EXAM 3 Material

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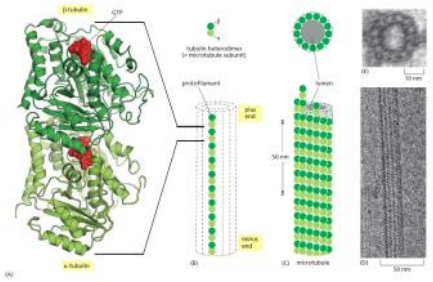
The Cytoskeleton

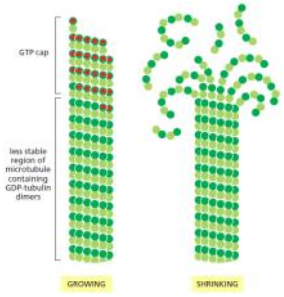
The Cytoskeleton: An Overview of The Filament Systems

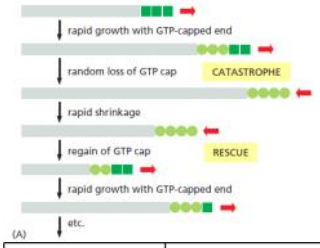
- Migration of cells, mitosis, cytokinesis, and membrane transport/mechanical integrity all rely on the cytoskeleton to function.
- The cytoskeleton consists of three major filament systems: microtubules, microfilaments, and intermediate filaments.
- Many prokaryotic cells are simple shaped whereas eukaryotic cells can take on more complex shapes. This difference can be accounted for by the diverse range of filaments and cytoskeletal components which contribute to a complex cell structure.
- Microtubules
  - Protein subunit is **tubulin**
  - Function: intracellular motility
  - Form from the assembly of the tubulin heterodimer into 13 protofilaments which line the circumference of the microtubule, as shown in the schematic to the right. The dimerized structure of microtubules allow for an establishment of polarity, with beta-tubulin being exposed at one end and alpha-tubulin being exposed at the other.
    - The side which *beta-tubulin is exposed is referred to as the plus end*, and the side which *alpha-tubulin is exposed is referred to as the minus end.*
  - Tubulin has two distinct binding sites to GTP, one at the beta-tubulin monomer and one at the alpha tubulin monomer.
  - Typically, the GTP off the beta-tubulin is hydrolyzed to GDP, and the GTP bound to the alpha tubulin monomer is not hydrolyzed.
  - The site at which microtubules are centralized, or stem from, in a cell, is known as the **microtubule organizing center (MTOC).** This structure is typically perinuclear, where there is a template for the microtubules to originate from.
  - The plus ends are typically directed toward the outer membrane of the cell, whereas the minus ends are directed toward the inner membrane of the cell, originating at the MTOC.
  - During mitosis, two MTOCs, one in each cell, facilitate the separation of chromosomes and the division of the cell cytoplasm.
- Microfilaments / Filamentous Actin
  - Protein subunit is **G-Actin**
  - Function: Retain cell shape and increase motility.
  - Smaller than microtubules
  - Forms from monomers, rather than dimers, as opposed to their microtubule counterparts.
  - Binds to ATP, which can also be hydrolyzed.
  - Also has a polarity, with one end being positive and another being negative
  - Unlike microtubules, forms from 2 protofilaments (instead of 13).
  - Localizes at the periphery of the cell, near the cell membrane, and is responsible for cell migration from its assembly.
- Intermediate Filaments
  - Protein subunit consists of **coil-coil proteins** encoded by many different genes.
  - Function: mechanical strength
  - Interacts between junctions of cells to prevent breaking/lysage of cell when introduced to stress from a change in shape.
  - Intermediate filaments are used to position things correctly within the cell. Nuclear intermediate filaments, also known as **lamins**, give the nucleus its shape. In cells with mutated or absent intermediate filaments, the positioning of organelles in the cell is more loose, and will impact the cell negatively.
  - Keratins are the most diverse form of intermediate filaments, present in many different cells in the body, including the skin and nails, vimentin in muscles and blood vessels, and neurofilaments in neurons.
  - Defects in intermediate filaments can result in devastating human disease
    - ALS (Amyotrohpic Lateral Sclerosis) is a result of abnormal assembly of neurofilaments.
    - Progeria is caused by a defect in the nuclear lamin A, resulting in an unstable and abnormalized nuclear envelope which causes early aging.
- Fundamentally, the formation of all 3 filament systems have 2 fundamental properties in common:
  - The polymers of smaller protein subunits allows for dynamic remodeling
  - Individual filaments consist of protofilament bundles enhancing mechanical strength

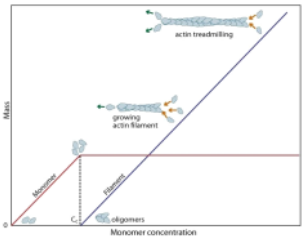
Critical Concentration, Microtubule and Actin Growth / Polymerization.

- At the positive end of both microtubules and actin, the rate of polymerization is much greater than that at the negative end.
- The presence of a GTP cap prevents the disassembly of microtubules, even in the presence of a less stable region containing GDP-tubulin dimers below the cap. The constant growth and shrinkage of microtubules is known as **dynamic instability**, and may be explained by the hydrolysis and exchange for GTP of GTP/GDP respectively at the beta-tubulin monomer in microtubules.
  - Once enough of the beta-tubulin monomers hydrolyse its GTP to GDP, the conformational change in shape of the GTP cap results in the microtubule's disassembly, beginning at the end originally guarded by the GTP cap. This phase is known as the **catastrophe** phase.
  - When the GTP cap is regained and microtubule polymerization continues, **rescue** occurs.
- Dynamic instability and the critical concentration
  - There are three distinct phases of actin assembly, the **lag phase**, the **growth phase**, and the **equilibrium phase**, or the **steady state**.
  - Lag Phase (Nucleation phase)
    - The monomers of G-actin need to come together and form a template, or nucleus, for further actin polymerization. This template can be produced from the random thermodynamic movement of G-actin.
  - Growth Phase
    - Actin is added on to the formed actin template much faster than the rate at which actin is coming off of the template, and the actin filament begins to elongate.
  - Equilibrium phase
    - The rate at which actin monomers associate with the actin polymer is equal to the rate at which the monomers dissociate with the polymer. Typically, however, this phase is rare as the cell needs to respond to different environmental stimuli, causing actin polymers to be disassembled and reassembled.
  - At the **critical concentration**, or the concentration of free monomers when the filament is in the equilibrium phase, the actin polymer will not have net polymerization or depolymerization.
    - If the concentration of G-actin monomers is greater than the critical concentration, polymerization will occur. On the other hand, if the concentration of G-actin is lower than the critical concentration, then depolymerization should occur.
    - Filaments begin to grow once the free G-actin monomer concentration rises above the critical concentration level. We can determine the critical concentration level from a plot of monomer mass vs. monomer concentration in the cell.

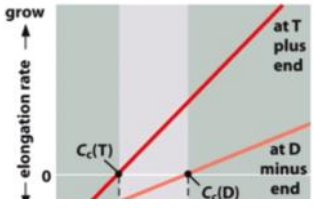






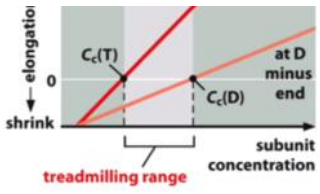


	Microtubules	Actin Filaments	Intermediate Filaments
basic subunit	α and β ubulin	g actin	coiled-coil, filamentous proteins
nucleating complex	γ TURC	ARP 2/3 and formins	none
subcellular localization	cytosolic emanating from MTOC	associated with plasma membrane	cytoplasmic associated with PM and junctions; nuclear
general functions	vesicle and organelle transport; chromosome segregation	cell motility; cytokinesis; structure	tension bearing; help maintain shape and stability



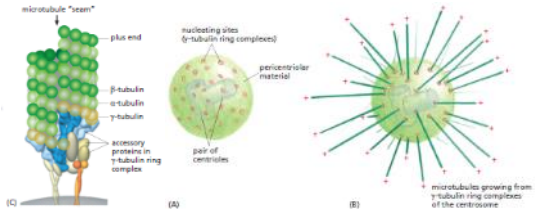
polymerization will occur. On the other hand, if the concentration of G-actin is lower than the critical concentration, then depolymerization should occur.

- Filaments begin to grow once the free G-actin monomer concentration rises above the critical concentration level. We can determine the critical concentration level from a plot of monomer mass vs. monomer concentration in the cell.
  - The point at which the monomer mass stops increasing and the filament mass begins to increase is the critical concentration, since the monomer is now contributing to the formation of filament.
  - If a hypothetical drug stabilizes filamentous actin, we would see a decrease in the critical concentration required for the actin to polymerize. Conversely, if a drug destabilizes f-actin, then we would see a shift rightward for critical concentration.
    - The drug would impact the lag phase of actin assembly by making it shorter.



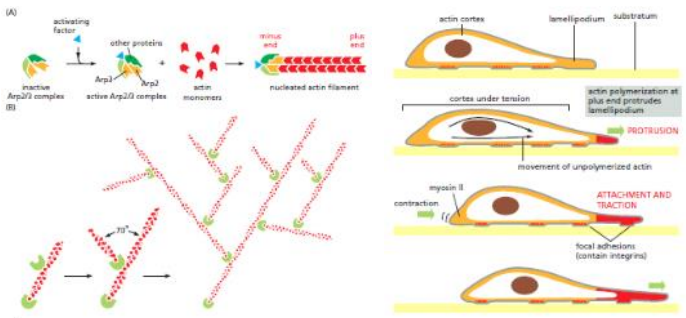
Treadmilling

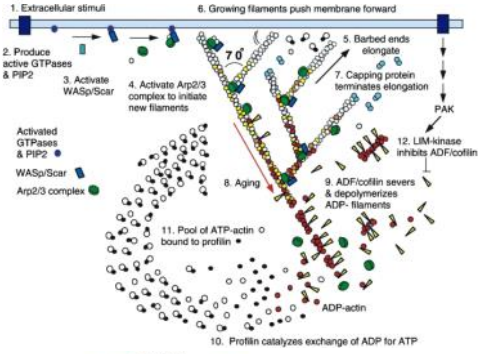
- Actin filament treadmilling is a consequence of differential polymerization at the plus and minus ends of the actin filament. Graphically, the treadmilling range of an actin filament is represented as the region between the critical concentration of the plus end and the critical concentration of the minus end. Note that the plus end will have a greater slope due to an ATP cap.
  - During treadmilling, the rate at which the actin shrinks from G-Actin falling off is replaced by the rate at which actin adds onto the plus end, giving the appearance and therefore the name of "treadmilling" to the actin.
  - Treadmilling rarely occurs naturally within a cell, due to the negative end of the actin often being anchored somewhere. However, this process is observed in isolated actin in test tubes.



Effector Proteins

- MT and actin dynamics are controlled by many different effector proteins which can nucleate assembly, stabilize polarized ends, induce catastrophe, and facilitate binding to other filaments.
- Microtubules are nucleated at the MTOC, often times the centrosome in eukaryotic cells, by association with the **gamma tubulin ring complex (γ-TURC)**.
  - Ring complexes are anchored to the centrosome on many parts of the pericentriolar material, and act as anchors for microtubules to grow off of.
- Branched actin microfilaments are nucleated at the cortex by the **ARP2/3 complex**.
  - ARP2/3 binds to existing filaments and acts as a nucleation site for actin monomers, which allows for the branching of actin to form complex structures and the protrusion of new actin filaments at the positive end of the actin filament.
  - Branched actin structures are found quite often in the **lamellipodia** at the leading end of the cell, as the branched structure allows for the propelling of the cell across a substrate.
  - *Listeria monocytogenes* can manipulate the branching nature of actin to increase its motility in a host cell. When actin filaments and bacteria are properly stained and viewed under a microscope, the actin filaments can be identified as "comet tails" that enhance the motility of *Listeria monocytogenes* in its host cell.
- **Profilin** is responsible for the catalysis of the exchange of ADP for ATP in depolymerized ADP-filament monomers in actin disassembly, recycling ATP-actin for a steady pool of monomers.
- **Formins** are important for the nucleation of unbranched actin filaments at the barbed end.
  - Formin is typically found as a dimeric protein which attaches to the barbed end of actin filaments and facilitates the nucleation of a filamentous actin.

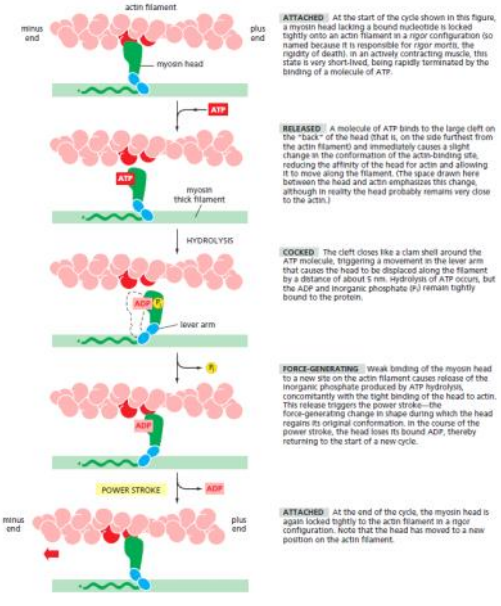




Cell Motility

Model for polarized cell motility

- In order for cells to move, the cells must establish polarity by determining which end is the front and which end is the back.
- The process by which actin filaments grow branchwise to push out the cell membrane at the leading end of the cell is known as the **dendritic nucleation hypothesis**.
  - Actin polymerization at the lamellipodia result in the protrusion of the forward end, with the attachment and traction of a cell at focal adhesion sites with integrins to move the cell.
    - **Integrins** are proteins which form a connection between the interior of a cell and the extracellular matrix to provide traction to move a cell
    - The integrin binds to an actin filament which is connected to other actin filaments via myosin, entering an "engaged state" and facilitates traction by the protrusion of the barbed end of the actin.
    - Without integrin binding to the actin, the system is "disengaged" and retrograde flow of the cell membrane can occur, as the actin itself is not strong enough to move the cell.
  - Capping proteins will stop the elongation of the branching actin filaments if they get too long, which may seem counterproductive, but actually contributes to more efficient movement, as if the actin filament becomes too long, the force which it exerts on te membrane will cause the filament to buckle and break.
- On the other side of the cell, **myosin II**, a form of non-muscle myosin, contracts and pulls the back end of the cell toward the direction the cell moves in through a pinching force which warps the cell membrane. This pulling motion also decreases strain on the cell.
- In conclusion, actin filaments are found within the corona of the cell toward the outer edges while myosin is found more centralized within the cell body.

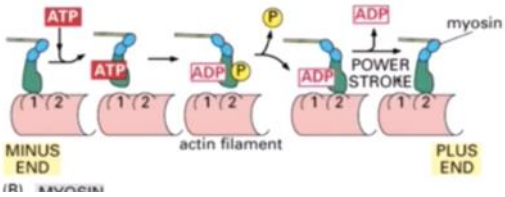


Skeletal Muscle Contraction

- Skeletal muscle contraction is dependent on myofibrils composed of actin and myosin.
- The **sarcomere** is the contractile unit of the myofibril.
- Each muscle cell is a bundle of many **myofibrils**, which itself is a highly ordered array of actin and myosin fibers. The myosin fibers are known as the thick filaments, whereas the actin filaments along with tropomyosin and troponin are commonly referred to as the thin filaments.
  - The depolarization of the membrane which covers the myofibrils, known as the **sarcolemma** causes the contraction of the sarcomeres of the cell.
  - Sarcolemma are associated with T-tubules which are connected to the sarcoplasmic reticulum, another organelle of the muscle cell, which acts similarly to an ER.
    - The **sarcoplasmic reticulum** is especially important for muscle contraction. When acetylcholine signals pass the synaptic cleft of a neuron to depolarize the sarcolemma, the signal is transferred to the SR, which releases calcium ions to help contract muscle.
    - Without calcium, the myosin heads can still associate with the actin filament, but will do so at a slower rate.
- Process of calcium activation of actin filaments for contraction:

1. Neuron sweeps an action potential signal down the plasma membrane of the muscle cell
2. Depolarization signals travel down the sarcolemma into the T-tubules
3. Voltage sensitive proteins detect the change in electrochemical potential and activate a calcium release channel in the sarcoplasmic reticulum membrane
4. Calcium is released from the SR.
5. Calcium interacts with the troponin complex found on the actin fibers comprising of a sarcomere.
6. **Tropomyosin** is dissociated from the myosin binding sites on the actin, allowing for myosin heads to bind to the actin, if the myosin head is not associated with ATP.

- Role of ATP Binding and hydrolysis in myosin motors
  - Myosin is bound to actin until ATP binds to myosin.
  - When myosin heads are bound to ATP, they are unable to associate with actin.
  - After ATP hydrolysis, the myosin is bound to ADP and an inorganic phosphate. A conformational change results, causing the motor head to move forward.
  - When the inorganic phosphate is released, the ADP-myosin complex can bind to actin at a site



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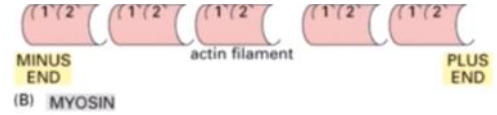
  - When the inorganic phosphate is released, the ADP-myosin complex can bind to actin at a site ahead of the original binding (when myosin was unbound to ATP).
  - ADP dissociates from myosin, causing a conformational change, known as a "power stroke" in which the myosin head pulls the actin filament in a direction to contract the sarcomere.

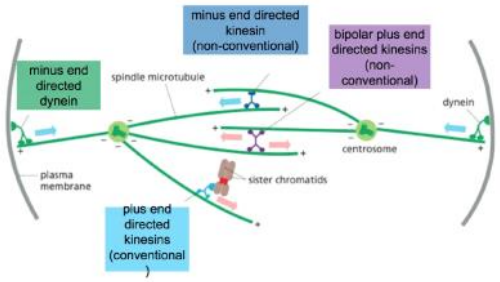


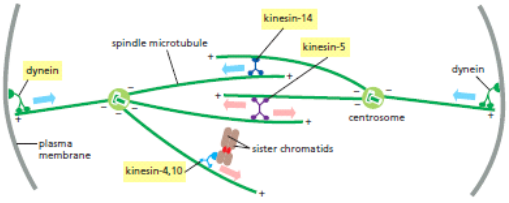
Types of Motor Proteins

- **Kinesin-I**, also known **as conventional kinesin**, is a type of motor protein which contains a motor domain part which binds to microtubules and a tail binding domain which binds to cargo the kinesin is carrying around. Between these two binding regions, the protein is stabilized by a coiled-coil domain containing many hinges for flexibility of the kinesin protein.
  - Conventional kinesin typically moves from the negative end of the microtubule to the positive end of the microtubule, away from the MTOC in a cell.
  - The movement of kinesin is regulated by the exchange of ATP and its hydrolysation in both the leading and lagging head of the kinesin protein.
    - Microtubule binding of one kinesin head causes ADP to be released from that head, and ATP replaces ADP's spot in the kinesin head.
    - This exchange causes the neck linker of the kinesin to "zipper" onto the catalytic core, thrusting the other kinesin head ahead of the bound kinesin head further along the microtubule of the kinesin.
    - When the leading head binds to the microtubule, the lagging head phosphorylates its ATP, and the leading head replaces its ADP with an ATP, restarting the cycle.
- The kinesin super family is diverse in structure although the motor domains of the protein are evolutionarily conserved.
- Kinesin is vital for transport of cell components during mitosis.
  - **Kinesin-V** for example, is composed of two structurally similar Kinesin-I proteins opposite to one another, and is useful for the cell during cytokinesis to separate the cytoplasm of the cell as the two components eventually separate from one another.
  - Kinesin-4,10 is responsible for carrying the sister chromosomes to their respective cells during mitosis.
- **Dynein** is a massive microtubule motor protein which moves in the opposite direction of kinesin, from the positive end of the microtubule to the negative end of the microtubule, toward the MTOC.
  - One example of dynein's use in organisms is the aggregation of melanosomes in fish pigment cells which change the color of the organism. Since dynein and kinesin work against each other, the aggregation of melanosomes near the center of the cell is caused by dyenin prevailing in a "tug of war" against kinesin, allowing the melanosomes to move to the center.

Molecular Motor Basics

	Myosin	Kinesin	Dynein
filament track	f-actin	microtubules	microtubules
model for movement	rocking of lever arm	hand over	rotation of head and stalk
rigor state	ADP-bound	ATP-bound	ADP+Pi-bound
directionality	generally + end directed	generally + end directed	- end directed
major families	**myosin II** (muscle and non-muscle) **unconventional**	**kinesin kinesin-related**	**cytoplasmic axonemal or ciliary**
processivity	attached / detached / hydrolysis cycle	attached / detached / hydrolysis cycle	attached / detached / hydrolysis cycle ?





Control of Cell Crawling

- **Non muscle myosin**, also known as myosin II, is responsible for the pinching of trailing ends of a cell to aid cell movement.
  - Non muscle myosin II assembles into a bipolar filament of 15-20 molecules when they are phosphorylated by myosin light chain kinase (MLCK). The main structural difference between muscle myosin and non muscle myosin is their thickness. Muscle myosin is considerably thicker than non muscle myosin.
- **RhoGTPases** control cytoskeletal reorganization during migration. The assembly of Rho factors leads to the assembly of actin filaments and the regulation of myosin.
  - **Cdc42** is a common RhoGTPase found in the filipodia protrusions of cells during migration
  - Rac1 is a RhoGTPase responsible for the pushing outward of the cell, and is typically organized as a coronal structure around the cell.
  - **Rho** is a RhoGTPase responsible for polymerization of cytoskeletal stress fibers, which are typically found organized parallel to each other.
  - **Rac** will be highly concentrated in the leading end of the cell whereas Rho will be found in the lagging end of the cell, and both these RhoGTPases inhibit each other. Initially, in response to a chemical signal, Rac will cause polymerization of cytoskeletal structures in the leading end and inhibit Rho from polymerizing stress fibers, then Rho will inhibit Rac to move the lagging end of the cell forward.

Cell Cycle

Introduction

- There are three main phases in the cell cycle: A cell growth phase where chromosomes are replicated, a chromosome segregation phase (karyokinesis), and a cell division phase (cytokinesis).
- More specifically, the cell cycle consists of four phases, named the G1, S, G2, and M phases. The G1, S, and G2 phases are collectively known as "interphase" whereas the M phase is the mitotic phase.
  - The G stands for Gap.
  - During the G phases, biosynthesis occurs (RNA, organelles, proteins are created), and the extracellar conditions are being monitored for conditions ideal to mitosis. (i.e. are there enough nutrients to support the new cell, enough space, etc)
- Cells are typically distinguished by their capacity to grow and divide. For example, epithelial cells and stem cells display high frequency of mitotic activity.
  - **Post-mitotic cells** are highly specialized cells which do not go through the cell cycle and are instead organized to a terminally differentiated state known as G0. Examples of these cells include neurons and skeletal muscle cells.
  - Other cells may also exit the cell cycle to a G0 state in what is known as a **resting state** before entering back into the cell cycle. Examples include liver cells, which will normally not divide, but will enter the cell cycle if there is damage to the liver. Fibroblasts, important for wound healing, are also an example of cells that can undergo a G0 resting state.

The Mitotic Phase

- One prevailing challenge for cells during the M phase is the replication of a large amount of DNA. The solutions to having so much DNA include:
  - Packaging the DNA into pieces (smaller segments).
  - Replication of DNA before cell division.
  - Keep the sister chromatids coupled until it is time for division for order to be maintained
  - Chromosomes are condensed by many fold before division begins
  - Development of the mitotic spindle to help segregation of chromosomes.
- During the M phase, the highly condensed sister chromatids are held together by a large protein complex known as **cohesin**. Cohesin is targeted during the metaphase to anaphase transition, when the separation of the sister chromatids are necessitated.
- Prophase
  - The parent centrosomes separate to opposite poles of the cell.
    - Centrosome duplication occurs during the S phase for most cells. This duplication process is closely related to DNA replication - i.e. inhibiting DNA replication inhibits centriole duplication.
- Prometaphase
  - Key event is the breakdown of the nuclear envelope.
  - Chromosomes now attach to spindle microtubules via their kinetochores and undergo active movement guided by the microtubules.

- At the end of prophase, the lamins within the nucleus are phosphorylated to allow the chromosomes to exit.
  - During telophase, the lamins are dephosphorylated and the nuclear envelope fragments are able to re-form around the chromosomes.
  - Ran GTPases are found to localize to the condensed chromosomes, which is essential for the reestablishing of the Ran GTPase gradient in the newly formed cell.

- Metaphase
  - The chromosomes are aligned at the equator and the spindles attach midway of the poles at the kinetochore of the sister chromatids
  - The bipolar attachment of kinetochores to their spindles are achieved by *trial and error*. It is important for one centromere each to be associated with one kinetochore each.
    - A low tension in the microtubule results in an inhibitory signal being sent to the attachment site, allowing for the misbound microtubule to dissociate.
    - A high tension in the microtubules results in the shutting off of the inhibitory signal, and the consequential stable conformation of microtubule binding.
    - The lack of mechanical tension at the kinetochore in at least one chromosome is enough to prevent entry into anaphase.
- Anaphase
  - The sister chromatids synchronously separate to form two daughter chromosomes, and each is pulled toward the spindle pole it faces.
  - Cohesin rings are degraded by **separase** during this process, allowing for the sister chromatids to separate to their respective cells.
  - Depolymerization of microtubules at the kinetochore generates a force to pull the associated chromosome towards the pole.
- Telophase
  - The two sets of daughter chromosomes arrive at the poles of the spindle and decondense while a nuclear envelope forms around each set, marking the end of mitosis.
  - A contractile ring consisting of actin and myosin filaments pinches the cell into two to create two daughter cells, each with their nucleus.

The Cell Cycle Control System

- The cell cycle is a highly regulated process, and the control system of this regulation is from **cyclin-dependent kinases (Cdks)** and **cyclin**.
  - Cdks phosphorylate target proteins to drive the cell through different phases of the cell cycle, and is only active in the presence of cyclin.
  - Different Cdk-cyclin complexes propogate different parts of the cell cycle, allowing for the cell to progress to different phases of the cycle at correct times. For example, an S-Cdk cyclin complex drives the cell through the S phase of the cell cycle, whereas an M-Cdk cyclin complex drives the cell through the M phase of the cell cycle.
    - The Cdk is the same Cdk throughout in budding yeast cells, but the difference in the type of cyclin is what causes different changes at specific times in the cell cycle.
    - Vertebrates have a more refined and complex system, where many varieties of Cdk exist and bind to different cyclins. However, the same cyclin-Cdk complexes will still regulate the cell cycle.
  - Cyclin destruction is required for the progression of the cell past the M phase. Experimentally shown decreases in cyclin concentrations during the mitotic phase affirms this hypothesis. Cyclin destruction is the job of the **cyclosome**, or the **APC (Anaphase promoting complex)**
- Anaphase Promoting Complexes (APCs) are responsible for the destruction of cyclins during mitosis.
  - APCs are not protein kinases, but rather, a form of ubiquitin protein ligase. Therefore, the role of APC is to attach a ubiquitin to the cyclin to mark it for degradation in the proteosome.
    - Once cyclins are marked for destruction by APCs, Cdk is deactivated, and substrates of Cdk can now be dephosphorylated, which is essential for progression through mitosis.
  - Another target for APCs is securin, which is a tight binding inhibitor of a protein called **separase**. Separase is liberated once securin is destroyed, and is able to cleave cohesin, allowing for the sister chromatids to be segregated.
  - APC is activated by Cdc20 when it forms a complex with inactive APC, and APC is repressed until the kinetochores are attached to the spindle fibers.

Cell Checkpoints

- There are three main checkpoints to the cell cycle: the Start, or G1/S checkpoint, the G2/M checkpoint, and the metaphase/anaphase transition checkpoint.
  - At any of these stages, the cell cycle may be arrested if conditions are not met to progress to the next checkpoint. For example, if a chromosome is somehow not fully replicated, the cell will be able to detect this abnormality and arrest the cell cycle at the G2/M checkpoint, preventing any further progression into the mitotic phase.
- There are 5 main ways to control the activity of proteins involved in cell cycle regulation:
  - Protein synthesis
  - Phosphorylation
  - CDK inhibitors
  - Subcellular localization (Cdc25 nuclear/cytosolic localization)
  - Protein degradation
- G1 (Start) Checkpoint
  - This checkpoint primarily checks for enough space in the cell for growth and replication, growth factors for progress into the S phase, and potential DNA damage.
  - p53 plays a vital role in this checkpoint. p53 is activated if there is DNA damage, and Cdk inhibitor proteins (CKIs) and repair proteins will be activated as a result to attempt to repair damage to DNA and arrest the cell cycle.
    - **Cdk inhibitor proteins (CKIs)**, also known as **p21** are a G1 specific inhibitor protein upregulated by transcription factor p53 which is responsible for the deactivation of cyclin-Cdk complexes and the arrest of cell progression to the S phase.
    - If the DNA damage is too severe, p53 triggers apoptosis.
  - Retinoblastoma (Rb) proteins directly prevent the transcription of S phase genes by inactivating E2F proteins.
    - E2F proteins are involved in a positive feedback loop. More active E2F proteins trigger the creation of more G1/S-Cdk complexes from s-phase gene transcriptions, which in turn phosphorylate and inactivate Rb proteins to free up more E2F proteins.
    - If Rb is unable to be phosphorylated, then the cell cycle would be arrested at the G1 checkpoint, as E2F is unable to propogate S-phase gene transcription when it is suppressed by active Rb proteins.
- G2 / M Checkpoint
  - This checkpoint checks for the cell to be the correct size and whether or not there are any basepair mismatches or other DNA replication errors.
  - Three key kinases are at play at this checkpoint, an inhibitory kinase known as **Wee I**, an activating kinase known as **CDK activating kinase,** or **CAK,** and another activating kinase known as **Cdc25 phosphatase**. Collectively, these three kinases regulate the M-Cdk complex.
    - CAK adds an activating phosphate to the M-Cdk complex whereas Wee1 adds an inhibitory phosphate to the complex. Cdc25 removes the inhibitory phosphate to activate the Cdk-Cyclin M complex.
    - If there is DNA damage detected or any other factor causing the failure of passing the G2 checkpoint, Wee1 activation will be promoted while Cdc25 is exported from the nucleus

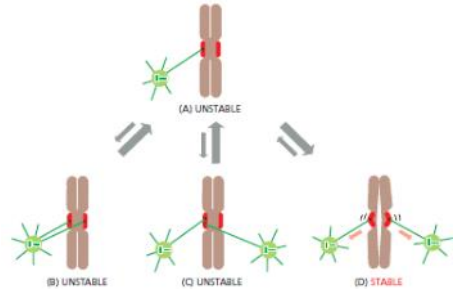
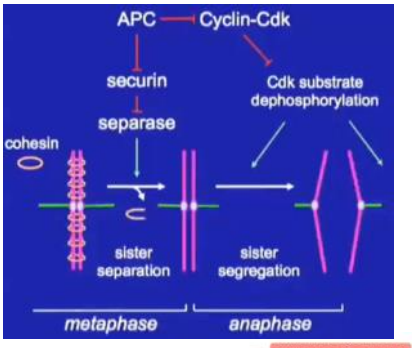
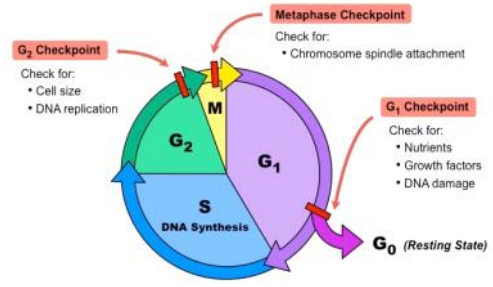


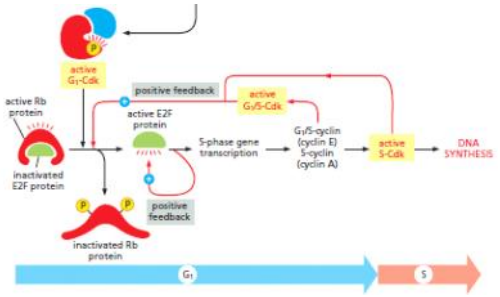
TABLE 17–1 The Major Cyclins and Cdks of Vertebrates and Budding Yeast

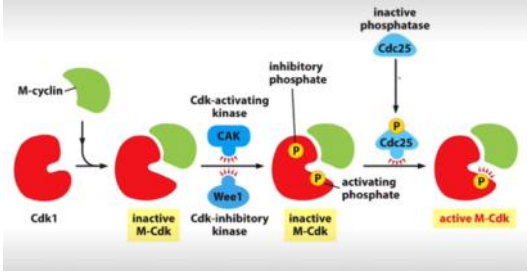
	Vertebrates		Budding yeast	
Cyclin–Cdk complex	**Cyclin**	**Cdk partner**	**Cyclin**	**Cdk partner**
G_1-Cdk	Cyclin D*	Cdk4, Cdk6	Cln3	Cdk1**
G_1/S-Cdk	Cyclin E	Cdk2	Cln1, 2	Cdk1
S-Cdk	Cyclin A	Cdk2, Cdk1**	Clb5, 6	Cdk1
M-Cdk	Cyclin B	Cdk1	Clb1, 2, 3, 4	Cdk1

* There are three D cyclins in mammals (cyclins D1, D2, and D3).
** The original name of Cdk1 was Cdc2 in both vertebrates and fission yeast, and Cdc28 in budding yeast.

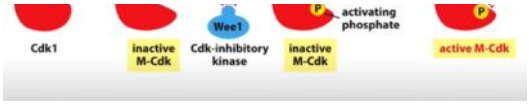








known as **Cdc25 phosphatase**. Collectively, these three kinases regulate the M-Cdk complex.
    - CAK adds an activating phosphate to the M-Cdk complex whereas Wee1 adds an inhibitory phosphate to the complex. Cdc25 removes the inhibitory phosphate to activate the Cdk-Cyclin M complex.
    - If there is DNA damage detected or any other factor causing the failure of passing the G2 checkpoint, Wee1 activation will be promoted while Cdc25 is exported from the nucleus (where it is used) to the cytosol to prevent activation of the M-Cdk complex, leading to cell cycle arrest before the mitotic phase.
        - Once the error is repaired, Cdc25 can be imported back into the nucleus.
    - Active M-Cdk is also involved in positive feedback mechanisms by its inhibition of Wee1 and its activation of Cdc25.



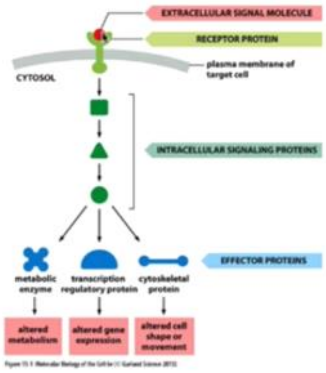
- Metaphase - Anaphase Checkpoint
    - This checkpoint mainly is concerned with whether the spindle fibers are correctly attached to the kinetochores for anaphase separation.
    - The lack of mechanical tension at the kinetochore in at least one chromosome is enough to prevent entry into anaphase. Tension is the driving factor for continuing past this checkpoint.

Cell Signaling

Introduction

- A typical cell in a multicellular organism is exposed to hundreds of different signaling ligands or molecules in different combinations to create different responses in the cell. If a cell lacks signaling ligands, the cell will die.
- Each cell is programmed to respond to specific combinations of extracellular signals. For example, from an acetylcholine signal, three different cells may have three different responses to the stimulus:
    - In a pacemaker cell of the heart, the acetylcholine will decrease the heart rate.
    - In a salivary gland cell, acetylcholine binding can cause the release or secretion of saliva.
    - In a skeletal muscle cell, acetylcholine binding causes contraction of the muscle cell.
- The general principle of cell communication through cell signaling is outlined as the following:

1. Extracellular signaling molecule (ligand) binds to receptor protein embedded in the cell membrane.
2. Activated receptor triggers the activation of many intracellular signaling proteins, also known as second messenger proteins. This is the stage at which the signal of the ligand is amplified.
3. Activated signaling proteins activate effector proteins
4. Effector proteins can alter many aspects of the cell, including:
    a. Altered metabolism
    b. Altered gene expression
    c. Altered shape or movement



Types of Intracellular Signaling

- Intracellular signaling can occur over short and long distances using distinct mechanisms.
    - **Contact dependent signaling** occurs between the shortest distance - a membrane bound signal molecule binds to a receptor on a target cell and initiates a response in the target cell.
    - **Paracrine signaling** occurs at a short distance - a signaling cell produces large amounts of a signal so that cells in the immediate vicinity may bind to and respond to the receptor.
    - **Synaptic signaling** occurs at intermediate distances, and occurs via neurotransmitters traveling to a target cell through the synaptic gap between the neuron and the target cell.
    - **Endocrine signaling** occurs at great distances, and is typically used to target cells all throughout the body. The signals of endocrine cells are known as hormones, and can travel through the bloodstream to bind to cells at the extremities of the body.
- **Autocrine signaling** occurs when a cell responds to a signal it produces itself.
    - This process is rare in normal cells, but is quite common in cancer cells to increase proliferation of malignant tumors.

Nuclear Receptors

- Receptors localized to the nucleus of the cell and any other intracellular receptor require hydrophobic ligands to work effectively.
    - Without the hydrophobic nature of the ligand, the ligand would not be able to travel past the cell membrane, and cannot reach the receptor.
    - Since the presence of hydrophobic ligands in a mainly water based extracellular matrix is not entropically favorable, the transport of hydrophobic ligands is mediated by carrier proteins, which transport the ligand to target cells to diffuse through the amphipathic lipid bilayer.

Ligand Receptor Interactions

- The **Ligand-Receptor Affinity**, or the **disassociation constant**, is a useful metric to measure how strongly a ligand binds to a receptor. The equation for the dissasociation constant is given as:

$$Kd = \frac{[L][R]}{[LR]}$$

  where [L] represents concentration of ligand, [R] represents concentration of receptor, and [LR] represents concentration of ligand-receptor complex.
    - The greater the Kd value, the lower the affinity of the ligand, as a large Kd value would indicate high concentrations of L and R but low concentrations of the complex.
    - Similarly, the lower the Kd value, the higher the affinity of the ligand.
    - Different receptors and ligands have different affinities for each other, and these different affinities can be mainly explained due to the necessary sensitivity of the cell to detect these changes in the environment. For example, take the analogy of a smoke detector vs. a carbon monoxide detector. A smoke detector would have a lower sensitivity to smoke as smoke can commonly be present in cooking or from a fireplace. A carbon monoxide detector, however, is highly sensitive to carbon monoxide.
    - Typically, contact dependent signaling, paracrine signaling, and synaptic signaling ligands typically have a lower affinity for their respective ligand due to the high local concentration of the ligand in the receptor's environment.
    - Endocrine signaling typically has a much higher affinity as they appear in much lower concentrations near target cells (as hormones typically target many cells).

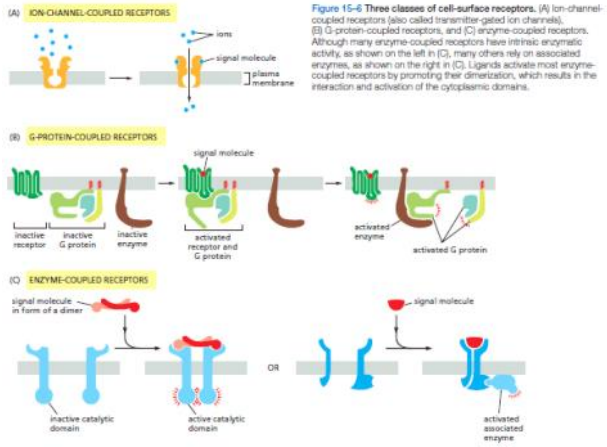


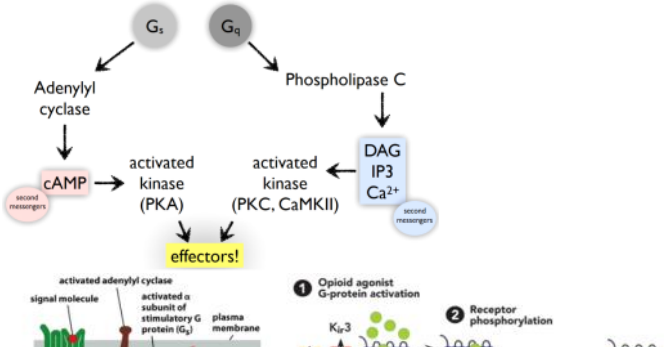
Figure 15–6 **Three classes of cell-surface receptors.** (A) Ion-channel-coupled receptors (also called transmitter-gated ion channels), (B) G-protein-coupled receptors, and (C) enzyme-coupled receptors. Although many enzyme-coupled receptors have intrinsic enzymatic activity, as shown on the left in (C), many others rely on associated enzymes, as shown on the right in (C). Ligands activate most enzyme-coupled receptors by promoting their dimerization, which results in the interaction and activation of the cytoplasmic domains.

Cell Surface Receptor Proteins: an Overview

- There are three main classes of cell surface receptor proteins. These include:
    - Ion-channel coupled receptors
    - G-protein coupled receptors (GPCR)
    - Enzyme coupled receptors

G Protein Coupled Receptors (GPCR)

- **GPCRs** are a huge family of receptors that comprise of 7 transmembrane domain regions and reside in the plasma cell membrane. The ligands and intracellular effector proteins for a GPCR receptor can be very diverse. For example, in humans, all 5 senses depend on GPCR receptors.
    - **Agonists** are ligands which activate the receptor.
    - **Antagonists** are ligands which deactivate the receptor.
- Each GPCR is, as the name suggests, coupled with a G protein complex.
    - The g protein complex is a trimeric protein complex comprising of an alpha-subunit, a beta-subunit, and a gamma subunit held together by a GDP bound to the alpha subunit. The alpha subunit acts as a GTPase, allowing for the protein to act as a GTP switch.
    - GPCRs can be further subdivided into many types of GPCRs, including Gs, Gi, and Gq GPCRs.
        - Gs GPCRs stimulate the production of cAMP
        - Gi GPCRs inhibit the production of cAMP
        - Gq GPCRs enhances the production of signaling phospholipids.
    - Gs GPCRs and Gq GPCRs activate two different pathways, as shown to the right. The Gs



- GPCRs can be further subdivided into many types of GPCRs, including Gs, Gi, and Gq GPCRs.
  - Gs GPCRs stimulate the production of cAMP
  - Gi GPCRs inhibit the production of cAMP
  - Gq GPCRs enhances the production of signaling phospholipids.
- Gs GPCRs and Gq GPCRs activate two different pathways, as shown to the right. The Gs pathway involves the production of cylic amp with adenylyl cyclase, whereas the Gq pathway involves the cleaving of PI(4,5)P2 from IP3 to stimulate an ER response.

- The mechanism for activation of the inactive G-protein complex is in the schematic to the right.
- The fight or flight response is a prime model of the Gs GPCR pathway. The binding of the adrenaline ligand, also known as epinephrine, triggers adrenergic receptors that bind to adenylyl cyclase to produce cyclic AMP for the signaling cascade. The cyclic amp then binds to the regulatory subunit of PKA, which releases activated PKA to activate effector proteins.
  - The effector proteins increase heart rate, constrict blood vessels, promote the breakdown of glycogen into glucose, and increase contractions in the skeletal muscle cells.
- Oxytocin is an agonist which is unique in that its binding to target receptors on cells promote the secretion of more oxytocin from the cell, an example of autocrine signaling.
- Certain opioids also activate the Gi pathway and regulate ion channel activation.

Attenuating Signaling Pathways

- The phosphorylation on the cytosolic side of GPCR by a kinases causes receptor desensitization by by GPCR kinase (GRK). Once the GPCR is phosphorylated, an inhibitory protein known as arrestin can bind to the GPCR and prevent its interaction with a trimeric g protein complex. Arrestin binding completely stops the signal from further being transferred.
- The levels of receptors and ligands may also be adjusted or attenuated via sequestration of receptors and down regulation of receptors and ligands.
  - If a signal is too powerful, the receptor-ligand complex can be endocytosed into the cell and broken down by a lysosome to prevent further activation of the pathway.
  - A variant of receptor down regulation involving the recycling of the receptor protein and the degradation of only the ligand also exists.
  - Receptor inactivation is analogous to arrestin deactivation of the GPCR.

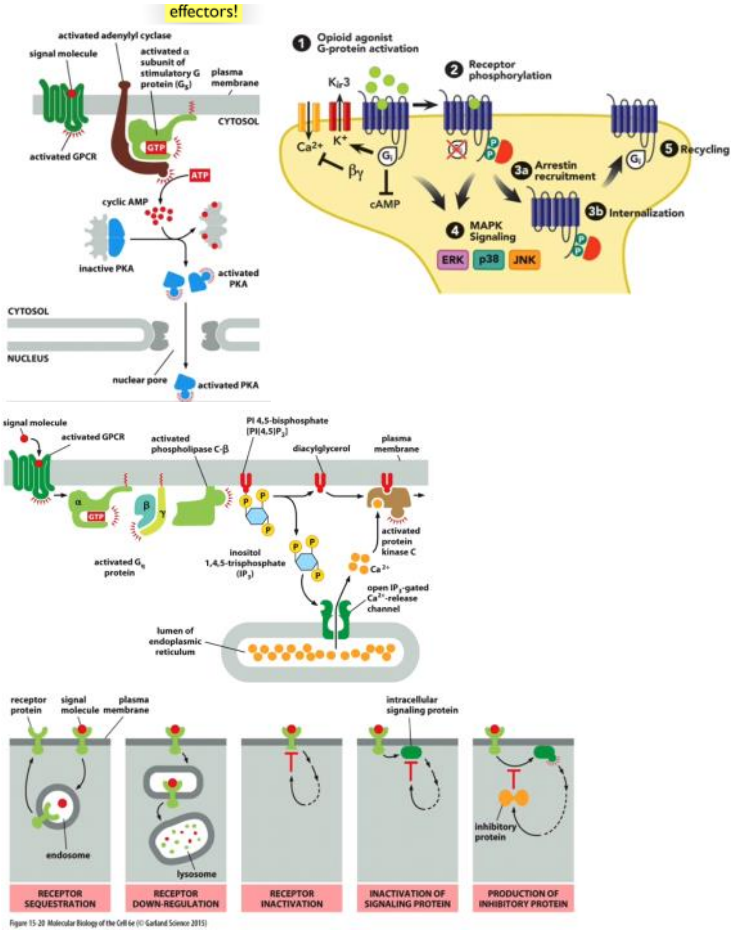


Figure 15-20 Molecular Biology of the Cell 6e (© Garland Science 2015)