

CHAPTER 15 CELL COMMUNICATION

Cells communicate with each other through signaling molecules. **Fig. 15-1**

Cells sense signaling molecules with receptor proteins.

These are intracellular receptors

There are several classes of cell surface receptors

1. kinases
2. phosphatases
3. GTP-binding proteins
4. others

Each class is evolutionarily and structurally related

Each class has a type of activity associated with it (e.g. kinases phosphorylate other proteins)

Each receptor (even within a class) is specific for a particular signal

Some receptors are located on the cell surface

Others are located inside the cell

Receptor proteins often transduce their signal to another intracellular signal transduction protein.

How do they know which protein to relay their signal to?

Ultimately the signal transduction cascade ends by phosphorylating a particular target protein, which alters its activity.

Examples:

- gene regulatory proteins
- ion channel proteins
- metabolic enzymes
- cytoskeleton

Thus, signaling molecules change what cells can do.

GENERAL PRINCIPLES OF CELL COMMUNICATION

Cells do not need to be part of a multi-cellular organism to communicate

Yeast cells (used to make bread and beer) normally are single cells. Fig. 15-2

There are “boy” yeast cells and “girl” yeast cells.

Each sends out “boy” and “girl” pheromones.

“Boy” meets “girl”, they mate (fuse their cells), and become diploid.

Extracellular Signal Molecules Bind to Specific Receptors

Signaling molecules are also generically called **ligands**.

Types of signaling molecules

- proteins
- small peptides
- amino acids
- nucleotides
- steroids
- retinoids
- fatty acid derivatives
- nitric oxide
- carbon monoxide

How do they get out of the signaling cell?

Diffusion if very small

Exocytosis, if big

How do signaling molecules move away from the signaling cell?

Some don't. They remain bound to the cell surface and act locally.

Some just diffuse away.

Some are transported by carrier proteins.

How do signaling molecules find their target cell?

Do they move directly to their target cell? Nope!

They diffuse or are transported to all cells.

Only those cells having a matching receptor have the potential to respond to the signal

Appropriate signal transduction apparatus needs to be in place

Hydrophobic signaling molecules can diffuse through the membrane. Why?

Hydrophilic signaling molecules require cell surface receptors.

Extracellular Signal Molecules Can Act Over Either Short or Long Distances

Short distance (acting upon nearby cells)

Contact-dependent signaling **Fig.15-4a**

Signaling molecules that remain bound to the surface of the signaling cell must act locally right?

So the target cell must come in contact with the signaling cell

Paracrine signaling **Fig.15-4b**

Signaling molecules move away from the signaling cell, but nearby cells have a matching receptor

Long distance (acting upon cells on the other side of the body)

Synaptic signaling **Fig. 15-4c**

The cell “reaches out” to the other side of the body.

Nerve cells do this.

The signaling molecules secreted at the end of the nerves “arm” (axon) are called neurotransmitters.

A nerve axon can deliver its signal to a single specific target cell

Endocrine signaling

The signal is sent into the blood stream which allows it to permeate the entire body.

Endocrine cells do this.

The signaling molecule is called a **hormone**.

While all cells are “bathed” in the hormone, only cells having a matching receptor will respond to the hormone.

Autocrine Signaling Can Coordinate Decisions by Groups of Identical Cells **Fig. 15-6**

Ever hear of “Horton Hears A Who”?

Well the autocrine system is a bit like that.

Nearby cells cannot “hear” a few cells “shouting”, but can hear a concerted blast from a group of cells.

This becomes self-reinforcing.

Early stages of animal development behave this way, giving rise to patterns of cell types that ultimately give rise to different body parts.

Gap Junctions Allow Signaling Information to Be Shared by Neighboring Cells

Rather than “shouting” so that all your neighbors can hear, you could try whispering in your friends ear.

This is what **gap junctions** do.

They are channels between two adjacent abutted cells. **Fig. 15-7**

The channels are constructed of protein.

But proteins are too big to pass through, so only small molecules pass through

Calcium ions

Cyclic AMP (or cAMP)

This type of direct and directed signaling is also important for development of multicellular organisms.

Each Cell Is Programmed to Respond to Specific Combinations of Extracellular Signal Molecules

All cells in an organism are constantly bathed in hundreds of different signaling molecules.

However, groups of cells are programmed to “hear” and respond to only a subset of signals.

What are they “hearing”? **Fig. 15-8**

A minimum subset of signals is required for the cell stay alive.

No signal → “I’m all alone...”, suicide, cell death, scientifically we call this **apoptosis** /ay-pop-tosis/

Apoptosis is a cellular program designed to destroy and recycle cellular components (more on this later).

Certain signals cause cells to **proliferate**.

Other signals cause cells to **differentiate** or engage in a specialized function.

Example: red blood cells make hemoglobin; undifferentiated stem cells can turn into brain cells

Different Cells Can Respond Differently to the Same Extracellular Signal Molecule

Not all cells respond to a particular signal or set of signals.

Cells that do respond might respond differently.

Cells within the same group will respond the same.

Cells that are different and thus programmed differently will respond differently to the same signal.

Example: **Fig. 15-9**

Muscle cells contract in response to acetylcholine (a neurotransmitter)

Heart muscle cells relax in response to acetylcholine

Different receptors to the same signal can elicit a different response.

Alternatively, same receptors but different internal signal transduction machinery

Heart muscle cell vs. salivary gland cell

The Concentration of a Molecule Can Be Adjusted Quickly Only If the Lifetime of the Molecule Is Short

Rapid control requires rapid turnover of signaling molecules.

Imagine driving a car in which your ability to take your foot off the accelerator is very slow.

Nitric Oxide Gas Signals by Binding Directly to an Enzyme Inside the Target Cell

Nitric oxide (NO) is a very small molecule that is produced by cells and is used as a diffusible signaling molecule.

Nitric oxide readily diffuses across membranes and binds to a nitric oxide receptor.

Nuclear Receptors Are Ligand-activated Gene Regulatory Proteins

Small hydrophobic signaling molecules (hormones) **Fig. 15-12**

Steroids – made from **cholesterol**

examples: testosterone, estradiol, cortisol, estrogen, progesterone, glucocorticoids

Vitamin D – made from cholesterol

Thyroid hormones

Example: thyroxine

Retinoids – made from **Vitamin A**

Example: retinoic acid

Hydrophobic means “water fearing”, so they need carrier proteins when moving around the blood.

They tend to last a long time, and thus are involved in long duration responses (like tissue development).

They freely diffuse across the hydrophobic cell membrane.

Require intracellular receptors.

Liganded receptors bind to certain promoter DNA in the nucleus and regulate gene expression. **Fig. 15-13b,c**

Certain unliganded receptors lie dormant in the cytoplasm until bound by the ligand

Others reside in the nucleus bound to their target DNA, but are inactive until liganded.

Called **nuclear receptors**

Nuclear receptors form a large gene family.

They are all structurally similar.

Each recognizes a different ligand and a different promoter DNA sequence

Some ligands can be quite similar.

Glucocorticoid receptor binds glucocorticoids

Vitamin D receptor binds Vitamin D

Orphan nuclear receptors have not had their signaling ligands determined yet.

Maybe some day you will figure out what the ligands are.

Promoter-bound liganded receptors activate transcription of the adjacent gene by recruiting component of the transcription machinery.

The Three Largest Classes of Cell-Surface Receptor Proteins Are Ion-Channel-linked, G-Protein-linked, and Enzyme-linked Receptors

All three classes are transmembrane proteins

Domain external to the cell binds the ligand

Hydrophobic transmembrane domain keeps it in the membrane

Cytosolic domain inside the cell is the “business” end.

Ion channel-linked receptors **Fig. 15-15**

Involve nerve cells and neurotransmitter signaling molecules.

G-protein-linked receptors

Receptor transmits its signal to a **G protein** (= **trimeric GTP-binding protein**).

G-protein is turned on, which then activates a target protein also located at the cell membrane.

Target protein then opens up an ion channel or catalyzes the production of a second messenger signaling molecule.

Enzyme-linked receptors

The receptor itself is an enzyme or associates with an enzyme.

Ligand binding activates the enzyme, which catalyzes the production of a **second messenger**

Most Activated Cell-Surface Receptors Relay Signals Via Small Molecules and a Network of Intracellular Signaling Proteins

Ligand binding on the cell surface receptor triggers the production of second messengers which diffuse throughout the cell.

These are small molecules (not protein)

Some are water soluble

cAMP, Calcium

Some are hydrophobic and thus diffuse throughout the membranes only

Diacylglycerol

Other receptors transduce their signals to other proteins, which transduce their signal to other proteins, and so on until the final target is reached.

You do not need to know the different functional classes of proteins described in **Fig 15-16** and on pp. 844-845. But do get a sense of the range different activities involved in the signal transduction process.

Some Intracellular Signaling Proteins Act as Molecular Switches **Fig. 15-17**

Molecular switches are inactive proteins that become active in response to a signal.

Important factor for a switch: how long to stay on.

Two important classes:

Protein kinases

GTP-binding proteins

Protein phosphorylation/dephosphorylation is a major switching mechanism

Protein kinases put phosphates on proteins.

Two major classes:

Serine/threonine protein kinases – guess what amino acid gets phosphorylated?

Tyrosine protein kinase

Protein phosphatases take phosphates off of proteins.

Each kinase phosphorylates its target amino acid (ser/thr, tyr) at a specific location on a specific protein.

GTP binding proteins

Active when bound to GTP

$GTP \rightarrow GDP + P_i$

Inactive when bound to GDP

Two types: **Fig. 15-18**

Trimeric G-proteins

Monomeric G-proteins

Multiple different signaling events must be integrated into a coordinated response.

Intracellular Signaling Complexes Enhance the Speed, Efficiency, and Specificity of the Response

A single type of extracellular signal can initiate multiple distinct signal transduction cascades, leading to multiple outcomes (change in cell shape, and cell movement) **Fig. 15-19**

Interactions Between Intracellular Signaling Proteins Are Mediated by Modular Binding Domains **Fig. 15-20**

SH2 domains

- Src homology domain = SH2 domain
- A family of related protein domains that have the common property of binding to phospho-tyrosine on certain proteins.
 - The tertiary protein structure surrounding the phospho-tyrosine determines whether or not a SH2 domain will dock with it.
- Found on many oncoproteins

Cells Can Respond Abruptly to a Gradually Increasing Concentration of an Extracellular Signal

- Cooperative assembly of an intracellular signaling complex by signaling molecules can lead to all or none responses. **Fig. 15-23**

A Cell Can Remember The Effect of Some Signals

Cells Can Adjust Their Sensitivity to a Signal

- Cells respond to changes in signal levels rather than absolute concentration.
- Called **adaptation** (not to be confused with genetic adaptation during evolution).
- This allows a signal transduction cascade to work over a wide range of concentrations which cannot always be maintained at the same level throughout the body.
- Through a variety of different negative feedback mechanisms, cells become desensitized to the signaling molecule **Fig. 15-25**

Summary

- All cells in all multicellular organisms are programmed to respond to specific extracellular signals.
- Different signals and different combinations of signals elicit different cellular response (changes in gene expression typically).
- Signals are typically small molecules that bind to cell surface receptors or intracellular receptors.
- Three types of cell surface receptors: ion channel, G-protein-linked, Enzyme-linked.
- Intracellular signal transduction pathways relay cell-surface signals throughout the cell and typically involve protein phosphorylation or GTP-binding as molecular switches.
- A variety of mechanisms exist to turn gradients of signal concentrations that exist throughout an organism into “on/off” switches.

SIGNALING THROUGH G-PROTEIN-LINKED CELL-SURFACE RECEPTORS

- G-protein-linked receptors form the largest family of cell surface receptors found in eukaryotes.
- Odors constitute signaling molecules. There are about a thousand different G-protein-linked receptors that respond to particular odor molecules giving us the ability to smell lots of different things.
- Other signals that activate G-protein-linked receptors include hormones and neurotransmitters.
- All G-proteins have similar structure. **Fig. 15-26**
 - The polypeptide snakes through the membrane 7 times.
- Most drugs (legal and illegal) work by binding to G-protein-linked receptors.

Trimeric G Proteins Disassemble to Relay Signals from G-Protein-linked Receptors

- **Trimeric G protein** transduce signals from G-protein-linked receptors.
- They are attached to the cytoplasmic side of the cell membrane. **Fig. 15-27**
- When a signaling ligand binds to the G-protein-linked receptor, a conformational change in the protein is induced through plasma membrane.
- The altered conformation of the receptor activates the trimeric G protein.
- There are a lot of related G proteins that are specific for certain receptors.
- G proteins are composed of three subunits: α , β , γ .
- The default state is inactive, and has a **GDP** (guanosine diphosphate) bound.
- Interactions with an activated receptor cause the alpha subunit to release its bound GDP. **Fig. 15-28**
- GTP then binds the alpha subunit which induces it to change conformation so that it can no longer bind $\beta\gamma$.
- Alpha then is activate to transduce its signal to a different protein molecule.
- Contact of alpha with its target protein induces alpha to hydrolyze GTP to GDP, returning it to the inactive state. **Fig. 15-29**
- Other proteins, generically called GAPs (GTPase activating proteins) also cause GTP binding protein to hydrolyze GTP.
 - GAPs are often specific for a certain G-proteins

Some G Proteins Signal By Regulating the Production of Cyclic AMP

- Activated alpha subunit of G-protein turns on membrane-bound **adenylyl cyclase**.
- cAMP acts as second messenger by binding to and activating other proteins.
- **cAMP phosphodiesterase** destroys cAMP.
- **Cholera** toxin causes modification of G-protein alpha subunit so it can't hydrolyzed GTP.
 - Caused by bacterial infection – bacteria usually present in bad drinking water.
 - Signal is always “on”, causing ion channels to open.
 - Leads to efflux of chloride ions, dehydration, diarrhea, death
- Many hormone-induced cell responses mediated by cAMP
 - Look over Table 15-1

Cyclic-AMP-dependent Protein Kinase (PKA) Mediates Most of the Effects of Cyclic AMP

- What does cAMP do?
- cAMP-dependent protein kinases (PKA) bind cAMP, causing dissociation of inhibitory subunits. **Fig. 15-32**
 - PKA phosphorylate target proteins at specific serine and threonine amino acids
- What are these target proteins?
 - One example is **CREB** (cAMP-response element binding protein)
 - CREB is a gene-specific transcriptional activator
 - Phospho-CREB recruits **CBP** (CREB binding protein) which is a chromatin remodeling factor
- So where are we? **Fig. 15-33** Specific example:
 - Signal binds receptor, which binds G protein, which activates adenylyl cyclase, which makes cAMP, which binds PKA, which phosphorylates promoter-bound CREB, which recruits CBP, which acetylates histones, which makes the promoter more accessible for transcription complex assembly (i.e. activates transcription)!!!!

Protein Phosphatases Make the Effects of PKA and Other Protein Kinases Transitory

- Phosphatases remove phosphates from proteins

Some G Proteins Activate the Inositol Phospholipid Signaling Pathway by Activating Phospholipase C- β

- When certain G-proteins get activated, rather than activating adenylyl cyclase they activate another membrane-bound enzyme called phospholipase C- β .
- Phospholipase C- β cleaves PIP_2 into DAG + IP_3
 - **PI(4,5)P_2** = phosphatidyl inositol bisphosphate
 - **DAG** = diacylglycerol
 - **IP_3** = inositol trisphosphate
- DAG activates **Protein Kinase C (PKC)**

Ca^{2+} Functions as a Ubiquitous Intracellular Messenger

- Some events triggered by a change in intracellular Ca^{2+} levels
 - Fertilization of an egg by sperm initiates a wave of calcium influx that ultimately establishes the body plan. **Fig. 15-37**
 - Muscle contraction
- Cytosolic calcium levels are kept low, while levels in the endoplasmic reticulum (ER) and outside the cell are high.
 - Calcium ion pumps are transmembrane protein the kick calcium out of the cytoplasm
- Opening of calcium ion channels in these membranes result in an influx of calcium.
- IP_3 is one signaling molecule that opens calcium channels

The Frequency of Ca^{2+} Oscillations Influences a Cell's Response

Ca^{2+} /Calmodulin-dependent Protein Kinases (CaM-Kinases) Mediate Many of the Actions of Ca^{2+} in Animal Cells

- Intracellular calcium receptors (examples):
 - **Troponin C** (muscle contraction)
 - **Calmodulin**
- Ca^{2+} -liganded calmodulin binds target proteins, thereby altering their activity.
- Targets include calmodulin-dependent protein kinases (CaM-kinases).
 - Examples:
 - myosin-light chain kinase \rightarrow muscle contraction
 - phosphorylase kinase \rightarrow glycogen breakdown

Some G Proteins Directly Regulate Ion Channels

Smell and Vision Depend on G-Protein-linked Receptors That Regulate Cyclic-Nucleotide-gated Ion Channels

- How are humans able to discern over 10,000 different smells?
- Inside the nose, there are specialized odor-smelling cells called olfactory neurons.
- On the surface of these neurons are **olfactory receptors**. Fig. 15-43
- Specific olfactory receptors bind to specific odor molecules.
 - Each olfactory neuron displays only one kind of olfactory receptor (but in large quantities)
- This triggers a special G protein to activate adenylyl cyclase.
- cAMP opens cAMP-gated ion channels, resulting in a wave of ion flux (nerve impulse).

- Vision works in a similar way, except that **rhodopsin** is the light sensor (a G-protein-linked receptor), and changes in **cGMP** (rather than cAMP) create the nerve impulse

Extracellular Signals Are Greatly Amplified by the Use of Small Intracellular Mediators and Enzymatic Cascades

G-Protein-linked Receptor Desensitization Depends on Receptor Phosphorylation

Summary

- Many cell surface receptors transduce their signals to trimeric G-proteins.
- G-proteins are activated by GTP, and dissociate into subunits that become regulators of other proteins such as adenylyl cyclase or phospholipase C.
- cAMP, cGMP, IP₃, DAG, and Ca₂₊ are second messengers.

SIGNALING THROUGH ENZYME-LINKED CELL-SURFACE RECEPTORS

- Enzyme-linked receptors are often involved in cell growth and movement.
- Ligands are typically growth factors (short peptides)
- Some ligands are bound to the cell surface over which the target cell is crawling.
- Cytosolic domain is either an enzyme or directly associates with an enzyme.
- Genetic defects in these receptors can lead to uncontrolled cell proliferation (cancer).
- Six classes of **enzyme-linked receptors**:
 - **Tyrosine kinase**
 - **Tyrosine kinase-associated**
 - **Tyrosine phosphatases**
 - **Serine/threonine kinase**
 - **Histidine-associate kinase**
 - **Guanylyl cyclase**

Activated Receptor Tyrosine Kinases Phosphorylate Themselves

- Ligands are secreted peptide growth factors and peptide hormones.
 - EGF – Epidermal growth factor
 - NGF – Nerve growth factor
 - PDGF – Platelet-derived growth factor
 - FGF – Fibroblast growth factor
 - M-CSF – Macrophage colony stimulatory factor
- Other ligands are bound to the cell surface upon which the target cell crawls
 - **Ephrins**
 - Ephrin receptors are tyrosine kinases
 - Important for development, including brain development
- Often the name of the receptor is the name of the ligand plus the word ‘receptor’.
- Receptor tyrosine kinases have their kinase domain inside the cell and ligand binding domain outside of the cell.
- Ligand-bound receptor tyrosine kinases phosphorylate themselves and other proteins that bind to the phosphorylated receptor.
- Since enzyme-linked receptors only have a single transmembrane pass (in contrast to the seven for G-protein-associated receptors), ligand binding cannot transmit a conformational change across the membrane.
- Enzyme-linked receptors often oligomerize (form dimers or bigger aggregates) upon ligand binding, allowing their cytosolic kinase domains to phosphorylate each other. **Fig. 15-50**
 - Sometimes the ligand itself simultaneously binds two receptors, causing dimerization.
- Autophosphorylation does two things:
 - It stimulates its own kinase activity
 - It provides docking sites for other proteins. **Fig. 15-52**

Phosphorylated Tyrosines Serve as Docking Sites For Proteins With SH2 Domains

- **Phospholipase C- γ** (PLC- γ) is one enzyme that binds certain phosphorylated receptors. **Fig. 15-53a**
- **Src** is a certain protein kinase that also binds phosphorylated receptors.
 - Certain defective versions of Src cause cancer, and thus is an **oncogene**.
- **Phosphatidylinositol-3' kinase** (PI3 kinase)
- These target proteins that bind phosphotyrosine are structurally very different, but they all possess a similar domain called an SH2 domain (Src homology domain), which binds to phosphotyrosine. **Fig. 15-53c**
 - Docking specificity is achieved through interactions with surrounding amino acids.

Ras Is Activated by a Guanine Nucleotide Exchange Factor

- Remember trimeric G-proteins?
 - Activated GTP-bound α subunit dissociates from $\beta\gamma$ allowing them to find their targets
- There are also monomeric G-proteins, the most notable is called **Ras**.
- There are three major subfamilies related in structure and function.
 - Ras
 - Rho – regulates cytoskeleton function
 - Rab – intracellular transport of vesicles
- There are many different versions, each with different targets
- These proteins are attached to the inside of the plasma membrane.
 - Covalently attached to a membrane lipid.
- Ras is activated when it binds GTP.
- It then engages its target, like a protein kinase.
 - These targets are often involved in processes like cell proliferation.
 - Ras mutants that fail to hydrolyze GTP (always ‘on’) are oncogenic
- How is Ras regulated? **Fig. 15-54**
 - Ras is a molecular switch
 - GTP-bound \rightarrow active
 - GDP-bound \rightarrow inactive
 - **Guanine nucleotide exchange factors (GEFs)** promote GDP dissociation so that GTP can bind.
 - GEFs therefore activate Ras
 - **GTPase-activating proteins (GAPs)** promoter GTP hydrolysis.
 - GAPs therefore inactivate Ras
 - Ras is regulated mostly by a GEF call **Sos**.
- How is Sos regulated? **Fig. 15-55**
 - Certain activated receptor tyrosine kinases are bound directly by Grb-2.
 - Do you think Grb-2 has an SH2 domain?
 - Other activated receptor tyrosine kinases do not present their phosphotyrosines in a way that Grb-2 can bind.
 - They require an adaptor protein called **Shc**.
 - Shc has an SH2 domain.
 - Grb-2 binds and activates Sos.

Ras Activates a Downstream Serine/Threonine Phosphorylation Cascade That Includes a MAP-Kinase **Fig. 15-56**

- Ras → MAP kinase kinase kinase (Raf) → MAP kinase kinase (MEK) → MAP kinase
- So remember: **Ras → Raf → MEK → MAP kinase** → lots of targets
- Targets include transcription factors, cell proliferation factors, other protein kinases.
- MAP kinase moves into the nucleus upon phosphorylation.
- MAP kinase activation requires two different phosphorylation events.
 - One on tyrosine
 - One on threonine
- What turns off these kinases?
- Different signaling molecules might employ distinct but parallel kinase cascades.
- Others might share some of the same kinases.
- How is **cross talk** prevented?
 - A → B → C
 - X → B → Z
- Cross talk is prevented by physically attaching members of a pathway together. **Fig. 5-57**
- Stress activates MAP kinase pathways.
 - Ultraviolet light
 - Heat shock
 - Osmotic shock (high external salt concentration)
 - Infection
 - Starvation

PI 3-Kinase Produces Inositol Phospholipid Docking Sites in the Plasma Membrane

- Cell proliferation needs two things:
 - Cell growth (increase in mass), induced by **growth factors**.
 - Driven by PI3 kinase.
 - Many different PI3 kinases.
 - Cell division ($1 \rightarrow 2$), induced by **mitogens**.
 - Driven by Ras – MAP kinase pathway.
- PI3 kinase phosphorylates a certain lipid located only on the inside of the plasma membrane. **Fig. 15-58**
 - The lipid is called **phosphatidylinositol (PI)**.
 - The phosphorylated products are called **PI(3,4)P₂** and **PI(3,4,5)P₃**.
 - Contains 2 and 3 phosphates, respectively.
- These PIPs remain in the membrane and become docking/activation sites for downstream target proteins. **Fig. 15-59a, Fig. 15-59b**
 - They do not get cleaved.
 - Eventually they do get dephosphorylated, which shuts down the mitogenic signal.
 - What would happen if the phosphatase was inactivated.
 - PTEN is one such phosphatase
- What are the downstream targets? **Fig. 15-59c**
 - Tyrosine kinases (e.g. BTK in B lymphocytes)
 - PLC- γ
 - BTK activates/phosphorylates PLC- γ .
 - PLC- γ cleaves the PI(4,5)P₃ to make IP₃.
 - A conserved domain on these downstream targets binds the PIPs
 - The conserved domain is called a PH domain
- Remember PI(4,5)P₃ ?
 - Its different
 - Gets cleaved into DAG + IP₃.
 - DAG \rightarrow activates protein kinase C (PKC); IP₃ \rightarrow intracellular calcium release.

The PI 3-Kinase/Protein Kinase B Signaling Pathway Can Stimulate Cells to Survive and Grow **Fig. 15-60**

- PI3 kinase \rightarrow PI(3,4,5)P \rightarrow binds PK-B (protein kinase B)
 - Do you think PK-B has a PH domain?
- PI3 kinase \rightarrow PI(3,4,5)P \rightarrow binds PDK1 (phosphatidylinositol-dependent protein kinase)
- PDK1 phosphorylates PK-B.
- PK-B moves into the cytoplasm, away from the plasma membrane.
- PK-B phosphorylates BAD.
 - Unphosphorylated BAD induces cell death (apoptosis).
 - Phospho-BAD is inactive.
- So if a cell is not receiving a continuous signal that is propagated through PI3 kinase, it will die.
 - So death is the default state for many cells.
 - Constant and proper signaling is a prerequisite for survival.
 - So if a cell finds itself in the wrong place in your body, it will die.
- Summary: Growth factors \rightarrow \rightarrow PI3 kinase \rightarrow \rightarrow PK-B \rightarrow cell growth/survival.
- “ “ \rightarrow S6 kinase \rightarrow stimulates ribosomes.
 - S6 is a ribosomal subunit.

Know **Fig. 15-61!**

Tyrosine-Kinase-associated Receptors Depend on Cytoplasmic Tyrosine Kinases for Their Activity

- The cytoplasmic domain of the receptor associates with a tyrosine kinase rather than being a kinase itself.
- These tyrosine kinases come in many flavors, each working in a different pathway.
 - Examples: *Src*, *Yes*, *Fyn*, *Lck*, *Lyn*.
- They have SH2 domains.
 - What do SH2 domains bind?

Cytokine Receptors Activate the Jak–STAT Signaling Pathway, Providing a Fast Track to the Nucleus

- Cytokines are locally-acting peptide signaling molecules.
- Here are some names of cytokines that you should know.
 - γ -interferon – activates macrophages (a type of phagocytic immune cell).
 - α -interferon – activates neighboring cells to resist viral infection.
 - Erythropoietin – stimulates production of red blood cells.
 - Prolactin – stimulates milk production.
 - Growth Hormone – stimulates growth
 - Interleukins – stimulate blood cell development
 - IL-3, IL-6, etc.
- Cytokine receptors are cell surface enzyme-linked receptors.
- **Janus tyrosine kinases (Jaks)** associate with the cytosolic side of these receptors.
 - Examples of Jaks
 - Jak1
 - Jak2
 - Tyk2
 - Certain Jaks work with certain receptors.
- Jaks phosphorylate themselves, the receptor, and STATs. **Fig. 15-63**
- **STATs** (signal transducers and activators of transcription) associate with the phosphorylated receptor to become phosphorylated.
 - Do STATs have an SH2 domain?
 - STATs are direct transcriptional activators!
 - Examples of STATs
 - STAT1
 - STAT2
 - STAT5
 - Certain STATs work with certain receptors.
- The phosphorylation of the STAT causes it to dimerize.
 - Dimerization occurs via SH2 domains and phosphotyrosine.
 - Homodimers and heterodimers can form.
 - If SH2 domains interact with phosphotyrosines then will all proteins that contain these features dimerize with each other?
 - How is specificity achieved?
- Dimerized STAT then moves into the nucleus and binds to specific promoter elements.
 - In combination with other transcriptional regulators certain genes are turned on.
- Summary example:

Prolactin → Prolactin receptor → Jak1/Jak2 → STAT5 → target genes

target genes: whey acidic protein; lactoglobulin; casein; other milk proteins
- The Jak-stat pathway is turned on by phosphorylation (kinases) and off by dephosphorylation (phosphatases)

Some Protein Tyrosine Phosphatases May Act as Cell-Surface Receptors

- Protein tyrosine phosphatases (PTPs) are very specific. Fig. 15-64
 - So there are lots of them.
 - Some are membrane-bound receptors.
 - Others are cytoplasmic
 - In contrast, ser/thr specific phosphatases act on a wide variety of proteins.
- They function to down-regulated Jak-STAT signaling.
 - Examples: SHP-1; SHP-2
 - PTPs bind to phosphotyrosines, so what must they have?

Signal Proteins of the TGF- β Superfamily Act Through Receptor Serine/Threonine Kinases and Smads Fig. 15-65

- **TGF- β** is a signaling peptide.
 - Transforming growth factor beta
 - There are many different homologous classes of TGF- β
 - TGF- β
 - Activins
 - BMPs – bone morphogenic proteins
 - Each doing different things.
 - Body patterning during development
 - Cell proliferation
 - Extracellular matrix production
 - Apoptosis
 - Tissue repair and immune regulation
- **TGF- β receptor** is a transmembrane receptor linked to a ser/thr kinase.
- The kinase phosphorylates latent cytoplasmic transcription factors called **Smads**.
 - Smads move into the nucleus and bind to target promoters.
 - Other regulatory proteins also needed.
 - Examples of Smads:
 - Smad1
 - Smad2
 - Smad5
- TGF- β \rightarrow TGF- β receptor \rightarrow ser/thr kinase \rightarrow Smad \rightarrow target genes

Receptor Guanylyl Cyclases Generate Cyclic GMP Directly

Bacterial Chemotaxis Depends on a Two-Component Signaling Pathway

Activated by Histidine–Kinase-associated Receptors

Summary

- Must know 3 of the 5 classes of enzyme-linked receptors.
 - Receptor tyrosine kinases
 - Tyrosine-kinase associated receptors
 - Receptor serine/threonine kinases
- Ligand binding induces receptors to dimerize and cross phosphorylate each other.
- Kinases and GTPases bind to phospho-receptors.
- A protein phosphorylation internal signaling cascade ensues.
- Ultimately, transcriptional activators that bind promoter elements become active by this cascade.

SIGNALING PATHWAYS THAT DEPEND ON REGULATED PROTEOLYSIS

- Signaling pathways to be covered here:
 - Notch
 - Wnt
 - Hedgehog
 - NF- κ B
- Ever wonder where they come up with these names?
 - Many like notch and hedgehog are names associated with a phenotype (typically in *Drosophila*) when the gene is mutated.
 - That is, the result of genetic investigation
 - Often the phenotype is observed first, then responsible gene is isolated.
 - Names like NF- κ B are derived from biochemical activities, then the responsible gene is isolated.
 - So NF- κ B was identified as an activity from fractionated cell extracts that stimulated transcription of the immunoglobulin κ light gene.
 - It was found in the nuclear fraction rather than the cytoplasmic fraction.
 - It was originally isolated from B-lymphocytes.
 - Can you now see where the name came from?

The Receptor Protein Notch Is Activated by Cleavage

- This is a major signaling pathway in animal development.
 - Particularly in nerve cell development
- **Delta** is the signaling protein.
- **Notch** is the receptor.
 - Quick, is this a cell surface receptor or an internal receptor?
- Delta is presented on the cell surface of the nerve cell.
 - Actually it's a transmembrane protein.
- Nearby cells contain Notch.
- When the nerve cells grows and moves around adjacent cells, Delta binds to Notch.
- This activation of Notch in the neighboring cells prevents these cells from developing into neurons.
- Called **lateral inhibition**.
- What are the down stream events upon Notch activation? **Fig. 15-71**
 - An intracellular protease cleaves off a cytoplasmic domain of Notch.
 - This domain is a transcriptional co-activator.
 - The Notch tail binds to a promoter-bound repressor called CSL, turning it into a transcriptional activator of genes that code for transcriptional inhibitors of neural genes.
- Notch and Alzheimer's disease
 - Notch is subjected to proteolytic cleavage at both its intra- and extra-cellular domain by a protease call *presenilin-1*.
 - Presenilin-1 cleaves other membrane proteins as well, such as β -amyloid precursor protein (APP).
 - Too much cleavage of APP results in accumulation of protein fragments outside of the cell.
 - The protein fragments aggregate into plaques which are thought to kill the nerve cell.
 - This nerve cell death cause senility and Alzheimer's disease.

Wnt Proteins Bind to Frizzled Receptors and Inhibit the Degradation of β -Catenin

- **Wnt** signaling controls many aspects of animal development.
 - Wnt is a local acting signaling peptide.
 - So is its receptor going to be located on the cell surface?
- The Wnt receptor is called Frizzled. **Fig. 15-72**
- The way Wnt works is to set up a cascade of events that protects a co-activator of Wnt-responsive genes from getting degraded by **proteolysis**.
 - Proteolysis
 - Proteolysis is the enzymatic removal of amino acids from proteins.
 - The coactivator of Wnt-responsive genes is called **β -catenin** (or Armadillo in flies).
 - β -catenin normally associated with **cadherins** which reside at cell-cell junctions.
 - Cell-cell junctions are where cells attach to each other.
 - β -catenin attaches cadherins to the cell's actin cytoskeleton.
 - Any β -catenin not part of this structural framework of the cell is rapidly degraded.
- When Wnt signaling is not present, β -catenin is phosphorylated by a protein complex having the following components.
 - The kinase component is **GSK-3 β** (glycogen synthase kinase-3b).
 - Wonder where it got that name?
 - Only β -catenin that is not part of cellular cytoskeleton gets phosphorylated.
 - **APC** (adenomous polyposis coli)
 - APC helps GSK-3 β bind to β -catenin.
 - Defects in APC prevent β -catenin degradation, which leads to activation of Wnt-responsive genes (even in the absence of Wnt)
 - When this happens at the wrong place, at the wrong time cell proliferation ensues.
 - In the colon (intestines), polyps (outgrowths) arise, that could be come cancerous.
 - 80% of all colon cancer is due to defects in APC
 - **Axin** – a scaffolding protein that hold GSK-3 β , APC, and β -catenin together.
- Phosphorylated β -catenin is then rapidly degraded by the proteasome
 - Phosphorylated β -catenin is rapidly ubiquitinated.
 - The proteasome recognizes the ubiquitin and degrades β -catenin.
- When Wnt signaling is present the bound Frizzled receptor sends an intracellular signal via a protein called Dishevelled to inhibit GSK-3 β .
- Free β -catenin migrates into the nucleus where it displaces a repressor protein (called Groucho) from the LEF-1/TCF transcriptional activator, which is bound at Wnt-responsive genes.
- Wnt \rightarrow Frizzled \rightarrow Dishevelled \neg (axin/APC/GSK-3 β \neg β -catenin)
 β -catenin \neg (LEF-1/Groucho \neg Wnt-responsive genes)
 β -catenin + LEF-1 \rightarrow Wnt-responsive genes
 '–' means inhibit, whereas ' \rightarrow ' means stimulates
- c-myc is a Wnt-responsive gene.

- c-myc is a potent transcriptional activator of genes involved in cell proliferation.
- So, can you explain how defects in APC lead to cancer?
- How is Wnt signaling and colon cancer related?

Hedgehog Proteins Act Through a Receptor Complex of Patched and Smoothed, Which Oppose Each Other Fig. 15-73

- **Hedgehog** proteins are signaling molecules.
 - *Drosophila* larvae having mutations in Hedgehog reminded the student working on this of a hedgehog.
- They act locally.
- **Patched** is the receptor that binds Hedgehog.
- Like the Wnt receptor Frizzled, liganded Patch prevents the degradation of a transcriptional activator of Hedgehog target genes.

SIGNALING IN PLANTS

Multicellularity and Cell Communication Evolved Independently in Plants and Animals

Receptor Serine/Threonine Kinases Function as Cell-Surface Receptors in Plants

Ethylene Activates a Two-Component Signaling Pathway

Phytochromes Detect Red Light, and Cryptochromes Detect Blue Light

Summary

CHAPTER 16 THE CYTOSKELETON

- Cells need to move, be structurally robust, and adopt certain shapes to conduct their function.
 - Think of
 - muscle fiber contraction in muscle cells
 - a nerve cell that extends from your foot to your head
 - a sperm cell trying to find its way
 - a macrophage crawling throughout your body
 - skin cells that protect your body
- Large “work projects” like moving chromosomes, vesicles, and protein complexes requires large structural machines.
- The cytoskeleton provides this.

THE SELF-ASSEMBLY AND DYNAMIC STRUCTURE OF CYTOSKELETAL FILAMENTS

- There are three major types of cytoskeleton filaments
 - Intermediate filaments – provides mechanical strength to the cell
 - Actin filaments – important for cell shape and motility
 - Microtubules (made up of tubulin) – Tracks for intracellular transport
- Motor proteins move vesicles and other large complexes along actin filaments and microtubules.
- Cytoskeletal filaments are dynamic – constantly assembling and disassembling. **Fig. 16-2**
- Proteins must regulate these dynamics.

Each Type of Cytoskeletal Filament Is Constructed from Smaller Protein Subunits

Structure	Subunit
Actin filament	actin
Microtubules	tubulin
Intermediate filaments	lamins vimentin keratins

-
- Each subunit is a protein.
- Thousands (millions?) of subunits line up head-to-tail via noncovalent interactions.
 - Remember from BMB/Micrb 251 what these noncovalent interactions are?
- Also side-to-side interactions make for a very stable structure. **Fig. 16-3**

Filaments Formed from Multiple Protofilaments Have Advantageous Properties

Nucleation Is the Rate-limiting Step in the Formation of a Cytoskeletal Polymer

The Tubulin and Actin Subunits Assemble Head-to-Tail, Creating Filaments that Are Polar **Fig. 16-6**

- The repeating unit in microtubules is a heterodimer of α -tubulin and β -tubulin.
 - Both are structurally related.
 - Arrangement is head-to-tail
 - Both subunit bind GTP, but only one can hydrolyze its GTP to GDP
 - GTP hydrolysis regulates microtubule stability.
 - Microtubules are cylinders (hollow tubes).
 - Structurally they are very stiff
-
- The repeating unit in actin filaments is a monomer of actin. **Fig. 16-7**
 - Each actin monomer binds ATP (not GTP).
 - ATP hydrolysis controls the dynamics.
 - Actin filaments are not hollow, but have a helical twist.
 - They are quite flexible.
 - Actin filaments can be 'lashed' together to make strong actin bundles.

The Two Ends of a Microtubule and of an Actin Filament Are Distinct and Grow at Different Rates

Filament Treadmilling and Dynamic Instability Are Consequences of Nucleotide Hydrolysis by Tubulin and Actin

- A growing microtubule involves addition of GTP-bound tubulin to one end.
- Within the microtubule, GTP is hydrolyzed to GDP.
- GDP-tubulin dissociates from the microtubule end more rapidly.
 - However, once GDP-tubulin is internal to the filament, it no longer dissociates.
- Net polymerization/depolymerization is a race between GTP hydrolysis at the end and addition of new GTP-tubulin subunits.
- This is called **dynamic instability**. Fig. 16-11b
 - Allows filaments to grow and contract.
- What factors contribute to net polymerization? Net depolymerization?

- Same with actin but with ATP.
- Also rather than assembling and disassembling from one end, actin assembles from one end and disassembles at the opposite end of the filament.
- ADP-actin dissociates faster than ATP-actin
- Called **treadmilling**.
- Treadmilling allows actin filaments to move, which allows cells to move.

- So what is the purpose of ATP and GTP hydrolysis with respect to filament dynamics?

Treadmilling and Dynamic Instability Require Energy but Are Useful

Other Polymeric Proteins Also Use Nucleotide Hydrolysis to Couple a Conformational Change to Cell Movements

Tubulin and Actin Have Been Highly Conserved During Eukaryotic Evolution

Intermediate Filament Structure Depends on The Lateral Bundling and Twisting of Coiled Coils **Fig. 16-16**

- Intermediate filaments impart structural strength to cells.
- Things that tend to undergo mechanical stress tend to be anchored to the intermediate filaments.
- Unlike actin and tubulin, intermediate filaments have subunits that are elongated and alpha-helical.
- Monomers dimerize via coiled coils.
- Dimers come together to form tetramers, and so on to form long ‘cables’.
- There is no head-tail arrangement, no dynamic instability, no treadmilling, no nucleotide binding.
- Assembly/disassembly is regulated by protein phosphorylation.

Intermediate Filaments Impart Mechanical Stability to Animal Cells

- Keratins
 - Finger nails, hair, scales, and skin are made up of **keratins**.
 - There are many different kinds of keratin intermediate filaments.
- Nuclear lamina
 - Lamins A, B, and C
 - Make up the inner lining and structure of the nucleus.
- Neurofilaments
 - Give structural stability to neuronal axons.
 - ALS (Lou Gehrig’s disease)
 - Results from accumulation and abnormal assembly of neurofilaments.

Filament Polymerization Can Be Altered by Drugs

- Plants and fungi make toxins that alter the assembly/disassembly of the cytoskeleton.
- Fungal *phalloidins* stabilizes actin filaments.
- *Colchicine* from a crocus cause tubulin depolymerization.
- *Taxol* from the yew tree stabilizes microtubules.
 - Used to treat cancer.
- *Acrylamide* disassembles neurofilaments.
 - Neurotoxin
 - Used in the laboratory to make polyacrylamide gels.

Summary

- *Microtubules are made up of globular tubulin subunits and form long structural cylinders.*
- *GTP hydrolysis controls rates of net assembly and disassembly.*
- *Microtubules function in intracellular transport.*
- *Actin filaments are made up of globular actin subunits.*
- *ATP hydrolysis controls the dynamics of assembly and disassembly.*
- *Actin filaments provide cell shape and is the treadmill for cell movement.*
- *Neurofilaments provide mechanical strength to the cell.*
- *Subunit structure of neurofilaments are unlike actin and tubulin.*
- *Drugs can affect cytoskeleton assembly/disassembly.*

HOW CELLS REGULATE THEIR CYTOSKELETAL FILAMENTS

Microtubules Are Nucleated by a Protein Complex Containing γ -tubulin

Fig. 16-22a

- Microtubule assembly is initiated at the **microtubule organizing center (MTOC)**
- A number of proteins are part of MTOC. One in particular is γ -tubulin.
- γ -tubulin is related to α - and β -tubulin, but serves only to anchor one end of the growing microtubule filament.

Microtubules Emanate from the Centrosome in Animal Cells Fig. 16-23

- **Centrosomes** and MTOC are essentially the same thing in animals.
 - Plants lack centrosomes but have numerous locations corresponding to MTOC
- Within the centrosome are many copies of the **γ -tubulin ring complex**, which are responsible for nucleating each microtubule.
- Also embedded in the centrosome is a pair of centrioles.
- The centrioles are orientated at right angles to each other.
- **Centrioles** organize the centrosome matrix.
- During cell division, they duplicate, move to opposite sides of the cell, and help pull apart duplicated chromosomes at mitosis. Fig. 18-18
- What are centrioles made up of?
 - Modified microtubules and other proteins.

Actin Filaments Are Often Nucleated at the Plasma Membrane

- Actin filament nucleation occurs at the plasma membrane.
- So the highest density of actin filaments is just under the cell surface where it determines cell shape, plasticity, and movement.
- Nucleation of actin is regulated by external signals in response to changing environments.
- Nucleation is catalyzed by **ARPs** (actin-related proteins). Fig 16-28
 - Analogous to the γ -tubulin ring complex
- Directional polymerization results in cell movement. Fig. 16-90

Filament Elongation Is Modified by Proteins That Bind to the Free Subunits

- About half of the actin in the cell is in filaments. The rest is free monomer.
- Proteins like **thymosin** are inhibitory to monomer incorporation, and thus regulate actin assembly.
- **Profilin** competes with thymosin binding, and enhances monomer incorporation into the “+” end of the filament. **Fig. 16-30**
- Profilin is activated by phosphorylation and inositol phospholipids.
- Profilin is localized to the plasma membrane.
- Extracellular signaling molecules bind to a section of the cell exterior can lead to local profilin activation and a burst of actin filament assembly resulting in filopodia/lamellipodia formation and cell movement.
- Similar processes (different protein and different location) happen with tubulin.

Proteins That Bind Along the Sides of Filaments Can Either Stabilize or Destabilize Them

- These proteins tend to bind throughout the filaments.
- Protein that bind microtubules are generically called **MAPs** (microtubule-associated proteins).
 - Do not confuse with MAP kinase described earlier – they are very different.
- Some MAPs stabilize microtubules, including the formation of large microtubule bundles. **Fig. 16-33**
- Other MAPs link microtubules with other cellular components.
- **Tropomyosin** holds together actin bundles.
- **Cofilin** depolymerizes actin.
- Cofilin preferentially interacts with the ADP form of actin, which tends to be near the minus end, in the treadmilling process.

Proteins That Interact with Filament Ends Can Dramatically Change Filament Dynamics

- Capping proteins ‘cap’ the plus end of actin filaments.
 - Subject to regulation by signaling molecules via PIP₂
- ARPs cap the minus end.
- Microtubules have their own set of capping proteins as well.

Filaments Are Organized into Higher-order Structures in Cells

Intermediate Filaments Are Cross-linked and Bundled Into Strong Arrays

Cross-linking Proteins with Distinct Properties Organize Different Actin Assemblies

- Actin filaments can be arranged in bundles or in a weblike network.
- Specific proteins are designed to crosslink actin filaments in different ways.
 - **α -actinin** and **vimentin** make bundles. Fig. 16-40, Fig. 16-41
 - **Spectrin** makes weblike networks in blood cells. Fig. 10-31
 - **Filamen** also makes networks. Fig. 16-42
 - Important for making flat lamellipodia for crawling along surfaces.
 - Fig. 16-47b,c,d

Severing Proteins Regulate the Length and Kinetic Behavior of Actin Filaments and Microtubules

Cytoskeletal Elements Can Attach to the Plasma Membrane

- **ERM** proteins attach actin filaments to the plasma membrane. Fig. 16-48
- Actually, the ERM proteins attach to transmembrane glycoproteins such as CD44.
- Unlike the 'permanent' attachment that occurs in red blood cells and muscle cells, the ERM attachment must be dynamic and regulated.
- Phosphorylation and PIP₂ regulate this in response to external signals.

Special Bundles of Cytoskeletal Filaments Form Strong Attachments Across the Plasma Membrane: Focal Contacts, Adhesion Belts, and Desmosomes

- When cells are slithering across a surface they need to grab on to things.
- Cells do this through focal contacts.
- Integrins are transmembrane proteins that bind to the extracellular matrix.
- **Cadherins** allow cells to attach to each other.
 - Cadherins are attached to catenins which attach to actin filaments.
- Desmosomes are places of cell-cell contact that have attached intermediate filaments (not actin filaments).

Extracellular Signals Can Induce Major Cytoskeletal Rearrangements

- Monomeric GTPase (Rho) control actin filaments. **Fig. 16-50**

Summary

- *Microtubules nucleate at centrosomes*
- *Actin nucleates at the plasma membrane*
- *Microtubule and actin filament can be lashed into strong bundles by crosslinking proteins.*
- *Microtubule and actin filament assembly and disassembly is dynamic and is controlled by capping proteins*
- *Extracellular signaling molecules can control actin filament assembly/disassembly.*
- *Actin filaments are anchored to the cell membrane by integral membrane proteins, that also attach to extracellular matrix and other cells.*

MOLECULAR MOTORS

- Motor proteins
 - One end attaches to a particular type of cytoskeleton filament
 - The other end attaches to a particular cargo.
 - organelles like mitochondria
 - chromosomes
 - muscle contraction
 - ATP hydrolysis moves the motor protein relative to the filament.

Actin-based Motor Proteins Are Members of the Myosin Superfamily

- **Myosin** is a protein responsible for force generation during muscle contraction.
- Myosin structure is important in force generation. **Fig. 16-51**
 - Long extended alpha helical region that dimerizes (tails).
 - Globular head that hydrolyzes ATP
 - Called myosin II heavy chain
 - Bound to each heavy chain is another subunit called myosin light chain.
 - Two copies of myosin light chain are bound to each heavy chain.
- Heavy chain tails form bundles that are symmetric. **Fig. 16-52**
- Bundles get together to form myosin filaments.
- There are many different kinds of myosins each having different functions, but all have similar structural arrangement, particularly in the head region.
- Myosin moves along the '+' end of the actin filament upon ATP hydrolysis, one step at a time.

There Are Two Types of Microtubule Motor Proteins: Kinesins and Dyneins

- **Kinesin** is a motor protein that moves along microtubules.
- Kinesin is structurally similar to myosin. **Fig. 16-55, Fig. 16-57**
- Kinesin is '+' end directed

- **Dyneins** are motor proteins that move toward the '-' end of microtubules.
- They are unrelated to kinesin and are very fast.
- Used to move cilia and flagella.

The Structural Similarity of Myosin and Kinesin Indicates a Common Evolutionary Origin

Motor Proteins Generate Force by Coupling ATP Hydrolysis to Conformational Changes

- The **myosin cycle** is like paddling a canoe – well work with me on this one.

Fig. 16-58

step	ATP binding site	What happens to the myosin head
1	empty	Bound to actin filament, in a state of rigor (cause of <i>rigor mortis</i> in death)
2	ATP	Detaches from actin filament (at position 'n')
3	ADP + Pi	'Cocks' forward (conformational change induced by ATP hydrolysis)
4	ADP	Re-attaches to actin filament at n+1 position
1	empty	Shifts back to original conformation (power stroke)

Be able to identify what happens at each of these steps:

- ATP binding
 - ATP hydrolysis
 - Release of Pi (inorganic phosphate)
 - Release of ADP
- The **kinesin cycle** is like walking – well walk with me on this one. Fig. 16-59a

step	Head A	Head B	What happens to the kinesin heads
A1	ADP		Release of (A) from tubulin = 'free'
A1		empty	(B) bound to tubulin
A2	ADP	ATP	Throws 'free' rear head (A) forward past 'attached' leading head (B)
B1	empty		(A) bound to tubulin
B1		ADP	Release of (B) from tubulin = 'free'
B2	ATP	ADP	Throws 'free' rear head (B) forward past 'attached' leading head (A)

Be able to identify what happens at each of these steps.

Motor Protein Kinetics Are Adapted to Cell Functions

Motor Proteins Mediate the Intracellular Transport of Membrane-enclosed Organelles

- Microtubules emanate from the cell center (minus end) to the periphery (+ end).
- Kinesin moves organelles toward the plus end (periphery).
 - **Endoplasmic reticulum (ER)** splays out toward the periphery due to Kinesin
- Dynein moves things toward the minus end (toward cell center). **Fig. 16-63**
 - The **golgi** stays toward the cell center, due to dynein.
 - Interestingly, the structural attachment to the golgi is very similar to the underlying cytoskeletal structure of red blood cells. **Fig. 10-31a**
 - Golgi shape and red blood cell shape are similar. **Fig 10-27 vs. Fig. 13-22**

Motor Protein Function Can Be Regulated

Muscle Contraction Depends on the Sliding of Myosin II and Actin Filaments

- Muscles are derived from the fusion of many muscle cells.
- **Myosin filaments** are a strung out between two scaffolds (**Z disc**) by a massive protein called titin. **Fig. 16-72**
 - **Titin** is the largest known polypeptide chain.
 - 3,000,000 daltons! (25,000 amino acids)
 - Most proteins are ~50,000 daltons (500 amino acids)
 - It acts as a bungee cord, helping to recover overstretched muscles.
- The myosin filament is bipolar.
- Encircling each myosin filament are actin filaments, which are also attached to the Z disc scaffold. **Fig. 16-71**
 - A separate set of actin filaments encircles each end of the myosin filament.
 - The actin filaments are capped at one end by tropomodulin.
- **Myofibrils** are composed of actin/myosin filaments.
- Myofibrils are striated due to the repeating nature of the filament arrangement. **Fig. 16-69**
 - $(Z \text{ disk} - \text{actin} - \text{myosin} - \text{actin} - Z \text{ disk})_n = \text{sarcomere}$
 - Myofibrils are as long as the muscle itself.
- Lots of myofibrils form a **muscle fiber**, and are the product of many fused muscle cells. **Fig. 16-68**
- Muscle contraction occurs when myosin fibers slide past the actin via the power strokes described above.
 - So sarcomeres get smaller when a muscle contracts.
 - Each myosin filament has about 300 heads, each hydrolyzing ATP without coordinating it with the others.

Muscle Contraction Is Initiated by a Sudden Rise in Cytosolic Ca^{2+} Concentration

- What happens when you decide to contract a muscle?
- A nerve impulse travels from the brain to the muscle.
- The “wave of depolarization” moves along the plasma membrane to invaginations called **T-tubules**. Fig. 16-73a
- T-tubules wrap around the myofibrils.
 - But they are kind of one-dimensional phone lines
- The signal is transmitted from the T-tubule to the **sarcoplasmic reticulum**.
 - The sarcoplasmic reticulum is more like a two-dimensional endoplasmic reticulum lattice, wrapping around the myofibrils.
- The T-tubules and the sarcoplasmic reticulum are connected by voltage-gated calcium (Ca^{2+}) channels.
 - Calcium is stored in the sarcoplasmic reticulum and is released into the cytoplasm of the muscle cells by the wave of depolarization. Fig. 16-73c
- Calcium binds to the **Troponin complex** causing it to dissociate from **Tropomyosin**.
 - Tropomyosin is a filamentous protein that normally interacts along the actin filament groove in a way that does NOT interfere with myosin binding to actin. Fig. 16-74a,b
 - However, the troponin complex pulls tropomyosin over the region where myosin binds, thereby preventing muscle contraction.
 - So the key is to get the Troponin complex to release Tropomyosin, so that Tropomyosin can move out of the way.
 - The Troponin complex binds calcium, causing it to dissociate from Tropomyosin.
 - This now exposes the myosin binding sites on actin.
- Summary

Nerve impulse \rightarrow target muscle \rightarrow T-tubules \rightarrow Ca^{2+} -gated ion channels \rightarrow Ca^{2+} release from SR \rightarrow Ca^{2+} **-I** (Troponin \rightarrow Tropomyosin **-I** myosin/actin ATP hydrolysis).

Heart Muscle Is a Precisely Engineered Machine

Cilia and Flagella Are Motile Structures Built from Microtubules and

Dyneins

- The coordinated movement of tubulin within a microtubule filament relative to other parts of the filament can cause bending.
 - Requires microtubules to be tethered at the end
- This occurs in sperm flagella and protist flagella. Fig. 16-77 and Fig. 16-79
- But not in bacteria.
 - They use a different kind of protein filament that rotates like a propeller rather than undulate.

Summary

- *Motor proteins use ATP hydrolysis to move things along microtubules and actin filaments (but not intermediate filaments).*
- *Myosin moves along actin filaments by 'rowing'.*
- *Kinesin moves along microtubules by 'walking'.*

THE CYTOSKELETON AND CELL BEHAVIOR

Mechanisms of Cell Polarization Can Be Readily Analyzed in Yeast Cells
Specific RNA Molecules Are Localized by the Cytoskeleton

Many Cells Can Crawl Across A Solid Substratum

Plasma Membrane Protrusion Is Driven by Actin Polymerization

Cell Adhesion and Traction Allow Cells to Pull Themselves Forward

External Signals Can Dictate the Direction of Cell Migration

The Complex Morphological Specialization of Neurons Depends on The Cytoskeleton

Summary

CHAPTER 19 CELL JUNCTIONS, CELL ADHESION, AND THE EXTRACELLULAR MATRIX

- Major tissue types in vertebrates:
 - Nerve
 - Blood
 - Lymphoid
 - Connective
 - Make lots of extracellular matrix, including collagen.
 - Cell-cell junctions are rare.
 - Provides a lot of mechanical stress resistance.
 - Epithelial (skin) **Fig. 19-1**
 - Very little extracellular matrix made.
 - Many strong cell-cell junctions make a strong epithelial sheet.
 - Junctions are attached to the cytoskeleton providing resistance to mechanical stress.
 - Epithelial sheets include the skin and the lining of your gut.
 - Form barriers to water , other molecules, and cells.
 - Molecules must move through the cell.
 - Epithelial sheets rest on a bed of connective tissue.
- Extracellular matrix is formed from secreted macromolecules that provide the substratum upon which cells can attach and move around on.
- Cells are also bound to each other via cell-cell junctions.
- Groups of tissue that have specific purposes are called organs.

CELL JUNCTIONS

- Points of cell-cell attachment, and cell-matrix attachment.
- Highly abundant in epithelial cells.
- Three functional groups
 - **Occluding junctions**
 - Like 'zip-lock' bags, nothing gets past
 - **Anchoring junctions**
 - How cells hold on to each other and the matrix
 - Are attached to the cytoskeleton
 - **Communicating junctions**
 - Provides a passage for communication between cells.
 - Small molecules pass through the junctions.

Occluding Junctions Form a Selective Permeability Barrier Across Epithelial Cell Sheets

- Also called tight junctions in vertebrates.
- The 'zip-lock' seal prevent molecules and cells from permeating between cells from the gut of an animal. **Fig. 19-2**
 - Actually cells regulate this too, to let certain molecules (like amino acids, certain ions) past.
- The tight junctions also prevent transmembrane carrier proteins on basal and lateral side of the cell from migrating over to the luminal side (apical surface).
- Model for tight junctions **Fig. 19-4a, Fig. 19-5**
 - **Claudin** proteins are integral membrane proteins that form the tight junctions.

Anchoring Junctions Connect the Cytoskeleton of a Cell Either to the Cytoskeleton of Its Neighbors or to the Extracellular Matrix

- They attach to each other across membranes between cells. **Fig. 19-7**
- Inside the cell they are attached to the cytoskeleton.
- Note: the cell membrane does not provide any major structural stability to cells.
- Two major functional forms:
 - **Cadherins** form adherens junctions and desmosomes. **Fig. 19-9**
 - Adherens junctions usually attaching to actin filaments via anchoring proteins such as catenins, vinculin, and α -actinin.
 - Desmosomes attach to intermediate filaments like keratin filaments. **Fig. 19-11**
 - **Integrins** form focal adhesions and hemidesmosome attachments to the extracellular matrix. **Fig. 19-12b**
 - Focal adhesions attach to actin filaments via anchoring proteins.
 - Hemidesmosomes attach to intermediate filaments.

Summary

cell-cell

Adherens junctions – **cadherins** attach to **actin** filamentsDesmosomes – **cadherins** attach to **intermediate** filaments.

cell-matrix

Focal adhesions – **integrins** attach to **actin** filamentsHemidesmosomes – **integrins** attach to **intermediate** filaments.

Adherens Junctions Connect Bundles of Actin Filaments from Cell to Cell

Desmosomes Connect Intermediate Filaments from Cell to Cell

Anchoring Junctions Formed by Integrins Bind Cells to the Extracellular Matrix: Focal Adhesions and Hemidesmosomes

Gap Junctions Allow Small Molecules to Pass Directly from Cell to Cell

A Gap-Junction Connexon Is Made Up of Six Transmembrane Connexin Subunits

- Connexins form a 6-member connexon channel between two adjacent cells.
Fig. 9-15
- Gap junctions are used for communication via small molecule second messengers.

Gap Junctions Have Diverse Functions

The Permeability of Gap Junctions Is Regulated

In Plants, Plasmodesmata Perform Many of the Same Functions as Gap Junctions

Summary

- See **Fig. 19-19**
- *Multicellular animals use a variety of junctions as permeability barriers, structural support, cell-cell communication.*

CELL–CELL ADHESION

Animal Cells Can Assemble into Tissues Either in Place or After They Migrate

Dissociated Vertebrate Cells Can Reassemble into Organized Tissues Through Selective Cell–Cell Adhesion

Cadherins Mediate Ca^{2+} -dependent Cell–Cell Adhesion

- Any cell adhesion molecule is generically referred to a CAM.
- There are different kinds of cadherins.
- Virtually all cells in a multicellular organism express some sort of cadherin.
- Most cadherins are single-pass glycoproteins.
- Extracellular domains are often found in tandem repeats. Fig. 19-24
- These cadherin repeats are structurally related to antibodies (immunoglobulins).
- They bind calcium.

Cadherins Have Crucial Roles in Development

Cadherins Mediate Cell–Cell Adhesion by a Homophilic Mechanism

- Three different possibilities shown in Fig. 19-26
- Cells expressing one type of cadherin tend to seek out similar cells. Fig. 19-27
-

Cadherins Are Linked to the Actin Cytoskeleton by Catenins Fig. 19-29

Selectins Mediate Transient Cell–Cell Adhesions in the Bloodstream

- Blood cells need to move about the body.
- **Selectins** are cell surface proteins on blood cells that bind to carbohydrates (lectins). Fig. 19-30
- White blood cells also express their own lectins.
- When a cell is damaged it flags down a white blood cells by expressing lectins on its surface.
 - This interaction is weak, allowing the white blood cell to move around.
 - But this induces the expression of integrins which allow stronger binding and penetration of the white blood cell into the tissue.

Members of the Immunoglobulin Superfamily of Proteins Mediate Ca^{2+} -

independent Cell–Cell Adhesion

- The most notable one is N-CAM.
 - Homophilic interactions
- Another one is I-CAM
 - Heterophilic interactions.
- Modification of N-CAM with sialic acid prevent cell adhesion.
- N-CAM directed cell-cell interaction are not as strong as that generated by cadherins.
- N-CAMs might be important for nerve cell-cell interactions.

Multiple Types of Cell-Surface Molecules Act in Parallel to Mediate Selective Cell–Cell Adhesion

Nonjunctional Contacts May Initiate Cell–Cell Adhesions That Junctional Contacts Then Orient and Stabilize

- This allows cells to move past one another.
- If permanent residence is taken up then they need to set up cell junctions.

Summary see **Fig. 19-32**

- Calcium dependent cell-cell adhesion is mediate by cadherins, via homophilic interactions.
- N-CAMs play a role in neural development.

THE EXTRACELLULAR MATRIX OF ANIMALS

- A vast network of proteins and polysaccharides upon which cells move and attach.
- It is made by the cells that occupy and traverse the matrix. Fig. 19-35
- The matrix can be diverse in function.
 - Calcification of the matrix gives rise to bones and teeth.
 - It can become transparent to give rise to the eye cornea
 - Rope-like organization gives rise to tendons.

The Extracellular Matrix Is Made and Oriented by the Cells Within It

- **Fibroblast** cells secrete the matrix over much of the body
- **Chondroblasts** make cartilage
- **Osteoblasts** make bone.
- The extracellular matrix is made up of proteoglycans and fibrous proteins.
- **Glycosaminoglycans** (GAGs) are the polysaccharides on the proteoglycans.
- The GAGs create a gel-like matrix which provides a spongy protection and a milieu for cells to move around in.
 - The matrix in the knee joint provides a cushion during running.
- Fibrous proteins include:
 - collagen - provides mechanical strength
 - elastin – provides stretchability/elasticity
 - fibronectin
 - laminin

Glycosaminoglycan (GAG) Chains Occupy Large Amounts of Space and Form Hydrated Gels

- Fig. 19-37, Fig. 19-38

Hyaluronan Is Thought to Facilitate Cell Migration During Tissue Morphogenesis and Repair

- **Hyaluronan** is not attached to protein but is a highly hydrated polysaccharide.

Proteoglycans Are Composed of GAG Chains Covalently Linked to a Core Protein

Proteoglycans Can Regulate the Activities of Secreted Proteins

- Some signaling proteins bind to the extracellular matrix.

GAG Chains May Be Highly Organized in the Extracellular Matrix

- Fig. 19-41

Cell-Surface Proteoglycans Act as Co-Receptors

- Some cell surface receptors require co-association with proteoglycans embedded in the cell membrane.

Collagens Are the Major Proteins of the Extracellular Matrix

- Collagen forms an extend triple alpha helix. Fig. 19-43
- Collagen → collagen fibrils → collagen fibers Fig. 19-44
- Crosslinking of collagen helps provide mechanical strength

Collagens Are Secreted with a Nonhelical Extension at Each End

After Secretion, Fibrillar Procollagen Molecules Are Cleaved to Collagen Molecules, Which Assemble into Fibrils

Fibril-associated Collagens Help Organize the Fibrils

Cells Help Organize the Collagen Fibrils They Secrete by Exerting Tension on the Matrix

Elastin Gives Tissues Their Elasticity Fig. 19-52

- Skin, blood vessels, lungs, bladder lining, and the birth canal are examples of tissue that need to expand. Yet they need to be strong.
- **Elastin** is a protein that provides elasticity (much more than rubber).
- When mixed with collagen, you get both strength and elasticity.
- Crosslinking and the folding properties of elastin provide the molecular basis for their stretchability.

Fibronectin Is an Extracellular Protein That Helps Cells Attach to the Matrix

Fibronectin Exists in Both Soluble and Fibrillar Forms

Intracellular Actin Filaments Regulate the Assembly of Extracellular Fibronectin Fibrils

Glycoproteins in the Matrix Help Guide Cell Migration

Basal Laminae Are Composed Mainly of Type IV Collagen, Laminin, Nidogen, and a Heparan Sulfate Proteoglycan

Basal Laminae Perform Diverse and Complex Functions

The Extracellular Matrix Can Control Cell Shape, Survival, and Proliferation

The Controlled Degradation of Matrix Components Helps Cells Migrate

- Cells need to move through the matrix and not get caught up in it.
- They do this by secreting proteases that locally degrade the matrix.
- **Collagenase** is a protease that degrades collagen.
- When cancer cells metastasize (migrate to different parts of the body) they need to secrete proteases.
- Some cells secrete protease inhibitors which block cell movement in that area.
 - A class of inhibitors called **serpins** inhibit serine proteases.

Summary

- *The extracellular matrix is composed of polysaccharides and glycoproteins.*
- *The polysaccharides are highly hydrated providing a gel for movement of cells and a cushion against physical pressure.*
- *Some matrix proteins provide mechanical strength, like collagen.*
- *Others, like elastin, provide elasticity.*
- *Cells move through the matrix by secreting proteases.*

INTEGRINS

- **Integrins** are cell surface receptors of the matrix.
- Integrins connect the matrix to the cytoskeleton.
- They trigger intracellular signaling events in response to the type of matrix that they are touching.
- Unlike typical signaling receptors, integrins are much more abundant and have very low affinity for the matrix. Why?
 - A large number of low affinity contacts allow the cell to move rapidly over the matrix.
 - Otherwise its like trying to run in thick mud up to your ankles!

Integrins Are Transmembrane Heterodimers **Fig. 19-64**

- They depend on divalent cations for matrix binding.
 - The divalent cation helps integrins fold properly.

Integrins Must Interact with the Cytoskeleton to Bind Cells to the Extracellular Matrix

- Most interact with actin filaments via α -actinin, filamin, and other adaptor proteins.
- Clustering of integrins as a result of matrix binding results in re-organization of actin filament, and thus altered cell movement and shape.
- Clustering of the actin filament also re-enforces the clustering of integrins allowing them to bind the matrix more efficiently.

Cells Can Regulate the Activity of Their Integrins

- In some cells like white blood cells, the integrins are on the cell surface but many are not in a conformation that binds to the matrix.
 - This allows cells to move around un-impeded, until a signal triggers them to stick around.
- Signals emanating from damaged tissue bind to cell surface receptors and elicit an intracellular signal transduction cascade that results in activation of the integrins.
 - The white blood cell then sticks around the damaged tissue longer.
- This also happens with blood platelets, allowing them to form a blood clot at the wound site.

Integrins Activate Intracellular Signaling Pathways

- Integrins bound to the matrix may induce localized cytoplasmic changes.
- This is particularly true in axon guidance in developing nerves, where local interactions of transmembrane adhesion proteins affect the placement of actin fibers and the plasma membrane.

Summary

- *Integrins are the principle transmembrane receptor used to bind the matrix.*
- *They link the matrix to the cytoskeleton*
- *They can direct an intracellular signal transduction cascade that directs cell movement, localized growth, and survival.*

THE PLANT CELL WALL

The Composition of the Cell Wall Depends on the Cell Type

The Tensile Strength of the Cell Wall Allows Plant Cells to Develop Turgor Pressure

The Primary Cell Wall Is Built from Cellulose Microfibrils Interwoven with a Network of Pectic Polysaccharides

Microtubules Orient Cell-Wall Deposition

Summary

CHAPTER 17 THE CELL CYCLE AND PROGRAMMED CELL DEATH

- The cell cycle is the process by which a cell duplicates all of its components resulting in two cells.
 - In unicellular organisms like bacteria, yeast, protozoans, each round of the cell cycle generates a new organism.
 - In multicellular animals each cell division just adds more cells to the organism.
- An ordered series of events constitute the cell cycle.
- A control system monitors these events making sure that all the event occur in the proper order.
- Each cell also monitors things happening outside of the cell, so it know when to proliferate and when to remain quiescent.

AN OVERVIEW OF THE CELL CYCLE

- There are four phase of the cell cycle: Fig. 17-3
 1. **G₁**
 - a. “G” stands for “gap”.
 - b. Cells have a standard set of chromosomes
 - i. Diploid – 2n; Haploid – 1n
 - c. Cells accumulate mass (grow) in G₁. This is the phase cells normally step off the cell cycle when they are not proliferating.
 - d. The cells step out of the cell cycle and into G₀ “resting” phase (but they are hardly resting)
 - e. Extracellular growth factors are required for cells to move through G₁.
 2. **S**
 - a. “S” stands for synthesis.
 - b. DNA replication occurs here.
 - c. Chromosomes are duplicated.
 - d. So a normally 2n diploid becomes transiently 4n.
 3. **G₂**
 - a. “G₂” stands for 2nd gap phase.
 - b. Chromosomes have duplicated and now the cell is preparing to divide.
 4. **M**
 - a. “M” stands for mitosis.
 - b. The nuclear envelope breaks down.
 - c. Chromosomes condense.
 - d. Chromosomes align.
 - e. Chromosomes separate to opposite sides of the cell.
 - f. Cytokinesis takes place – physical division of the cell into two.
- After mitosis, we’re back to G₁.
- **Interphase** represents those phases in which the chromosomes are not condensed.
 - That includes everything but mitosis.
- As cells move through G₁, they get to a point of “no return” in late G₁, call **start** (in yeast) or the **restriction point** (mammalian cells).

The Cell-Cycle Control System Is Similar in All Eukaryotes

- The molecules that control the cell cycle are essentially the same in all eukaryotes.
- So, you can study cell cycle control in yeast to learn how it is done in humans.
- Cell cycle control is so important, because loss of control leads to cancer.

The Cell-Cycle Control System Can Be Dissected Genetically in Yeasts

- Fungi represent a diverse phylogenetic kingdom.
- Two fungi in particular have been studied intensively as an easy way to study the cell cycle.
 - *Schizosaccharomyces pombe*
 - Fission yeast
 - Used to brew African beer
 - *Saccharomyces cerevisiae*
 - Budding yeast
 - Baker's and brewers yeast
 - Cerveza means beer in Spanish
 - These two yeast species are about as far apart from each other, evolutionarily, as they are from humans!
- Advantages of using yeast as an experimental organism.
 - They grow fast (rapid cell cycle)
 - Yeast - 90 min
 - Bacteria – 20 min
 - Human cells – 24 hours or longer
 - Genome size is 0.1% the size of mammals
 - Very little 'junk' DNA.
 - Few introns, and little alternative splicing
 - Less genes
 - Short intergenic regions (control regions)
 - Many yeast species have had their genome sequenced and thus are amenable to **comparative genomics**.
 - Powerful genetic system
 - Easy to delete genes
 - Easy to mutate genes
 - Easy to set up screens
 - Cell proliferation occurs in the haploid (1n) state.
 - Problem with diploids is that you have to knock out both genes to see an effect.
 - With yeast, knock out a single gene.
- Isolation of yeast mutants that have defects in the cell cycle.
 - Why? If a mutant fails to move through the cell cycle than the wild type counterpart must be required to move through the cell cycle.
 - If a mutant fails to move through the cell cycle, how can you propagate it?
 - Need to isolate conditional mutants that fail to move through the cell cycle only at a certain temperature.
 - For example, at 30°C they grow and divide normally, but at 37°C they stop in one phase of the cell cycle. **Fig. 17-5**
 - How do you isolate such mutants?
 - Mutagenize cells with ultraviolet light or a chemical mutagen.
 - Plate out cells on agar media so that isolated colonies arise at 30°C.

- Transfer a sample of each colony to two new plates.
 - One is incubated 30°C (control)
 - The other at 37°C
 - Those that grow at 30°C but not at 37°C are temperature-sensitive mutants (ts).
 - For all ts mutants look under the microscope for cells that look like they are stuck in one phase of the cell cycle.
 - Grow cells at 30°C
 - Shift to 37°C for a few hours.
 - Look under the microscope for entire populations of cells stuck in one phase of the cell cycle.
 - Often various phases of the cell cycle can be visually identified as phase-specific phenotypes. **Fig. 17-6**
 - Normally, a population of wild type cells will have cells in all phases of the cell cycle
 - Such mutants have been called ***cdc*** mutants (cell-division-cycle), and their genes ***cdc*** genes
- Normal wild type cells are asynchronous in their cell cycle.
 - **Wild type** refers to the non-mutant form.
 - **Asynchronous** means that in a population, different cells will be in different phases of the cell cycle.

The Cell-Cycle Control System Can Be Analyzed Biochemically in Animal Embryos

- Yeast genetics provides a powerful means of identifying genes involved in the cell cycle.
 - But what do those genes do?
 - Need to turn to biochemistry.
 - Now-a-days yeast biochemistry is quite easy, but “back in my day” they were considered to be a sack of proteases and thus were unsuitable for protein isolation.
- Animal eggs are ideal for biochemical cell cycle studies.
 - They are huge in comparison to normal cells
 - About 100,000 times bigger, and thus with 100,000 times more cell cycle proteins.
 - Eggs are just poised to go into a flurry of cell division.
 - G₁ and G₂ phases are largely eliminated.
 - Thus, at the early stages of cell division there is no growth.
 - Just 11 rounds of S & M phases yielding $2^{11} = 4096$ cells. But no growth.
 - *Xenopus* (frog) eggs have been the favorite eggs to use. **Fig. 17-7**

Cell-Cycle Control in Multicellular Organisms Can Be Studied in Cultured Mammalian Cells

- Its easiest to watch individual cells go through the cell cycle rather than watching cells that are part of a multi-cellular organism.

Cell-Cycle Progression Can Be Studied in Various Ways

- The most popular method uses fluorescence activated cell sorting (FACS) using a flow cytometer.
 - A dye is added to a population of cells that stains the DNA.
 - The dye binds DNA and fluoresces.
 - Cells with twice the normal DNA content will fluoresce twice as bright.
 - FACS rapidly looks at thousands of cells and reports the amount of fluorescence in each.
 - A histogram report gives you sense of what portion of the cell population are in different phases of the cell cycle. **Fig. 17-12**

Summary

- *There are four phases of the cell cycle – know them, understand them.*
- *Yeast genetics have been used to identify cell cycle regulatory genes.*
- *Egg cells are used to isolate and study cell cycle regulatory molecules.*

COMPONENTS OF THE CELL-CYCLE CONTROL SYSTEM

The Cell-Cycle Control System Triggers the Major Processes of the Cell Cycle

- Essentials of cell cycle control
 1. A timing device
 2. Maintaining an order of events
 3. Once and only once per cell cycle
 4. Finish what you start
 5. Back-up systems
 6. Work under a variety of conditions

The Control System Can Arrest the Cell Cycle at Specific Checkpoints

- The cell cycle cannot proceed through checkpoints in the cell cycle unless the “checklist” of events since the previous checkpoint has been completed.
 - _The cell cycle will not proceed through the G_1 checkpoint unless appropriate signals are received from other cells.
 - _At the G_2 checkpoint, cells cannot enter mitosis unless the cell affirms that all the DNA is replicated.
- There are also other kinds of checkpoints that only come up under certain situations.
 - _DNA damage checkpoint occurs if the DNA has been damaged.
 - _The cell cycle is aborted and put on hold until the DNA is repaired and the checklist of repairs is complete.

Checkpoints Generally Operate Through Negative Intracellular Signals

- Signals emanate from incomplete jobs.
- Only when the job is complete does the negative signal cease.

The Cell-Cycle Control System Is Based on Cyclically Activated Protein Kinases

- These protein kinases are called **Cdks (cyclin-dependent kinases)**
- Their activity rises and falls with the cell cycle.
- Cdks are regulated by **cyclins**. Fig. 17-16
- Cyclins bind to Cdk and activate their protein kinase activity.
- Cyclins undergo cycles of synthesis and degradation during the cell cycle.
 - Cdks do not.
- There are four classes of cyclins.
 - G₁/S-cyclins
 - Bind Cdks at the end of G₁.
 - Commits the cell to DNA replication.
 - S-cyclins
 - Bind Cdks during S-phase.
 - Required for the initiation of DNA replication.
 - M-cyclins
 - Promote mitosis
 - G₁-cyclins
 - Promote passage through Start.
- Names of cyclins
 - Vertebrates – cyclin A, cyclin B, etc.
 - Yeast – Cln3, Clb5, etc.
- What do cyclins do?
 - Activate Cdks by altering the conformation of Cdks
 - Direct Cdks to their target proteins.
- Cdks also require activation by phosphorylation.
 - **Cdk-activating kinase (CAK)** does this but only after cyclins bind. Fig. 17-17

Cdk Activity Can Be Controlled Both by Inhibitory Phosphorylation and by Inhibitory Proteins

- The inhibitory phosphate is put on by the Wee1 kinase.
- It is removed by the Cdc25 phosphatase. Fig. 17-18
- Regulation of Cdks is a lot like an integration device on a computer. Fig. 3-66
 - Read p. 179 of the Alberts text.

The Cell-Cycle Control System Depends on Cyclical Proteolysis

- Cyclins are degraded by a ubiquitin system.
- The proteasome recognizes ubiquitinated cyclins and degrades them.
- During mitosis this is carried out by the **anaphase-promoting complex (APC)**. Fig. 17-20b

Cell-Cycle Control Also Depends on Transcriptional Regulation

Summary

- *Phosphorylation (no surprise) via Cdks controls the cell cycle*
- *Cyclins, phosphorylation, and phosphatases control the Cdks*
- *Cyclin levels are controlled by proteolysis.*

INTRACELLULAR CONTROL OF CELL-CYCLE EVENTS

S-Phase Cyclin–Cdk Complexes (S-Cdks) Initiate DNA Replication Once Per Cycle

- Passage through M-phase is required for a cell to regain the ability to go through S phase.
- Steps in DNA replication **Fig. 17-22**
 - The **ORC (origin recognition complex)** binds to replication origins on the DNA.
 - They remain bound throughout the cell cycle.
 - Unlike in bacteria.
 - **Cdc6** binds ORC early in G₁.
 - Cdc6 prevent premature firing of the origin.
 - **Mcm** proteins then bind the DNA to form a pre-replicative complex (pre-RC).
 - This Mcm complexes are helicases, that unwind the DNA in preparation for DNA replication.
 - S-Cdks (S-phase cyclins + Cdk) triggers DNA replication
 - Cdc6 is phosphorylated, dissociates from the pre-RC, and is degraded.
 - ORC is phosphorylated
 - S-Cdk activity remains high after DNA replication to prevent re-assembly of the pre-RC
 - After mitosis, Cdk activity is reduced to zero, allowing replication to begin anew.

The Activation of M-Phase Cyclin–Cdk Complexes (M-Cdks) Triggers Entry into Mitosis

- At the end of S-phase, the DNA is duplicated.
 - So a diploid (2n) now has 4n amount of DNA
 - The duplicated chromosomes are still attached to each other at their **centromere** (not to be confused with centrosome or centriole – cytoskeleton stuff)
- We're now in G₂.
- During G₂ the M-Cdk complex accumulates, but it is kept inactive via the **Wee1 kinase** that puts an inhibitory phosphate on Cdk.
- DNA replication signals are sent to the **Cdc25 phosphatase**, keeping it inactive until replication is complete.
- Entry into mitosis is triggered by a large cooperative transition involving the following positive feedback loop: **Fig. 17-23**
 - Inhibition of the Wee1 kinase
 - Activation/phosphorylation of the Cdc25 phosphatase.

Entry into Mitosis Is Blocked by Incomplete DNA Replication: The DNA Replication Checkpoint

- DNA replication checkpoint ensures no entry into mitosis until every nucleotide is replicated.
- Some unknown molecular sensor that emanates from unreplicated DNA shuts down M-Cdk (via inactivation of cdk25) until replication is complete.
- Any guesses as to what this could be?

M-Cdk Prepares the Duplicated Chromosomes for Separation

- M-Cdk, via phosphorylation, does the following:
 - Induce assembly of the mitotic spindle.
 - What is the mitotic spindle made of?
 - Chromosome condensation
 - Phosphorylation of the condensin complex.
 - Nuclear envelope breakdown
 - Via phosphorylation and dissociation of nuclear lamins (intermediate filaments).
 - Actin cytoskeleton rearrangement.
 - Reorganization of the ER and golgi.

Sister Chromatid Separation Is Triggered by Proteolysis

- During DNA replication (in what phase?) a protein called **cohesin** is laid down along the sister chromosomes (or chromatids as their called before separation).
 - Cohesin is related to but is different from condensin
 - One holds sister chromatids together
 - The other packages chromosomes in preparation for mitosis.
- Cohesin keeps the sister chromatid aligned.
- When the replicated chromosome is attached to the mitotic spindle the sister chromatids are pulled in opposite direction.
- But cohesin prevents this.
- APC (anaphase promoting complex) comes along and promotes cohesin destruction. **Fig. 17-26**
 - Cdc20 activates APC
 - APC ubiquitinates a protein called **securin**.
 - Securin inhibits a protease called **separase**.
 - Separase proteolyzes cohesin.
- Sister chromatids are then free to be pulled apart by the mitotic spindle.

M-Cdk → ? → Cdc20 → APC **-|** securin **-|** separase **-|** cohesin **-|** sister chromatid separation

Unattached Chromosomes Block Sister-Chromatid Separation: The Spindle-Attachment Checkpoint

- The sensor monitors whether the kinetichore is attached to spindle.
- The **kinetichore** is a specialized region of the chromosome attaching the centromere to the microtubule spindle.
- A negative signal is sent out unless the kinetichore is properly attached.

Exit from Mitosis Requires the Inactivation of M-Cdk

- Cdc20/APC complex → proteolysis of M-Cdk → loss of Cdc20/APC.
- Negative feedback loop in late mitosis.

The G₁ Phase Is a State of Stable Cdk Inactivity

- M-Cdk → Hct1 \downarrow APC
- Cdk inhibitor are also active
- M cyclin genes are turned down
- Accumulation of G₁ cyclins sets Cdk on down another cell cycle path.

The Rb Protein Acts as a Brake in Mammalian G₁ Cells

- G₁ is where cells generally hang out when not dividing.
- Therefore, in cancer cells something is screwed up in G₁.
- Transcriptional activator **E2F** turns on genes involved in S phase including S-Cdk. **Fig. 17-30**
- The **Retinoblastoma protein (Rb)** binds to and inhibits E2F, thus preventing exit from G₁.
- G₁-Cdk phosphorylates Rb, causing it to dissociate from E2F, leading to progression into S.
- Loss of Rb causes cancer in the eye.

Cell-Cycle Progression Is Somehow Coordinated With Cell Growth

Cell-Cycle Progression is Blocked by DNA Damage and p53: DNA Damage Checkpoints

- Prior to S-phase, if there is DNA damage you don't want to start replicating your chromosomes.
 - Replication machinery might stall at damaged DNA.
 - Want to wait till damage is repaired
- DNA damage causes activation of **p53**, a transcriptional activator of DNA damage response genes. **Fig. 17-33**
- One of the genes expressed by p53 is p21.
- p21 binds G₁/S-Cdk and S-Cdk and inhibits them.
- p53 is normally down-regulated by proteolysis by **Mdm2** ubiquitin ligase.
- **In almost half of all cancers, p53 is mutated!**
 - Without p53, cells accumulate DNA damage and continue on through DNA replication, causing the accumulation of stably inherited mutations.
 - Cells that accumulate mutations also accumulate mutations in checkpoint control genes, leading to their inactivation and ultimately to cancer.

DNA damage → protein kinases → phospho-p53 → p21 **-|** G₁/S-Cdk and S-Cdk
 Normal state of the cell → Mdm2 **-|** p53

Summary

- Know **Table 17-2**
- Review **Fig. 17-34**
- Cyclins-Cdks control events throughout the cell cycle.

PROGRAMMED CELL DEATH (APOPTOSIS)

- Cells die in your body all the time.
- Billions every hour.
- What's more, is that these cells commit suicide.
- They initiate a genetic program to destroy their cellular constituents.
- Why?
 - Cells that are no longer needed for development are destroyed. Fig. 17-36
 - To maintain a constant size, cell proliferation must be counter balanced with cell death (apoptosis).

Apoptosis Is Mediated by an Intracellular Proteolytic Cascade

- Apoptosis is not the same thing as cell necrosis due to trauma. Fig. 17-37
 - Busted open cells cause an inflammation response.
- In apoptosis, cells neatly package things up.
 - Chromosomes are cut up
 - Nuclear envelope breaks down via destruction of nuclear lamins.
 - A 'white flag' signal is placed on the cell surface, so that the 'undertaker' (phagocytic cells) can take them away.
- **Caspases** are the proteases responsible for carrying out the apoptotic pathway.
 - They act in a cascade of protease activation which serves to amplify and re-enforce their fateful decision.

Procaspases Are Activated by Binding to Adaptor Proteins

- Cells have death receptors on their cell surface. Fig. 17-39
 - **Fas** is a **death receptor**.
 - Binding of the Fas ligand (presented by lymphocytes) leads to death.
 - Other cells might present both the ligand and receptor, leading to suicide.
- Activation of the death receptors leads to caspase activation.
- There are also other pathways that lead to caspase activation.

Bcl-2 Family Proteins and IAP Proteins Are the Main Intracellular Regulators of the Cell Death Program

Summary

- Programmed cell death is a normal developmental fate of a cell.
- Apoptosis is mediated in part by cell surface death receptors or by intracellular signaling events.
- Caspase proteases are responsible for executing cell death.

EXTRACELLULAR CONTROL OF CELL DIVISION, CELL GROWTH, AND APOPTOSIS

Mitogens Stimulate Cell Division

Cells Can Delay Division by Entering a Specialized Nondividing State
Mitogens Stimulate G1-Cdk and G1/S-Cdk Activities

Abnormal Proliferation Signals Cause Cell-Cycle Arrest or Cell Death

Human Cells Have a Built-in Limitation on the Number of Times They Can Divide

Extracellular Growth Factors Stimulate Cell Growth

Extracellular Survival Factors Suppress Apoptosis

Neighboring Cells Compete for Extracellular Signal Proteins

Many Types of Normal Animal Cells Need Anchorage to Grow and Proliferate

Some Extracellular Signal Proteins Inhibit Cell Growth, Cell Division, and Survival

Intricately Regulated Patterns of Cell Division Generate and Maintain Body Form

Summary