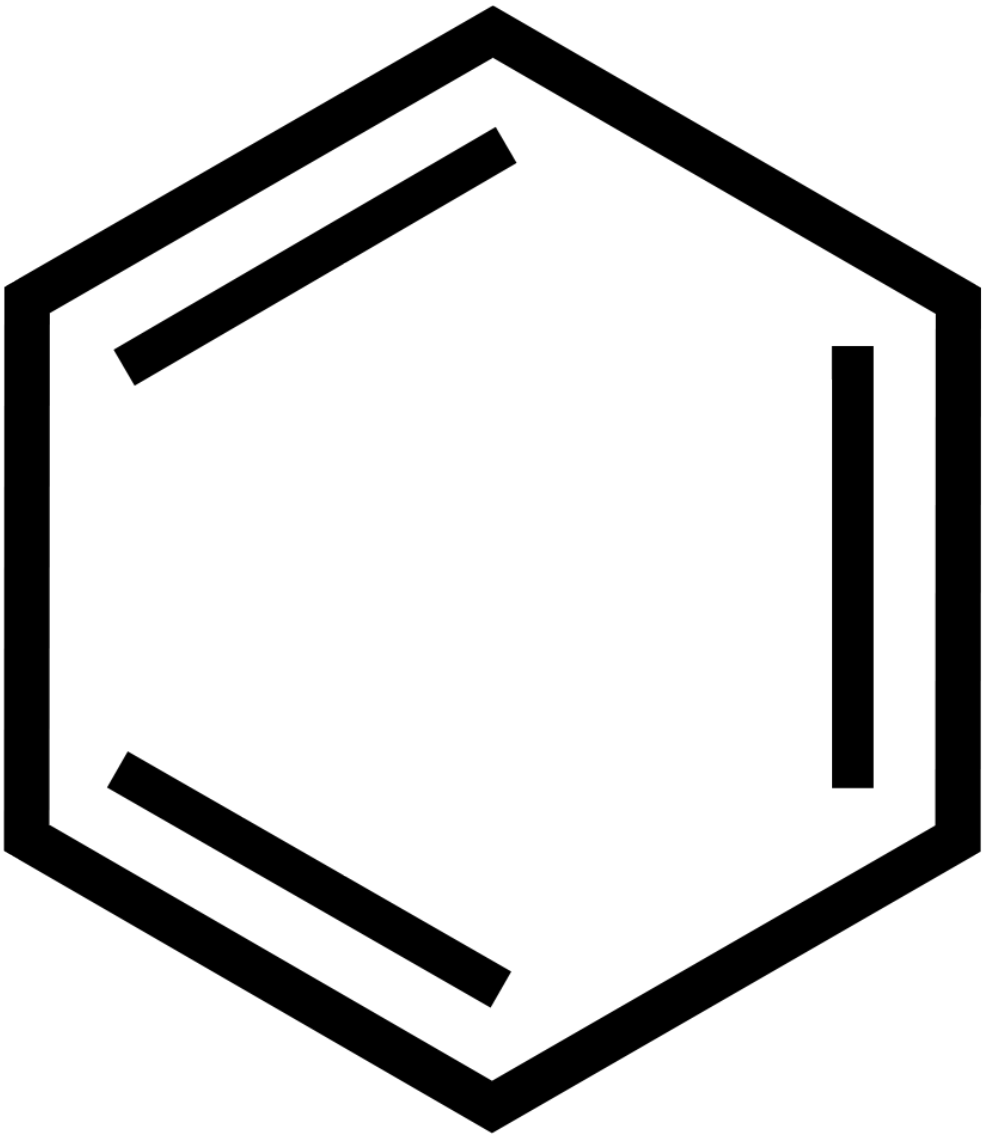

CLUTCH

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CONCEPT: OVERVIEW OF THE CELL CYCLE

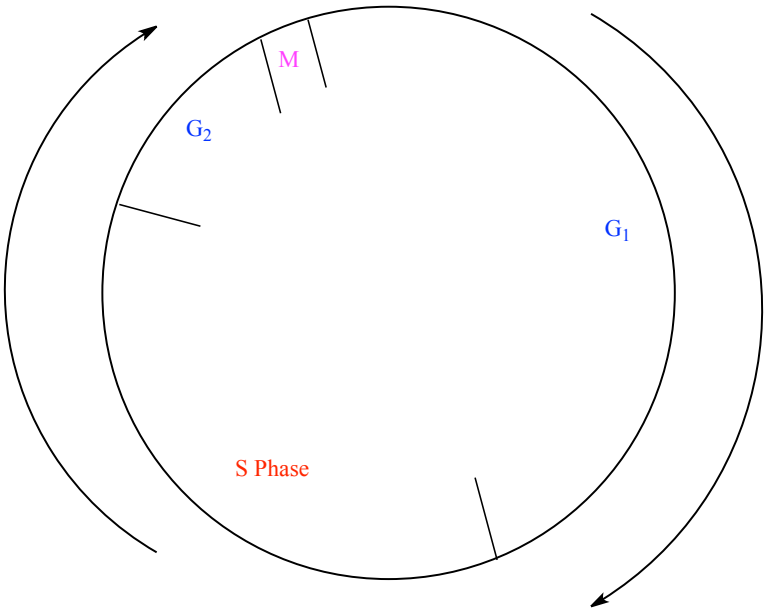
- There are three classes of cells based on whether they divide

CELL TYPE	EXAMPLE
Cells that do not divide	Nerve cells
Cells that normally do not divide, but can when induced	Liver cells
Cells that divide often	Stem cells, White blood cells

- All eukaryotic cell division is divided into four _____

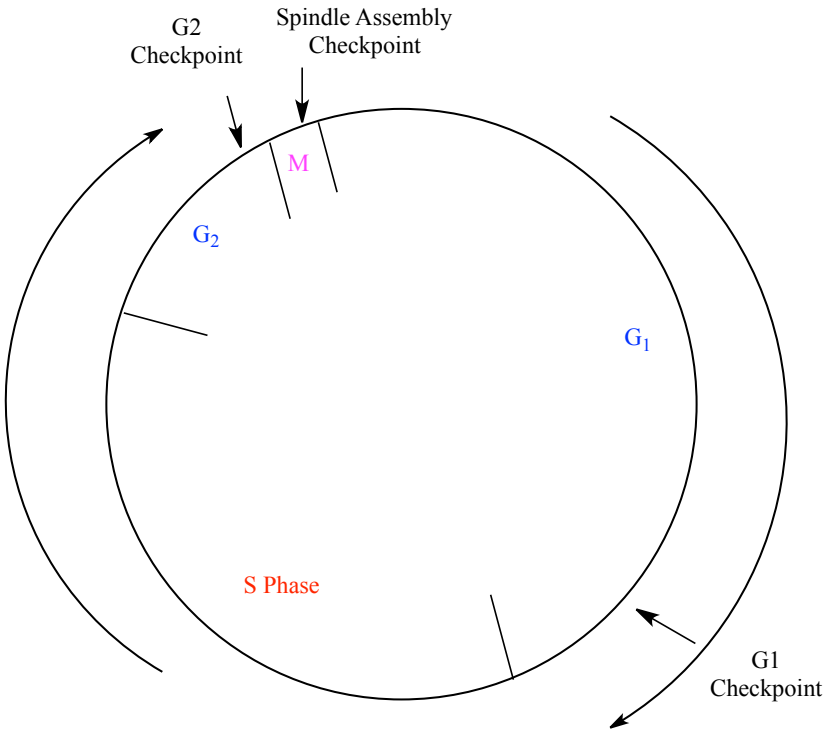
1. **Interphase**: Period between cell divisions
2. **Gap Phases**: Growth phases that precede and follow DNA replication
3. **S phase**: DNA replication
4. **M phase:** Cell division – includes **cytokinesis** which is the process of physically separating the two cells

EXAMPLE: Stages of cell cycle



- Cell cycle control is extremely important to ensure cell division _____
  - □ **Cell cycle control system** is a network of regulatory proteins that controls cell division
    - **Checkpoints** are molecular breaks where it pauses at certain points to ensure accuracy
  - □ Different cell types undergo division at different rates
    - **Mitotic index** measures the percentages of cells in mitosis at a given time
  - □ Eukaryotic cell division is extremely similar between multiple cell types

EXAMPLE: Cell cycle checkpoints



PRACTICE:

1. Which of the following is not a phase of cell division?
    a. GAP Phase
    b. M Phase
    c. S Phase
    d. L Phase

2. True or False: All cells undergo cell division at the same rate.
    a. True
    b. False

CONCEPT: CELL CYCLE CONTROL

Overview

● Cell cycle control relies on a core group of molecular _____ that turn on and off different steps of the cell cycle

□ **Cyclins** are proteins that turn protein kinases on and off

□ **Cyclin-dependent kinases (Cdk)** are cell cycle control protein kinases that are regulated by cyclins

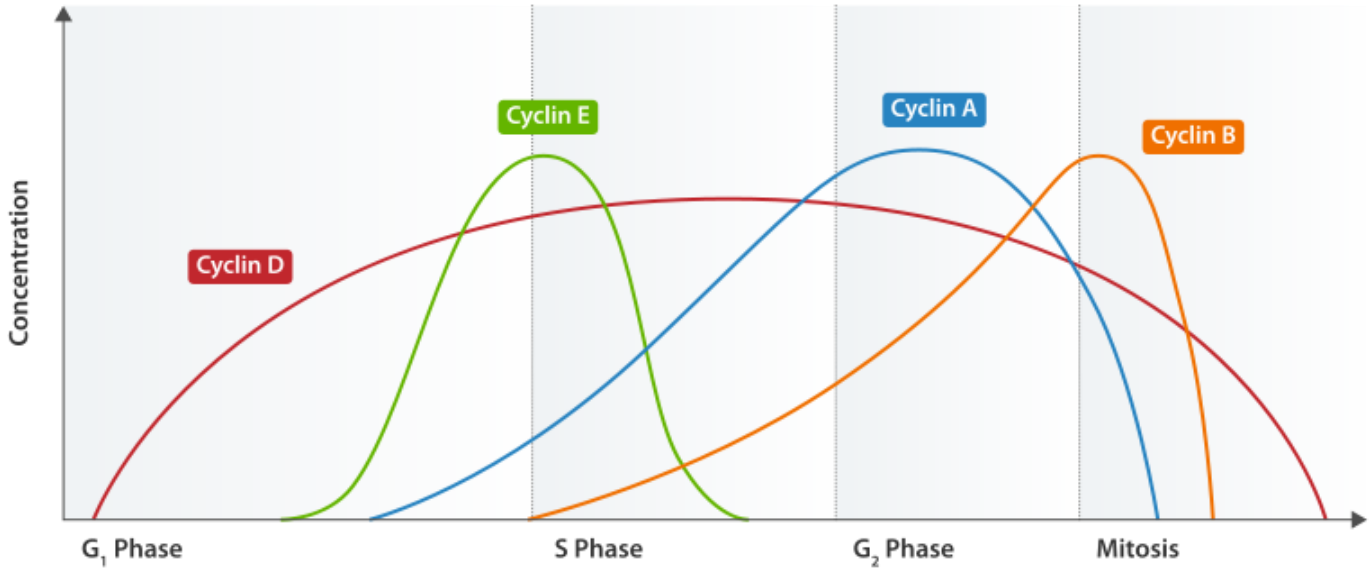
- Regulate cell cycle events by phosphorylating or dephosphorylated other proteins

□ Cdk protein levels remain the same – but cyclin levels vary, which controls activation/inactivation of Cdks

- Rise is due to slow increase in gene transcription

- Rapid fall is due to protein degradation

EXAMPLE: Rise and Fall of Cdks and cyclins



□ **Checkpoints** are _____ within the cell cycle that Cdks act

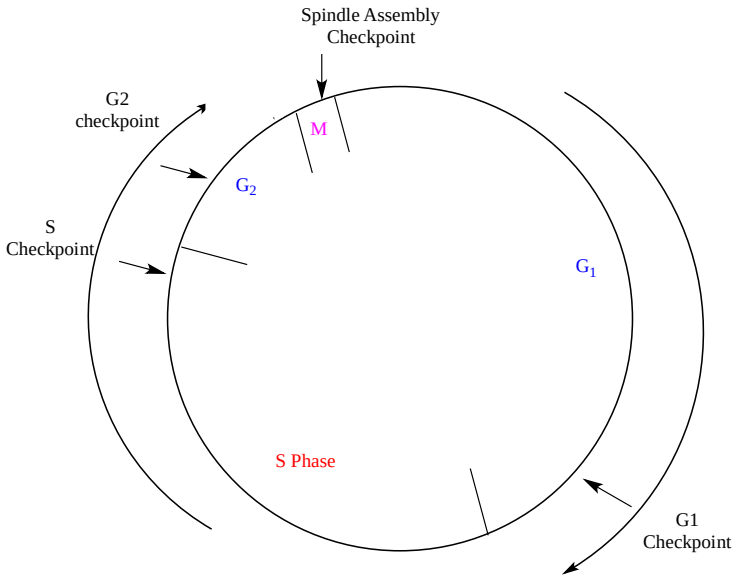
- **G_1 Checkpoint:** Cell cycle pauses to repair damaged DNA before S phase

- **START**: Point of no return when cell leaves G_1 and enters S phase

- **S Checkpoint:** Cell pauses to monitor integrity of DNA replication

- **G_2 Checkpoint:** Cell pauses to prevent division until DNA replication is complete

EXAMPLE: Cell Cycle Checkpoints



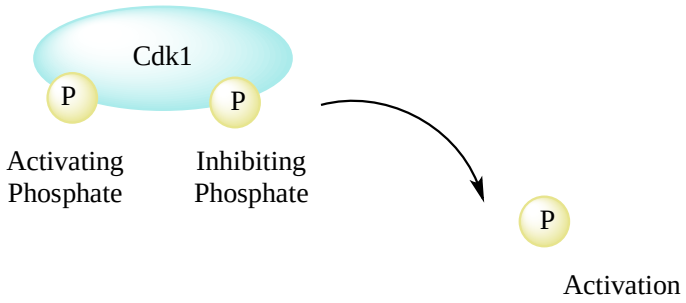
● There are a few important Cdks to know

Cyclins	Function
G1/S cyclin	Activate Cdks in late G1 – triggers START progression
G1 cyclins	Controls G1/S cyclins
S cyclins	Actiates Cdks after START to stimulate replication
M cyclins	Tiggers entry into Mitosis

Regulation

- Regulation of Cdks occurs in numerous ways
  - □ **Cdk inhibitor phosphate**: Cdks must be dephosphorylated at a specific site to become active
    - Cdks must be phosphorylated at one site and dephosphorylated at _____ to be active
    - **Cdc25** is responsible for removing the inhibitory phosphate
  - □ **Cdk inhibitors:** Bind and block cyclin-Cdk complexes
  - □ Cyclin levels: High = active Cdks, Low = inactive Cdks
    - **Anaphase-promoting complex (APC)** degrades M and S cyclins by labeling them with ubiquitin

EXAMPLE: Cdk1 activation through dephosphorylation



PRACTICE:

1. True or False: Often, Cdks have to be dephosphorylated to become activated.
    a. True
    b. False

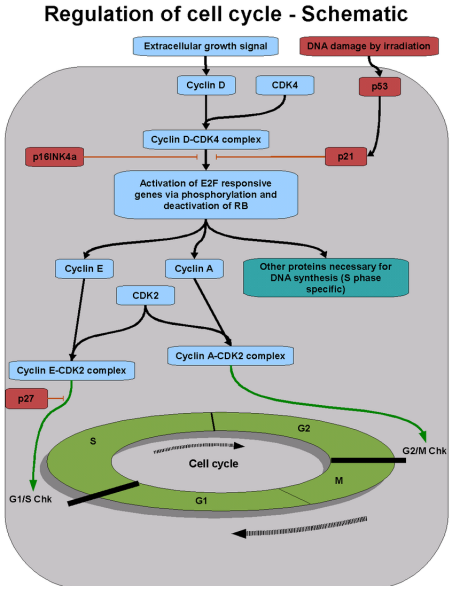
2. Which of the following cell cycle checkpoints occurs immediately before the start of S phase?
    a. G_1
    b. S
    c. M

3. Which of the following proteins is responsible for removing the inhibitory phosphate on Cdks?
    a. S cyclins
    b. APC
    c. M cyclins
    d. Cdc25

CONCEPT: G_1 PHASE AND S PHASE ENTRY

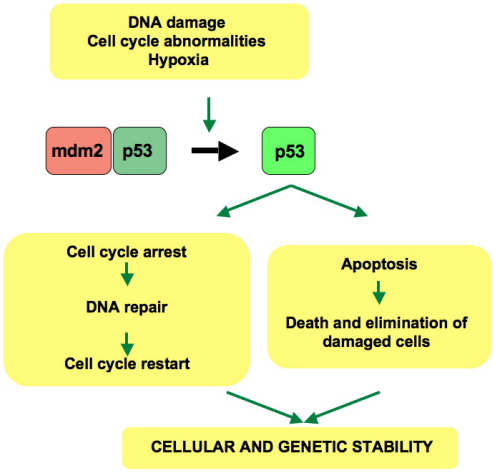
- G_1 phase is a _____ phase that occurs after interphase but before DNA replication and cell division
  - □ The G_1 to S phase transition is critical – because once it enters S phase the cell has to divide or die
    - Initiated by **START**
  - □ **Mitogens** are extracellular signals that the cell receives during interphase to signal for growth and division
    - Stimulate G_1 phase cylins and cyclin-dependent kinases
    - Also inhibits S and M phase cyclins to prevent a rapid re-division before the cell is ready

EXAMPLE: Mitogens stimulating the cell cycle



  - □ If the DNA is _____ the cell will halt in G_1
    - **p53** is a transcription regulator that halts entrance into S phase if the DNA is damaged
    - p53 is mutated in a large amount of cancers
  - □ If the cell is determined to not be ready for division it can enter into $\mathbf{G_o}$ which is a non-dividing state
    - The cell can remain here for prolonged periods of time
    - Terminally differentiates cells (like nerve cells) can stay in G_o for forever

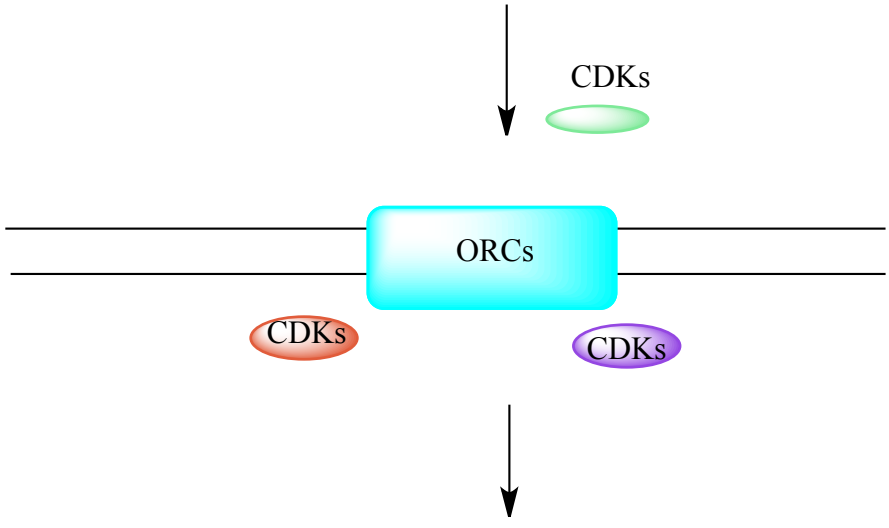
EXAMPLE: p53 and the Cell Cycle



S Phase Entry

- **S-Cdks** are the main driving proteins responsible for the cell _____ S phase
  - □ **S-Cdk** triggers S phase by:
    - Activating DNA helicases
    - Promoting replication fork formation
  - □ The **pre-replicative complex** is recruiting to *origins of replication* by S-Cdk
    - **Concentration gradients**: Concentrations of molecules differ on either side of a membrane

EXAMPLE: S-Cdks activation S phase



S Phase Activation

PRACTICE:

1. What protein halts entry into S phase when DNA is damaged?
    a. Mitogen
    b. p53
    c. S-Cdk
    d. M-Cdk

2. Which of the following is NOT a function of S-Cdks?
    a. Causing the entrance of the cell into S phase
    b. Recruiting DNA helicases
    c. Activating mitogens
    d. Promoting the formation of the replication fork

CONCEPT: DNA REPLICATION

Overview of DNA replication

● DNA replication begins by using one strand as a template (*semiconservative replication*)

□ **Replication origins** are specific DNA sequences where replication begins

- Initiation proteins bind these regions

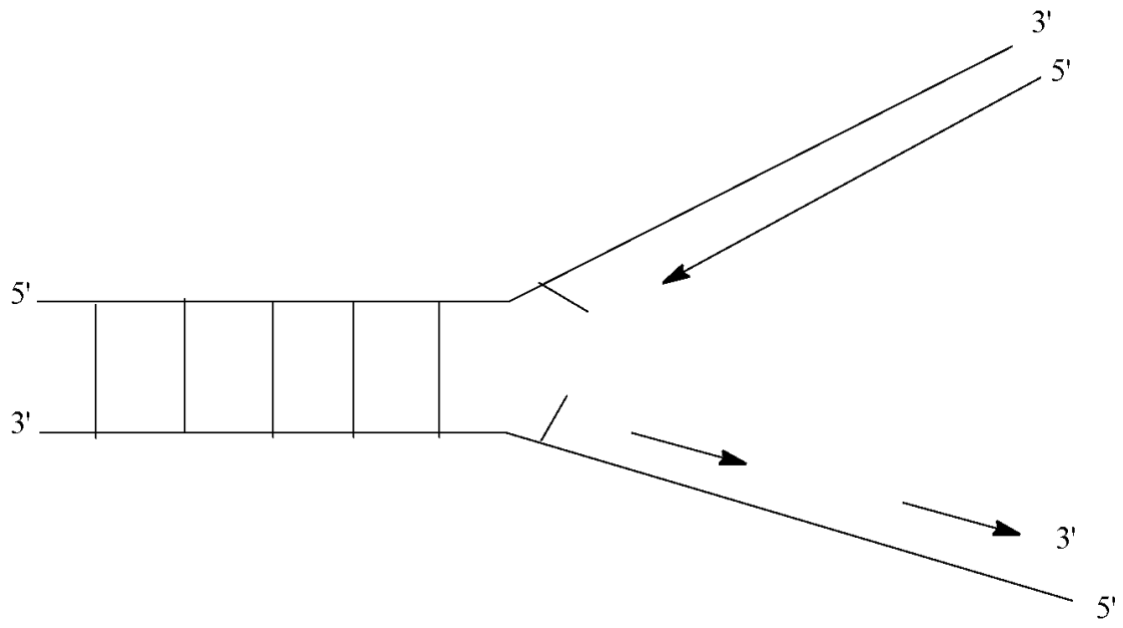
□ Two **replication forks** are formed at each replication origin

- **Bidirectional replication** occurs using each strand is used as a template strand

□ **DNA polymerase** catalyzes the replication of DNA

- Adds nucleotides to the 3' end of a growing DNA strand (replications forms new strand in 5' to 3' direction)

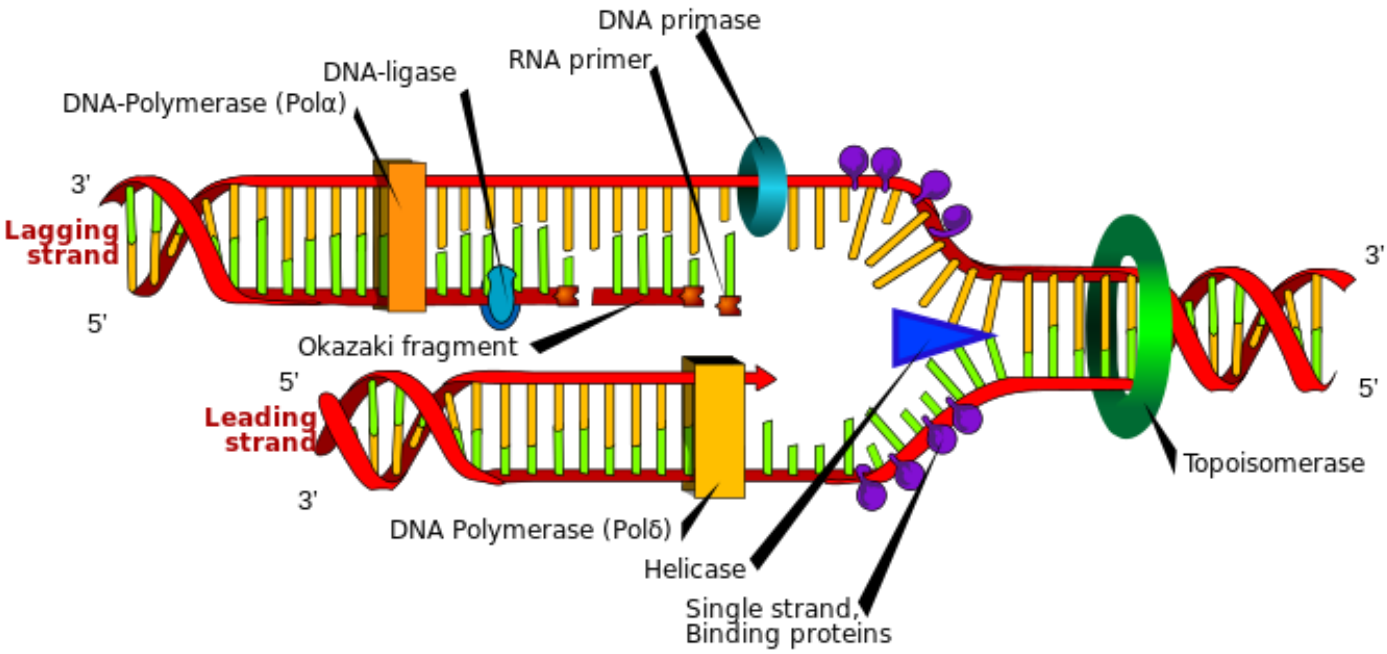
EXAMPLE: Structure of replication



DNA Replication Machinery

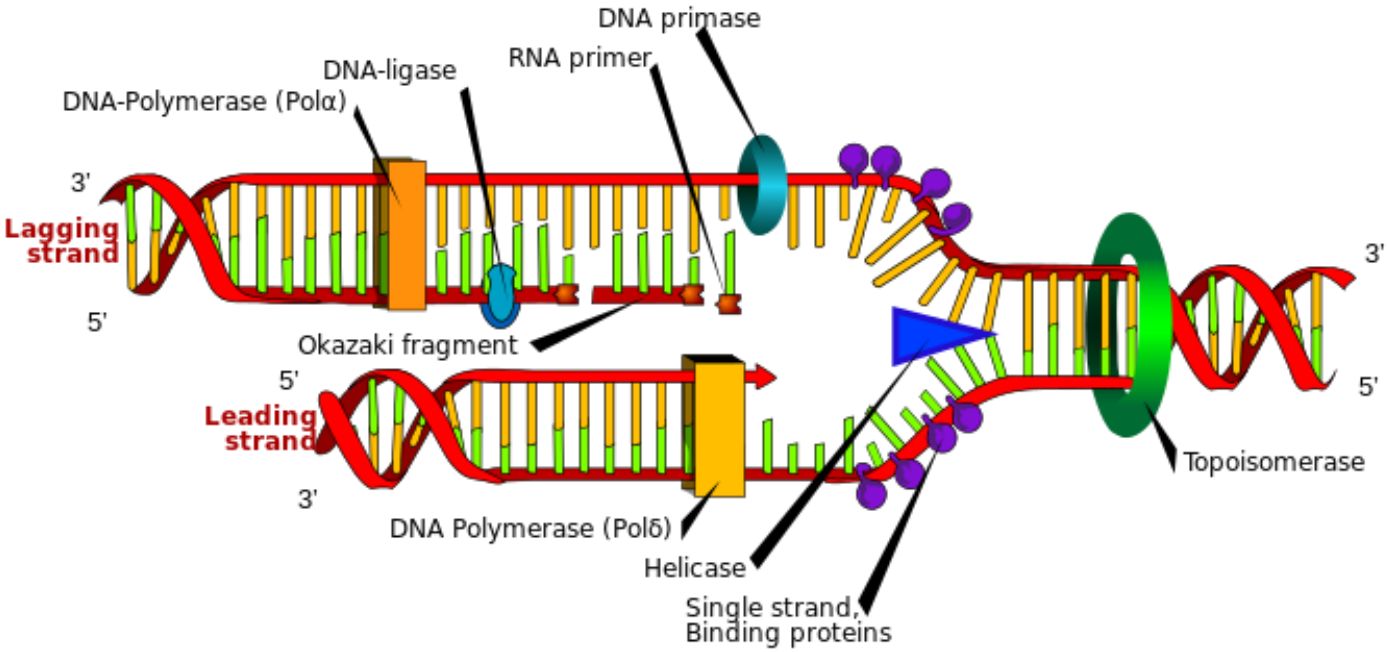
- DNA replication is characterized by bidirectional replication
  - □ Both strands are replicated at the same time
    - DNA Polymerase reads the template DNA strand in a 3' to 5' direction and synthesizes the new DNA strand in the 5' to 3' direction
    - **Leading strand** is *continuously* synthesized in the 5' to 3' direction
    - **Lagging strand** is *discontinuously* synthesized in the 5' to 3' direction
    - **Okazaki fragments** are the small fragments of replicated DNA that are bound together to form the lagging strand.
  - □ DNA Replication Machinery:
    - **RNA primer** is composed of around 10 RNA nucleotides and is used to begin DNA replication
    - **Primase** synthesizes the RNA primer utilizing the template DNA strand
    - *Repair polymerase* replaces the RNA with DNA
    - **DNA ligase** joins the Okazaki fragments together

EXAMPLE:



- DNA Replication Machinery (continued):
  - □ **DNA helicases** are enzymes that pry the two DNA strand apart (breaks hydrogen bonds between bases)
  - □ **Single-strand DNA binding proteins (SSB)** bind to single stranded DNA to prevent reforming a double helix
  - □ **DNA topoisomerases (DNA gyrase)** help prevent DNA supercoiling during replication
  - □ **Sliding clamp (beta clamp)** keeps DNA polymerase attached while it's replicating DNA
    - **Clamp loader** hydrolyzes ATP to clamp DNA (removed and reattached between Okazaki fragments)

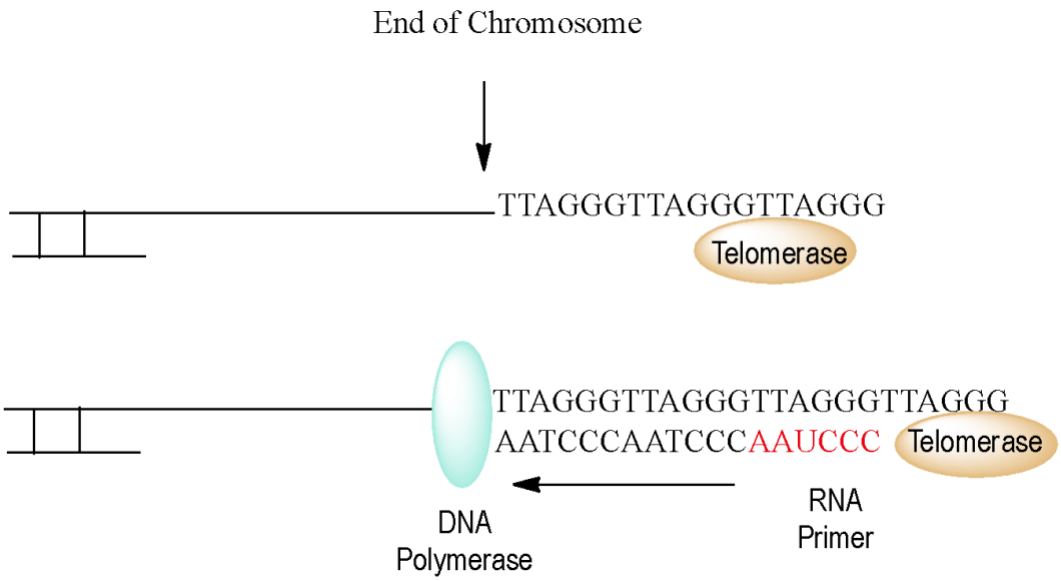
EXAMPLE:



Telomeres

- DNA replication occurs differently at telomeres (ends of the chromosomes)
    - □ Leading strand has no problems replicating the end of the chromosomes
    - □ Lagging strand can't replicate the end of the chromosome because the RNA primer can't bind
    - □ **Telomeres** are long repetitive nucleotide sequences at the end of the chromosome
        - **Telomerase** uses an RNA template (on the enzyme itself) to extend the lagging strand
            - Adds short repetitive DNA sequences to the DNA template – so lagging strand can finish

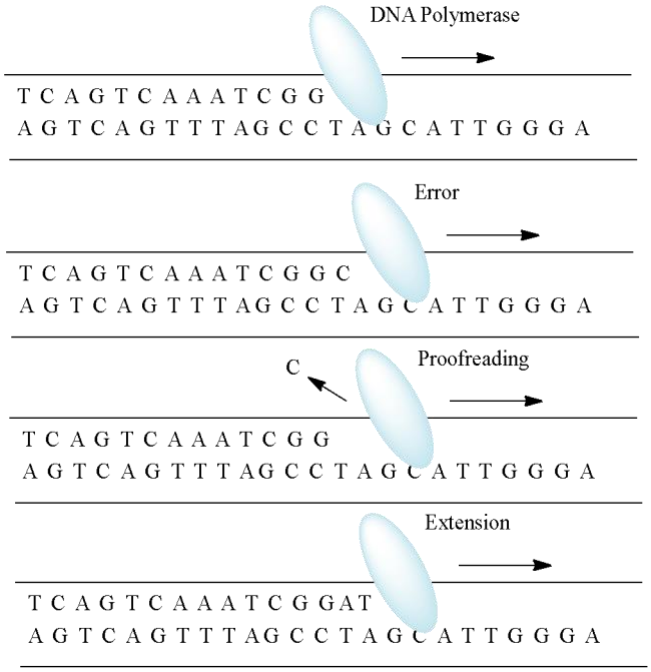
EXAMPLE: Telomerase allowing for replication of end of chromosome

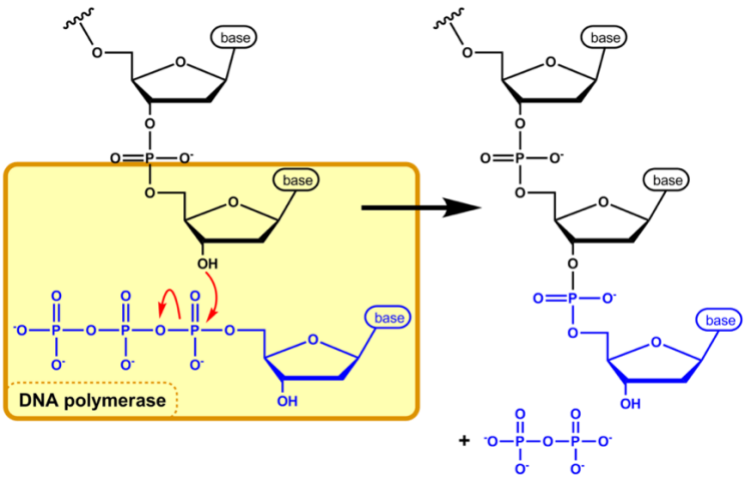


Replication Fidelity and Proofreading

- DNA replication is highly accurate
  - □ There is one error per every 10^7 (ten million) replicated nucleotide bases (human genome ~ 3 billion base pairs)
    - Very rarely, DNA Polymerase will match base pair incorrectly
    - **Proofreading** is the ability of the polymerase to double check and correct mismatched bases
  - □ Proofreading occurs before the next nucleotide is added
    - If the previous match was incorrect then it removes it and replaces it with the correct one
      - removal of mismatched bases is done by the DNA Polymerase's *3' to 5' exonuclease activity*
  - □ DNA can only be synthesized in the 5' to 3' direction:
    - **dNTPs** (deoxyribose nucleoside triphosphates) have 3 phosphate groups attached to a DNA nucleoside
      - 2 of the phosphate groups are removed releasing energy to power DNA synthesis
      - the 3' end of the growing DNA strand helps remove the 2 phosphates of the dNTPs
    - The 5' end wont undergo this reaction so the phosphate remains on the nucleoside instead of hydrolyzing

EXAMPLE: DNA polymerase proofreading & the addition of new dNTPs





PRACTICE

1. DNA replication occurs differently at telomeres.
    a. True
    b. False

2. DNA is replicated in which of the following directions?
    a. 5’ to 3’
    b. 3’ to 5’
    c. 5’ to 5’
    d. 3’ to 3’

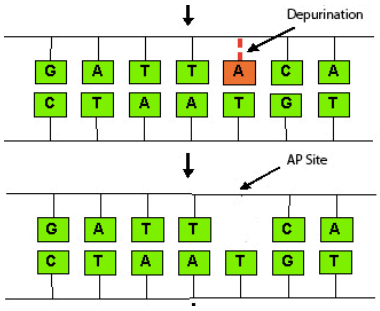
3. Which of the following proteins are responsible for unwinding the DNA double helix for replication?
    a. DNA helicases
    b. Single stranded binding proteins
    c. DNA Topoisomerases
    d. Sliding Clamp

4. Only the lagging strand uses telomerase to replicate the ends of the telomeres.
    a. True
    b. False

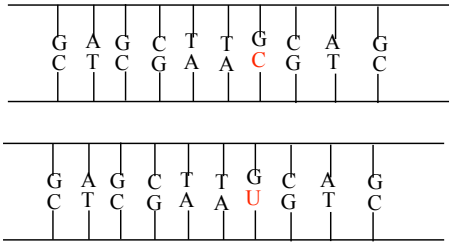
CONCEPT: DNA REPAIR AND RECOMBINATION

Overview

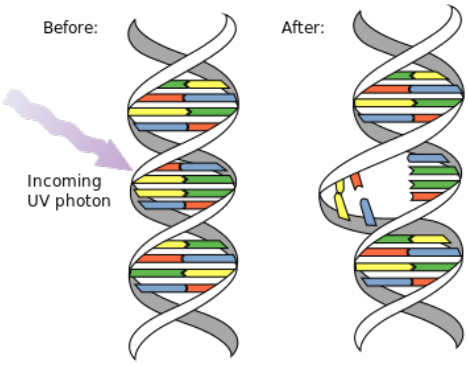
- There are many different _____ of DNA damage
  - □ **Deupriation** is when purine bases (A and G) are spontaneously lost (like missing teeth)



  - □ **Deamination** is when a base is chemically converted into a different base (cytosine to uracil)



  - □ **Thymine dimer** is when UV light exposure causes two adjacent thymines to dimerize



  - □ **Double Strand Break** is when both strands of a DNA double helix are damaged

- If damaged DNA is not repaired it can cause serious diseases
  - □ Xeoderma pigmentosum – "light allergy" – have inability to repair UV lesions

Repair mechanisms

● Each mutation is repaired by the cell in a _____ way

□ **Mismatch repair** fixes mismatched or lost bases

- Mismatched nucleotides cause distortion in double helix

□ **Base Excision Repair (BER)** removes nucleotide damage caused by chemicals (Ex: deamination)

- **DNA glycosylase** is the enzyme that cleaves out uracil for repair

□ **Nucleotide excision repair (NER)** fixes bulky lesions (Ex: thymine dimers)

- Repair involves a “cut and paste” method;

- DNA ligase reseals the cut

□ **Double Strand Break** is when both strands of a DNA double helix are damaged

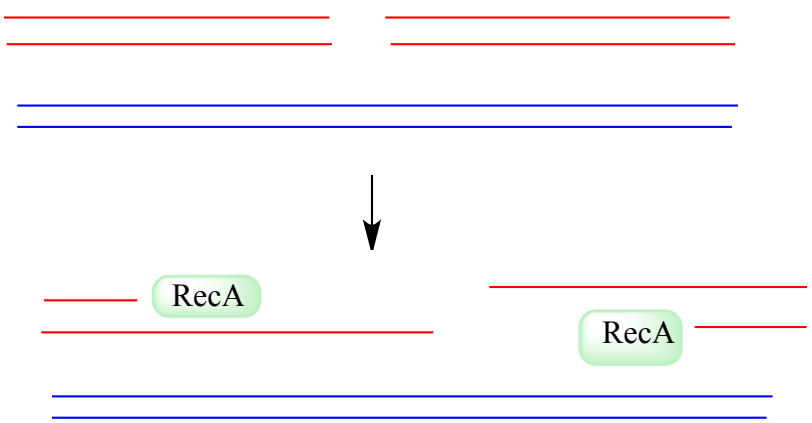
- **Nonhomologous end joining** is when the cell just sticks the broken ends back together

- **Homologous recombination** is when the cell uses undamaged DNA as a template to repair the break

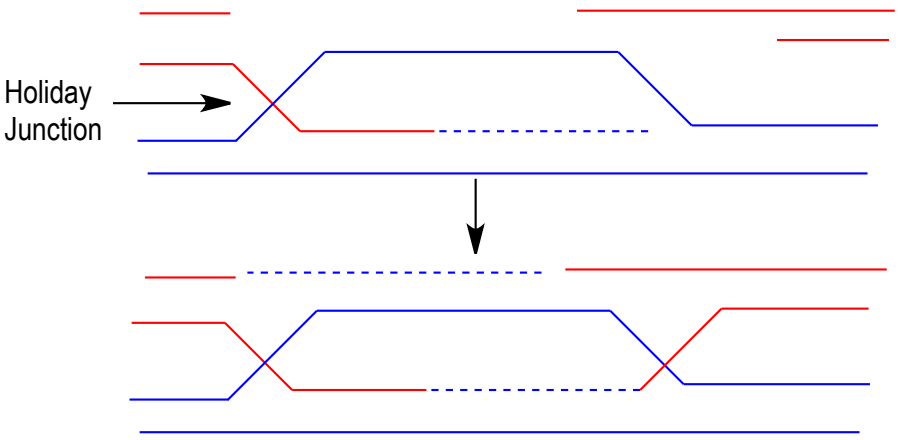
Homologous Recombination

- Homologous recombination repairs double strand breaks in 8 steps
  - □ Homologous recombination occurs shortly after DNA has been replicated
    - Undamaged copy can act as a template

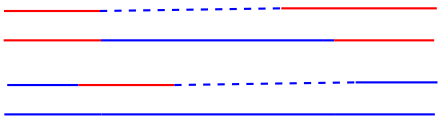
1. Double Strand Break occurs
2. **RecA** protein binds to a single strand of broken DNA
    - Also binds to single strand of undamaged DNA



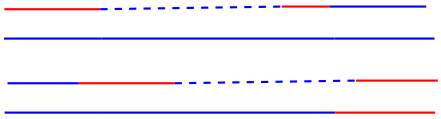
3. Single broken strand and single undamaged strand interact with their complementary regions
4. The DNA repair begins using the undamaged strand as a template
5. **Holiday junctions** form. These are connections between four DNA strands on two helices
    - Sometimes called **cross-strand exchange**



6. **Branch migration** occurs when the cross-strand point (holiday junction) moves down the DNA
    - Movement increases the amount of DNA template available for repair
7. DNA repair is completed
8. Holiday junction is cleaved and the two DNA strands are rejoined to form two separate DNA helices
    - Cleavage can result **crossing-over** causing DNA exchange outside of the damaged area (break point)
    - Cleavage can result in non-complementary regions between the two helices where holiday junctions were
        - Can stay in genome OR be corrected through base excision repair



Non-Crossover



Crossover

PRACTICE:

1. Which of the following is not a type of DNA damage repair?
    a. Mismatch repair
    b. Base Excision repair
    c. Lost nucleotide repair
    d. Double Strand Break repair

2. Which of the following types of DNA damage occurs when a base is chemically converted into another base?
    a. Depurination
    b. Deaminatino
    c. Thymine Dimer

3. Which type of DNA repair is responsible for fixing bulky lesions through a “cut and paste” method?
    a. Base excision repair
    b. Mismatch Repair
    c. Nucleotide Excision Repair
    d. Homologous Recombination

4. True or False: Homologous recombination occurs directly after DNA replication?
    a. True
    b. False

CONCEPT: MITOSIS

Mitosis Entry

● Cells must pass through interphase, G_1 phase, S phase and G_2 phase before _____ into mitosis

□ **M-cyclins and Cdks** are responsible for entering the cell into mitosis

- These M-Cdks are activated by **Cdc25**

- Cdc25 is a phosphatase enzyme that removes inhibitory phosphates from the Cdk active site

□ M-Cdks instigate chromosomal condensation – which is _____ for mitosis

- **Condensins** are protein complexes that assist in chromosomal condensation and segregation

- **Sister chromatids** are identical copies of a replicated chromosome attached via a centromere

- **Cohesins** are protein complexes that hold two sister chromatids together and regulate their separation during anaphase

EXAMPLE: Cohesin and Condensin on sister chromatids

Steps of Mitosis

- Mitosis occurs in 6 steps
  - □ **Prophase** is when the *mitotic spindle* forms
    - The **mitotic spindle** is a network of **asters** (microtubules) and centrosomes that control mitosis
    - It is organized into two distinct **spindle poles** where microtubules are connected to centrosomes
    - Mitotic spindle forms via centrosome duplication (S phase) which move to opposite side of nucleus
  - □ **Prometaphase** is when the nuclear envelope is disassembled
    - Microtubules extending from the mitotic spindle attach to duplicated chromosomes (**kinetochores)**
    - Sister chromatids have a **bi-orientation,** meaning that they are attached to opposite spindle poles

  - □ **Metaphase** is when duplicated chromosomes align at the spindle equator
    - **Metaphase plate** forms with a line of chromosomes along the equator
    - **Spindle assembly checkpoint** check to see if the chromosomes align properly at metaphase plate
      - Delays entry into anaphase if they aren't aligned properly

□ **Anaphase** is when cohesion is broken by *separase* and each sister chromatid is pulled to the spindle pole

- **A:** Sister chromatids begin moving towards the poles

- **B:** The spindle poles move apart – further segregating the sister chromatids

- **Anaphase promoting complex** begins to form to degrade M phase cyclins (to prevent repeat of mitosis)

□ **Telophase** is when the nuclear envelope reforms and mitotic spindle disassembles

EXAMPLE: Overview of Mitosis

PRACTICE:

1. Which of the following is the correct order of mitosis?
    a. Prophase → Prometaphase → Anaphase → Metaphase → Telophase
    b. Prophase → Prometaphase → Metaphase → Anaphase → Telophase
    c. Prophase → Telophase → Anaphase → Metaphase → Prometaphase
    d. Prophase → Anaphase → Prometaphase → Metaphase → Telophase

2. In which of the following steps do the sister chromatids separate?
    a. Prophase
    b. Prometaphase
    c. Metaphase
    d. Anaphase
    e. Telophase

3. In which of the following steps does the cell cross the spindle assembly checkpoint?
    a. Prophase
    b. Prometaphase
    c. Metaphase
    d. Anaphase
    e. Telophase

4. The nuclear envelope begins to reform in which of the following steps?
    a. Prophase
    b. Prometaphase
    c. Metaphase
    d. Anaphase
    e. Telophase

CONCEPT: CYTOKINESIS

● **Cytokinesis** completes M phase by cleaving the cytoplasm into _____ cells

□ **Mitotic spindle** is disassembling in cytokinesis BUT it positions the *cleavage furrow*

- The **cleavage furrow** is a puckering of the plasma membrane where the cell will split into two

- Mostly results in symmetrical division – but not always (developing into different cell types)

- **Contractile ring** is formed during anaphase and is made of actin and myosin filaments

- It exerts a force on the plasma membrane to assist cytokinesis

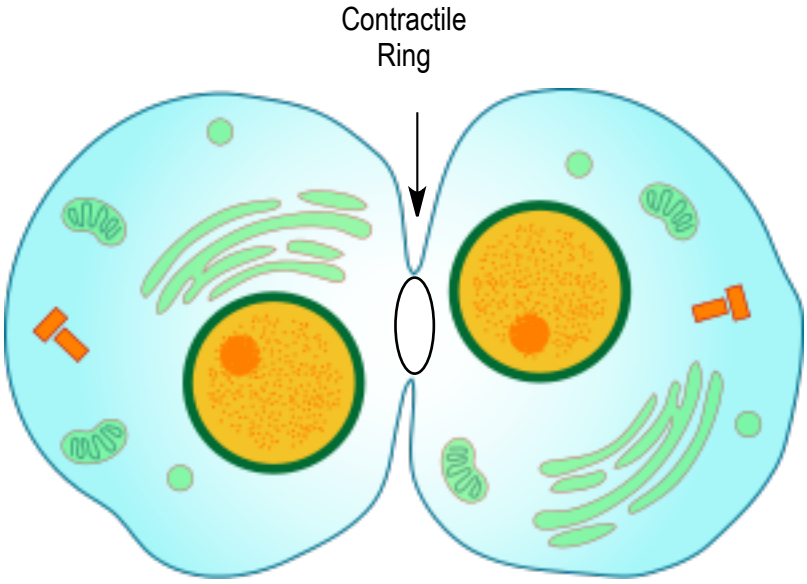
- **RhoA** is a GTPase that triggers contractile ring formation

□ Plant cells need to also create a new _____ after division

- **Phragmoplast** is a structure formed by microtubules which helps assemble the new cell wall

- The phragmoplast forms a **cell plate** (cell wall pre-cursor) inside the cell

EXAMPLE: Contractile ring in two dividing cells



PRACTICE:

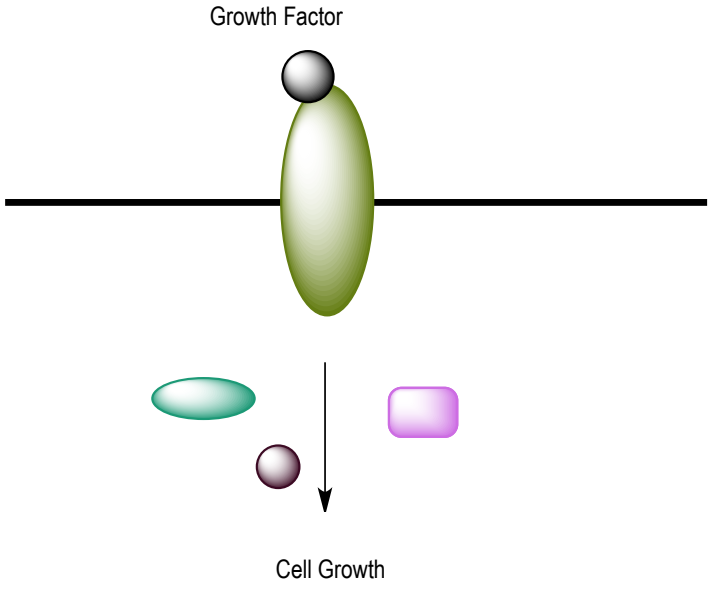
1. Which of the following structures is responsible for cleaving the cell into two cells?
    a. Phragmoplast
    b. Cleavage Furrow
    c. Contractile Ring
    d. Cell Plate

2. Which of the following structures is a precursor to a cell plate in plant cells?
    a. Phragmoplast
    b. Cleavage Furrow
    c. Contractile Ring
    d. Cell Plate

CONCEPT: CONTROL OF CELL SIZE

- There are three _____ factors that play a role in cell division, size, and survival
  - □ **Mitogens** stimulate cell division by removing negative controls that block cell cycle progression
    - Trigger wave of G_1/S Cdk activity
  - □ **Growth factors** stimulate cell growth by promoting protein synthesis and inhibiting degradation
  - □ **Survival factors** stimulate cell survival by suppressing apoptosis
  - □ It is important to understand the difference between these three and not confuse them
    - Cell growth does not mean the same thing as cell _____

EXAMPLE: Growth factor stimulates cell growth

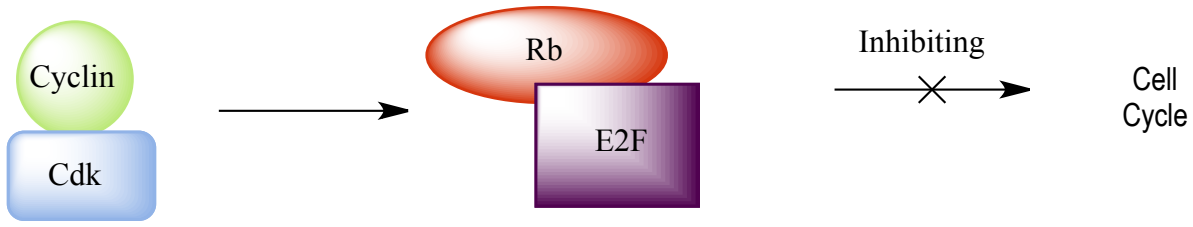


- Mitogens and Growth factors stimulate a variety of intracellular proteins that control cell size and the cell cycle
  - □ Mitogens stimulate G_1 Cdks which enter the cell into the growth phase of the cell cycle
    - Without them the cell enters into G_0
  - □ **E2F** transcription factor is activated by a variety of mitogens and growth factors (PDGF, EF)
    - E2F is a transcription factor that actives genes to promote S-phase entry
    - Inhibited by *retinoblastoma protein* (Rb) binding
  - □ **RAS-MAPK** signaling pathway can also activate transcription factors that support growth

□ DNA damage repair pathway can pause the cell cycle and result in cell growth

- PI3K-AKt pathway

EXAMPLE: E2F and retinoblastoma protein inhibit the cell cycle and cell growth



PRACTICE:

1. Which of the following factors is not responsible for controlling cell division, size, or survival?
   a. Growth factors
   b. Survival factors
   c. Integrins
   d. Mitogens

2. Which of the following proteins is a transcription factor that activates genes to promote entrance into S phase?
    a. Mitogens
    b. RAS-MAPK
    c. PI3K-AKT
    d. E2F

CONCEPT: CONTROL OF CELL DEATH

Overview

● **Apoptosis** is the process of _____ cell death

□ Important because it balances cell division

□ It's regulated death (neat and clean – doesn't damage any other cells)

1. The cell begins to degrade into blebs
2. Nuclear envelope degrades
3. DNA degrades
4. Cytoskeleton collapses
5. Cell is dismantled into small **apoptotic bodies**

□ Controlled extrinsically and intrinsically

EXAMPLE: Apoptosis vs. necrosis

● **Caspases** are the proteins responsible for _____ different parts of the cell

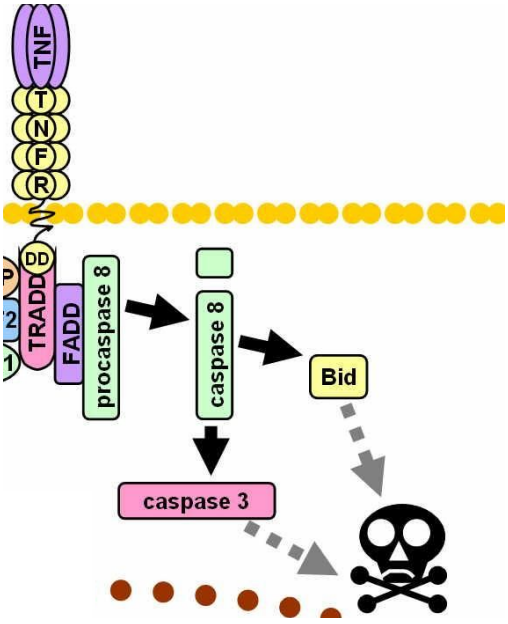
□ **Procaspases** are the precursor forms of caspase proteins

- The procaspase must be activated via cleavage

- Activated caspases can cleave and activate other caspases

□ **Inhibitors of apoptosis (IAPs)** bind and inhibit or cause degradation of caspases to block apoptosis

EXAMPLE: Caspases promoting apoptosis



Triggering Apoptosis

● **Intrinsic pathway** regulates apoptosis based on intracellular _____

□ **Bcl2 family** of proteins plays a major role in inhibiting apoptosis

- Cytochrome C is released from mitochondria into cytosol

- Cytochrome C then binds to a variety of proteins that trigger apoptosis

- DNA damage results in release of Bax and Bad – which act to release cytochrome C

- Bcl2 can bind to Bax and Bad to prevent cytochrome C release and apoptosis

EXAMPLE: Intrinsic pathway of apoptosis

● **Extrinsic pathway** regulates apoptosis based on extracellular _____

□ **Death receptors** trigger apoptosis when activated

- Ex: Fas receptor – binds Fas (ligand)

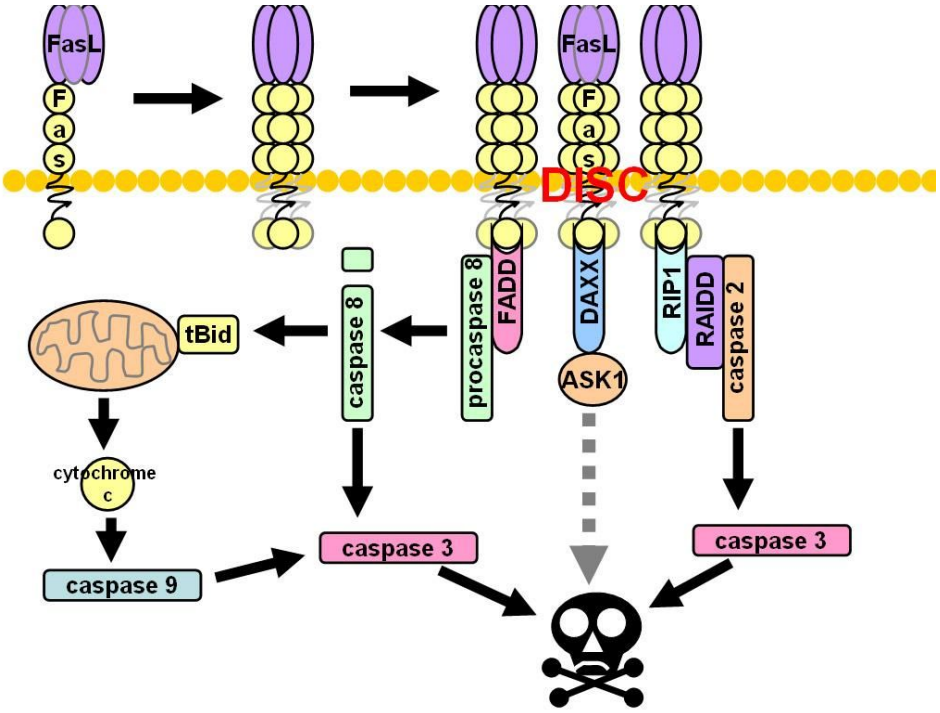
- Activates the *death-inducing signaling complex (DISC)* which stimulates apoptosis

□ **Survival factors** suppress apoptosis when activated

- Can inhibit Bad (which triggers intrinsic pathway)

- Can regulate Bcl2 family proteins

EXAMPLE: Fas receptor induction of apoptosis



PRACTICE:

1. True or False: Apoptosis can only be stimulated through intracellular signals.
    a. True
    b. False

2. In the intrinsic pathway of regulating apoptosis, Bcl2 controls what?
    a. It releases cytochrome C from the mitochondria
    b. It binds to cytochrome C and prevents its release
    c. It binds to Bad and Bax and prevents cytochrome C release
    d. It binds to Bad and Bax and triggers cytochrome C release

3. Which of the following suppresses apoptosis?
    a. Release of cytochrome C from the mitochondria
    b. Survival Factors
    c. Death Receptors
    d. Caspases