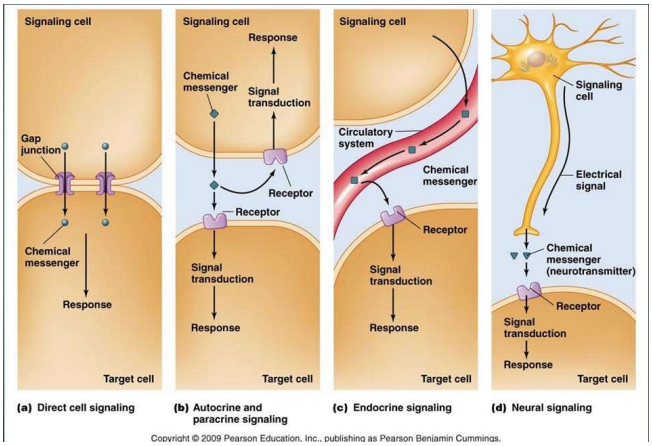
BIO1140 Midterm 2 Notes
Mitochondria: Apoptosis/Cell Communication
Why do cells die?

- The size of an organ or organism depends on the Total Cell Mass; the cell number must be controlled
    - Cells can grow → Total cell number…
    - Cell can divide or die → Total cell number…
        - Important to regulate
        - More useful to have one huge cell or many small cells? → SA vs. V
        - How do we tell cells whether they should divide or die? → cell communication



Cell Communication Contributes to Control

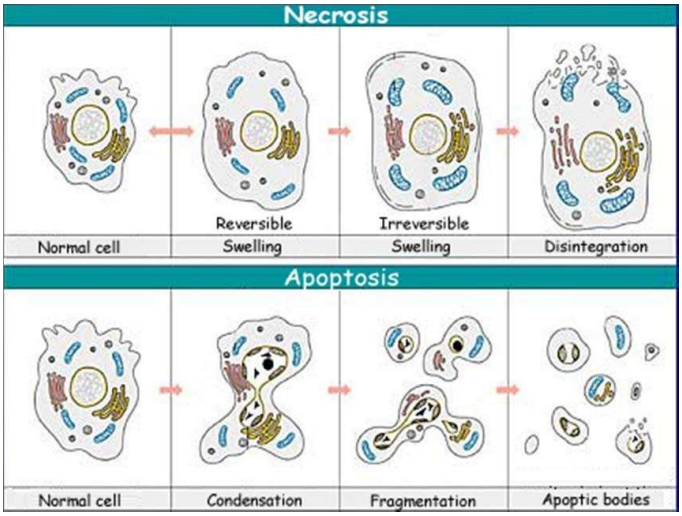
- Direct signalling (gap junctions)
- Sending one molecule from one cell to another
- Neuronal signalling
- *What's common to all these paths of communication?*
    - Chemical messenger – cell must use a molecule of some sort and send it to next cell; receiving cell must receive chemical messenger, interpret it & act on it

What are the triggers of cell death?

- **Chemical messengers**
    - Growth factors
    - Mitogens (stimulate cell division)
    - Survival factors
        - If there is no messenger telling a cell to grow or divide it may remain stagnant or be a trigger to die
- **Toxicity**
- **Damage (to cell or to DNA)**
- **Cell cycle checkpoints**
    - If one of the steps is not carried out properly, might be a trigger to tell the cell to die (**i.e.** cancer)

All 'reasons' for cell death lead to changes in the cell, which lead to its demise. The mechanisms 'how' are different.

Cell death: Necrosis vs. Apoptosis



What is the most striking difference between the two?

Necrosis: the cell swells so much that it literally explodes; the membrane disintegrates. Membrane's permeability to water and ions has changed – lots of stuff in and out of cell – cell is no longer able to cope, cell lyses. Neighbouring cells receive collateral damage.

Apoptosis: breaking apart of the cell into smaller, non-functional compartments. Not dangerous to neighbouring cells, "apoptotic bodies" are always encompassed by membrane – no lysosomes or enzymes (degrading material), going to neighbouring cells; theses bodies will be phagocytized.

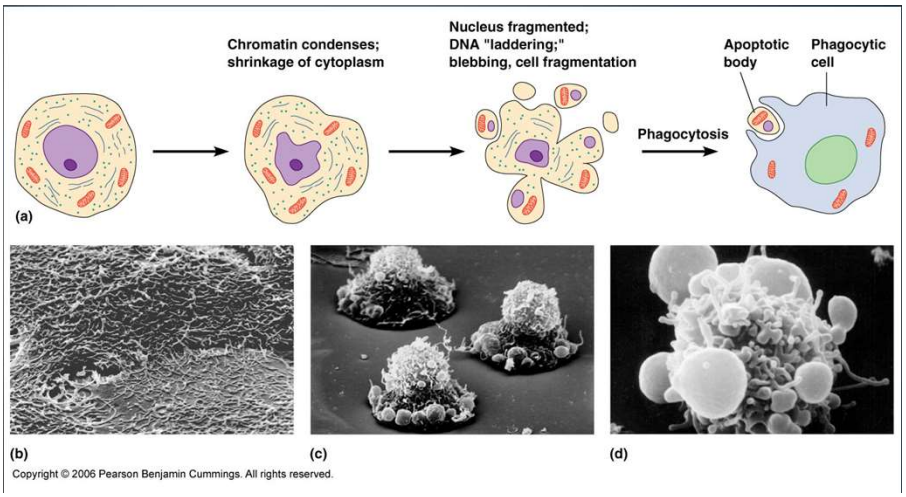
NECROSIS

- Damage
- Ischemia, excitotoxicity
- Ca^{2+} surplus
- Loss of ATPase activity, loss of electrochemical gradient, decreased ATP
- Membrane swells, inflammation, diffusion
- Loss of membrane integrity
- Cell death, lysis and consequences to neighbours

APOPTOSIS

- Stress signal (intrinsic or extrinsic)
- Increase in Ca^{2+} (cell and mitochondria)
- Pro-apoptotic protein: mitochondria
- Opening of PTP (Permeability transition pore)
- Cytochrome c release (changes to cristae)
- Activation of apoptosomes
- Signalling cascade (Caspases)
- *Programmed cell death* (autophagy)

Structural Changes During Apoptosis



Cell decides it needs to die, message is interpreted by the cell and initiates in a cascade of events. 2 basic pathways: exterior or interior signal.

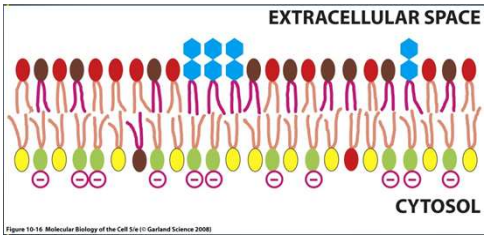
1. Condense chromatin in nucleus
2. Cytoplasm starts shrinking; nuclear envelope disintegrates → nucleus = fragmented
3. Long pieces of DNA cut into smaller portions → DNA laddering, specific enzymes are activated and it cuts DNA in smaller chunks → blebbing (forms blebs, apoptotic bodies) (NO nuclear envelope)
4. Smaller portions are engulfed by membrane (there is loss of adhesion, anchoring junctions not working)
5. Apoptotic bodies engulfed by macrophages and sent for degradation

How do neighbouring cells detect apoptotic bodies for phagocytosis?

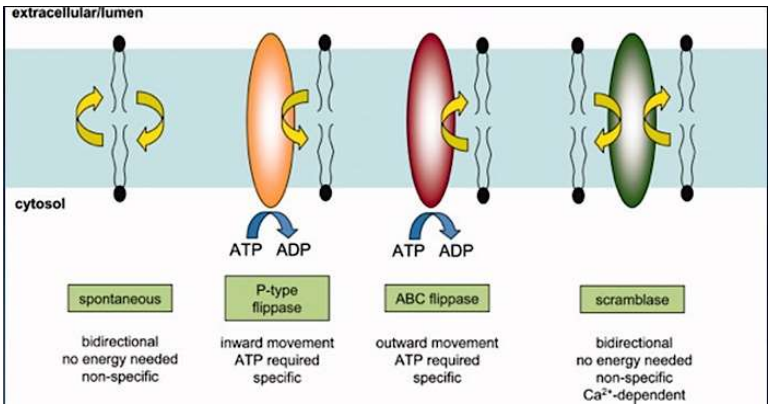
Their PM presents more phosphatidylserine (on the outside) **it is negatively charged (normal cell would want negative charge on the inside)

Phagocytosis

- Asymmetric distribution of plasma membrane is lost (recall: the flip flop in PM – requires energy)
- Negatively charges phosphatidylserine then becomes exposed on the OUTSIDE of cell – membrane is no longer asymmetric → this "marks" the cell
- The cell is then marked for phagocytosis by a macrophage



What makes this arrangement possible?

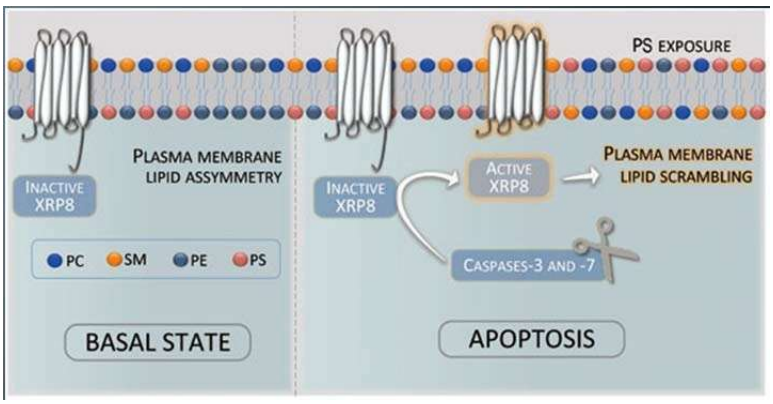


Scramblase: requires activation: Ca^{2+} and caspase dependent (protein enzymes); large integral trans membrane protein present in the membrane but inactive until activity of Ca^{2+} & caspase; once activated can translocate phospholipids in both directions, it will favour shipping phosphatidyleserine on the OUTER leaflet

Spontaneous: occurs rarely; there are 3 diff. types of proteins that allow to translocate phosphatidyleserine from the inner to the outer leaflet

P-type flippase: outside to inside leaflet – active transport; translocate to the inner leaflet (not used for apoptosis but used for membrane asymmetry)

ABC Flippase: Flip phosphatidyleserine *outwards* – active transport that uses ABC binding casset



Apoptosis – Signaling Pathways

2 Main apoptic signaling pathways:

- Intrinsic pathway**
- Extrinsic pathway

THE INTRINSIC PATHWAY OF APOPTOSIS

- Something disrupts the cell so that it is internally stressed; internally it triggers a cascade of events that leads to the programmed cell death → apoptosis
- **Possible triggers:** loss binding of survival factor (no longer a message to keep surviving to maintain healthy cells), DNA damage (missing cell cycle check point, decide to not divide) – *internal* stressor
- Leads to dephosphorylation and activation of Bad (*pro-apoptic*)
    - When a mechanisms triggers the pathway BAD will be activated
- Bad inhibits Bcl2 (*anti-apoptic*)

THERE IS A BALANCE IN THE CELL – put with a trigger leading to the pro survival protein becoming inactive and the pro apoptotic protein being activated you have "let go of the leash, and opened the door"

- Bad also activated Bax and Bak (changes to calcium regulation in ER and mitochondria)
    - These proteins regulate Ca inside the cell
- Caspase cascade

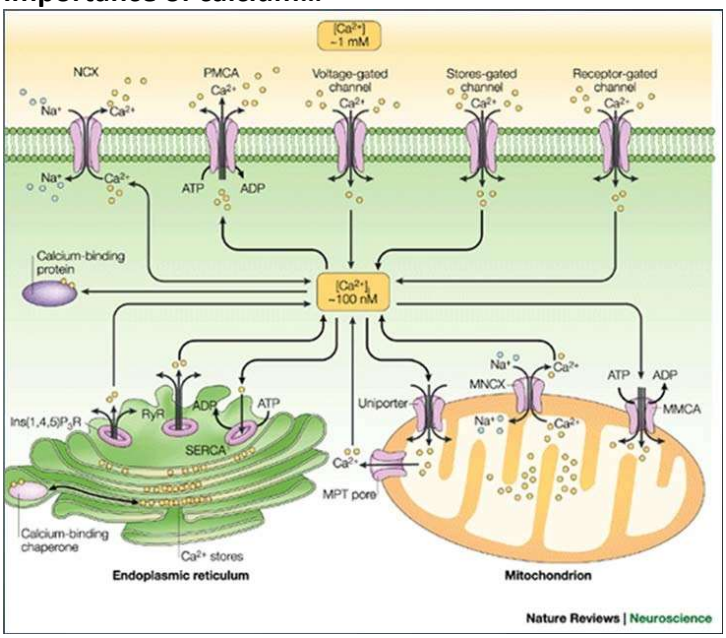
Caspases

- Caspases are a family of proteases (enzymes that cleave (chew up) protein)
- They are split in 2 groups: initiator (CASP2, 8, 9, 10) → first ones to become activated and go to wake up the executioner caspases; they put the breaks on various protein kinases → cell adhesion mechanisms are becoming inefficient, cell starts to detach and favour blebbing, also puts the break on lamins (keep nuclear membrane in tact)…
- And Executioner (CASP3, 6, 7) Caspases
- Caspases activate scramblase***

Caspases

Protein kinases	Lamins	Cytoskeleton	DNAse
Disrupt cell adhesion	Disassembly of nuclear envelope	Inhibit and deactivate elements of the cytoplasm. Change cell shape and size (change to apoptotic bodies)	DNA fragmentation

Importance of calcium…



- Where do we hide/store Ca? Mitochondria, Ca binding proteins and ER
- What happens if they become overfilled with Ca? Mitochondria become so filled with Ca (become toxic), will react and punch a hole in the membrane (has a double membrane), between the membrane there are important elements/proteins
    - Cristae will rearrange & punch a hole in outer membrane (permeability transition pore – PTP), allows protein cytochrome C to exit the mitochondria and trigger the caspase cascade
    - Controlling the Ca so that the mitochondria remain functional
    - Apoptosis is an ACTIVE PROCESS

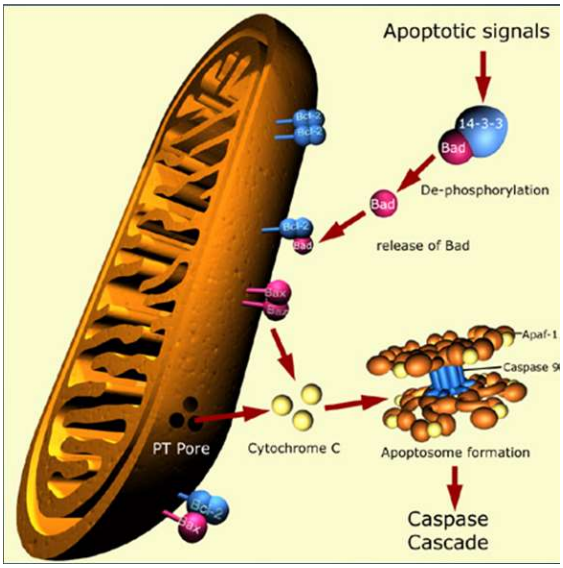
- Mitochondria and ER interact via Ca^{2+}
- During stress, ER releases CA^{2+} via IP3-dependant channel (Inosotoltriphosphate)
    - BCL2 prevents the ER from releasing Ca – prevents IP3 from binding to the IP3 ligand-gated Ca channel
    - When its inactivated by BAD – activated BAX and BAK → facilitates binding of the IP3 ligand-gated channel
        - Ca being released into cytoplasm from ER
- Mitochondria absorb Ca^{2+} to protect cell → facilitates reaching the transition (PTP) for mitochondria to punch the whole

Ca is essential for cell survival and for apoptosis.

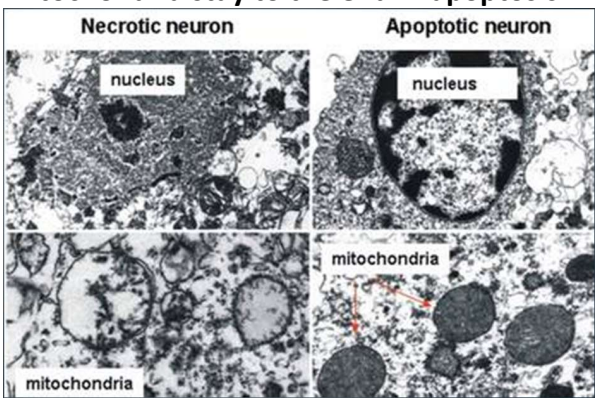
- Excess Ca^{2+} in mitochondria leads to PTP
- Bcl2 can inhibit IP3 (stops release of Ca^{2+}) while BAX/BAK activate IP3 (promotes Ca^{2+} release)

Mitochondria in Apoptosis

1. NO, toxins, hormones, cytokines, etc.
2. (BAD is activated) Inhibition of BCL2 and activation of BAX and BAK
3. Increase in Ca^{2+}, opening of PTP (permeability transition pore), release of CytoC (forms a large structure = apoptosome; binds with APAF1 and caspase 9)
4. Apoptosomes are formed → allows to activate initiator caspase, which initiates the executioner caspase which leads to the whole sequence…
5. Nuclear condensation DNA fragmentation, cytoplasmic shrinkage, apoptic bodies, phagocytosis



Mitochondria stay to the end in apoptosis



Necrosis and Apoptosis

- Apoptosis does not occur only in response to negative stressors → it can also be an *essential* part of development
    - Could be a good way to reduce the amount of cells
    - Apoptosis is favourable because necrosis is damaging to neighbouring cells

Start with tadpole

Hormones (Thyroxine)

↓

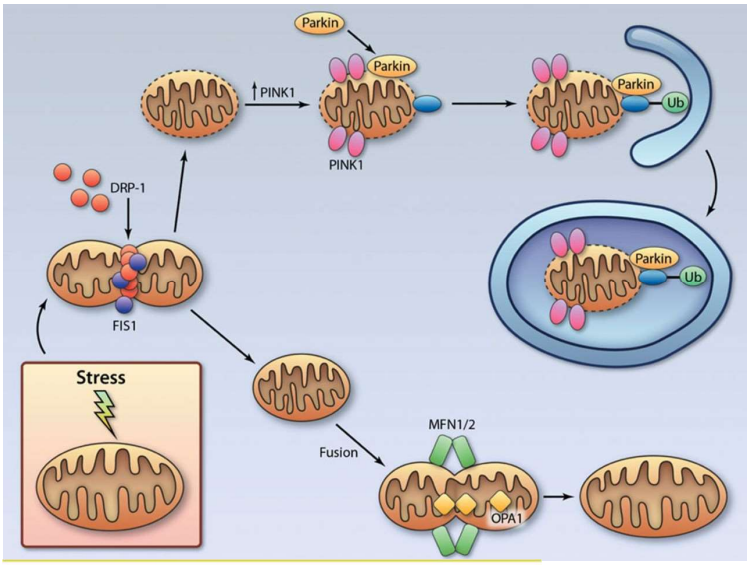
Apoptosis

↓

Tadpole loses tail in favour of legs as a frog

Mitophagy

- The number of mitochondria can vary throughout the life cycle of a cell
- Controlled regulation of the number of mitochondria according to the metabolic requirements
    - Can reduce
    - Or can get rid of damaged portion of mitochondria
        - Need to express some extra membrane proteins to allow a signalling cascade in side the cell to initiate “mitochondria apoptosis”
- Process that involves recruiting various signalling protein and lysosomes
- The process how mitochondria are ‘chosen’ remains unclear
- Important for aging, development, and certain pathologies (AD, Parkinson’s, etc.)



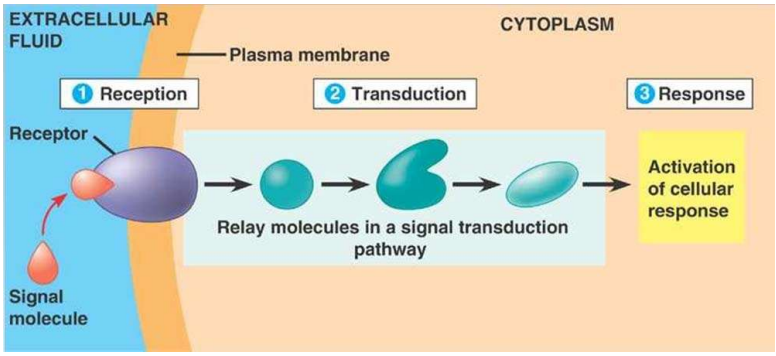
1) PINK is a kinase; it recruits Parkin
2) Parkin is an E3 ubiquitin-ligase; it promotes ubiquitination of membrane protein, signalling autophagocytosis (signalling that organelle to go towards lysosome for degradation)
Something will damage mitochondria or name it as useless – too many in cell

What happens when the mitochondrial membrane starts to degrade? Everything in the membrane will be released and lead to the cells demise

Cell Signaling (pt. 1) **readings: ch. 5.7

Cell Communication

- Conserved mechanisms (unicellular and multicellular organisms)
- Essential during: **everything the cell does involves cell signaling → coordination
    - Development
    - Hormonal regulation
    - Muscle contraction
    - Immunity and recognition of self
    - Cancer
    - Apoptosis, etc.



3 STEPS

1. **Reception**: there has to me a message (initial stage) – choose chemical messenger & means to send
    a. Message must be received → depends on receptors (what type, how many?)
    b. The binding of a signal molecule with a specific receptor
2. **Transduction**: how the cell interprets the message, how will the cell relay & interpret the message?
    a. Series of chemical & biochemical rxns

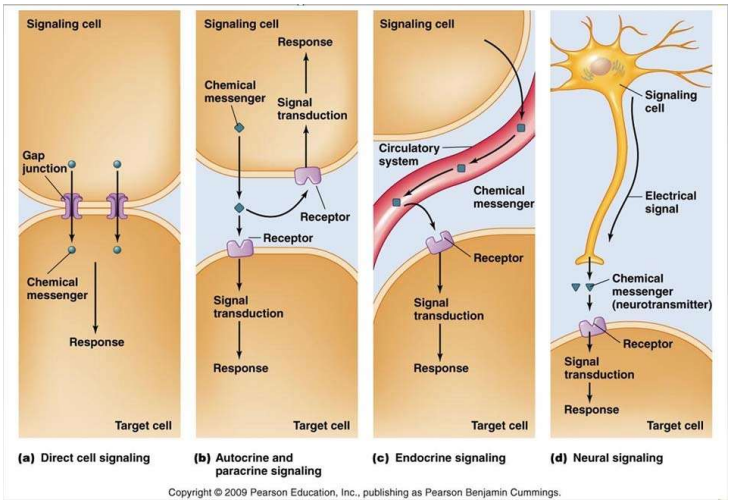
   b. Signal reception triggers other changes within the cell to cause a cellular response
3. **Response:** RESULT, does the response match the message, was the goal achieved?
   a. Cellular response

A conversation…

- Change in cell or environment (stimuli, loss of homeostasis, lack/need, etc.)
- The cell sends a message
  - 6 classes of chemical messengers
    - Messages sorted by chemical make-up (**i.e.** cholesterol & steroids can be used to send message)
    - Proteins
    - What type of molecule is the messenger?
- Message reaches target cell
- Message is received
- *Signal transduction* (response to signal)

Cell Communication Paths

Main communication paths – be able to differentiate & explain*



- **Autocrine vs. paracrine (indirect):**
  - **Autocrine:** produces by signaling cells that can also bind to the ligand that is released
    - Cell secretes a hormone/chemical messenger (autocrine agent) that binds to autocrine receptors on that same cell, leading to changes in the cell
  - **Paracrine:** occurs between local cells, quick responses that last a short time
    - The target cell is near the signal-releasing cell
- **Endocrine signaling (indirect):** using blood to send message
- **Exocrine (indirect):** secretion of a substance out through a duct (salivary gland, sweat glands, etc.**)**

Which communication path is the fastest?

Gap junctions (direct)

Neuronal (travel great distances in short time) → electrical transfer (indirect)

Messengers – 6 Classes

- Their structure will determine:
  - Their chemical properties (hydrophobic vs. hydrophilic)
  - The communication path taken to reach the target cell
  - Their mode of action/interaction with the cell and receptors
- 6 Classes:
  - Steroids
  - Eicosanoids (lipids)
  - Peptides/proteins
  - Purines
  - Amines
  - Gases

Steroids

- Derived from cholesterol
- Lipophilic – cannot be stored in vesicles (Endocrine path)
    - Will diffuse OR be bound to transport protein (**i.e.** albumin)

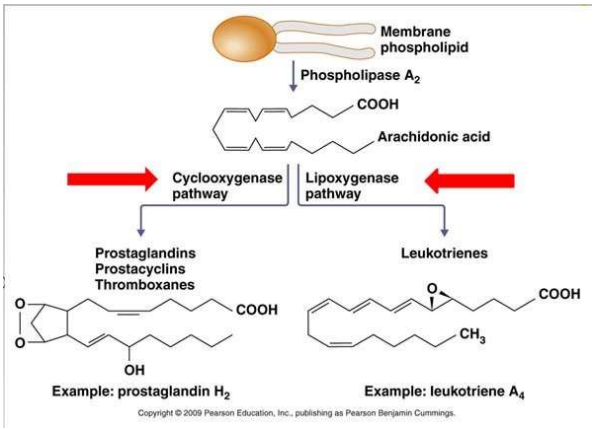
Why do they need a carrier? What type of carrier will they use?

→ Lipophilic – will diffuse across the membrane; need to use a transport protein to get it from point A to point B in the body

→ Needs to be a globular, soluble protein

→ Use endocrine pathway (bloodstream) to bring to target

- Membrane permeable – can reach intracellular receptors
- Act as gene transcription factors
    - Can diffuse into nucleus; receptors are inside the cell → act directly on DNA
- 3 Classes of Steroids:
    - Mineralocortocoids (Aldosterone) – act on minerals
    - Glucocorticoids (Cortisol) – act on sugar mechanisms; cortisol = stress hormone
    - Sex hormones (Testosterone/Estrogen)



Eicosanoids (lipids) – The Local Hormones

- Most are derived from Arachidonic Acid (lipid) → produced from membrane phospholipids
- Lipophilic
- Paracrine path
- 2 Main pathways lead to 2 more classes: depend on *enzymes*
    - **Pain**: Prostaglandins (*cyclooxygenase*)
    - **Inflammation**: Leukotrienes (*lipoxygenase*)
- Important molecules for managing cell response

Main communication paths?

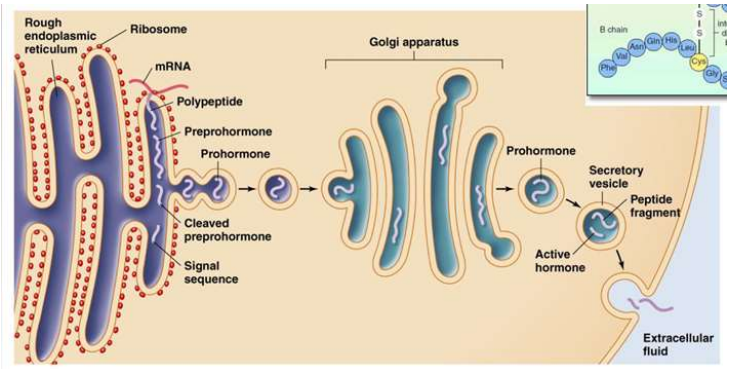
→ Too big for gap junctions

→ Response must be quick & local

→ Paracine & autocrine

Peptides/Protein

- Few to many amino acids (more than ~50 = protein)
- Hydrophilic
    - Able to store in vesicles → can produce a lot and store it, have a reserve
- In vesicles
- Secreted by exocytosis
- Most indirect paths: endocrine, neuronal, NO exocrine, autocrine/paracrine



*Enzymes are packaged with inactive proteins in vesicles. Enzymes can turn into an active protein and then secrete by exocytosis (**i.e.** insulin)*

Amines

- Have an amine NH_2 group
- Usually biosynthesized from an amino acid
- Most are hydrophilic (reserve pool in vesicles)
- Many neurotransmitters are amines
    - Neurons use a lot (neuronal pathway)

- **Examples:** Epinephrine, histamine, GABA
- Thyroid hormones (they are HYDROPHOBIC)
    - Need a carrier protein and CANNOT be packaged → produced on demand

Purines

- Derived from nitrogenous bases adenine or guanine
- NEED a transporter or use exocytosis
    - Can be packaged in vesicles
    - Can use transporters (secreted faster) → facilitated diffusion
- Mainly use paracrine and neuronal pathways
- **Examples:** caffeine, chocolate

Gases

- Small molecules, short half-life
- Passively diffuse
    - Can be used as messengers
    - Very quick to act on neighbouring cells
- Direct, and paracrine, neuronal, endocrine pathways
- **Examples:** NO, O_2, CO, etc.

Vascular homeostasis: NO diffuses from RBCs, triggers relaxation of smooth muscle cells lining blood vessels (vasodilation)

What is the advantage for the cell to have these different types of ligands?

→ Communication with different types of receptors

→ Communication with different types of cells

→ FLEXIBILITY

→ Cell is very efficient, multiplies uses for a series of messengers

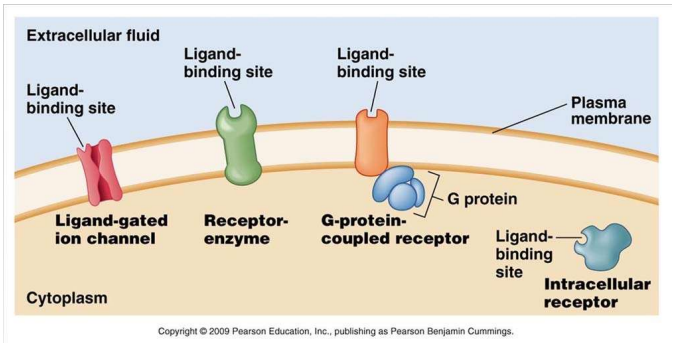
Receiving the Message

- The interaction with the receptor is the first level of discrimination (sets the path to the cell's response)
- Receptors are proteins; they can be on cell surface or inside cell
- Ligand (messenger) binding will induce a change in conformation of the receptor protein
- This acts as the trigger for the 2nd phase – transduction

Receptors – 4 Classes

TRANSMEMBRANE RECEPTORS – ligand NEVER enters the cell, change in conformation when ligand binds

- Span the membrane
- Receptor on OUTSIDE of cell to bind chemical messengers
- Lots of specificity
    - Influences how tightly ligand is bound (affinity), how message is transmitted/how long it stays & how long message will last



- **Ligand-gated ion channel:** only open or close once they bind a chemical messenger
    - Many types
- **Receptor-enzyme:** integral proteins, when messenger fits in ligand-binding domain it provides receptor with enzymatic activity in the INTRACELLULAR side
    - 3 main classes
- **G-protein coupled receptor:** large integral proteins that span membrane 7 times (just receptor portion), when ligand binds it recruits a G-protein from intracellular side – allows interaction with receptor and triggers a series of events inside the cell
    - GPCRs, many types

- Ligands are mostly hydrophilic (proteins, amines)
- Ligand binding induces a change in conformation that relays the message inside the cell – 2nd messengers

INTRACELLULAR RECEPTORS – change in conformation when ligand binds

- **Intracellular receptor:** found in cytoplasm or in nucleus (steroids use)
    - Ligand must enter the cell

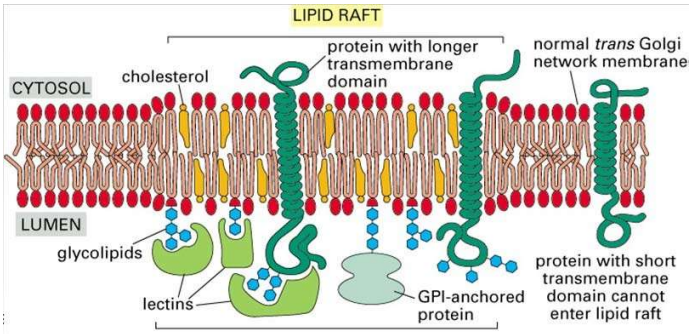
  Ligand binding domains is the area on the receptors that bind the chemical messenger.
- Lipophilic or very small ligands will diffuse and reach these receptors
- **The ligand-receptor complex acts as a transcription factor**
    - In the nucleus, bind to specific areas of DNA (the gene promoter sequence)
    - Activate or inhibit gene transcription

Lipid Rafts

- *Sphingolipids* and *cholesterol* form highly-ordered *microdomains* or *rafts*
- SL hydrocarbon tails are LONGER and SATURATED (**i.e.** NOT kinked)
    - Longer tails = thicker membrane
- Rafts are produced in the ER and sent to the PM
- Rafts can accommodate proteins with long transmembrane domains
- Organize and cluster proteins to function together (**i.e.** for receptor-mediated signaling)

Why more cholesterol?

→ Increases fluidity of membrane
→ Maintain constant degree of fluidity
→ Does NOT affect membrane's permeability
→ Accommodate larger sphingolipids



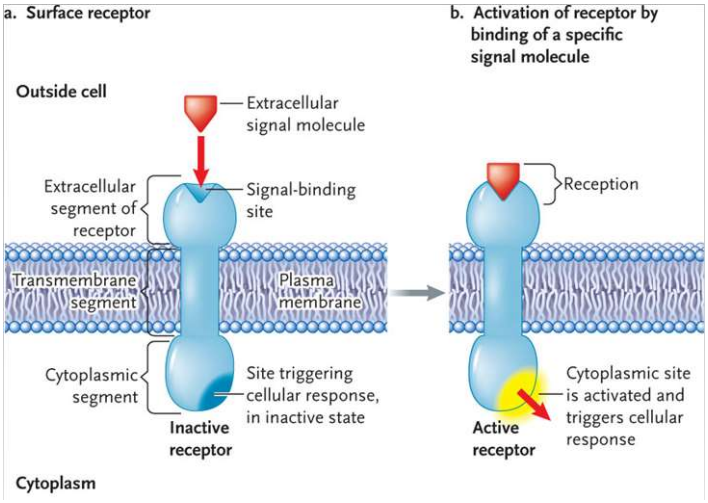
Cell Signaling (pt. 2) **Readings: Ch. 5.7

How can one cell alter the gene expression of another cell?

By sending a chemical messenger that will trigger a signal transduction cascade → chemical messenger can leave one cell and make it to another cell

- There is an area in the receptors that can bind ligands – initiates a change in receptor

- Receptor activation leads to relaying AND amplifying the signal inside the cell (intracellular)
    - When the receptor becomes active by binding the ligand it must relay the message inside the cell → 2nd messengers come in to interact with receptor and relay the message in the cell
    - Amplification comes from an active receptor being able to interact with more than one 2nd messenger as long as the ligand remains bound to the receptor (receptor remains *active*)
    - Amplification is dependent on the time that the ligand is bound to the receptor



    - As long as you have 2nd messenger available you can continue to amplify the message – it is also a rate limiting step
        - ***What can act as a second messenger?*** Kinases, GTP, Ca
- Maximum cellular response with minimal ligand

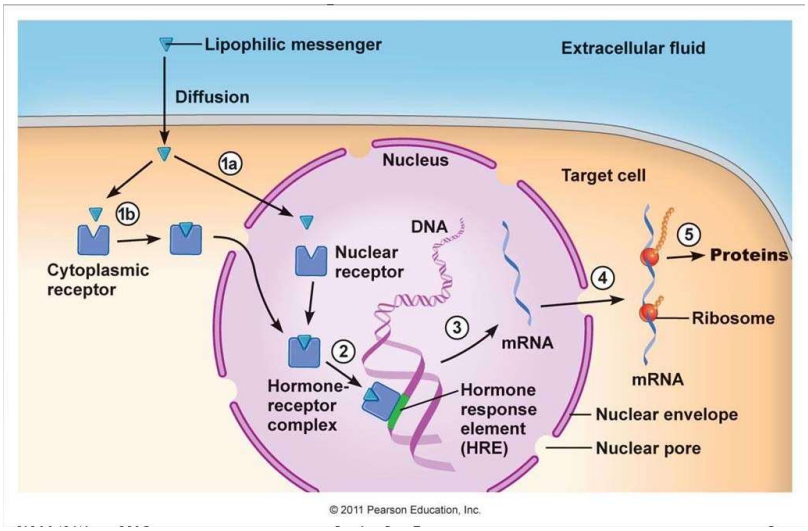
Receptors – 4 Classes (recall)

TRANSMEMBRANE RECEPTORS

- **Ligand-gated ion channel**
- **Receptor-enzyme**
- **G-protein-coupled receptor**

INTRACELLULAR RECEPTORS

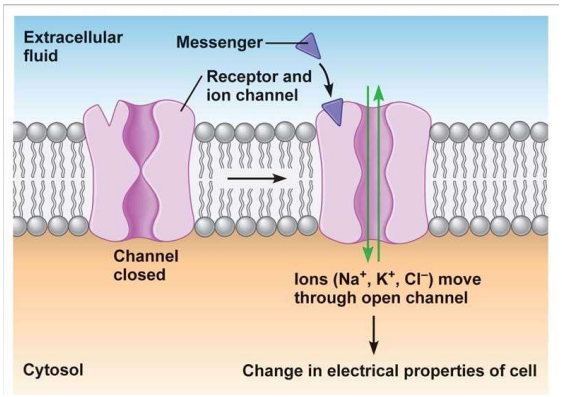
- **Intracellular receptor**
    - Need a messenger → can get into the cell by diffusion across the membrane (lipophilic – can freely pass through the membrane, **i.e.** steroids)
    - Can reach an intracellular receptor in cytoplasm or one already present in the nucleus



    - **Reach receptor in cytoplasm:** bind to, change conformation, complex will move to nucleus and get inside the nucleus
    - **Reach receptor in nucleus:** binds, changes conformation and forms a complex
        - Both outcomes form a complex in the nucleus that has gained activity/properties that act a gene transcription factor → looks for genes
        - How does it choose which gene to interact with? Looks for the promoter (switch to turn genes on or off), within promoter there are response elements (specific sequence) → transcription factors bind here, once promoter is activated it turns the gene on or off
        - This helps to increase or to reduce the amount of protein that the cell produces

Glucocorticoids

- Cortisol (stress hormone) is secreted by adrenal glands; reduces insulin synthesis (cells will stop producing insulin until the relationship with the complex formed – gene transcription factor – and the receptor element is broken)
- Sequence of amino acids to Functional domains

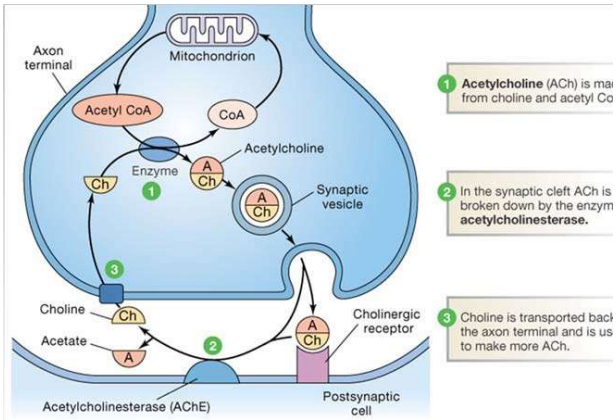


TM receptors 1: Ligand-gated ion channels

- Messenger = ligand, will bind to a specific area of the transmembrane protein
    - When the ligand binds to a specific subunit of the receptor, it creates an opening in the membrane or it will close this channel (3D structure of subunits has been rearranged) – ions can now pass (in or out)
- Very important for nervous system – used by many NTs
- Some channels are specific to certain ions, others are simply open passage
    - What happens when ions are let through? The electrochemical gradient will change
        - Influence membrane potential → can influence permeability,

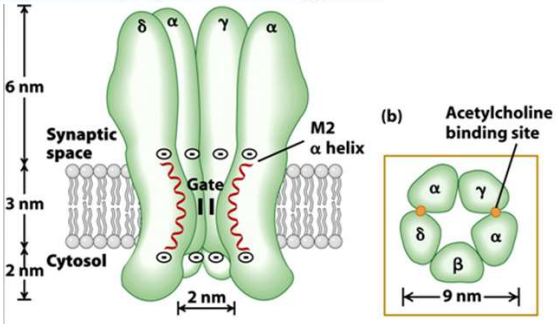
Back to Mitochondria

- Acetylcholine (Ach) is a very important neurotransmitter
- *Excitatory* or *inhibitory* depending on tissue
- Can cause 2 types of receptors (AChR): ligand-gated ion channels (nicotinic receptors) or GPCRs (muscarinic receptors)



Acetylcholine: Ligand-gated ion channels

- Ion-channel receptor is a large unit formed of multiple subunits, Ach must bind to two alpha subunits forming a channel
- When binding sites are filled, conformation changes, pore opens and lets ions through; mostly Na^+ and Ca^+ in to cell, some K^+ out (not enough to compensate for the amount of + charge coming into the cell)
    - Muscle contractions, Ach allows Ca into muscle cell → binds troponin to reveal myosin binding site
    - How does the muscle cell relax? Stop entry of Ca & Na = no membrane potential, get rid of all Ca, tropomyosin slides back on myosin binding site on actin
- Changes the electrochemical gradient = membrane potential

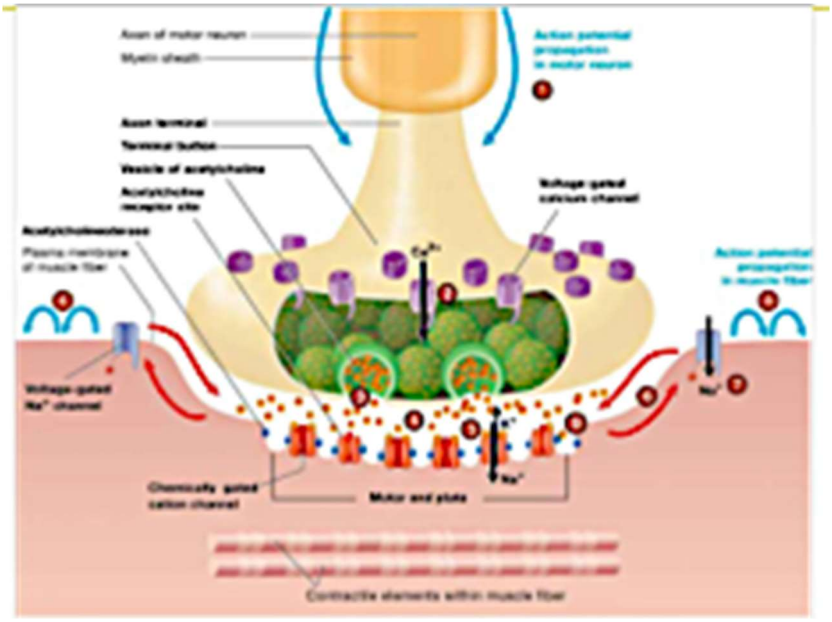


How is the message ACh brings interpreted by the muscle cell?

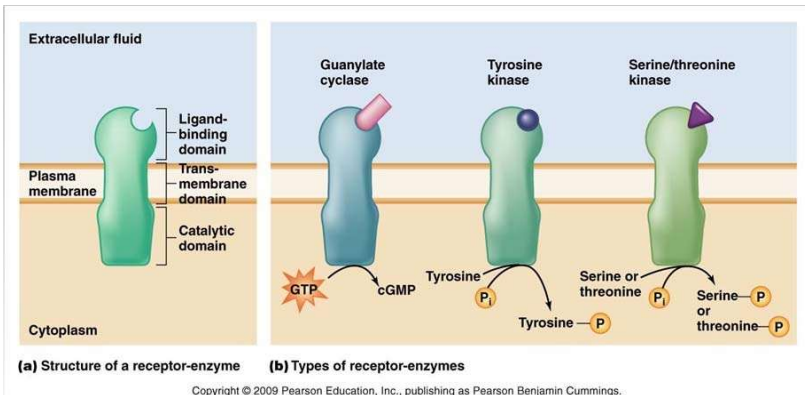
Change in membrane potential in the muscle cell, membrane allows entry of more ions, which triggers and amplifies the cellular response

ACh is similar to amines, signaling elements lead to a cascade of events

1. An action potential in a motor neuron is propagated to the terminal button
2. The presence of an action potential in the terminal button triggers the opening of voltage-gated Ca^{2+} channels and the subsequent entry of Ca^{2+} into the terminal button
3. Ca^{2+} triggers the release of acetylcholine by exocytosis from a portion of the vesicles
4. Acetylcholine diffuses across the space separating the nerve and muscle cells and binds with receptor sites specific for it on the motor end plate of the muscle cell membrane
5. This binding brings about the opening of cation channels, leading to a relatively large movement of Na^+ into the muscle cell compared to a smaller movement of K^+ outward



6. The result is an end-plate potential. Local current flow occurs between the depolarized end plate and adjacent membrane
7. This local current flows opens voltage-gated Na^{2+} entry reduces the potential to threshold, initiating an action potential, which is propagated throughout the muscle fiber
8. Acetylcholine is subsequently destroyed by acetylchloinesterase, an enzyme located on the motor end-plate membrane, terminating the muscle cell's response



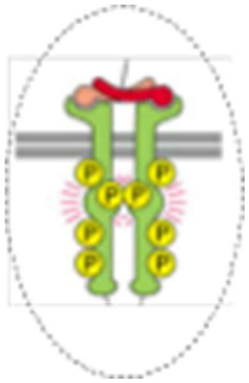
TM receptors 2: Receptor Enzymes

- Intracellular catalytic domain acts as an enzyme
- Initiates a cascade of phosphorylation-dephosphorylation that amplifies the ligand's impact on target cell
- 3 main classes – we focus on Tyrosine Kinase receptors

Tyrosine Kinase Receptors (Tyr-K; RTK)

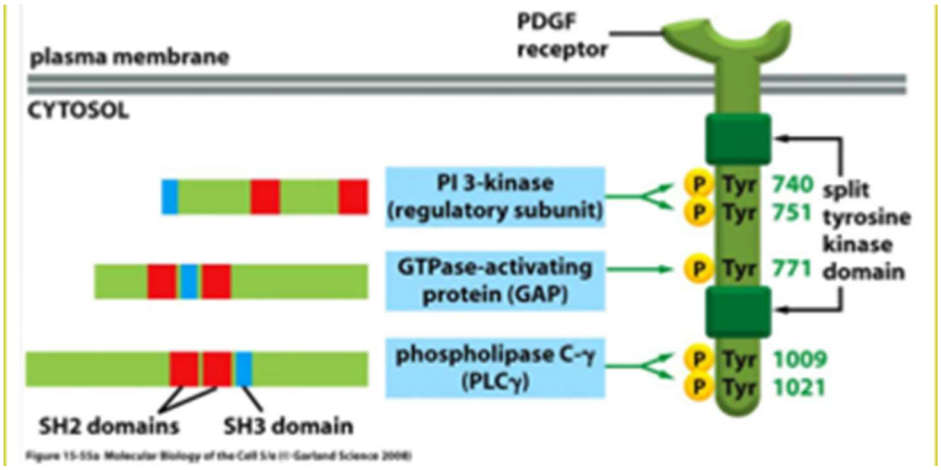
- Largest family of enzyme receptors (over 20 types)
- Involved in pathways linked to survival, growth, proliferation, and metabolism
  - Cascades that lead to cells growing, dividing & thriving
- Activation requires dimerization and autophosphorylation
  - Must form dimers → 2 receptors bind their ligand and bind together (dimerization)
  - Autophosphorylates its catalytic domain
  - Can now recruit protein enzymes that act as the 2nd messenger
- Signaling is initiated via an area called SH2 domain (Src *homo*logy)

- **Main second messengers**: Ras (*Rat* sarcoma protein) and phospholipids
- Ligand examples:
  - Insulin
  - Growth factors (NGF, EGF, VEGF, PDGF)

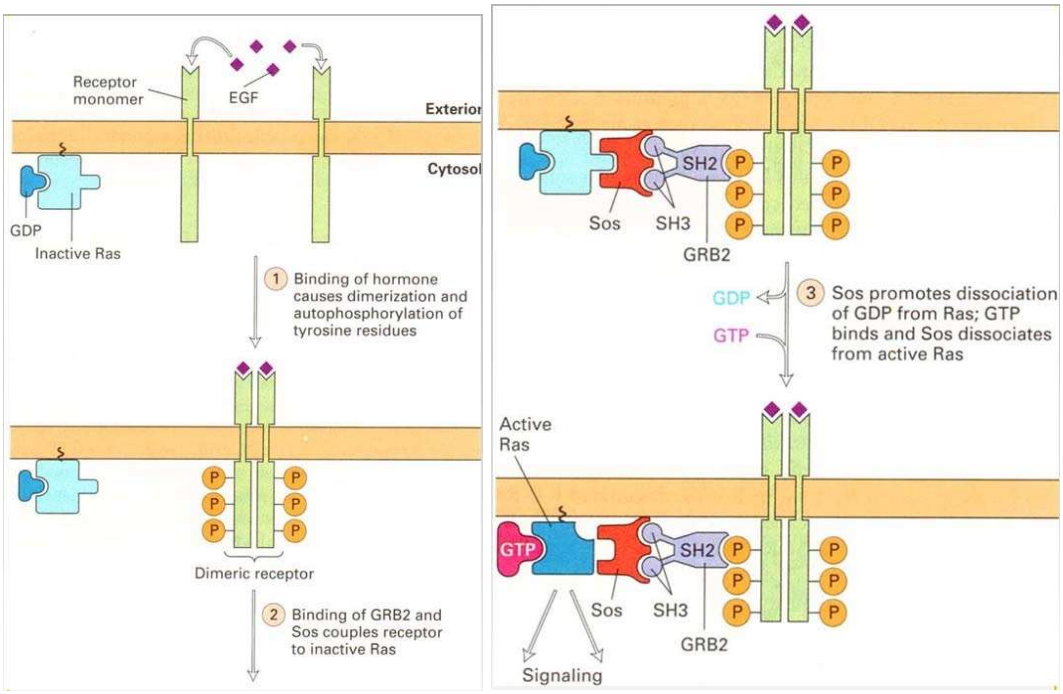


The Case of NGF

- NGF = nerve growth factor
- Rita Levi-Montalcini won the Nobel Prize in 1986
  - Used embryos in tumor models
  - Principles discovered applied to healthy tissue aswell
  - NGF at the root of any nerve cells growing
  - Need to bind to 2 monomers, must dimerize, autophosphorylate…etc.
- Mouse sarcoma tissue released "neurotrophins"



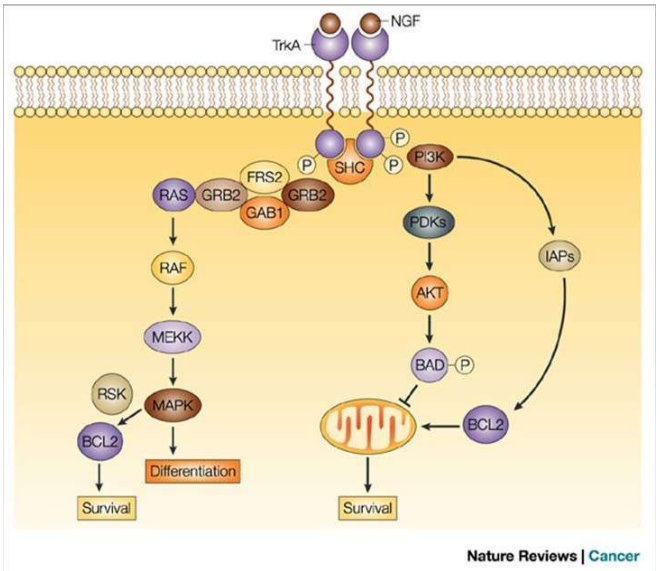
- SH2 and SH3 (SH = Sarc homology) domains are conserved regions that bind to the P-Tyr
- SH2 and SH3 match/fit in phosphorylated area of the catalytic receptor
- Dimers can phosphorylate the catalytic domain 6-10 tyrosines
  - Depending on which tyrosine is phosphorylated, it's going to orient which 2nd messenger you will recruit to interact with activated receptor so that you can initiate the cascade inside the cell
- Protein that you're recruiting must have proper shape/conformation to fit with phosphorylated dimer
- Proteins have the ability to interact with phosphorylated tyrosine → SH2 domain
- SH3 domain allows them to interact with the next step in the cascade



<u>GRB2:</u>
SH2 domain – Trk
SH3 domain – Sas

- Receptors in membrane are inactive (monomers)
- A growth factor will bind to receptor on each monomer, once GF binds → induces a change in conformation which allows the 2 monomers to come closer in the membrane and form a dimer
- Can now autophosphorylate → gives receptor the ability to interact with proteins on the intracellular side of the membrane
- Interacts with Ras → needs an adaptor protein to interact with the receptor; NEED an SH2 domain to interact with receptor and a portion to interact with Ras = GRB2 and Sos
    - **Sos can activate Ras**
    - Ras is a small protein that has GTPase activity → can hydrolyze G-protein (when inactive it is bound to GDP), when Sos interacts with it this induces a change in conformation to EXHANGE the GDP for a GTP → now activated and changes shape (can no longer remain bound to Sos)
    - Ras is now active and loaded with GTP to relay the message in the cell
        - Ras relays the message inside the cell → carries it to the various targets inside the cell

Growth Factor NGF



The Ras (MAPK) Pathway

IAP = inhibitors of apoptosis

- Bind monomers of receptors to form dimer, autophosphorylate and recruit adapter proteins → two GRB2 and Ras
- Ras is active and interacts with other proteins inside the cell
    - MAPK (MAP Kinase)**
        - Activates transcription factors
        - Also makes sure that BCL2 remains active and happy (inhibits apoptosis)

**PI3 Kinase doesn't need an adapter protein because it has an SH2 domain that interacts with phosphorylated tyrosine

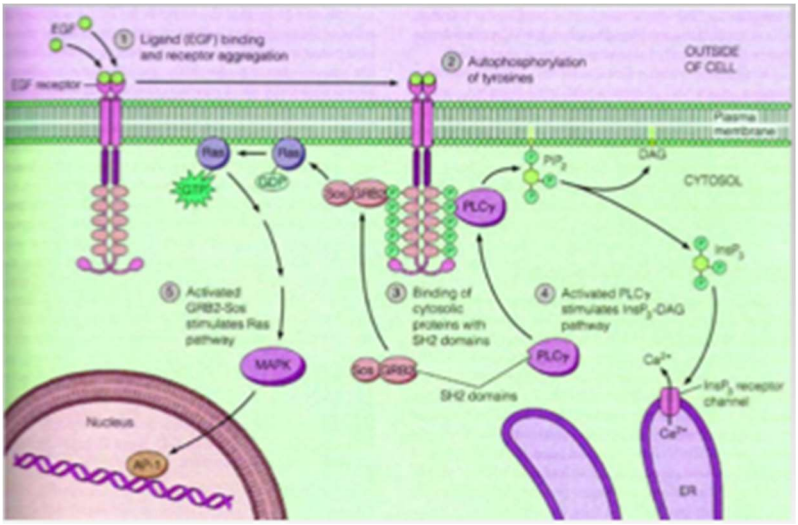
- Keeps BAD phosphorylated and inactive (activates apoptosis)
- Promoting survival → activating proteins that are inhibitors of apoptosis

3-6 phosphorylation steps
30% of cancers arise in one

DAG = diacylglycerol
PIP2 = Phosphatidyl inositol bi-phosphate
IP3 = inositol tri-phosphate

- Phospholypase C (PLC_γ) works with anchored protein, PIP2 → gets cleaved to DAG (still part of the membrane) and IP3
    - IP3 is cleaved off of PIP2
    - Binds ligand-gated Ca^{2+} channel on ER (lets Ca IN)
    - Ca^{2+} can act as 2nd messenger (can activate kinases, channels, proteins, etc.)
- Growth factor can activate a cascade of events leading to the cell thriving and growing, keeping apoptosis in check

Why does DAG remain in the membrane after cleavage from PIP2? Because of hydrophobic interactions in the layer (it is a fatty acd)



TM Receptors 3: GPCRs

- 2012 Chemistry Nobel for their work on structure and function of GPCRs (researched adrenaline)
    - Brian Kobilka – Crystallography structure of receptors
    - Robert Lefkowitz MD – biochemist signalization adrenergic receptors

X-Ray Crystallography

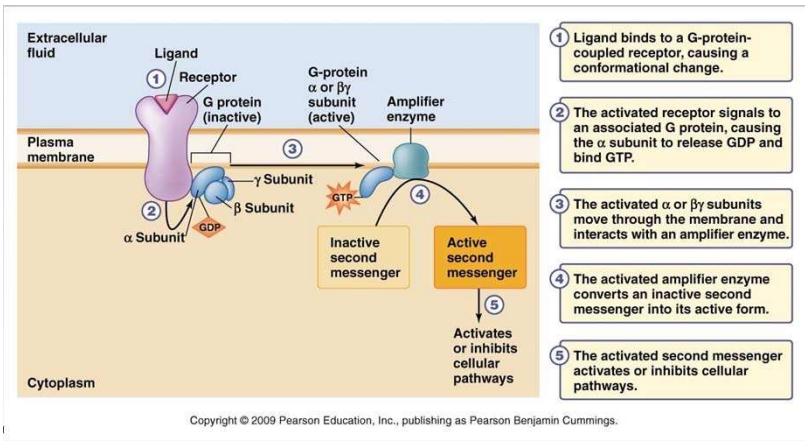
- Structure – function
- Macromolecules – arrangement
- Atomic resolution
- X-rays (0.1 nm ~ diam. H atom)
- Crystallized proteins
- Bombardment and diffraction
- **I.e.** interference patterns

Problems:

- Time-consuming (first structure → 22 yrs)
- Large amount of material required
- Insoluble protein crystallization (**i.e.** membranes)

GPCRs:

- 6 classes according to their sequence and their role/function
- Can be activators or inhibitors or cellular response
- Receptor portion is 7 transmembrane domains / Ligand binding recruits G-proteins on the intracellular side that will activate second messengers
- Intracellular loops interact with G-protein
    - **G-protein:** 3 protein subunits attached together = inactive
        - When ligand binds to G-protein receptor it changes the conformation and gives it the ability to recruit and interact with G-protein
        - Alpha subunit can EXCHANGE GDP for GTP → dissociates from beta and gamma subunits
        - Can no go one and act on another component → relays the message
        - Will go to activate 1 of 2 of the pathways



- In both cases an amplifier enzyme must be activated (responsible for producing 2nd messenger in large quantities as long as GTP remains bound)
- Multiple opportunities for amplification
- Amplifier enzyme active = 2nd messenger active = cascade of events

- Extracellular interact with ligand

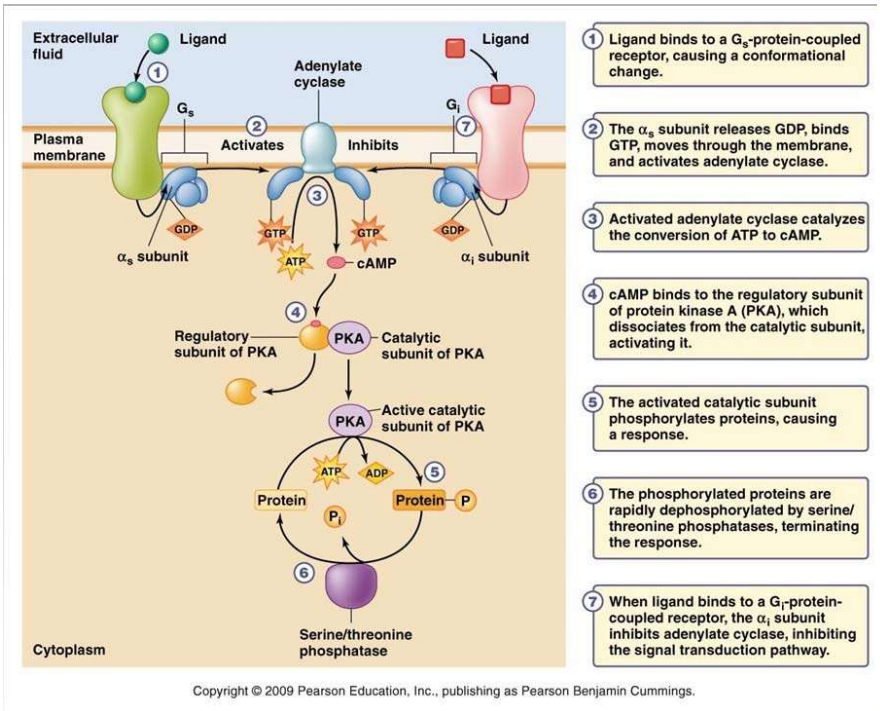
G-protein Coupled Receptors

- Largest family of receptors (metabotropic)
- Multiple ligands (can interact with many signals) – some bind to many different GPCRs
  - Ach can bind 5 different types of GPCRs
  - Adrenalin (4 main receptors)
- Mediate responses to a diverse range of ligands (**i.e.** neurotransmitters, hormones, odorants, tastants, and photons of light)
- Diversity of receptors for given ligand translates to multiple responses
- They interact with GTP-activating proteins (G-proteins)
- 2 Main pathways:
  - cAMP
  - PIP (IP3)

Second Messengers

cAMP Pathway

- TARGET: adenylate cyclase
- Gs are activators; Gi are inhibitor
- The [cAMP] determines PKA activity
- PKA can also translocate to the nucleus and activate genes via the CRE (cAMP response element)
  - cAMP is required to activate PKA
- **Ligand binding domains outside the cell
- Inside – recruit G-proteins when ligand binds to receptor
  - 3 protein subunits bound together
- Alpha subunit it a molecular switch
  - Inactive when bound to GDP
  - Trigger to exchange GDP for GTP → active status, changes conformation and dissociates from other 2 subunits

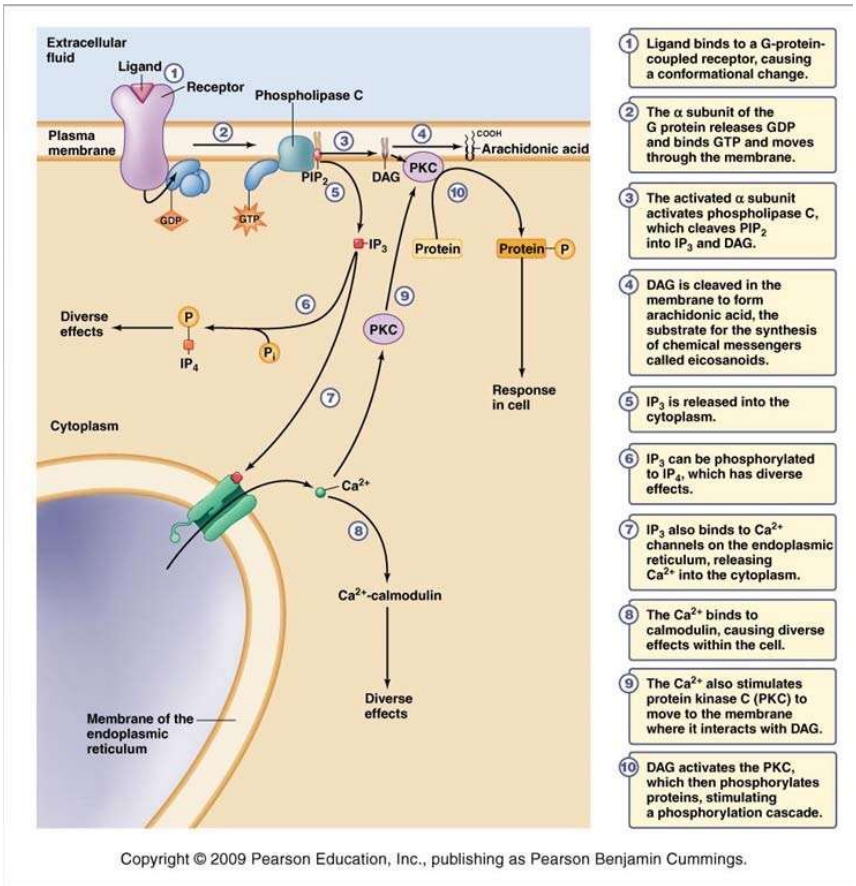


- Target = adenylate cyclase (amplifier enzyme)
  - When activated – catalytic activity is on
- 2nd messenger = cyclic AMP
  - binds to protein kinase A
    - Becomes active and phosphorylates other targets inside the cell
- G-protein receptors can also bind an inhibitor protein instead of ligand
  - Shuts down activating enzyme

- Difference = alpha subunit
- Alpha subunit geared towards inhibition

- Both proteins are present in the cell to regulate responses
- Protein kinase A can transcribe to the nucleus → acts as transcription factor and bind to specific genes that regulate cAMP

The PIP/IP3 Pathway

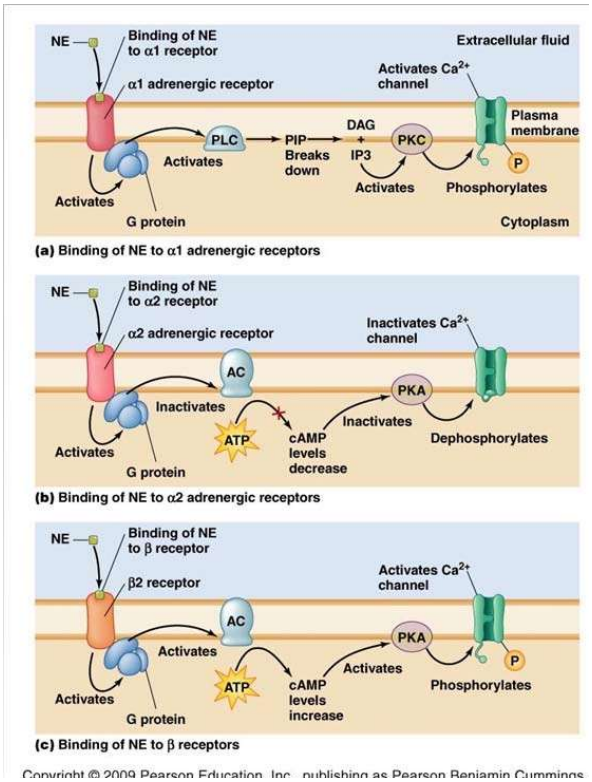


- Leads to IP3 and DAG and Ca as 2nd messengers
- Bind primary messenger or ligand to G-protein coupled receptor, recruits G-protein, alpha subunit exchanges GDP for GTP, activated alpha subunit goes on to activate amplifier enzyme (PLC – phospholipase C)
  - When it is active it cleaves (chops off polar head) PIP2 and produces DAG and IP3
  - IP3 can go on and bind to the IP3 gated calcium channel on the endoplasmic reticulum
  - Calcium released
  - Calcium can bind and activate protein kinase C
  - Protein kinase C can go on and activate different targets (some targets acting as transcription factors – indirect transcription)
  - DAG produces arachidonic acid leading to eicosanoids
- *Different pathways lead to different responses*
- *2 contradictory events in the cell?* Growth and death

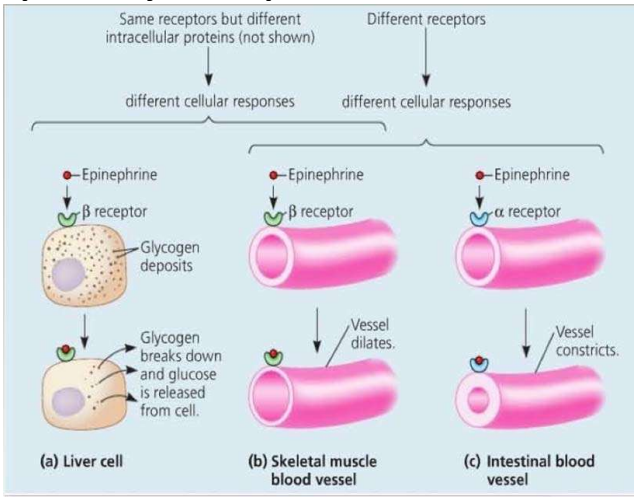
One Ligand – Many Responses

- Norepinephrine (NE) is a catecholamine (NT and hormone)
- Responsible for concentration
- Binds to adrenergic receptors (3)
- Stimulate NS
- Activate glycogenolysis and gluconeogenesis
- Pupil dilation
- Maximize blood flow to skeletal muscle and survival organs

- Same messenger can lead to entirely different responses – this is common
    - Depends on which cascade/pathway that they activate
        - What do they bind to and activate?
- Norepinephrine is able to bind to G-protein coupled receptors
    - Different subunits
    - All get activated in same mechanism but don't interact with same alpha subunit
    - Lead to same pathways in different manners
    - Binds to Alpha 1 – triggers PIP cascade
        - DAG and IP3 activate protein kinase C
        - Leads to activating Ca channel on PM
        - Calcium can rush into cell – can cause muscle contraction
    - Binds to Alpha 2 – G-protein activates inhibitory alpha subunit
        - Goes to adenylate cyclase and shuts it down
        - No protein kinase A
        - Calcium channel is not activated
        - Inhibiting calcium entry
        - Favour relaxation of muscle – important if you're trying to dilate a vessel
    - Binds to beta subunit
        - Alpha subunit still activated
        - Calcium still enters the cell using cAMP



Specificity of Responses

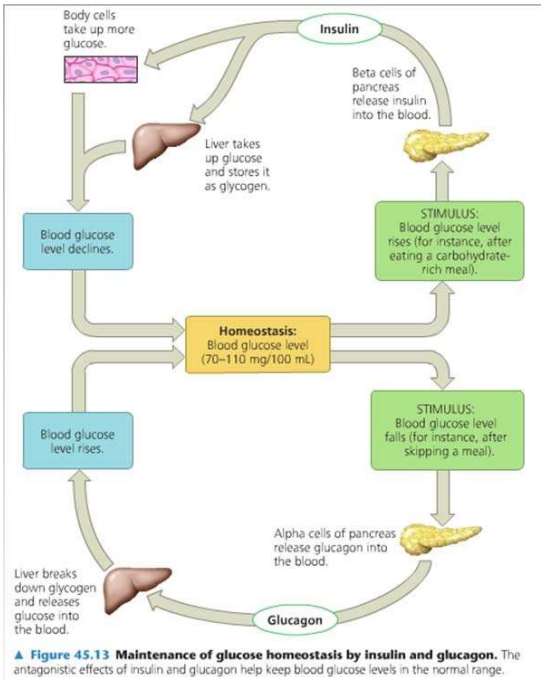


- Using different cell types – same ligand (messenger) and same receptor but completely different responses
- Activating the same cascade but causes a completely different response
- Different cell types can present different proportions of receptors → different response
- Example of how an organism can obtain a multitude of responses but keep the messengers to a minimum

Coordination of Response

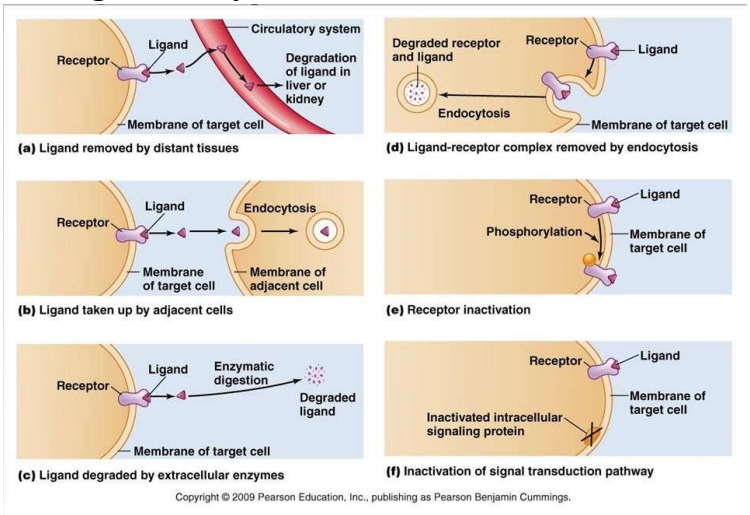
- Antagonism between insulin and glucagon on the pancreas avoids a futile cycle of increasing and dropping blood glucose concentration and maintain homeostasis

- Glucose at good level; no need for production of insulin – receptors are taken in by endocytosis
- Is pancreas is releasing insulin pancreas cannot release glucagon & vice versa
- Glucagon works using cAMP
- When two pathways oppose each other = antagonistic
- Coordination of cell activity***



▲ Figure 45.13 **Maintenance of glucose homeostasis by insulin and glucagon.** The antagonistic effects of insulin and glucagon help keep blood glucose levels in the normal range.

Ending the Response



- How do we end the conversation? Cell has be able to shut this down
- **be familiar with 6 ways – integrate with what's going on in cell
- ligand can be removed
    - reaches target by diffusion
    - can diffuse back
- ligand taken up by adjacent cells
- enzymes can come and degrade ligand – receptor is no longer activated
- ligand-receptor complex removed by endocytosis
    - lysosomes come into play
- phosphorylate receptor & dephosphorylating → turning it on and off
- inactivate one of the signalling steps in pathways
    - cascade ends regardless of if you have ligand bound to the receptor

you can manipulate and tweak these steps

LECTURE TOOLS:
Q: An amino acid derived ligand binds to a receptor. A cytosolic protein with an SH2 domain was recruited and the cascade initiated resulted in release of calcium from the ER. Which type of receptor was involved?
A: tyrosine kinase enzyme receptor

look for steps of an acetylcholine-mediated cascade in chronological order

- F B A E G C D
    - Look for actual steps**

Q: What if the GPCR had been coupled to the cAMP pathway?
A: cAMP & protein kinase would be activated

DNA Replication **Readings: Ch. 12

What is DNA?

Deoxyribonucleic Acid (DNA): The Hereditary Molecule (READ: 12.1)

- In 1911, Thomas Hunt Morgan was able to associate traits (genes) to a chromosome by studying sex-linked inheritance in *Drosophila melanogaster*
- In 1928, Frederick Griffith identified properties of strains of *Steptococcus pneumonia* related to their virulence in mammals
- In 1940, Oswold Avery determined that these properties were related to DNA (not RNA or protein)
- In 1952, Alfred Hershey was able to finally demonstrate that DNA was indeed the hereditary molecule by using phages

Solving the Mystery: DNA's structure

- In 1953, James Watson and Francis Crick determined the molecular structure of DNA
- They were awarded the Nobel Prize in 1962 (with Maurice Wilkins, but not Rosalind Franklin…)
- 50 years later, we were able to sequence entire genomes

How did they solve the structure?

- They knew DNA was made of A, T, G, C (purines and pyrimidines)
- They were trying to figure out the 3D structure and had different possibilities of arrangements
    - It has to be a helix, of ~2mm diameter
    - The only possible arrangement was A-T and G-C

How are Nucleotides linked – orientation of DNA strands

- Nitrogenous bases (A, T, G, C) are linked to a 5C sugar (deoxyribose) and a phosphate group
- Each nucleotide is attached by a phosphodiester linkage between the 3rd C of the sugar and the P group of the next nucleotide
- This gives orientation to the strand; the **first nucleotide is the 5' end**; it presents a phosphate group
    - The last nucleotide is 3' end, with an OH on the 3rd C of the sugar, ready to link the next nucleotide

ELONGATION IS ALWAYS 5' TO 3'

The Double Helix

- Both strands are in OPPOSITE DIRECTIONS
- Nitrogenous bases are facing INSIDE the helix and provide stability by hydrogen bonding
- Sugars and phosphate forming a ribbon backbone

Organizing DNA: Fitting millions (or billions) or BP (~2m) into a nucleus 6 um diameter

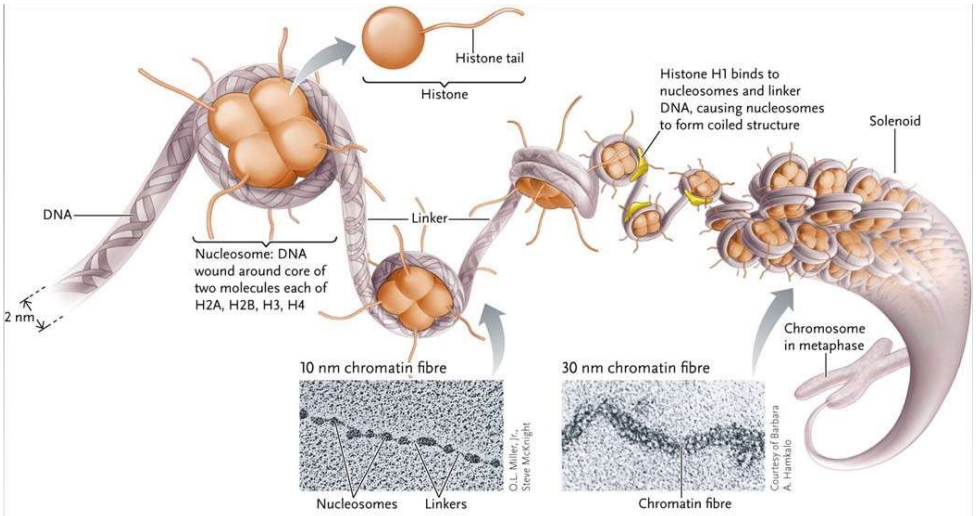
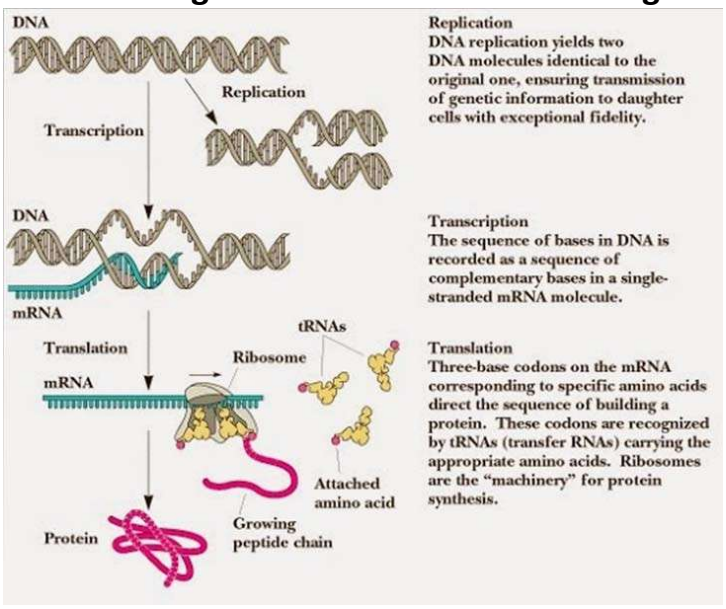


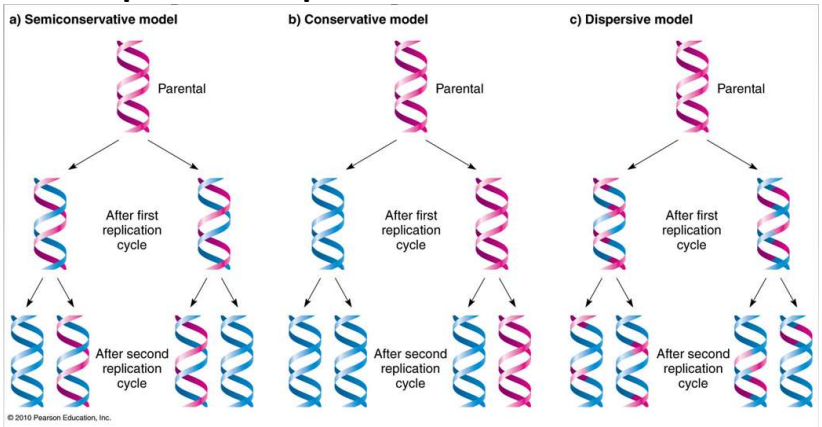
Figure 12.21
Levels of organization in eukaryotic chromatin and chromosomes.

The Flow of genetic information – The Dogma of molecular Biology



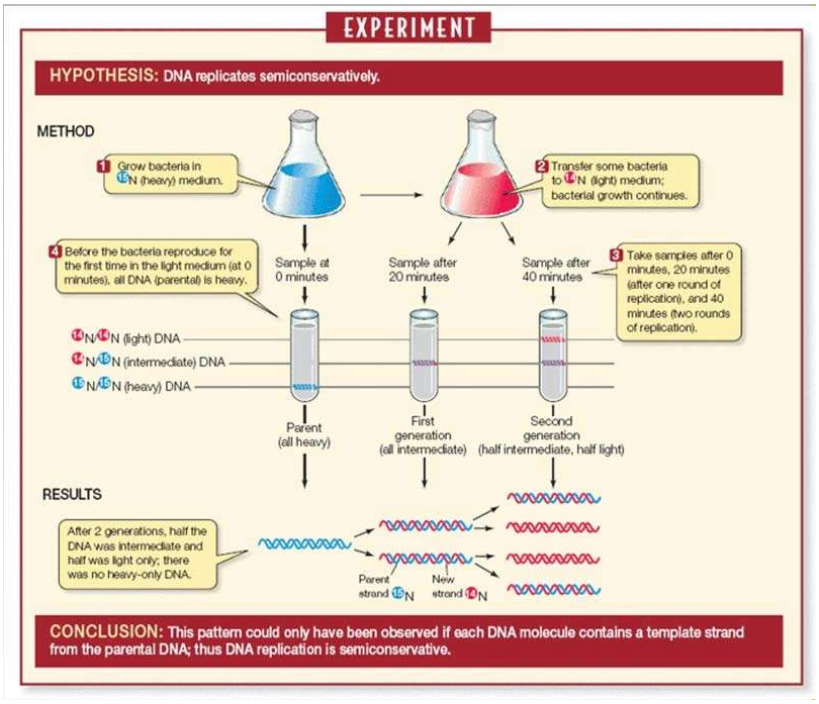
Unidirectional. Always from DNA to RNA to protein

DNA Replication: 3 possible models



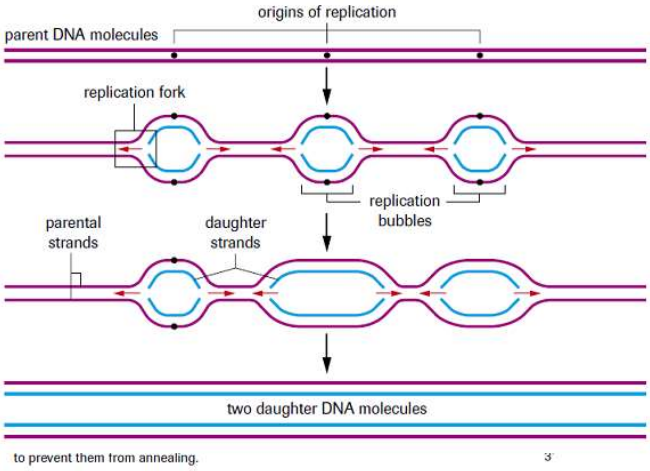
Lacking the technical tools to prove which model is followed…
5 years later…

Meselon – Stahl Experiment



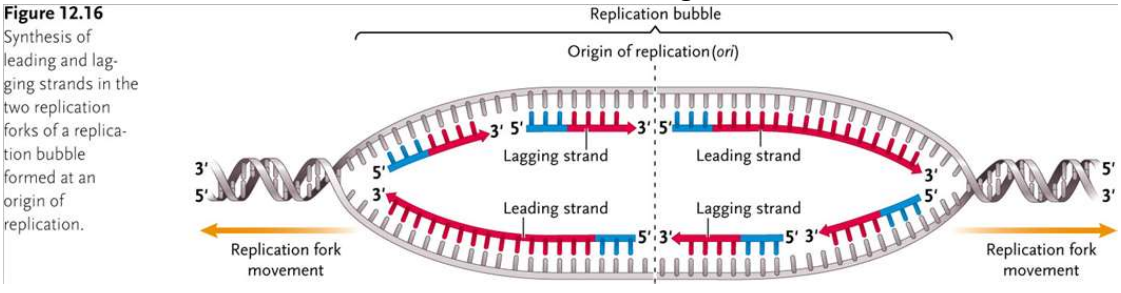
DNA Replication

- The **circular genome** of bacteria is replicated from a single point of origin (ori)
- In **eukaryotes**, there can be multiple simultaneous replication forks (autonomous replication sequences)
- **Fig. 12.22 for prokaryotic (bacterial) cells



<u>Overview of Replication Requirments:</u>

- Unwind DNA – and make sure it does not supercoil on the other side
- 2 separate anti-parallel mother strands – make 2 complimentary daughter strands (respect orientation of elongation)
- Obtain 2 identical sets of DNA, each with a mother-daughter strand, in a double helix



Replication

- Elongation occurs ONLY 5’ to 3’: simple for one strand, but what about the other strand?
- **Leading strand**: orientation of elongation of daughter strand is 5’ to 3’ so it is replicated in a continuous fashion
- **Lagging strand**: orientation of elongation of daughter strand would be 3’ to 5’ which is IMPOSSIBLE – it is therefore accomplished by sections in 5’ to 3’

Enzymes involved in replication

Table 12.1 Major Enzymes of DNA Replication

Enzyme	Activity
Helicase	Unwinds DNA helix
Single-stranded binding proteins	Stabilize single-stranded DNA and prevent the two strands at the replication fork from reforming double-stranded DNA
Topoisomerase	Avoids twisting of the DNA ahead of the replication fork (in circular DNA) by cutting the DNA, turning the DNA on one side of the break in the direction opposite to that of the twisting force, and rejoining the two strands
Primase	Assembles RNA primers in the 5′ → 3′ direction to initiate a new DNA strand
DNA polymerase III	Main replication enzyme in *E. coli;* extends the RNA primer by adding DNA nucleotides to it
DNA polymerase I	*E. coli* enzyme that uses its 5′ → 3′ exonuclease activity to remove the RNA of the previously synthesized Okazaki fragment, and uses its 5′ → 3′ polymerization activity to replace the RNA nucleotides with DNA nucleotides
Sliding clamp	Tethers DNA polymerase III to the DNA template, making replication more efficient
DNA ligase	Seals nick left between adjacent bases after RNA primers replaced with DNA

Setting the Stage: Primase

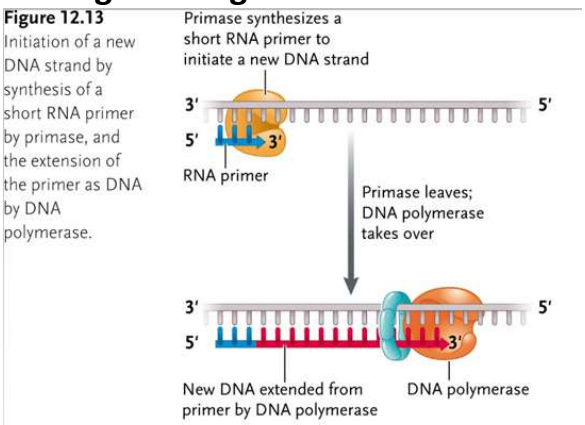


Figure 12.13
Initiation of a new DNA strand by synthesis of a short RNA primer by primase, and the extension of the primer as DNA by DNA polymerase.

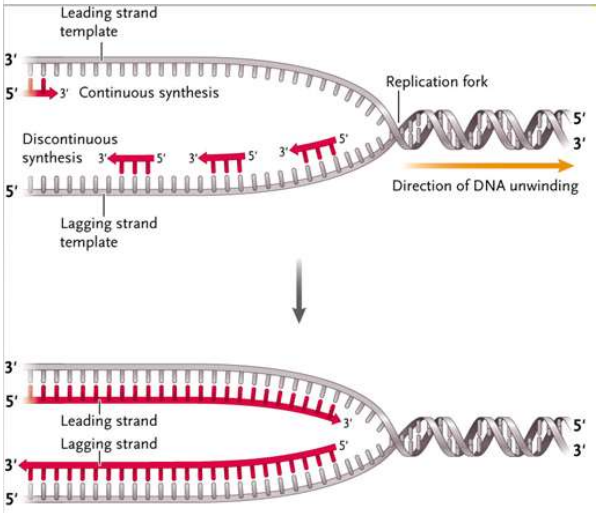
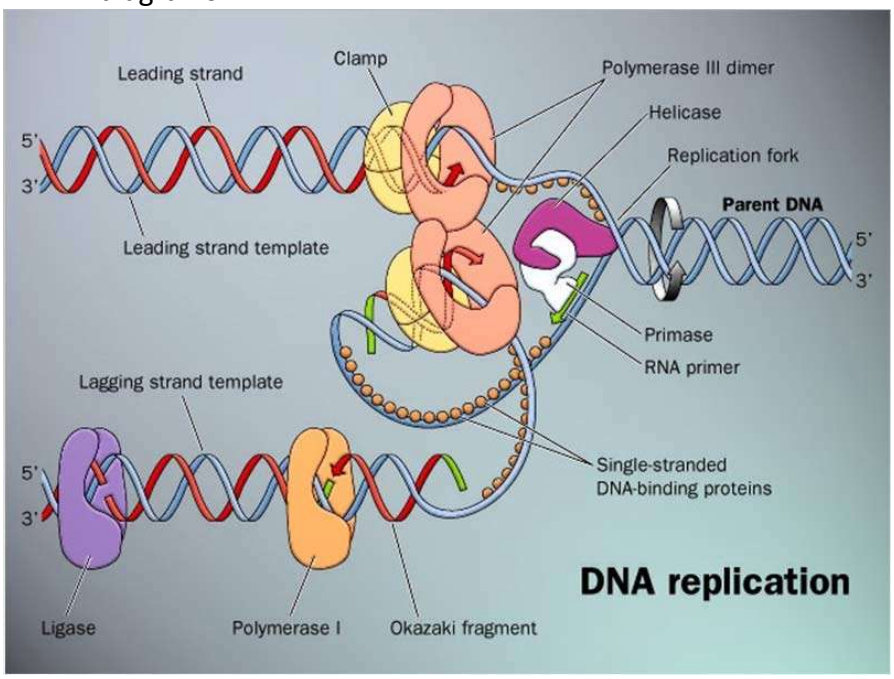
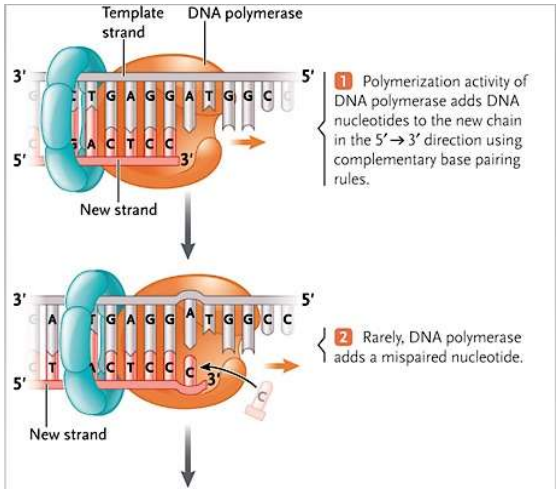


Figure 12.14
Replication of antiparallel template strands at a replication fork. Synthesis of the new DNA strand on the top template strand is continuous. Synthesis on the new DNA strand on the bottom template strand is discontinuous—short lengths of DNA are made, which are then joined into a continuous chain. The overall effect is synthesis of both strands in the direction of replication fork movement.

SLIDES 19 & 20 → diagrams



Proofreading: DNApol III can correct mistakes



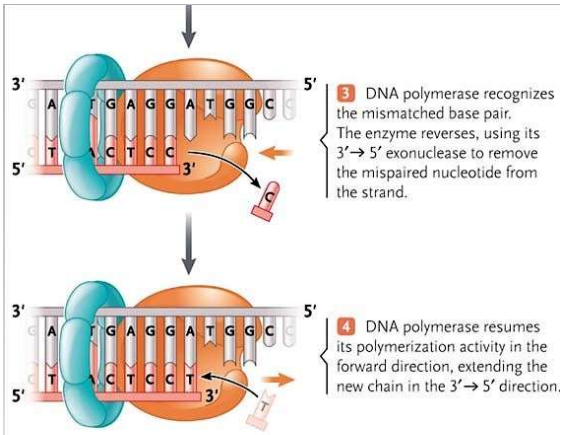


Figure 12.19
Proofreading by a DNA polymerase.

Corrections of Mistakes – Nucelotide excision repair

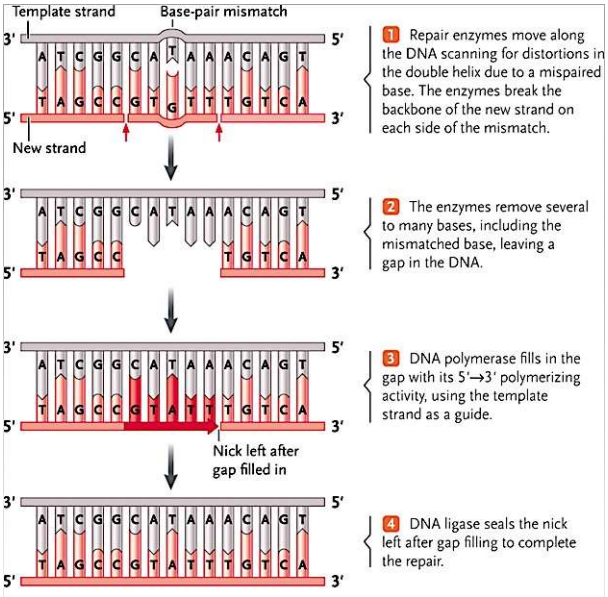
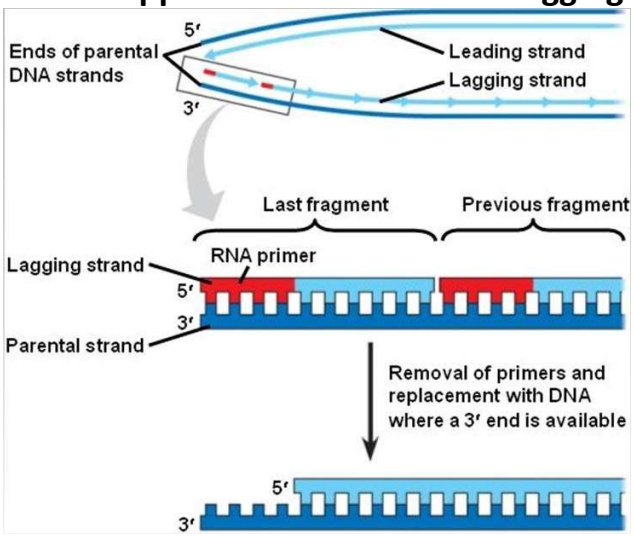


Figure 12.20
Repair of mismatched bases in replicated DNA.

What Happens at the End of the Lagging Strand?



Telomeres

- Additional DNA, the sequence TTAGGG (humans) repeated thousands of times
- With replication, that sequence shortens, but protects the coding regions of our chromosomes

Protection in Eukaryotes: Telomeres

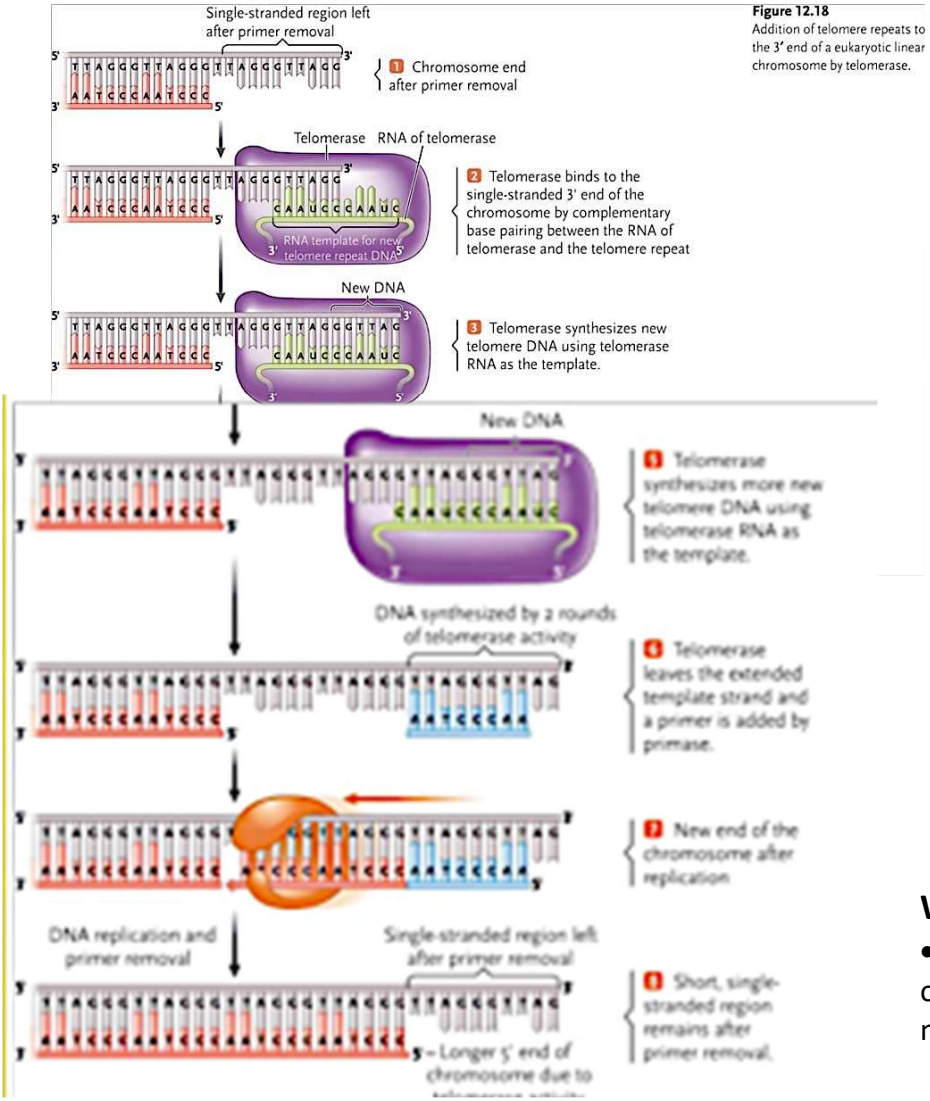


Figure 12.18
Addition of telomere repeats to the 3′ end of a eukaryotic linear chromosome by telomerase.

Working with DNA

- Knowing the entire sequence of a organism's genome has opened the door to numerous genomics advances

- <u>3 Techniques that are essential to work on DNA or specific genes:</u>
  - DNA cloning
  - Restriction enzymes
  - Polymerase chain reaction

Cloning Genomic DNA in a bacterial plasmid

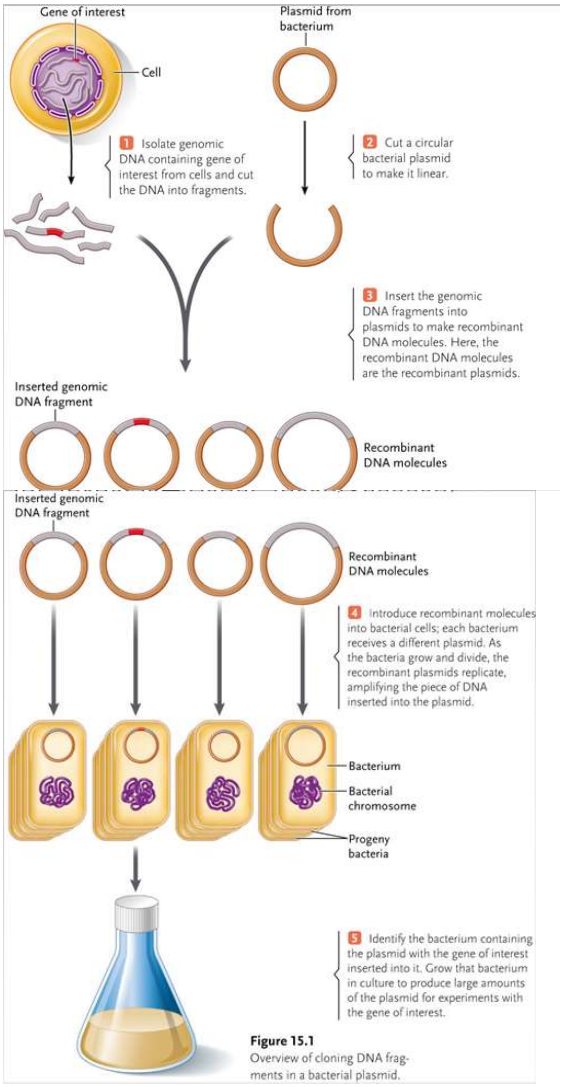


Figure 15.1
Overview of cloning DNA fragments in a bacterial plasmid.

Restriction Enzymes

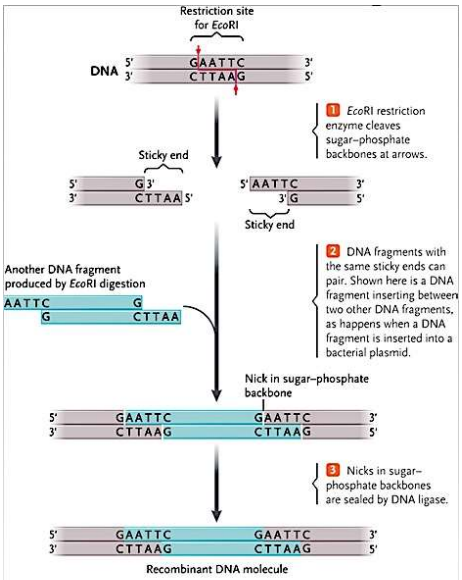


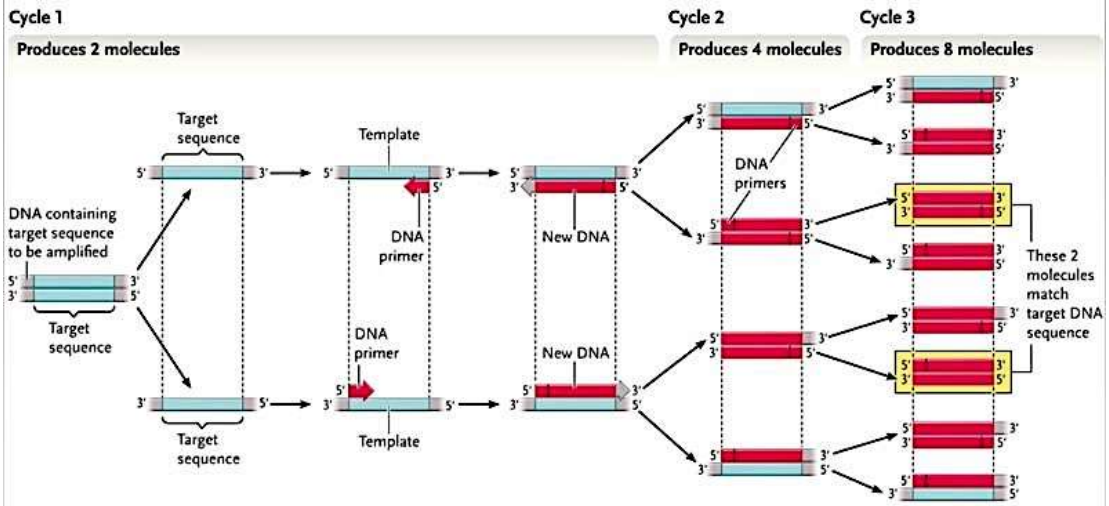
Figure 15.2
The restriction site for the restriction enzyme *Eco*RI, and the generation of a recombinant DNA molecule by complementary base-pairing of DNA fragments produced by digestion with the same restriction enzyme.

Polymerase Chain Reaction (PCR)

Figure 15.5
The polymerase chain reaction (PCR).

PURPOSE: To amplify—produce large numbers of copies of—a target DNA sequence in the test tube without cloning.

PROTOCOL: A polymerase chain reaction mixture has four key elements: (1) the DNA with the target sequence to be amplified; (2) a pair of DNA primers, one complementary to one end of the target sequence and the other complementary to the other end of the target sequence; (3) the four nucleoside triphosphate precursors for DNA synthesis (dATP, dTTP, dGTP, and dCTP); and (4) DNA polymerase. Since PCR uses high temperatures that would break down normal DNA polymerases, a heat-stable DNA polymerase is used. Heat-stable polymerases are isolated from microorganisms that grow in a high-temperature area such as a thermal pool or near a deep-sea vent.



1 Denaturation: Heat DNA containing target sequence to 95°C to denature it to single strands.

2 Annealing: Cool the mixture to 55–65°C (depending on the primers) to allow the two primers to anneal their complementary sequences at the two ends of the target sequence.

3 Extension: Heat to 72°C, the optimal temperature for DNA polymerase to extend the primers, using the four nucleoside triphosphate precursors to make complementary copies of the two template strands. This completes cycle 1 of PCR; the end result is two molecules.

4 Repeat the same steps of denaturation, annealing of primers, and extension in cycle 2, producing a total of four molecules.

5 Repeat the same steps in cycle 3, producing a total of eight molecules. Two of the eight match the exact length of the target DNA sequence (highlighted in yellow).