ligase. As we learn more about the APC/C and its functions, it is clear that the control of its activity is a key regulator of the cell cycle.

In multicellular eukaryotes, many Cdks and external signals act on the cell cycle

The major difference between more complex animals and single-celled eukaryotes such as fungi and protists is twofold: First, multiple Cdks control the cycle as opposed to the single Cdk in yeasts; and second, animal cells respond to a greater variety of external signals than do yeasts, which primarily respond to signals necessary for mating.

In higher eukaryotes there are more Cdk enzymes and more cyclins that can partner with these multiple Cdks, but their basic role is the same as in the yeast cycle. A more complex cell cycle is shown in figure 10.21. These more complex controls allow the integration of more input into control of the cycle. With the evolution of more complex forms of organization (tissues, organs, and organ systems), more complex forms of cell-cycle control evolved as well.

A multicellular body's organization cannot be maintained without severely limiting cell proliferation—so that only certain cells divide, and only at appropriate times. The way cells inhibit

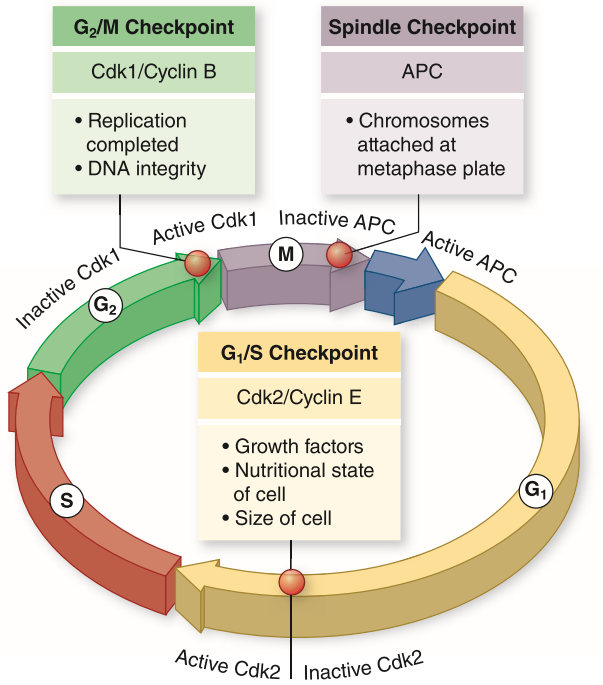


Figure 10.21 Checkpoints of the mammalian cell cycle.
The more complex mammalian cell cycle is shown. This cycle is still controlled through three main checkpoints. These integrate internal and external signals to control progress through the cycle. These inputs control the state of two different Cdk–cyclin complexes and the anaphase-promoting complex (APC).

individual growth of other cells is apparent in mammalian cells growing in tissue culture: A single layer of cells expands over a culture plate until the growing border of cells comes into contact with neighboring cells, and then the cells stop dividing. If a sector of cells is cleared away, neighboring cells rapidly refill that sector and then stop dividing again on cell contact.

How are cells able to sense the density of the cell culture around them? When cells come in contact with one another, receptor proteins in the plasma membrane activate a signal transduction pathway that acts to inhibit Cdk action. This prevents entry into the cell cycle.

Growth factors and the cell cycle

Growth factors act by triggering intracellular signaling systems. Fibroblasts, for example, possess numerous receptors on their plasma membranes for one of the first growth factors to be identified, **platelet-derived growth factor (PDGF).** The PDGF receptor is a receptor tyrosine kinase (RTK) that initiates a MAP kinase cascade to stimulate cell division (discussed in chapter 9).

PDGF was discovered when investigators found that fibroblasts would grow and divide in tissue culture only if the growth medium contained blood serum. Serum is the liquid that remains in blood after clotting; blood plasma, the liquid from which cells have been removed without clotting, would not work. The researchers hypothesized that platelets in the blood clots were releasing into the serum one or more factors required for fibroblast growth. Eventually, they isolated such a factor and named it PDGF.

Growth factors such as PDGF can override cellular controls that otherwise inhibit cell division. When a tissue is injured, a blood clot forms, and the release of PDGF triggers neighboring cells to divide, helping to heal the wound. Only a tiny amount of PDGF (approximately 10^{-10} M) is required to stimulate cell division in cells with PDGF receptors.

Characteristics of growth factors

Over 50 different proteins that function as growth factors have been isolated, and more undoubtedly exist. A specific cell surface receptor recognizes each growth factor, its binding site fitting that growth factor precisely. These growth factor receptors often initiate MAP kinase cascades in which the final kinase enters the nucleus and activates transcription factors by phosphorylation. These transcription factors stimulate the production of G_1 cyclins and the proteins that are necessary for cell-cycle progression (figure 10.22).

The cellular selectivity of a particular growth factor depends on which target cells bear its unique receptor. Some growth factors, such as PDGF and epidermal growth factor (EGF), affect a broad range of cell types, but others affect only specific types. For example, nerve growth factor (NGF) promotes the growth of certain classes of neurons, and erythropoietin triggers cell division in red blood cell precursors. Most animal cells need a combination of several different growth factors to overcome the various controls that inhibit cell division.

The G_0 phase

If cells are deprived of appropriate growth factors, they stop at the G_1 checkpoint of the cell cycle. With their growth and division arrested, they remain in this dormant G_0 phase.

The ability to enter G_0 accounts for the incredible diversity seen in the length of the cell cycle in different tissues. Epithelial cells lining the human gut divide more than twice a day, constantly renewing this lining. By contrast, liver cells divide only once every year or two, spending most of their time in the G_0 phase. Mature neurons and muscle cells usually never leave G_0.

Cancer is a failure of cell-cycle control

The unrestrained, uncontrolled growth of cells in humans leads to the disease called **cancer.** Cancer is essentially a disease of cell division—a failure of cell division control.

The p53 gene

One of the critical players in this control system has been identified. Officially dubbed ***p53,*** this gene plays a key role in the G_1 checkpoint of cell division.

The gene's product, the p53 protein, monitors the integrity of DNA, checking that it is undamaged. If the p53 protein detects damaged DNA, it halts cell division and stimulates the activity of special enzymes to repair the damage. Once the DNA has been repaired, p53 allows cell division to continue. In cases

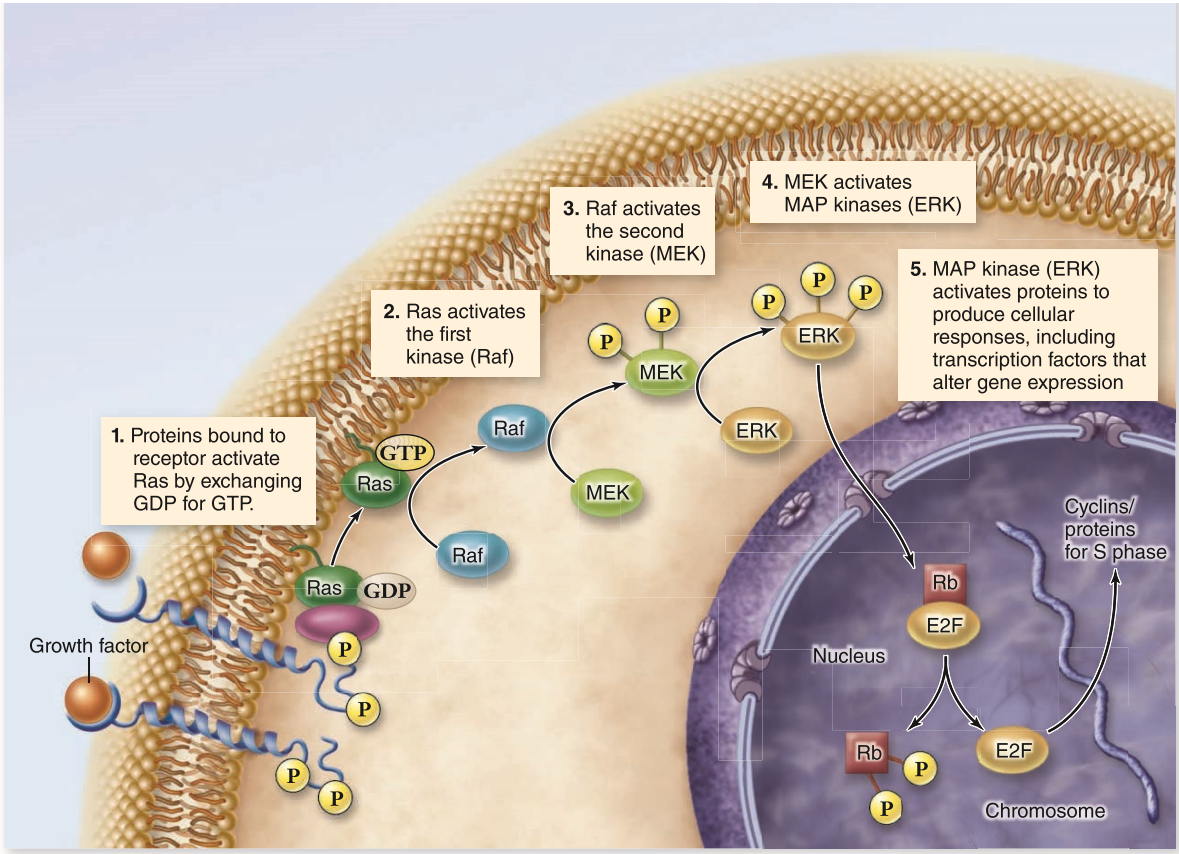


Figure 10.22 The cell proliferation-signaling pathway. Binding of a growth factor sets in motion a MAP kinase intracellular signaling pathway (described in chapter 9), which activates nuclear regulatory proteins that trigger cell division. In this example, when the nuclear retinoblastoma protein (Rb) is phosphorylated, another nuclear protein (the transcription factor E2F) is released and is then able to stimulate the production of cyclin and other proteins necessary for S phase.

where the DNA damage is irreparable, p53 then directs the cell to kill itself.

By halting division in damaged cells, the *p53* gene prevents the development of many mutated cells, and it is therefore considered a **tumor-suppressor gene** although its activities are not limited to cancer prevention. Scientists have found that *p53* is entirely absent or damaged beyond use in the majority of cancerous cells they have examined. It is precisely because *p53* is nonfunctional that cancer cells are able to repeatedly undergo cell division without being halted at the G_1 checkpoint (figure 10.23).

Proto-oncogenes

The disease we call cancer is actually many different diseases, depending on the tissue affected. The common theme in all

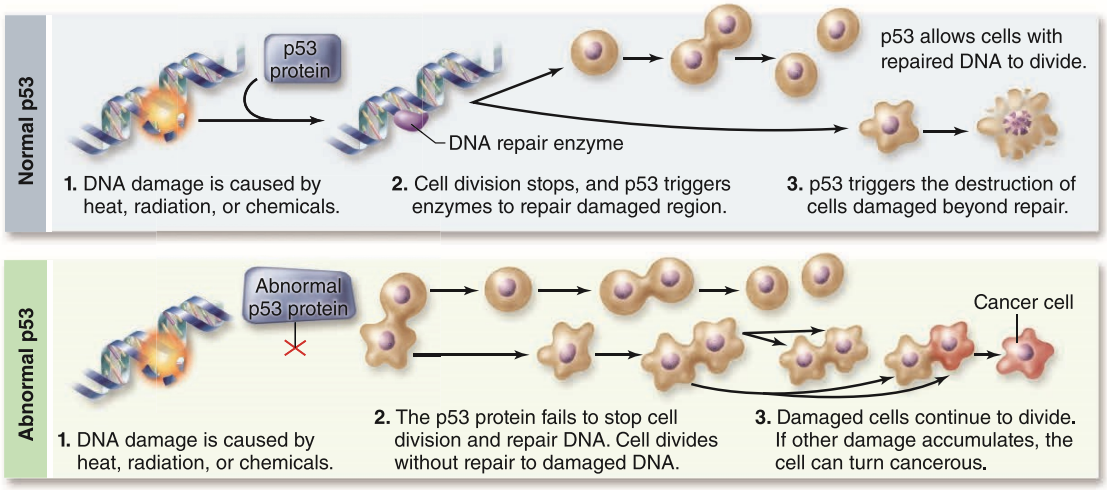


Figure 10.23 Cell division, cancer, and p53 protein. Normal p53 protein monitors DNA, destroying cells that have irreparable damage to their DNA. Abnormal p53 protein fails to stop cell division and repair DNA. As damaged cells proliferate, cancer develops.