CELL CYCLE AND GROWTH REGULATION

6.29.1 Introduction

Key Terms

The **cell cycle** is the set of stages through which a cell progresses from one division to the next.

Interphase is the period between mitotic cell divisions; divided into G1, S, and G2.

Mitosis (M phase) is the process by which the cell nucleus divides, resulting in daughter cells that contain the same amount of genetic material as the parent cell.

The two cells that result from a cell division are referred to as **daughter cells.** In budding yeast only the cell derived from the bud is called the daughter cell.

G1 is the period of the eukaryotic cell cycle between the last mitosis and the start of DNA replication.

S phase is the restricted part of the eukaryotic cell cycle during which synthesis of DNA occurs.

G2 phase is the period of the cell cycle separating the replication of a cell's chromosomes (S phase) from the following mitosis (M phase).

The **spindle** guides the movement of chromosomes during cell division. The structure is made up of microtubules.

The act of division is the culmination of a series of events that have occurred since the last time a cell divided. The period between two mitotic divisions defines the somatic **cell cycle**. The time from the end of one mitosis to the start of the next is called **interphase**. The period of actual division, corresponding to the visible mitosis, is called **M phase**.

In order to divide, a eukaryotic somatic cell must double its mass and then apportion its components equally between the two **daughter cells**. Doubling of size is a continuous process, resulting from transcription and translation of the genes that code for the proteins constituting the particular cell phenotype. By contrast, reproduction of the genome occurs only during a specific period of DNA synthesis.

Mitosis of a somatic cell generates two identical daughter cells, each bearing a diploid complement of chromosomes. Interphase is divided into periods that are defined by reference to the timing of DNA synthesis, as summarized in **Figure 29.1**:

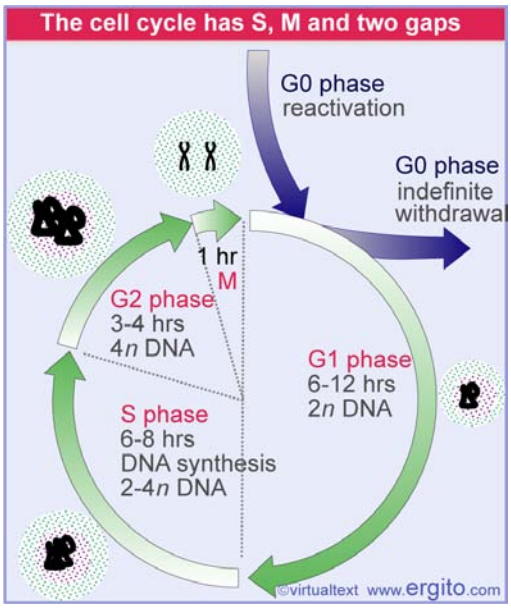

Figure 29.1 Overview: interphase is divided into the G1, S, and G2 periods. One cell cycle is separated from the next by mitosis (M). Cells may withdraw from the cycle into G0 or reenter from it.

- Cells are released from mitosis into **G1** phase, when RNAs and proteins are synthesized, but there is no DNA replication.
- The initiation of DNA replication marks the transition from G1 phase to the period of **S phase**. S phase is defined as lasting until all of the DNA has been replicated. During S phase, the total content of DNA increases from the diploid value of 2*n* to the fully replicated value of 4*n*.
- The period from the end of S phase until mitosis is called **G2** phase; during this period, the cell has two complete diploid sets of chromosomes.

[S phase was so called as the synthetic period when DNA is replicated, G1 and G2 standing for the two "gaps" in the cell cycle when there is no DNA synthesis (823).]

The changes in cellular components are summarized in **Figure 29.2**. During interphase, there is little visible change in the appearance of the cell. The more or less continuous increase of RNA and protein contrasts with the discrete doubling of DNA. The nucleus increases in size predominantly during S phase, when proteins accumulate to match the production of DNA. Chromatin remains a compact mass in which no change of state is visible.

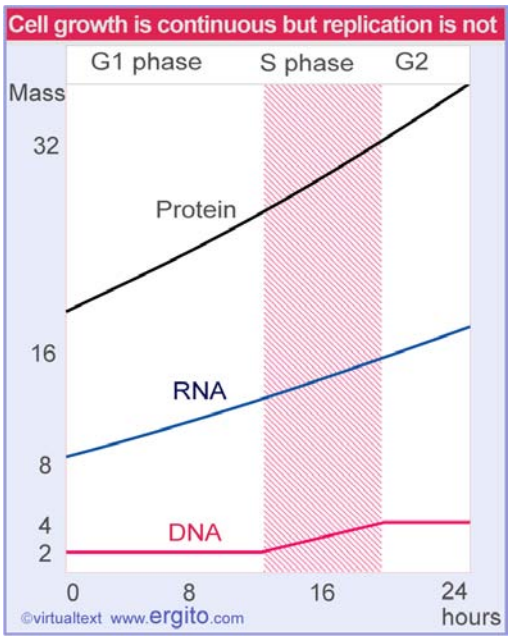

Figure 29.2 Synthesis of RNA and proteins occurs continuously, but DNA synthesis occurs only in the discrete period of S phase. The units of mass are arbitrary.

Mitosis segregates one diploid set of chromosomes to each daughter cell. Individual chromosomes become visible only during this period, when the nuclear envelope dissolves, and the cell is reorganized on a **spindle**. The mechanism for specific segregation of material applies only to chromosomes, and other components are apportioned essentially by the flow of cytoplasm into the two daughter cells. Virtually all synthetic activities come to a halt during mitosis.

In a cycling somatic animal cell, this sequence of events is repeated every 18-24 hours. **Figure 29.1** shows that G1 phase usually occupies the bulk of this period, varying from ~6 h in a fairly rapidly growing animal cell to ~12 h in a more slowly growing cell. The duration of S phase is determined by the length of time required to replicate all the genome, and a period of 6-8 h is typical. G2 phase is usually the shortest part of interphase, essentially comprising the preparations for mitosis. M phase (mitosis) is a brief interlude in the cell cycle, usually <1 h in duration.

References

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CELL CYCLE AND GROWTH REGULATION

6.29.2 Cycle progression depends on discrete control points

Key Terms

START (restriction point) is the point during G1 at which a cell becomes committed to division.

G0 is a noncycling state in which a cell has ceased to divide.

Key Concepts

- START in yeast cells and the restriction point in animal cells define the time in G1 when a cell makes a commitment to divide.

There are two points at which a decision may be taken on whether to proceed through another cell cycle. These are superimposed on the cycle in **Figure 29.3**:

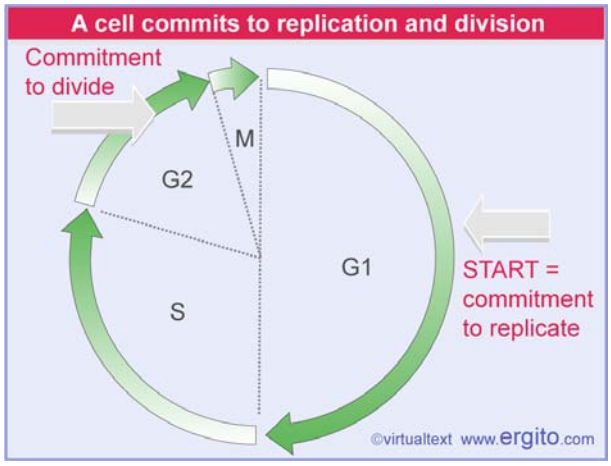

Figure 29.3 Commitment to replication occurs in G1, and commitment to division occurs in G2.

- *Commitment to chromosome replication occurs in G1 phase.* If conditions to pass a "commitment point" are satisfied, there is a lag period and then a cell enters S phase. The commitment point has been defined most clearly in yeast cells, where it is called **START**. The comparable feature in animal cells is called the **restriction point**.

- *Commitment to mitotic division occurs at the end of G2.* If the cell does not divide at this point, it remains in the condition of having twice the normal complement of chromosomes.

How do cells use these two control points?

For animal cells growing in culture, G1 control is the major point of decision, and G2/M control is subsidiary. Cells spend the longest part of their cycle in G1, and it is the length of G1 that is adjusted in response to growth conditions. When a cell proceeds past G1, barring accidents it will complete S phase, proceed through G2, and divide. Cultured cells do not halt in G2. Control at G1 is probably typical of most diploid cells, in culture or *in vivo*.

Some cell phenotypes do not divide at all. These cells are often considered to have withdrawn from the cell cycle into another state, resembling G1 but distinct from it because they are unable to proceed into S phase. This noncycling state is called **G0**. Certain types of cells can be stimulated to leave G0 and reenter a cell cycle. Withdrawal from, or reentry into, the cell cycle can occur before the restriction point in G1 (see **Figure 29.1**).

Some cell types do halt in G2. In the diploid world, these are usually cells likely to be called upon to divide again; for example, nuclei at some stages of insect embryogenesis divide and rest in the tetraploid state. In the haploid world, it is more common for cells to rest in G2; this affords some protection against damage to DNA, since there are two copies of the genome instead of the single copy present in G1. Some yeasts can use either G1 or G2 as the primary control point, depending on the nutritional conditions. Some (haploid) mosses usually use G2 as the control point.

CELL CYCLE AND GROWTH REGULATION

6.29.3 Checkpoints occur throughout the cell cycle

Key Terms

A **checkpoint** is an event in the cell cycle that can only proceed if some earlier event has been completed.

Key Concepts

- Orderly progress through the cell cycle is controlled by checkpoints, which prevent one stage from proceeding unless necessary earlier stages have been completed.

The upper part of **Figure 29.4** (in blue) identifies the critical points in the cell cycle:

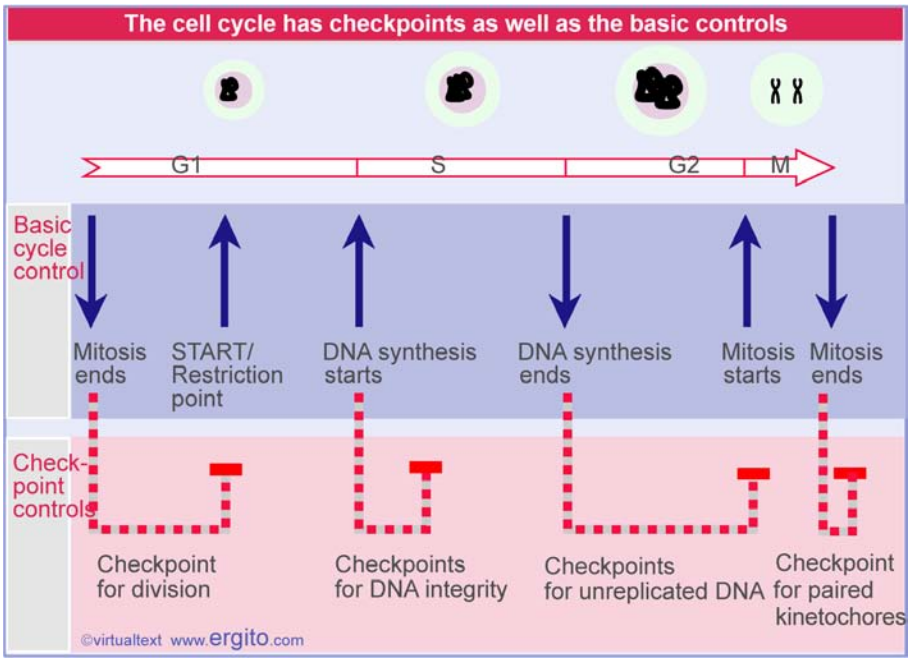

Figure 29.4 Checkpoints control the ability of the cell to progress through the cycle by determining whether earlier stages have been completed successfully. A horizontal red bar indicates the stage at which a checkpoint blocks the cycle.

- For a cell with a cycle determined through G1 control, START marks the point at which the cell takes the basic decision: do I divide? Various parameters influence the ability of a cell to take this decision, including the response to external stimuli (such as supply of nutrients), and an assessment of whether cell mass is sufficient to support a division cycle. (Generally a cell is permitted to divide at a mass that is not absolute but is determined by a control that itself responds to growth rate.)

- The beginning of S phase is marked by the point at which the replication apparatus begins to synthesize DNA.
- The start of mitosis is identified by the moment at which the cell begins to reorganize for division.

Each of these events represents a discrete moment at which a molecular change occurs in a regulatory molecule. Once a cell has taken the decision to proceed at START, the other events will follow in order as the result of the cell cycle pathway.

Checkpoints that assess the readiness of the cell to proceed are superimposed on the pathway. The lower part of **Figure 29.4** (in red) shows that *each checkpoint represents a control loop that makes the initiation of one event in the cell cycle dependent on the successful completion of an earlier event.* A checkpoint works by acting directly on the factors that control progression through the cell cycle.

Figure 29.5 summarizes the events that trigger some important checkpoints (for review see 319). DNA damage is checked at every stage of the cycle. Checkpoints for specific events related to the individual stage occur at S phase and at mitosis.

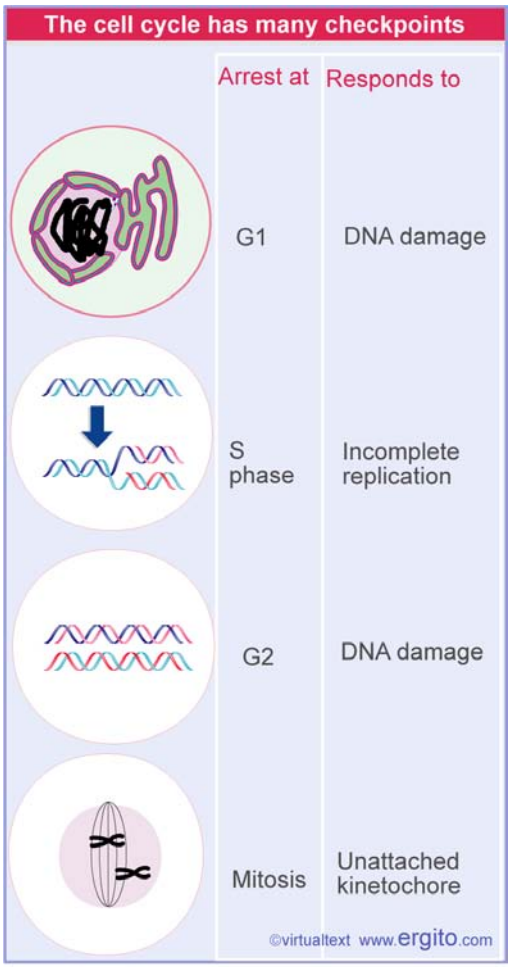

Figure 29.5 Checkpoints can arrest the cell cycle at many points in response to intrinsic or extrinsic conditions.

Checkpoints operate within S phase to prevent replication from continuing if there are problems with the integrity of the DNA (for example, because of breaks or other damage). One important checkpoint establishes that all the DNA has been replicated. This explains why a common feature in the cycle of probably all somatic eukaryotic cells is that completion of DNA replication is a prerequisite for cell division

Several checkpoints operate at mitosis, to ensure that the cell does not try to divide unless it has completed all of the necessary preceding events. There is a checkpoint that confirms that mitosis has been successfully completed before a cell can proceed through START to commit itself to another cycle.

Two types of cycle must be coordinated for a cell to divide:

- A cell must replicate every sequence of DNA once and only once. This is controlled by licensing factor (see *Molecular Biology 4.14.20 Licensing factor controls eukaryotic rereplication*). Having begun replication, it must complete

it; *and it must not try to divide until replication has been completed.* This control is accomplished by the checkpoint at mitosis.

- The mass of the cell must double, so that there is sufficient material to apportion to the daughter cells. So *a cell must not try to start a replication cycle unless its mass will be sufficient to support division.* Cell mass influences ability to proceed through START and may also have a checkpoint at mitosis.

Some embryonic cycles bypass some of these controls and respond instead to a timer or oscillator. So the control of the cell cycle can be coupled as required to time, growth rate, mass, and the completion of replication.

Reviews

319. Hartwell, L. H. and Weinert, T. A. (1989). *Checkpoints: controls that ensure the order of cell cycle events.* Science 246, 629-634.

CELL CYCLE AND GROWTH REGULATION

6.29.4 Cell fusion experiments identify cell cycle inducers

Key Terms

A **heterokaryon** is a cell containing two (or more) nuclei in a common cytoplasm, generated by fusing somatic cells.

The **S phase activator** is the cdk-cyclin complex that is responsible for initiating S phase.

M phase kinase (MPF) was originally called the maturation promoting factor (or M phase-promoting factor). It is a dimeric kinase, containing the p34 catalytic subunit and a cyclin regulatory subunit, whose activation triggers the onset of mitosis.

Cyclins are proteins that accumulate continuously throughout the cell cycle and are then destroyed by proteolysis during mitosis. A cyclin is one of the two subunits of the M-phase kinase.

Key Concepts

- The S phase activator is responsible for the ability of an S phase nucleus to induce DNA replication in a G1 nucleus in a heterokaryon.
- The M phase inducer is responsible for the ability of a mitotic cell to induce pseudo-mitosis in an interphase nucleus upon fusion of two cells.
- The M phase inducer is produced by activating the M phase kinase, which consists of a catalytic subunit and a cyclin regulatory subunit.

The existence of different regulators at different stages of the cell cycle was revealed by early experiments that fused together cells in different stages of the cycle. As illustrated in **Figure 29.6**, cell fusion is performed by mixing the cells in the presence of either a chemical or viral agent that causes their plasma membranes to fuse, generating a hybrid cell (called a **heterokaryon**) that contains two (or more) nuclei in a common cytoplasm.

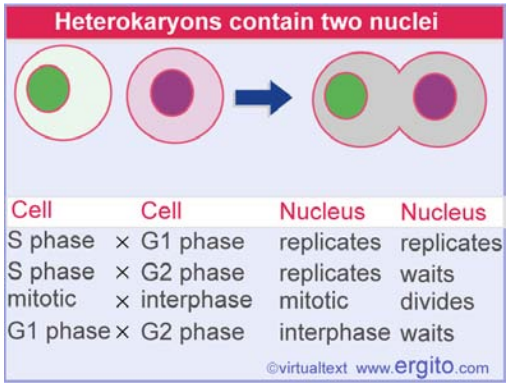

Figure 29.6 Cell fusions generate heterokaryons whose nuclei behave in a manner determined by the states of the cells that were fused.

When a cell in S phase is fused with a cell in G1, both nuclei in the heterokaryon replicate DNA. This suggests that *the cytoplasm of the S phase cell contains an activator of DNA replication.* The *quantity* of the activator may be important, because in fusions involving multiple cells, an increase in the ratio of S phase to G1 phase nuclei increases the rate at which G1 nuclei enter replication. The regulator identified by these fusions is called the **S phase activator**.

When a mitotic cell is fused with a cell at any stage of interphase, it causes the interphase nucleus to enter a pseudo-mitosis, characterized by a premature condensation of its chromosomes. This suggests that *an* M phase inducer *is present in dividing cells*.

Both the S phase and M phase inducers are present only transiently, because fusions between G1 and G2 cells do not induce replication or mitosis in either nucleus of the heterokaryon.

Figure 29.7 relates the cell cycle to the molecular basis for the regulatory cyclical events that control transitions between phases. Some of these events require the synthesis of new proteins or the degradation of existing proteins; other events occur by reversible activation or inactivation of pre-existing components. A minimum of three molecular activities must exist, and the striking thread that connects them is that all of these activities are controlled by phosphorylation:

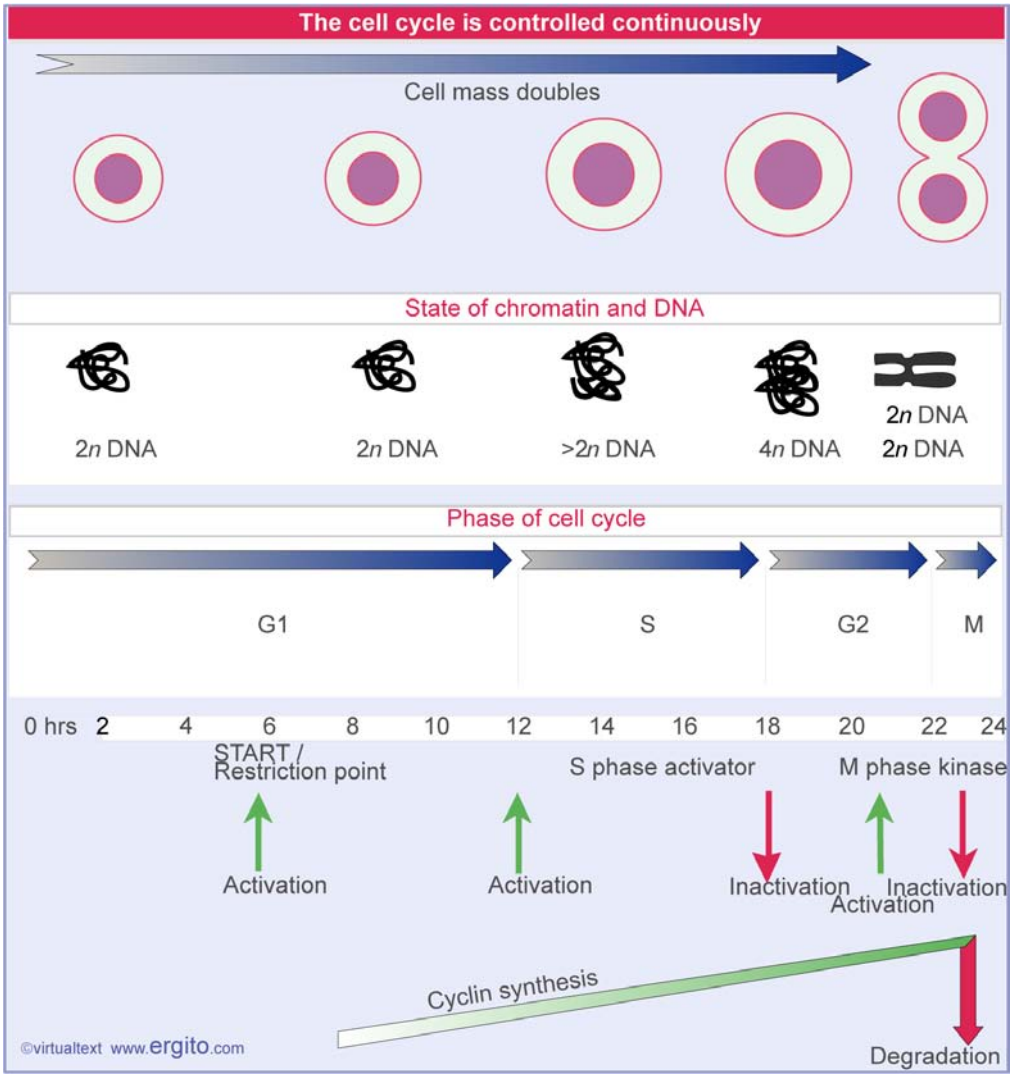

Figure 29.7 The phases of the cell cycle are controlled by discrete events that happen during G1, at S phase, and at mitosis.

- During G1, the cell passes START and becomes committed to a division cycle. They key event is a phosphorylation. The target protein is called RB, and its nonphosphorylated form represses transcription of genes that are needed for the cell cycle to advance. The repression is released when RB is phosphorylated.

- The period of S phase is marked by the presence of the S phase activator. This is a protein kinase. It is related to the kinase that activates mitosis (which was identified first and is better characterized).

- Mitosis depends upon the activation of a pre-existing protein, the **M phase kinase**, which has two subunits. One is a kinase catalytic subunit that is activated by modification at the start of M phase. The other subunit is a **cyclin**, so named because it accumulates by continuous synthesis during interphase, but is destroyed during mitosis. Its destruction is responsible for inactivating M phase kinase and releasing the daughter cells to leave mitosis.

A striking feature of cell cycle regulation is that similar regulatory activities are employed in (probably) all eukaryotic systems. Some of these systems have cycles that superficially appear quite different from the normal somatic cycle. So very rapid divisions in which S phase alternates directly with mitosis characterize the development of the *Xenopus* egg, where entry into mitosis is controlled by M phase kinase, the very same factor that controls somatic mitosis. Yeast cells exist in a unicellular state, and certain species divide by an asymmetrical budding process; but control of entry into S phase and control of mitosis are determined by pathways that are related to those employed in the *Xenopus* egg. Homologous genes play related roles in organisms as distant as yeasts, insects, and mammals.

CELL CYCLE AND GROWTH REGULATION

6.29.5 M phase kinase regulates entry into mitosis

Key Terms

The fertilized eggs of some species are very large and initially undergo several rounds of cell division without any growth of the cells between successive mitoses. As a result each embryo is progressively divided into into smaller and smaller cells. This process is the **cleavage stage** of embryogenesis.

M phase kinase (MPF) was originally called the maturation promoting factor (or M phase-promoting factor). It is a dimeric kinase, containing the p34 catalytic subunit and a cyclin regulatory subunit, whose activation triggers the onset of mitosis.

Key Concepts

- MPF was originally identified as a factor that can induce G2 stage oocytes to enter meiosis.
- The same factor is responsible for inducing somatic cells to enter mitosis.
- Maturation promoting factor and M phase promoting factor are both manifestations of the M phase kinase.

The early development of eggs of the toad *X. laevis* has provided a particularly powerful system to analyze the features that drive a cell into mitosis. **Figure 29.8** summarizes the early divisions of *Xenopus* embryogenesis.

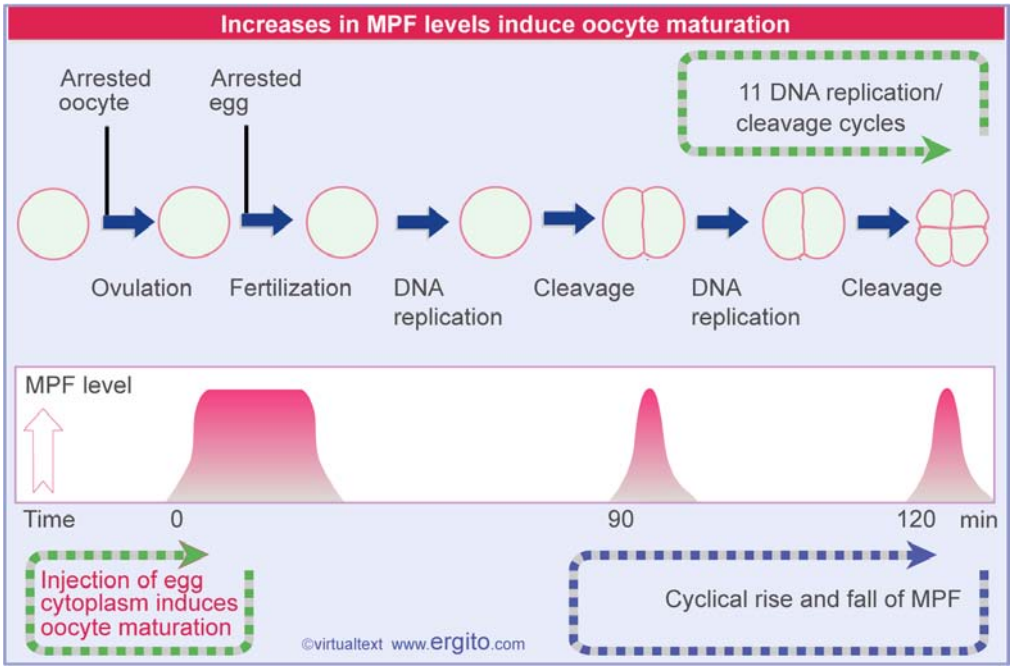

Figure 29.8 MPF induces M phase in early *Xenopus* development.

In its immature form, the *Xenopus* oocyte is arrested in its first meiotic division cycle, just at the initial stage of chromosome condensation. The closest correspondence to a somatic cycle is to the G2 stage. Ovulation occurs when the hormone progesterone releases the arrest, and the egg proceeds into meiosis. When the egg is laid, it is arrested towards the end of the second meiosis in a condition that corresponds to a somatic M phase.

The egg is a large structure (~1 mm. diameter in the case of *X. laevis*), which contains a vast store of material needed for early divisions. Fertilization triggers a series of very rapid division cycles. The initial division takes ~90 minutes; then during the **cleavage stage**, another 11 divisions occur synchronously, each lasting ~30 minutes. These divisions in effect represent an alternation of S phase and mitosis; the major synthetic activity of the cleavage egg is the replication of DNA, all the required proteins having been previously synthesized and stored in the oocyte.

The size of the egg allows material to be purified from it. It is particularly useful that arrested oocytes (equivalent to G2 somatic cells) and arrested eggs (equivalent to M phase somatic cells) can be readily obtained. After eggs have been treated with progesterone, a factor can be obtained from their cytoplasm that, when injected into arrested oocytes, causes them to enter meiosis. (The original work is reviewed in *Great Experiments 8.1 The discovery of MPF*.) The active component of the extract was called *maturation promoting factor* (**MPF**). The same factor can induce mitosis in somatic cells.

Because MPF turns out to have a general responsibility for causing somatic cells to enter M phase, MPF is now understood to stand for *M phase promoting factor*. The importance of MPF in inducing mitosis can be seen from its cyclical increase and decrease at the next stage of development, in the cleaving egg. As a synchronous wave of mitoses occurs in the cleavage egg, the level of MPF activity rises; as the

mitoses are completed and S phase occurs, the MPF activity disappears.

MPF causes germinal vesicle (nuclear envelope) breakdown when injected into *Xenopus* oocytes, and induces several mitotic events in a cell-free system, including nuclear envelope disaggregation, chromosome condensation, and spindle formation. MPF is a kinase that can phosphorylate a variety of protein substrates. This immediately suggests a mode for its action: *by phosphorylating target proteins at a specific point in the cell cycle, MPF controls their ability to function.* In fact, the activity of the enzyme is most often assayed by its ability to phosphorylate target proteins (rather than by the induction of mitosis), and so the name *M phase kinase* provides a better description.

CELL CYCLE AND GROWTH REGULATION

6.29.6 M phase kinase is a dimer of a catalytic subunit and a regulatory cyclin

Key Concepts

- Cdc2 is the catalytic subunit of M phase kinase and phosphorylates target proteins on Ser or Thr.
- The regulatory subunit is cyclin A or cyclin B.
- Activation results from covalent modification of the Cdc2 subunit.
- Inactivation results from degradation of the cyclin subunit.

M phase kinase consists of two types of subunit with different functions:

- Cdc2 is the *catalytic subunit* which phosphorylates serine and threonine residues in target proteins. It is named for the homologous protein in *S. pombe*.
- Its partner is a cyclin; this is a *regulatory subunit* which is necessary for the kinase to function with appropriate substrates.

The crystal structure of a dimer shows that the cyclin induces a change in the conformation of its partner that is necessary to create the active kinase site. So a catalytic subunit by itself is inactive, and can be active only when joined by a cyclin partner.

An M phase kinase typically has a single type of catalytic subunit (Cdc2), but may have any one of several alternative cyclin partners. There are two general types of mitotic cyclins, A and B. They are characterized by the sequence of a stretch of ~150 amino acids (sometimes known as the cyclin box). In mammals and frogs, the B cyclins can be divided into the subtypes B1 and B2.

So there are (at least) two general forms of the M phase kinase: Cdc2-cyclin A and Cdc2-cyclin B. The common properties of the cyclins suggest that they have the same type of function: to influence the activity of the catalytic subunit of M phase kinase. Yet the two classes of cyclins have only weak similarity and follow a different temporal and spatial pattern of behavior. Although they are involved in the timing and localization of M phase kinase activity, we do not know how they function as regulatory subunits in determining the specificity of the catalytic subunit. We should like to know in particular whether Cdc2-cyclin A and Cdc2-cyclin B recognize different proteins as substrates.

The events that activate M phase kinase at G2/M and inactivate it during M phase identify crucial points in the cell cycle. Activation and inactivation are achieved by different types of action:

- *Activation requires modification of the catalytic subunit.* In most cells, the level of Cdc2 remains constant through the cycle, and is in excess compared to the cyclins. Cyclins are necessary to turn on the M phase specific kinase activity of Cdc2. However, they cannot provide the activating event because they accumulate to a maximum level before the kinase activity appears. The intact Cdc2-cyclin dimer accumulates in an inactive form; modification of Cdc2 is the critical event that triggers the G2/M transition (833; 836).

- *Inactivation is achieved by the physical destruction of the cyclin*. Cyclins were originally named for their property of accumulating continuously through the cell cycle; then they are destroyed abruptly by proteolysis during mitosis (see **Figure 29.7**). The timing of cyclin destruction is characteristic; typically A precedes B (by a few minutes in embryonic divisions, by rather longer in a cultured cell cycle), and this difference appears to be common to all cells (825; 830; 832).

What activates the catalytic activity of the M phase kinase? Cdc2 is itself a phosphoprotein, and its state of phosphorylation is a crucial determinant of activity. The events involved in the cycle of activation and inactivation of M phase kinase are summarized in **Figure 29.9**. For M phase kinase to be active, phosphate groups must be absent at some positions, but present at another position (826).

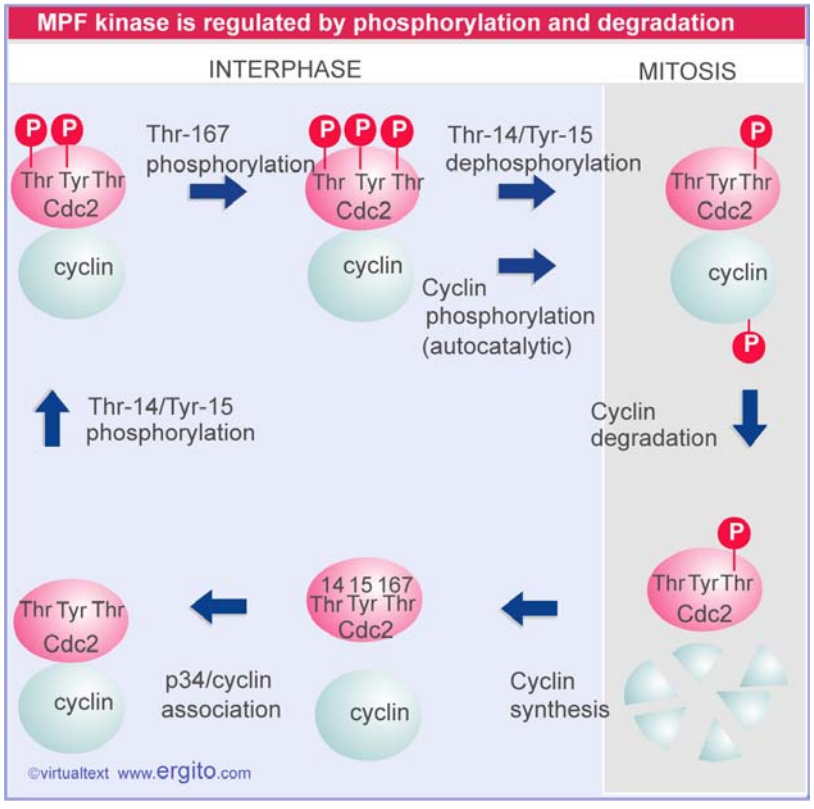

Figure 29.9 The activity of M phase kinase is regulated by phosphorylation, dephosphorylation, and protein proteolysis. The 3 phosphorylated amino acids are: Thr-14, Tyr-15, Thr-161. The first two are in the ATP-binding site.

Two residues located within the ATP-binding site of Cdc2 must be *dephosphorylated* in order to activate the kinase (in mouse cells). The phosphates are located on Thr-14 and Tyr-15; both are removed by the same (dual specificity) phosphatase. M phase kinase is autocatalytic, that is, the activation of a small amount of the kinase is sufficient to trigger activation of the rest. This could be explained if M phase kinase itself activates the phosphatase.

Another phosphorylation occurs on Thr-161 of Cdc2. This phosphate group is added in G2 and removed at the end of mitosis. The phosphate is *required* for activity of Cdc2; mutations introducing an amino acid that cannot be phosphorylated at this site inactivate the kinase activity.

Association of the catalytic and cyclin subunits, and phosphorylation and dephosphorylation, occur in a specific order. Cyclin B associates specifically with the tyrosine-*dephosphorylated* form of Cdc2 *in vitro*. This generates a potentially active dimer. However, formation of the dimer causes Thr-14/Tyr-15 to be phosphorylated. So *association with cyclin induces the inactivating event*. The dimer is then maintained in its inactive form until the phosphates are removed.

Cyclins A and B both have a short motif near the N-terminus – the cyclin destruction box – which is required to make the cyclin a target for proteolysis. Cyclins are degraded by a common proteolytic system, the proteasome (a complex containing proteolytic activities that recognizes its targets when ubiquitin is added to them; see *Molecular Biology Supplement 8.32 The proteasome is a large machine that degrades ubiquitinated proteins*).

Destruction of the cyclin subunits is responsible for inactivating M phase kinase during mitosis, and is necessary for cells to exit mitosis. A truncated cyclin B that lacks the N-terminal region is resistant to proteolysis. When this protein is synthesized in *Xenopus* eggs, or in a cell-free extract that undertakes some of the typical cycling reactions, it causes anaphase arrest. This is the basis for concluding that *loss of kinase activity is a prerequisite for completing mitosis*.

An important question is how the roles of cyclin A and cyclin B differ in mitosis. A hint that their functions are different is provided by differences in the timing of their synthesis and destruction, but there is as yet no direct evidence to show whether both cyclins are required for passage through mitosis in an animal cell or whether they are redundant with one another.

References

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CELL CYCLE AND GROWTH REGULATION

6.29.7 Protein phosphorylation and dephosphorylation control the cell cycle

Key Concepts

- M phase kinase triggers mitosis by phosphorylating a wide variety of substrates.
- Mitosis is terminated by dephosphorylating the substrates.
- One of the best substrates for M phase kinase, which is often used to assay its activity, is histone H1.

Phosphorylation (catalyzed by kinases) and dephosphorylation (catalyzed by phosphatases) are the critical events that regulate the cell cycle. *They are used both to control the activities of the regulatory circuit itself and to control the activities of the substrates that execute the decisions of the regulatory circuit.*

The cell cycle regulatory circuit consists of a series of kinases and phosphatases that respond to external signals and checkpoints by phosphorylating or dephosphorylating the next member of the pathway. The ultimate readout of the circuit is to determine the activity of M phase kinase (or the S phase kinase) by controlling its state of phosphorylation.

Activation of M phase kinase is the event that triggers onset of M phase. Inactivation is necessary to exit M phase. This suggests that the events regulated by M phase kinase are reversible: *phosphorylation of substrates is required for the reorganization of the cell into a mitotic spindle, and dephosphorylation of the same substrates is required to return to an interphase organization.*

What are the targets for M phase kinase? A major reorganization of the cell occurs at mitosis, and the ability of MPF to induce mitosis implies that the M phase kinase triggers these activities. Does M phase kinase acts directly or indirectly upon the various potential substrates? Two general models could be proposed for its role:

- It may be a "master regulator" that phosphorylates target proteins that in turn act to regulate other necessary functions – a classic cascade.
- Or it may directly phosphorylate the proteins that are needed to execute the regulatory events or cell reorganization involved in the cycle.

The only common feature in substrates that are phosphorylated by M phase kinase is the presence of the duo Ser-Pro, flanked by basic residues (most often in the form Ser-Pro-X-Lys). Potential substrates, based upon the ability of M phase kinase preparations to phosphorylate targets *in vitro*, include H1 histone (perhaps required to condense chromosomes), lamins (possibly required for nuclear envelope breakdown), nucleolin (potentially involved in the arrest of ribosome synthesis), and

other structural and enzymatic activities. The strength of the evidence varies as to which of these targets is phosphorylated *in vivo* in a cyclic manner and whether M phase kinase is in fact the active enzyme. From the variety of substrates, however, it seems likely that M phase kinase acts directly upon many of the proteins that are directly involved in the change in cell structure at mitosis.

How can we determine whether a potential substrate is an authentic target for Cdc2 in the cell cycle? The same sites should be phosphorylated by Cdc2 *in vitro* that are cyclically phosphorylated *in vivo* at time(s) when Cdc2 is known to be active. Ideally it should be possible to show that a mutation in Cdc2 kinase activity blocks the phosphorylation *in vivo*, but this is at present practical only in yeast. To conclude that the phosphorylation is a significant event in the cycle, some function of the protein must be altered by the presence of phosphate. This can be tested by making mutations at the amino acid that is phosphorylated to determine whether the absence of phosphorylation blocks a mitotic function.

The best characterized substrate for Cdc2 kinase is the H1 histone (one of the 5 histones that are the major protein constituents of chromatin; see *Molecular Biology 5.20 Nucleosomes*). It has been known for a long time that H1 is phosphorylated during the cell cycle, with 2 phosphate groups added during S phase, and 4 further phosphate groups added during mitosis. The major H1 kinase activity of the cell is provided by M phase kinase (827; 831).

What purpose the phosphorylation of H1 serves in the cell cycle remains a matter for speculation, since no effects upon chromatin structure have been directly demonstrated. It is reasonable to suppose that it might be connected with chromosome condensation at M phase. Not enough is known about the timing of modification at S phase to wonder whether it is concerned with preparations for replication (which might require uncoiling) or with the consequences of replication (when preparations for mitosis could begin). But H1 histone is an exceedingly good substrate for kinases based on the Cdc2 engine, with the result that H1 kinase activity has become the usual means by which this enzyme is assayed *in vitro.* An illustration of the appeal of this assay is its application to *S. cerevisiae*, where H1 kinase activity is routinely measured to assess the cyclic activity of M phase kinase, although this yeast in fact is unusual in containing no H1 histone!

References

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831. Labbe, J. C., Picard, A., Peaucellier, G., Cavadore, J. C., Nurse, P., and Doree, M. (1989). *Purification of MPF from starfish: identification as the H1 histone kinase p34^{cdc2} and a possible mechanism for its periodic activation*. Cell 57, 253-263.

CELL CYCLE AND GROWTH REGULATION

6.29.8 Many cell cycle mutants have been found by screens in yeast

Key Terms

Some species of yeast, the most well known of which is *Saccharomyces cerevisiae*, reproduce by forming a **bud.** The bud is formed off the side of the mother cell and gradually enlarges over the course of the cell cycle. Its interior is initially continuous with the cytoplasm of the mother cell, but after a copy of the genome is segregated into the bud during mitosis a wall is constructed between the two and the bud breaks free to become a separate cell.

The **cell division cycle** is the entire sequence of events required to reliably replicate the cell's genetic material and separate the two copies into new cells. The term "cell division cycle" has been largely replaced by the term "cell cycle".

cdc is an abbreviation for "cell division cycle". It is most frequently used as part of the names of a large collection of yeast mutants isolated in the 1970s in which the cell cycle arrested at a specific point in each type of mutant.

Key Concepts

- Blocking the cell cycle is likely to kill a cell, so mutations in the cell cycle must be obtained as conditional lethals.
- Screens for conditional lethal cell cycle mutations identify ~80 genes in either *S. cerevisiae* or *S. pombe*.

To define the circuit for cell cycle control, we need to identify the genes that regulate progression through the cycle. We define a mutation in the cell cycle as one that *blocks the cell cycle at a specific stage*, a definition that excludes mutations in genes that control continuous processes of growth and metabolism. But the approach of characterizing mutants that are unable to proceed through the cycle has not been straightforward to develop. A mutation that prevents a cell from dividing will be lethal; and the reverse is also true, insofar as any lethal mutation will stop cells from dividing. It has therefore been difficult to devise procedures to isolate cell cycle mutants of animal cells, or, indeed, to demonstrate that potential mutants have specific blocks in the cell cycle.

Because of their nature, cell cycle mutants must be obtained as conditional lethals, so that although they are unable to grow under the conditions of isolation, they can be maintained by growth in other conditions. A series of such mutants has been isolated in two yeasts, in which the block to the cell cycle can be seen to affect the visible phenotype of the cell.

The mitotic cycles of fission yeast (*S. pombe*) and baker's yeast (*S. cerevisiae*) are summarized in **Figure 29.10**.

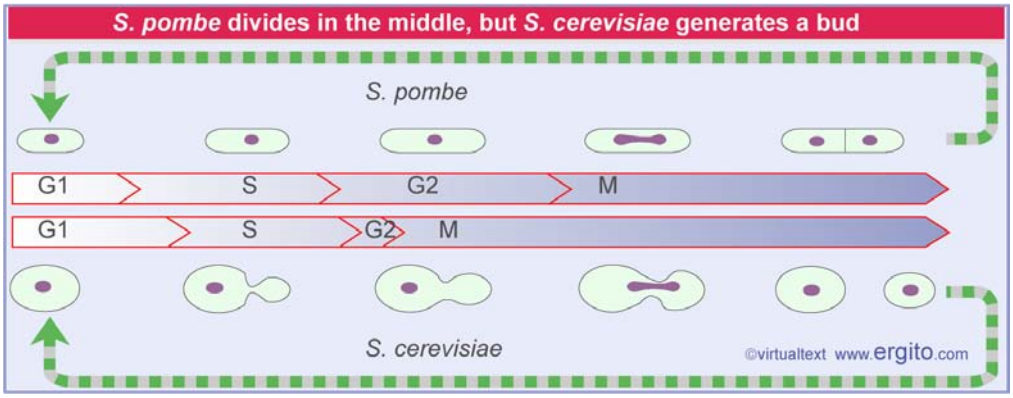

Figure 29.10 *S. pombe* lengthens and then divides during a conventional cell cycle, while *S. cerevisiae* buds during a cycle in which G2 is absent or very brief, and M occupies the greatest part.

The cell cycle of *S. pombe* is divided into the usual phases (G1, S, G2, M). The cell grows longitudinally and then divides; progress through the cell cycle can be assessed (approximately) by the physical length of the cell (which doubles from 7-8 µm to 14-16 µm).

The cycle of *S. cerevisiae* is unusual. Cells proceed almost directly from S phase into division, so there is effectively very little or no G2 phase. (A short G2 phase is shown in this and subsequent figures for the purpose of localizing the relative timing of events concerned with the transition into M phase.) And instead of an equal division of the cell, the daughter cell grows as a **bud** off the mother cell, eventually obtaining its independence when it is released as a small separate cell. Again the cell cycle can be followed visually in terms of the growth of the bud. Mitosis itself shares some unusual features in both types of yeast; the nuclear membrane does not break down, and segregation of chromosomes therefore is compelled to occur within the nucleus.

Extensive screens for **cell division cycle** or **cdc** mutants have been performed in both yeasts. Initial isolation relies upon the criterion that cells accumulate at a particular stage of the cell cycle at an elevated temperature (36°C), but continue normally through the cycle at 23°C. The mutant phenotype allows cells to continue growing larger while the cycle is blocked, causing an obvious aberration; in *S. pombe* the cells become highly elongated, and in *S. cerevisiae* they fail to bud. **Figure 29.11** compares cell cycle mutants with wild-type *S. pombe*. The left panel shows a group of normal cells; the center panel shows a *cdc* mutant that is blocked in the ability to divide, and has therefore become elongated, because it has continued to grow (824).

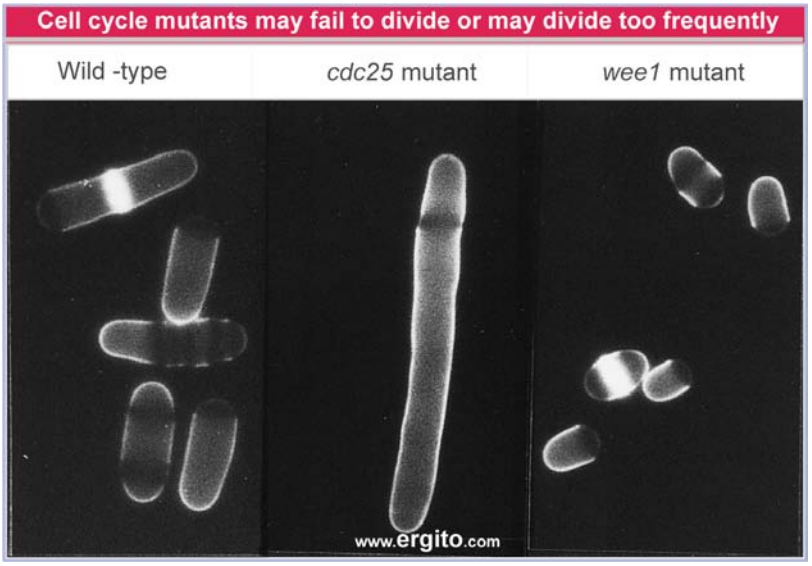

Figure 29.11 *S. pombe* cells are stained with calcofluor to identify the cell wall (surrounding the yeast cell) and the septum (which forms a central division when a cell is dividing). Wild-type cells double in length and then divide in half, but *cdc* mutants grow much longer, and *wee* mutants divide at a much smaller size. Photograph kindly provided by Paul Nurse.

Because the mutants are temperature sensitive, the time at which a mutation takes effect can be determined by temperature shift protocols in which cells are shifted up in temperature at a specific point in the cycle. If this point is prior to the point at which the gene product acts, the cells halt at the execution point, but if the point is past the time when the protein function is needed, the cells continue their cycle.

Of the order of 80 *cdc* genes have been identified in each species, but not all of these loci are concerned with regulating the cell cycle. Many represent genes whose products are needed for metabolic purposes; for example, absence of enzymes that replicate DNA or synthesize the nucleotide precursors can block progression through S phase.

References

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CELL CYCLE AND GROWTH REGULATION

6.29.9 Cdc2 is the key regulator in yeasts

Key Terms

The **mating type** is a property of haploid yeast that makes it able to fuse to form a diploid only with a cell of the opposite mating type.

Key Concepts

- *cdc* mutants of *S. pombe* fall into two groups that block the cell cycle either at G2/M or at START in G1.
- Different *cdc2* mutant alleles may block the cycle at either of these stages.
- *CDC28* is the homologous gene in *S. cerevisiae*.
- Homologues of *cdc2* are found in all eukaryotic organisms.
- The active form of M phase kinase is phosphorylated on Thr-161; the inactive form is phosphorylated on Tyr-15 (and in animal cells also on Thr-14).

Yeast cells may exist in either haploid or diploid form. They have two forms of life cycle, as illustrated in **Figure 29.12**. Haploid cells double by mitosis. A haploid cell has a **mating type** of either *a* or α. Haploids of opposite types enter the sexual mating pathway, in which they conjugate (fuse) to form diploid cells. The diploid cells in turn sporulate to form haploid cells by meiosis (see *Molecular Biology 4.18.2 The mating pathway is triggered by pheromone-receptor interactions*).

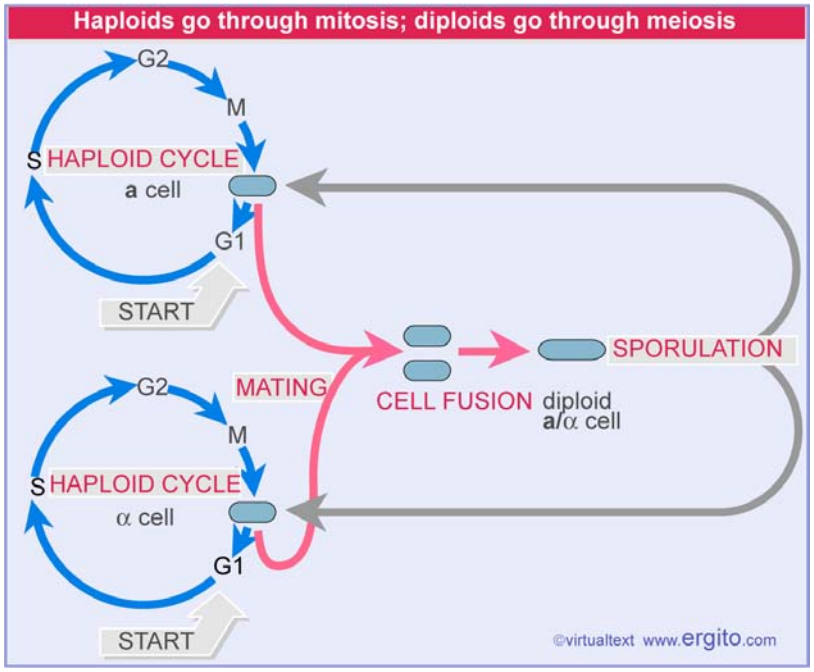

Figure 29.12 Haploid yeast cells of either *a* or α mating type may reproduce by a mitotic cycle. Cells of opposite type may mate to form an a/ α diploid. The diploid may sporulate to generate haploid spores of both *a* and α types.

A crucial point in the cell cycle is defined by the behavior of haploid cells. A haploid cell decides at a point early in G1 whether to proceed through a division cycle or to mate. The decision is influenced by environmental factors; for example, cells of opposite mating type must be present for conjugation to occur. In fact, a mating factor (a polypeptide hormone) secreted by a cell of one type causes a cell of the other type to arrest its cycle (see *Molecular Biology 4.18.2 The mating pathway is triggered by pheromone-receptor interactions*). But a mating factor can divert a cell into the mating pathway *only early in G1;* later in G1 the cell becomes committed to the division cycle and cannot be stimulated to enter the mating pathway. This assay was how the commitment point in G1 (START) was originally identified.

The events and genes involved in the cycle of *S. pombe* are summarized in **Figure 29.13**. Various *cdc* mutants of *S. pombe* block the cell cycle at one of two stages: at the boundary between G2 and M phase; or in G1 at START.

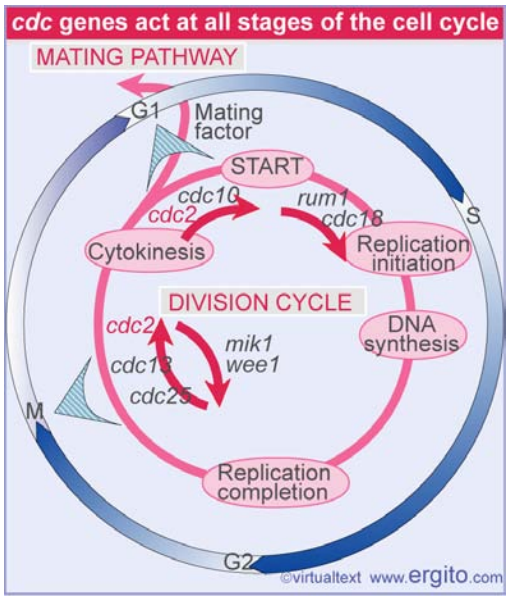

Figure 29.13 The cell cycle in *S. pombe* requires *cdc* genes to pass specific stages, but may be retarded by genes that respond to cell size (*wee1*). Cells may be diverted into the mating pathway early in G1.

cdc2 is identified as a crucial regulator by its involvement at *both* stages of cell cycle block: mutants of *cdc2* may be blocked prior to START or prior to M phase (depending on the point a cell had reached in the cycle when the mutation took effect).

The homologous gene in *S. cerevisiae* is called *CDC28*. A deficiency in the gene of either type of yeast can be corrected by the homologue from the other yeast. In both yeasts, proteins that resemble B-type cyclins associate with Cdc2 or CDC28 at G2/M.

The crucial breakthrough in understanding the nature and function of M phase kinase was the observation that *Xenopus Cdc2* is the homologue of *cdc2* of *S. pombe* and *CDC28* of *S. cerevisiae*. [When this discovery was made, the *Xenopus* protein was called p34, after its size, but then it was renamed Cdc2 after its name in *S. pombe* (828; 829).]

The activity of the Cdc2 catalytic subunit in these dimers (and of the equivalent CDC28 in *S. cerevisiae*) is controlled by phosphorylation in the same way as Cdc2 in animal cells. A difference is that in yeast there is no Thr-14, so there are only two relevant sites: Tyr-15 where phosphorylation is inhibitory; and Thr-161 where phosphorylation is required.

The existence of a Cdc2 catalytic subunit, in organisms as diverse in evolution as yeasts, frogs, and mammals, identifies the key feature of cell cycle control. Conservation of function is indicated by the ability of the cloned human gene to complement the deficiency in *cdc2* mutants of *S. pombe*. This was the crucial experiment that identified Cdc2 as the universal regulator (see *Great Experiments 8.2 The discovery of cdc2 as the key regulator of the cell cycle*).

(Ability to compensate for the deficiency of a specific yeast mutant has been used with great effect to identify higher eukaryotic genes homologous to several cell cycle regulators of yeast. The assay introduces a cloned animal gene into a yeast mutant and then identifies cells that resume growth. It is quite remarkable that the control of the cell cycle has been so well conserved as to make this possible. However, it should be remembered that this assay does not impose very rigorous demands, and sometimes leads to the identification of a gene that is only loosely related to the mutant function.)

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CELL CYCLE AND GROWTH REGULATION

6.29.10 Cdc2 is the only catalytic subunit of the cell cycle activators in *S. pombe*

Key Terms

G1 is the period of the eukaryotic cell cycle between the last mitosis and the start of DNA replication.

Cyclins are proteins that accumulate continuously throughout the cell cycle and are then destroyed by proteolysis during mitosis. A cyclin is one of the two subunits of the M-phase kinase.

A **mitotic cyclin (G2 cyclin)** is a regulatory subunit that partners a kinase subunit to form the M phase inducer.

Key Concepts

- During mitosis Cdc2 exists in a dimer with one of the mitotic cyclins (A or B).
- During G1 Cdc2 forms a dimer with a G1 cyclin.
- G1 cyclins are distantly related to mitotic cyclins but are not regulated by degradation.
- The Cdc2-G1 cyclin dimer must be activated in order to enter S phase.

One of the key concepts of cell cycle regulation is that each stage is controlled by a dimer consisting of a Cdc2-like catalytic subunit and a cyclin-like regulatory subunit. Either or both of these subunits may be changed between the regulatory stages, and in some cases there may in fact be several alternative versions of the dimer at one stage.

In yeast, there is only one type of catalytic subunit, and the differences between regulatory stages are determined by its partner. Thus Cdc2 in *S. pombe* has different partners at mitosis and during G1. At mitosis, its partner is the product of *cdc13*, generating an M phase kinase that resembles the Cdc2-B cyclin dimer of animal cells. During G1, the active form of Cdc2 is associated with a B-like cyclin, cig2 (there is also a related cyclin, cig1).

Figure 29.14 summarizes changes in phosphorylation of the alternative dimers during the cell cycle.

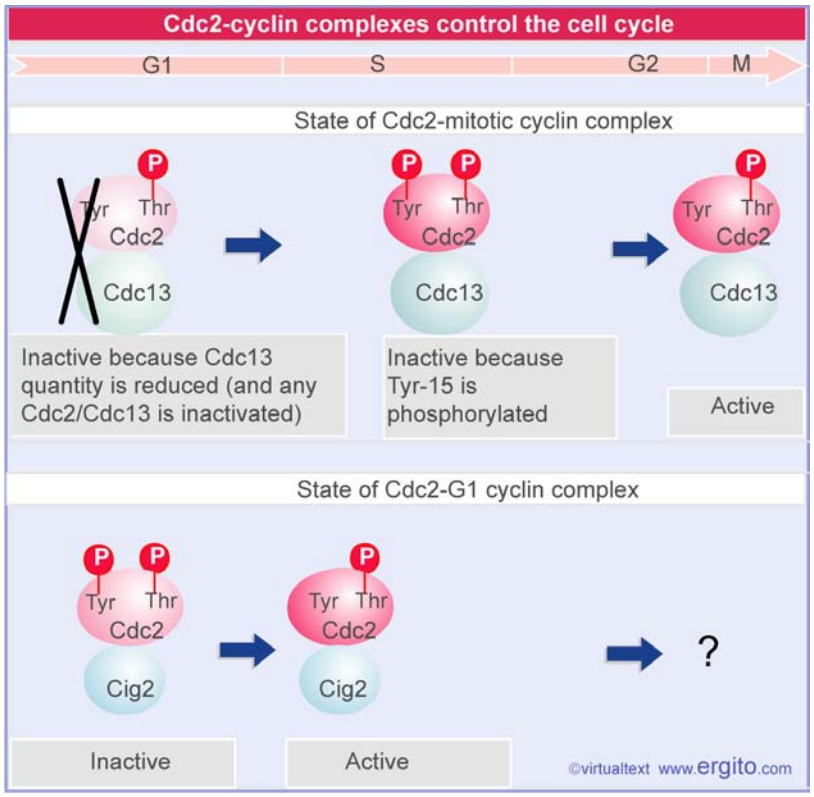

Figure 29.14 The state of the cell cycle in *S. pombe* is defined by the forms of the Cdc2-cyclin complexes.

The upper part of the figure summarizes the condition of the M phase kinase. At mitosis, the Cdc2 subunit of the Cdc2/Cdc13 dimer is in the active state that lacks the phosphate at Tyr-15 and has the phosphate at Thr-161. At the end of mitosis, kinase activity is lost when Cdc13 is degraded. The state of phosphorylation of Cdc2 does not change at this point. As new Cdc13 is synthesized, it associates with Cdc2, but the dimeric complex is maintained in an inactive state by another protein (see **Figure 29.19**). After START, however, activity of the Cdc2/Cdc13 dimer is inhibited by addition of the phosphate to Tyr-15. Removal of the inhibitory phosphate is the trigger that activates mitosis.

The lower part of the figure shows that Cdc2 forms a dimer with cig2 during G1. This forms a kinase with the same general structure as the M phase kinase. This kinase is in effect a counterpart to the M phase kinase, and it controls progression through the earlier part of the cell cycle. cig2 resembles a B-type cyclin. The dimer is converted from the inactive state to the active state by dephosphorylation of the Tyr-15 residue of Cdc2 at or after START. We do not know what happens to the Cdc2-cig2 dimer at the transition from S phase into G2.

The existence of alternative dimers during the cell cycle suggests the concept that *cyclins can be classified according to their period of active partnership with cdc2* as **G1cyclins** or **mitotic cyclins** (also known as **G2 cyclins**). (Note that the cyclins defined in this way by their partnership with Cdc2 or a Cdc2-like protein do not necessarily have the property of cyclic degradation by which the original cyclins were defined. Cyclic degradation is a general property specifically for mitotic cyclins

in animal cells.)

We may take the concept of cyclin classification further and suggest that *the phase of the cell cycle is defined by the nature of the active cdc2-cyclin dimer*. The period of G1 and S phase is defined by the presence of an active Cdc2-G1 cyclin dimer. Progression through G1 into S is controlled by activation of the Cdc2-G1 cyclin. The period of G2 and mitosis is characterized by an active Cdc2-mitotic cyclin dimer. The transition from G2 into mitosis is controlled by activation of the Cdc2-mitotic cyclin.

The original model for the employment of Cdc2 proposed that the available form of the Cdc2-cyclin dimer was regulated simply by replacement of one cyclin with another. However, it seems now that the mitotic and G1 forms may coexist in the yeast cell, but that their activities are differently regulated at each stage of the cycle.

CELL CYCLE AND GROWTH REGULATION

6.29.11 *CDC28* acts at both START and mitosis in *S. cerevisiae*

Key Terms

Functionally redundant genes fulfill the same function in the same time and place, so that mutation of every member of the set is necessary to show a deficient phenotype.

Key Concepts

- CDC28 forms dimers with each of 4 B-type cyclins at mitosis.
- It forms dimers with each of 3 G1 cyclins, any one of which is sufficient to activate START.

The analysis of *cdc* mutants in *S. cerevisiae* shows that more than one type of cycle is required to proceed from one division to the next, although the cycles are connected at crucial points. (Remember that mitosis in *S. cerevisiae* is unusual morphologically, and chromosome segregation occurs within the intact nucleus; see **Figure 29.10**.) **Figure 29.15** shows how the three cycles of *S. cerevisiae* relate to the conventional phases of the overall cell cycle:

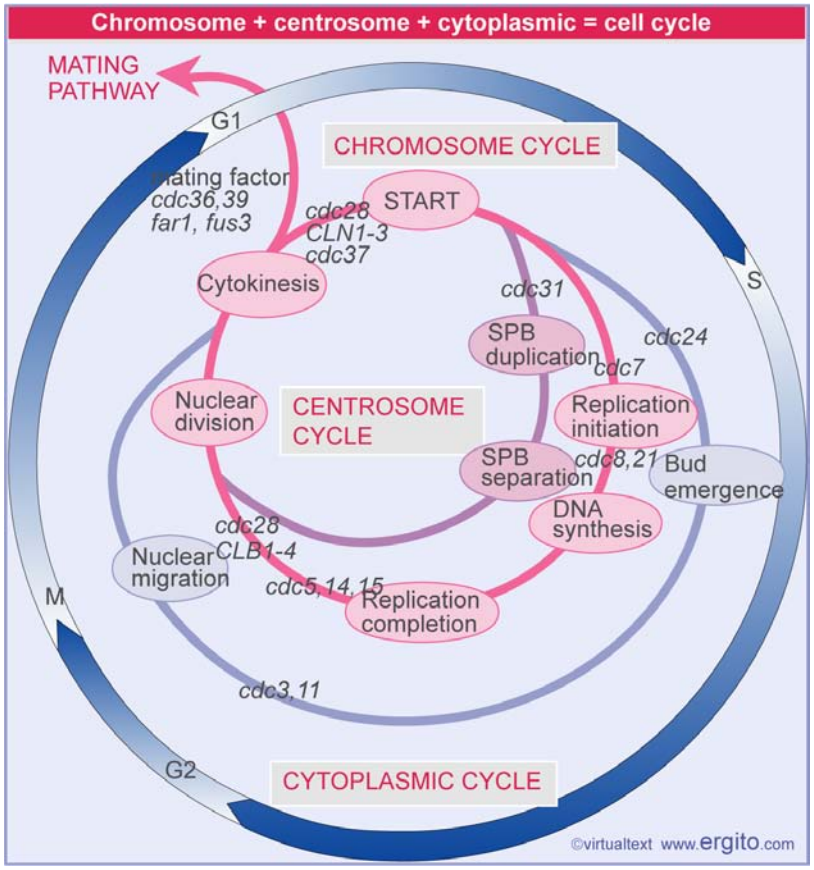

Figure 29.15 The cell cycle in *S. cerevisiae* consists of three cycles that separate after START and join before cytokinesis. Cells may be diverted into the mating pathway early in G1.

- The *chromosome cycle* comprises the events required to duplicate and separate the chromosomes, consisting of the initiation, continuation, and completion of S phase, and nuclear division. A mutation such as *cdc8* stops this cycle in S phase.
- Mutations in the chromosome cycle do not stop the *cytoplasmic cycle*, which consists of bud emergence and nuclear migration into the bud (visualized at the start of M phase in **Figure 29.10**). This cycle can be halted before bud emergence by *cdc24*, but the mutation does not prevent chromosome replication.
- The *centrosome cycle* consists of the events associated with the duplication and then separation of the spindle pole body (SPB), which in effect substitutes for the centrosome and organizes microtubules to allow chromosome segregation within the nucleus. Blocking this cycle, for example with *cdc31*, does not prevent S phase or bud emergence.

Completion of an entire cell cycle requires all three constituent cycles to be functional, since nuclear division requires both the chromosome and centrosome cycles, and cytokinesis requires these and the cytoplasmic cycle.

The decision on whether to initiate a division cycle is made before the point START.

The crucial gene in passing START is *CDC28* (the homologue of *cdc2* of *S. pombe*). Mutations in *CDC28* prevent all three cycles from proceeding. The ability to pass START is determined by environmental conditions, since stationary phase populations that are limited by nutrients are arrested at START. Morphologically, the cells of such a population are arrested at point after cell separation and prior to SPB duplication, bud emergence, and DNA replication, which is to say that when *S. cerevisiae* exhaust their nutrients, they complete the current cycle and arrest all three cycles before START. In terms of phases of the cell cycle, this corresponds to a point early in G1.

Most mutants in *CDC28* are blocked at START, which has therefore been characterized as the major point for *CDC28* action. This contrasts with the better characterized function of M phase kinase at M phase. However, one mutant in *CDC28* passes START normally, but is inhibited in mitosis, suggesting that CDC28 is required to act at both points in the cycle. In fact, the function of *CDC28* is required at three stages: classically before START; then during S phase; and (of course) prior to M phase.

There is a difference in emphasis concerning control of the cell cycle in fission yeast and bakers' yeast, since in *S. pombe* we know most about control of mitosis, and in *S. cerevisiae* we know most about control of START. However, in both yeasts, the same principle applies that a single catalytic subunit (Cdc2/CDC28) is required for the G2/M and the G1/S transitions. It has different regulatory partners at each transition, using B-like (mitotic) cyclins at mitosis and G1 cyclins at the start of the cycle. A difference between these yeasts is found in the number of cyclin partners that are available at each stage (for review see 323).

A single regulatory partner for Cdc2 is used at mitosis in *S. pombe*, the product of *cdc13*. In *S. cerevisiae*, there are multiple alternative partners. These are coded by the *CLB1-4* genes, which code for products that resemble B cyclins and associate with CDC28 at mitosis. Sequence relationships place the genes into pairs, *CLB1-2* and *CLB3-4*. Mutation in any one of these genes fails to block division, but loss of the *CLB1-2* pair of genes is lethal. Constitutive expression of *CLB1* prevents cells from exiting mitosis.

The state of CDC28 in the *S. cerevisiae* cell cycle is summarized in **Figure 29.16**. The G1 cyclins were not immediately revealed by mutations that block the cell cycle in G1. The absence of such mutants has been explained by the discovery that three independent genes, *CLN1, CLN2, CLN3 all must be inactivated* to block passage through START in *S. cerevisiae*. Mutations in any one or even any two of these genes fail to block the cell cycle; thus the *CLN* genes are **functionally redundant**. The *CLN* genes show a weak relationship to cyclins (resembling neither the A nor B class particularly well), although they are usually described as G1 cyclins.

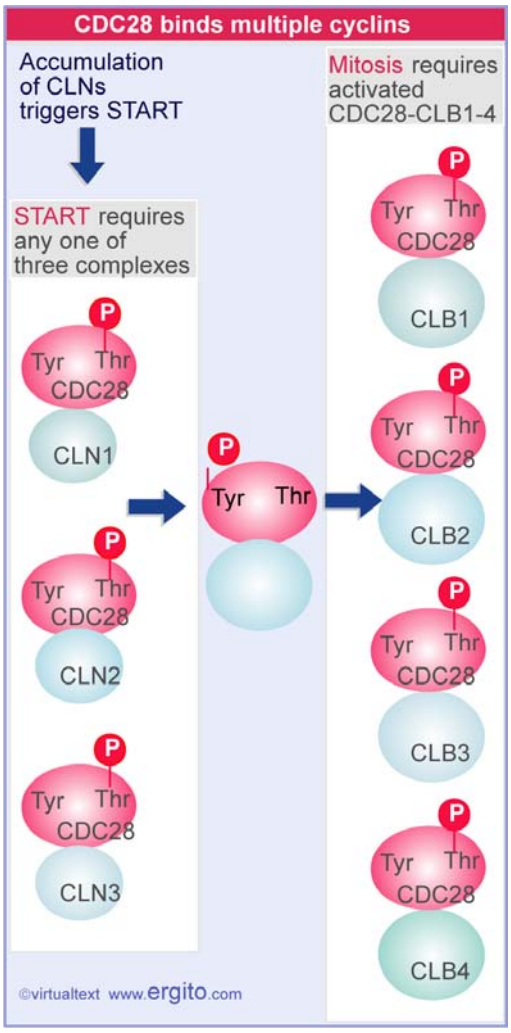

Figure 29.16 Cell cycle control in *S. cerevisiae* involves association of CDC28 with redundant cyclins at both START and G2/M.

Accumulation of the CLN proteins is the rate-limiting step for controlling the G1/S transition. Blockage of protein synthesis arrests the cycle by preventing the proteins from accumulating. The half-life of CLN2 protein is ~15 minutes; its accumulation to exceed a critical threshold level could be the event that triggers passage. This instability presents a different type of control from that shown by the abrupt destruction of the cyclin A and B types. Dominant mutations that truncate the protein by removing the C-terminal stretch (which contains sequences that target the protein for degradation) stabilize the protein, and as a result G1 phase is basically absent, with cells proceeding directly from M phase into S phase. Similar behavior is shown by the product of *CLN3*.

The redundancy of the *CLN* genes and the *CLB* genes is a feature found at several other stages of the cell cycle. In each case, the hallmark is that deletion of an individual gene produces a cell cycle mutant, but deletion of both or all members of the group is lethal. Members of a group may play overlapping rather than identical functions. This form of organization has the practical consequence of making it

difficult to identify mutants in the corresponding function.

Reviews

323. Forsburg, S. L. and Nurse, P. (1991). *Cell cycle regulation in the yeasts S. cerevisiae and S pombe.* Annu. Rev. Cell Biol. 7, 227-256.

CELL CYCLE AND GROWTH REGULATION

6.29.12 Cdc2 activity is controlled by kinases and phosphatases

Key Concepts

- Cdc25 is a phosphatase that removes the inhibitory phosphate from Tyr-15 of Cdc2 in order to activate the M phase kinase.
- Wee1 is a kinase that antagonizes Cdc25 by phosphorylating Tyr-15 when the cell size is small.

We can divide *cdc* genes into two classes, defined by the stage of the cell cycle at which the effects of a mutation are manifested. We know most about the circuit that controls the state of the Cdc2/Cdc13 dimer at mitosis.

The states of Cdc2 complexes and the enzymes that act upon them during the *S. pombe* cell cycle are summarized in **Figure 29.17**. The Cdc2-cig2 dimer is phosphorylated during G1. By G2, the predominant form is the Cdc2/Cdc13 dimer whose activity is controlled by antagonism between kinases and phosphatases that respond to environmental signals or to checkpoints.

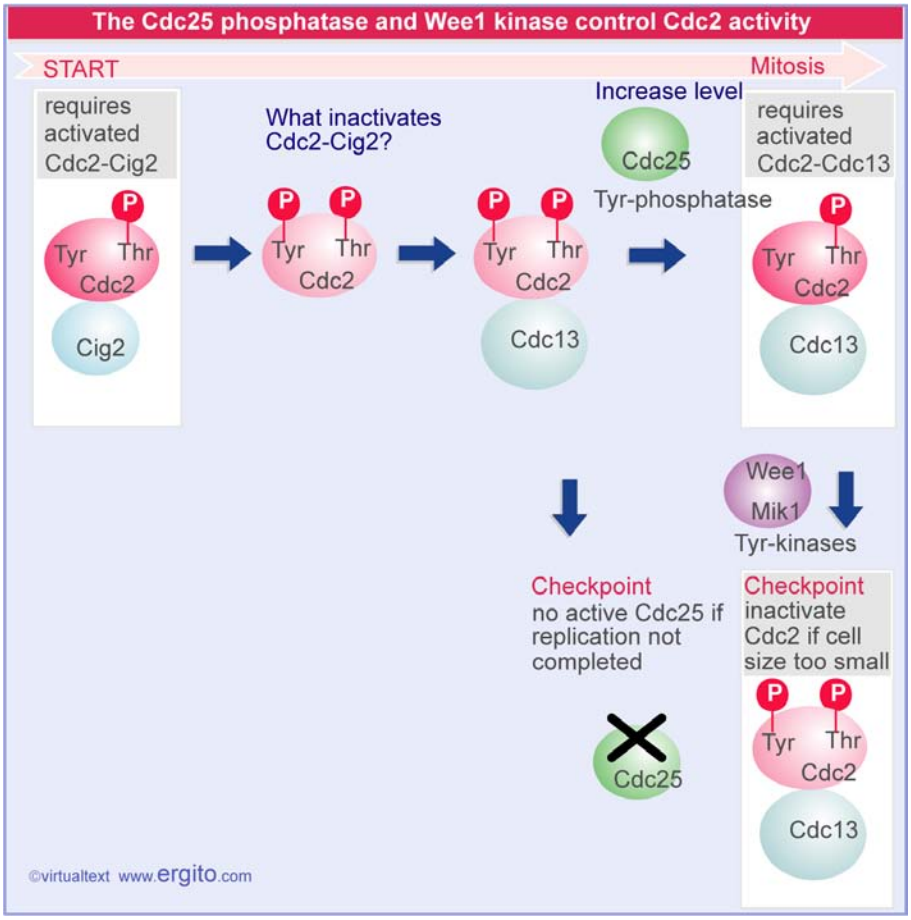

Figure 29.17 Cell cycle control in *S. pombe* involves successive phosphorylations and dephosphorylations of Cdc2. Checkpoints operate by influencing the state of the Tyr and Thr residues.

cdc25 codes for a tyrosine phosphatase that is required to dephosphorylate Cdc2 in the Cdc2/Cdc13 dimer. It is responsible for the key dephosphorylating event in activating the M phase kinase. It is not a very powerful phosphatase (its sequence is atypical), and the quantities of Cdc25 and Cdc2 proteins are comparable, so the reaction appears almost to be stoichiometric rather than catalytic.

The level of Cdc25 increases at mitosis, and its accumulation over a threshold level could be important. Cdc25 executes the checkpoint that ensures S phase is completed before M phase can be activated (837). In mutants of *cdc2* that do not require *cdc25*, or in strains that over-express *cdc25*, blocking DNA replication does not impede mitosis. (Wild-type cells arrest in the cell cycle if DNA synthesis is prevented, for example, by treatment with hydroxyurea. But these mutant cells attempt to divide in spite of the deficiency in replication, with lethal consequences.)

Under normal conditions, the cell division cycle is related to the size of the cell. In poor growth conditions, when the cells increase in size more slowly, G1 becomes longer, because START does not occur until the cells attain a critical size. This is a protection against starting a cell division cycle and risking division before the amount of material is adequate to support two daughter cells. *cdc* mutants typically

delay the onset of mitosis and lead either to cell cycle arrest or to division at increased size (as shown for *cdc25* in **Figure 29.11**).

Genes involved in cell size control are identified by mutations with the opposite property: they advance cells into mitosis and therefore divide at reduced size. The *wee1* gene takes its name from this phenotype (see the right panel of **Figure 29.11**). This behavior suggests that *wee1* usually inhibits cells from initiating mitosis until their size is adequate. This identifies a checkpoint that prevents the activation of Cdc2 until an adequate mass has been attained. *wee1* codes for a "dual specificity" kinase: it can phosphorylate serine/threonine and tyrosine. It inhibits Cdc2 by phosphorylating Tyr-15. Another gene, *mik1*, has similar effects. The deletion of both *wee1* and *mik1* is lethal, suggesting that either gene can fulfill the same function. Redundancy of this sort is a common theme in the yeast cell cycle.

The products of *wee1* and *cdc25* play antagonistic roles, as shown in **Figure 29.17**. The kinase activity of *wee1* acts on Tyr-15 to inhibit Cdc2 function. The phosphatase activity of *cdc25* acts on the same site to activate Cdc2. Mutants that over-express *cdc25* have the same phenotype as mutants that lack *wee1*. Regulation of Cdc2 activity is therefore important for determining *when* the yeast cell is ready to commit itself to a division cycle; inhibition by wee1 and activation by Cdc25 allow the cell to respond to environmental or other cues that control these regulators.

It is striking that all of the genes known to affect the G2/M boundary appear to have been widely conserved in evolution. Extending beyond the conservation of the components of the M phase kinase, *cdc25* has a counterpart in the *string* gene of *D. melanogaster*, and analogous proteins are found in amphibian and mammalian cells.

The basic principle established by this work is that the activity of the key regulator, Cdc2, is controlled by kinase and phosphatase activities that themselves respond to other signals. Cdc2 is the means by which all of these various signals are ultimately integrated into a decision on whether to proceed through the cycle (for review see 321; 322).

Activation of the G1/S form of the kinase (Cdc2/Cig2 in *S. pombe*) is required to enter S phase, but inactivity of the M phase form (Cdc2/Cdc13) is also required. Mutants in *cdc13* fail to enter mitosis (of course), but also undergo multiple cycles of DNA replication, suggesting that the M phase kinase usually inhibits S phase. This provides a checkpoint that ensures the alternation of S phase and mitosis. Activation of the M phase kinase during G2 prevents further rounds of DNA replication occurring before mitosis; and inactivation of the M phase kinase prevents another mitosis from occurring before the next S phase has occurred (835).

The target protein through which this circuit functions is probably Cdc18. Transcription of *cdc18* is activated as a consequence of passing START, and Cdc18 is required to enter S phase. Overexpression of *cdc18* allows multiple cycles of DNA replication to occur without mitosis. If Cdc18 is a target for Cdc2/Cdc13 M phase kinase, and is inactivated by phosphorylation, the circuit will take the form shown in **Figure 29.18**. For Cdc18 to be active, Cdc2/Cdc13 must be inactive. When the M phase kinase is activated, it causes Cdc18 to be inactive (possibly by phosphorylating it), and thereby prevents initiation of another S phase.

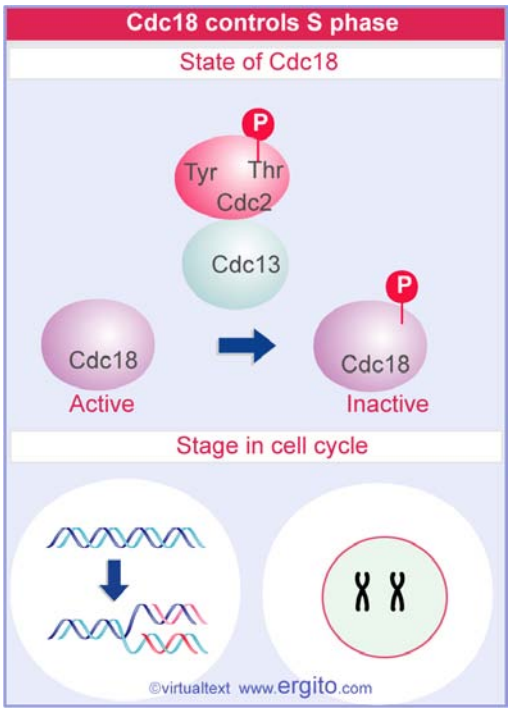

Figure 29.18 Cdc18 is required to initiate S phase and is part of a checkpoint for ordering S phase and mitosis. Active M phase kinase inactivates Cdc18, generating a reciprocal relationship between mitosis and S phase.

Activity of the Cdc2/Cdc13 M phase kinase is itself influenced by the factor Rum1, which controls entry into S phase. When *rum1* is over-expressed, multiple rounds of replication occur, and cells fail to enter mitosis. When *rum1* is deleted, cells enter mitosis prematurely. These properties suggest that rum1 is an inhibitor of the M phase kinase. It is expressed between G1 and G2 and keeps any M phase kinase in an inactive state. (This is important during G1, before the inhibitory phosphate is added to Tyr-15; see **Figure 29.14**.) It also represses the level of Cdc13 protein. The overall effect is to minimize M phase kinase activity, which is necessary to allow S phase to proceed. The consequences of the production of Rum1 on the state of M phase kinase, and consequently on the state of cdc18, are illustrated in **Figure 29.19**, which suggests a model for the overall circuit to control S phase.

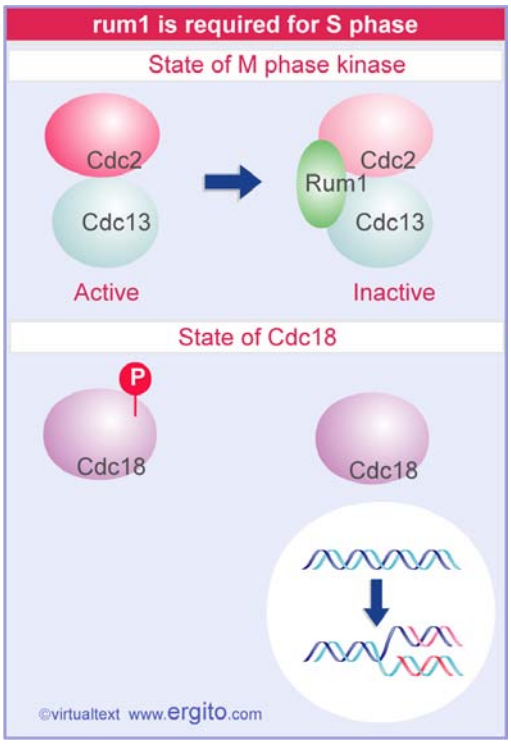

Figure 29.19 Rum1 inactivates the M phase kinase, preventing it from blocking the initiation of S phase.

A general theme emerges from these results: the circuits that control the cell cycle have interlocking feedback loops to ensure orderly progression. And giving dual roles to a single component in which its activity is necessary to promote one event but to block another creates an intrinsic alternation of events. So an active M phase kinase simultaneously promotes mitosis as a legitimate event and inhibits S phase as an illegitimate event. This creates an intrinsic checkpoint: one event cannot be initiated until the state of the component responsible for the prior event has been reversed. By contrast, the pathway of **Figure 29.23** is an example of an extrinsic checkpoint: in response to specific conditions, a pathway is activated whose end result is to alter the state of regulatory components in the cell cycle pathway.

A checkpoint is most often executed by controlling the state of phosphorylation of Cdc2. This may be achieved by activating or inhibiting the phosphatases and kinase that act on the activating or inhibitory tyrosines. Similar circuitry controls progress through meiosis as well as mitosis. For example, failure in recombination or in formation of synaptonemal complexes activates the pachytene checkpoint, which halts the cell during meiotic prophase. In *S. cerevisiae*, this is accomplished by activating the kinase Swe1 that phosphorylates the inhibitory tyrosine on Cdc28.

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CELL CYCLE AND GROWTH REGULATION

6.29.13 DNA damage triggers a checkpoint

Key Concepts

- Damage to DNA triggers a checkpoint that blocks the cell cycle until the damage has been repaired.
- The protein kinase ATM is a key component of the checkpoint pathway; it phosphorylates Chk2, which phosphorylates Cdc25, which is inactive in this form.
- The response pathway also activates genes that code for some repair proteins and directly activates other repair proteins.

Some of the most important cell cycle checkpoints are triggered by DNA damage. Cells contain many pathways to respond to DNA damage. A key aspect of the response is the control of the cell cycle. Cells are extremely sensitive to double-strand breaks in DNA—even a single break in the genome of *S. cerevisiae* can trigger a checkpoint (2858).

Figure 29.20 illustrates the basic principle of the response to DNA damage. A complex of proteins binds to damaged DNA, and then triggers signaling pathways. The best characterized signaling pathways lead to a block in the cell cycle, which allows time for response genes to be activated and then for the damage to be repaired (1402). An alternative response is to trigger death of the cell (thus preventing the sites of damage from inducing mutations) by apoptosis (see *Molecular Biology 6.29.25 Apoptosis is a property of many or all cells*).

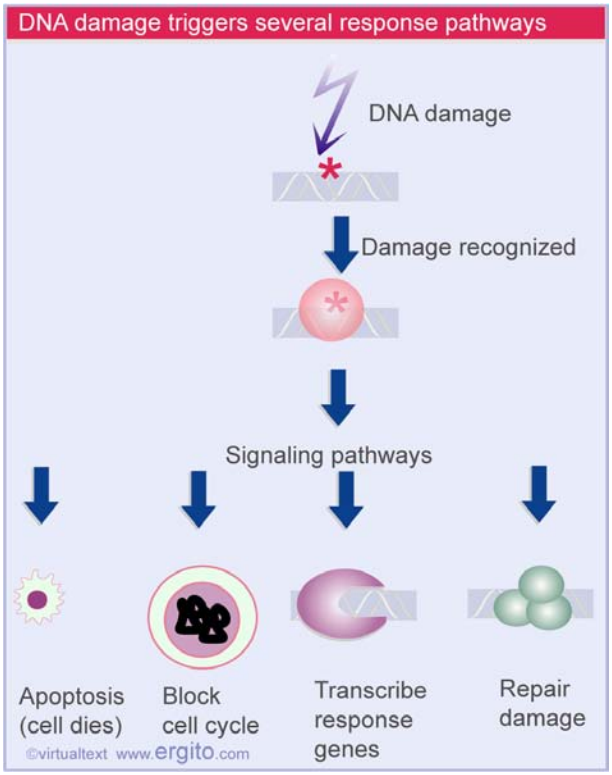

Figure 29.20 Damage to DNA triggers a response system that blocks the cell cycle, transcribes response genes, and repairs the damage, or that causes the cell to die.

Checkpoints respond to DNA damage at every stage of the cell cycle. There are also checkpoints to detect whether appropriate progress has been made through specific stages of the cycle. For example, replication must be completed before division is allowed to occur. All kinetochores must be paired before metaphase can give way to anaphase (see *Molecular Biology 6.29.19 Cohesins hold sister chromatids together*). **Figure 29.21** summarizes the checkpoints in *S. cerevisiae*. Note the distortion when the cycle is plotted in terms of checkpoints instead of real time. Although G1 is the longest part of the cycle, the checkpoints are concentrated in the later phases. This is because once a cell has left G1, it is committed to proceeding, but cannot be allowed to do so if the result will be to produce daughter cells with damaged DNA or other problems. So there are many checkpoints to ensure quality control.

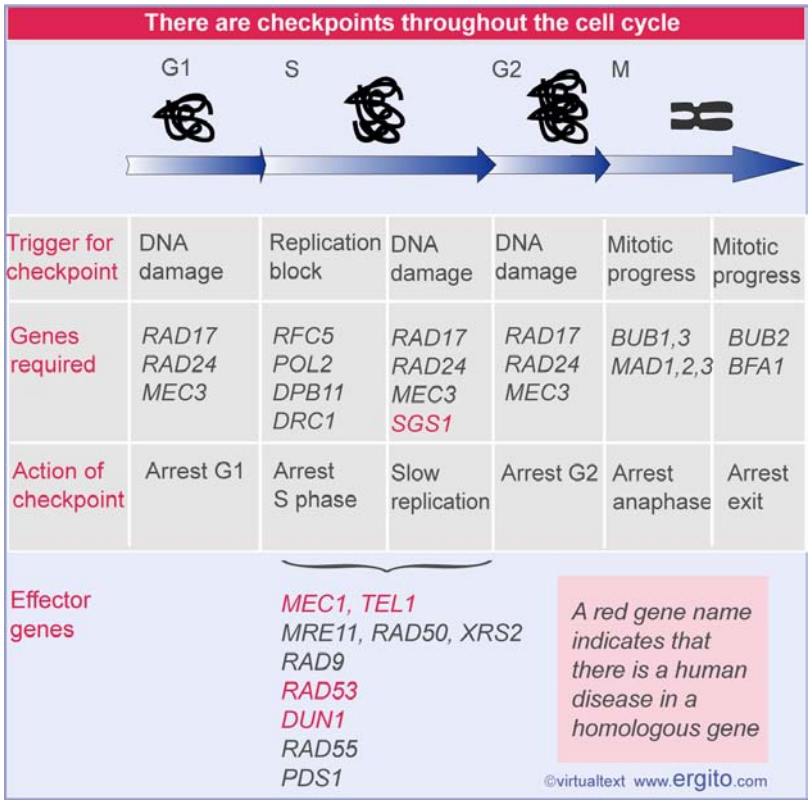

Figure 29.21 Checkpoints function at each stage of the cell cycle in *S. cerevisiae*. When damage to DNA is detected, the cycle is halted. The products of *RAD17, RAD24, MEC3* are involved in detecting the damage. During S phase, there is a checkpoint for the completion of replication. During mitosis, there are checkpoints for progress, for example, for kinetochore pairing.

A checkpoint pathway typically involves three groups of proteins:

- Sensor proteins recognize the event that triggers the pathway. In the case of a checkpoint that responds to DNA damage, they bind to the damaged structure in DNA, typically to a double-strand break or to single-stranded DNA.

- Transducer proteins are activated by the sensor proteins. They are usually kinases that amplify the signal by phosphorylating the next group of proteins in the pathway.

- Effector proteins are activated by the transducer kinases. They execute the actions that are required by the particular pathway. They often include kinases whose targets are the final proteins in the pathway.

The three genes RAD17, RAD24, and MEC3 code for a group of sensor proteins that are important in detecting DNA damage at each stage of the cell cycle in *S. cerevisiae* (for review see 3243). At replication, there is a second checkpoint, which also includes SGS1, that activates the same effector pathway. The least well

characterized stage of the process is in fact the initial binding to DNA, but we believe that many of the early events in the pathway occur at a complex that assembles at the site of damage (for review see 2855). **Figure 29.22** shows that this complex includes the three proteins RAD17, RAD24, and MEC3. They are joined by two transducer kinases, MEC1 and TEL1, that play partially redundant roles. These kinases phosphorylate and thereby activate the effector kinases RAD53 (called Chk2 in Man) and CHK1, which then phosphorylate proteins required to execute the pathway. The importance of this pathway is emphasized by the fact that many of its genes have human homologues that are implicated in genetic diseases.

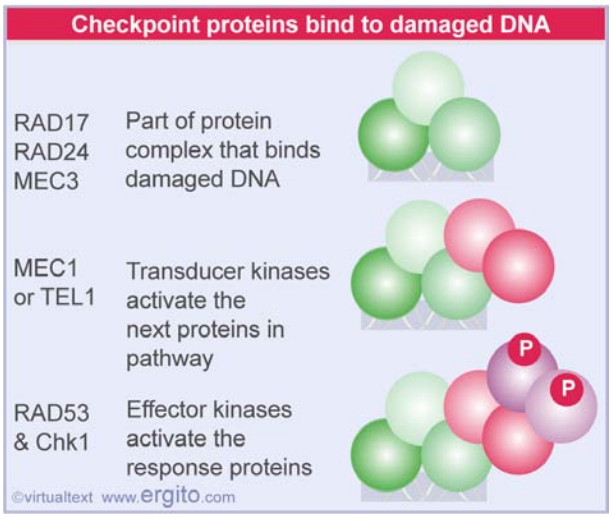

Figure 29.22 A complex assembles at a damaged DNA site. It includes the proteins required to detect damage, trigger the checkpoint, and execute the early stages of the effector pathway.

One of the effectors of the response pathway in mammalian cells is the protein kinase ATM (the homologue of yeast TEL1). This is a central component of the DNA damage response. Loss of ATM function is responsible for the human disease ataxia telangiectasia. Patients with this disease are not only abnormally sensitive to agents that damage DNA, but also have many cellular defects that result from errors that occur during normal cell division, but which fail to be repaired because they did not induce the checkpoint. The protein kinase ATR (homologue of yeast MEC1) is related to ATM and also triggers this response.

ATM exists in unirradiated cells as a dimer in which its enzymatic activity is suppressed. DNA damage causes phosphorylation of a serine, which causes the dimer to dissociate (3602). The monomer is an active kinase. The activation event may be the consequence of changes in chromatin structure that result from breaks in DNA caused by irradiation.

The ATR kinase exists in a complex with the another protein (ATRIP).ATRIP is recruited to single-stranded DNA by the RPA protein complex (a component of the replication fork that binds to free single-stranded DNA). This localizes ATR at the single-stranded DNA, where it acts on Chk enzymes to trigger the damage response (3995).

Figure 29.23 shows that DNA damage triggers a G2 checkpoint that involves both kinases and phosphatases. ATM activates (directly or indirectly) the kinase Chk2. Chk2 (and also the related kinase Chk1) phosphorylates Cdc25 on the residue Ser216. This causes Cdc25 to bind to the protein 14-3-3s, which maintains it in an inactive state. As a result, Cdc25 cannot dephosphorylate Cdc2, so M phase cannot be activated. A similar pathway is found in animal cells and in *S. pombe*, but in *S. cerevisiae* it is different.

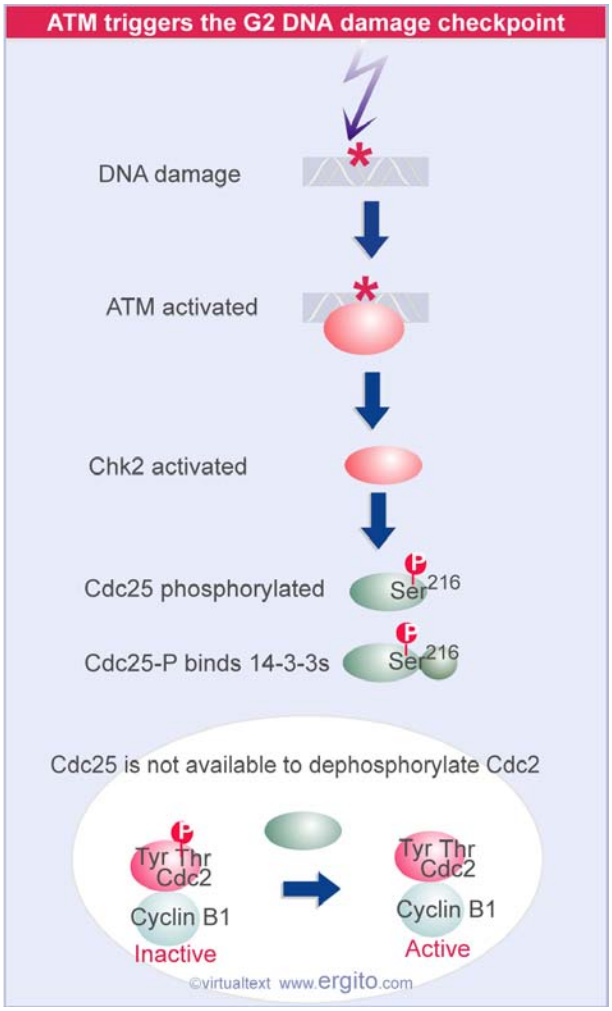

Figure 29.23 DNA damage triggers the G2 checkpoint.

Figure 29.24 shows that repair of the damaged DNA requires a combination of products of genes that are activated in response to damage and proteins that are activated by phosphorylation. Response genes that are transcribed as the result of DNA damage produce proteins that are involved directly in repairing DNA (such as the excision repair protein p48) and proteins that are needed in an ancillary capacity (such as the enzymes required for dNTP synthesis). Direct targets for phosphorylation by ATM include proteins involved in homologous recombination and the protein Nbs1 which is required for nonhomologous end-joining.

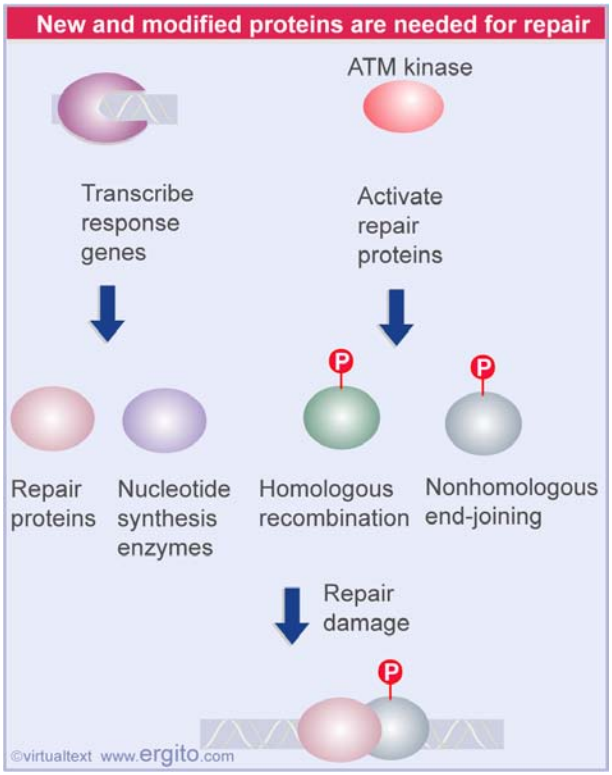

Figure 29.24 Proteins required to repair damaged DNA are provided by transcribing response genes to synthesize new proteins and by phosphorylating existing proteins.

The *RAD9* mutant of *S. cerevisiae* reveals another connection between DNA and the cell cycle. Wild-type yeast cells cannot progress from G2 into M if they have damaged DNA. This can be caused by X-irradiation or by the result of replication in a mutant such as *cdc9* (DNA ligase). Mutation of *RAD9* allows these cells to divide in spite of the damage. RAD9 therefore exercises a checkpoint that inhibits mitosis in response to the presence of damaged DNA. The reaction may be triggered by the existence of double-strand breaks. RAD9-dependent arrest and recovery from arrest can occur in the presence of cycloheximide, suggesting that the RAD9 pathway functions at the post-translational level. At least 6 other genes are involved in this pathway; its ultimate regulatory target remains to be found (843).

Last updated on 7-16-2003

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CELL CYCLE AND GROWTH REGULATION

6.29.14 The animal cell cycle is controlled by many cdk-cyclin complexes

Key Terms

A **cyclin-dependent kinase (cdk)** is one of a family of kinases which are inactive unless bound to a cyclin molecule. Most cyclin-dependent kinases participate in some aspect of cell cycle control.

Key Concepts

- Many dimeric kinases with a Cdc2-like catalytic subunit (cdk) are found in animal cells.
- cdk2 and cdk4 are the catalytic subunits that are usually bound to G1 cyclins.

Control of the cell cycle uses the same types of components in animal cells and in yeasts, although there is more diversity in each component in animal cells. The unifying theme is that progression to the next stage of the cell cycle is controlled by a kinase that consists of a catalytic subunit and a regulatory (cyclin) partner.

The components of the regulatory kinases for both G1/S and G2/M in yeasts and animal cells are summarized in **Figure 29.25**. The major difference is that animal cells have more variation in the subunits of the kinases. Instead of using the same catalytic subunit at both START and G2/M, animal cells use different catalytic subunits at each stage. They also have a larger number of cyclins (for review see 325).

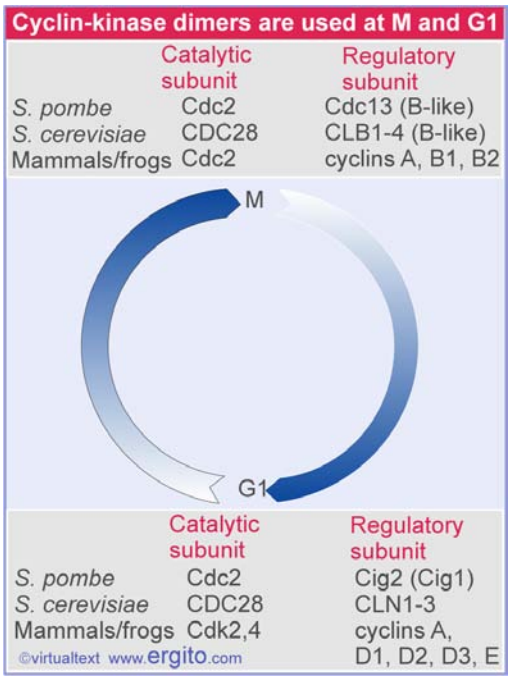

Figure 29.25 Similar or overlapping components are used to construct M phase kinase and a G1 counterpart.

At mitosis in animal cells, the Cdc2 catalytic subunit is provided by a single gene. The regulatory partner at mitosis usually is not unique, but is provided by a family of B-type cyclins, and sometimes also A-type cyclins.

During G1, animal cells have multiple kinases involved in cell cycle control, and they vary in *both* the catalytic subunit and the regulatory (cyclin) subunit. This contrasts with the retention of a common catalytic subunit in yeasts.

Just as families of cyclins can be defined by ability to interact with Cdc2, so may families of catalytic subunits be defined by the ability to interact with cyclins. Catalytic subunits that associate with cyclins are called **cyclin-dependent kinases** (**cdks**). Higher eukaryotes possess a large number of genes (~10) related to the true *cdc2* homologue. It is not entirely clear how many of these gene products are involved with the cell cycle and how many code for kinases with other functions. The cdk/cyclin dimers have the same general type of kinase activity as the Cdc2-cyclin dimers, and are often assayed in the same way, by H1 histone kinase activity. The involvement of the Cdc2/cdk kinase engines (and/or others related to them) at two regulatory points *in vivo* is consistent with an increase in H1 kinase activity at S phase as well as at M phase.

The pairwise associations between the catalytic and regulatory subunits are not exclusive, and a particular cyclin may associate with several potential catalytic subunits, while a catalytic subunit may associate with several potential cyclins. The trick is to determine which of these pairwise combinations form in the cell and are concerned with regulating the cell cycle.

Two of the *cdk* genes, *cdk2* and *cdk4*, code for proteins that form pairwise combinations with potential G1 cyclins. The first and best characterized is *cdk2*; it has 66% similarity to the *cdc2* homologue in the same organism.

G1 cyclins were originally identified as genes that could overcome the deficiency of *CLN* mutants in *S. cerevisiae.* Several new types of cyclins (including D and E) were identified by this means. They are distantly related to one another and to other cyclins. Cyclin E accumulates in a periodic manner through the cycle, but is not regulated by periodic destruction of protein. There are 3 D-type cyclins; they form dimers with cdk4 and cdk6.

Proteins described as "cyclins" are therefore now significantly more diverse than the A and B classes encompassed by the original definition. For working purposes, we class as cyclins various proteins that have some sequence relationship to the original class, and which can participate in formation of a kinase by pairing with a Cdc2 or cdk2 or related catalytic subunit.

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CELL CYCLE AND GROWTH REGULATION

6.29.15 Dimers are controlled by phosphorylation of cdk subunits and by availability of cyclin subunits

The timing of activity of the various forms of cdk- and Cdc2-cyclins during the animal cell cycle suggests a model in which cdk2-G1 cyclin dimers function to regulate progression through G1 and S phase, while Cdc2-cyclinA,B dimers regulate passage through mitosis. We know most about the details of controlling the G2/M transition, but the principles are likely to be similar for G1/S, since the Cdc2 and cdk catalytic subunits conserve the residues that are involved in regulation (for review see 328; 329).

Figure 29.26 shows that the regulatory events in an animal cell mitosis are similar to those in yeast cells (compare with **Figure 29.17**). The lower part of the figure shows the changes in M phase kinase; the upper part shows the enzymes that catalyze these changes. A cell leaves mitosis with Cdc2 monomers and no mitotic cyclins (because cyclins A and B were degraded during mitosis). Cyclins are then resynthesized. After a lag period, their level reaches a threshold at which they form dimers with Cdc2. But this does not activate the kinase activity; as we saw previously in **Figure 29.9**, the activity of the dimer is controlled by the state of certain Tyr and Thr residues:

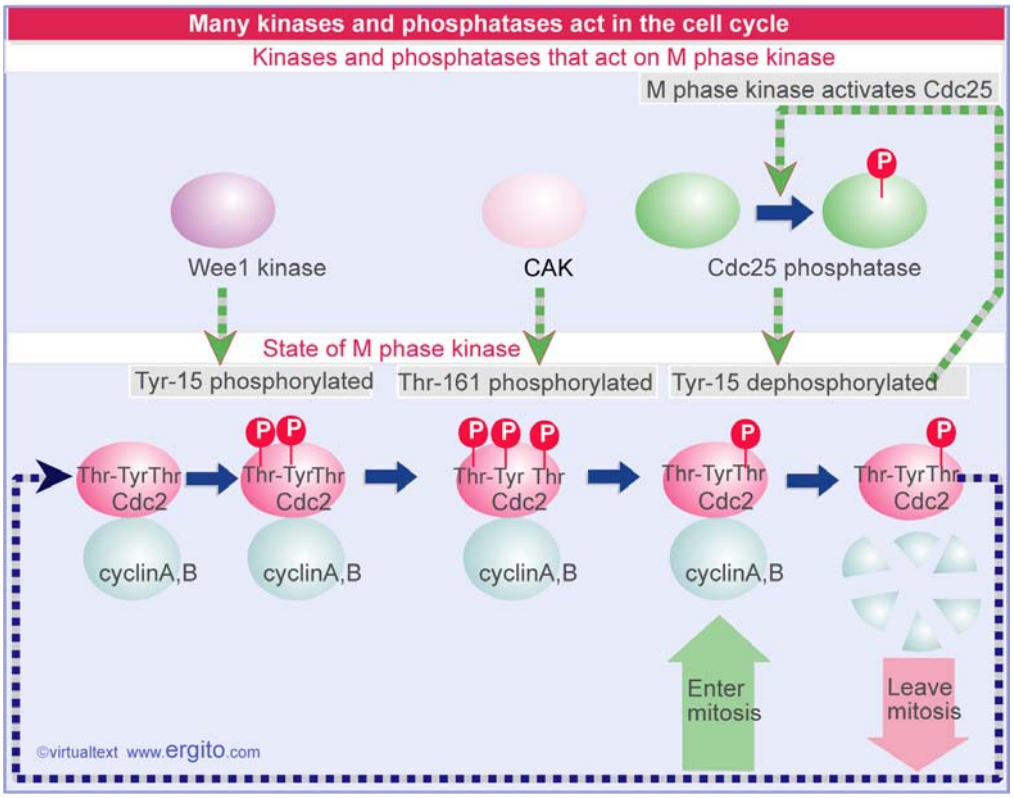

Figure 29.26 Control of mitosis in animal cells requires phosphorylations and dephosphorylations of M phase kinase by enzymes that themselves are under similar control or respond to M phase kinase.

- The phosphate that is necessary at Thr-161 is added by CAK (the Cdc2-activating kinase). CAK activity is probably constitutive (839; 840).
- The Wee1 kinase is a counterpart to the enzyme of *S. pombe* and phosphorylates Tyr-15 to maintain the M phase kinase in inactive form.
- The Cdc25 phosphatase is a counterpart to the yeast enzyme and removes the phosphate from Tyr-15. Cdc25 is itself activated by phosphorylation; and M phase kinase can perform this phosphorylation, creating a positive feedback loop. Removal of the phosphate from Tyr-15 is the event that triggers the start of mitosis. Cdc25 is itself regulated by several pathways, including phosphatases that inactivate it, but these pathways are not yet well defined.
- Separate kinases and phosphatases that act on Thr-14 have not been identified; in some cases, the same enzyme may act on Thr-14 and Tyr-15.

This means of control is common, but not universal, since in some cells tyrosine phosphorylation does not seem to be critical, and in these cases other events must be used to control the activity of M phase kinase.

Various cdk-cyclin dimers regulate entry into S and progression through S in animal cells. Some may be concerned with entering the cycle from G0 or exiting to it. The pairwise combinations of dimers that form during G1 and S are summarized in **Figure 29.27**. All of these dimers require phosphorylation on Thr-161 by CAK to generate the active form (834; for review see 326; 327).

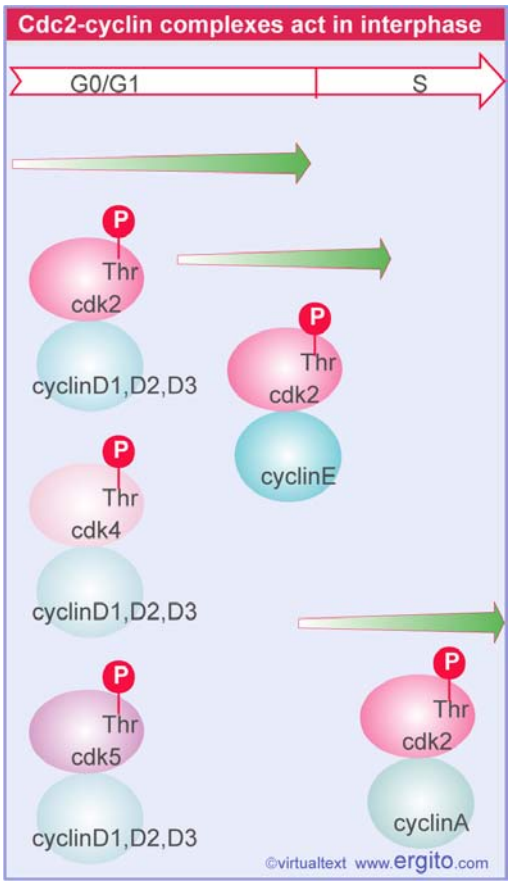

Figure 29.27 Several cdk-cyclin complexes are active during G1 and S phase. The shaded arrows show the duration of activity.

The synthesis of D cyclins is activated when growth factors stimulate cells to reenter the cycle from G0. The D cyclins have short half-lives, and their levels decline rapidly when the growth stimulus is removed. They may be involved with triggering reentry of quiescent cells into the cycle. Loss of D cyclins could be a trigger for a cell to leave the cell cycle for the G0 state.

The activity of D cyclins is required during the latter part of G1, but not close to the G1/S boundary. Their functions may be partly redundant, but there are some differences between the D cyclins in their susceptibilities to inhibitors of the cell cycle. The significance of the ability of each D cyclin to associate with 3 different cdk subunits is not clear.

Activity of the cdk2-cyclin E complex is necessary to enter S phase. Cyclin E is synthesized during a period that spans the G1/S transition, but we do not yet know how and when it is inactivated or at what point it becomes dispensable. Cyclin E clearly has a unique role.

Progression through S phase requires the cdk2-cyclin A complex. Cyclin A is also required to associate with Cdc2 for entry into mitosis. The dual use of cyclin A in animal cells appears to be the only case in which a cyclin is used for both G1/S and

G2/M transitions.

The states of cyclin-cdk complexes may influence the licensing system that prevents reinitiation of replication (see **Figure 14.39**). Cyclin B-cdk complexes prevent Cdc6 from loading on to the origin. This results in an orderly succession of events, because the degradation of cyclin B in mitosis releases the block and allows the procedure to start for forming a prereplication complex at the origin.

Both the beginning and end of S phase are important points in the cell cycle. Just as a cell must know when it is ready to initiate replication, so it must have some means of recognizing the successful completion of replication. This may be accomplished by examining the state of DNA.

Inhibitors of DNA replication can block the cell cycle. The effect may depend on the presence of replication complexes on DNA. We do not know exactly how they trigger the checkpoint that blocks the activation of M phase kinase.

Controls that ensure an orderly progression through the cell cycle and that can be visualized in the context of checkpoints include monitoring the completion of DNA replication and checking cell size. It may be necessary to bypass some of these checkpoints in early embryogenesis; for example, early divisions in *Drosophila* in effect are nuclear only (because there are no cellular compartments: see *Molecular Biology 6.31 Gradients, cascades, and signaling pathways*). It may be significant that the product of *string* (the counterpart of *S. pombe cdc25* which exercises a size-dependent control) is present at high levels in these early cycles. After the 14th cycle, division becomes dependent on *string* expression. It is at this point that cellular compartments develop, and it becomes appropriate for there to be a checkpoint for cell size. *string* may provide this checkpoint.

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CELL CYCLE AND GROWTH REGULATION

6.29.16 RB is a major substrate for cdk-cyclin complexes

Key Concepts

- RB is an important target for cdk-G1 cyclin complexes; its phosphorylation is required for initiation of S phase.

An important insight into control of the cell cycle at G1 has been provided by the identification of tumor suppressor genes that code for products that interact with cdk-cyclin complexes or with the downstream circuitry. Tumor suppressors are generally identified as genes in which loss of function causes tumor formation, either as seen by transformation of cells in culture, or by association of loss-of-function mutations with tumors in animals (see *Molecular Biology 6.30.3 Oncogenes and tumor suppressors have opposite effects*). The tumor suppressor RB is a key component in controlling the cell cycle (see also *Molecular Biology 6.30.19 RB is a tumor suppressor that controls the cell cycle*).

RB is a substrate for cdk-cyclin D complexes, and exerts its effects during the part of G1 that precedes the restriction point. **Figure 29.28** shows the basic circuit. In quiescent cells, or during the first part of G1, RB is bound to the transcription factor E2F. This has two effects. First, some genes whose products are essential for S phase depend upon the activity of E2F. By sequestering E2F, RB ensures that S phase cannot initiate. Second, the E2F-RB complex represses transcription of other genes. This may be the major effect in RB's ability to arrest cells in G1 phase (for review see 333).

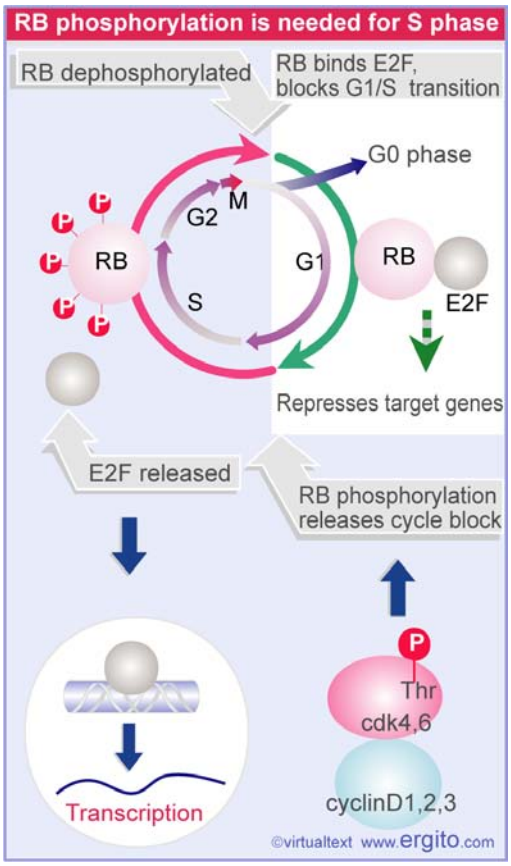

Figure 29.28 A block to the cell cycle is released when RB is phosphorylated by cdk-cyclin.

RB may exert its repressive effects by interacting with chromatin (for review see 1863). It binds histone deacetylases, which raises the possibility that it functions by causing them to remove acetyl groups from the histones at target promoters, thus inactivating the promoters (see *Molecular Biology 5.23.8 Deacetylases are associated with repressors*). It also interacts with components of a chromatin remodeling complex.

The nonphosphorylated form of RB forms a complex with cdk-cyclins. The complex with cdk4,6-cyclin D1,2,3 is the most prominent, but RB is also a substrate for cdk2-cyclin E. At or close to the restriction point, RB is phosphorylated by cdk4,6-cyclin D kinases. The phosphorylation causes RB to release E2F, which then activates transcription of the genes whose functions are required for S phase, and also releases repression of genes by the E2F-RB complex. The importance of E2F is seen by the result that expression of E2F in quiescent cells enables them to synthesize DNA.

There is an especially close relationship between RB and cyclin D1. Overexpression of D1 causes cells to enter S phase early. Inhibition of expression of D1 arrests cells before S phase. The sole role of cyclin D1 could be to inactivate RB and permit entry into S phase.

There are several related transcription factors in the E2F family, sharing the property

that all recognize genes with the same consensus element. RB binds three of these factors. Two further proteins, p107 and p130, which are related to RB, behave in a similar way, and bind the other members of the E2F group. So together RB and p107 may control the activity of the E2F group of factors.

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CELL CYCLE AND GROWTH REGULATION

6.29.17 G0/G1 and G1/S transitions involve cdk inhibitors

Key Terms

Cyclin-dependent kinase inhibitors (cki) are a class of proteins which inhibit cyclin-dependent kinases by binding to them. Inhibition lasts until the cki is inactivated, often in response to a signal for the cell cycle to progress.

Key Concepts

- CKI proteins are inhibitors of cdk-cyclin dimers.
- Different CKI proteins have specificities for different cdk-cyclin complexes.

RB is a target for several pathways that inhibit growth, and may be the means by which growth inhibitory signals maintain cells in G1 (or G0). Several of these signals, including the growth inhibitory factor TGF β , act through inhibitors of cdk-cyclin kinases. The inhibitors are called **CKIs**. They are found as proteins bound to cdk-cyclin dimers in inactive complexes, for example, in quiescent cells. By maintaining the cdk-cyclin complexes in inactive form, they prevent the phosphorylation of RB, making it impossible to release cells to enter S phase.

The CKI proteins fall into two classes. The INK4 family is specific for cdk4 and cdk6, and has four members: $p16^{INK4A}$, $p15^{INK4B}$, $p18^{INK4C}$, and $p19^{INK4D}$. The Kip family inhibit all G1 and S phase cdk enzymes, and have three members: $p21^{Cip1/WAF1}$, $p27^{Kip1}$, and $p57^{Kip2}$. [Each protein is identified by its size, with the casual name used as a superscript; for review see 332].

INK4 protein binds specifically to cdk4 and cdk6. This suggests a connection with the G0/G1 transition. p16 cannot inhibit proliferation of cells that lack RB, which suggests that it functions by preventing cdk-cyclin kinase activity from using RB as a substrate, as illustrated in **Figure 29.29**. By binding to the cdk subunits, INK4 proteins inhibit both cdk4-cyclin D and cdk6-cyclin D activities. As exemplified by p16 and p19, they bind next to the ATP binding site of cdk6. This both inhibits catalytic activity and triggers a conformational change that prevents cyclin from binding (the conformational change is propagated to the cyclin-binding site).

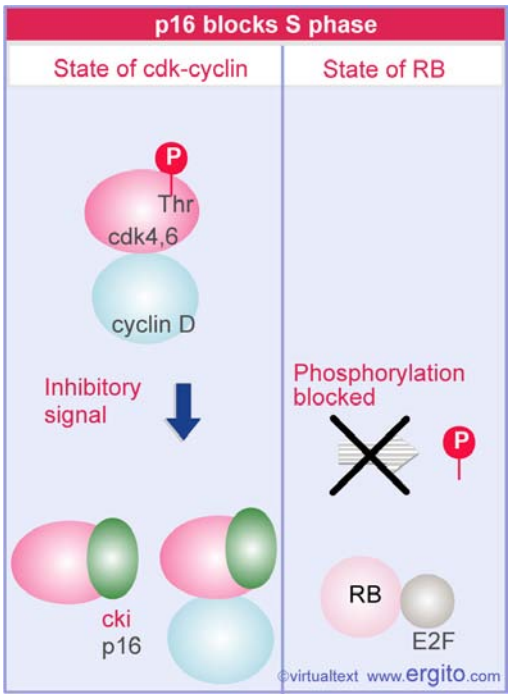

Figure 29.29 p16 binds to cdk4 and cdk6 and to cdk4,6-cyclin dimers. By inhibiting cdk-cyclin D activity, p16 prevents phosphorylation of RB and keeps E2F sequestered so that it is unable to initiate S phase.

p21 is a universal cdk inhibitor, binding to all complexes of cdk2, 4, 6. This suggests that it is likely to block progression through all stages of G1/S. In primary cultured cells (taken directly from the animal), cdk-cyclin dimers are usually found in the form of quaternary complexes that contain two further components. One is PCNA, a component of DNA polymerase δ, which may provide a connection with DNA replication. The other is the inhibitor p21. It may seem paradoxical that an inhibitor is consistently associated with the cdk-cyclin dimer, but it turns out that at a stoichiometry of 1:1 the p21 is not inhibitory. An increase in the number of p21 subunits associated with the cdk-cyclin dimer inhibits kinase function. In transformed cells (from lines that have been successfully perpetuated in culture), cdk-cyclin complexes lack p21 and PCNA. This suggests the possibility that p21 is involved in G1/S control, and that relaxation of this control is necessary for cells to be perpetuated in culture.

p27 has a sequence that is partly related to p21, and also binds promiscuously to cdk-cyclin complexes. Overexpression of p27 blocks progression through S phase, and levels of p27 are increased when cells are sent into a quiescent state by treatment with TGF β . p21 and p27 block the catalytic subunit of cdk-cyclin dimers from being a substrate for activation by phosphorylation by CAK. They also prevent catalytic activity of the cdk-cyclin complex. The stages at which they function are illustrated in the summary of inhibitory pathways in **Figure 29.30**.

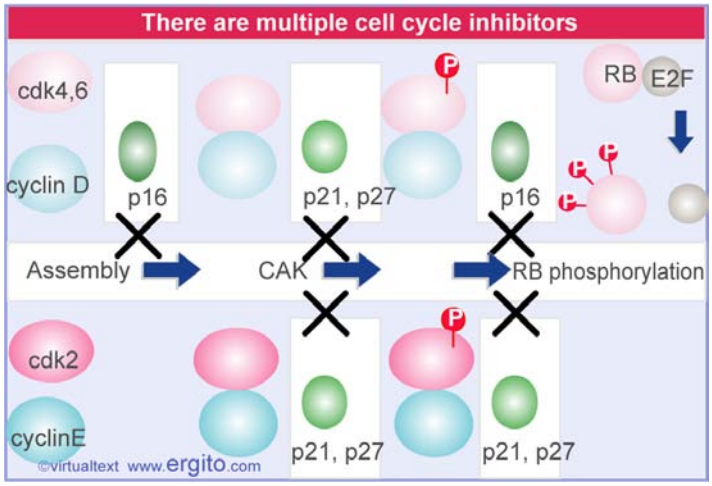

Figure 29.30 p21 and p27 inhibit assembly and activity of cdk4,6-cyclin D and cdk2-cyclin E by CAK. They also inhibit cycle progression independent of RB activity. p16 inhibits both assembly and activity of cdk4,6-cyclin D.

p21 and p27 are probably partially redundant in their functions. The pathway by which they inhibit the cell cycle is not entirely clear, but we know that it does not depend on controlling RB, because they can inhibit proliferation of cells that lack RB. This may mean that their inhibition of cdk2-cyclin E dimers is critical. Since both are present in proliferating cells, the normal progress of the cell cycle may require the levels of the cdk-cyclin dimers to increase to overcome an inhibitory threshold. p27 appears to be the major connection between extracellular mitogens and the cell cycle, with an inverse correlation between p27 activity and ability to proliferate.

The importance of the pathway from CKI proteins to RB is emphasized by the fact that tumor suppressors are found at every stage, including CKI proteins, cyclins D1,2, and RB. The implication is that the CKI proteins are needed to suppress unrestrained growth of cells. In terms of controlling the cell cycle, this pathway is clearly central. It may be the key pathway by which cells are enabled to undertake a division cycle (by passing the restriction point in G1 if they are already cycling, or by reentering G1 if they are quiescent in G0; for review see 331).

The CKI proteins are also involved in another level of control. In *S. cerevisiae*, the CKI Sic1 is bound to CDC28-CLB during G1, and this maintains the kinase in an inactive state. Entry into S phase requires degradation of Sic1 to release the kinase. **Figure 29.31** shows how Sic1 is targeted for degradation by a ubiquitinating system (see **Figure 8.68** for a general description). The Sic1 target is recognized by a complex called the SCF, which functions as an E3 ligase (the component that selects the target). The SCF complex includes Cdc53, Skp1, and Cdc4. Cdc4 is the targeting component, which, together with Skp1, binds to Sic1. For this reason, the complex is described as SCF^{Cdc4}. Skp1 is the connection to Cdc53, which interacts with the E2 ligase (Cdc34). The E2 ligase adds ubiquitin to Sic1, causing it to be degraded.

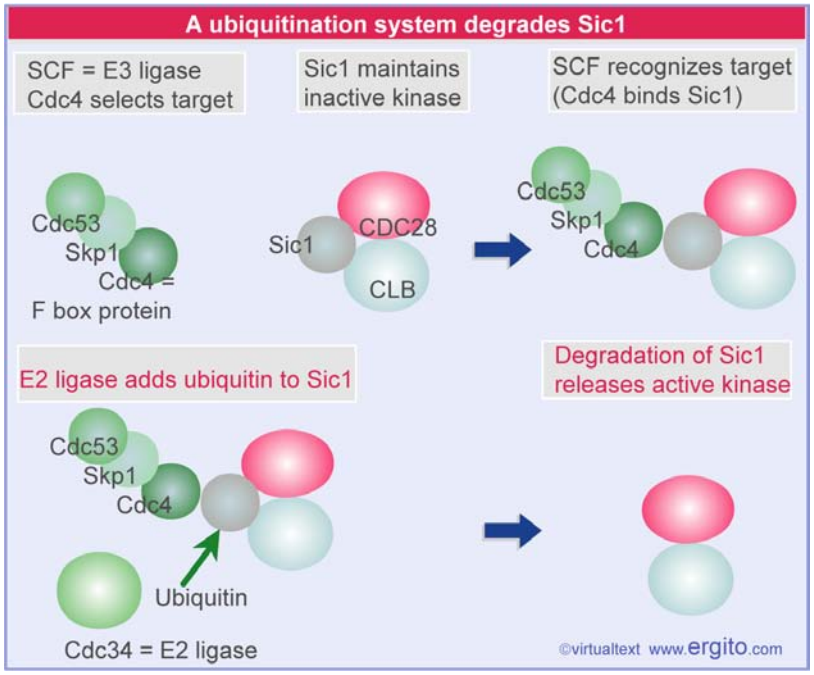

Figure 29.31 The SCF is an E3 ligase that targets the inhibitor Sic1.

Cdc4 is a member of a class of proteins called F-box proteins. It uses the F-box motif to bind to Skp1. This is a general paradigm for the construction of SCF complexes (for review see 1006). Other SCF complexes exist in which the targeting subunit is a different F-box protein, but the Cdc53 and Skp1 components remain the same. An example relevant to the cell cycle is SCF^{Grr1} in which the F-box protein Grr1 provides the targeting subunit, and causes the degradation of G1 cyclins (850).

There are further layers of control in this system. The substrates for the SCF must be phosphorylated to be recognized. The kinases that perform the phosphorylation are the cdk-cyclin complexes that are active at the appropriate stage of the cell cycle. The abundance of the SCF complexes is itself controlled by degrading the F-box subunits. SCF^{Cdc4} targets Cdc4, thus creating an autoregulatory limitation on its activity. The consequence of such feedbacks is to maintain a supply of the Cdc53-Skp1 cores that can be recruited as appropriate by the F-box subunits.

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CELL CYCLE AND GROWTH REGULATION

6.29.18 Protein degradation is important in mitosis

Key Terms

The **anaphase promoting complex (APC)** is a set of proteins that triggers proteolysis or targets required to allow chromosomes to separate.

The **cyclosome** is a multisubunit complex which initiates anaphase and the exit of cells from mitosis by promoting the ubiquitination and proteolysis of a variety of proteins. These include the mitotic cyclins, several proteins required to hold sister chromatids together, and other proteins which control the dynamics of the mitotic spindle.

Key Concepts

- Cyclins are degraded at two points during mitosis, cyclin A during metaphase, and B cyclins to terminate mitosis.
- The APC (anaphase promoting complex) is an E3 ubiquitin ligase that targets a substrate for degradation.

The timing of the events that regulate mitosis is summarized in **Figure 29.32**. Mitosis is initiated by the activation of M phase kinase. Progress through mitosis requires degradation of cyclins, and also of other proteins (845). Several degradation events play different roles in mitotic progression:

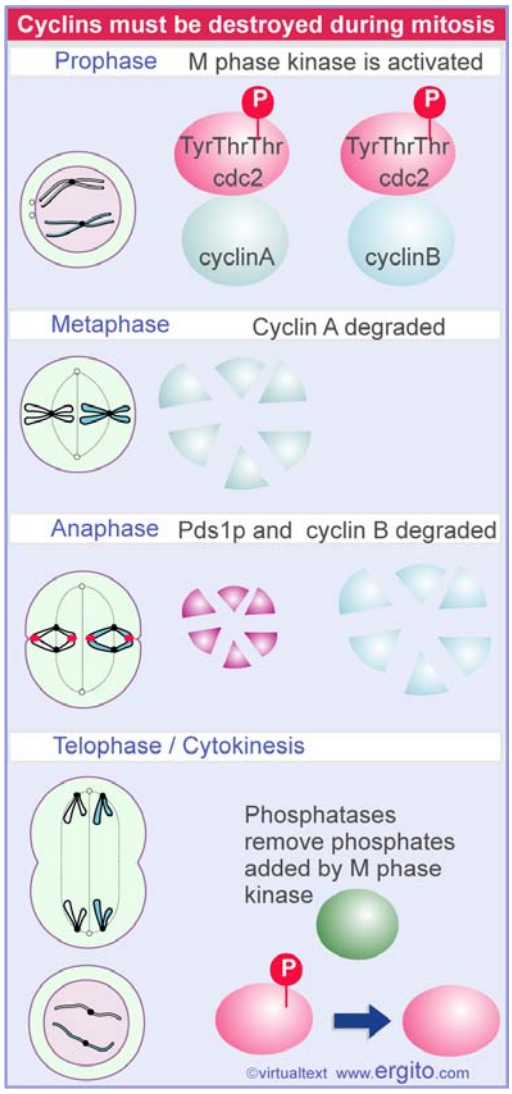

Figure 29.32 Progress through mitosis requires destruction of cyclins and other targets.

- The first event to occur is the degradation of cyclin A at metaphase.
- The separation of chromosomes at anaphase requires the activity of the proteasomal system for protein degradation (but does not require cyclin degradation). The target for this event is the protein Pds1p, whose degradation triggers the pathway that enables sister chromatids to separate.
- Later during anaphase, B cyclins must be degraded in order to inactivate M phase kinase. This is necessary to allow the phosphorylations of substrate proteins that were catalyzed by M phase kinase to be reversed at the end of mitosis.

A large complex of 8 subunits is responsible for selecting the substrates that are degraded in anaphase. It is called the **anaphase promoting complex** (**APC**)

(sometimes also known as the **cyclosome**). Similar complexes are found in both yeasts and vertebrates (for review see 3026). The APC becomes active specifically during mitosis (846; for review see 1438). It functions as an E3 ubiquitin ligase (see **Figure 8.68**), which is responsible for binding to the substrate protein so that ubiquitin is transferred to it. The ubiquitinated substrate is then degraded by the proteasome.

There are two separate routes to activating the APC, and each causes it to target a particular substrate. **Figure 29.33** shows that the regulatory factors CDC20 and Cdh1 bind to the APC and activate its ubiquitination activity. CDC20 is necessary for the APC to degrade Pds1p, which controls the transition from metaphase to anaphase. Cdh1 is necessary for the APC to degrade Clb2 (yeast cyclin B), which is necessary to exit mitosis. The timing of activation and persistence of each type of complex is different (847; 849).

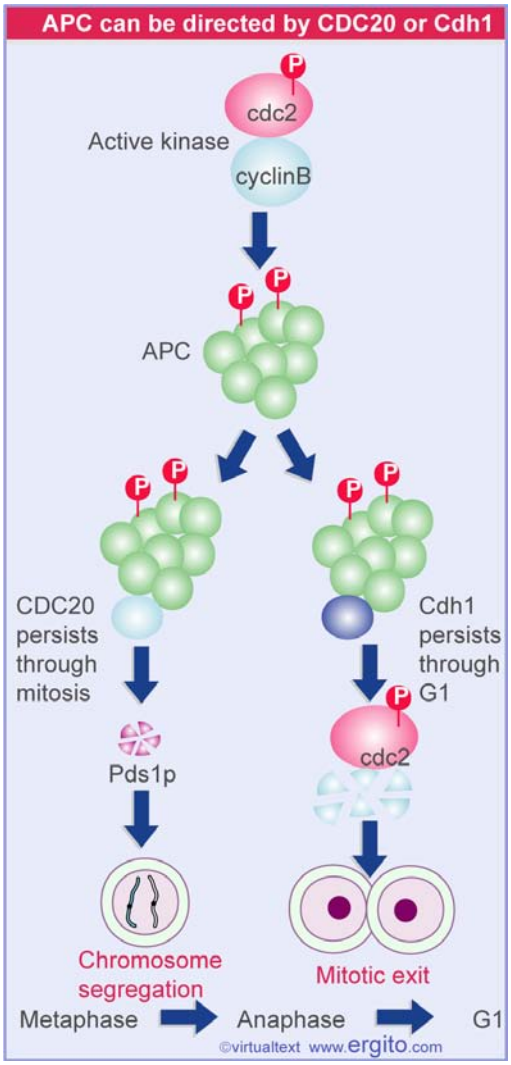

Figure 29.33 Two versions of the APC are required to pass through anaphase.

Last updated on 10-16-2002

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CELL CYCLE AND GROWTH REGULATION

6.29.19 Cohesins hold sister chromatids together

Key Terms

Cohesin proteins form a complex that holds sister chromatids together. They include some SMC proteins.

Separins are proteins which play a direct role in initiating anaphase by cleaving and inactivating a component (a cohesin) that holds sister chromatids together.

Securins are a class of proteins that prevent the initiation of anaphase by binding to and inhibiting separin, a protease which cleaves the structural component required for holding sister chromatids together. Inhibition of separin by securin ends when securin is itself proteolyzed as a result of activation of the anaphase promoting complex (APC).

Key Concepts

- Cohesins associate with chromatin at S phase and hold sister chromatids together.
- A securin is a protein that sequesters a separin and keeps it in an inactive state.
- Anaphase in yeast is triggered when CDC20 causes the APC to degrade the securin Pds1p.
- Degradation of the securin releases the separin (Esp1 in yeast) which is a protease that cleaves the cohesin Scc1p.
- Cleavage of Scc1p releases the sister chromatids.

After replication occurs at S phase, the products of each chromosome (sister chromatids) remain associated with one another, although this becomes evident only later at the beginning of mitosis. This is a crucial aspect of segregating the sister chromatids to different daughter cells, as shown in **Figure 19.22**. The sister chromatids are held together by a complex of **cohesin** proteins, which functions as a sort of "glue". The cohesins are the key players in assuring chromatid association at the start of mitosis, and dissociation during mitosis (for review see 2859).

Figure 29.34 shows that cohesins are located at various sites along a pair of sister chromatids, with the appearance of being centrally localized between the chromatids. At metaphase, each sister chromatid pair is in equilibrium on the spindle, being connected to both poles by microtubules. When the connecting glue dissolves, the sister chromatids are released from one another and pulled toward opposite poles. This marks the transition from metaphase to anaphase.

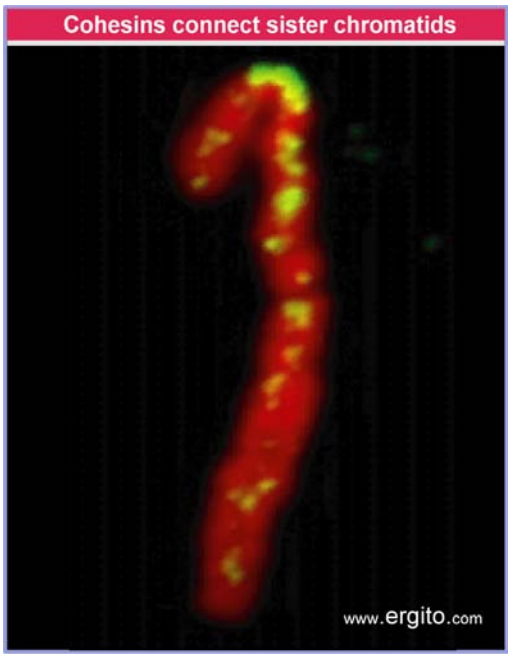

Figure 29.34 Cohesins are located sporadically along a pair of sister chromatids early in mitosis. DNA is in red; cohesins are in yellow. Photograph kindly provided by Ana Losada and Tatsuya Hirano.

At the metaphase-anaphase transition, the route to releasing sister chromatids so that they may segregate to opposite poles is indirect, as illustrated in **Figure 29.35**. There are three components in the system (for review see 2297). The cohesins connect the sister chromatids. A **separin** releases the cohesins. And the separin is controlled by a **securin**.

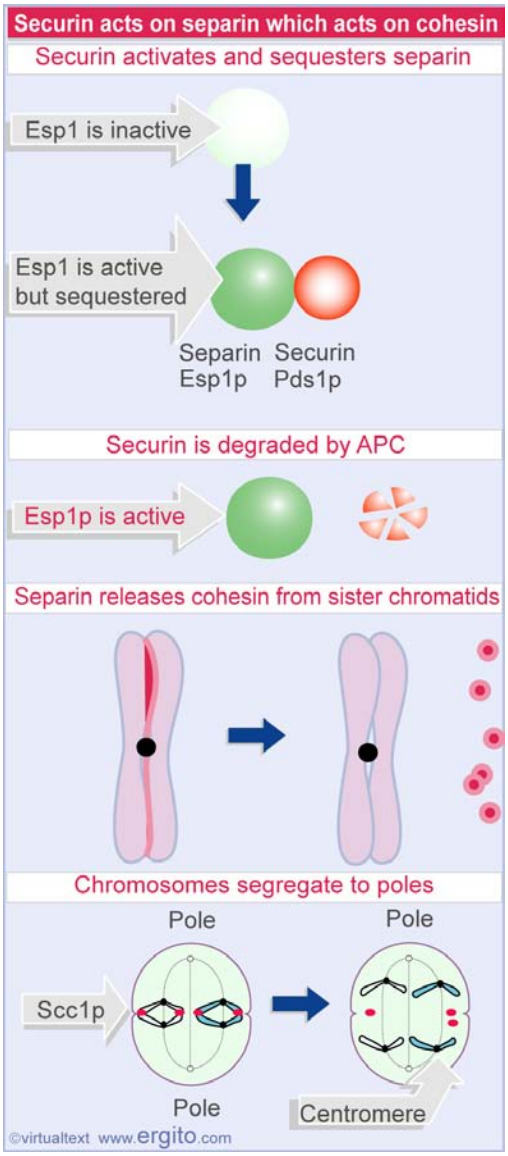

Figure 29.35 Anaphase progression requires the APC to degrade Pds1p to allow Esp1p to remove Scc1 from sister chromatids.

Prior to anaphase in yeast, the securin Pds1p binds to the separin Esp1p. When the APC degrades Pds1p (securin), Esp1p (separin) is released. The separin is a protease. It cleaves the protein Scc1p, which is a component of the cohesin complex (848; 1430). When Scc1p is released by the action of Esp1p, the cohesin complex can no longer hold the chromatids together, and they therefore become free to segregate on the spindle.

If securin simply sequesters separin, we would expect the loss of securin to cause premature chromosome separation. However, the opposite happens. If the securin gene is inactivated, the chromosomes have difficulty in separating, and the delay causes abnormalities in chromosome segregation (1958). This suggests that securin plays two roles. Suppose that the separin is in an inactive state before it binds to

securin. The securin activates it, but keeps it sequestered. When securin is degraded, the separin is released in the active state. If securin is absent altogether, the separin never becomes activated, so that the cohesins are not destroyed.

Scc1p is only one component of the cohesin complex. The core of the complex is a heterodimer of SMC proteins. (SMC stands for structural maintenance of chromosome; other SMC proteins form the condensins, which are involved in controlling chromosome condensation; see *Molecular Biology 5.23.18 Chromosome condensation is caused by condensins*). The cohesin complex contains Scc1p, Scc3p, and a heterodimer of the two SMC proteins Smc1p and Smc3p (2483). However, loss of Scc1p is sufficient to abolish the ability to hold sister chromatids together. The cohesins may function by cross-linking DNAs as shown in **Figure 23.30** (for review see 2323; 2262).

Smc3p is involved in meiosis as well as mitosis. It is required for sister chromatid cohesion, together with a protein (Rec8p) that is related to Scc1p. This suggests that a cohesin complex, related to that of mitosis, may form at meiosis. Both of these components are required for synaptonemal complexes to form (see *Molecular Biology 4.15.5 Recombining chromosomes are connected by the synaptonemal complex*). The metaphase-anaphase transition at the first meiotic division in yeast is triggered in the same way as at mitosis, when the cohesin Rec8 is cleaved by separin to allow disjunction of homologous chromosomes (1431).

The situation in higher eukaryotic cells is more complex. The cohesin Scc1p is mostly released from the chromosomes during prophase, although it is left in the centromeric regions. It is degraded at the start of anaphase, and its loss from the centromeric regions may be the trigger for chromosome separation (1432).

The major target of the APC^{Cdh1} complex is the mitotic cyclin component of the cdk-cyclin kinase. Its destruction makes it possible to reverse the phosphorylations that triggered mitosis, which is necessary to exit mitosis. However, there is some overlap in the actions of the two APC complexes, and APC^{CDC20} may also act on mitotic targets. The relative timing of activation of the two APC complexes may be determined by a circuit in which a target of APC^{CDC20} is needed to activate APC^{Cdh1}.

Formation of the cohesin complex (or possibly its association with the chromosomes) occurs during S phase. Mutants in the locus *ctf7* have sister chromatids that never associate. The gene acts during the period of DNA replication, but does not code for a component of the cohesin complex (2485). It is possible that the establishment of cohesion is triggered by replication in some way. So far as we can tell from examining individual sequences, the replicated copies remain tightly associated from replication until cell division (2484).

The cohesion system is the target for a checkpoint. Progression through mitosis requires all kinetochores to be paired with their homologues. **Figure 29.36** shows that the checkpoint consists of a surveillance system that is triggered by the presence of an unattached kinetochore. Mutations in the *Mad* and *Bub* genes allow mitosis to continue (aberrantly) in the presence of unpaired kinetochores. The Mad proteins control the system for chromatid segregation. They bind to CDC20 and prevent it from activating the APC. When the kinetochores are all attached, some (unidentified) signal causes Mad proteins to be released from CDC20, which now activates the

APC, allowing anaphase to continue. A similar role is played by Bub proteins in controlling the ability of Cdh1 to activate the APC.

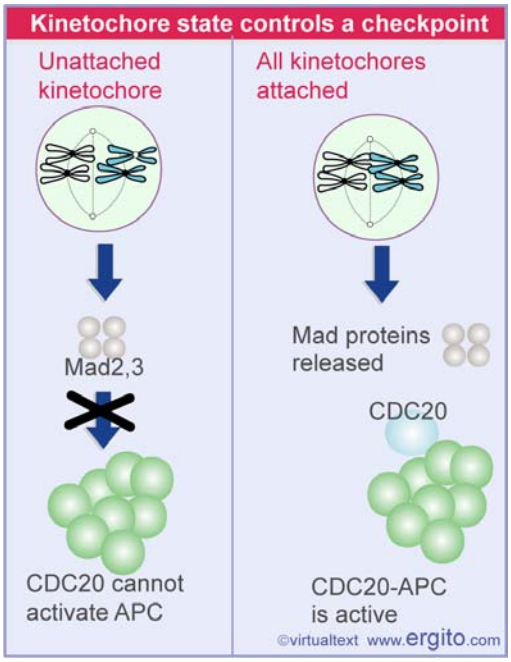

Figure 29.36 An unattached kinetochore causes the Mad pathway to inhibit CDC20 activity

Last updated on 1-30-2002

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CELL CYCLE AND GROWTH REGULATION

6.29.20 Exit from mitosis is controlled by the location of Cdc14

Key Concepts

- During interphase the phosphatase Cdc14 is held in the nucleolus.
- When a spindle pole body migrates into the bud of *S. cerevisiae*, it carries the protein Tem1.
- Tem1 is a monomeric G protein that is activated by the local concentration of the exchange factor Lte1 in the bud.
- Activation of Tem1 triggers release of Cdc14.
- The action of Cdc14 triggers exit from mitosis.

The key event in leaving mitosis is the activation of the phosphatase Cdc14 (908). **Figure 29.37** shows that during interphase, Cdc14 is sequestered in the nucleolus (because it binds to a nucleolar protein variously called RENT/Cfi1/Net1) (1202; 1203). When it is localized in the nucleolus, it cannot find any of its substrates, and therefore is inactive. When it is released from the nucleolus, it acts on (at least) two substrates. Dephosphorylation of Cdh1 is necessary to activate the APC_{Cdh1} complex. And dephosphorylation of Sic1 enables it to inactivate mitotic cyclins. This is another example of belts and braces in cell cycle control, where parallel pathways lead to the same outcome.

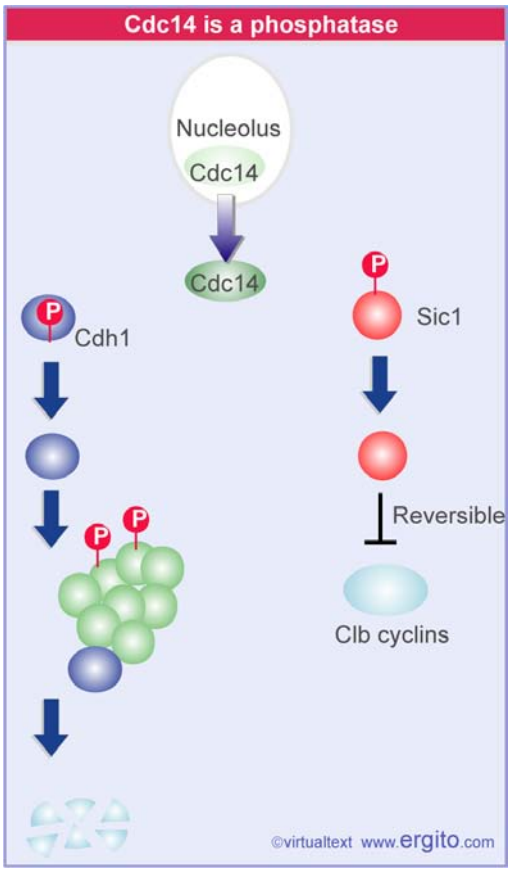

Figure 29.37 Cdc14 dephosphorylates both Cdh1 and Sic1. The first action leads to activation of the APC that degrades mitotic cyclins. The second action enables Sic1 to reversibly inactivate mitotic cyclins.

What triggers the release of Cdc14 from the nucleolus? The pathway for leaving mitosis has many genes, and genetic relationships suggest that two key components are the GTP-binding protein Tem1 and the exchange factor Lte1. Like other monomeric G proteins, Tem1 is active when bound to GTP, and inactive when bound to GDP. The exchange factor activates it by causing bound GDP to be replaced with GTP (see *Molecular Biology Supplement 32.10 G proteins*). The ability of Lte1 to activate Tem1 is controlled in an interesting way by the locations of the two components in the yeast cell.

Recall that *S. cerevisiae* has an asymmetrical division in which the daughter cell forms as a bud of the mother cell (see **Figure 29.10**). Lte1 protein is present throughout the cell cycle, and when the bud forms at the beginning of S phase, the Lte1 is localized in it. By contrast, Tem1 is synthesized only at late S phase. At mitosis, the Tem1 is localized with one of the spindle pole bodies (the structures identifying the ends of the spindle where microtubules are nucleated). **Figure 29.38** shows that this is the spindle pole body that migrates into the bud! When Tem1 arrives in the concentration of Lte1 in the bud, it is activated (1204). This triggers the release of Cdc14 from the nucleolus, which in turn triggers exit from mitosis.

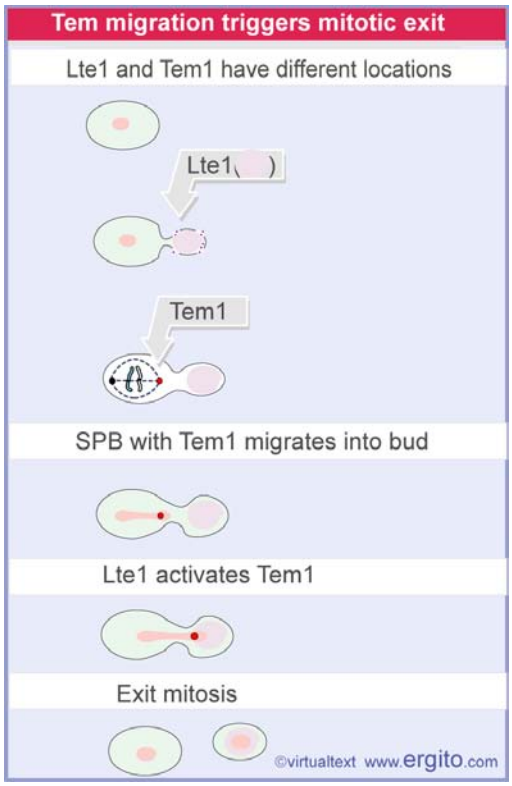

Figure 29.38 Exit from mitosis is triggered when the Tem1 that is localized on a spindle poly body migrates into the bud where Lte1 is localized.

Last updated on 10-18-2000

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CELL CYCLE AND GROWTH REGULATION

6.29.21 The cell forms a spindle at mitosis

Key Terms

The **spindle** guides the movement of chromosomes during cell division. The structure is made up of microtubules.

A **microtubule** is a filament consisting of dimers of tubulin. It serves as a track for the movement of chromosomes during cell division and for the movements of vesicles and organelles in nondividing cells. It is also a dynamic component of cilia and flagella.

Key Concepts

- The activity of M phase kinase is responsible for condensation of chromatin into chromosomes, dissolution of the nuclear lamina and envelope breakdown, reorganization of actin filaments, and (by unknown means) reorganization of microtubules into the spindle.

The culmination of the cell cycle is the act of division, when the chromosomes segregate into two diploid sets and the other components of the cell are partitioned between the two daughter cells. The change in cell structure is dramatic, as summarized in **Figure 29.39**. The division between nucleus and cytoplasm is abolished, and the cytoskeleton is entirely reorganized. The relevant events include:

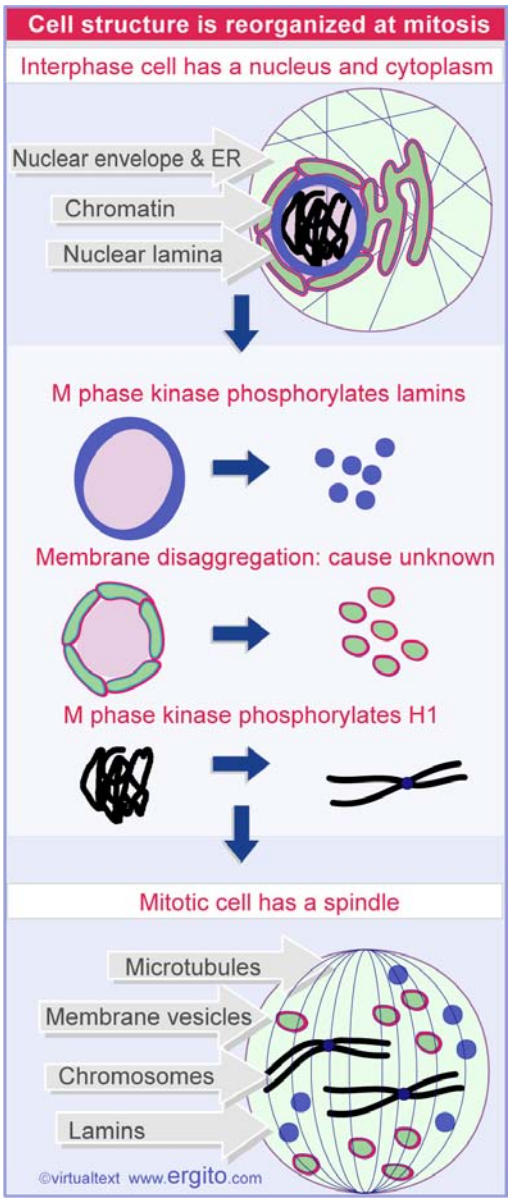

Figure 29.39 Major changes in the cell at mitosis involve the chromosomes, nuclear envelope, nuclear lamina, and microtubules.

- Condensation of chromatin to give recognizable chromosomes.
- Dissolution of the nuclear lamina and breakdown of the nuclear envelope. The lamina dissociates into individual lamin subunits, and the nuclear envelope, endoplasmic reticulum, and Golgi apparatus break down into small membrane vesicles. (Nuclear dissolution is typical of animal cells but does not occur in some lower eukaryotes, including yeasts, where mitosis involves nuclear division.)
- Dissociation and reconstruction of microtubules into a **spindle**. **Microtubules**

dissociate into tubulin dimers, which reassemble into microtubules extended from the mitotic microtubule organizing centers.

- Reorganization of actin filaments to replace the usual network by the contractile ring that pinches the daughter cells apart at cytokinesis.

All of these changes are reversible; following the separation of daughter cells, the actin filaments resume their normal form, the microtubular spindle is dissolved, the nuclear envelope reforms, and chromosomes take a more dispersed structure in the form of interphase chromatin. Modification of appropriate substrate proteins (which could be either the structural subunits themselves or proteins associated with them) provides a plausible means to control the passage of mitosis. The question then becomes how the mitotic changes and their reversal depend upon the activation and inactivation of M phase kinase (for review see 320; 330).

The best characterized substrate for M phase kinase is histone H1. As noted previously, we do not know what role the phosphorylation of H1 plays at either G1/S or M phase transitions. It is a reasonable assumption, however, that M phase kinase acts directly on chromatin by phosphorylating H1 and other target proteins, and that this is the cause of chromosomal condensation.

Two types of event have been implicated in nuclear envelope breakdown (sometimes abbreviated to NEBD). They affect the two components of the envelope: the membrane and the underlying lamina.

Mechanical effects are caused by the growth of microtubules into the envelope. This occurs when the centrosomes (the microtubule nucleating centers) move into the envelope at the start of mitosis. The motor dynein pulls the envelope along the microtubules. The stress causes the envelope to tear, and may also disrupt the structure of the lamina (2401; 2402).

Nuclear integrity is abandoned when the lamina dissociates into its constituent lamins. The lamina is a dense network of fibers just underneath the inner nuclear membrane. It is responsible for maintaining the shape of the nucleus (for review see 2425). The components of the lamina are three types of lamins, each of which has a domain structure similar to that of the protein subunits of the intermediate filaments that are found in the cytoplasm. The importance of lamins is emphasized by the existence of human diseases resulting from mutations in lamin genes, although we cannot yet relate the phenotypes of the diseases to the molecular functions of the lamins.

Lamins are phosphorylated at the start of mitosis, and the presence of phosphate groups on only two serine residues per lamin is sufficient to cause dissociation of the lamina. Mutations that change these serines into alanines prevent the lamina from dissociating at mitosis. So the reversible phosphorylation of these two serine residues induces a structural change in the individual lamin subunits that controls their ability to associate into the lamina (841; 842).

The combined effects of tearing the membrane and dissociating the lamina generate a series of vesicles containing the membranes. The process is reversed at the end of

mitosis, when lamins are closely involved in reassembly of the nuclear membrane.

Nuclear pore complexes dissociate from the envelope during mitosis. Nuclear pore components are phosphorylated, and as a result are released from the pores and dispersed in the cell (2403).

These phosphorylation events are the direct responsibility of the M phase kinase, which can cause the nuclear lamina to dissociate *in vitro.* The reorganization of the endoplasmic reticulum and Golgi is not well defined.

Last updated on 4-18-2002

Reviews

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CELL CYCLE AND GROWTH REGULATION

6.29.22 The spindle is oriented by centrosomes

Key Terms

Centrosomes are the regions from which microtubules are organized at the poles of a mitotic cell. In animal cells, each centrosome contains a pair of centrioles surrounded by a dense amorphous region to which the microtubules attach.

The **kinetochore** is the structural feature of the chromosome to which microtubules of the mitotic spindle attach. Its location determines the centromeric region.

A **centriole** is a small hollow cylinder consisting of microtubules. It occurs in the centrosome (a type of microtubule organizing center) and is thought to play a role in organizing the microtubules.

The **procentriole** is an immature centriole, formed in the vicinity of a mature centriole.

Key Concepts

- Microtubule assembly is nucleated by complexes located within the centrosomes that contain γ-tubulin.
- The centrioles duplicate by a mechanism involving orientation of the daughter relative to the parent.

The reorganization of microtubules into the spindle has been extensively described, but cannot yet be connected to the action of M phase kinase. Some microtubules extend from pole to pole, others connect the chromosomes to the poles. The ends of each microtubules are attached to MTOCs – microtubule organizing centers. The MTOCs at the poles lie in the regions of the **centrosomes**. The MTOC on a chromosome is located at the **kinetochore**.

The structure of centrosomes is not well defined, but in animal cells a centrosome contains a pair of **centrioles**, surrounded by a dense amorphous region. The centriole is a small hollow cylinder whose wall consists of a series of triplet fused microtubules. A centriole is shown in cross-section in **Figure 29.40**.

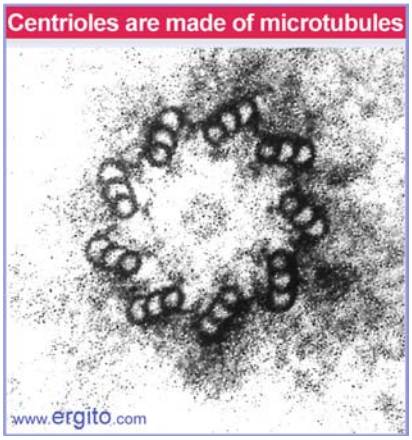

Figure 29.40 The centriole consists of nine microtubule triplets, apparent in cross-section as the wall of a hollow cylinder. Photograph kindly provided by A. Ross.

The function of the centriole in mitosis is not clear. Originally it was thought that it might provide the structure to which microtubules are anchored at the pole, but the fibers seem instead to terminate in the amorphous region (the pericentriolar material) around the centrioles. It is possible that the centriole is concerned with orienting the spindle; it may also have a role in establishing directionality for cell movement. However, there are cell types in which centrosomes do not appear to contain centrioles.

Centrioles have their own cycle of duplication. When born at mitosis, a cell inherits two centrioles. During interphase they reproduce, so that at the start of mitosis there are four centrioles, two at each pole. Probably only the parental centriole is functional.

The centriole cycle is illustrated in **Figure 29.41**. Soon after mitosis, a **procentriole** is elaborated perpendicular to the parental centriole. It has the same structure as the mature parental centriole, but is only about half its length. Later during interphase, it is extended to full length. It plays no role in the next mitosis, but becomes a parental centriole when it is distributed to one of the daughter cells. The orientation of the parental centriole at the mitotic pole is responsible for establishing the direction of the spindle.

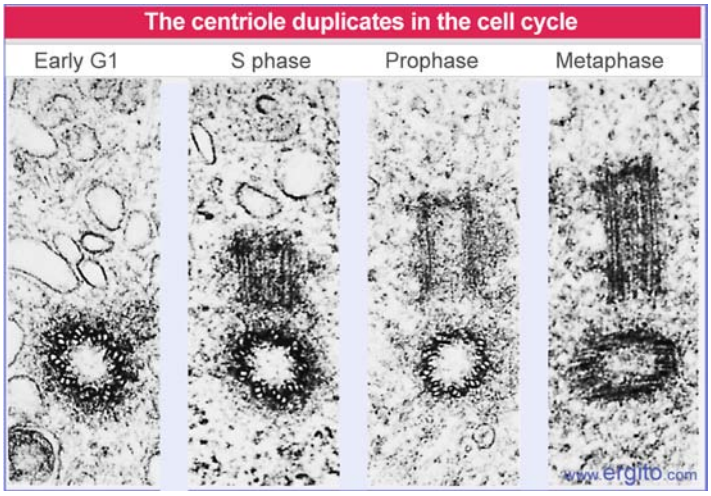

Figure 29.41 A centriole reproduces by forming a procentriole on a perpendicular axis; the procentriole is subsequently extended into a mature centriole. Photograph kindly provided by J. B. Rattner and S. G. Phillips.

How are centrioles reproduced? The precise elaboration of the procentriole adjacent to the parental centriole suggests that some sort of template function is involved. The parental centriole cannot itself be seen to reproduce or divide, but it could provide some nucleating structure onto which tubulin dimers assemble to extend the procentriole. Could a centriole be assembled in the absence of a pre-existing centriole?

Microtubules consist of hollow filaments made of 13 protofilaments that are constructed from dimers of α-tubulin and β-tubulin. Within the centrosome there is a related protein, γ-tubulin, which is part of a complex that provides the actual nucleating source for the microtubules (for review see 317; 318). The complex is large, sedimenting at ~25S, and contains several other proteins in addition to γ-tubulin. The complex can nucleate the formation of microtubules from α-tubulin and β-tubulin *in vitro*. The complex takes the form of a ring, and probably the γ-tubulin-containing complex nucleates microtubules through some sort of end-binding mechanism (1591).

The spindle is generally nucleated by the centrosomes, although cells can in fact generate spindles by other pathways in the absence of centrosomes. In addition to its mechanical role in cell reorganization, a centrosome is a regulatory target. Centrosome duplication is regulated during the cell cycle, and there is a checkpoint to stop the cycle proceeding until centrosome duplication has occurred. The components of the centrosome involved in nucleating microtubules are beginning to be defined, but the components involved in regulation mostly remain to be described (for review see 3424).

Last updated on 1-30-2003

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CELL CYCLE AND GROWTH REGULATION

6.29.23 A monomeric G protein controls spindle assembly

Key Concepts

- The active form of the G protein Ran (Ran-GTP) causes importin dimers to release proteins that trigger microtubule nucleation.
- The Ran-activating protein RCC is localized on chromosomes, generating a high local concentration of Ran-GTP.
- The proteins released by the importins have several different functions that assist microtubule nucleation.

The trigger for the reorganization of microtubules from the interphase network into the spindle may be the breakdown of the nuclear envelope, which exposes nuclear components to cytoplasmic components. Indirect evidence has been available for some time to indicate a connection, but only recently has a molecular mechanism been suggested. The important point here is that the ability of an MTOC to nucleate microtubules must be *controlled*, so that it happens only in the right time and place.

The critical component is a monomeric G protein called Ran, which controls the direction of protein transport through the nuclear envelope. Like all members of its class, Ran is active when bound to GTP, and inactive when bound to GDP (see *Molecular Biology Supplement 32.10 G proteins*). Conditions in the nucleus and cytosol differ so that typically there is Ran-GTP in the nucleus, but there is Ran-GDP in the cytosol. Protein export complexes are stable in the presence of Ran-GTP, whereas import complexes are stable in the presence of Ran-GDP. So export complexes are driven to form in the nucleus and dissociate in the cytosol, whereas the reverse is true of import complexes (see *Molecular Biology 2.8.28 Transport receptors carry cargo proteins through the pore*).

Mutations in some proteins that bind to Ran cause the spindle to malfunction, and overexpression of the protein RanBPM (another Ran-binding protein) causes the formation of ectopic asters – arrays of microtubules emanating from centrosomes. The usual assay for these experiments is to inject demembranated sperm into *Xenopus* eggs. The sperm centrioles assemble into centrosomes that nucleate microtubule asters. Using this assay identifies proteins that can stimulate nucleation. These include a mutant of Ran and the protein RCC that maintains Ran in the GTP-bound active state (1592; 1593). The most likely explanation is that the breakdown of the nuclear envelope releases Ran-GTP, which then triggers microtubule nucleation by centrosomes.

Does Ran act directly or indirectly? One of the targets for Ran in the nuclear transport process is the import receptor importin- β, which (in combination with importin- α) transports cargo proteins from the cytoplasm to the nucleus. It turns out that the importin dimer binds to proteins that affect microtubules. One of these

proteins is Xklp2, which connects a motor (a protein that moves other proteins) to microtubules at the poles; another is NuMA which cross links microtubules at the poles during mitosis. When the complex of importins with either of these proteins is exposed to Ran-GTP, it dissociates, releasing the cargo protein, which can then act to trigger microtubule nucleation (1594; 1595).

How does the exposure of the importins to Ran-GTP change at mitosis? **Figure 29.42** shows that the situation in the cytoplasm of the interphase cell, and then correspondingly in the spindle, is that Ran is predominantly in the form of Ran-GDP, and therefore does not affect the importin complex. But there are localized areas where Ran-GTP is formed. The Ran-activating protein RCC is located on chromatin, so Ran-GTP forms in the vicinity of the chromosomes. This releases the proteins that are bound to the importin dimer, which activate the kinetochores to connect to microtubules.

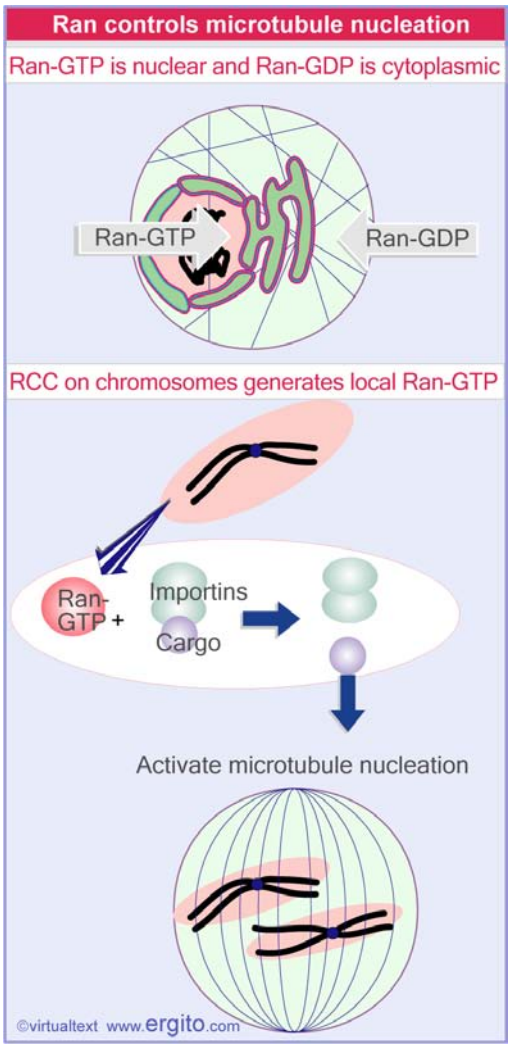

Figure 29.42 The Ran-activating protein RCC is localized on chromosomes. Ran-GTP is high in the nucleus in the interphase cell. When the nuclear envelope breaks down, RCC maintains a high level of Ran-GTP in the vicinity of the chromosomes. This causes importin dimers to release the proteins bound to them. These proteins cause microtubule nucleation.

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CELL CYCLE AND GROWTH REGULATION

6.29.24 Daughter cells are separated by cytokinesis

Key Terms

Cytokinesis is the process involved in separation and movement apart of daughter cells. Cytokinesis occurs at the end of mitosis.

The constriction in the cell cortex that separates newly reformed nuclei after mitosis and results in the formation of two cells is the **cleavage furrow**.

The **contractile ring** is a ring of actin filaments that forms around the equator at the end of mitosis and is responsible for pinching the daughter cells apart.

The **midbody** is the last connection between two cells as they separate at the end of cytokinesis. A parallel array of microtubules derived from the mitotic spindle enters the midbody from each cell, and the two arrays interdigitate across its whole width.

Once the two sets of daughter chromosomes have been separated at the poles, the cell must complete its division by physically separating into two parts. This process is called **cytokinesis**, and it passes through the stages illustrated in **Figure 29.43** (for review see 2303).

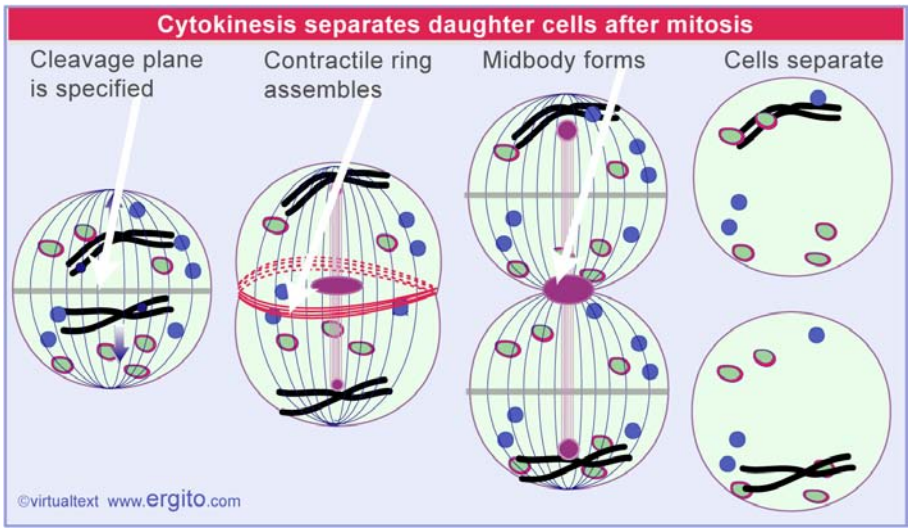

Figure 29.43 The spindle specifies the cleavage plane where the contractile ring assembles, the midbody forms in the center, and then the daughter cells separate.

The plane for division forms in the center of the spindle. We do not know exactly how its position is defined, but it seems to depend on the microtubules arrays that run to the poles. A local event that may be needed is the activation of the RhoA monomeric G protein. (This is one of the monomeric G proteins that controls actin filament behavior in the interphase cell; see **Figure 28.35** in *Molecular Biology 6.28.15 The activation of Ras is controlled by GTP*).

An invagination called the **cleavage furrow** appears in the plasma membrane soon after the start of anaphase. This is caused by the formation of the **contractile ring**, which forms from actomyosin fibers. It extends around the equator of the dividing cell and then pinches inward until it contacts a group of microtubules that run between the poles. This forms a structure connecting the future daughter cells that is called the **midbody**.

The final step in cytokinesis is to cut the cytoplasmic connection between the two cells by "resolving" the midbody. This requires changes in the organization of the plasma membrane, but we cannot yet account for these events at the molecular level.

Last updated on 2-6-2002

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CELL CYCLE AND GROWTH REGULATION

6.29.25 Apoptosis is a property of many or all cells

Key Terms

Apoptosis (Programmed cell death) is the capacity of a cell to respond to a stimulus by initiating a pathway that leads to its death by a characteristic set of reactions.

Key Concepts

- All cells possess the pathways that can cause death by apoptosis, which requires RNA and protein synthesis by the dying cell, but the pathway is activated only by appropriate stimuli.

During development of a multicellular eukaryotic organism, *some cells must die*. Unwanted cells are eliminated during embryogenesis, metamorphosis, and tissue turnover. This process is called **programmed cell death** or **apoptosis**. It provides a crucial control over the total cell number. In the worm *C. elegans* (in which somatic cell lineages have been completely defined), 131 of the 1090 adult somatic cells undergo programmed cell death – cells die predictably at a defined time and place in each animal. Similar, although less precisely defined, cell deaths occur during vertebrate development, most prominently in the immune system and nervous system. The proper control of apoptosis is crucial in probably all higher eukaryotes.

Apoptosis involves the activation of a pathway that leads to suicide of the cell by a characteristic process in which the cell becomes more compact, blebbing occurs at the membranes, chromatin becomes condensed, and DNA is fragmented (see **Figure 29.44**). *The pathway is an active process that depends on RNA and protein synthesis by the dying cell*. The typical features of a cell as it becomes heteropycnotic (condensed with a small, fragmented nucleus) are shown in **Figure 29.45**, and the course of fragmentation of DNA is shown in **Figure 29.46**. Ultimately the dead cells become fragmented into membrane-bound pieces, and may be engulfed by surrounding cells (for review see 324).

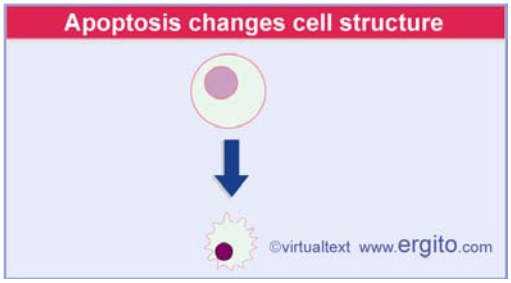

Figure 29.44 The nucleus becomes heteropycnotic and the cytoplasm blebs when a cell apoptoses.

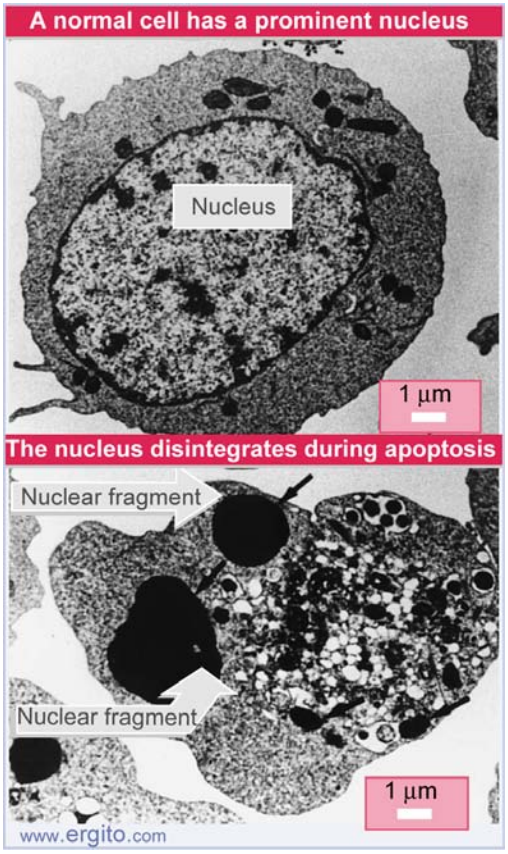

Figure 29.45 Cell structure changes during apoptosis. The top panel shows a normal cell. The lower panel shows an apoptosing cell; arrows indicate condensed nuclear fragments. Photographs kindly provided by Shigekazu Nagata.

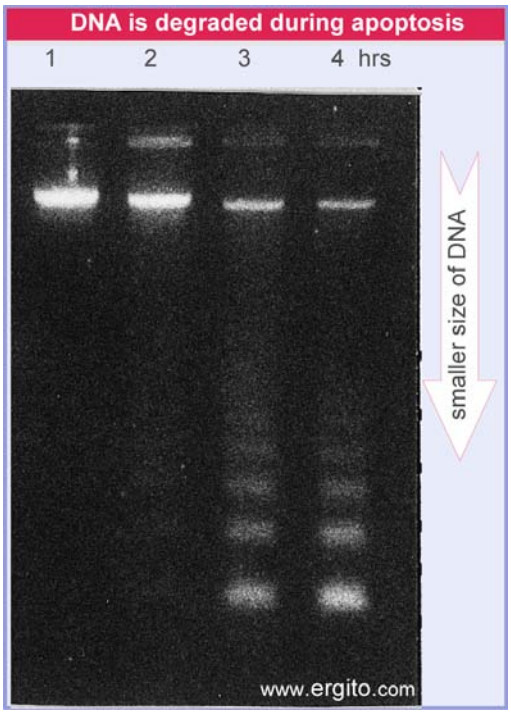

Figure 29.46 Fragmentation of DNA occurs ~2 hours after apoptosis is initiated in cells in culture. Photograph kindly provided by Shigekazu Nagata.

Apoptosis can be triggered by a variety of stimuli, including withdrawal of essential growth factors, treatment with glucocorticoids, γ-irradiation, and activation of certain receptors, as summarized in **Figure 29.47**. These all involve a molecular insult to the cell. Another means of initiating apoptosis is used in the immune system, where cytotoxic T lymphocytes attack target cells. Apoptosis is also an important mechanism for removing tumorigenic cells; the ability of the tumor suppressor p53 to trigger apoptosis is a key defense against cancer (see *Molecular Biology 6.30.20 Tumor suppressor p53 suppresses growth or triggers apoptosis*). Apoptosis is important, therefore, not only in tissue development, but in the immune defense and in the elimination of cancerous cells. Also, inappropriate activation of apoptosis is involved in neurodegenerative diseases.

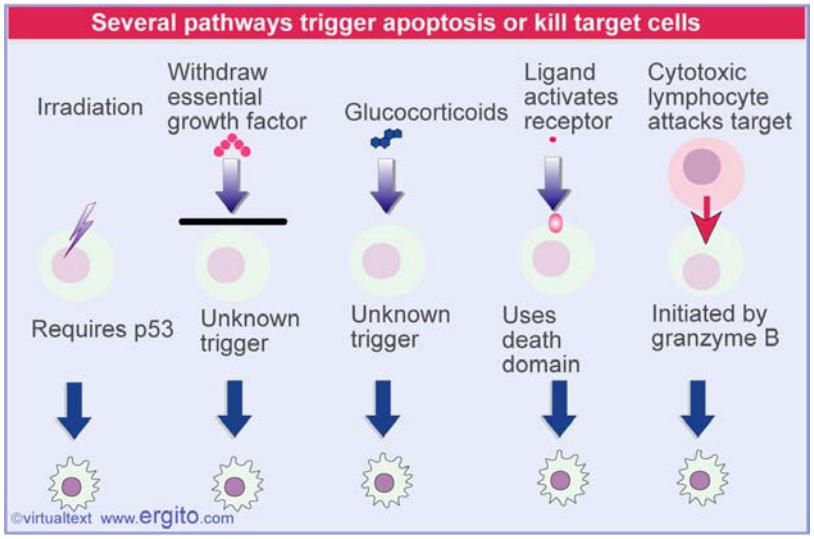

Figure 29.47 Apoptosis is triggered by a variety of pathways.

Reviews

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CELL CYCLE AND GROWTH REGULATION

6.29.26 The Fas receptor is a major trigger for apoptosis

Key Terms

The **death domain** is a protein-protein interaction motif found in certain proteins of the apoptotic pathway.

Key Concepts

- The Fas receptor on a target cell is activated by interaction with the FasL protein on an activating cell plasma membrane.
- Fas is related to TNF receptor, and FasL is related to TNF.
- Fas is a trimer that aggregates upon interaction with FasL.
- Fas has an cytoplasmic domain called the "death domain" which is involved in protein-protein interactions.

The Fas receptor (called Fas or FasR) and Fas ligand (FasL) are a pair of plasma membrane proteins whose interaction triggers one of the major pathways for apoptosis. **Figure 29.48** shows that the cell bearing the Fas receptor apoptoses when it interacts with the cell carrying the Fas ligand (for review see 969).

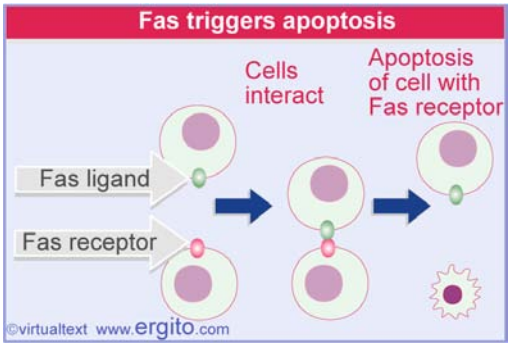

Figure 29.48 The Fas receptor and ligand are both membrane proteins. A target cell bearing Fas receptor apoptoses when it interacts with a cell bearing the Fas ligand.

Activation of Fas resembles other receptors in involving an aggregation step. However, **Figure 29.49** shows that there are some interesting differences from the growth receptor model. First, Fas forms a homomeric trimer. Second, the trimer assembles *before* the interaction with ligand. The effect of ligand may be to cause the trimers to cluster into large aggregates (1067; 1068). At all events, when FasL interacts with Fas, there is an aggregation event that enables Fas to activate the next stage in the pathway.

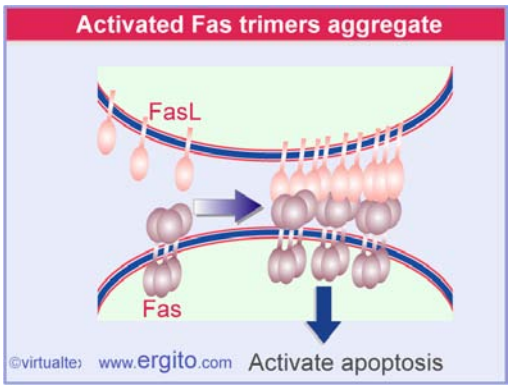

Figure 29.49 Fas forms trimers that are activated when binding to FasL causes aggregation.

The names of the two proteins (Fas receptor and Fas ligand) reflect the way the system was discovered. An antibody directed against Fas protein kills cells that express Fas on their surface. The reason is that the antibody-Fas reaction activates Fas, which triggers a pathway for apoptosis. This defines Fas as a receptor that activates a cellular pathway.

Fas is a cell surface receptor related to the TNF (tumor necrosis factor) receptor. The FasL ligand is a transmembrane protein related to TNF. A family of related receptors includes two TNF receptors, Fas, and several receptors found on T lymphocytes. A corresponding family of ligands comprises a series of transmembrane proteins. This suggests that there are several pathways, each of which can be triggered by a cell-cell interaction, in which the "ligand" on one cell surface interacts with the receptor on the surface of the other cell. Both the Fas- and TNF-receptors can activate apoptosis.

Both of the Fas and TNF ligands are initially produced as membrane-bound forms, but can also be cleaved to generate soluble proteins, which function as diffusible factors. The soluble form of TNF is largely produced by macrophages, and is a pleiotropic factor that signals many cellular responses, including cytotoxicity. Most of its responses are triggered by interaction with one of the TNF receptors, TNF-R1. FasL is cleaved to generate a soluble form, but the soluble form is much less active than the membrane-bound form, so the reaction probably is used to reduce the activity of the cell bearing the ligand.

An assay for the capacity of the ligand-receptor interaction to trigger apoptosis is to introduce the receptor into cultured cells that do not usually express it. On treatment with the ligand, the transfected cells die by apoptosis, but the parental cells do not. Using this assay, similar results are obtained with FasL/Fas receptor and with TNF/TNF-R1. Mutant versions of the receptor show that the apoptotic response is triggered by an ~80 amino acid intracellular domain near the C-terminus. This region is loosely conserved (~28%) between Fas and TNF-R1, and is called the **death domain** (854).

An assay for components of the apoptotic pathway in the cell is to see whether their overexpression causes apoptosis. This is done by transfecting the gene for the protein into the cell (which results in overexpression of the protein). This assay identifies several proteins that interact specifically with the Fas and/or TNF receptors. All of

these proteins themselves have death domains, and it is possible that a homomeric interaction between two death domains provides the means by which the signal is passed from the receptor to the next component of the pathway.

The validity of this pathway *in vivo* was demonstrated by the discovery of the mouse mutation *lpr*. This is a recessive mutation in the gene for Fas. It causes proliferation of lymphocytes, resulting in a complex immune disorder affecting both B cells and T cells. Another mutation with similar effects is *gld* (generalized lymphoproliferative disease). This turns out to lie in the gene that codes the FasL ligand. The related properties of these two loci suggest that this apoptotic pathway is triggered by an interaction between the FasL ligand (*gld* product) and Fas (*lpr* product). The pathway is required for limiting the numbers of mature lymphocytes (851; 852; 853).

Last updated on 8-22-2000

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CELL CYCLE AND GROWTH REGULATION

6.29.27 A common pathway for apoptosis functions via caspases

Key Terms

Caspases comprise a family of protease some of whose members are involved in apoptosis (programmed cell death).

Key Concepts

- Caspases are proteases that are involved in multiple stages of the apoptotic pathway.
- Caspases are synthesized as inactive procaspases that are activated by autocleavage to form the active dimer.
- A complex forms at the Fas or TNF receptor that activates caspase-8 to initiate the intracellular pathway.

The "classical" pathway for apoptosis is summarized in **Figure 29.50**. A ligand-receptor interaction triggers the activation of a protease. This leads to the release of cytochrome *c* from mitochondria. This in turn activates a series of proteases, whose actions culminate in the destruction of cell structures (for review see 1003).

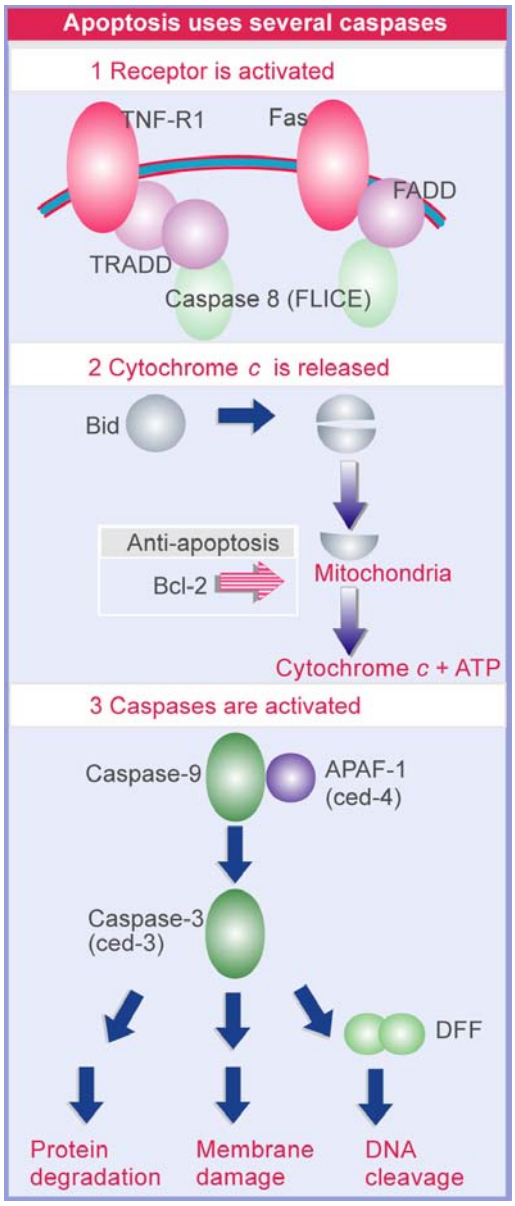

Figure 29.50 Apoptosis can be triggered by activating surface receptors. Caspase proteases are activated at two stages in the pathway. Caspase-8 is activated by the receptor. This leads to release of cytochome *c* from mitochondria. Apoptosis can be blocked at this stage by Bcl-2. Cytochrome *c* activates a pathway involving more caspases.

A complex containing several components forms at the receptor. The exact components of the complex depends on the receptor (for review see 2264). TNF receptor binds a protein called TRADD, which in turn binds a protein called FADD. Fas receptor binds FADD directly.**Figure 29.51** shows that, in either case, FADD binds the protein caspase-8 (also known as FLICE), which has a death domain as well as protease catalytic activity. The activation of caspase-8 activates a common pathway for apoptosis. The trigger for the activation event is the oligomerization of the receptor. In the case of the Fas system, the interaction of FasL with Fas causes

the Fas trimers to interact, activating the pathway.

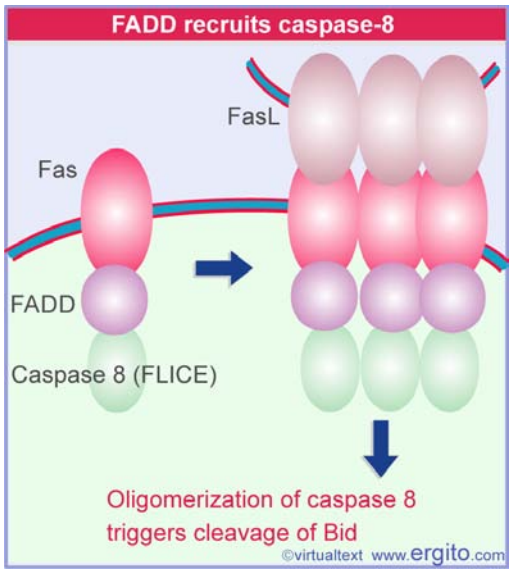

Figure 29.51 The TNF-R1 and Fas receptors bind FADD (directly or indirectly). FADD binds caspase-8. Activation of the receptor causes oligomerization of caspase-8, which activates the caspase.

Members of the **caspase** family (cysteine aspartate proteases) are important downstream components of the pathway. Caspases have a catalytic cysteine, and cleave their targets at an aspartate. Individual enzymes have related, but not identical targets. For example, caspase-3 and ICE both cleave at tetrapeptide sequences in their substrates, but caspase-3 recognizes YVAD and ICE recognizes DEVD. There are ~14 mammalian members of the caspase family.

Caspases fall into two groups. The caspase-1 subfamily is involved in the response to inflammation. The caspase-3 subfamily (consisting of caspase 3 and caspases 6-10) is involved in apoptosis. All caspases are synthesized in the form of inactive procaspases, which have additional sequences at the N-terminus. **Figure 29.52** shows that the activation reaction involves cleavage of the prodomain followed by cleavage of the caspase sequence itself into a small subunit and large subunit. All procaspases except procaspase-9 probably exist as dimers.

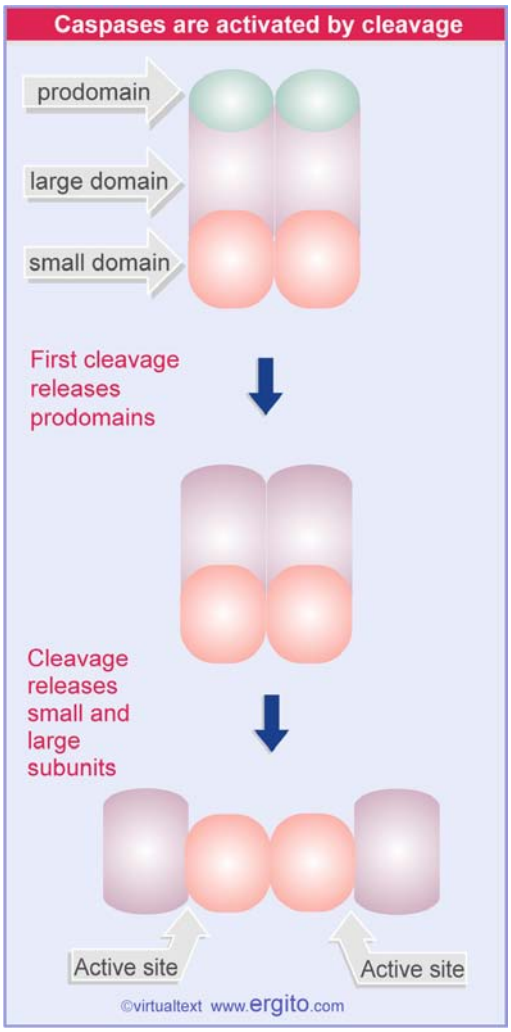

Figure 29.52 Caspase activation requires dimerization and two cleavages.

Caspases with large prodomains are involved in initiating apoptosis. Dimerization causes an autocatalytic cleavage that activates the caspase (for review see 992). The prodomain of caspase-8 has two death domain motifs that are responsible for its association with the receptor complex. Cleavage to the active form occurs as soon as procaspase-8 is recruited to the receptor complex (1007; 1008).

Caspases with small prodomains function later in the pathway. The first in the series is activated by an autocleavage when it forms an oligomer. Others later in the pathway typically are activated when another caspase cleaves them .

The first caspase to be discovered (ICE=caspase-1) was the IL-1 β-converting enzyme, which cleaves the pro-IL-1 β precursor into its active form. Although this caspase is usually involved with the inflammatory response, transfection of ICE into cultured cells causes apoptosis. The process is inhibited by CrmA (a product of cowpox virus). All caspases are inhibited by CrmA, although each caspase has a characteristic sensitivity. CrmA inhibits apoptosis triggered in several different ways, which demonstrates that the caspases play an essential role in the pathway,

irrespective of how it is initiated. However, it turns out that ICE is not itself the protease commonly involved in apoptosis, because inactivation of the gene for ICE does not block general apoptosis in the mouse. [The ability of ICE to cause apoptosis demonstrates a danger of the transfection assay: overexpression may allow it to trigger apoptosis, although usually it does not do so. But ICE may be needed specifically for apoptosis of one pathway in lymphocytes (855).]

Last updated on 1-31-2002

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CELL CYCLE AND GROWTH REGULATION

6.29.28 Apoptosis involves changes at the mitochondrial envelope

Key Concepts

- Caspase-8 cleaves Bid to release a C-terminal domain that translocates to the mitochondrion.
- Bid is a member of the Bcl2 family and acts together with other members of the family to cause mitochondria to release cytochrome *c*.
- Some members of the family, including Bcl2, inhibit the release of cytochrome *c*.

Changes in mitochondria occur during apoptosis (and also during other forms of cell death). These are typically detected by changes in permeability. The breakthrough in understanding the role of mitochondria was the discovery that cytochrome *c* is released into the cytosol (see *Great Experiments 7.1 The role of cytochrome c in apoptosis*) (1009). **Figure 29.53** summarizes the central role of the mitochondrion (for review see 2431). In addition to releasing cytochrome *c*, it also releases other proteins from its intermembrane space that may either promote or inhibit apoptosis.

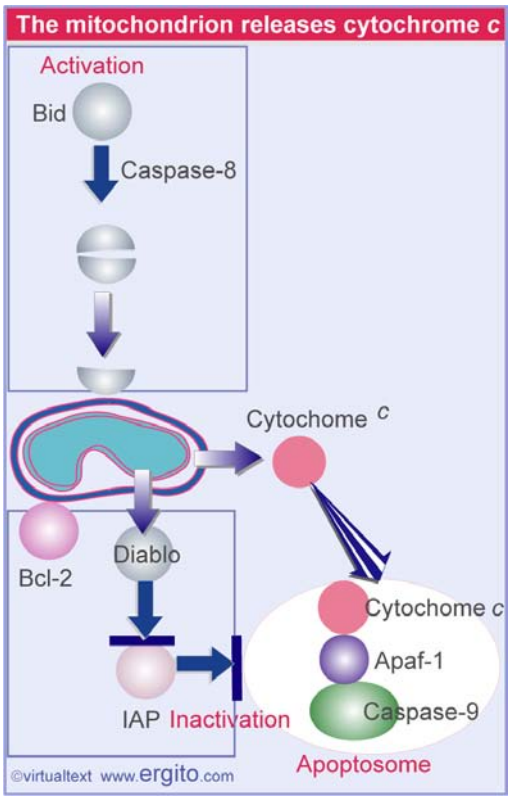

Figure 29.53 The mitochondrion plays a central role in apoptosis by releasing cytochrome *c*. This is activated by BID. It is inactivated by Bcl-2. Cytochrome *c* binds to Apaf-1 and (pro)-Caspase-9 to form the apoptosome. The proteolytic activity of caspase-9 (and other caspases) can be inhibited by IAP proteins. Proteins that antagonize IAPs may be released from the mitochondrion.

The pathway moves from the plasma membrane to the mitochondrion when caspase-8 cleaves a protein called Bid. The cleavage releases the C-terminal domain, which then translocates to the mitochondrial membrane. The action of Bid causes cytochrome *c* to be released (1011; 1012).

Bid is a member of the important Bcl2 family. Some members of this family are required for apoptosis, while others counteract apoptosis. The eponymous Bcl2 inhibits apoptosis in many cells. It has a C-terminal membrane anchor, and is found on the outer mitochondrial, nuclear, and ER membranes. It prevents the release of cytochrome c, which suggests that in some way it counteracts the action of Bid.

bcl2 was originally discovered as a proto-oncogene that is activated in lymphomas by translocations resulting in its overexpression. (As discussed in more detail in *Molecular Biology 6.30 Oncogenes and cancer*, this means that Bcl2 is a member of a class of proteins that causes proliferation or tumorigenesis when inappropriately expressed.) Its role as an inhibitor of apoptosis was discovered when it was shown that its addition protects cultured lymphoid and myeloid cells from dying when the essential factor IL-3 is withdrawn.

Mammalian cells that are triggered into apoptosis by a wide variety of stimuli, including activation of the Fas/TNF-R1 pathways, can be rescued by expression of Bcl2. This suggests that these pathways converge on a single mechanism of cell killing, and that Bcl2 functions at a late, common stage of cell death. There are some systems in which Bcl2 cannot block apoptosis, so the pathway that it blocks may be common, but is not the only one.

Bcl2 belongs to a family whose members can homodimerize and heterodimerize. Two other members are bcl-x (characterized in chicken) and Bax (characterized in man). bcl-x is produced in alternatively spliced forms that have different properties. When transfected into recipient cells, bcl-x_L mimics Bcl2, and inhibits apoptosis. But bcl-x_S counteracts the ability of Bcl2 to protect against apoptosis. Bax behaves in the same way as bcl-x_S. This suggests that the formation of Bcl2 homodimers may be needed to provide the protective form, and that Bcl2/Bax or Bcl2/bcl-x_S heterodimers may fail to protect. Whether Bax or bcl-x_S homodimers actively assist apoptosis, or are merely permissive, remains to be seen. The general conclusion suggested by these results is that combinatorial associations between members of the family may produce dimers with different effects on apoptosis, and the relative proportions of the family members that are expressed may be important. The susceptibility of a cell to undergo apoptosis may be proportional to the ratio of Bax to Bcl2 (for review see 334).

The mitochondrion is a crucial control point in the induction of apoptosis (for review see 4520. The release of cytochrome *c* is preceded by changes in the permeability of the mitochondrial membrane (1013). Bcl2 family members act at the mitochondrial membrane, and although their mode of action is not known, one possibility is that they form channels in the membrane. Apoptosis involves localization (or perhaps increased concentration) of Bcl2 family members at the mitochondrial membrane, including Bid (required to release cytochrome *c*) and Bax (perhaps involved in membrane permeability changes).

Last updated on 4-30-2002

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CELL CYCLE AND GROWTH REGULATION

6.29.29 Cytochrome *c* activates the next stage of apoptosis

Key Terms

IAPs are inhibitors of apoptosis. They function by antagonizing the actions of caspases.

Key Concepts

- Cytochrome *c* causes Apaf-1 to aggregate with procaspase-9 to form the autosome, which then activates caspase-9 by autocleavage.
- Caspase-9 cleaves caspase-3 and other caspases to trigger the effector phase of apoptosis, when cellular structures are destroyed.

The release of cytochrome *c* is a crucial control point in the pathway (for review see 2431). The basic role of cytochrome *c* is to trigger the activation of caspase-9 (858). **Figure 29.54** shows the stages between cytochrome *c* release and caspase-9 activation. Cytochrome *c* triggers the interaction of the cytosolic protein Apaf-1 with caspase-9 in a complex called the apoptosome. The reaction takes place in several stages. Cytochrome *c* binds to Apaf-1. This enables Apaf-1 to bind ATP. This in turn enables it to oligomerize, which causes a change of conformation that exposes the caspase-binding domain; then Apaf-1 binds procapase-9. The incorporation of procaspase-9 into the apoptosome triggers the auto-activating cleavage (1010).

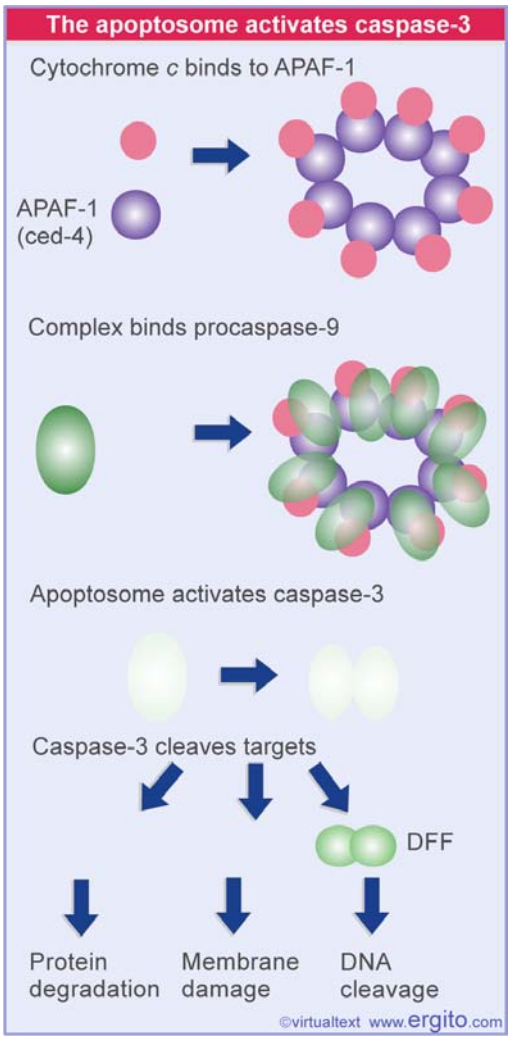

Figure 29.54 Cytochrome *c* causes Apaf-1 to interact with caspase-9, which activates caspase-3, which cleaves targets that cause apoptosis of the cell.

The properties of mice lacking Apaf-1 or caspase-9 throw some light upon the generality of apoptotic pathways. Lack of caspase-9 is lethal, because the mice have a malformed cerebrum as the result of the failure of apoptosis. Apoptosis is also reduced in thymocytes (immune precursors to lymphocytes). Apaf-1 deficient mice have less severe defects in brain development, implying that there are alternative means for activating caspase-9. Both types of deficient mice continue to show Fas-mediated apoptosis, implying that Fas has alternative means of triggering apoptosis.

Caspase-9 in turn cleaves procaspase-3 to generate caspase-3 (which is in fact the best characterized component of the downstream pathway. Caspase-3 is the homologue of the *C. elegans* protein ced-3; see below). Caspase-9 also activates caspases-6 and 7.

Caspase-3 acts at what might be called the effector stage of the pathway. We have not identified all of the targets of the protease activity that are essential for apoptosis.

One known target is the enzyme PARP (poly[ADP-ribose] polymerase). Its degradation is not essential, but is a useful diagnostic for apoptosis.

One pathway that leads to DNA fragmentation has been identified. Caspase-3 cleaves one subunit of a dimer called DFF (DNA fragmentation factor). The other subunit then activates a nuclease that degrades DNA (856). However, the degradation of DNA by this pathway does not appear to be necessary for cell death, which continues in mice that lack the enzyme (1926).

Another pathway for DNA degradation is triggered directly by release of an enzyme from the mitochondrion. The normal function of endonuclease G within the mitochondrion is concerned with DNA replication. However, in apoptosing cells it is released from the mitochondrion, and then degrades nuclear DNA (1925). Interference with the function of the corresponding gene in *C. elegans* reduces DNA degradation and delays the appearance of cell corpses (1924). This enzyme therefore appears to be important at least for the time course of apoptosis, even if it is not necessary for the eventual death of the cell.

The control of apoptosis involves components that inhibit the pathway as well as those that activate it. This first became clear from the genetic analysis of cell death in *C. elegans*, when mutants were found that either activate or inactivate cell death. Mutations in *ced-3* and *ced-4* cause the survival of cells that usually die, demonstrating that these genes are essential for cell death. *ced-3* codes for the protease activity (and was in fact the means by which caspases were first implicated in apoptosis). It is the only protease of this type in *C. elegans*. *ced-4* codes for the homologue to Apaf-1.

ced-9 inhibits apoptosis. It codes for the counterpart of Bcl2. A mutation that inactivates *ced-9* is lethal, because it causes the death of cells that should survive. This process requires *ced-3* and *ced-4*, and this was the original basis for the idea that *ced-9* blocks the apoptotic pathway(s) in which *ced-3* and *ced-4* participate. This relationship makes an important point: *ced-3* and *ced-4* are not expressed solely in cells that are destined to die, but are expressed also in other cells, where normally their action is prevented by *ced-9*. The proper control of apoptosis may therefore involve a balance between activation and inhibition of this pathway.

The apoptotic pathway can also be inhibited at the stages catalyzed by the later caspases. Proteins called **IAP** (inhibitor of apoptosis) can bind to procaspases and activated caspases to block their activities (see **Figure 29.53**). The blocking activities of the AIPs need to be antagonized in order for apoptosis to proceed (for review see 3435). Vertebrate cells contain a protein called Diablo/Smac, which is released from mitochondria at the same time as cytochrome c, and acts by binding to IAPs (1060; 1061).

The existence of mechanisms to inhibit as well as to activate apoptosis suggests that many (possibly even all) cells possess the intrinsic capacity to apoptose. If the components of the pathway are ubiquitous, the critical determinant of whether a cell lives or dies may depend on the regulatory mechanisms that determine whether the pathway is activated or repressed.

Last updated on 4-30-2002

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CELL CYCLE AND GROWTH REGULATION

6.29.30 There are multiple apoptotic pathways

Key Concepts

- Fas activates apoptosis via the caspase pathway and also via the activation of the JNK kinase.

The pathway shown in **Figure 29.50** is the prototypical pathway for activation of apoptosis via a protease cascade. However, Fas can also activate apoptosis by a pathway that involves the kinase JNK, whose most prominent substrate is the transcription factor c-Jun (see **Figure 28.43**). This leads by undefined means to the activation of proteases. **Figure 29.55** shows that this pathway is mediated by the protein Daxx (which does not have a death domain). Binding of FADD and Daxx to Fas is independent: each adaptor recognizes a different site on Fas. The two pathways function independently after Fas has engaged the adaptor. The TNF receptor also can activate JNK by means of distinct adaptor proteins (857).

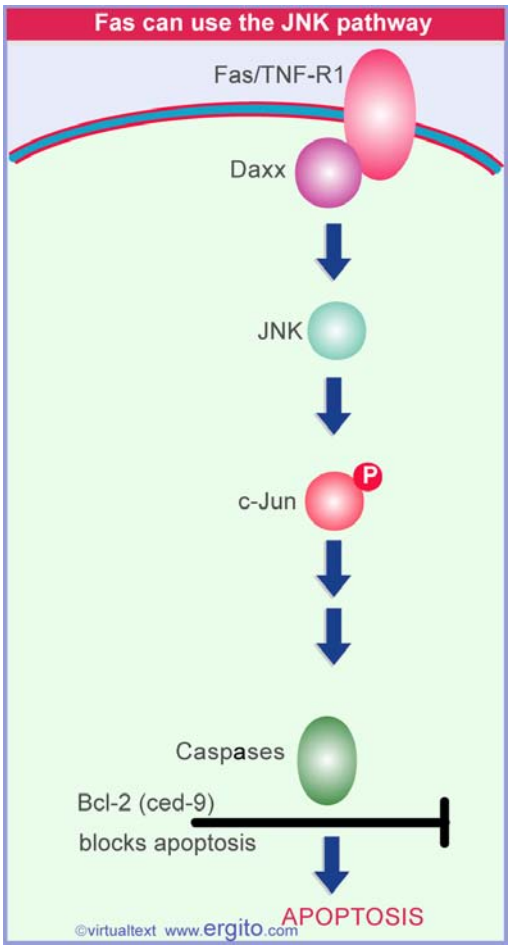

Figure 29.55 Fas can activate apoptosis by a JNK-mediated pathway.

In the normal course of events, activation of Fas probably activates both pathways. Overexpression experiments show that either pathway can cause apoptosis. The relative importance of the two pathways may vary with the individual cell type, in response to other signals that affect each pathway. For example, JNK is activated by several forms of stress independently of the Fas-activated pathway. This pathway is not inhibited by Bcl2, which may explain the variable ability of cells to resist apoptosis in response to Bcl2.

Another apoptotic pathway is triggered by cytotoxic T lymphocytes, which kill target cells by a process that involves the release of granules containing serine proteases and other lytic components. One such component is perforin, which can make holes in the target cell membrane, and under some conditions can kill target cells. The serine proteases in the granules are called granzymes. In the presence of perforin, granzyme B can induce many of the features of apoptosis, including fragmentation of DNA. It activates a caspase called Ich-3, which is necessary for apoptosis in this pathway.

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CELL CYCLE AND GROWTH REGULATION

6.29.31 Summary

The cell cycle consists of transitions from one regulatory state to another. The change in regulatory state is separated by a lag period from the subsequent changes in cell phenotype. The transitions take the form of activating or inactivating a kinase(s), which modifies substrates that determine the physical state of the cell. Checkpoints can retard a transition until some intrinsic or extrinsic condition has been satisfied.

The two key control points in the cell cycle are in G1 and at the end of G2. During G1, a commitment is made to enter a replication cycle; the decision is identified by the restriction point in animal cells, and by START in yeast cells. After this decision has been taken, cells are committed to beginning an S phase, although there is a lag period before DNA replication initiates. The end of G2 is marked by a decision that is executed immediately to enter mitosis.

A unifying feature in the cell cycles of yeasts and animals is the existence of an M phase kinase, consisting of two subunits: Cdc2, with serine/threonine protein kinase catalytic activity; and a mitotic cyclin of either the A or B class. Homologous subunits exist in all eukaryotic cells. The genes that code for the catalytic subunit in yeasts are *cdc2* in *S. pombe* and *CDC28* in *S. cerevisiae*. Animal cells usually contain multiple mitotic cyclins (A, B1, B2); in *S. pombe*, there is only a single cyclin at M phase, a B class coded by *cdc13*, although *S. pombe* has several CLB proteins.

The activity of the M phase kinase is controlled by the state of phosphorylation of the catalytic subunit. The active form requires dephosphorylation on Tyr-15 (in yeasts) or Thr-14/Tyr-15 (in animal cells) and phosphorylation on Thr-161. The cyclins are also phosphorylated, but the significance of this modification is not known. In animal cells, the kinase is inactivated by degradation of the cyclin component, which occurs abruptly during mitosis. Cyclins of the A type are typically degraded before cyclins of the B type. Destruction of at least the B cyclins, and probably of both classes of cyclin, is required for cells to exit mitosis.

A comprehensive analysis of genes that affect the cell cycle has identified *cdc* mutants in both *S. pombe* and *S. cerevisiae*. The best characterized mutations are those that affect the components or activity of M phase kinase. Mutations *cdc25* and *wee1* in *S. pombe* have opposing effects in regulating M phase kinase in response to cell size (and other signals). Wee1 is a kinase that acts on Tyr-15 and maintains Cdc2 in an inactive state; Cdc25 is a phosphatase that acts on Tyr-15 and activates Cdc2. The existence of *wee1* and *cdc25* homologues in higher eukaryotes suggests that the apparatus for cell cycle control is widely conserved in evolution.

By phosphorylating appropriate substrates, the kinase provides MPF activity, which stimulates mitosis or meiosis (as originally defined in *Xenopus* oocytes). A prominent substrate is histone H1, and H1 kinase activity is now used as a routine assay for M phase kinase. Phosphorylation of H1 could be concerned with the need to condense chromatin at mitosis. Another class of substrates comprises the lamins, whose phosphorylation causes the dissolution of the nuclear lamina. A general

principle governing these (and presumably other) events is that the state of the substrates is controlled reversibly in response to phosphorylation, so that the phosphorylated form of the protein is required for mitotic organization, while the dephosphorylated form is required for interphase organization. Phosphatases are required to reverse the modifications introduced by M phase kinase.

Transition from G1 into S phase requires a kinase related to the M phase kinase. In yeasts, the catalytic subunit is identical with that of the M phase kinase, but the cyclins are different (the combinations being CDC28-cig1,2 in *S. cerevisiae*, Cdc2-CLN1,2,3 in *S. pombe*). Activity of the G1/S phase kinase and inactivity of the M phase kinase are both required to proceed through G1. Initiation of S phase in *S. pombe* requires rum1 to inactivate cdc2/cdc13 in order to allow the activation of Cdc18, which may be the S phase activator.

In mammalian cells, a family of catalytic subunits is provided by the *cdk* genes, named because they code for the catalytic subunits of cyclin-dependent kinases. There are ~10 *cdk* genes in an animal genome. Aside from the classic Cdc2, the best characterized product is cdk2 (which is well related to Cdc2). In a normal cell cycle, cdk2 is partnered by cyclin E during the G1/S transition and by cyclin A during the progression of S phase. cdk2 and cdk4 both partner the D cyclins to form kinases that are involved with the transition from G0 to G1. These cdk-cyclin complexes phosphorylate RB, causing it to release the transcription factor E2F, which then activates genes whose products are required for S phase. A group of CKI (inhibitor) proteins that are activated by treatments that inhibit growth can bind to cdk-cyclin complexes, and maintain them in an inactive form.

Checkpoints control progression of the cell cycle. One checkpoint responds to the presence of unreplicated or damaged DNA by blocking mitosis. Others control progress through mitosis, for example, detecting unpaired kinetochores.

Apoptosis is achieved by an active pathway that executes a program for cell death. The components of the pathway may be present in many or all higher eukaryotic cells. Apoptosis may be triggered by various stimuli. A common pathway involves activation of caspase-8 by oligomerization at an activated surface receptor. Caspase-8 cleaves Bid, which triggers release of cytochrome *c* from mitochondria. The cytochrome c causes Apaf-1 to oligomerize with caspase-9. The activated caspase-9 cleaves procaspase-3, whose two subunits then form the active protease. This cleaves various targets that lead to cell death. The pathway is inhibited by Bcl2 at the stage of release of cytochrome c. An alternative pathway for triggering apoptosis that does not pass through Apaf-1 and caspase-9, and which is not inhibited by Bcl2, involves the activation of JNK. Different cells use these pathways to differing extents. Apoptosis was first shown to be necessary for normal development in *C. elegans*, and knockout mutations in mice show that this is also true of vertebrates. Every cell may contain the components of the apoptotic pathway and be subject to regulation of the balance between activation and repression of cell death.

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