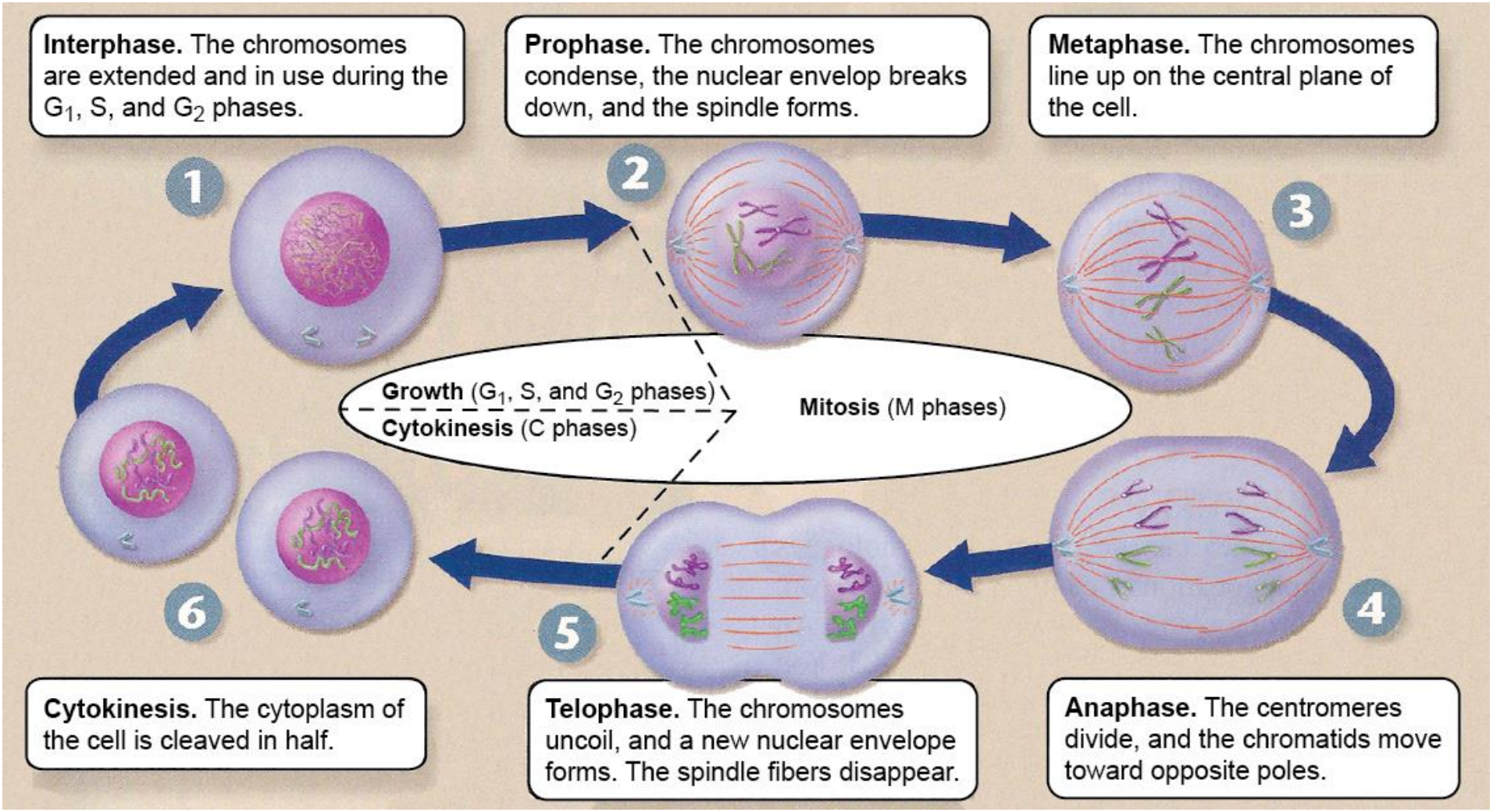
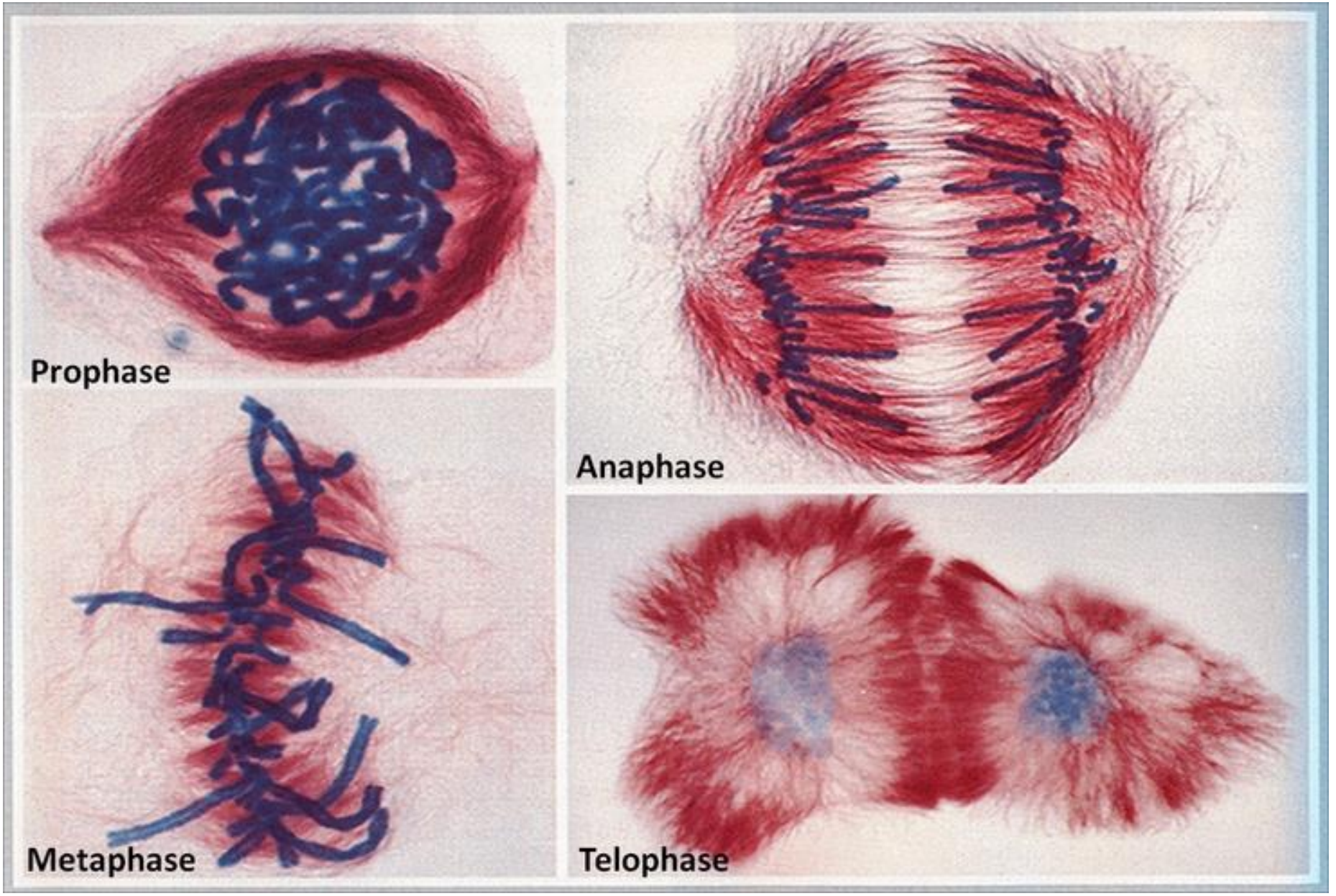
Cell Cycle

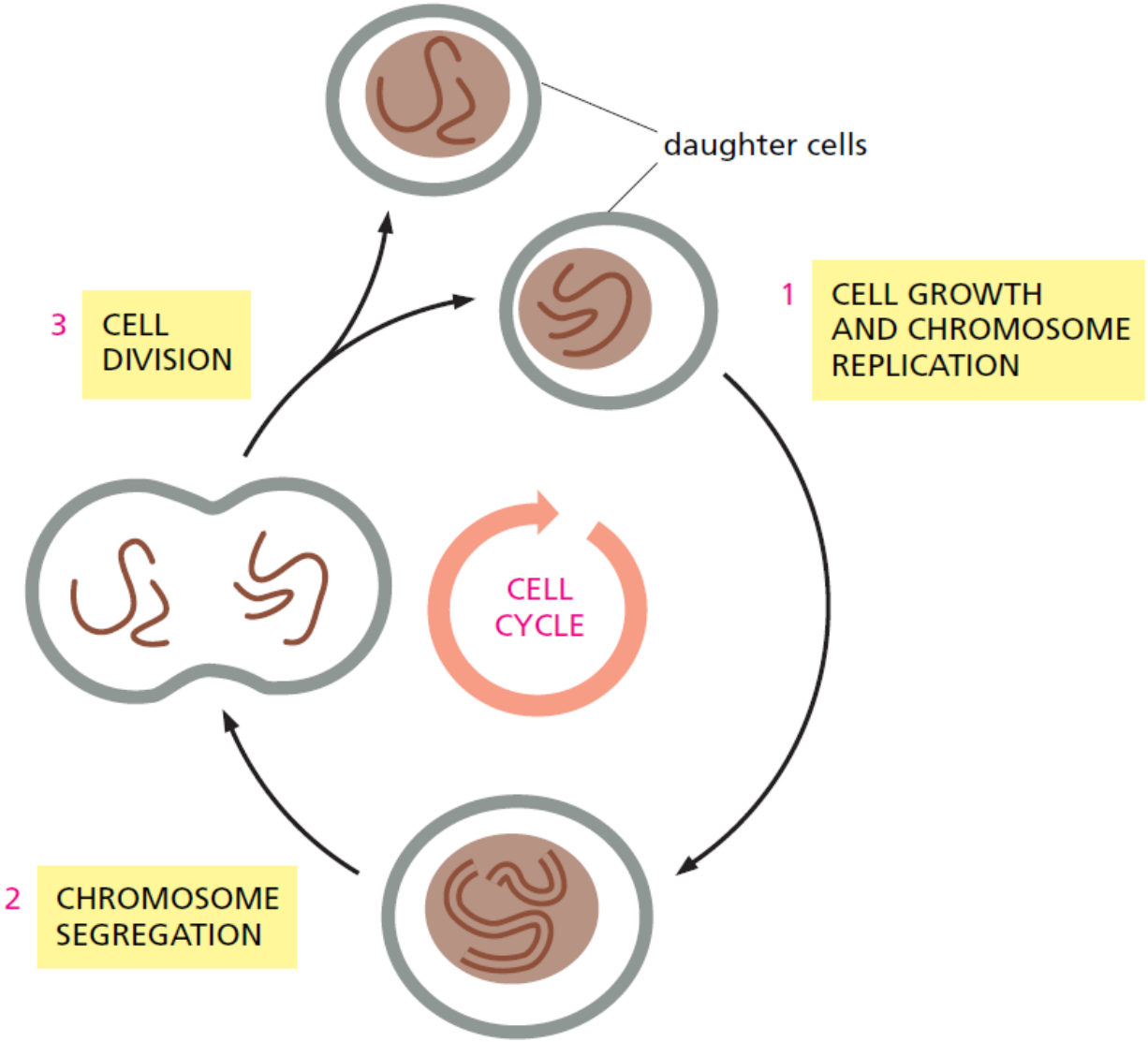

Interphase. The chromosomes are extended and in use during the G_1, S, and G_2 phases.
Prophase. The chromosomes condense, the nuclear envelop breaks down, and the spindle forms.
Metaphase. The chromosomes line up on the central plane of the cell.
1
2
3
Growth (G_1, S, and G_2 phases)
Cytokinesis (C phases)
Mitosis (M phases)
6
5
4
Cytokinesis. The cytoplasm of the cell is cleaved in half.
Telophase. The chromosomes uncoil, and a new nuclear envelope forms. The spindle fibers disappear.
Anaphase. The centromeres divide, and the chromatids move toward opposite poles.

Cell Cycle

- The only way to make a new cell is to duplicate a cell that already exists.
- This simple fact, first established in the middle of the nineteenth century, carries with it a profound message for the continuity of life.
- All living organisms, from the unicellular bacterium to the multicellular mammal, are products of repeated rounds of cell growth and division extending back in time to the beginnings of life on Earth over three billion years ago.
- A cell reproduces by performing an orderly sequence of events in which it duplicates its contents and then divides in two.
- This cycle of duplication and division, known as the cell cycle, is the essential mechanism by which all living things reproduce.

- In unicellular species, such as bacteria and yeasts, each cell division produces a complete new organism.
- In multicellular species, long and complex sequences of cell divisions are required to produce a functioning organism.
- Even in the adult body, cell division is usually needed to replace cells that die.
- The details of the cell cycle vary from organism to organism and at different times in an organism's life.
- Certain characteristics, however, are universal.
- The minimum set of processes that a cell has to perform are those that allow it to accomplish its most fundamental task: the passing on of its genetic information to the next generation of cells.


Prophase
Anaphase
Metaphase
Telophase


daughter cells
1 CELL GROWTH AND CHROMOSOME REPLICATION
2 CHROMOSOME SEGREGATION
3 CELL DIVISION
CELL CYCLE

- Eucaryotic cells have evolved a complex network of regulatory proteins, known as the cell-cycle control system, that governs progression through the cell cycle.

- The core of this system is an ordered series of biochemical switches that initiate the main events of the cycle, including chromosome duplication and segregation.

- In addition to duplicating their genome, most cells also duplicate their other organelles and macromolecules; otherwise, daughter cells would get smaller with each division.

- To maintain their size, dividing cells must coordinate their growth (that is, their increase in cell mass) with their division.

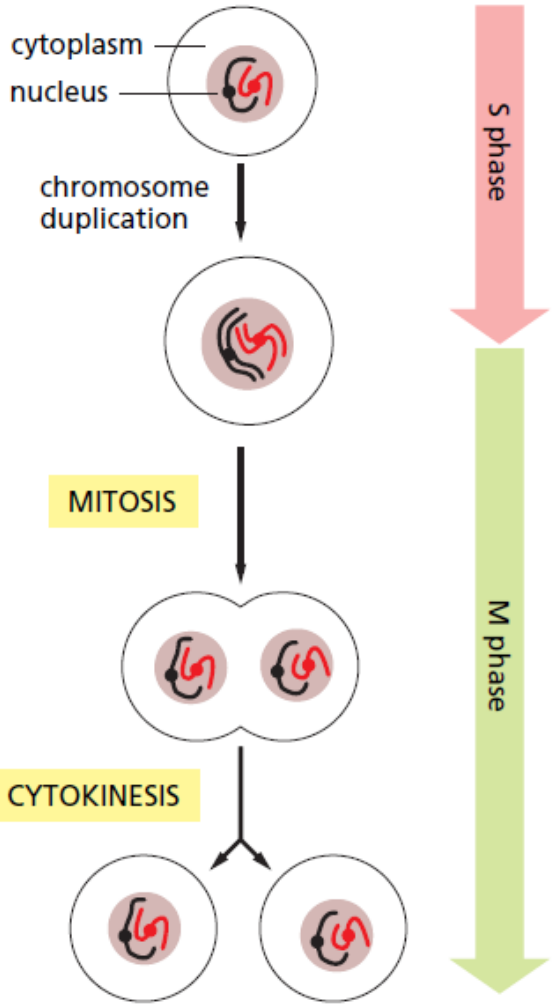
- The eucaryotic cell cycle is divided into four phases.

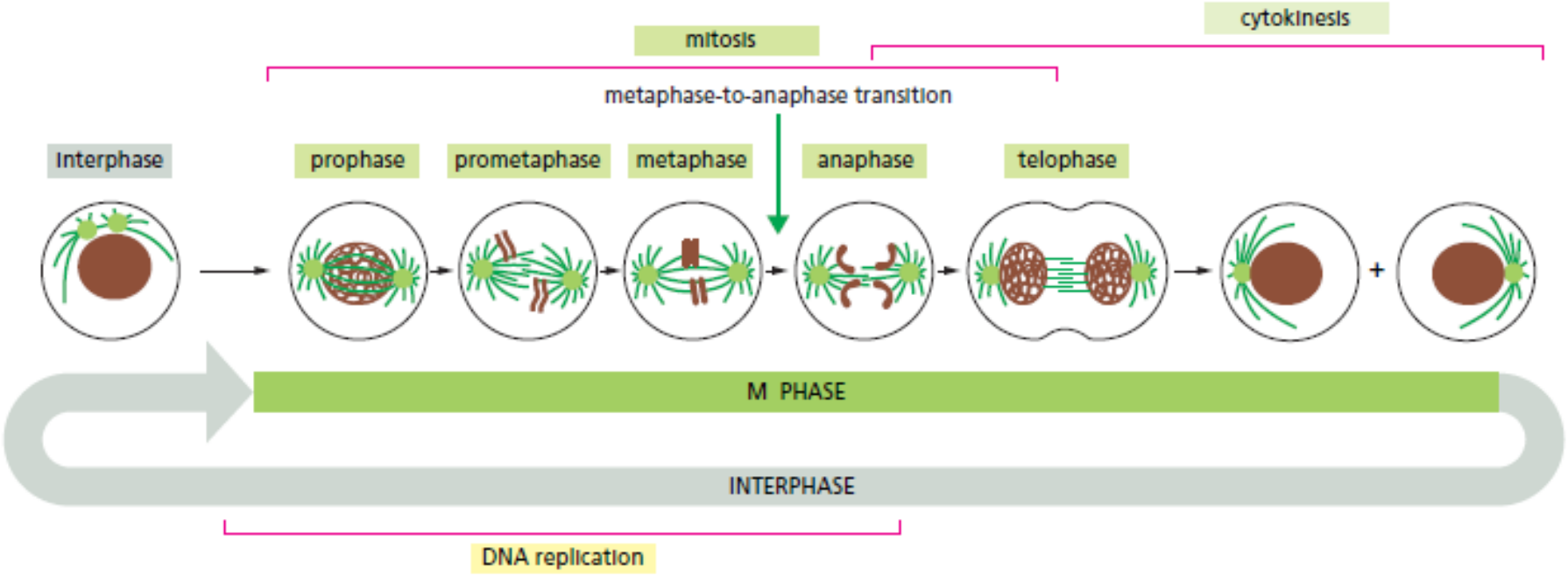
- The most basic function of the cell cycle is to duplicate accurately the vast amount of DNA in the chromosomes and then segregate the copies precisely into two genetically identical daughter cells.

- Chromosome duplication occurs during S phase (S for DNA synthesis), which requires 10–12 hours and occupies about half of the cell-cycle time in a typical mammalian cell.

- After S phase, chromosome segregation and cell division occur in M phase (M for mitosis), which requires much less time (less than an hour in a mammalian cell).

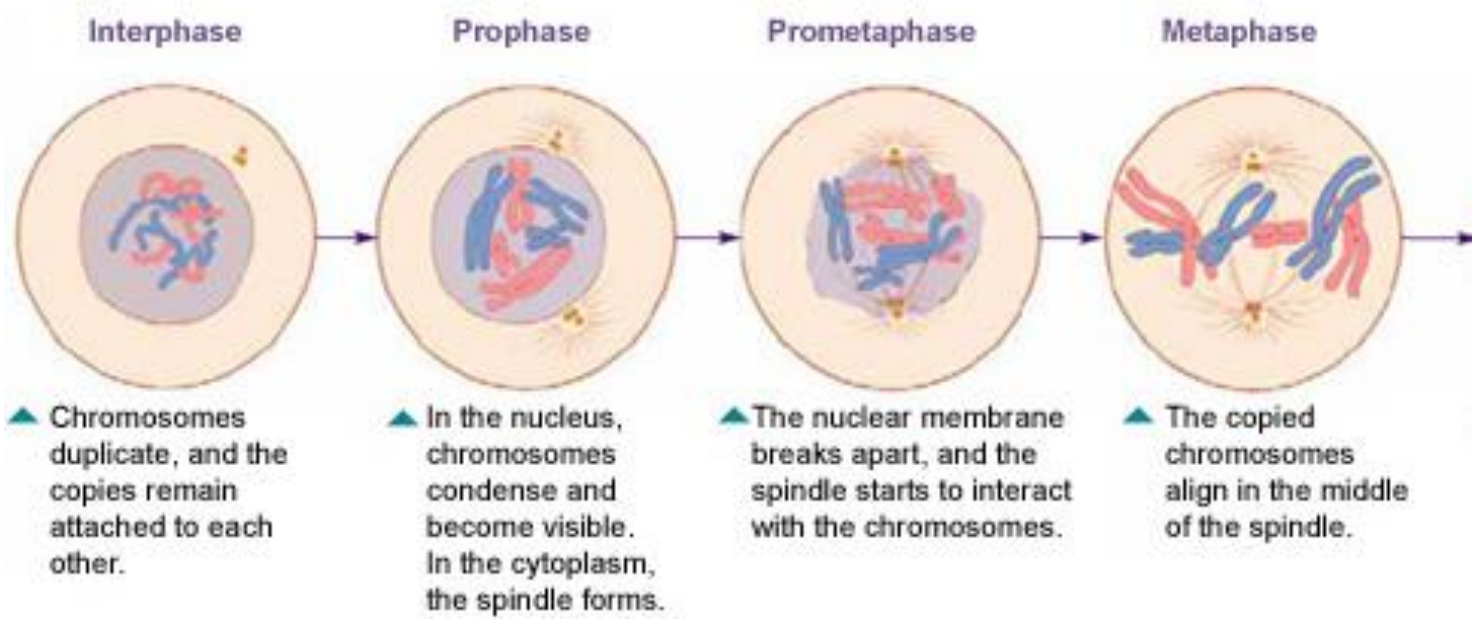
- M phase comprises two major events: nuclear division, or mitosis, during which the copied chromosomes are distributed into a pair of daughter nuclei; and cytoplasmic division, or cytokinesis, when the cell itself divides in two.





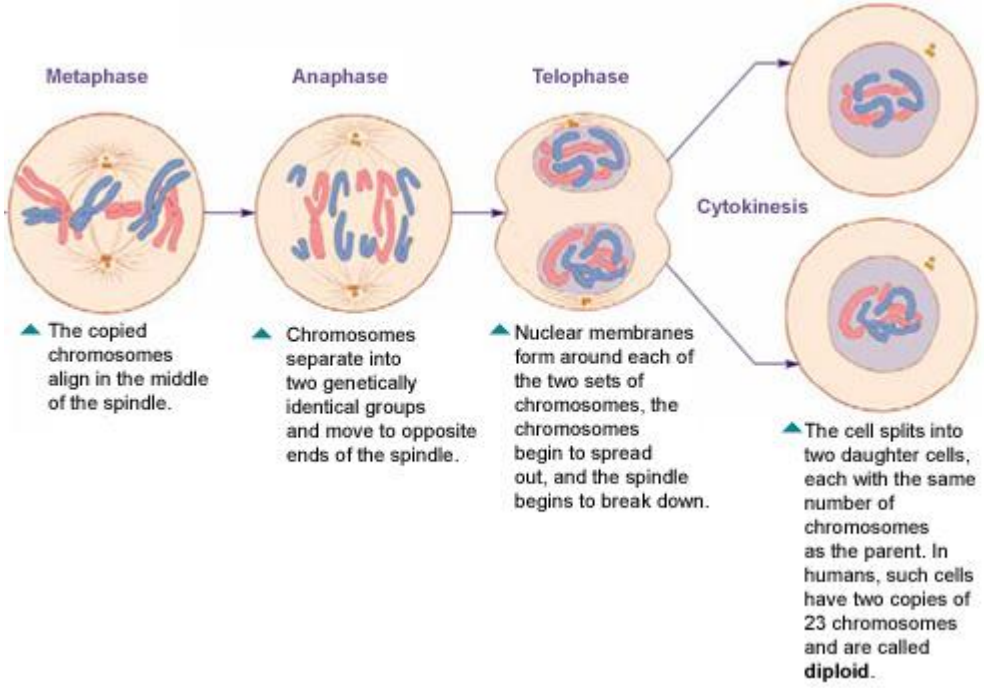
- At the end of S phase, the DNA molecules in each pair of duplicated chromosomes are intertwined and held tightly together by specialized protein linkages.

- Early in mitosis at a stage called prophase, the two DNA molecules are gradually disentangled and condensed into pairs of rigid and compact rods called sister chromatids, which remain linked together by sister-chromatid cohesion.



- When the nuclear envelope disassembles later in mitosis, the sister chromatid pairs become attached to the mitotic spindle, a giant bipolar array of microtubules.

- Sister chromatids are attached to opposite poles of the spindle, and, eventually, all sisters align at the spindle equator in a stage called metaphase.



- The destruction of sister-chromatid cohesion at the start of anaphase separates the sister chromatids, which are pulled to opposite poles of the spindle.

- The spindle is then disassembled, and the segregated chromosomes are packaged into separate nuclei at telophase.

- Cytokinesis then cleaves the cell in two, so that each daughter cell inherits one of the two nuclei.

- Most cells require much more time to grow and double their mass of proteins and organelles than they require to duplicate their chromosomes and divide.

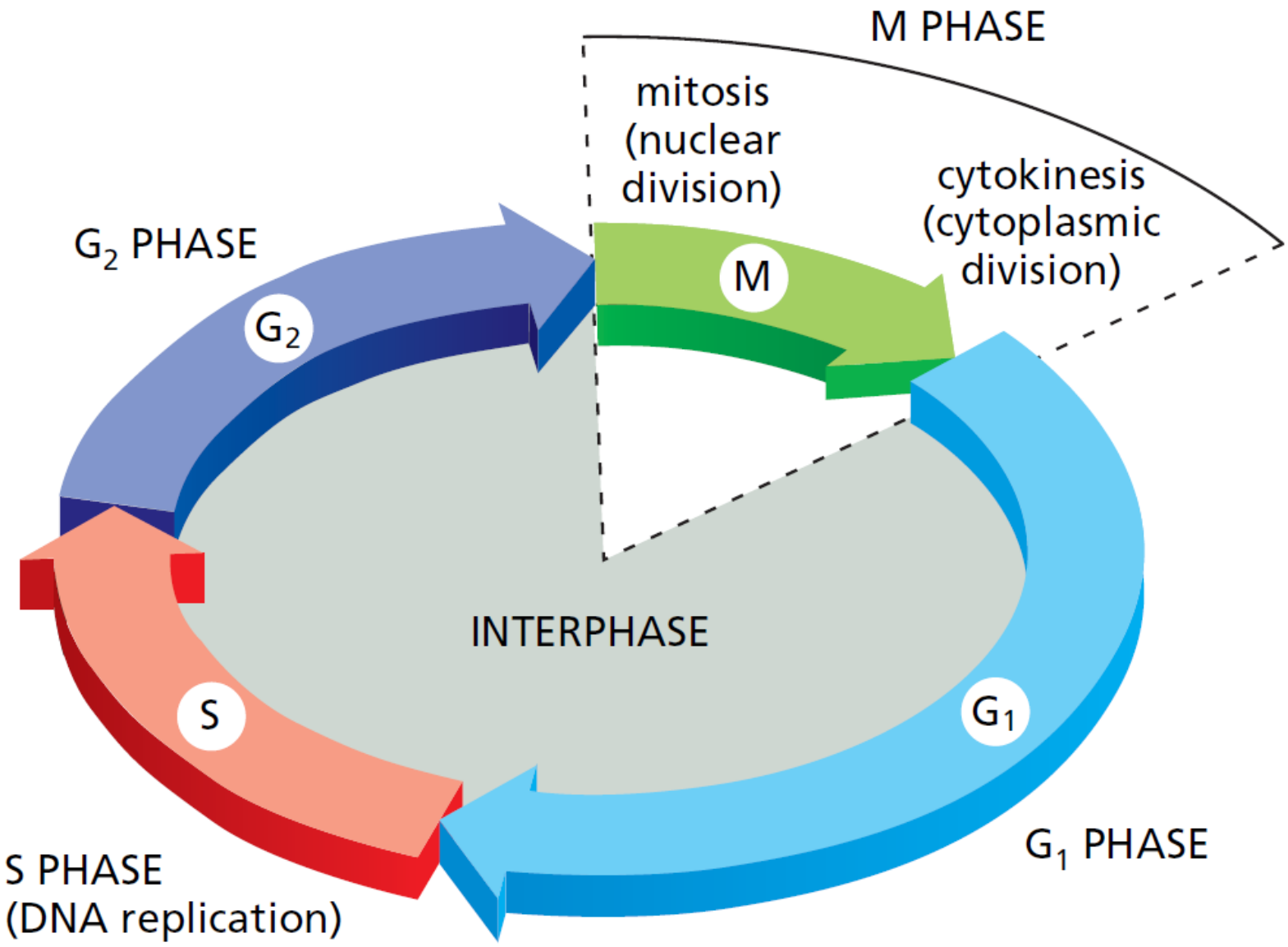
- Partly to allow more time for growth, most cell cycles have extra gap phases—a G1 phase between M phase and S phase and a G2 phase between S phase and mitosis.

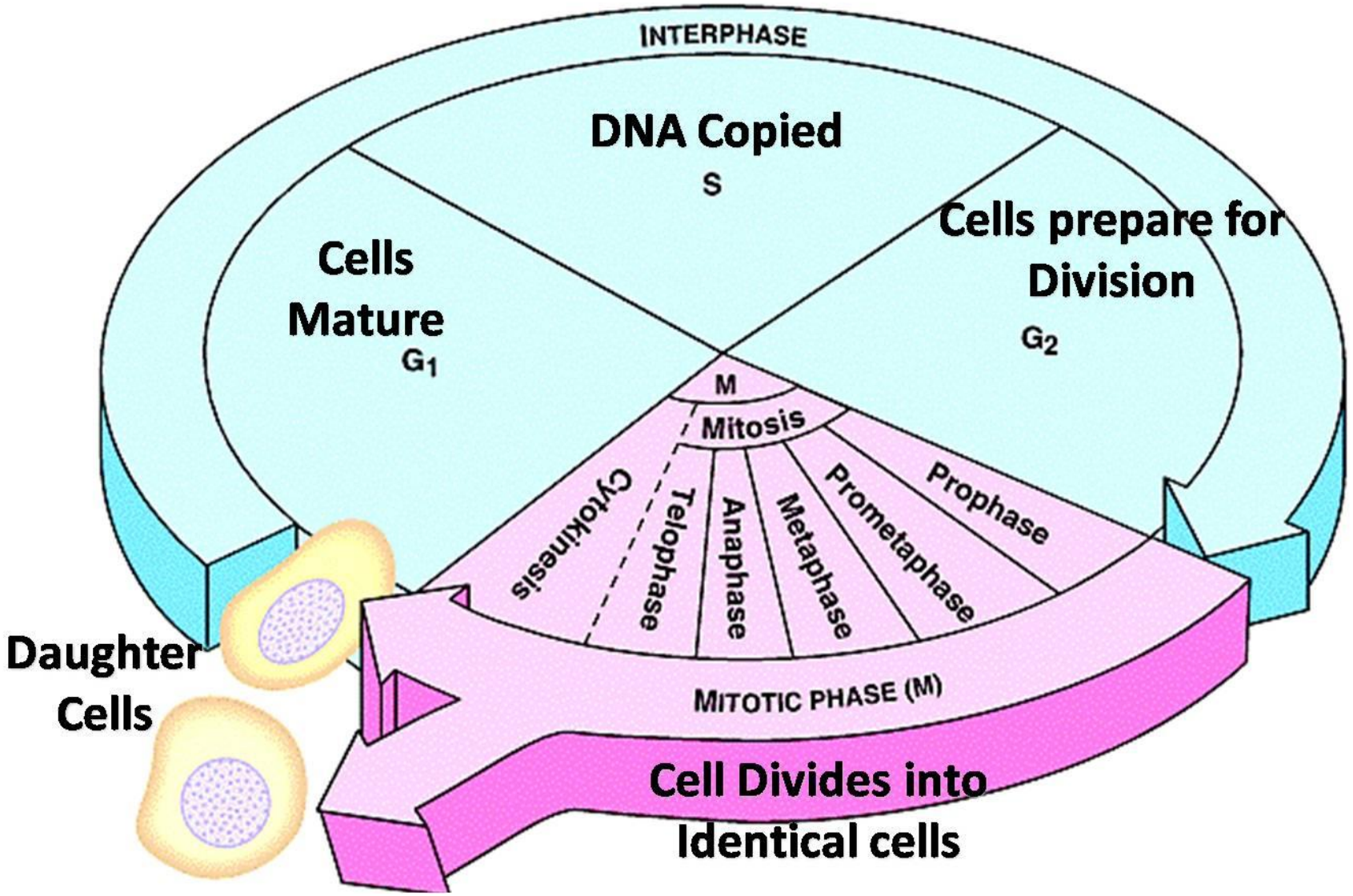
- Thus, the eucaryotic cell cycle is traditionally divided into four sequential phases: G1, S, G2, and M.

- G1, S, and G2 together are called interphase.

- In a typical human cell proliferating in culture, interphase might occupy 23 hours of a 24-hour cycle, with 1 hour for M phase.

- Cell growth occurs throughout the cell cycle, except during mitosis.


M PHASE
mitosis (nuclear division)
cytokinesis (cytoplasmic division)
M
G2 PHASE
G2
INTERPHASE
S
G1
S PHASE (DNA replication)
G1 PHASE


INTERPHASE
DNA Copied
S
Cells
Mature
G1
Cells prepare for
Division
G2
M
Mitosis
Cytokinesis
Telophase
Anaphase
Metaphase
Prometaphase
Prophase
MITOTIC PHASE (M)
Daughter
Cells
Cell Divides into
Identical cells

- The two gap phases are more than simple time delays to allow cell growth.

- They also provide time for the cell to monitor the internal and external environment to ensure that conditions are suitable and preparations are complete.

- The G1 phase is especially important in this respect. If extracellular conditions are unfavorable, cells delay progress through G1 and may even enter a specialized resting state known as G0 (G zero), in which they can remain for days, weeks, or even years before resuming proliferation.

- Indeed, many cells remain permanently in G0 until they or the organism dies.

- If extracellular conditions are favorable and signals to grow and divide are present, cells in early G1 or G0 progress through a commitment point near the end of G1 known as Start (in yeasts) or the restriction point (in mammalian cells).

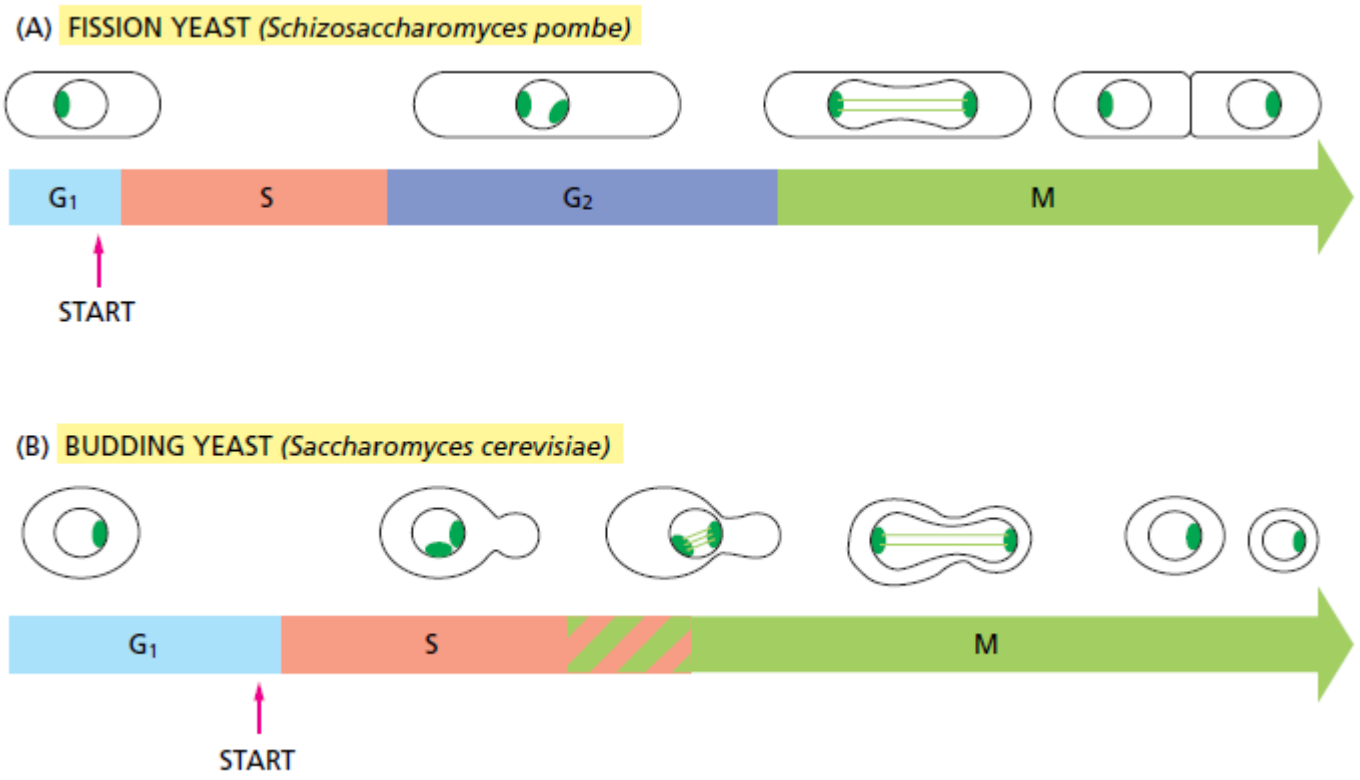
- After passing this point, cells are committed to DNA replication, even if the extracellular signals that stimulate cell growth and division are removed.

- The basic organization of the cycle, is essentially the same in all eucaryotic cells, and all eucaryotes appear to use similar machinery and control mechanisms to drive and regulate cell-cycle events.

- The proteins of the cell cycle control system first appeared over a billion years ago. And remarkably, they have been so well conserved over the course of evolution that many of them function perfectly when transferred from a human cell to a yeast cell.

❖ Yeasts are tiny, single-celled fungi, with a cell-cycle control system remarkably similar to our own.

❖ Two species are generally used in studies of the cell cycle.



❖ Despite their outward differences, the two yeast species share many features that are extremely useful for genetic studies.

- They reproduce almost as rapidly as bacteria and have a genome size less than 1% that of a mammal.

- They are amenable to rapid molecular genetic manipulation, in which genes can be deleted, replaced, or altered.

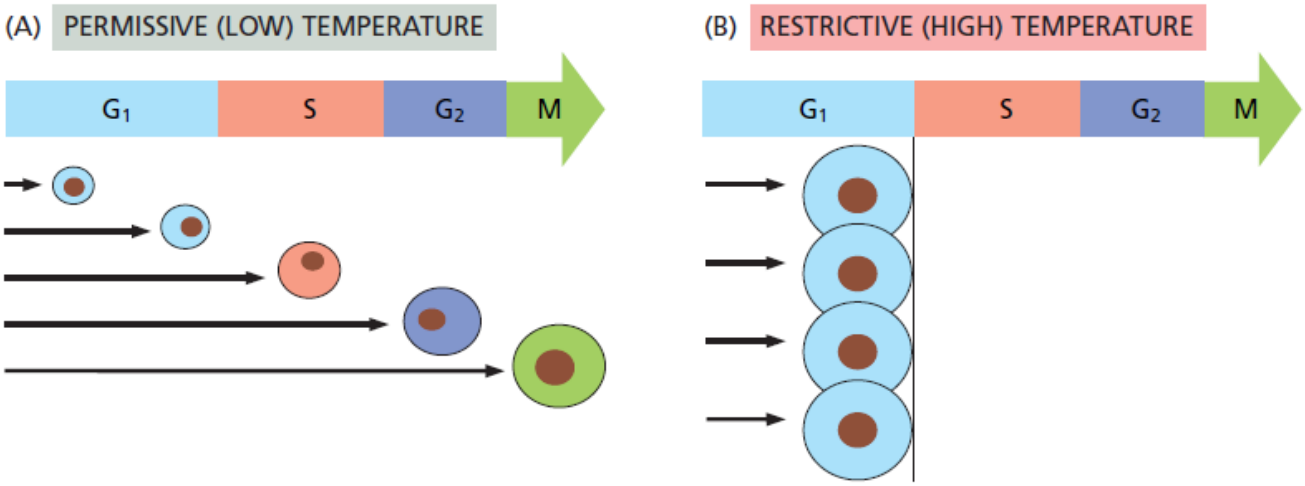
- Most importantly, they have the ability to proliferate in a haploid state, with only a single copy of each gene present in the cell.

- When cells are haploid, it is easy to isolate and study mutations that inactivate a gene, because we avoid the complication of having a second copy of the gene in the cell.

- Many important discoveries about cell-cycle control have come from systematic searches for mutations in yeasts that inactivate genes encoding essential components of the cell-cycle control system.

- The genes affected by some of these mutations are known as cell-division-cycle genes, or *Cdc* genes.

- Many of these mutations cause cells to arrest at a specific point in the cell cycle, suggesting that the normal gene product is required to get the cell past this point.



- A temperature-sensitive *Cdc* mutant can be propagated at a low temperature (the permissive condition) and then raised to a higher temperature (the restrictive condition) to switch off the function of the mutant gene.

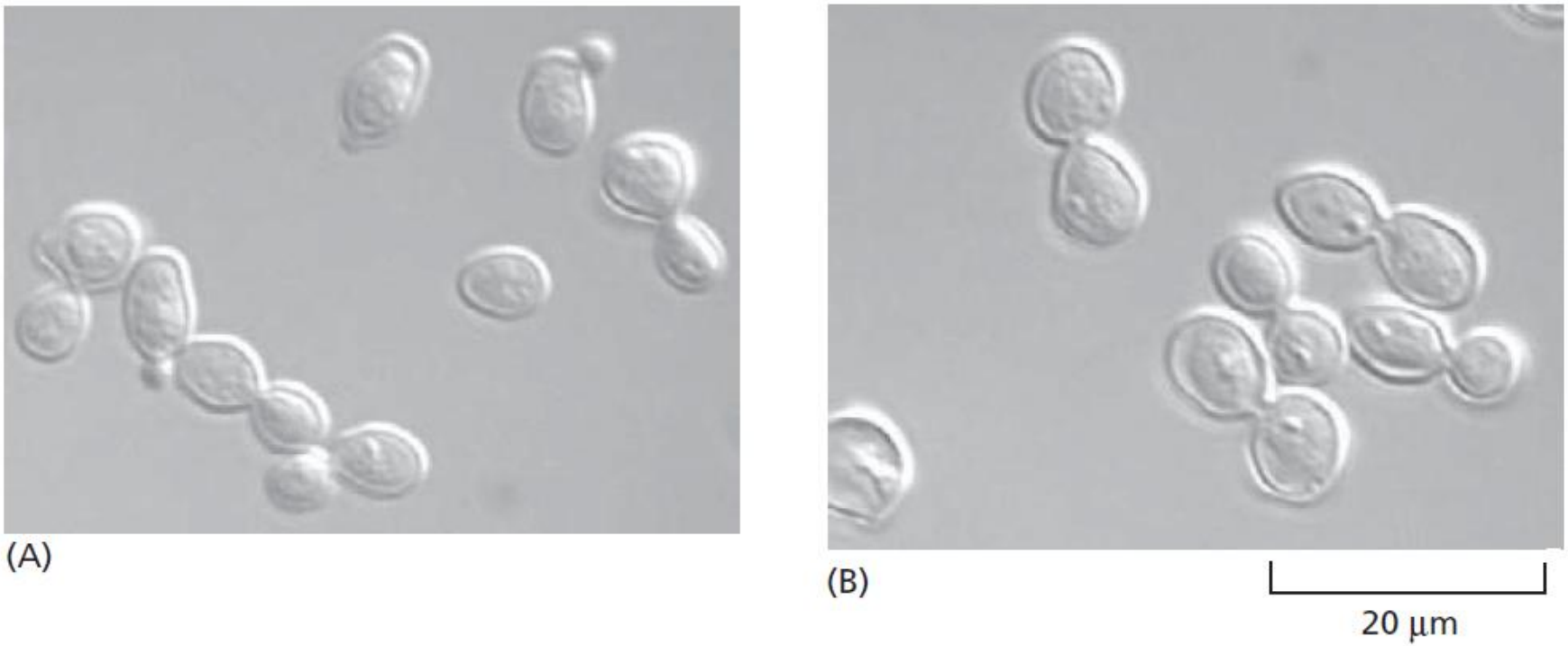
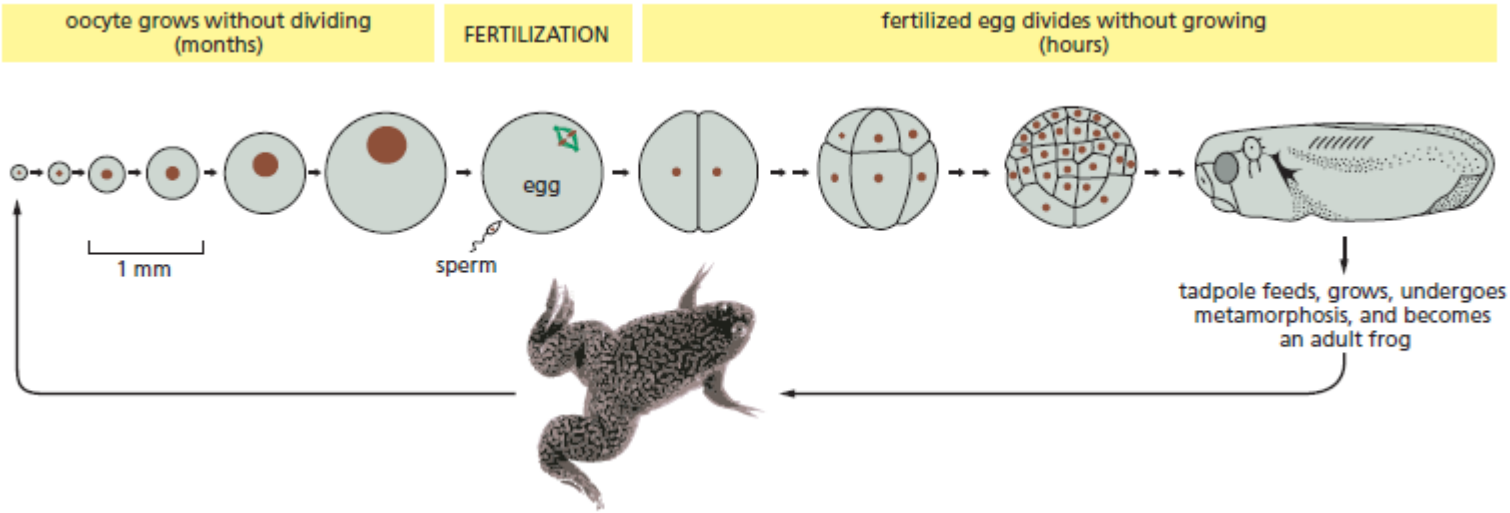


Figure 17–7 The morphology of budding yeast cells arrested by a *Cdc* mutation. (A) In a normal population of proliferating yeast cells, buds vary in size according to the cell-cycle stage. (B) In a *Cdc15* mutant grown at the restrictive temperature, cells complete anaphase but cannot complete the exit from mitosis and cytokinesis. As a result, they arrest uniformly with large buds, which are characteristic of late M phase. (Courtesy of Jeff Ubersax.)

- The biochemical features of the cell cycle are readily analyzed in the giant fertilized eggs of many animals, which carry large stockpiles of the proteins needed for cell division.

- The egg of the frog *Xenopus*, for example, is over 1 mm in diameter and contains 100,000 times more cytoplasm than an average cell in the human body.

- Fertilization of the Xenopus egg triggers an astonishingly rapid sequence of cell divisions, called cleavage divisions, in which the single giant cell divides, without growing, to generate an embryo containing thousands of smaller cells

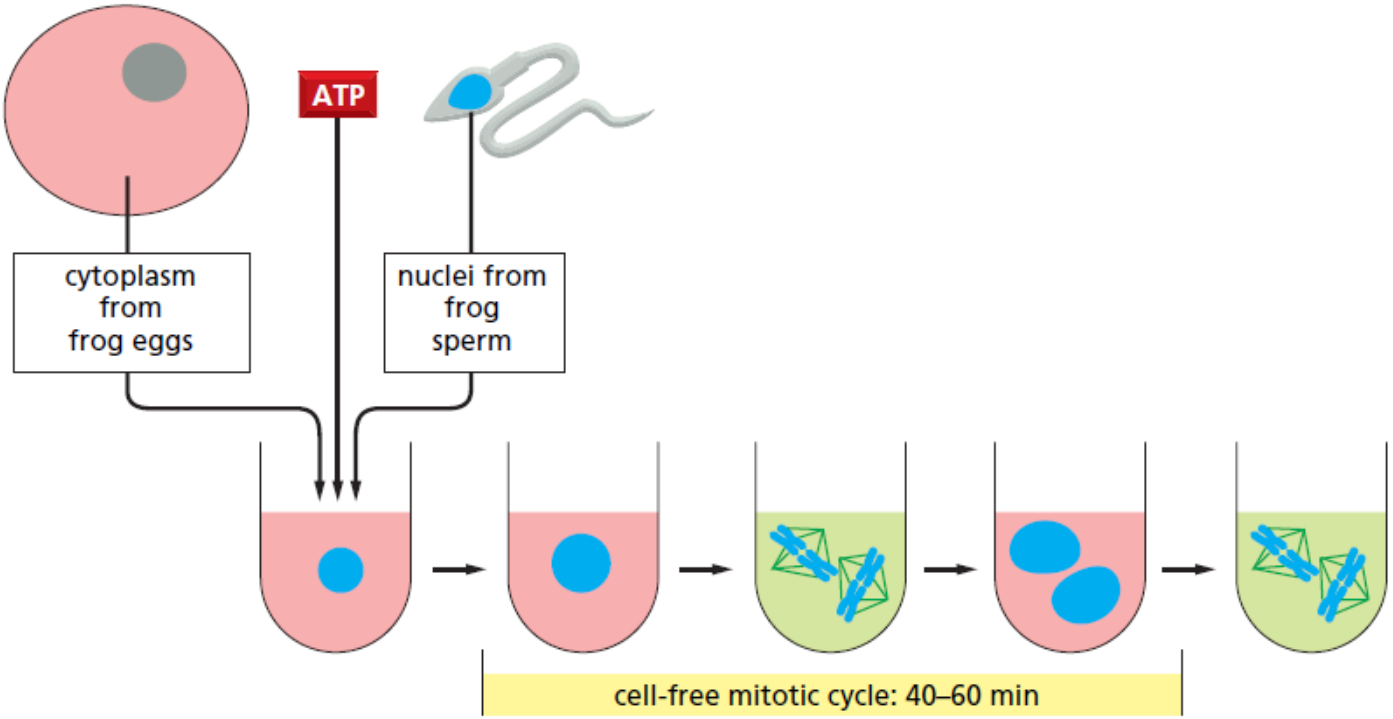


- After a first division that takes about 90 minutes, the next 11 divisions occur, more or less synchronously, at 30-minute intervals, producing about 4096 (2^{12}) cells within 7 hours.

- Each cycle is divided into S and M phases of about 15 minutes each, without detectable G1 or G2 phases.

- The early embryonic cells of *Xenopus*, as well as those of the clam *Spisula* and the fruit fly *Drosophila*, are thus capable of exceedingly rapid division in the absence of either growth or many of the control mechanisms that operate in more complex cell cycles.

- Another advantage of these early embryos for cell-cycle analysis is their large size. It is relatively easy to inject test substances into an egg to determine their effect on cell-cycle progression.



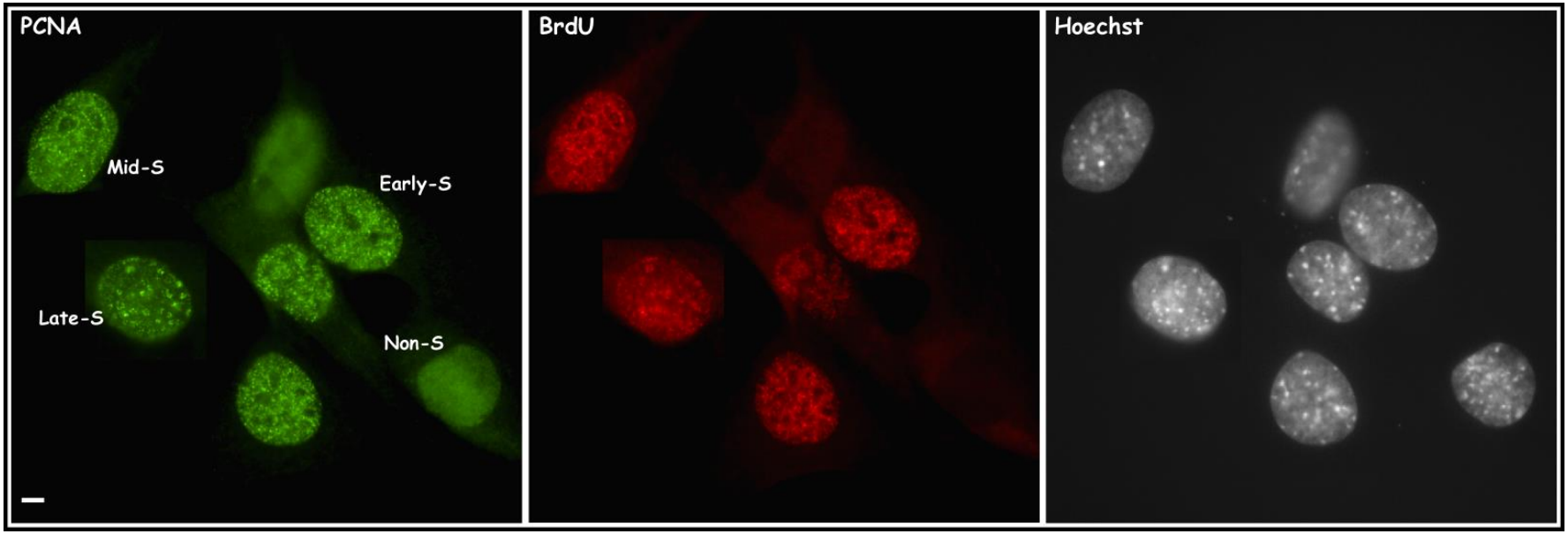
- How can we tell what stage an animal cell has reached in the cell cycle? One way is simply to look at living cells with a microscope.

- A glance at a population of mammalian cells proliferating in culture reveals that a fraction of the cells have rounded up and are in mitosis. Others can be observed in the process of cytokinesis.

- We can gain additional clues about cell-cycle position by staining cells with DNA-binding fluorescent dyes or with antibodies that recognize specific cellular components such as the microtubules (revealing the mitotic spindle).

- Similarly, S-phase cells can be identified in the microscope by supplying them with visualizable molecules that are incorporated into newly synthesized DNA, such as the artificial thymidine analog bromo-deoxyuridine (BrdU).

- Cell nuclei that have incorporated BrdU are then visualized by staining with anti-BrdU antibodies


PCNA
Mid-S
Early-S
Late-S
Non-S
BrdU
Hoechst

- Typically, in a population of cells that are all proliferating rapidly but asynchronously, about 30–40% will be in S phase at any instant and become labeled by a brief pulse of BrdU.

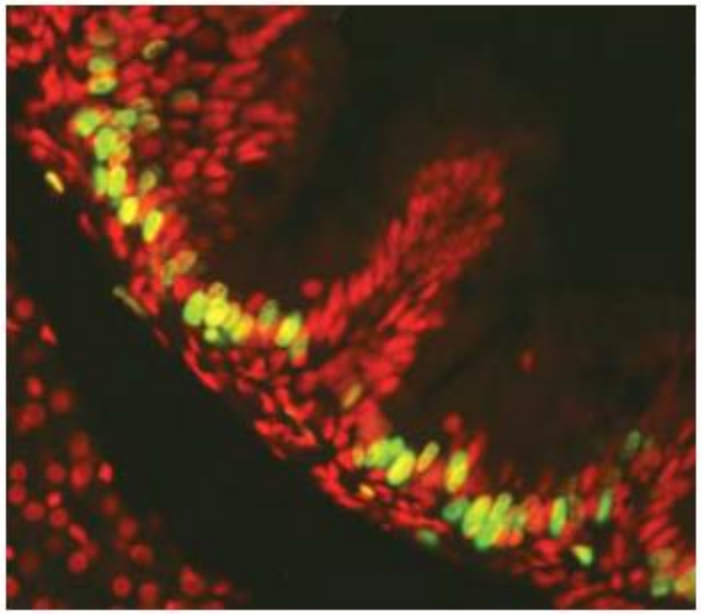
- From the proportion of cells in such a population that are labeled (the labeling index), we can estimate the duration of S phase as a fraction of the whole cell-cycle duration.

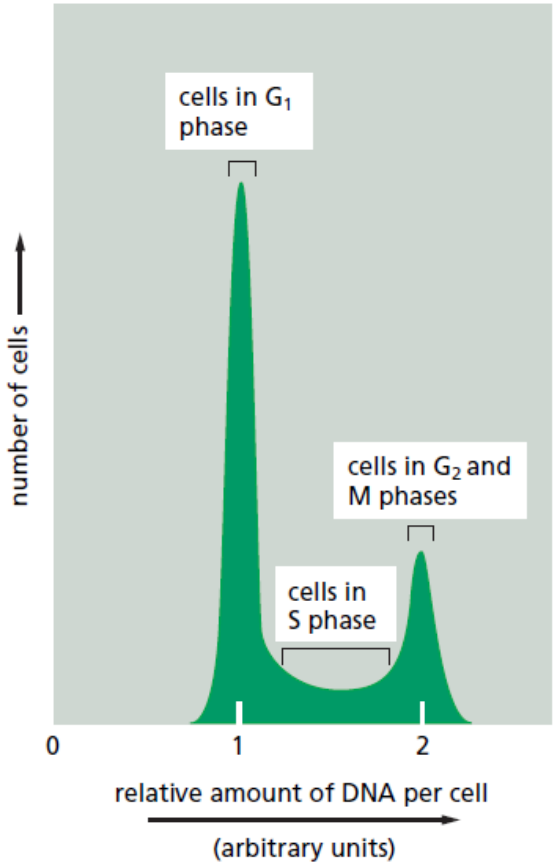
- Similarly, from the proportion of cells in mitosis (the mitotic index), we can estimate the duration of M phase.

- In addition, by giving a pulse of BrdU and allowing the cells to continue around the cycle for measured lengths of time, we can determine how long it takes for an S phase cell to progress through G2 into M phase, through M phase into G1, and finally through G1 back into S phase.

- Another way to assess the stage that a cell has reached in the cell cycle is by measuring its DNA content, which doubles during S phase.

- This approach is greatly facilitated by the use of fluorescent DNA-binding dyes and a flow cytometer, which allows the rapid and automatic analysis of large numbers of cells





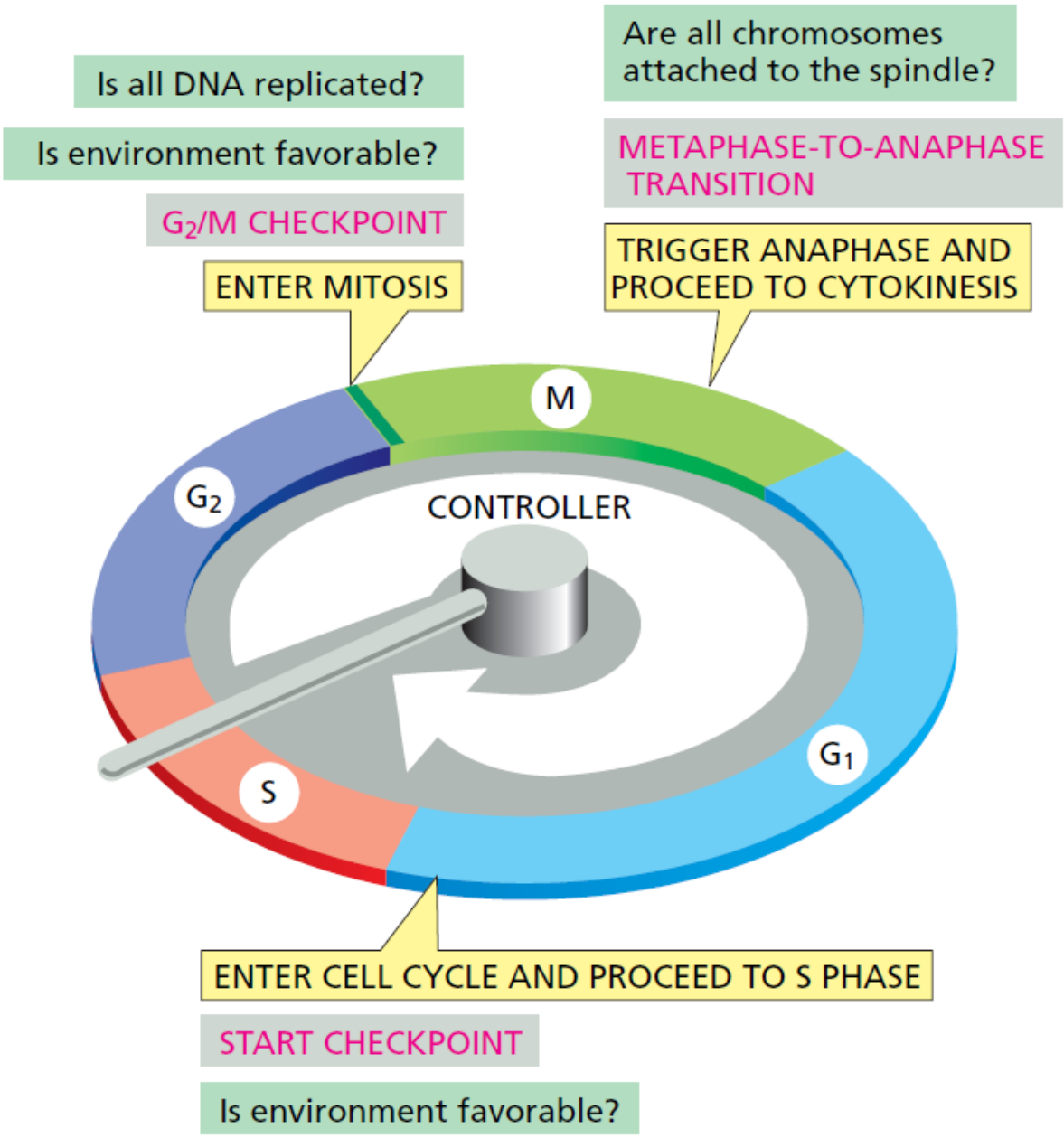
THE CELL-CYCLE CONTROL SYSTEM

- For many years cell biologists watched the puppet show of DNA synthesis, mitosis, and cytokinesis but had no idea of what lay behind the curtain controlling these events.

- A major breakthrough came in the late 1980s with the identification of the key proteins of the control system, along with the realization that they are distinct from the proteins that perform the processes of DNA replication, chromosome segregation, and so on.

- The cell-cycle control system operates much like a timer or oscillator that triggers the events of the cell cycle in a set sequence.

- In its simplest form—as seen in the stripped-down embryonic cell cycles discussed earlier— the control system is like a rigidly programmed timer that provides a fixed amount of time for the completion of each cell-cycle event


Is all DNA replicated?
Is environment favorable?
G_2/M CHECKPOINT
ENTER MITOSIS
Are all chromosomes attached to the spindle?
METAPHASE-TO-ANAPHASE TRANSITION
TRIGGER ANAPHASE AND PROCEED TO CYTOKINESIS
M
G_2
CONTROLLER
G_1
S
ENTER CELL CYCLE AND PROCEED TO S PHASE
START CHECKPOINT
Is environment favorable?

- The control system in these cells is independent of the events it controls, so that its timing mechanisms continue to operate even if those events fail.

- In most cells, however, the control system does respond to information received back from the processes it controls.

- Sensors, for example, detect the completion of DNA synthesis, and if some malfunction prevents the successful completion of this process, signals are sent to the control system to delay progression to M phase.

- Such delays provide time for the machinery to be repaired and also prevent the disaster that might result if the cycle progressed prematurely to the next stage—and segregated incompletely replicated chromosomes, for example.

- The cell-cycle control system is based on a connected series of biochemical switches, each of which initiates a specific cell-cycle event.

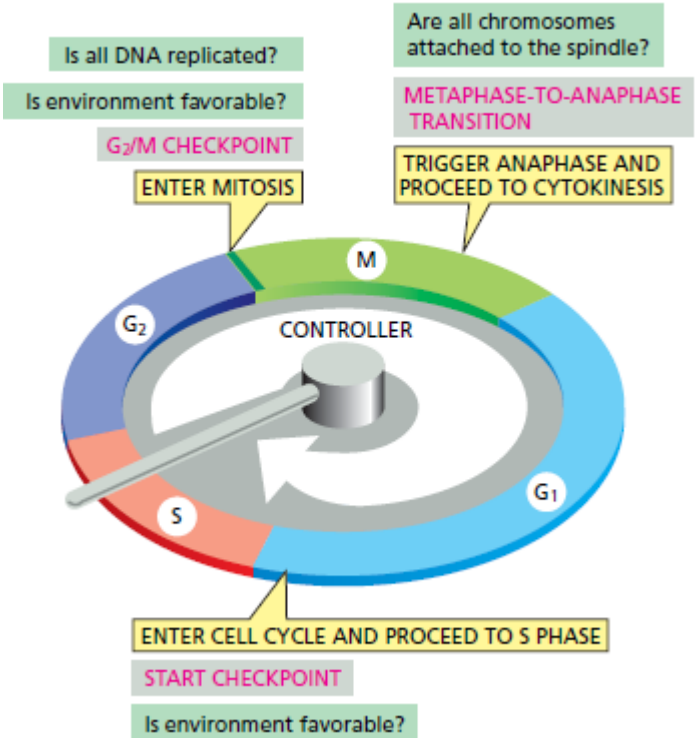
- This system of switches possesses many important engineering features that increase the accuracy and reliability of cell-cycle progression.

- First, the switches are generally binary (on/off) and launch events in a complete, irreversible fashion. It would clearly be disastrous, for example, if events like chromosome condensation or nuclear envelope breakdown were only partially initiated or started but not completed.

- Second, the cell-cycle control system is remarkably robust and reliable, partly because backup mechanisms and other features allow the system to operate effectively under a variety of conditions and even if some components fail.

- Finally, the control system is highly adaptable and can be modified to suit specific cell types or to respond to specific intracellular or extracellular signals.

- In most eucaryotic cells, the cell-cycle control system triggers cell-cycle progression at three major regulatory transitions, or checkpoints.



- The first checkpoint is Start (or the restriction point) in late G1, where the cell commits to cell-cycle entry and chromosome duplication, as mentioned earlier.

- The second is the G2/M checkpoint, where the control system triggers the early mitotic events that lead to chromosome alignment on the spindle in metaphase.

- The third is the metaphase-to-anaphase transition, where the control system stimulates sister-chromatid separation, leading to the completion of mitosis and cytokinesis.

- The control system blocks progression through each of these checkpoints if it detects problems inside or outside the cell.

- If the control system senses problems in the completion of DNA replication, for example, it will hold the cell at the G2/M checkpoint until those problems are solved.

- Similarly, if extracellular conditions are not appropriate for cell proliferation, the control system blocks progression through Start, thereby preventing cell division until conditions become favorable.

- Central components of the cell-cycle control system are members of a family of protein kinases known as cyclin-dependent kinases (Cdks).

- The activities of these kinases rise and fall as the cell progresses through the cycle, leading to cyclical changes in the phosphorylation of intracellular proteins that initiate or regulate the major events of the cell cycle.

- Cyclical changes in Cdk activity are controlled by a complex array of enzymes and other proteins that regulate these kinases.

- The most important of these Cdk regulators are proteins known as cyclins.

- Cdks, as their name implies, are dependent on cyclins for their activity

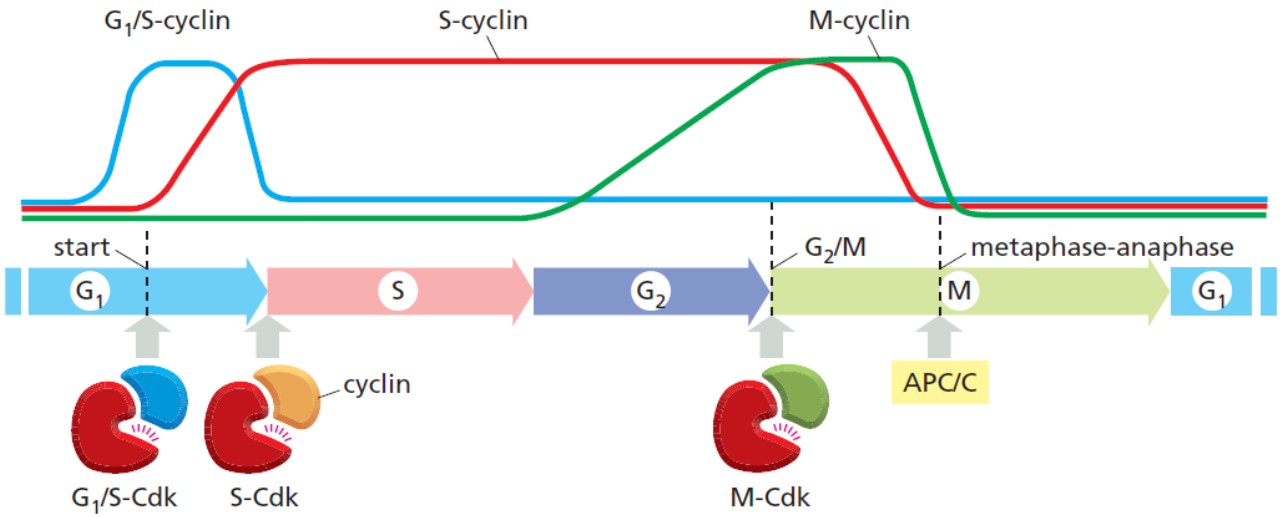
- There are four classes of cyclins, each defined by the stage of the cell cycle at which they bind Cdks and function.

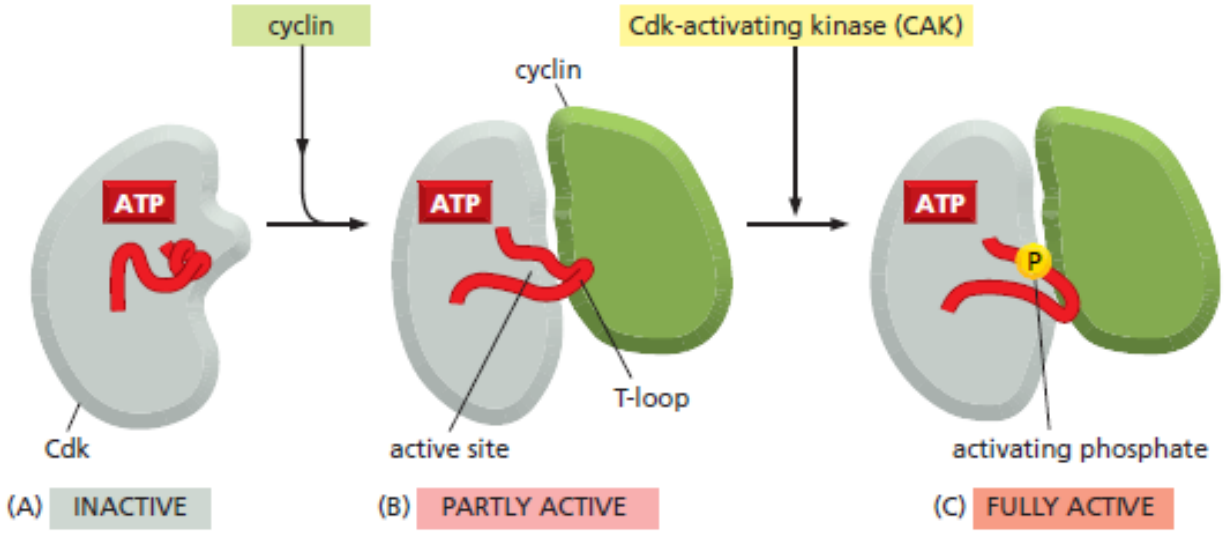
- 1. **G1/S-cyclins** activate Cdks in late G1 and thereby help trigger progression through Start, resulting in a commitment to cell-cycle entry.

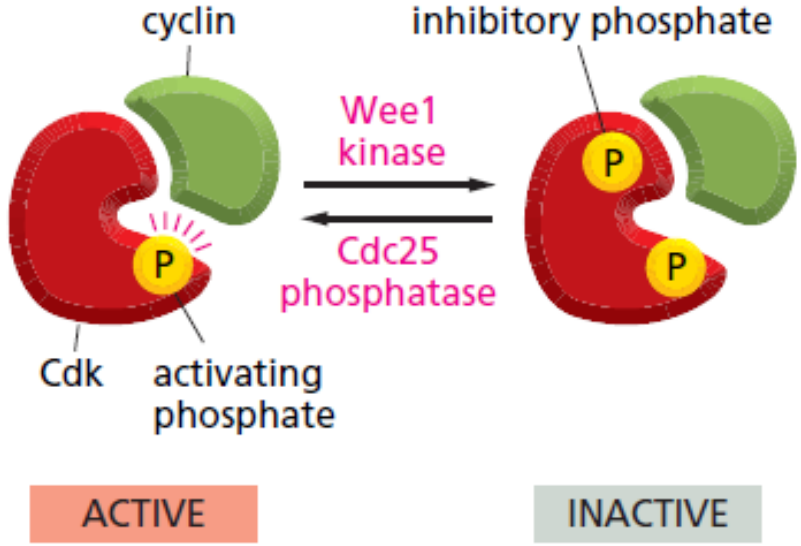
- 2. **S-cyclins** bind Cdks soon after progression through Start and help stimulate chromosome duplication. S-cyclin levels remain elevated until mitosis.

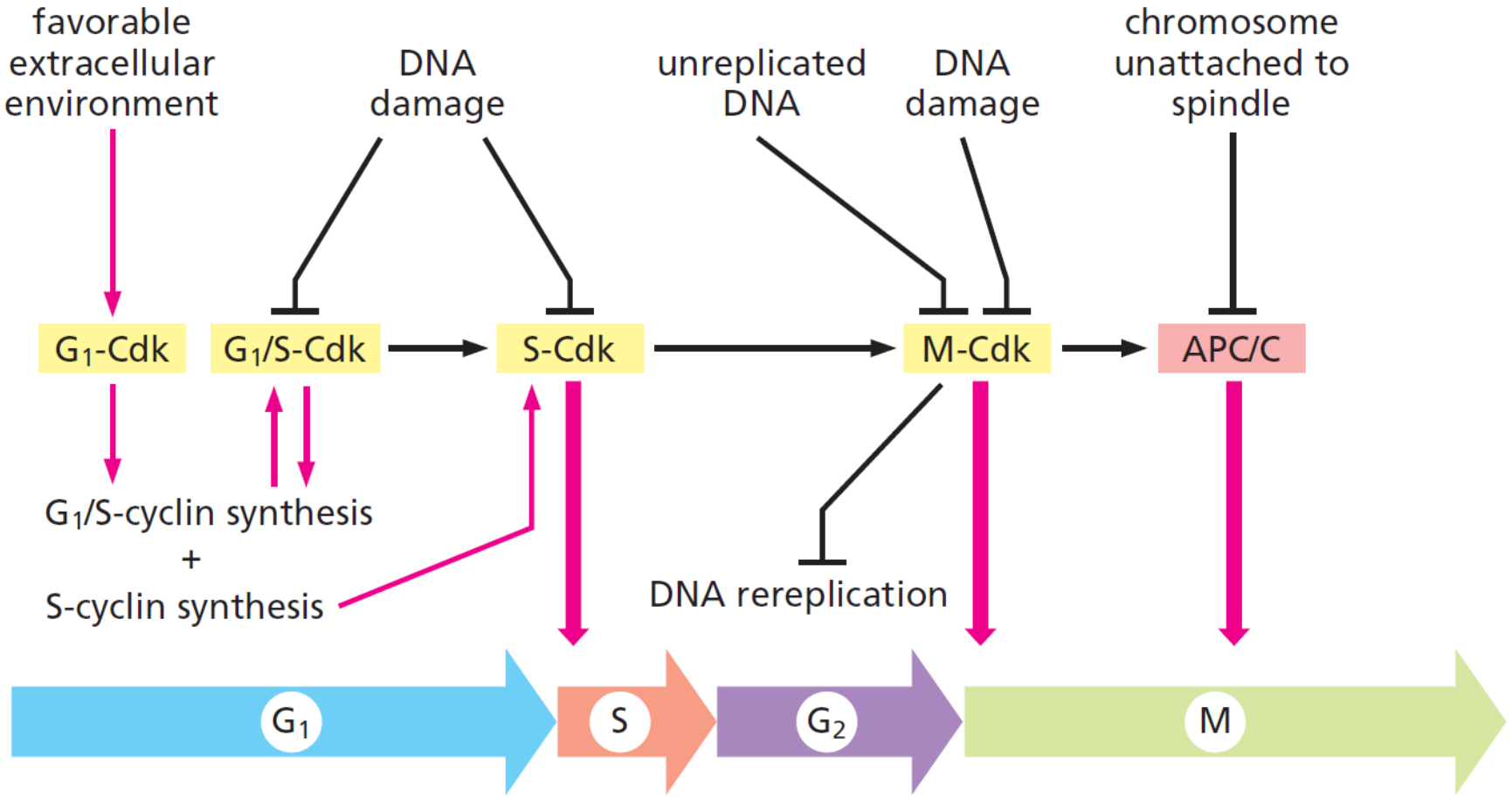
- 3. **M-cyclins** activate Cdks that stimulate entry into mitosis at the G2/M checkpoint. Mechanisms that we discuss later destroy M-cyclins in midmitosis.

- 4. In most cells, a fourth class of cyclins, the **G1-cyclins**, helps govern the activities of the G1/S cyclins, which control progression through Start in late G1.




cyclin
Cdk-activating kinase (CAK)
cyclin
ATP
ATP
ATP
P
T-loop
Cdk
active site
activating phosphate
(A) INACTIVE
(B) PARTLY ACTIVE
(C) FULLY ACTIVE


cyclin
inhibitory phosphate
Wee1
kinase
Cdc25
phosphatase
P
P
P
Cdk
activating
phosphate
ACTIVE
INACTIVE


favorable extracellular environment
DNA damage
unreplicated DNA
DNA damage
chromosome unattached to spindle
G1-Cdk
G1/S-Cdk
S-Cdk
M-Cdk
APC/C
G1/S-cyclin synthesis
+
S-cyclin synthesis
DNA rereplication
G1
S
G2
M

- Following the completion of S phase and transition through G2, the cell undergoes the dramatic upheaval of M phase.

- This begins with mitosis, during which the sister chromatids are separated and distributed (segregated) to a pair of identical daughter nuclei, each with its own copy of the genome.

- Mitosis is traditionally divided into five stages—prophase, prometaphase, metaphase, anaphase, and telophase—defined primarily on the basis of chromosome behavior as seen in a microscope.

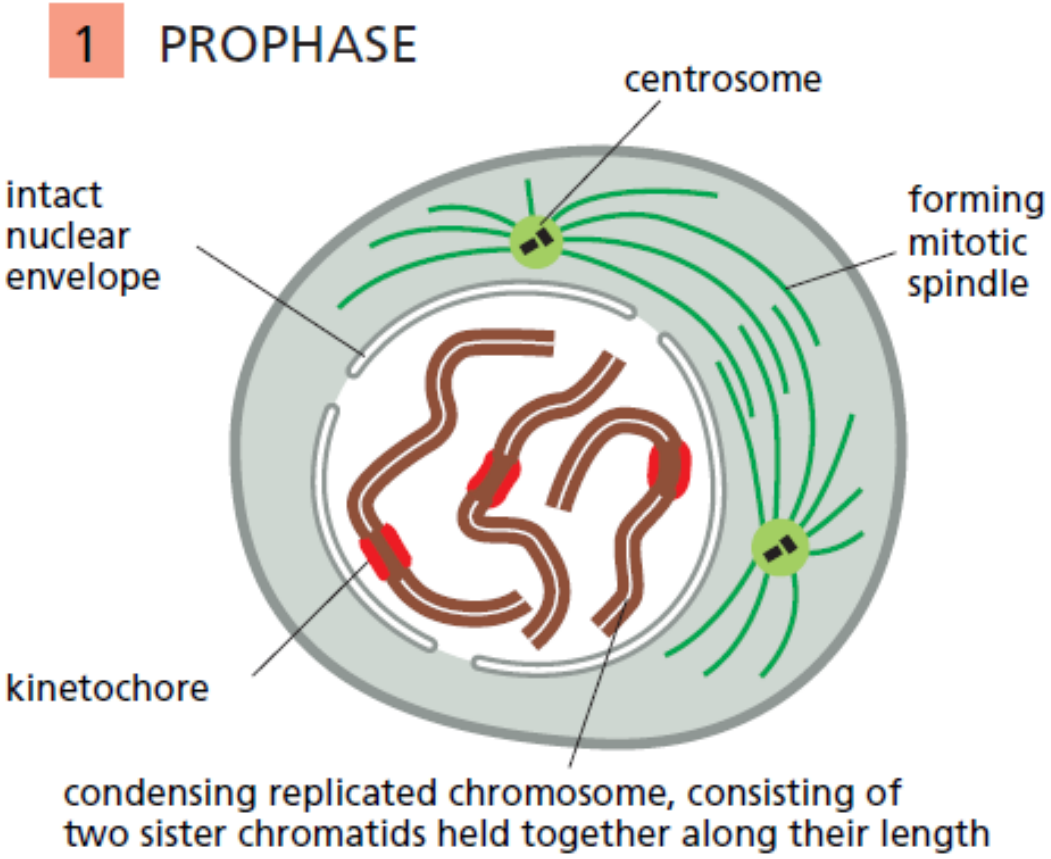
- As mitosis is completed, the second major event of M phase—cytokinesis—divides the cell into two halves, each with an identical nucleus.

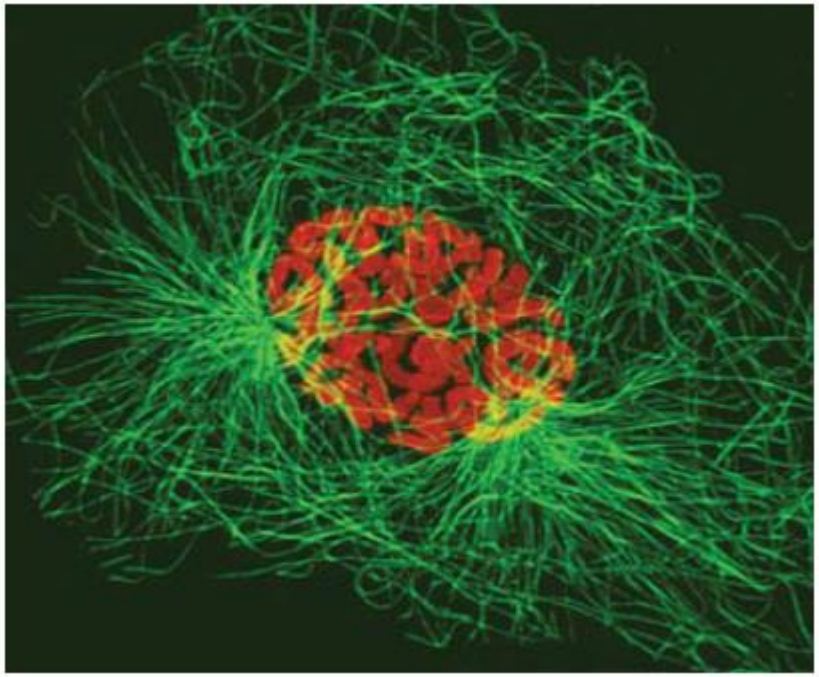
- From a regulatory point of view, mitosis can be divided into two major parts; M-Cdk and APC/C

- At **prophase**, the replicated chromosomes, each consisting of two closely associated sister chromatids, condense.

- Outside the nucleus, the mitotic spindle assembles between the two centrosomes, which have replicated and moved apart.

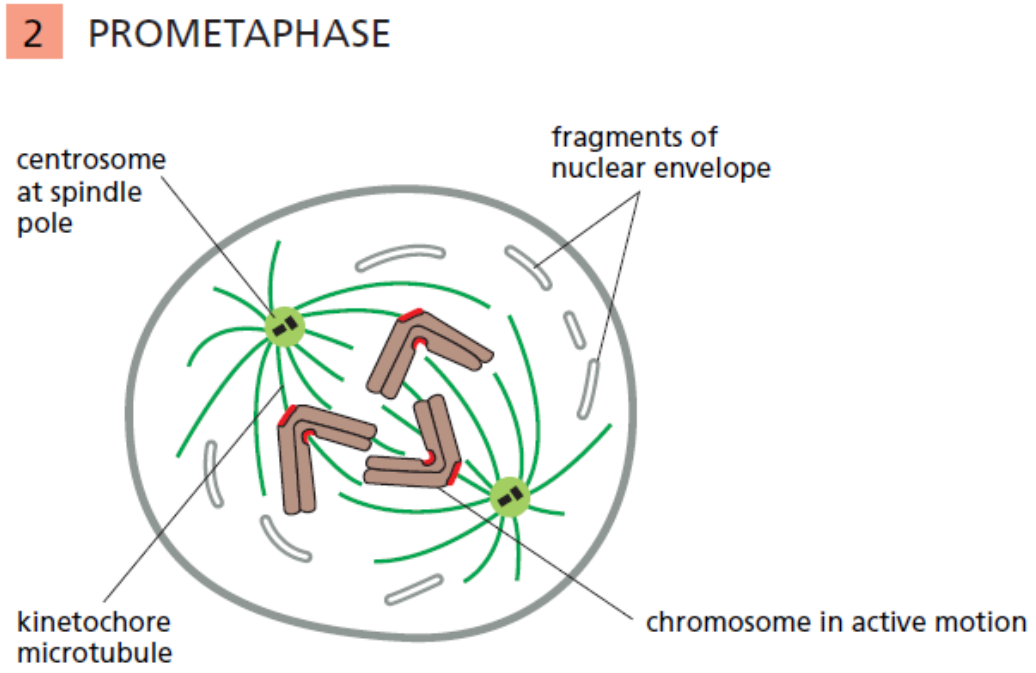
- In the photomicrograph, chromosomes are stained orange and microtubules are green.

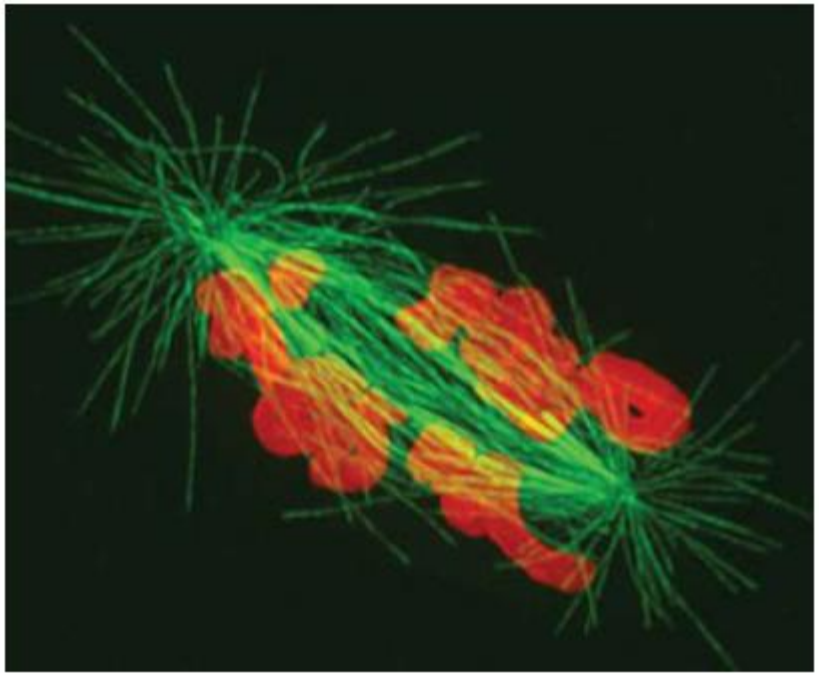




- **Prometaphase** starts abruptly with the breakdown of the nuclear envelope.

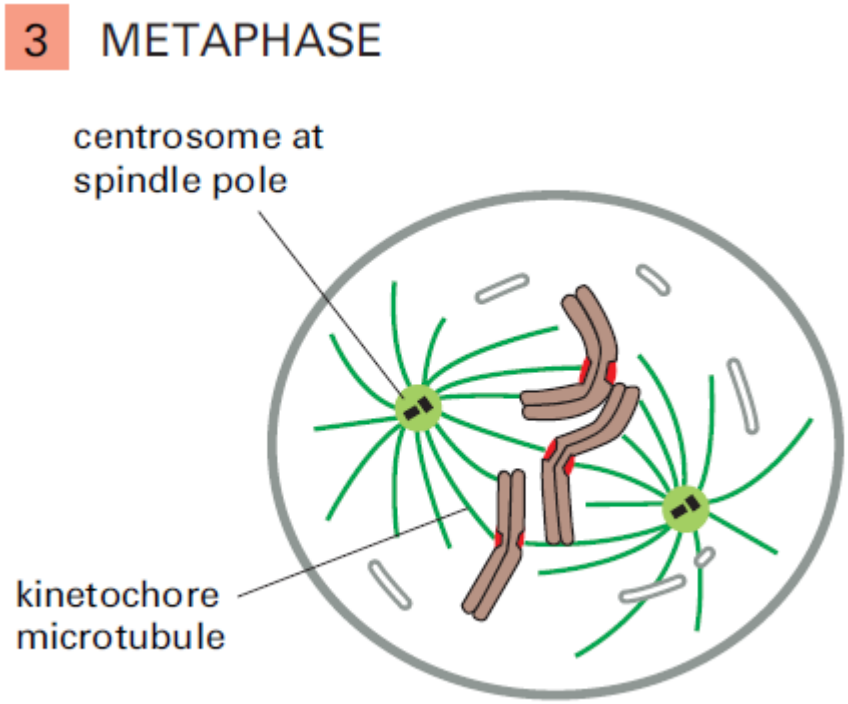
- Chromosomes can now attach to spindle microtubules via their kinetochores and undergo active movement.

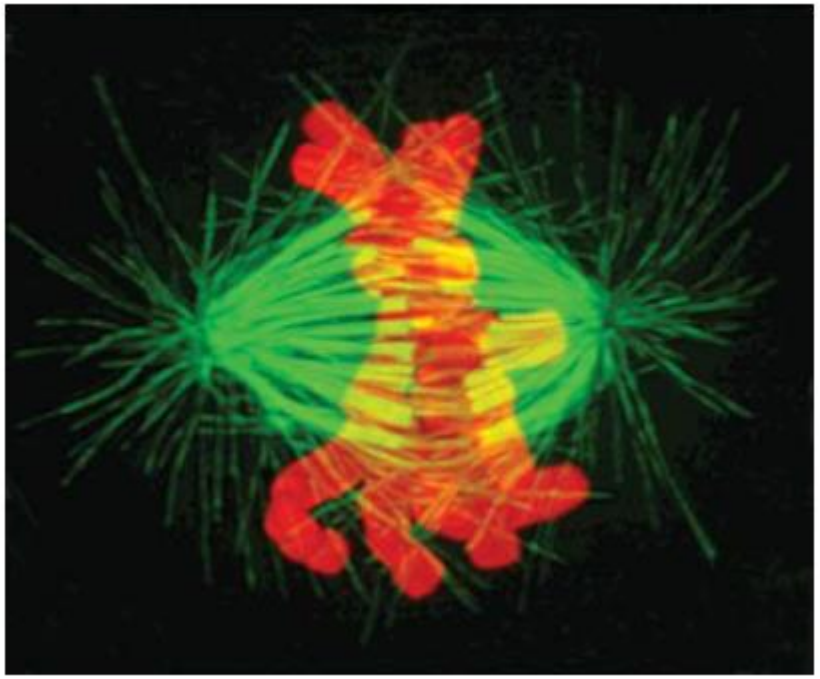




- At **metaphase**, the chromosomes are aligned at the equator of the spindle, midway between the spindle poles.

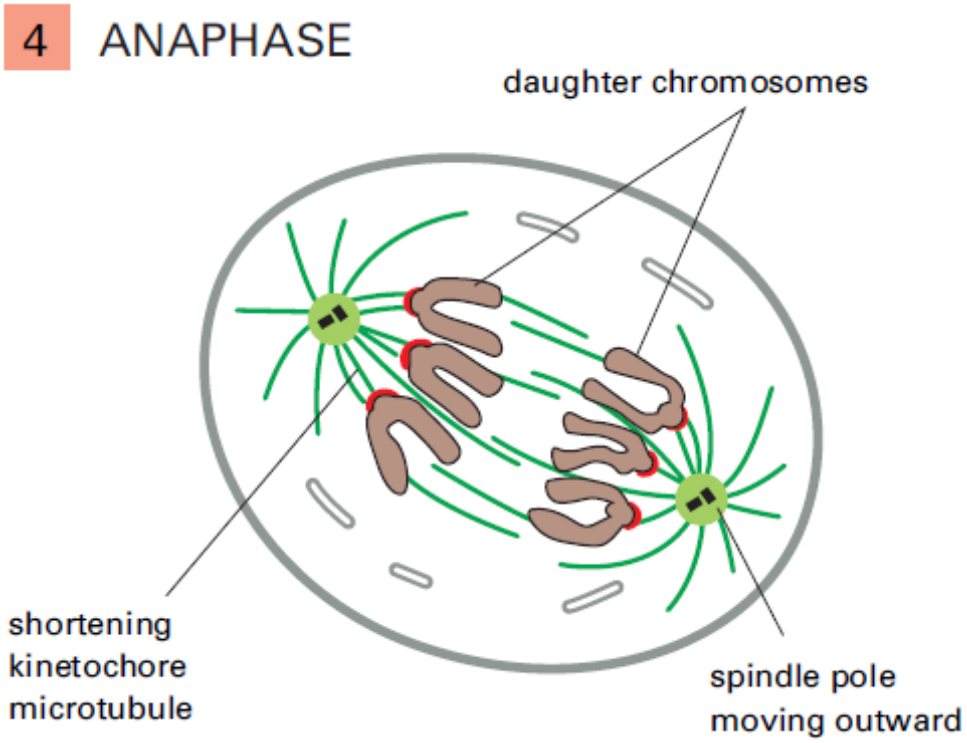
- The kinetochore microtubules attach sister chromatids to opposite poles of the spindle.

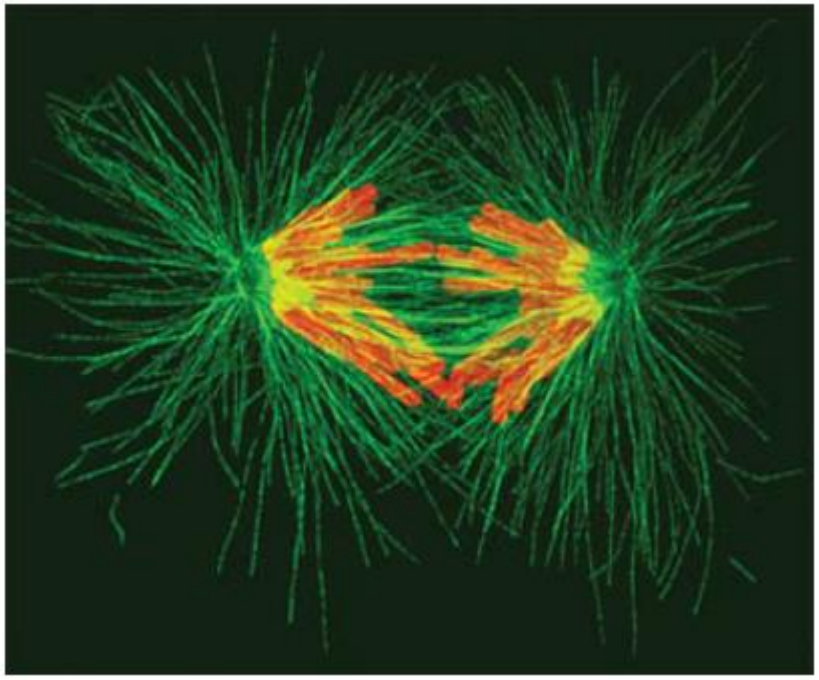




- At anaphase, the sister chromatids synchronously separate to form two daughter chromosomes, and each is pulled slowly toward the spindle pole it faces.

- The kinetochore microtubules get shorter, and the spindle poles also move apart; both processes contribute to chromosome segregation.

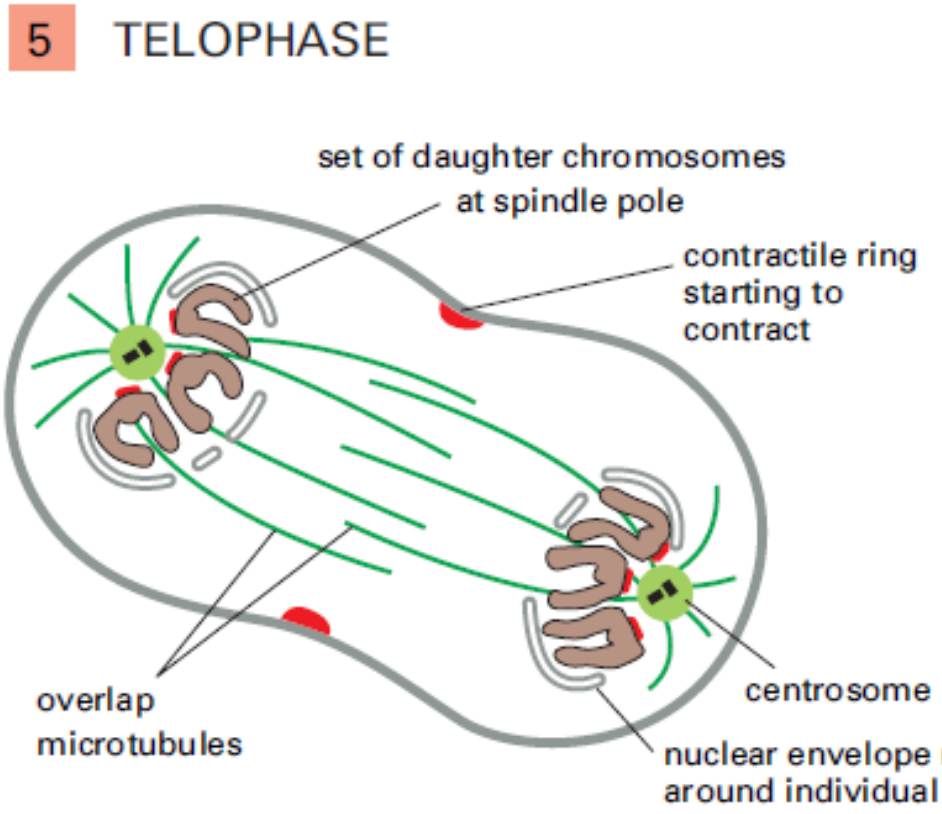


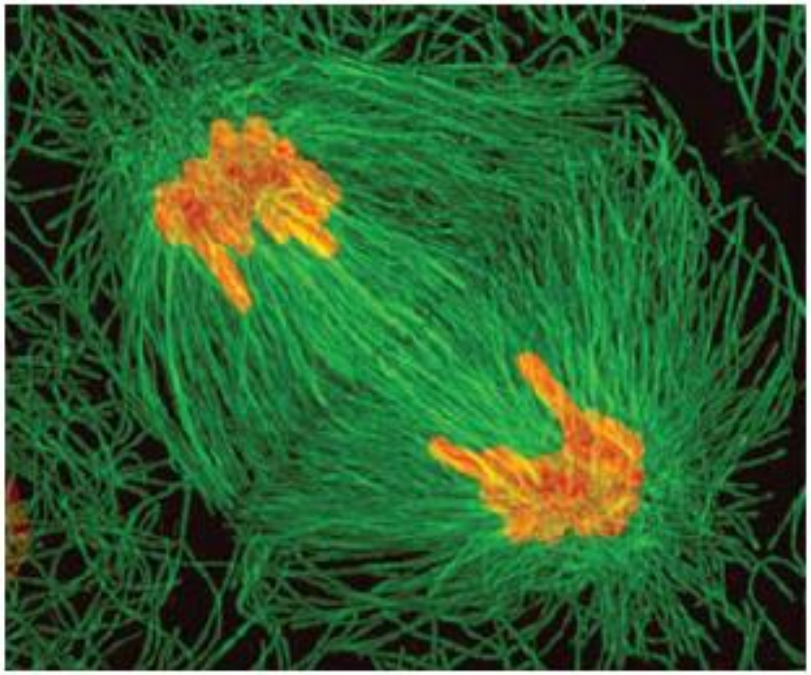


- During **telophase**, the two sets of daughter chromosomes arrive at the poles of the spindle and decondense.

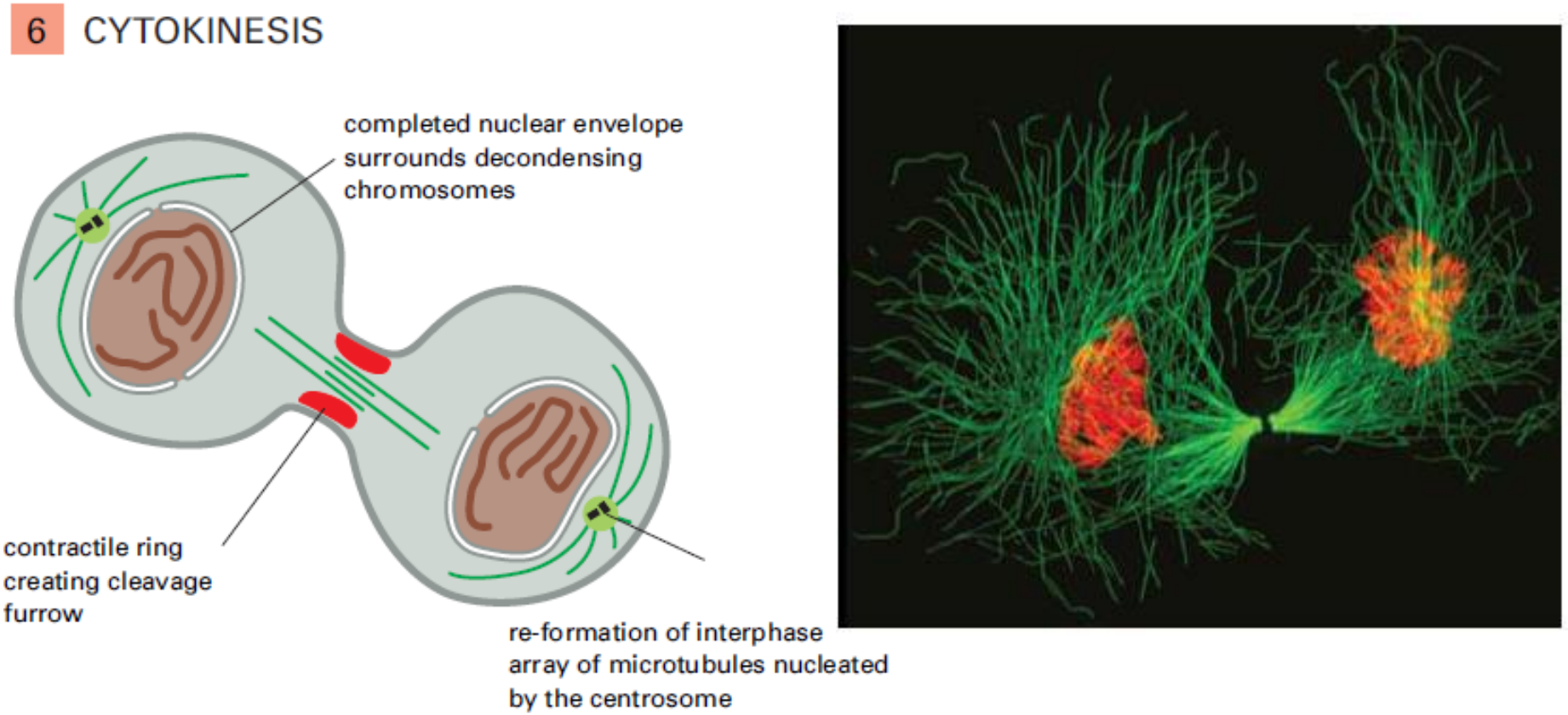
- A new nuclear envelope reassembles around each set, completing the formation of two nuclei and marking the end of mitosis.

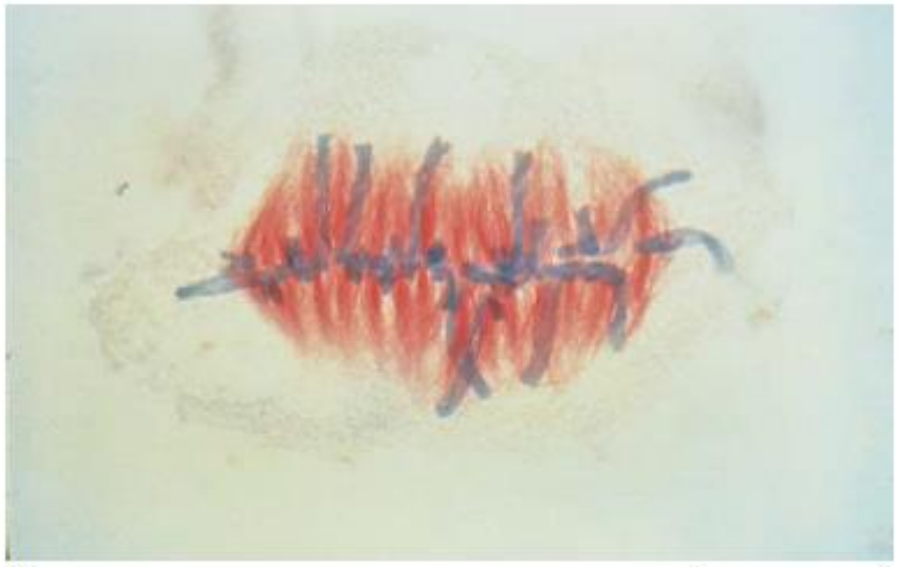
- The division of the cytoplasm begins with contraction of the contractile ring.





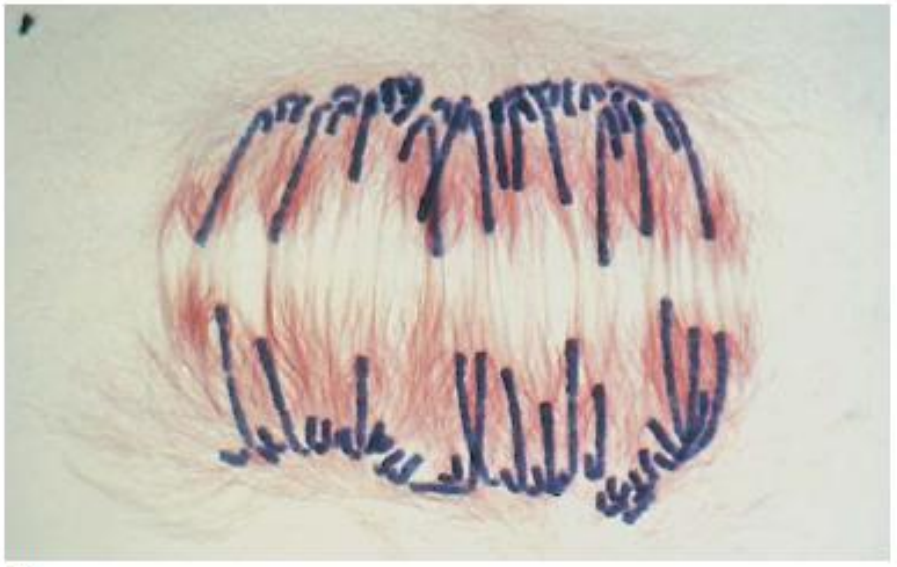
- During cytokinesis, the cytoplasm is divided in two by a contractile ring of actin and myosin filaments, which pinches the cell in two to create two daughters, each with one nucleus.



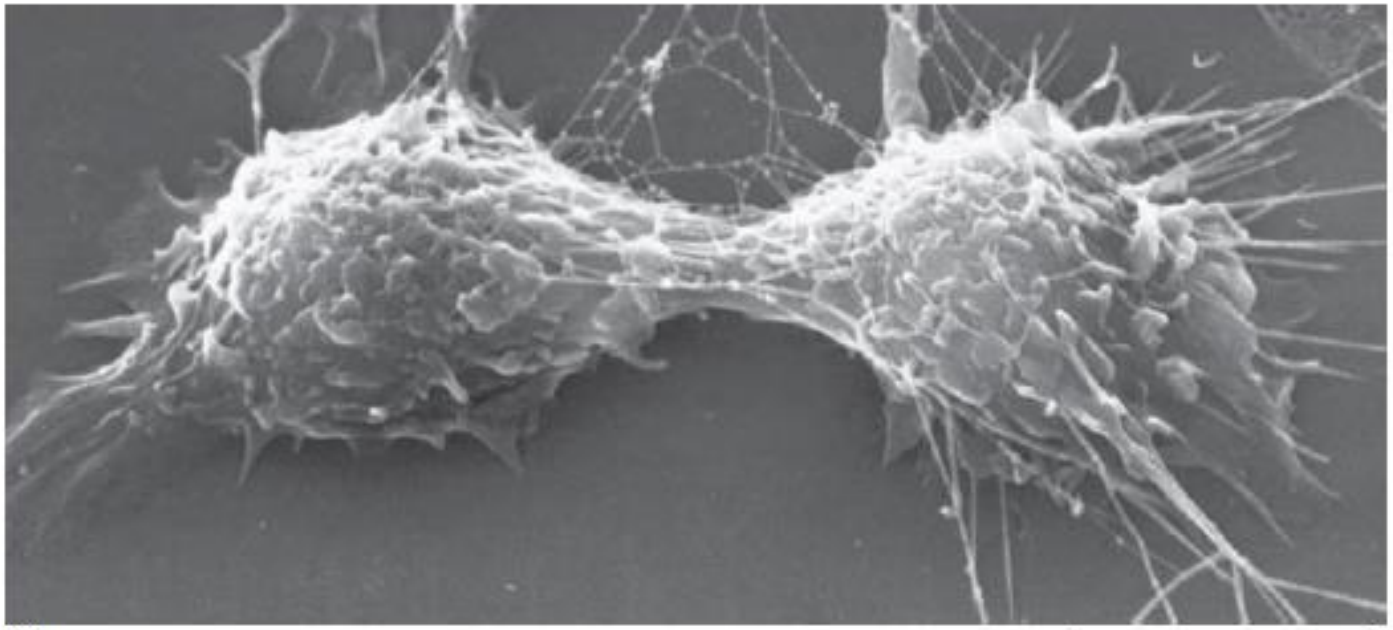


(A)

20 µm



(B)



(A)

10 µm

- Most eucaryotic organisms reproduce sexually: the genomes of two parents mix to generate offspring that are genetically distinct from either parent .

- The cells of these organisms are generally diploid: that is, they contain two slightly different copies, or homologs, of each chromosome, one from each parent.

- Sexual reproduction depends on a specialized nuclear division process called meiosis, which produces haploid cells carrying only a single copy of each chromosome.

- In many organisms, the haploid cells differentiate into specialized reproductive cells called gametes—eggs and sperm in most species.

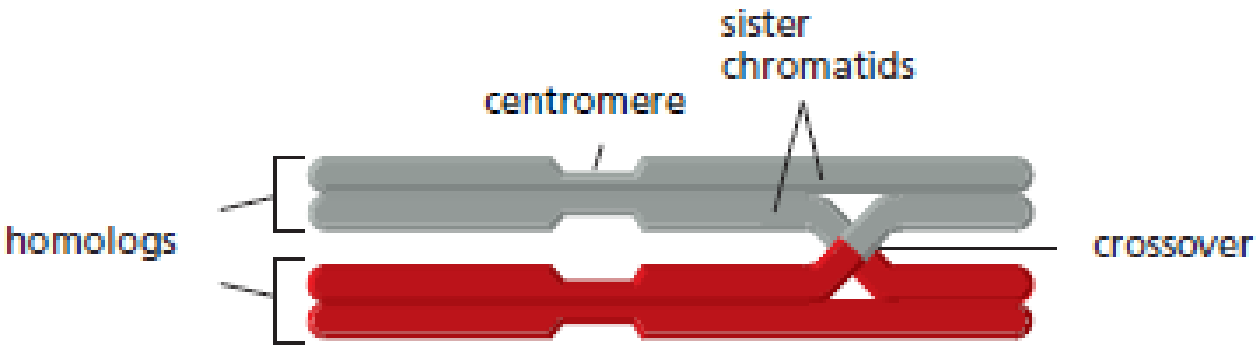
- In these species, the reproductive cycle ends when a sperm and egg fuse to form a diploid zygote with the potential to form a new individual.

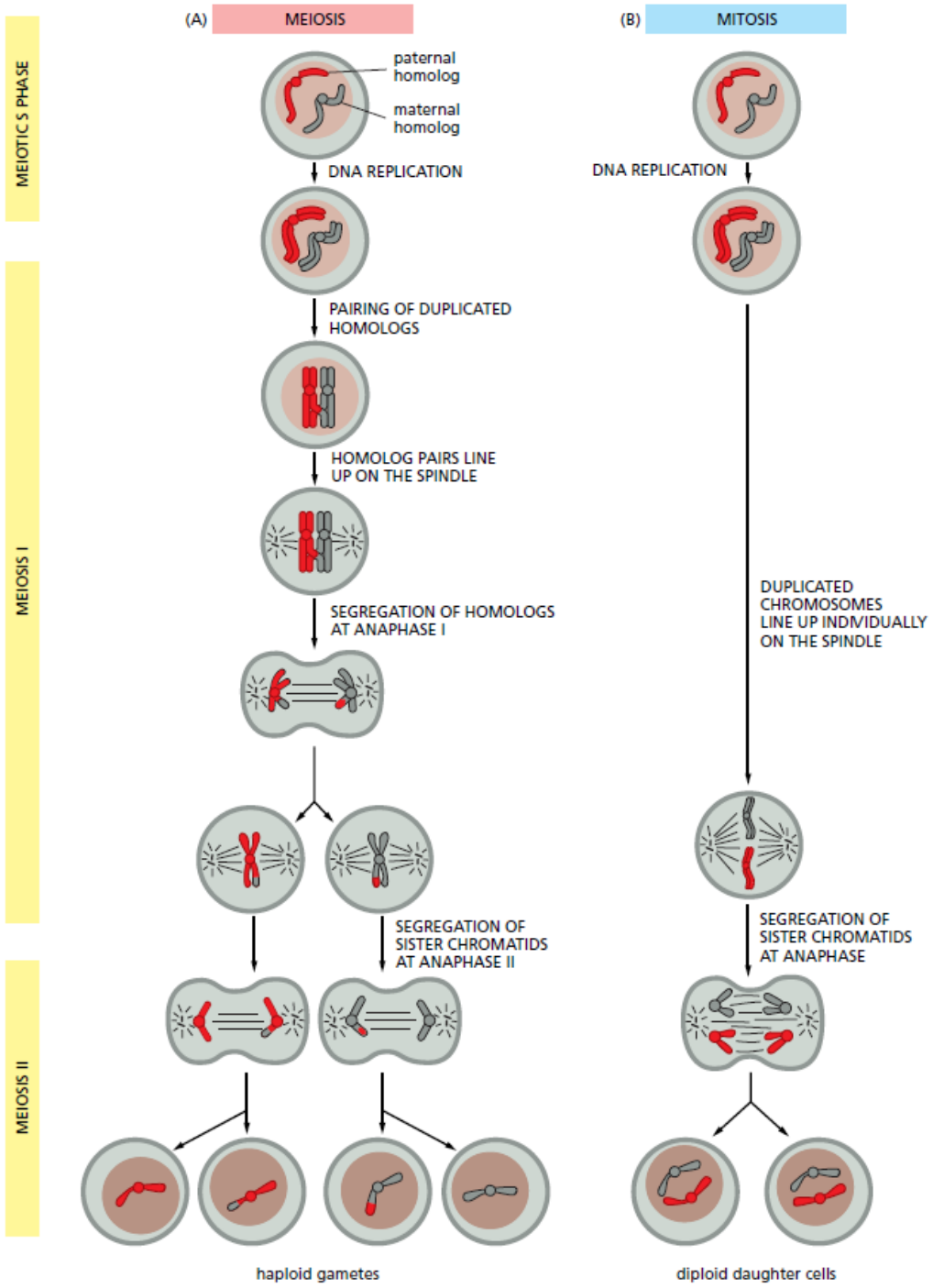
- Meiosis begins with a round of chromosome duplication, called meiotic S phase, followed by two rounds of chromosome segregation, called meiosis I and II.

- Meiosis I segregates the homologs (each composed of a tightly linked pair of sister chromatids).

- Meiosis II, like conventional mitosis, segregates the sister chromatids of each homolog.

- In the homolog pairing phase interactions between complementary DNA sequences in the two homologs, occur.




(A) MEIOSIS
(B) MITOSIS
MEIOTIC S PHASE
MEIOSIS I
MEIOSIS II
paternal homolog
maternal homolog
DNA REPLICATION
DNA REPLICATION
PAIRING OF DUPLICATED HOMOLOGS
HOMOLOG PAIRS LINE UP ON THE SPINDLE
SEGREGATION OF HOMOLOGS AT ANAPHASE I
DUPLICATED CHROMOSOMES LINE UP INDIVIDUALLY ON THE SPINDLE
SEGREGATION OF SISTER CHROMATIDS AT ANAPHASE II
SEGREGATION OF SISTER CHROMATIDS AT ANAPHASE
haploid gametes
diploid daughter cells

- Cell death plays a crucially important part in animal and plant development, and it usually continues into adulthood.

- In a healthy adult human, billions of cells die in the bone marrow and intestine every hour.

- Our tissues do not shrink because, by unknown regulatory mechanisms, cell division exactly balances the cell death.

- We now know that these “normal” cell deaths are suicides, in which the cells activate an intracellular death program and kill themselves in a controlled way—a process known as programmed cell death.

- The idea that animal cells have a built-in death program was proposed in the 1970s, but its general acceptance took another 20 years and depended on genetic studies in the nematode *C. elegans* that identified the first genes dedicated to programmed cell death and its control.

- Programmed cell death in animals usually, but not exclusively, occurs by **apoptosis** (from the Greek word meaning “falling off,” as leaves from a tree).

- Although apoptosis is only one form of programmed cell death, it is by far the most common and best understood, and, confusingly, biologists often use the terms programmed cell death and apoptosis interchangeably.

- Cells dying by apoptosis undergo characteristic morphological changes.

- They shrink and condense, the cytoskeleton collapses, the nuclear envelope disassembles, and the nuclear chromatin condenses and breaks up into fragments.

- The cell surface often blebs and, if the cell is large, often breaks up into membrane-enclosed fragments called apoptotic bodies.

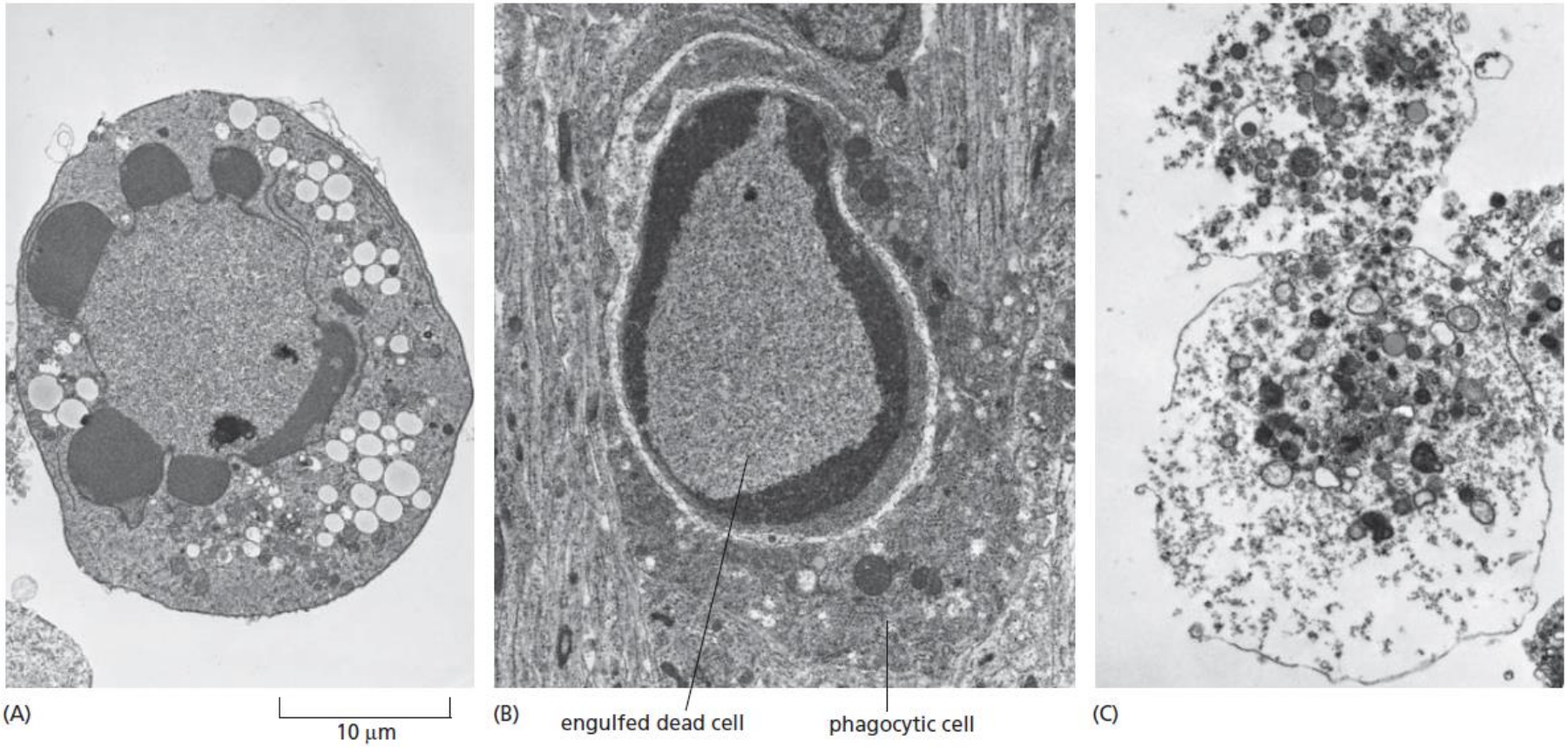
- Most importantly, the surface of the cell or apoptotic bodies becomes chemically altered, so that a neighboring cell or a macrophage (a specialized phagocytic cell) rapidly engulfs them, before they can spill their contents.

- In this way, the cell dies neatly and is rapidly cleared away, without causing a damaging inflammatory response.

- Because the cells are eaten and digested so quickly, there are usually few dead cells to be seen, even when large numbers of cells have died by apoptosis.

- By contrast to apoptosis and other less well characterized forms of programmed cell death, animal cells that die accidentally in response to an acute insult, such as trauma or a lack of blood supply, usually do so by a process called cell **necrosis**.

- Necrotic cells swell and burst, spilling their contents over their neighbors and eliciting an inflammatory response


(A)
10 μm
(B)
engulfed dead cell
phagocytic cell
(C)

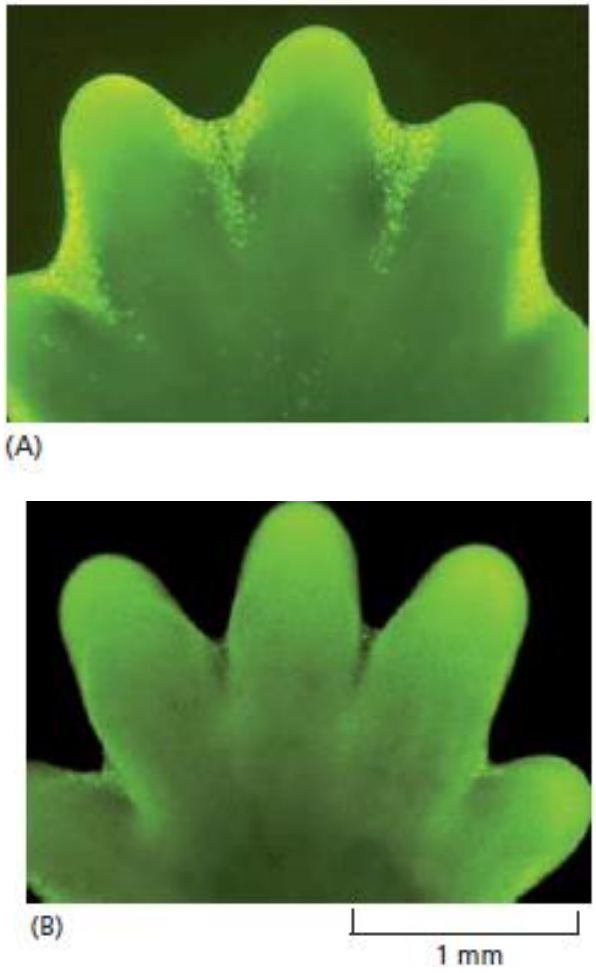
- The amount of programmed cell death that occurs in developing and adult animal tissues can be astonishing. In the developing vertebrate nervous system, for example, more than half of many types of nerve cells normally die soon after they are formed.

- It seems remarkably wasteful for so many cells to die, especially as the vast majority are perfectly healthy at the time they kill themselves.

- What purposes does this massive cell death serve?

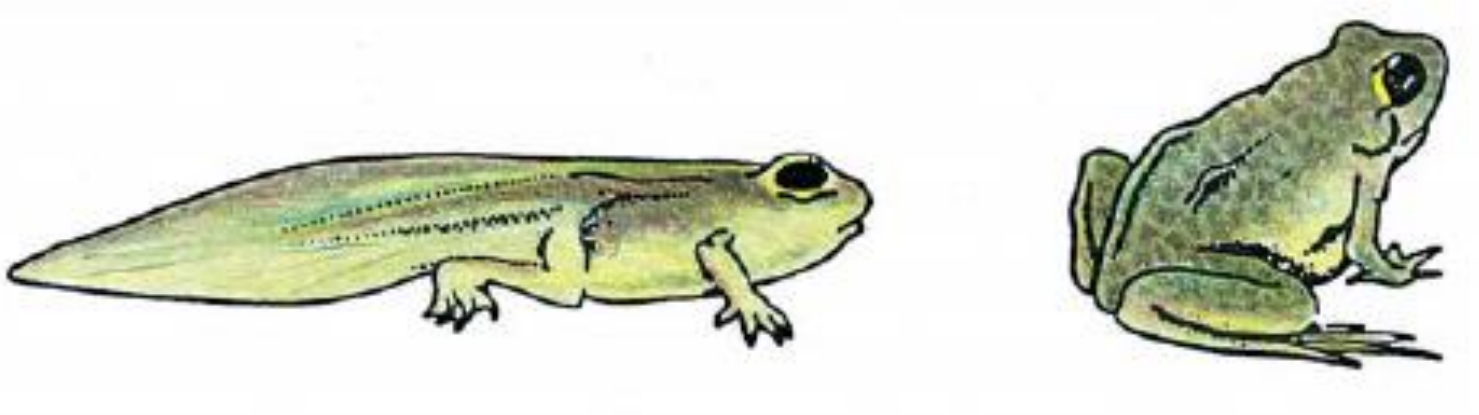
- In some cases, the answer is clear. In animal development, programmed cell death eliminates unwanted cells, usually by apoptosis.

- Cell death, for example, helps sculpt hands and feet during embryonic development: they start out as spade-like structures, and the individual digits separate only as the cells between them die



- In other cases, cells die when the structure they form is no longer needed.

- When a tadpole changes into a frog at metamorphosis, the cells in the tail die, and the tail, which is not needed in the frog, disappears.



- In many other cases, cell death helps regulate cell numbers. In the developing nervous system, for example, cell death adjusts the number of nerve cells to match the number of target cells that the nerve cells connect to.

- Programmed cell death also functions as a quality-control process in development, eliminating cells that are abnormal, misplaced, nonfunctional, or potentially dangerous to the animal.

- Striking examples occur in the vertebrate adaptive immune system, where apoptosis eliminates developing T and B lymphocytes that either fail to produce potentially useful antigen-specific receptors or produce self-reactive receptors that make the cells potentially dangerous; it also eliminates most of the lymphocytes activated by an infection, after they have helped destroy the responsible microbes.

- In adult tissues that are neither growing nor shrinking, cell death and cell division must be tightly regulated to ensure that they are exactly in balance.,

- If part of the liver is removed in an adult rat, for example, liver cell proliferation increases to make up the loss.

- Conversely, if a rat is treated with the drug phenobarbital— which stimulates liver cell division (and thereby liver enlargement)— and then the phenobarbital treatment is stopped, apoptosis in the liver greatly increases until the liver has returned to its original size, usually within a week or so.

- Thus, the liver is kept at a constant size through the regulation of both the cell death rate and the cell birth rate, although the control mechanisms responsible for such regulation are largely unknown.

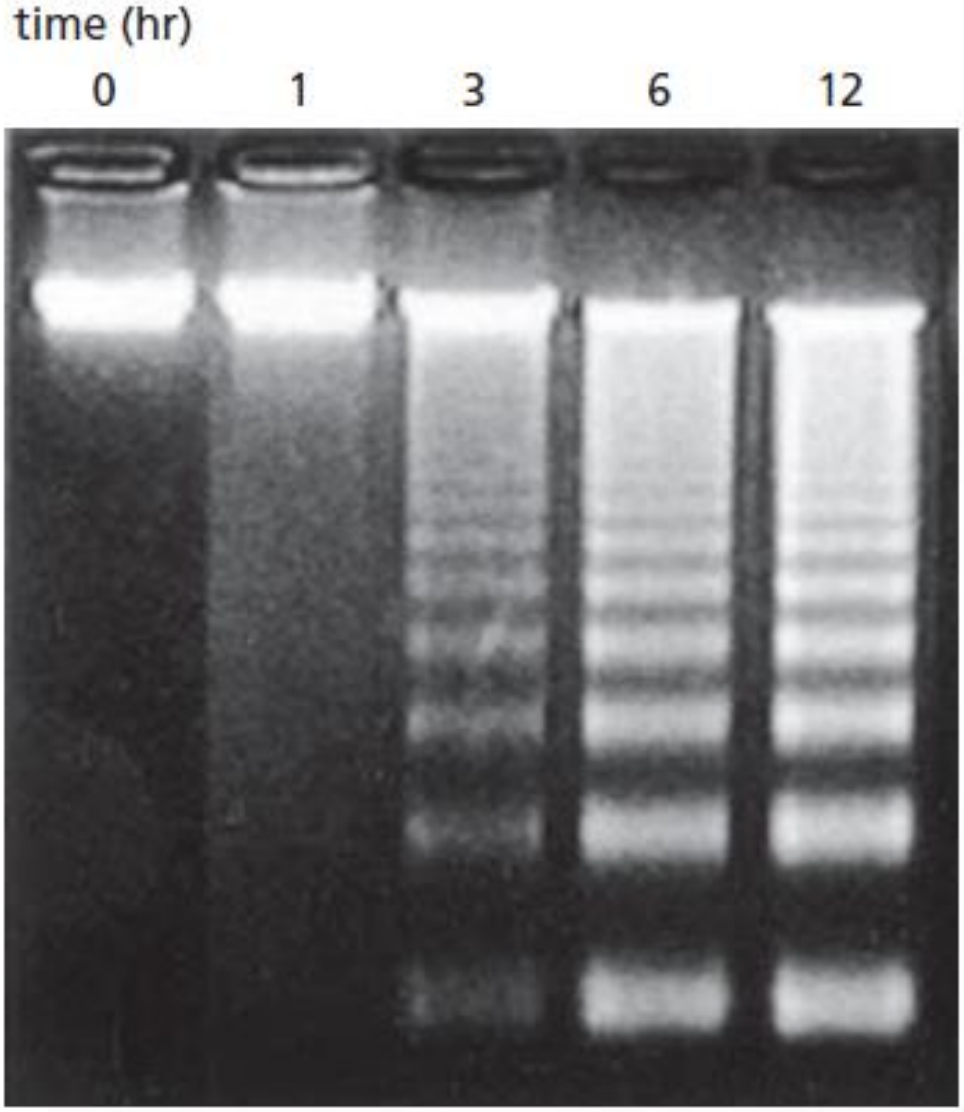
- Apoptosis occurs at a staggeringly high rate in the adult human bone marrow, where most blood cells are produced. p.

- Cells undergoing apoptosis not only have a characteristic morphology but also display characteristic biochemical changes, which can be used to identify apoptotic cells.

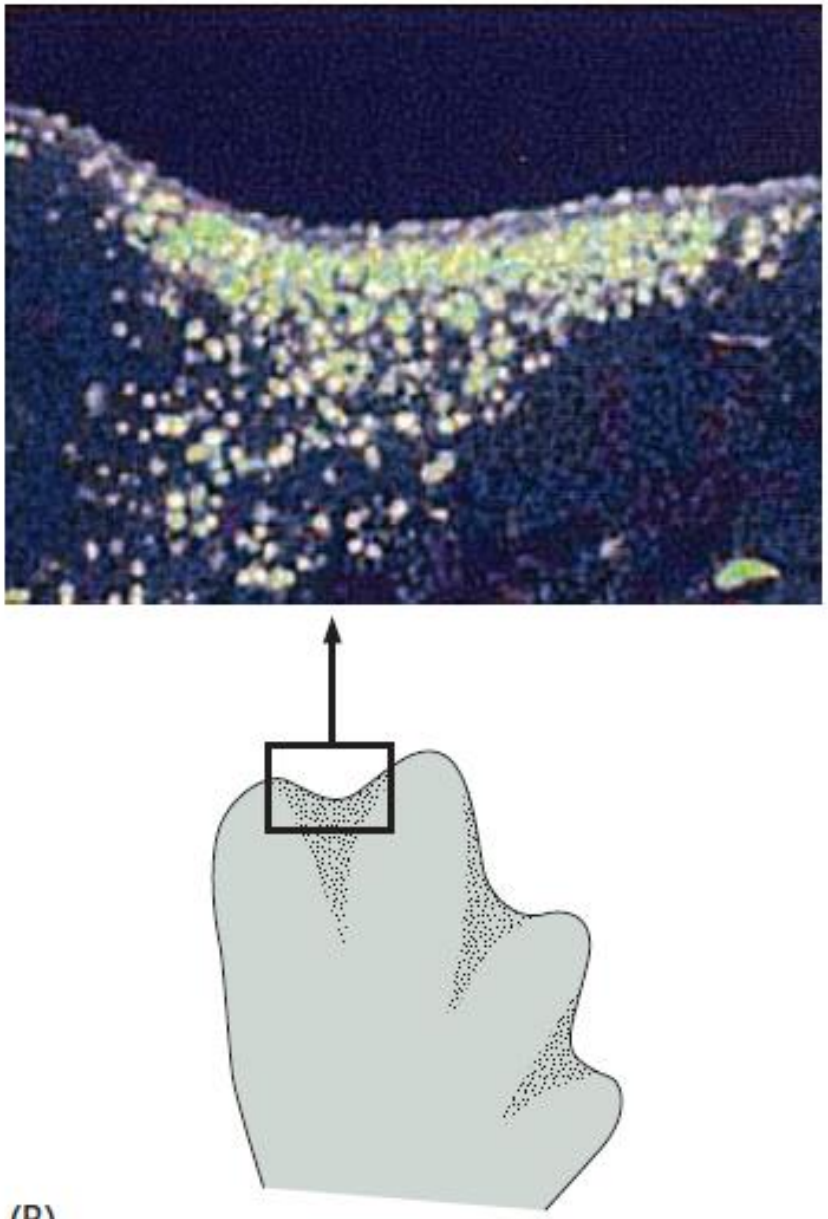
- During apoptosis, for example, an endonuclease cleaves the chromosomal DNA into fragments of distinctive sizes; because the cleavages occur in the linker regions between nucleosomes, the fragments separate into a characteristic ladder pattern when analyzed by gel electrophoresis.

- Moreover, the cleavage of DNA generates many new DNA ends, which can be marked in apoptotic nuclei by using a labeled nucleotide.

- An especially important change occurs in the plasma membrane of apoptotic cells. The negatively charged phospholipid phosphatidylserine is normally exclusively located in the inner leaflet of the lipid bilayer of the plasma membrane), but it flips to the outer leaflet in apoptotic cells, where it can serve as a marker of these cells.

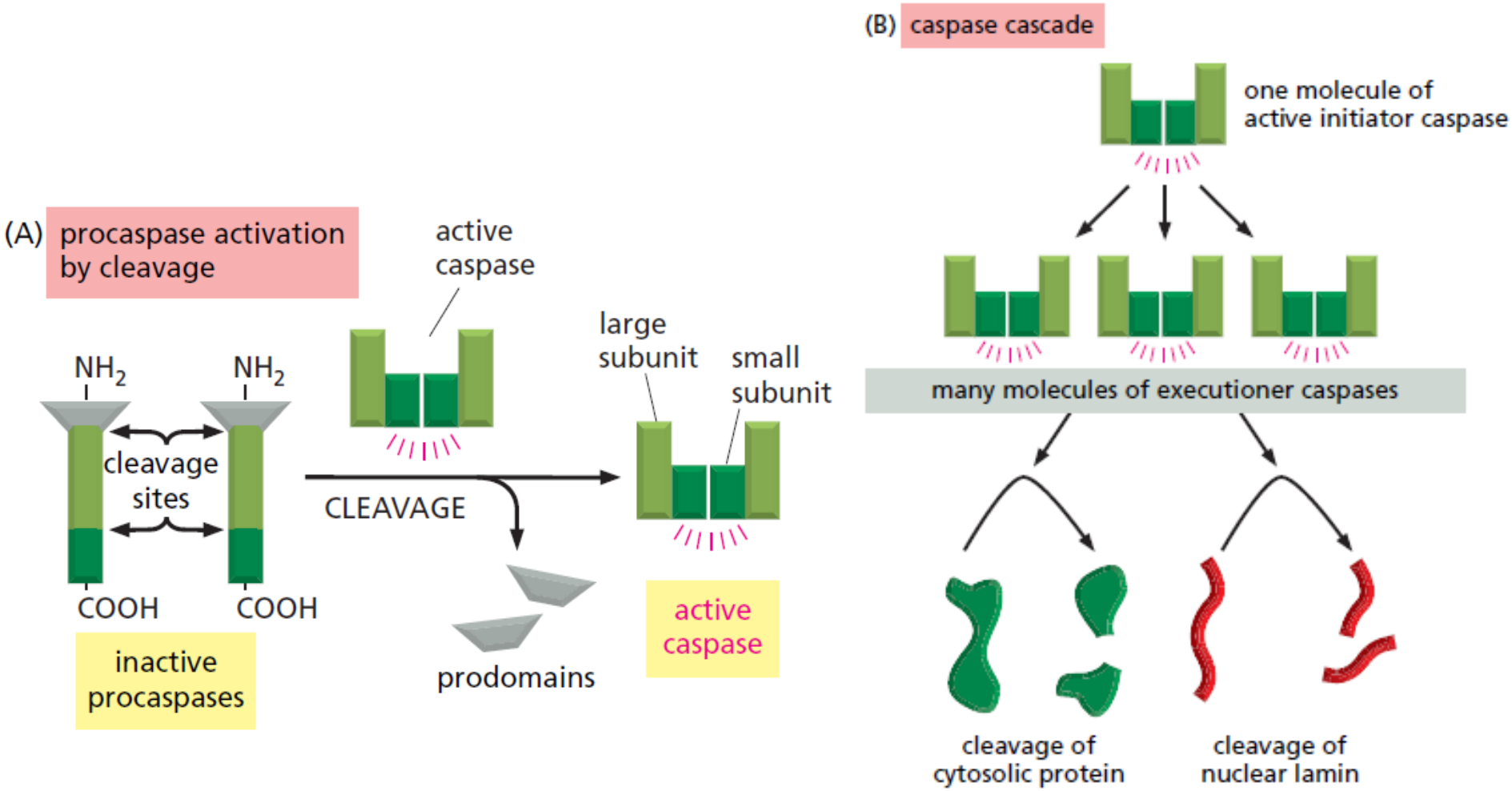


(A)



(B)

- The intracellular machinery responsible for apoptosis is similar in all animal cells.
- It depends on a family of proteases that have a cysteine at their active site and cleave their target proteins at specific aspartic acids.
- They are therefore called caspases (c for cysteine and asp for aspartic acid).
- Caspases are synthesized in the cell as inactive precursors, or procaspases, which are typically activated by proteolytic cleavage.
- Once activated, caspases cleave, and thereby activate, other procaspases, resulting in an amplifying proteolytic cascade.


(A) procaspase activation by cleavage
active caspase
large subunit
small subunit
NH_2
NH_2
cleavage sites
CLEAVAGE
COOH
COOH
inactive procaspases
prodomains
active caspase
(B) caspase cascade
one molecule of active initiator caspase
many molecules of executioner caspases
cleavage of cytosolic protein
cleavage of nuclear lamin

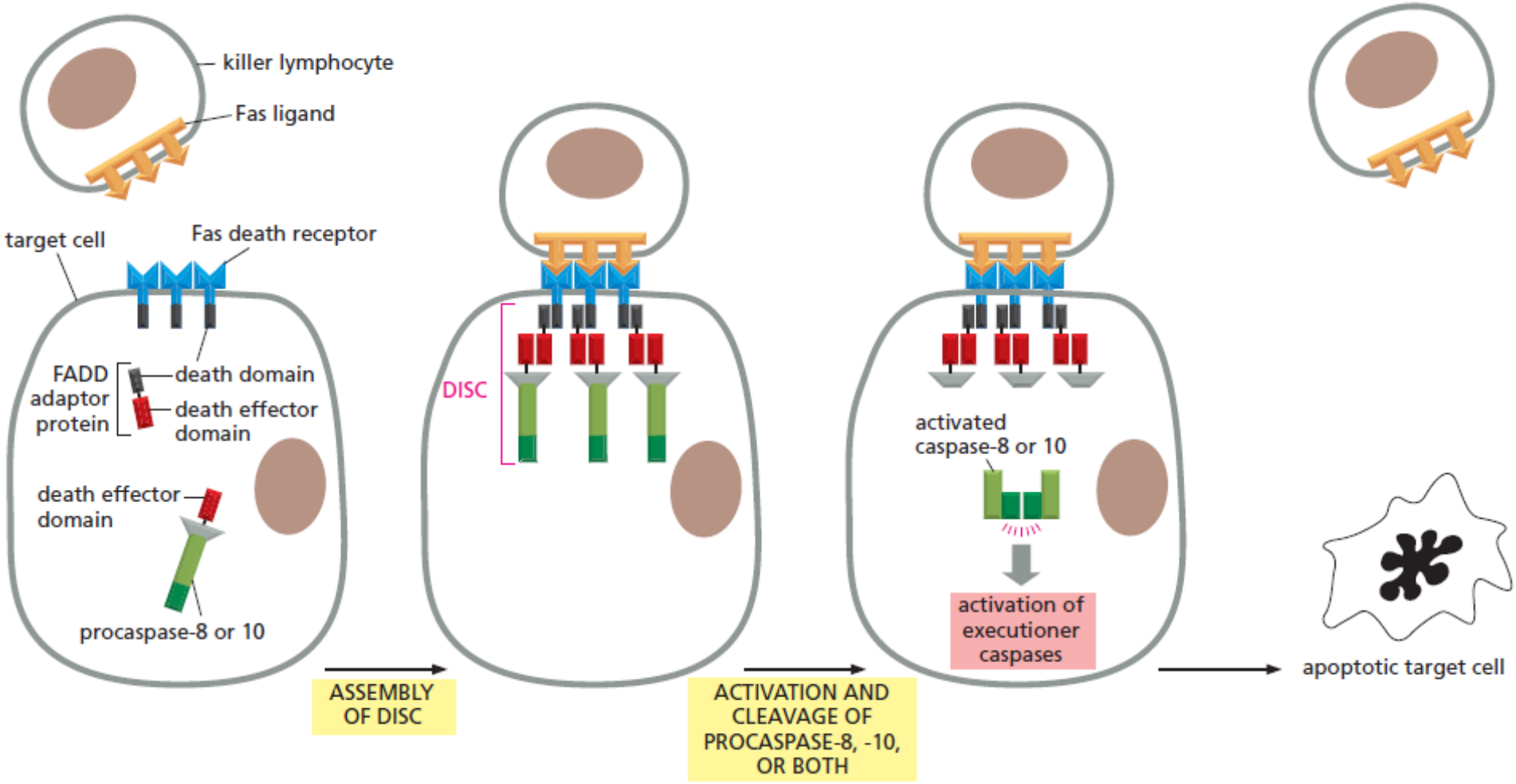
- The two best understood signaling pathways that can activate a caspase cascade leading to apoptosis in mammalian cells are called the extrinsic pathway and the intrinsic pathway.

- Each uses its own initiator procaspases and activation complex.

- When activated by the binding of Fas ligand, the death domains on the
- cytosolic tails of the Fas death receptors recruit intracellular adaptor proteins, which in turn recruit initiator procaspases (procaspase-8, procaspase-10, or both), forming a death-inducing signaling complex (DISC).

- Once activated in the DISC, the initiator caspases activate downstream executioner procaspases to induce apoptosis.

- In some cells the extrinsic pathway must recruit the intrinsic apoptotic pathway to amplify the caspase cascade in order to kill the cell.


killer lymphocyte
Fas ligand
target cell
Fas death receptor
FADD adaptor protein
death domain
death effector domain
death effector domain
procaspase-8 or 10
DISC
activated caspase-8 or 10
activation of executioner caspases
apoptotic target cell
ASSEMBLY OF DISC
ACTIVATION AND CLEAVAGE OF PROCASPASE-8, -10, OR BOTH

- Many cells produce inhibitory proteins that act either extracellularly or intracellularly to restrain the extrinsic pathway.

- For example, some produce cellsurface decoy receptors, which have a ligand-binding domain but not a death domain; because they can bind a death ligand but cannot activate apoptosis, the decoys competitively inhibit the death receptors.

- Cells can also produce intracellular blocking proteins such as FLIP, which resembles an initiator procaspase but lacks the proteolytic domain; it competes with procaspase-8 and procaspase-10 for binding sites in the DISC and thereby inhibits the activation of these initiator procaspases.

- Such inhibitory mechanisms help prevent the inappropriate activation of the extrinsic pathway of apoptosis.

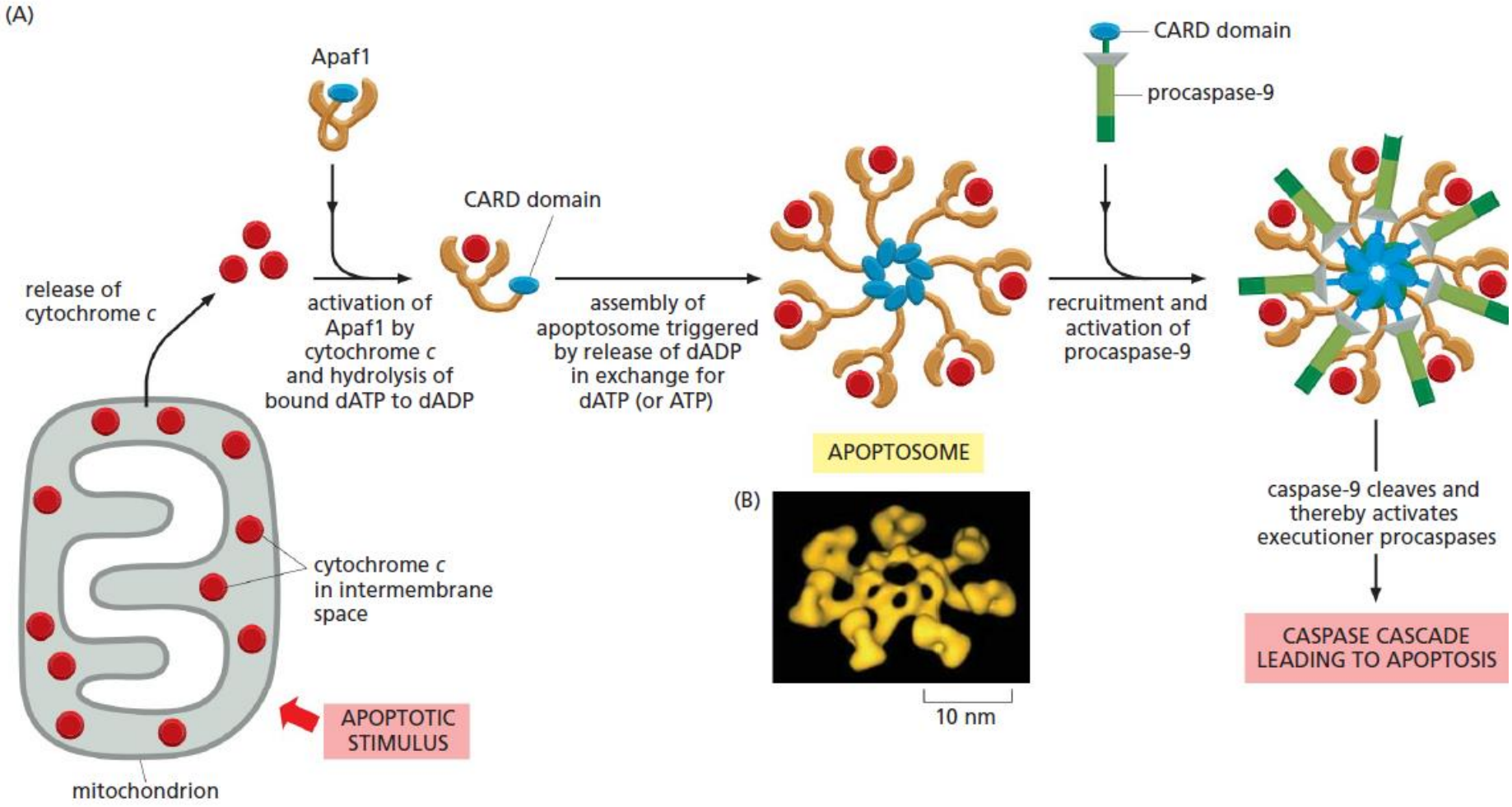
- Cells can also activate their apoptosis program from inside the cell, usually in response to injury or other stresses, such as DNA damage or lack of oxygen, nutrients, or extracellular survival signals.

- In vertebrate cells, such intracellular activation of the apoptotic death program occurs via the intrinsic pathway of apoptosis, which depends on the release into the cytosol of mitochondrial proteins that normally reside in the intermembrane space of these organelles.

- Some of the released proteins activate a caspase proteolytic cascade in the cytoplasm, leading to apoptosis.

- A crucial protein released from mitochondria in the intrinsic pathway is cytochrome c, a water-soluble component of the mitochondrial electron-transport chain.

- When released into the cytosol, it has an entirely different function: it binds to a procaspase-activating adaptor protein called Apaf1 (apoptotic protease activating factor-1), causing the Apaf1 to oligomerize into a wheel-like heptamer called an apoptosome.


(A)
Apaf1
CARD domain
procaspase-9
release of cytochrome c
activation of Apaf1 by cytochrome c and hydrolysis of bound dATP to dADP
assembly of apoptosome triggered by release of dADP in exchange for dATP (or ATP)
recruitment and activation of procaspase-9
APOPTOSOME
(B)
10 nm
caspase-9 cleaves and thereby activates executioner procaspases
CASPASE CASCADE LEADING TO APOPTOSIS
cytochrome c in intermembrane space
APOPTOTIC STIMULUS
mitochondrion

- Inhibitors of apoptosis (IAPs) were first identified in certain insect viruses (baculoviruses), which encode IAP proteins to prevent a host cell that is infected by the virus from killing itself by apoptosis.

- Virus-infected animal cells frequently kill themselves to prevent the virus from replicating and infecting other cells.

- It is now known that most animal cells also make IAP proteins.

- All IAPs have one or more BIR (baculovirus IAP repeat) domains, which enable them to bind to and inhibit activated caspases.

- Some IAPs also polyubiquitylate caspases, marking the caspases for destruction by proteasomes.

- In this way, the IAPs set an inhibitory threshold that activated caspases must overcome to trigger apoptosis.

- Intercellular signals regulate most activities of animal cells, including apoptosis. These signals are part of the normal “social” controls that ensure that individual cells behave for the good of the organism as a whole—in this case, by surviving when they are needed and killing themselves when they are not.

- Some extracellular signal molecules stimulate apoptosis, whereas others inhibit it.

- Signal proteins such as Fas ligand that activate death receptors and thereby trigger the extrinsic pathway of apoptosis.

- Other extracellular signal molecules that stimulate apoptosis are especially important during animal development: a surge of thyroid hormone in the bloodstream, for example, signals cells in the tadpole tail to undergo apoptosis at metamorphosis, while locally produced bone morphogenic proteins (BMPs) stimulate cells between developing fingers and toes to kill themselves.

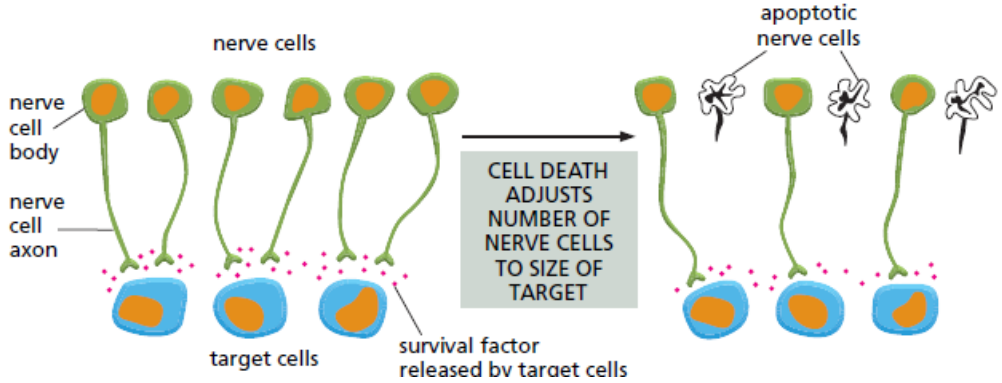
- Most animal cells require continuous signaling from other cells to avoid apoptosis.

- This surprising arrangement apparently helps ensure that cells survive only when and where they are needed.

- Nerve cells, for example, are produced in excess in the developing nervous system and then compete for limited amounts of survival factors that are secreted by the target cells that they normally connect to.

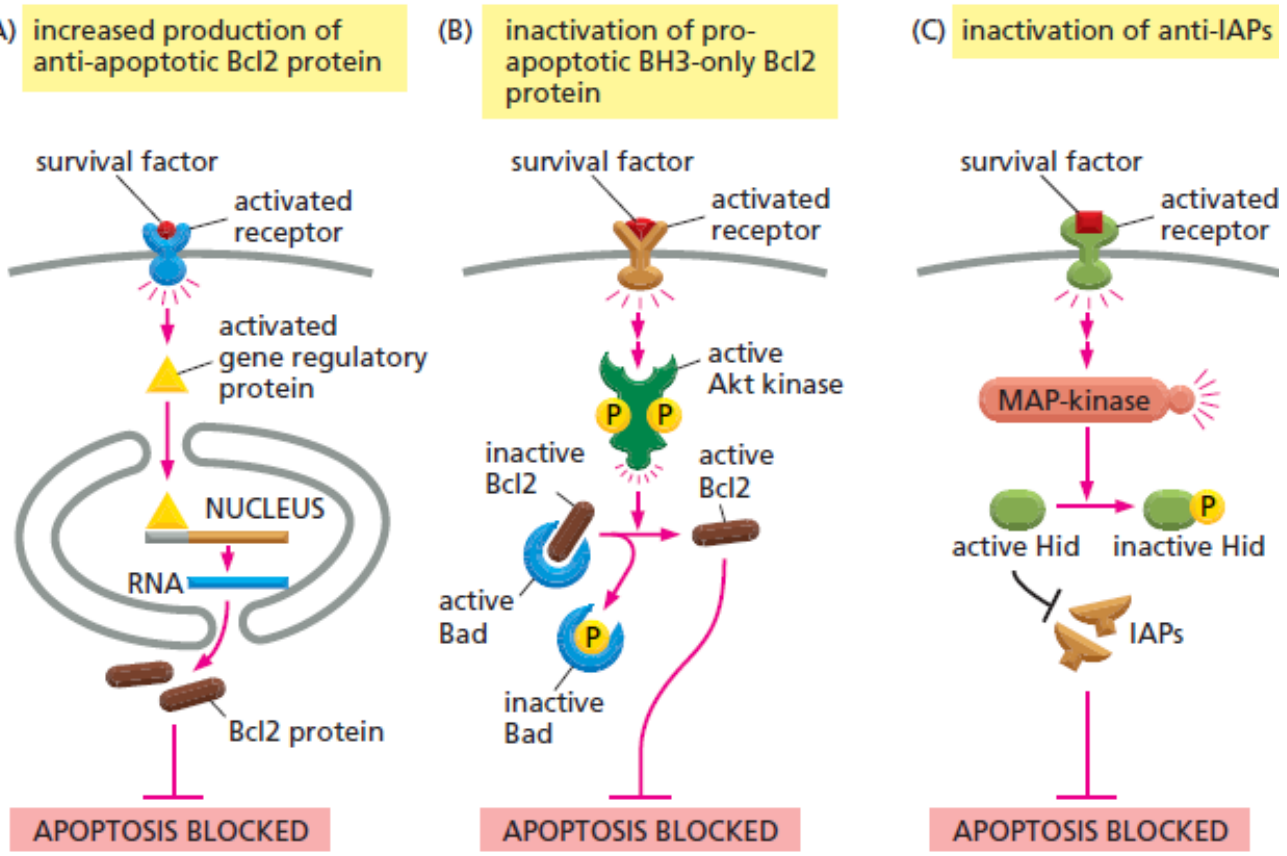
- Nerve cells that receive enough of the appropriate type of survival signal live, while the others die.

- In this way, the number of surviving neurons is automatically adjusted so that it is appropriate for the number of target cells they connect with.



- Survival factors usually bind to cell-surface receptors, which activate intracellular signaling pathways that suppress the apoptotic program.

- When mammalian cells are deprived of survival factors, they kill themselves by producing and activating pro-apoptotic BH3-only proteins, which activate the intrinsic pathway of apoptosis by overriding the anti-apoptotic Bcl2 proteins that are required to keep the cells alive.



- There are many human disorders in which excessive numbers of cells undergo apoptosis and thereby contribute to tissue damage.

- Among the most dramatic examples are heart attacks and strokes.

- In these acute conditions, many cells die by necrosis as a result of ischemia (inadequate blood supply), but some of the less affected cells die by apoptosis. It is hoped that, in the future, drugs such as caspase inhibitors that block apoptosis will prove useful in saving cells in these conditions.

- There are other conditions where too few cells die by apoptosis.

- Mutations in mice and humans, for example, that inactivate the genes that encode the Fas death receptor or the Fas ligand prevent the normal death of some lymphocytes, causing these cells to accumulate in excessive numbers in the spleen and lymph glands.

- In many cases, this leads to autoimmune disease, in which the lymphocytes react against the individual's own tissues.

- Decreased apoptosis also makes an important contribution to many tumors, as cancer cells often regulate the apoptotic program abnormally.

- The high level of Bcl2 protein in the lymphocytes that carry the translocation promotes the development of cancer by inhibiting apoptosis, thereby prolonging cell survival and increasing cell numbers; it also decreases the cells' sensitivity to anticancer drugs, which commonly work by causing cancer cells to undergo apoptosis.

- Similarly, the gene encoding the tumor suppressor protein p53 is mutated in 50% of human cancers so that it no longer promotes apoptosis or cell-cycle arrest in response to DNA damage.

- The lack of p53 function therefore enables the cancer cells to survive and proliferate even when their DNA is damaged; in this way, the cells accumulate more mutations, some of which make the cancer more malignant