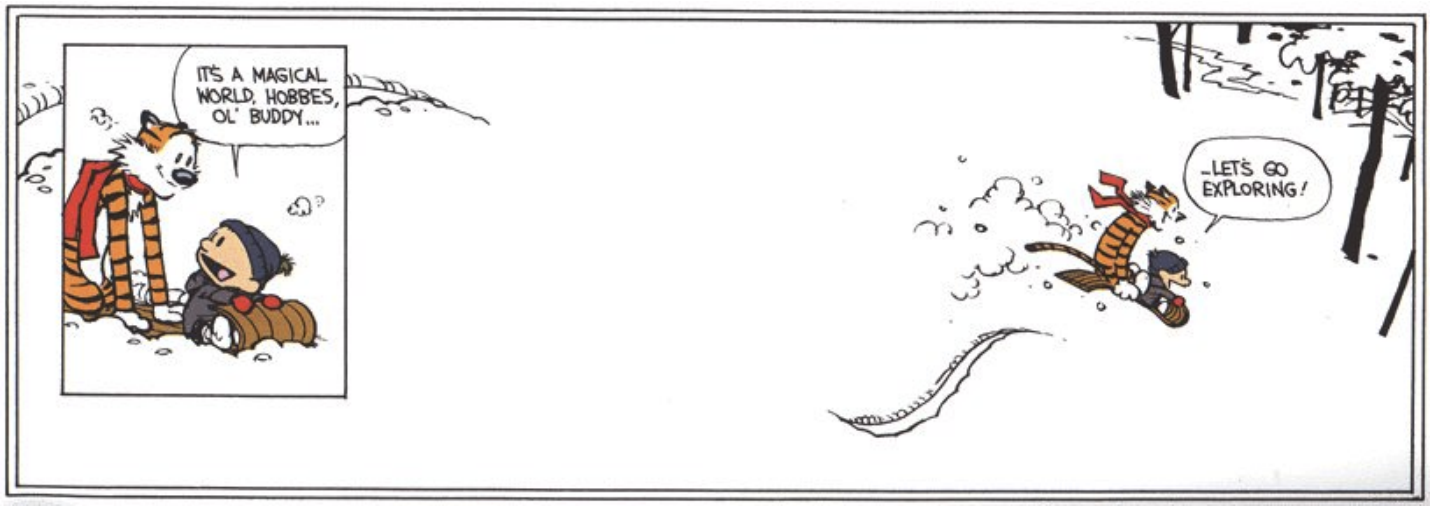
bio 42 notes | winter 2013

biafra ahanonu*

Discussion 2 Mon 14:15-15:05 | Encina West 202
Discussion 3 Mon 15:15-16:05 | 160-326
Office Hours Sunday 17:00-19:00 | Falconer
Email bahanonu@stanford.edu
Overview These section notes contain material from the class, additional information (in appendix), some references (textbooks[4,10] and additional readings), and an index of terms/concepts. A short handout is provided in class, the full notes are on coursework.
Section Each week is broken down into key concepts and terms students should know. In addition, more in-depth discussion of material is covered along with several problems provided at the end.
Appendix Background material and other content that should improve a students understanding of core concepts.
References Additional papers that students might find useful to get a more in-depth understanding of content covered in the course.



How you should feel about biology!

Dept. of Biology, Stanford University, Stanford, California
Correspondence: bahanonu@stanford.edu

Notes

Thanks to all the bio TAs that came before me for advice and helpful notes.
If any errors are seen in the text, shoot me an email with suggested corrections.

cheers!
-biafra

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Week 1 Cell Organization

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Overview

This week Prof. Shen talked about cellular organization and protein targeting to organelles. This section will cover signal sequences and protein membrane orientation. We will do several problems to check our understanding of the material.

Concepts

- Protein targeting components: signal sequences, recognition, translocation, and processing.
- Understand techniques (EM, microscopy, pulse-chase, etc.), both how they work, what they tell you, and their limitations.
- Protein membrane orientation.
- Necessity and sufficiency of biological molecule (think nucleoplasmin experiment).
- ON-OFF molecular switches and (un)binding of proteins, e.g. GTP or Pho4 phosphorylation.

Terms

- endoplasmic reticulum
- ER-Golgi intermediate compartment
- SRP receptor
- translocon
- stop-transfer sequence
- signal sequence
- KDEL sequence
- ER retention signal
- nuclear localization sequence
- nuclear export sequence
- golgi apparatus
- cisternae
- glycosylation
- constitutive secretion
- secretory vesicles
- lysosome
- mannose 6-phosphate receptor
- endosome
- clathrin coated pits
- adaptins
- clathrin
- consensus sequence
- LDL particle
- Ran-GDP/GTP
- plasma membrane
- ribosome
- cytoskeleton
- nuclear envelope
- nucleolus
- inner membrane
- outer membrane
- perinuclear space
- nuclear pore complex
- nuclear ring
- nucleoplasmin
- karyopherin
- importins
- exportins
- NF-κB
- Pho4
- RER
- smooth ER
- ER lumen
- pancreatic acinar cells
- signal sequence
- microsomes
- signal recognition particle
- SRP receptor
- translocon
- Sec61
- signal peptidase
- Sec62/62
- posttranslational
- Hsp70
- BiP
- stop-transfer sequence
- internal signal sequence
- glycosylation
- KDEL
- KKXX
- cis golgi network
- golgi stack
- medial
- trans
- trans golgi network
- mannose-6-phosphate
- signal patches
- constiuitive secretory pathway
- lateral sorting
- vacuole
- vacuolar targeting signal
- cell-free systems
- COPI
- COPII
- lysosomes
- endocytosis
- mitocondria
- cristae
- matrix
- presequence
- matrix processing peptidase
- chloroplasts
- thylakoid membrane
- grana
- stroma
- transit peptide
- stromal processing peptidase
- thylakoid signal sequence
- stromal signal recognition particle
- peroxisomes
- peroxins
- catalase
- PTS1
- PTS2

Experiments

- Pulse-chase
- Autoradiography
- Electron microscopy
- Fluorescence microscopy
- Western blot
- Centrifugation

Protein Targeting

The best way to understand protein targeting is by looking at a figure of the entire process. Fig. 6 shows the trafficking signals

and locations proteins with those signals travel to. In addition, Table 1 contains a list of all the relevant signal sequences.

Nucleus

Nucleoplasmin was used to identify that proteins needed a specific amino acid sequence.[5,8] Further studies identified the following sequence of events for exporting and importing nuclear proteins.

Import Cargo protein with NLS in the cytoplasm is bound by importin. This complex binds to the nuclear pore complex and is transported across. Then Ran-GTP binds the importin-cargo protein-nuclear pore complex and causes importin to release the cargo protein. Ran-GTP stays bounds to importin and is trafficked to the cytosol via a nuclear pore. Ran-GAP (GTPase-activating protein) remove a phosphate from GTP to turn Ran-GTP into Ran-GDP. Ran-GDP is then released from importin.

Export Cargo protein with NES binds to Exportin and Ran-GTP. The complex travels to the cytosol through the nuclear pore complex where Ran-GAP turns Ran-GTP into Ran-GDP, causing exportin to release the cargo protein. Ran-GTP returns to the nucleus via NTF2 receptor.

ER

Proteins are targeted to the ER via the following series of events: protein with signal peptide begins to get translated on free ribosome, SRP binds signal peptide and holds translation, SRP binds SRP receptor on rough ER, ribosome binds translocon, SRP is released from complex and translation restarts with protein moving across membrane. Transfer through the ER requires multiple signals to help ensure the protein either makes it to the lumen or is correctly orientated in the membrane. Fig. 8 has a good overview of the different sequences, we will discuss this in class.

Golgi Apparatus

The Golgi consists of several locations: cis network, golgi stack (medial and trans) and trans network. Proteins from the ER arrive in COPII coated vesicles and are processed. Further sugar modifications occur in the Golgi, the type of sugar modification on a protein tells you were it was in the Golgi at time of purification. The orientation of a membrane protein is fixed; though, that is not always the case.[2] Proteins with KDEL or KKXX signal sequences are returned to the ER via COPI-coated vesicles, with KDEL binding to the KDEL receptor.

Lysosome

Mannose-6-phosphate targets proteins to the lysosome via the mannose-6-P receptor. The receptor binds to an adaptor protein that in turn binds clathrin. Clathrin forms a cage-like structure around the membrane, eventually causing it to pinch off with the help of dynamin. Remember, yeast and plants mainly utilize the vacuole in place of lysosomes.

Mitochondria

Mitochondrial proteins contain a presequence that is recognized by the Tom complex, a translocase on the outer membrane. Because the intermembrane space is positively charged (remember electron transport chain!), the positively charged presequence is helped along through the Tim23 complex (a translocase) on the inner mitocondrial membrane. Hsp70, part of the PAM complex, keeps the proteins unfolded in both the cytosol and matrix until they are folded by Hsp60. These interactions with chaperones require ATP.

Hydrophobic stop-transfer sequences halt transfer of a protein through the Tom or Tim complex, causing their insertion into the outer or inner membrane. For the outer membrane, the Sorting and assembly machinery (SAM) complex takes over the task of Tom. In the inner membrane, Tim22 (as opposed to Tim23) acts as a translocase to insert carrier proteins.

Chloroplast

Chloroplasts use a similar mechanism to the mitocondria for protein import. In this case Hsp70 or TOC159 bind proteins with a transit peptide. They are then guided to the TOC complex on the outer envelope, a GTP depedent receptor. The protein is then passed through the TIC complex and into the stroma. There a stromal processing peptidase cleaves the transit peptide and the protein either folds in the stroma or is directed to the thylakoids.

Whether SRP and its receptor are released and then the ribosome binds translocon or vis-versa is not entirely clear given present experimental results.

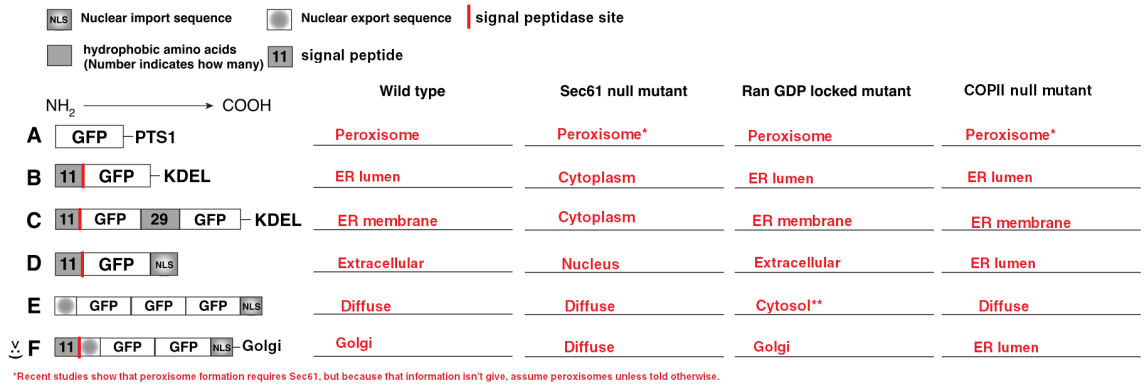
Peroxisome

Peroxisome proteins contains either a N-terminal PTS2 or a C-terminal PTS1 that are recognized by Pex7 and Pex5 receptors, respectively. The receptor and cargo move into the peroxisome where the cargo detaches from the receptor. The receptor is subsequently trafficked back into the cytosol. Some proteins piggy-back with PTS1 containing proteins into the peroxisome without themselves having a PTS1 or PTS2 signal.

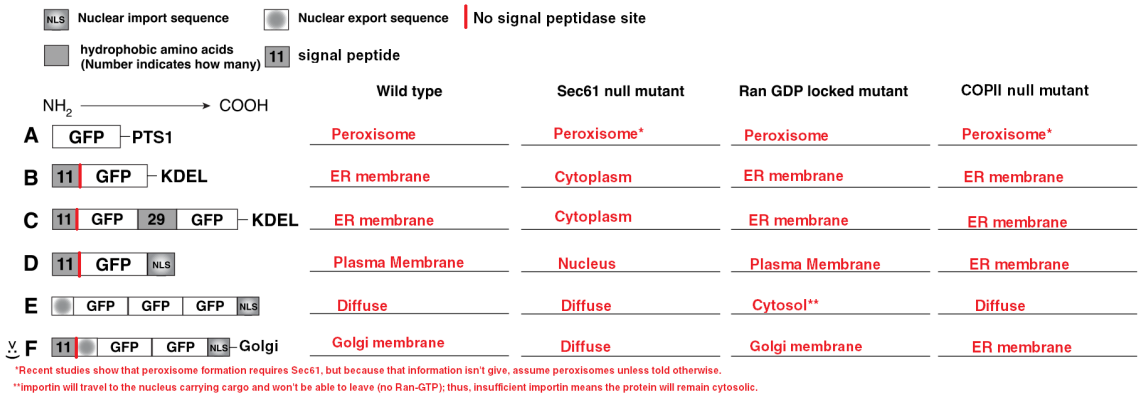
Problems

If there is any confusion about the questions or answers, shoot me an email.

1. See Fig. 2, and Fig. 3 for problems. Explanations in figure.
2. How would you visualize the movement of an individual group of proteins produced during a specific time?
3. Why would a protein need to have both an NLS and NES? What type of protein would require such a mechanism?
4. What would happen if there was no recycling of membranes between the ER and Golgi, and within the Golgi? How might you test your hypothesis?



(a) signal sequence is cleaved



(b) signal sequence is not cleaved

Figure 2 | Problem: protein targeting
Determine where the protein will go given the signal sequences and mutants. Note, diffuse means the protein is located in the cytosol and nucleus.

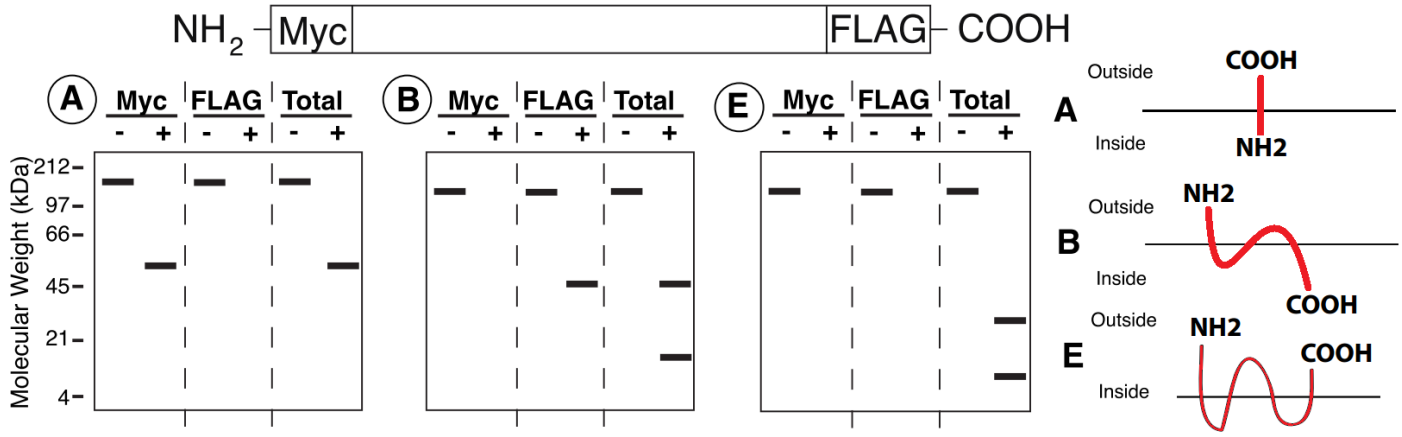
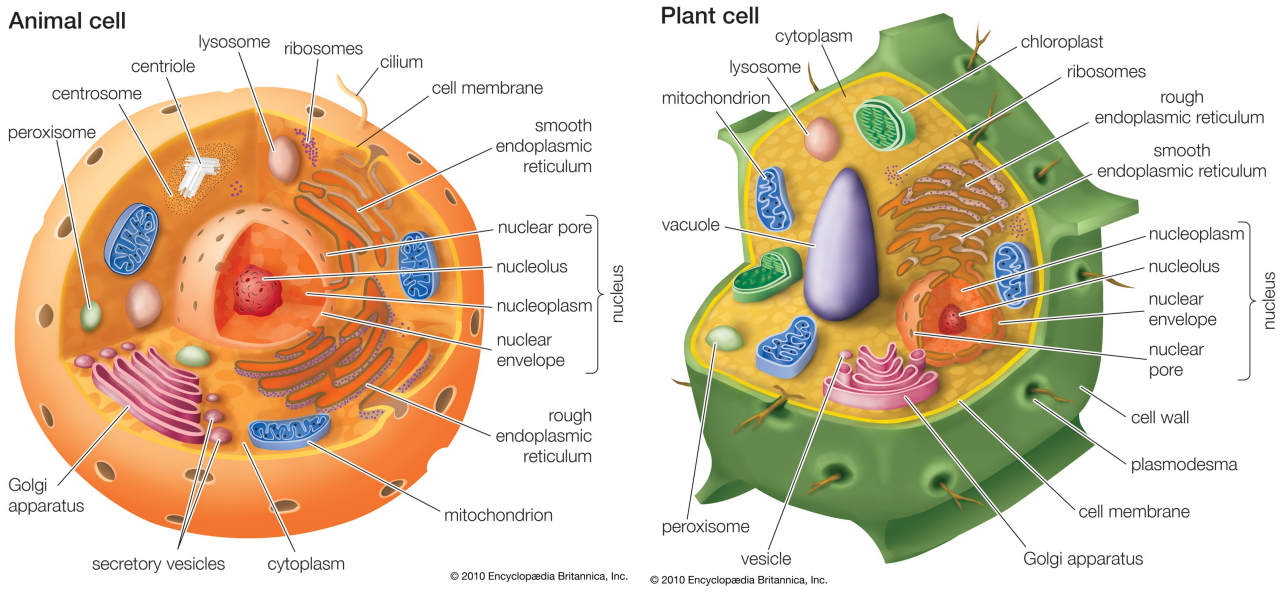


Figure 3 | Problem: membrane orientation

A protein that has a N-terminal MYC and C-terminal FLAG tags is expressed in cells and located on the plasma membrane. To test orientation, a protease is added to a dish containing cells expressing the MYC-FLAG protein; it chews up the extracellular portions of proteins but **cannot** get inside the cell.

In the gel, lanes with a (-) did not have protease added to the sample while those with (+) did. For example, if the only MYC side was outside the cell, that portion would be chewed up and you would not see a band on the gel lanes labeled MYC (+), but bands would appear on FLAG (+) bands. For cases A, B and E, determine the orientation of the protein based on information from the gel.

Figures and Tables



(a) animal cell (b) plant cell

Figure 4 | Cell Physiology

General structure and organelle location in animals and plants, note that plants and yeast have vacuoles.

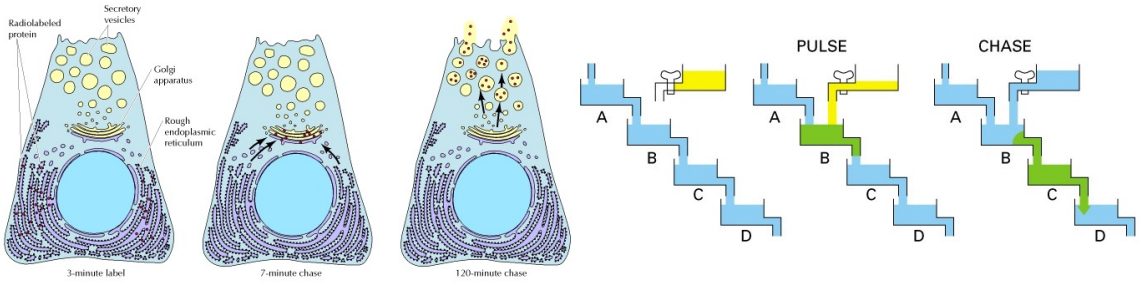


Figure 5 | Pulse-Chase and ER secretion
Left, Palade's classic pulse-chase experiment to determine the secretory pathway. Right, the pulse is applied for a short period, after which it is removed. You can thus visualize the portion of the flow that occurred during the pulse.

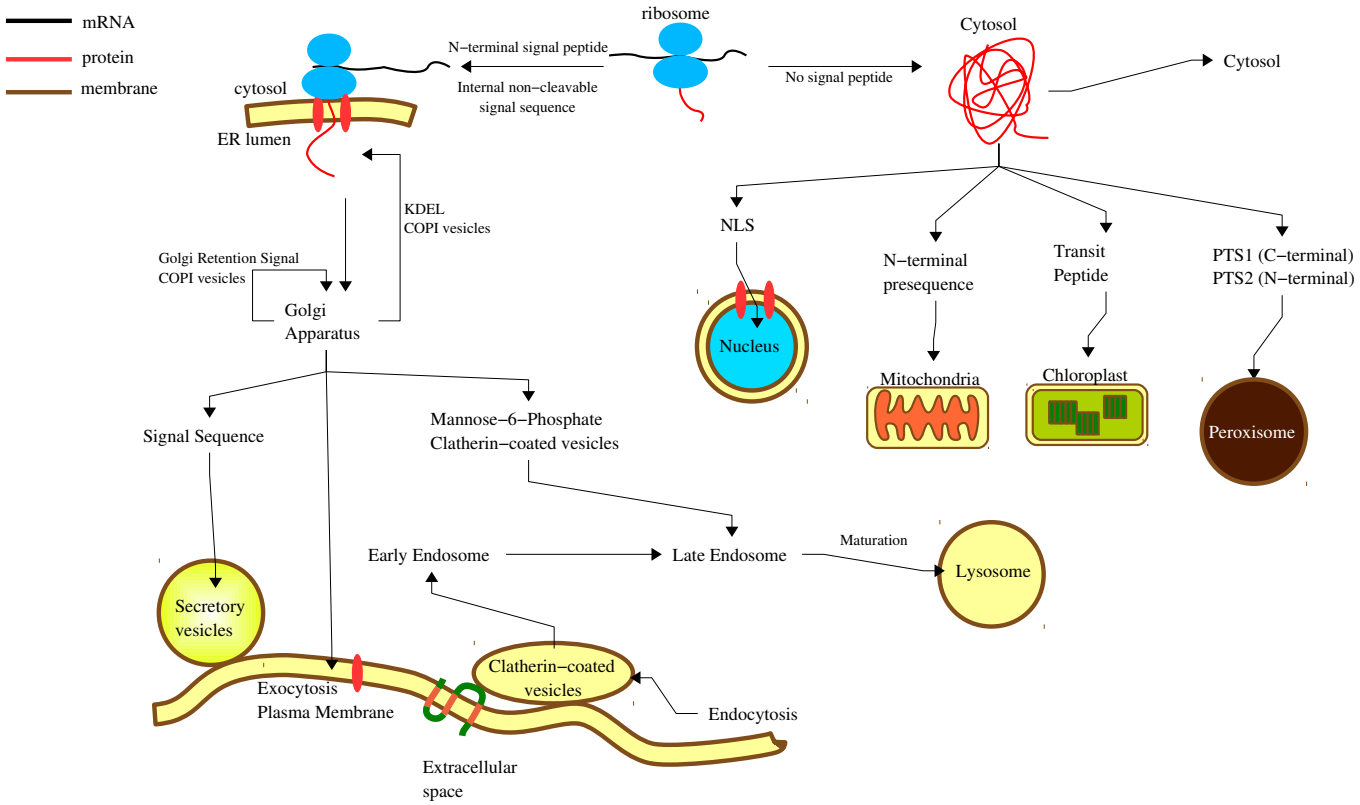


Figure 6 | Protein Trafficking Pathways
Overview of protein sorting in the cell.[3] Drawing the figure out is good practice, it is much better than memorizing a list.

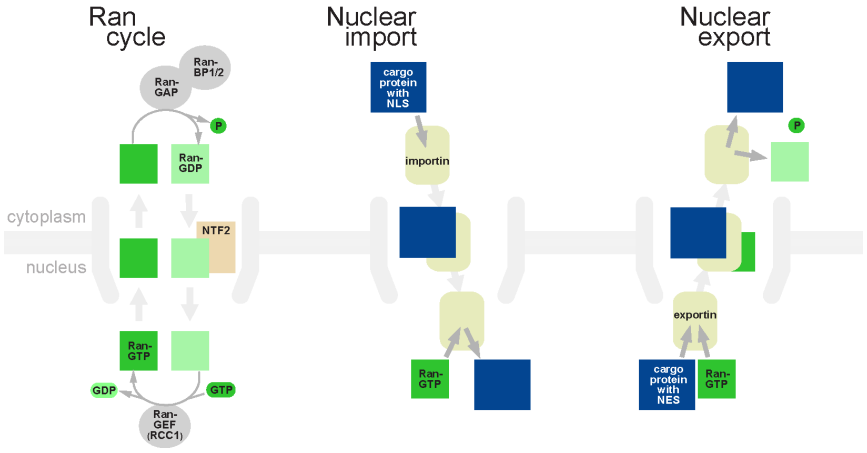
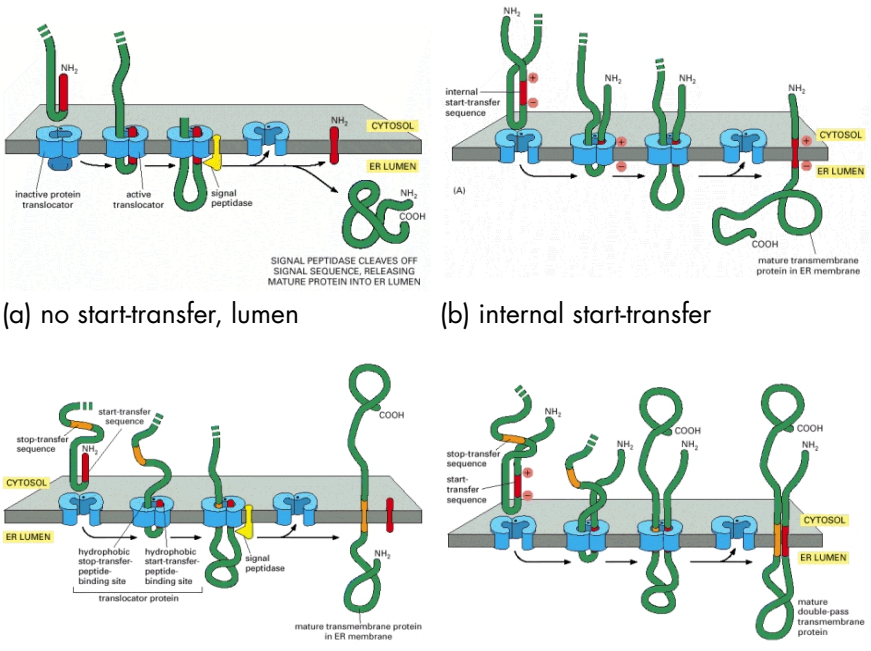


Figure 7 | Nuclear Import and Export
Overview of protein import and export.[3] Remember than Ran is shuttled back to the nucleus via its own receptor.



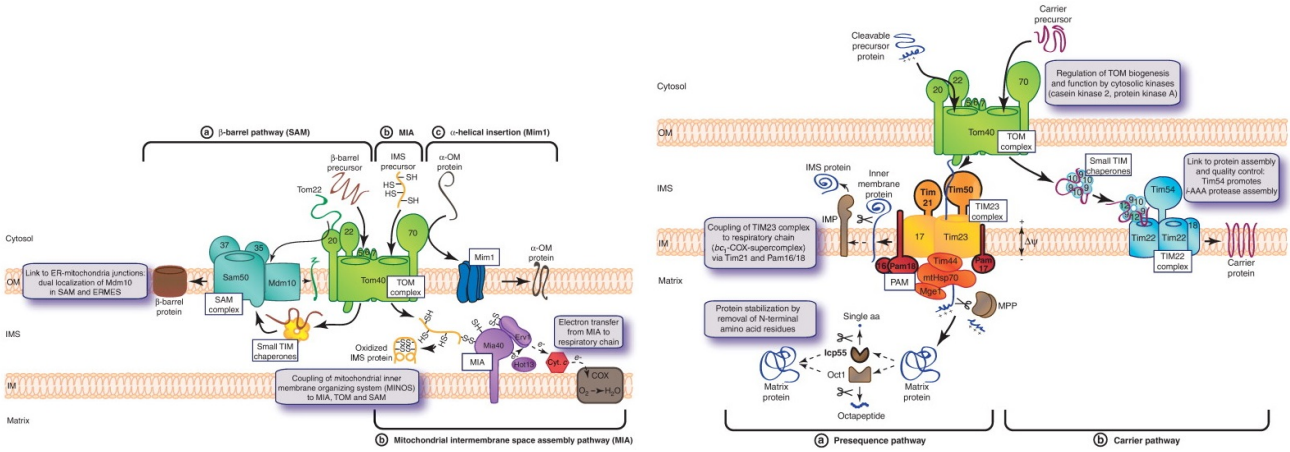
(a) no start-transfer, lumen

(b) internal start-transfer

(c) start-transfer and stop-transfer w/ peptidase signal

(d) start-transfer and stop-transfer w/ no peptidase signal

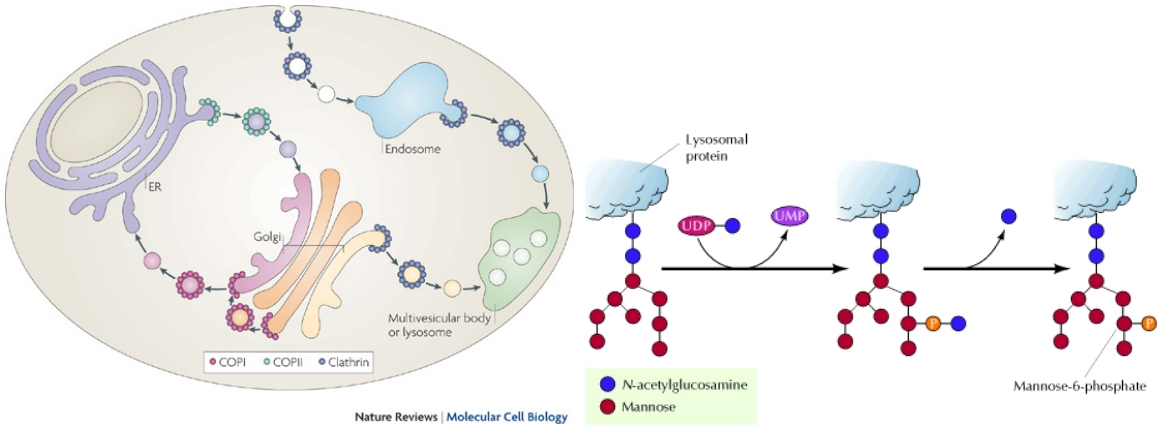
Figure 8 | ER Translocation
Different types of signal sequences ensure that the protein is orientated in the membrane correctly.



(a) outer membrane insertion [1]

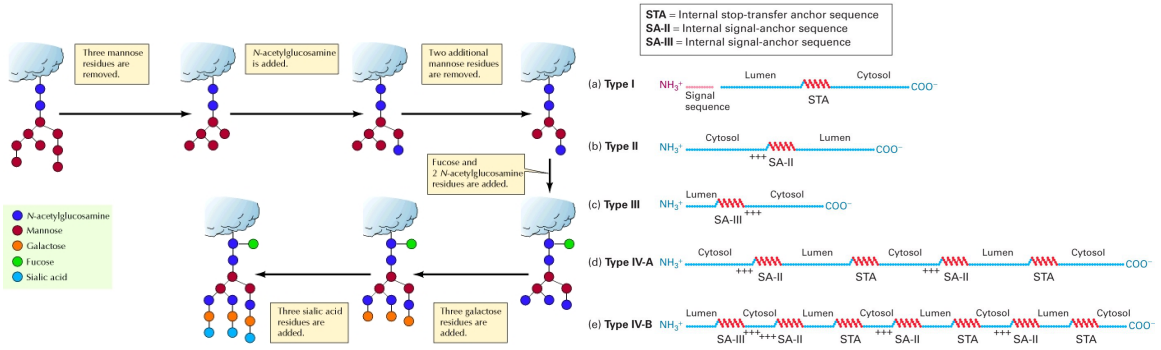
(b) matrix and inner membrane insertion

Figure 9 | Mitocondria Translocation
Example of protein translocation.



(a) ER-Golgi transport

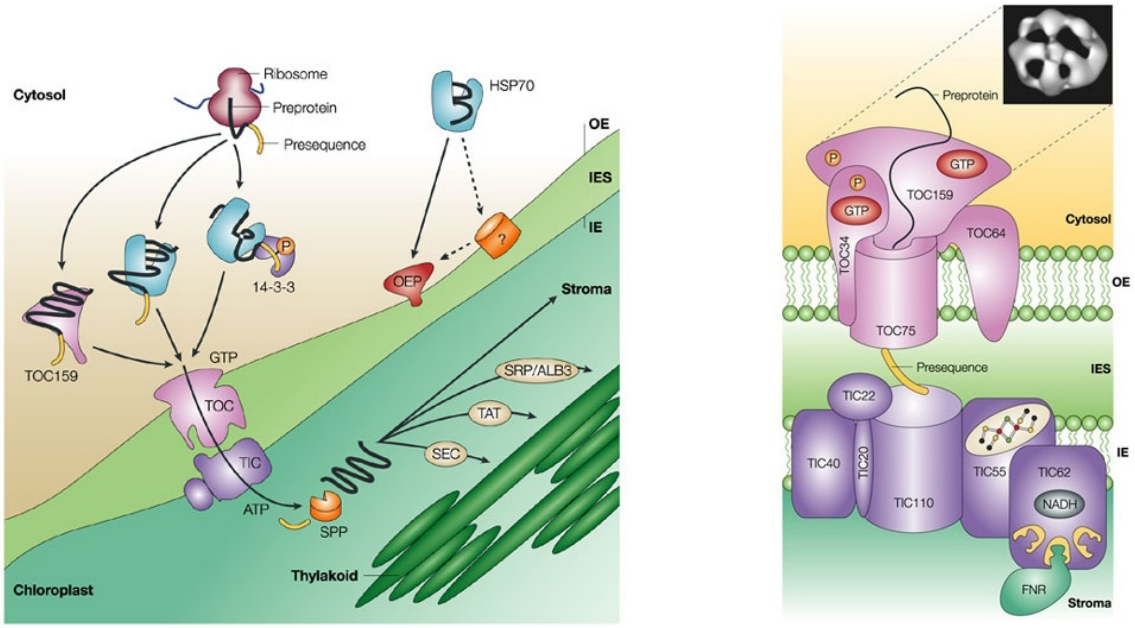
(b) Mannose-6-phosphate addition



(c) Sugar modifications

(d) Representative sequences of the different types of membrane proteins

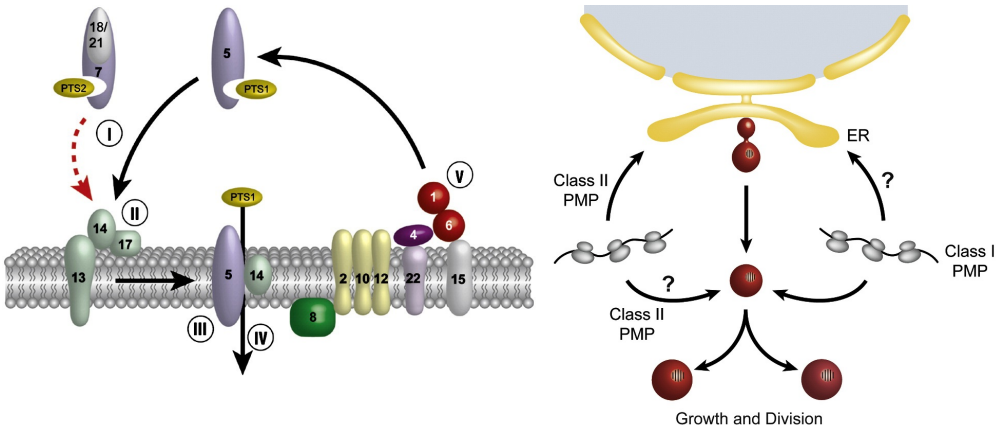
Figure 10 | Golgi Functions
The golgi performs a variety of functions, including sugar modification and addition of M6P for lysosome targeting. Lastly, KDEL receptors help transport proteins back to the ER



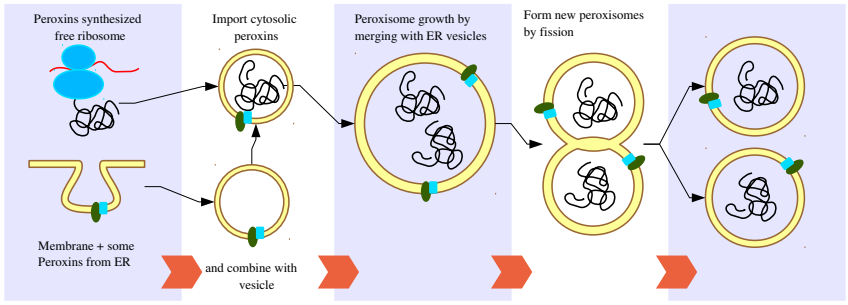
(a) cytosolic targeting[11]

(b) Tic-Toc Complexes

Figure 11 | Chloroplast Translocation
The chloroplast uses a mechanism similar to mitocondria to import proteins.



(a) Recycling of Pex receptors and formation of peroxisome.[9]



(b) Peroxisomes are formed by vesicles from the ER and cytosolic proteins containing a PTS1 or PTS2

Figure 12 | Peroxisome protein targeting
Peroxisome proteins contains either a N-terminal PTS2 or a C-terminal PTS1 that are recognized by Pex7 and Pex5 receptors, respectively. The receptor and cargo move into the peroxisome where the cargo detaches from the receptor. The receptor is subsequently trafficked back into the cytosol.

Signal	Organelle	Description
NLS	nucleus	Nuclear Localization Sequence, anywhere within the protein sequence
Presequence	mitocondria	N-terminus
Transit peptide sequence	chloroplasts	N-terminus
PTS-1	peroxisome	C-terminus
PTS-2	peroxisome	N-terminus
No signal sequence	cytosol	and translated on a free ribosome
Signal peptide	RER	N-terminus
Signal peptidase sequence	ER	between the signal peptide and remainder of cleaved ER-targeted protein following translocation. It is a separate entity from the signal peptide, but most often occurs with a signal peptide.
Signal peptide	plasma membrane	with internal non-cleavable sequence, transmembrane protein
Signal peptide	extracellular	no internal sequence, within lumen, will be secreted
Stop transfer sequence	ER	hydrophobic region of an ER-targeted protein that cannot pass fully through the translocon and exits sideways into the ER membrane, creating a membrane spanning region of the protein
Internal non-cleavable signal sequence	ER	internal hydrophobic region similar to the signal peptide that targets a protein to the ER for translation, but does not have an adjacent signal peptidase sequence for cleavage. These sequences can result in the N-terminus or C-terminus being translocated into the ER depending upon its orientation
KDEL	ER	ER retention signal, C-terminus of a protein that binds the KDEL receptor in the golgi signaling for that protein to be returned to the ER by the COPI vesicle recycling pathway
KKXX	ER	di-lysine ER retention signal, interacts with COPI
Golgi retention signal	golgi	sequence that determines that a protein should remain in the cis, medial or trans golgi
Secretory vesicles signals	vesicles	Sequences that sort RER synthesized proteins into specialized secretory vesicles
Mannose-6-phosphate	endosome & lysosome	added onto a protein in the cis golgi signals a protein, it signals the protein for the late endosome

Table 1 | List of signal sequences
Signal sequences, targeting location and short description. RER = rough endoplasmic reticulum, RERnoseq = RER protein sans additional sequence.

Week 2 Vesicles and Cell Dynamics

Readings

Cooper 4th: p. 473-525 556-567
Cooper 5th: p. 473-524 557-568

Overview

This week Prof. Shen discussed vesicle budding and fusion, endocytosis, and actin and microtubule dynamics along with related proteins. In additions, experiments related to each system were discussed. We will go over the general concept of a biological pathway then apply it to specific examples from class. Problems at the end test understanding of biological logic and ability to recall pathways diagrammatically.

Concepts

- Regulation: multiple control points, e.g. ATP and Ca^{2+} in muscle contraction.
- Biological pathways: a series of activations or inhibitions. Understand epistasis and how to determine if proteins are in the same pathway.
- Difference between dynamic equilibrium and static, e.g. actin.
- Signal transduction and secondary messangers, e.g. calmodulin and Ca^{2+}.
- Hypothesis testing, e.g. think about the entire system.
- Rescue experiments: think addition of cytoplasm to a system in which vesicles can't fuse and getting fusion. What does this tell you?
- Appropriate system for analysis, e.g. using immunofluorescence instead of pulse-chase to track a specific protein's localization.
- Relation between conformation and function: think calmodulin binding to MLC kinase.

Terms

- **Actin**
- globular actin
- filamentous actin
- nucleation
- spontaneous assembly
- equilibrium
- critical concentration
- treadmilling
- cytochalasin
- phalloidin
- actin-binding proteins
- formin
- Arp2/3
- tropomyosin
- ATP-ADP exchange protein
- severing protein
- capping protein
- filament-stabilizing protein
- cross-linking proteins
- ADF/Cofilin
- profilin
- actin bundles
- actin networks
- actin-bundling proteins
- fimbrin
- contractile bundles
- alpha-actinin
- filamin
- **Cell cortex**
- erythrocytes
- spectrin
- actin-binding domain
- ankyrin
- band 3
- glycophorin
- protein 4.1
- fodrin
- ERM proteins
- dystrophin
- filamin
- **Extracellular matrix**
- integrins
- focal adhesion
- stress fibers
- adherens junctions
- adhesion belt
- cadherins
- microvilli
- stereocilia
- villin
- calmodulin
- myosin I
- terminal web
- pseudopodia
- lamellipodia
- microspikes
- filopodia
- **Muscles**
- skeletal
- cardiac
- smooth
- muscle fibers
- myofibrils
- thick filaments
- thin filaments
- **Sarcomere**
- A bands
- Z disc
- I band
- H zone
- M line
- titin
- nebulin
- sliding filament model
- myosin II
- head region
- heavy chain
- regulatory light chain
- essential light chain
- sarcoplasmic reticulum
- transverse tubules
- Ca^{2+}-ATPase pump
- tropomyosin
- troponin
- cytokinesis
- contractile ring
- myosin light-chain kinase
- myosin I
- **Cell movement**
- pseudopodia
- lamellipodia
- filopodia
- WASP/Scar complex
- twinfilin

- focal adhesions
- ARF
- intermediate filaments
- keratins
- nuclear lamins
- nestin
- vimentin
- desmin
- neurofilament proteins
- desmosomes
- hemidesmosomes
- plakins
- desmoglein
- desmocollin
- dominant negative mutant
- **Microtubule**
- tubulin
- GTP-cap
- dynamic instability
- Colchicine
- Colcemid
- taxol
- centrosome
- catastrophe
- microtubule-organizing center
- γ-tubulin ring complex
- centrioles
- pericentriolar material
- delta-tubulin
- centrin
- microtubule-associated proteins
- kinesins
- dyneins
- dynactin
- cilia
- flagella
- axoneme
- nexin
- basal body
- **Mitotic spindle**
- kinetochore microtubules
- chromosomal microtubules
- polar microtubules
- astral microtubules
- anaphase A
- anaphase B
- **Endocytosis**
- phagocytosis
- pinocytosis
- phagosome
- phagolysosome
- receptor-mediated endocytosis
- clathrin-coated pits
- dynamin
- ligands
- **LDL**
- familial hypercholesterolemia
- LDL receptor
- statins
- HMG-CoA reductase
- internalization signals
- fluid phase endocytosis
- caveolae
- caveolin
- HDL
- macropinocytosis
- **Vesicle Fusion**
- Rab-GTP
- Rab-GEF
- Rab-GDI
- GDI dissociation factor
- v-SNARE
- t-SNARE
- NSF
- SNAP
- receptor down-regulation
- transcytosis
- Botulinum toxin

Techniques

For each technique, you should know when is appropriate to use it, what its limitations are, and a particular cellular process that was used to study it.

- Fluorescence Recovery After Photobleaching (FRAP) Inactivate GFP molecules in a particular area, watch the recovery to measure movement or diffusion of protein.
- Speckle biology Express a GFP-actin at low levels, observe direction of treadmilling in microscope.
- Radio-labeling Add radioactive Brown and Goldstein experiment, see page 560 textbook.
- Cell fusion assay The purpose of this assay is to allow testing of components involved in vesicle fusion. Isolate vesicles from two Golgi, one from mutant, the other wild-type. Mix together and see if fusion occurs, using a specific read-out to tell.
- Fractionation Squeeze out squid axon interior onto glass and add vesicles. Observe movement and add or remove fractions (e.g. cytoplasm) or molecules (e.g. ATP) to see what is required.
- Drugs How can drugs be used to test the cellular functions of a protein? e.g. cytochalasin blocking of actin polymerization. What are the caveats to consider when using a drug as opposed to a mutant? What are the advantages?
- Velocity centrifugation Create a sucrose gradient inside a vial, add your sample, and spin at high speeds. Can help separate molecules of different physical properties and thus help isolate *fractions*.

Drugs!

These are useful to know.

cytochalasin blocks growth at actin plus end.

phalloidin stabilizes actin filaments, prevents depolymerization.

colchicine binds to tubulin dimers and prevents them from polymerizing.

colcemid depolymerizes microtubules by binding to plus ends.

taxol stabilizes microtubules.

botulinum toxins Chews up synaptobrevin, syntaxin, and SNAP-25, neuronal SNAREs.

tetanus toxin Chew up synaptobrevin.

statins markted as Lipitor, a very succesful and helpful drug. Inhibits the enzyme HMG-CoA reductase, which plays a central role in the production of cholesterol in the liver.

antibodies Can inhibit the function of the protein they bind to.

Vesicles

The process of vesicle budding and fusion fits into a general framework encountered in biology: some molecule is switch on/off, binding is changed, and associated factors either fall off or start to associate. In the cell, there are three types of vesicles you need to know: clathrin, Golgi or plasma membrane to endosome; COPI, Golgi to ER; and COPII, ER to Golgi. To study these processes, we use velocity centrifugation to isolate vesicles, membrane, and cytoplasm.

Clathrin-coated vesicles

- ARF, a GTP-binding protein, binds to Golgi.
- ARF-GEF converts ARF-GDP to ARF-GTP.
- ARF-GTP binds adaptin (GGA and AP1).
- adaptin binds receptor (e.g. M6P receptor) to clathrin.
- clathrin deforms membrane, dynamin helps pinch vesicle off.
- ARF-GTP self-hydrolyzes to ARF-GDP, clathrin falls off.

Vesicle docking

- GDP-dissociation-inhibitor, Rab-GDI, prevents Rab-GDP to Rab-GTP.
- GDI-displacement factor blocks Rab-GDI, Rab-GDP adds prenyl group.
- Rab-GEF turns Rab-GDP into Rab-GTP, allowing it to bind Rab effector.
- Rab effector organizes SNARE protein in pre-fusion complex.
- *Remember*, there are specific and complementary Rab pairs for each vesicle and target membrane.

Vesicle fusion

- Vesicles contain v-SNARE proteins while membranes have t-SNARE proteins.
- When Rab effectors dock, allows v- and t-SNAREs to coil together.
- Membrane is brought close, allowing fusion of vesicle and membrane.
- Rab-GTP is hydrolyzed to Rab-GDP, Rab effectors no longer associate.
- NSF and SNAP bind to fusion complex, break apart SNAREs, *requires ATP hydrolysis*.
- **Example**: synaptobrevin, syntaxin, and SNAP-25 for vesicle fusion in neurons.

Vesicle fusion assay The purpose of this assay to later determine factors involved in vesicle fusion. See Fig. 14 for more.

- Take wild-type medial Golgi cells, where an N-acetyleglucosamine transferase resides. These have *no* G-protein in them.
- Make a mutant cell with G-protein but *no* N-Ac-Glc transferase.
- Mix wild-type and mutant cells, if wild-type Golgi contains enzymes needed, will get addition of N-acetylglucosamine to G-protein.
- Next, add cytosol, membrane and other fractions to determine where necessary proteins reside, e.g. NSF and SNAP.

LDL

Familial hypercholesterolemia affects X percent of the population and involves elevated levels of cholesterol in the bloodstream. Through a series of elegant experiments that won them each a Nobel prize, Michael Brown and Joseph Goldstein, then medical residents at MGH in Boston, showed that a mutation in the LDL receptor was responsible for this phenotype. They did this by careful radio-labeling of LDL particles and tracking their binding to, uptake, and degradation in FH and normal cells. Statins are a particularly useful class of drug that inhibits HMG-CoA reductase, altering synthesis of cholesterol.

Endocytosis LDL particles bind a LDL receptor and are endocytosed. Binding causes a clathrin-coated pit to form (what other proteins are involved?) and they travel to the endosome where the receptor unbinds the particle due to the low pH. The LDL receptor is recycled back to the surface and the LDL particle travels to the lysosome and is broken down, releasing cholesterol.

Actin

Actin is composed of a plus end and minus end, these have *nothing* to do with charge. In the cell, there are two pools of actin: F actin, in filaments, and G actin, free floating.

- ATP binds actin, causes a conformational change making it more likely to polymerize.
- Formation of actin-ATP *trimer* is limiting step.
- Actin-ATP binds more readily on the plus end, filament grows.
- Actin-ADP falls off at minus end, filament shortens.
- Treadmilling Actin is *dynamic*, actin subunits to move along the filament as actin-ADP falls off and actin-ATP binds.

Actin proteins

- Formin facilitates actin polymerization by providing faster nucleation of trimers.
- ADF/cofilin binds actin-ADP, causing dissociation.
- Twinfilin brings actin-ADP to plus end.
- Profilin exchanges ADP for ATP, actin binds plus end, filament extends.
- Arp2/3 promotes branching.
- Capping proteins help stabilize ends.

Actin bundles are promoted by villin and fibrin. Actin networks are facilitated by filamin, which has to form a dimer. These proteins *do not* promote actin polymerization. Actin is anchored to the plasma membrane by binding spectrin, which in turn binds ankyrin (binds membrane proteins) or directly to glycophorin.

Muscle

There are three different types of muscle: skeletal (voluntary), smooth (involuntary), and cardiac (heart). The skeletal muscle is composed of sarcomeres that contain an A band (myosin and actin), I band (actin), H band (myosin), Z-disc (actin, filamin, spectrin) and M line (myomesin, etc.).

Skeletal muscle contraction

- Nerve impulse reaches muscle, propagates to sarcoplasmic reticulum, which releases Ca^{2+}.
- Ca^{2+} binds troponin, changes conformation and moves tropomyosin from actin-binding site.
- Myosin (thick filament) binds actin (thin filament).
- Loss of ADP + Pi causes myosin to stroke forward.
- ATP binds myosin, detaching it from actin.
- ATP hydrolyzes to ADP + Pi, causing myosin to return to the ready form.
- Myosin binds actin and cycle repeats.
- This causes actin to move toward the M-line, so the I band shortens, *not* the A band.
- This causes the sarcomere to get shorter.
- Spring-like titin protein bound to myosin and Z disc, returns sarcomere to relaxed state when myosin not bound to actin.

Smooth muscle Thin and thick filaments still slide, but *no* sarcomeres. Actin filaments are attached to the plasma membrane and contraction causes the smooth muscle cells to become smaller.

- Nerve impulse arrives at smooth muscle cell.
- Ca2+ enters cytoplasm and binds calmodulin, activating it.
- Calmodulin binds myosin light-chain kinase, activating it.
- MLC kinase phosphorylates myosin, activating it.
- Active myosin binds to actin, initiating contraction.

Contractile Ring forms during cytokinesis, after cells have replicated and separated their nuclei. The membrane is pinched off as actin slides past myosin II, making the ring smaller. During contraction, actin is also depolymerizing and the ring size remains the same.

Myosin

Myosin I binds actin and membrane vesicles in cells. The binding to vesicles is thought to be due to direct interaction with membrane phospholipids. It moves toward the *plus end* (barbed) of actin.

Myosin II is used in muscles and is composed of a globular head region that both binds actin and is an ATPase. There are two regulatory light chains, ELC and RLC, that can be used to regulate myosin activity via phosphorylation. Two heavy chains coil together to produce dimers.

Cell movement Actin and myosin are involved in cell movement.

- Arp2/3 proteins, and their activator WASP/Scar, that bind actin at the plus end and anchor it to the membrane.
- Lamellipodia (protrusions) are formed.
- Actin depolymerizes at trailing edge, allowing movement.
- Cell movement blocked by cytochalasin, inhibits plus end, and phalloidin, stabilizes filaments.

Intermediate Filaments

Intermediate filaments are composed of a head and tail, which wind together to form a dimer then dimers align anti-parallel to make tetramers. Tetramers form protofilaments that come together to form filaments of eight protofilaments. Nuclear lamins help give nucleus structure, disassembled during mitosis. See Fig. 18. Even though we don't cover this protein, it is important, so don't forget it!

Microtubules

Structure Composed of α- and β-tubulin, β-tubulin binds GTP. Forms a tube of 13 protofilaments, α- and β-tubulin laid in a track end-to-end. Plus end favors tubulin-GTP addition while the minus end is anchored in MTOC. Tubulin-GTP acts as a `cap' at end of microtubule, leads to catastrophe if lost because tubulin-GDP rapidly dissociates (see Fig. 19).

MTOC is known as the centrosome in animals. It is composed of two centrioles, which are made of nine triplet MTs. It duplicates before mitosis.

Cell cycle There are several MTs involved in cell cycle: kinetochore, attached condensed chromosomes at centrosomes; chromosomal, attach at ends of chromosomes; polar, aligned with chromosomes, but doesn't interact; and astral, point from cell periphery. During anaphase, *dynein* at the kinetochore facilitates chromosome movement toward spindles along chromosomal MTs while the polar microtubules slide past each other due to *kinesins*.

Transport Dynein carries cargo toward the minus end of MTs while kinesin moves toward the plus end. In neurons, allows transport of organelles and vesicles along the axon. Why is mechanism particularly important in neurons (the best cell type)?

Problems

If there is any confusion about the questions, shoot me an email or talk to me after class.

1. A theater student, who performs in emotional plays like *A Midsummer Night's Dream*, believes she has too many wrinkles to play Hippolyta. In a panic, she comes to you, knowing you've taken bio42, asking whether she should inject botulinum toxins or tetanus toxin around her forehead and lips. What advice would you give her? Tell her not to do it, since botulinum and tetanus both cause paralysis due to destruction of synaptobrevin, synataxin, etc. This reduces the ability to show a range of emotions and explains why people with botox injections always seem…off.
2. Several nuclear proteins have been tagged with GFP. You perform FRAP and bleach *only* the nucleus. Unfortunately, you have mixed up your samples but have curves 1-3 seen in Fig. 13. Match each curve with the following: soluble nuclear protein; histone protein (part of nucleosome that binds DNA); and a protein that is part of two populations, a stable one that does not diffuse and a dynamic one that does. Lastly, in curve 4 draw what you would see if the *entire* cell was bleached. Curve 1: two population protein. Curve 2: Histone bound protein. Curve 3: soluble protein. Curve 4: would see no recover for awhile then around 5 minutes an increase would occur due to synthesis of new GFP-bound protein.
3. A collaborator gives you a mouse that has digestive and other problems related to smooth muscle contraction. Curious, you isolate calmodulin and find that it binds calcium several orders of magnitude less tightly than normal. Feeling bad for the mice, you decide to cure them. How would you do this? Assume you can express any protein of your choice in these mice or inject them with any drug of your choosing. Express wild-type calmodulin in the mice to rescue function. Do not inject active MLC kinase or other constitutively active proteins. This would cause the pathway to be ON at all times, leading to paralysis.
4. You have found a novel protein that is targeted to the mitocondria. After analyzing the sequence, it appears to have a signal peptide. Confused, you ask your advisor and he tells you to test whether this protein goes through the secretory pathway and then to the mitocondria, or is just transported from the cytoplasm to the mitocondria. The former would be a novel pathway and an instant *Nature* or *Science* paper. How would you test this hypothesis? Assume you have access to temperature sensitive mutants, a microscope, and the ability to tag a protein (e.g. with GFP). Tag the protein with GFP and observe where it localized in the cell. Alternatively, stain with an antibody against the protein then image. After, make a temperature sensitive mutant in COPII or clathrin and observe if localization changes upon raising the temperature. If the protein no longer locates to the mitocondria, you have an initial finding showing that it uses the secretory pathway.
5. Two proteins localize to different parts of the chloroplasts, protein A to the stroma and protein B to the thylakoid. In mutants of each (mutant A and B), both stay in the cytoplasm and never make it into the chloroplast. You wish to determine whether the two proteins go into the chloroplast by the same or different pathways, how would you do this? Observe the localization of protein B in mutant A and the localization of protein A in mutant B. If they don't localize normally in the other mutant, indicates that they are in the same pathway.
6. What is an advantage of using GFP over immunohistochemistry for tracking protein localization? What is a disadvantage? GFP allows the real-time imaging of protein diffusion throughout the cell. Using FRAP or photobleaching, one can test the diffusion or sequestration of a protein during different cellular processes. A disadvantage is that GFP is a 27 kDa protein and adding it onto your protein of choice might disrupt its normal activity.
7. List whether the following assays look at a single-cell or a population: western blot for protein orientation, immunofluorescence assay for protein orientation, radio-labeled pulse-chase asssay followed by lysis and fractionation, and vesicle fusion assay. Western, population; immunofluorescence, single-cell; pulse-chase followed by gel, population; and vesicle fusion, population.
8. A protein localizes to the outer nuclear membrane. Propose how it got there and what experiments you could do to test this hypothesis? The outer nuclear membrane is continuous with the ER membrane; therefore proteins inserted into the ER membrane can move to the outer nuclear membrane by diffusion. You can test this using a temperature sensitive mutant for Sec61, SRP, etc. or by pulse-chase experiments to see how these proteins move from the cytoplasm to the nuclear membrane.
9. You have developed a method to specifically label a transmembrane protein kinase receptor at time t=0. At t=20 minutes, 100% of this protein localizes to internal membrane compartments. At t=40 minutes, 90% of this protein localizes to the plasma membrane (i.e., only 10% to internal compartments). However, at t=60 minutes, 30% of the protein localizes to internal membrane compartments. Provide an explanation for these observations. At t = 20 minutes, newly synthesized

receptor is still being processed and has not yet been brought to the plasma membrane. At t = 40 minutes, most of the receptor is on the plasma membrane and 10% has either been internalized again or not yet made it to the membrane. At t = 60 minutes, 30% of the receptors have been internalized by endocytosis upon ligand binding, i.e. **receptor-mediated endocytosis**.

10. Explain the molecular mechanisms of rigor mortis, when muscles become rigid after death. What would happen if muscles were injected with ADF-cofilin after death? When the cells die, they no longer have ATP to maintain the ATPase that pumps Ca^{2+} into the sarcoplasmic reticulum. Thus, calcium levels rise, leading to troponin to permanently move tropomyosin off the actin binding site. This allows myosin to bind to actin and because no ATP is present (cells are dead), it won't eventually dissociate as it normally does. This will cause the muscles to become rigid. The muscles should relax if injected with ADF-cofilin because it would break apart the actin filaments.
11. What is the difference between smooth and skeletal muscle? Why do you think the systems were designed this way? Skeletal muscle involves the contraction of a sarcomere unit while smooth muscle involved reduction is smooth muscle cell size. Smooth muscles use calcium binding to calmodulin and activation of MLC kinase while skeletal muscles use movement of troponin and tropomyosin to allow myosin/actin interaction.
12. Why would you not want to just have Ca^{2+} or only ATP activate muscle contraction? Having a second layer of regulation allows the system to be more flexible. In addition, ATP is needed for other cellular processes, as is calcium, so needing both to initiate a reaction can protect against false-positives, which would be problematic in the case of muscle contraction.
13. If you block minus-end directed motor proteins, what phase of mitosis is affected? Plus-end directed? Blocking minus-end directed motor proteins prevents the kinetochore from moving the chromosomes toward the spindle poles during anaphase A. Blocking plus-end directed would affect polar microtubules from separating, halting anaphase B.
14. What would happen to vesicle trafficking if Rab-GTP could not be hydrolyzed to Rab-GDP? It would cause an slight increase in vesicle trafficking because Rab would no longer be under the regulation of Rab-GDI, GDI dissociation factor, and Rab-GEF.
15. Sar1 is mutated in a strain of *S. cer* (yeast) cells so that it no longer binds to the membrane. What defects will this cell have? How would you make these cells healthy again? You try several experiments and the cells remain sick. Talking to the grad student you work under, he tells you that these cell express a non-exchangeable GDP analog. Why are your cells still sick? This cell's secretory pathway would be blocked since COPII vesicles will no longer be able to form. You could overexpress normal Sar1, which could bind the membrane in response to being converted from Sar1-GDP to Sar1-GTP. If the cell express a non-exchangeable GDP analog, this would keep Sar1 in its GDP bound form no matter if it is mutated or normal, preventing membrane binding and recruitment of adaptins, etc.
16. While scuba diving on a trip in Malaysia, you happen upon a interesting looking microbe called *M. Herrin*. After taking it back to the lab, you discover that it appears to have abnormal cell movement. Instead of dragging its trailing edge, parts of the it break off and then the cell moves forward and gobbles up membranes in front of it left by other *M. Herrin* cells. Curious as to whether there is something different about its actin dynamics, you make an actin-GFP construct and express it in cells. What experiment would you do to test if actin filaments in this species form filaments like normal? Why would this mechanism of cell movement be detrimental to a cell in the long run? Express actin-GFP in the cells and photobleach both the trailing and leading edge. If you do not see polymerization at the front end (indicating by a static bleached area with increased fluorescence at the from) or depolymerization at the trailing edge (static fluorescence area with decreased fluorescence at trailing edge), this would indicate that a different mechanism is used to allow the cell to move. This type of cell movement would be detrimental because if no new membrane could be found, the cell would eventually die as all its membrane breaks off during movement.
17. Diagram the following, include components and ordering of events:For these problems see the figures in this handout and Prof. Shen's notes.
    - Actin polymerization and factors that facilitate nucleation, binding, branching and unbinding of actin. Properly label ATP- and ADP-actin. What would happen if you added cytochalasin?
    - Microtubule assembly, including nucleation site and its structure.
    - Vesicle budding, include the order of events.
    - Vesicle fusion, include order of events. What would happen if you had a temperature sensitive NSF and SNAP mutants and switched to the *non-permissive* temperature? Draw the location of vesicles right before, just after and a

long time after the switch.

- LDL receptor endocytosis, draw major players and what happens if LDL receptor cycling is inhibited.
- Draw the to and from locations of vesicles with the following coats: COPI, COPII, and clathrin. What would happen to LDL endocytosis if you had a shibire mutant?
- Sacromere and muscle components, both before and after contraction. Include location of Ca^{2+} binding.

Figures and Tables

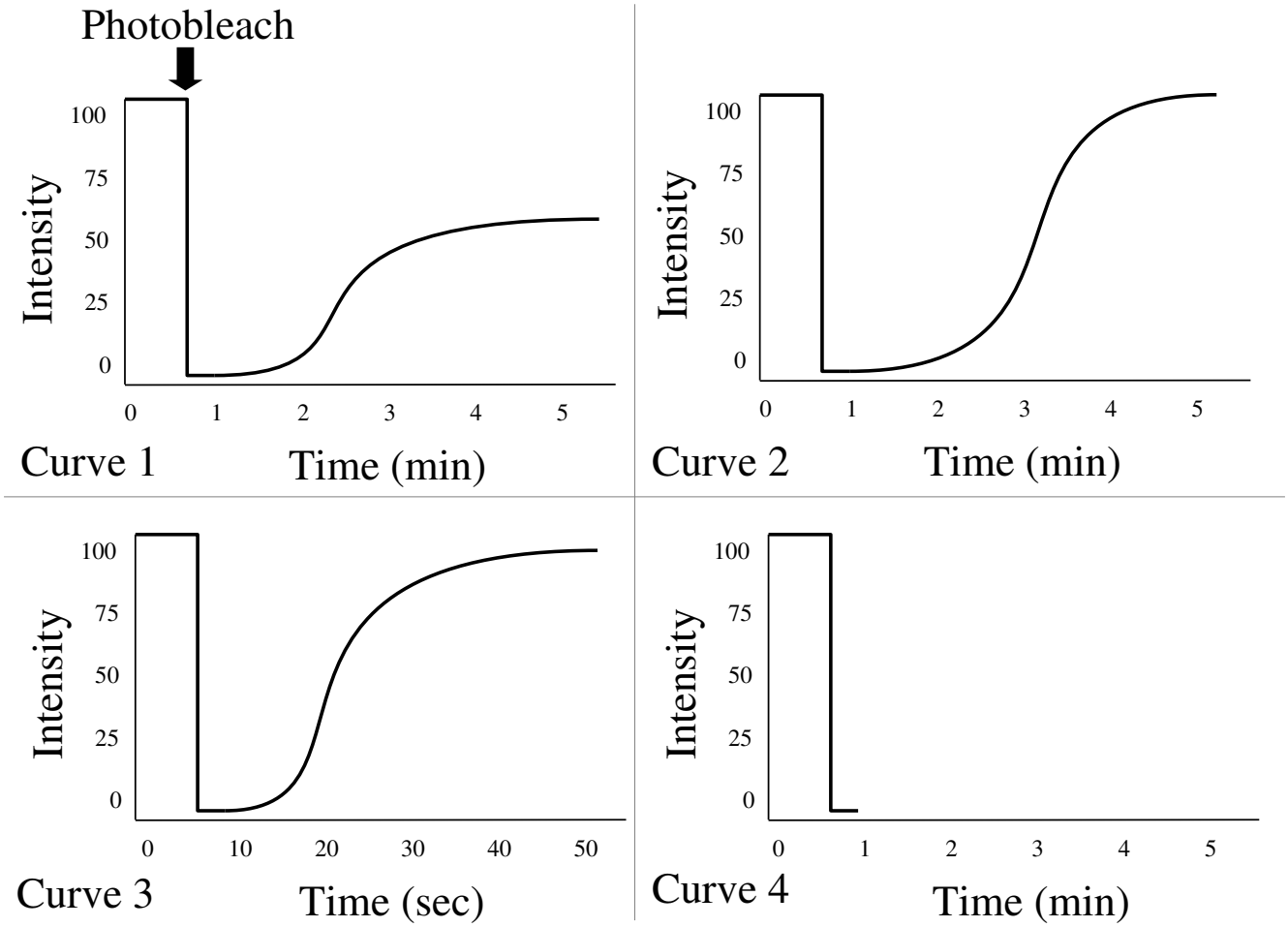


Figure 13 | Problem: FRAP of nuclear proteins
See text for details.

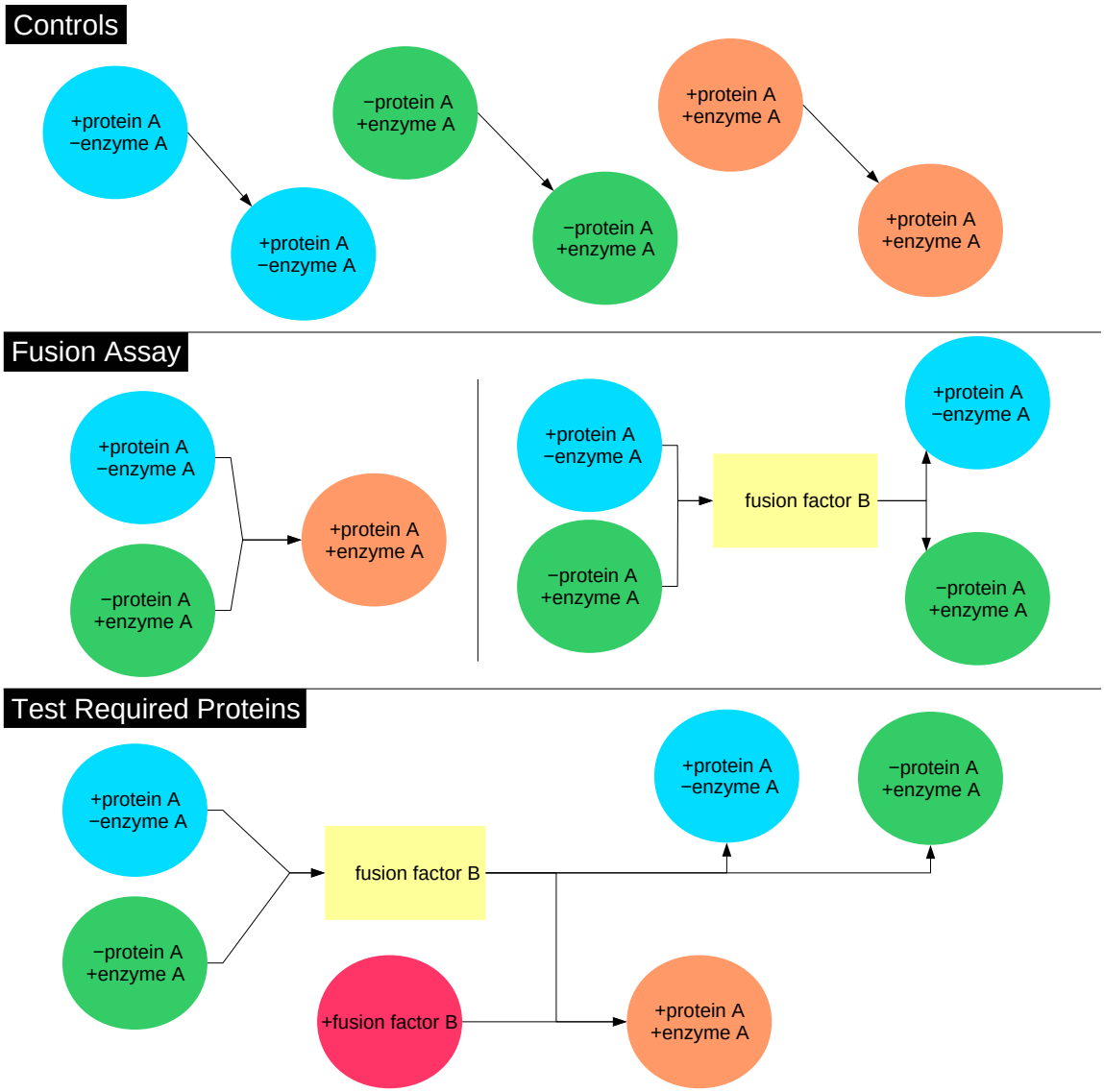
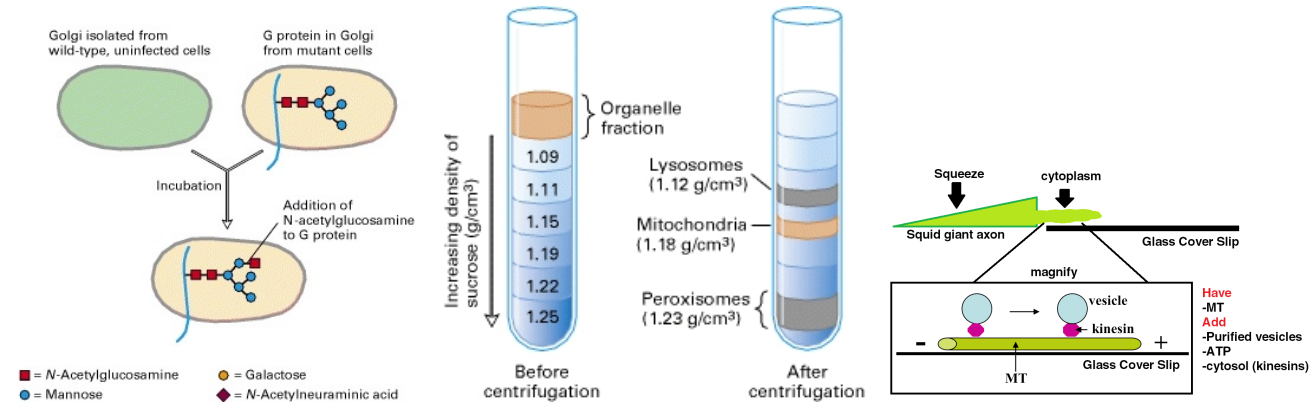


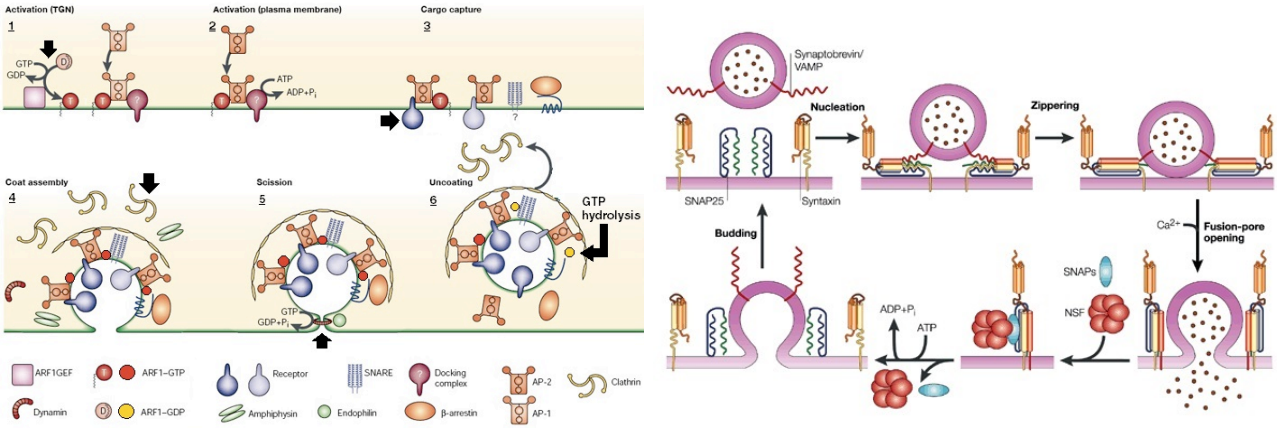
Figure 14 | Fusion Assay
Controls Check that a vesicle without enzyme A can't produce required protein A, conversely, vesicle without protein A can't produce required color because no protein A present. Normal cells with both produce a cell with a different color. **Fusion Assay** Put both vesicles missing either protein A or enzyme A in a tube and see if they fuse, if so, then vesicle fusion is normal. Now suppose fusion factor B is needed that these purified vesicles don't have, no fusion will occur. **Test required proteins** Add back in media with fusion factor B, vesicles should fuse and you can then say fusion factor B is required and study it more!



(a) cell fusion assay (b) velocity centrifugation (c) kinesin transport experiment

Figure 15 | Experiments

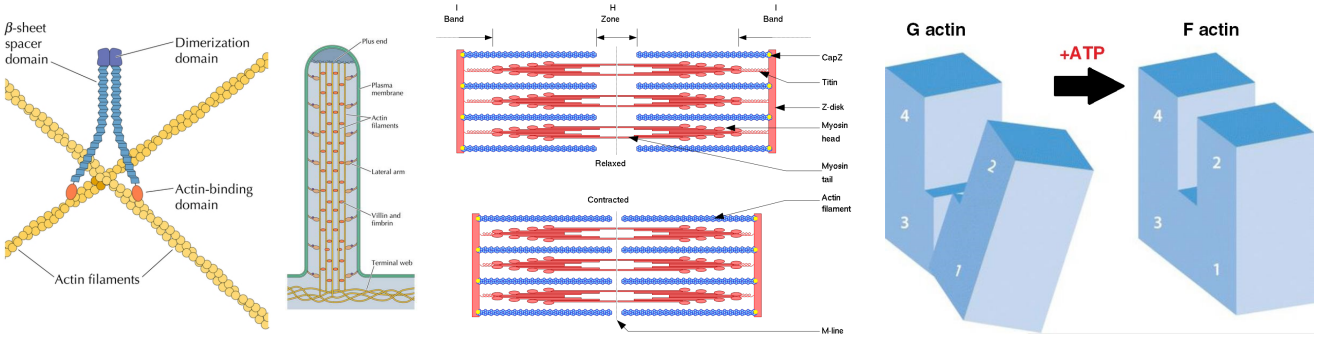
a| See text. b| Create a sucrose gradient that divides fractions based on size and composition, allows you to separate different proteins or parts of a cell. c| Squeeze out axoplasm and then observe vesicle movement in microscope. Add different factors to promote or inhibit vesicle movement.



(a) Vesicle Budding (b) vesicle fusion

Figure 16 | Vesicle Budding and Fusion

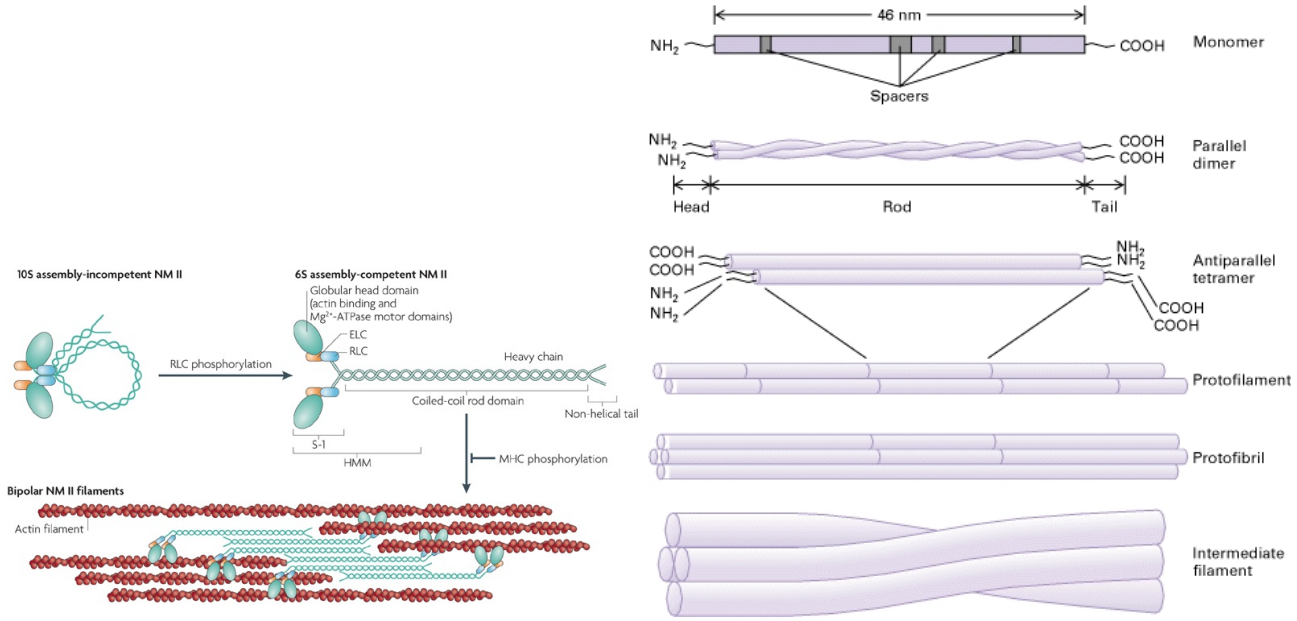
Vesicle budding involves ARF, clatherin, adaptins and a receptor protein. docking requires Rab, Rab effectors, and SNAREs. Vesicle undocking requires NSF, SNAP, and Rab-GTPase activity.



(a) actin bundle/network (b) muscle contraction (c) actin transition.

Figure 17 | Actin

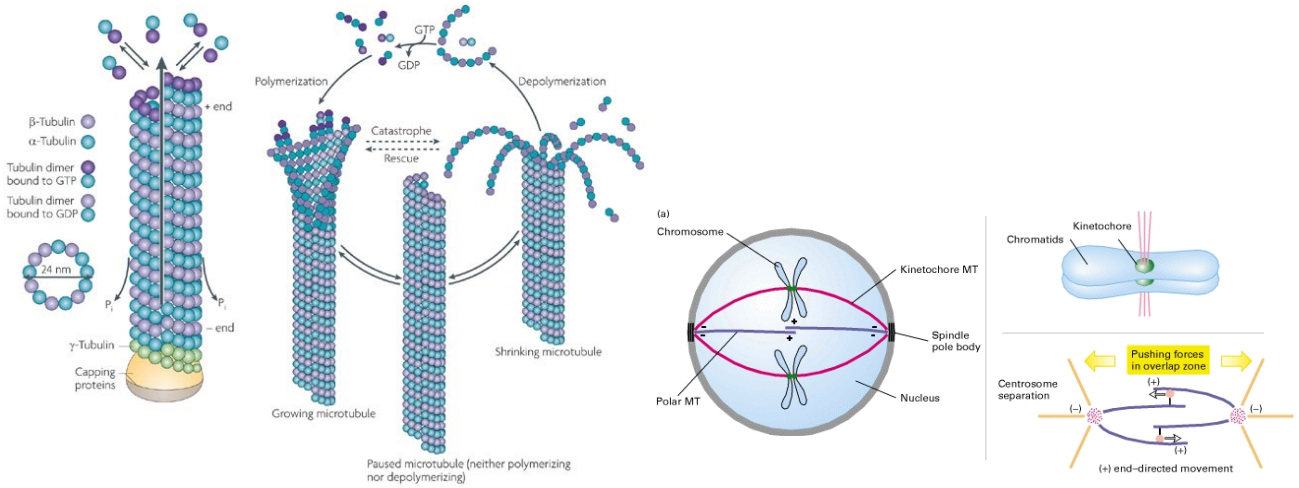
b: Muscle contracts when calcium removes tropomyosin block of actin binding, allowing myosin to slide actin past it. Titin functions as a spring to return the sarcomere back to its relaxed state. c: binding of ATP causes change in actin conformation.



(a) Myosin (b) intermediate filament assembly

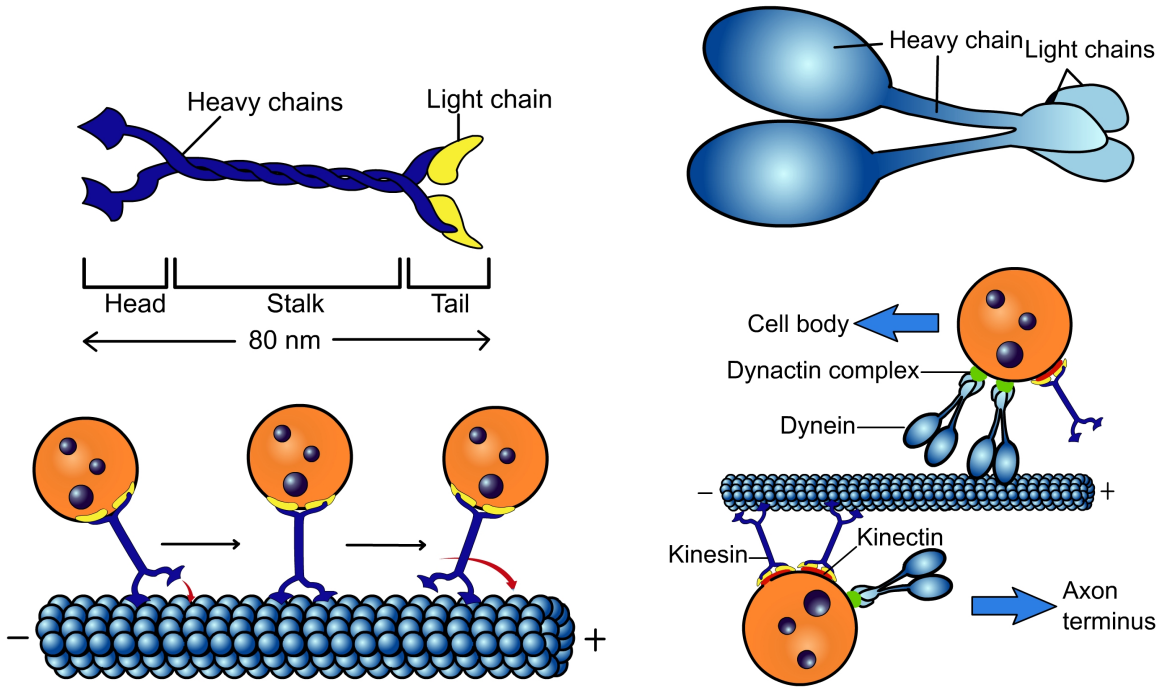
Figure 18 | Myosin and Intermediate Filaments

Myosin II is involved in muscle contraction. Intermediate filaments help with nuclear structure and other cell cytoskeleton.



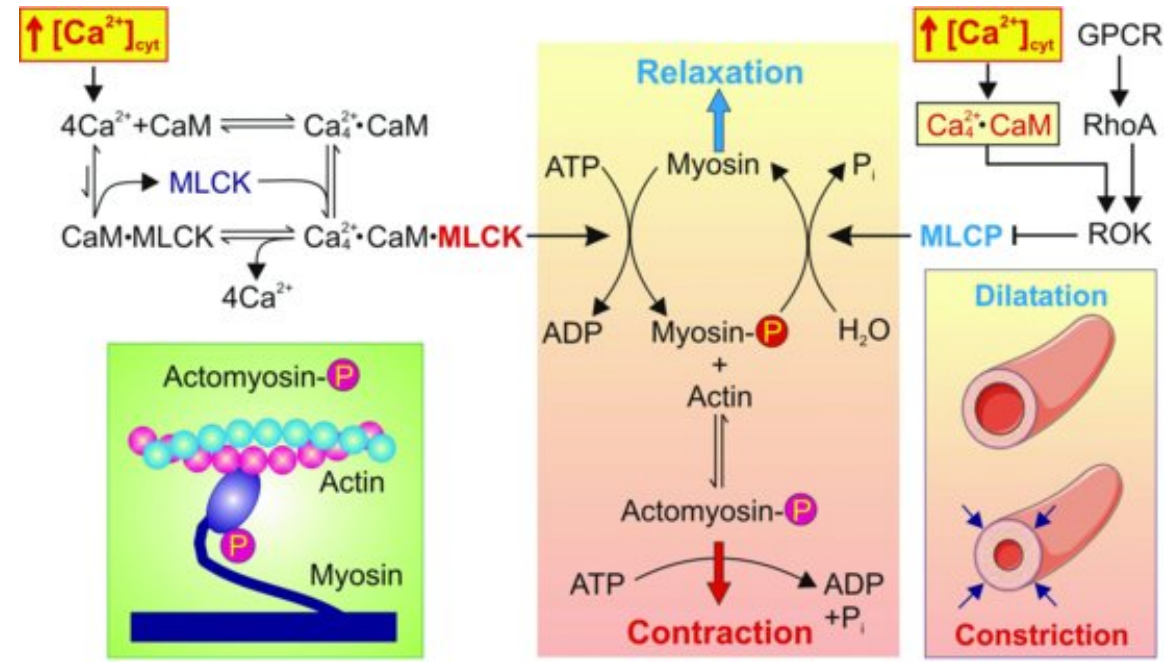
(a) microtubule assembly (b) MT in cell cycle

Figure 19 | Microtubules
Microtubules are assembled by β-tubulin-GTP binding at the plus end. The minus end is anchored in the MTOC.

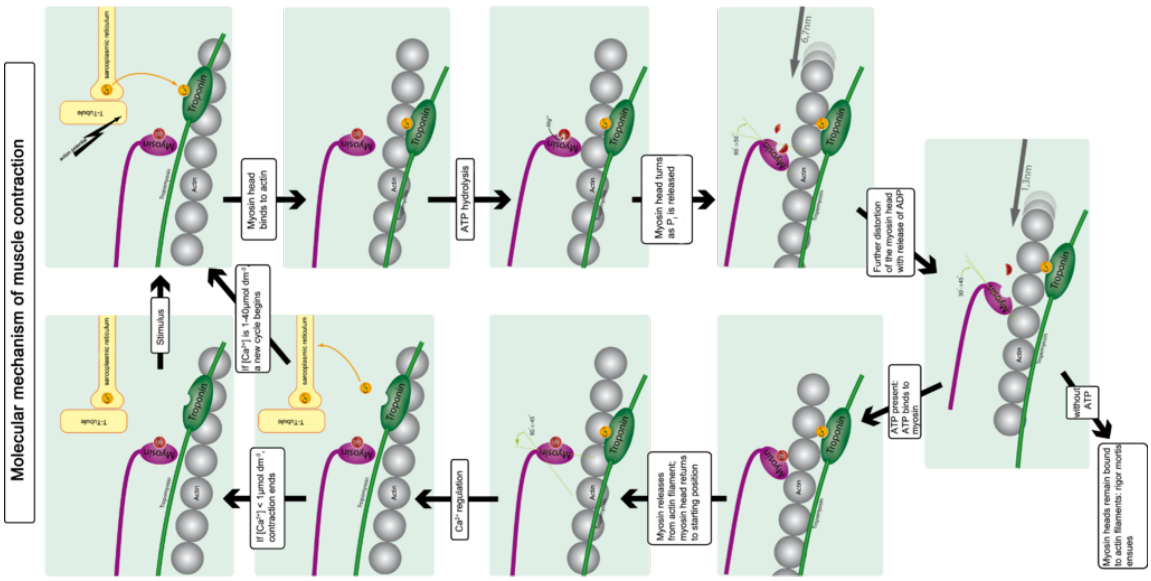


(a) Kinesin (b) Dynein

Figure 20 | Motor proteins
Structure or kinesin and dynein. Kinesin and dynein are plus and minus end directed motor proteins, respectively, that move along MTs.



(a) Smooth Muscle



(b) Myosin Mechanism

Figure 21 | Motor proteins
Calcium activates a cascade that eventually leads to muscle contraction. Myosin hydrolysis of ATP allows it to move actin past it.

Week 3 Cell Cycle and Signal Transduction

Readings

Cooper 4th: p. 649-678 605-637
Cooper 5th: p. 653-681 609-640

Overview

This week Prof. Shen discussed the cell cycle and signal transduction (in the context of EGFR). We will go over the basics of CDK, cyclin and microtubules in mitosis and make sure we understand certain techniques discussed in class such as cell fusion and immunoprecipitation.

Concepts

- CDK/cyclin regulation of and events in the cell cycle.
- Experimental (oocytes, cell fusion, yeast, etc.) determination of factors promoting or inhibiting stages of cell cycle.
- Signal transduction, e.g. know EGF and MAPK cascade. Also recall experiments (Drosophila, etc.) used to determine pathway.
- Signal amplification, regulation, and modularity in signal cascades.
- Second messenger (e.g. cAMP or Ca^{2+}).
- Feedback loop, e.g. CDK1/cyclin causing degradation of cyclin.

Terms

cell cycle

- mitosis
- interphase
- cytokinesis
- G_1 phase
- S phase
- G_2 phase
- M phase
- G_0 phase
- DNA content
- DAPI
- Hoechst 3342
- START
- restriction point
- nutrients
- mating factors
- cell size
- quiescent stage
- fission yeast
- oocytes

Model organisms

- Drosophila
- Yeast
- Sea Urchin

cell cycle checkpoints

- chromosome misalignment
- damaged DNA
- G_2 checkpoint
- unreplicated DNA
- checkpoint kinases
- p53
- spindle assembly checkpoint
- aneuploidy
- MCM helicase

cell cycle regulation

- maturation promoting factor
- cdc mutants
- Cdc28
- cdc2
- CDK
- cyclin
- Cdk1
- threonine-161
- tyrosine-15
- threonine-14
- Wee1
- Cdc25C
- G_1 cyclins
- Cln's
- Clb's
- Cdk1-7
- A-type cyclins
- B-type cyclins
- D-type cyclins
- E-type cyclins
- CAKs
- CKIs
- proteolysis
- tumor suppressor gene
- oncogene
- Fos
- Jun

M phase

- prophase
- prometaphase
- metaphase
- anaphase
- telophase
- sister chromatids
- centromere
- kinetochore
- centrosomes
- mitotic spindle
- nuclear envelope breakdown
- nuclear lamin phosphorylation
- chromosome condensation
- spindle formation
- Golgi fragmentation
- polar microtubules
- astral microtubules
- chromosomal microtubules
- kinetochore microtubules

cytokinesis

- contractile ring

signal transduction

- peptide hormones
- growth factors
- insulin
- NGF
- EGF
- PDGF
- cAMP
- second messenger
- adenylyl cyclase

receptor tyrosine kinases

- dimerization
- autophosphorylation
- SH2 domains

- EGFR
- Ras
- Raf
- MEK
- ERK
- SOS

- Sevenless
- Drk/Grb2
- Ras-GAP
- Ras-GEF
- cAMP
- signal amplification

- cGMP
- guanylyl cyclase
- phospholipids
- calmodulin
- protein-tyrosine phosphatase

- serine/threonine kinase
- intracellular signal transduction
- NF-κB
- IκB

Techniques

For each technique, you should know when is appropriate to use it, what its limitations are, and a particular cellular process that was used to study it.

- Immunoprecipitationttached antibody against protein A to beads, mix beads with lysed cells, spin down beads (pellet at bottom) and wash away cell contents. Run on gel to see what associated with protein A.
- Cell fusion assayake cells from different stages in cell cycle, fuse their membranes, and see what happens to each cells nuclei.
- Flow cytometryr fluorescence-activated cell sorting (FACS) allows you to dye cells based on a specific property (e.g. DNA content) and then sort them *quantitatively* based on that property (e.g. 2n or 4n DNA content).
- Microinjection studiesake cytoplasm from one source (say xenopus eggs) and inject it into another source (e.g. oocyte). See what happens. Used to purify MPF (CDK/cyclin complex).
- Temperature sensitive mutantsutants that exhibit a particular phenotype only when the temperature is raised or lowered. Normally due to point mutation that causes a protein to unfold more rapidly at different temperatures.
- Cell synchronizationdd nocodazole to cells, depolymerizes MTs arresting cells in M phase.
- Radioactive thymidinenly dividing cells incorporate thymidine into DNA, help mark which cells are in cell cycle.

Rao and Johnson Experiments

They first synchronized cells in different cell cycle stages and then fused them together and looked at what happened. See figure Fig. 26. They have several conclusions:

- Mitotic cells (only) contain an M-phase promoting factor (MPF), induces M phase events. e.g. CDK/cyclin complex present.
- S phase activating factor present in S phase cells.
- After passage through S, there is a block that prevents re-replication. e.g. no MCM helicase.
- S phase activating factor disappears during G2. e.g. degradation of cyclins.

Cell cycle

G_1 phase

- Cells grow and build up necessary proteins, etc. for division and replication.
- If sufficient nutrients are available and correct size reached, they pass through the restriction point (animals) or START (fungi).
- Cells halted in G_1 by mating or other factors are not necessarily G_0 if they can still divide once the inhibition is removed.

G_0 phase

- Cells that have stopped proliferating and are waiting for a growth or other signal to re-enter the cell cycle.
- Application of EGF or other growth factors/hormones can push cells back to G_1.

S phase

- Chromosomes are replicated and remain diffuse.
- MCM helicase prevent re-duplication before M phase finished.

G_2 phase

- Cell continue to grow!
- Nuclear envelope is intact.
- MTOC generates few microtubules that extend to the cell periphery.

Prophase

- CDK1/cyclin complex activated.
- Mitotic spindles begin to form and separate along poles.
- Two active MTOCs and more, shorter microtubules (in-

creased nucleation capacity).
- Chromosomes condense from CDK phosphorylation of condensins.
- Nuclear envelope breaks down, from CDK phosphorylation of nuclear lamins.
- Golgi fragments due to CDK1 phosphorylation of Golgi proteins.
- And much more!

Metaphase

- Chromosomes line-up on the metaphase plate.
- Sister chromatids are each connected to a spindle pole, when tension equal, anaphase starts.
- Nocodazole can destabilize MTs, causing arrest in metaphase.

Anaphase Two stages: A and B.

- A: Kinetochore mictotubules attached to chromosomes shorten, chromosomes pulled by minus-end directed microtubule motor protein (dynein-like). Remember FRAP experiment to prove this?
- A: Middle motor kinesin help depolymerize microtubules, then *bind* but *do not* move along microtubules.
- B: Polar microtubules elongate and kinesin push them apart, thus causing them to slide by one another.
- B: BimC is one such kinesin, forms anti-parallel dimers.
- B: Astral microtubules shorten, mitotic spindle pulled toward cell cortex by astral-attached dyneins.

Telophase

- Chromosomes decondense.
- Nuclear envelope reforms.
- Spindle disassembles.

Cytokinesis

- Animals: Contractile ring forms, splits cell in half.
- Plants: new cell wall forms, eventually creating two separate cells.

CDK and cyclin

CDK identified via micro-injection experiments with oocytes and through temperature sensitive mutants in yeast. Work with sea urchin embryos showed that cyclin oscillates with the cell cycle.

- CDK1 levels constant during cell cycle.
- Cyclin A levels oscillate, highest during mitosis.
- CDK1 starts dephosphorylated.
- CDK1 associates with cyclin A in G_2 and is phosphorylated at Thr-161 (activating) and at Thr-14/Tyr-15 (inactivating) by Wee1.
- Phosphatase (Cdc25C) removes Thr-14/Tyr-15 phosphates, activating CDK1/cyclin A.
- CDK1/cyclin A activate enzymes that destroy cyclin A, feedback. *Cyclin A must be degraded to progress from metaphase to anaphase.*
- CDK1/cyclin do the following: chromatin condensation, nuclear envelope breakdown, Golgi fragmentation, increase MT instability, activate proteases and other things.

CDK/cyclin regulators

- CKI binds CDK1/cyclin complex, inhibiting activity.
- CAK kinase phosphorylates Thr-161, promoting activity.
- Wee1 kinase phosphorylates Thr-14/Tyr-15, inhibiting activity.
- Cdc25 phosphatase removes Thr-14/Tyr-15 phosphate, promoting activity.

Cyclins all associated with a CDK (in yeast CDK1). See Fig. 27.

- G_1 cyclins: help pass through START.
- S phase cyclins: initiate DNA replication
- Mitotic, B-type cyclins: initiate M phase, accumulate in G_2. Need to degrade to finish mitosis.
- Cyclin-D (CDK4) promotes cell growth.
- Cyclin-E (CDK2) allows cell to pass restriction point.
- Cyclin-A (CDK2 then CDK1) induces replication (CDK2) then promotes M phase (CDK1).
- Cyclin-B (CDK1) promotes anaphase and mitosis finish.

Cell signaling

Non-proliferating cells arrest in G_0. Growth factors help cells in G_0 go to G_1 by promoting synthesis of cyclins amongst other proteins. PDGF and EGF are growth factors that can be isolated from serum, soluble protein fraction from blood, and induce cells to proliferate when added to media.

Let's talk about EGFR! Its one of many growth factor receptors, initiating a series a intracellular events:

- EGF binds to EGFR.
- EGFR dimerizes, bringing tyrosine kinase domains in close proximity.
- EGFR kinase domains autophosphorylate one another.
- Drk/Grb2 binds to EGFR via SH2 domains (recognize phosphorylated tyrosine).
- SOS associated with Drk/Grb2 via SH3 domain, binds Ras-GDP (on membrane).
- Ras-GDP converted to Ras-GTP via SOS (Ras-GEF) action.
- Ras-GTF binds Raf (MAPKKK), activating it.
- Raf phosphorylates MEK (MAPKK), activating it.
- MEK phosphorylates ERK (MAPK), activating it.
- MAPK translocates to the nucleus, phosphorylating transcription factors and inducing growth.
- e.g. Cyclin-Ds are synthesized, promoting passages through restriction point via CDK/cycD complex. Cell cycle starts!

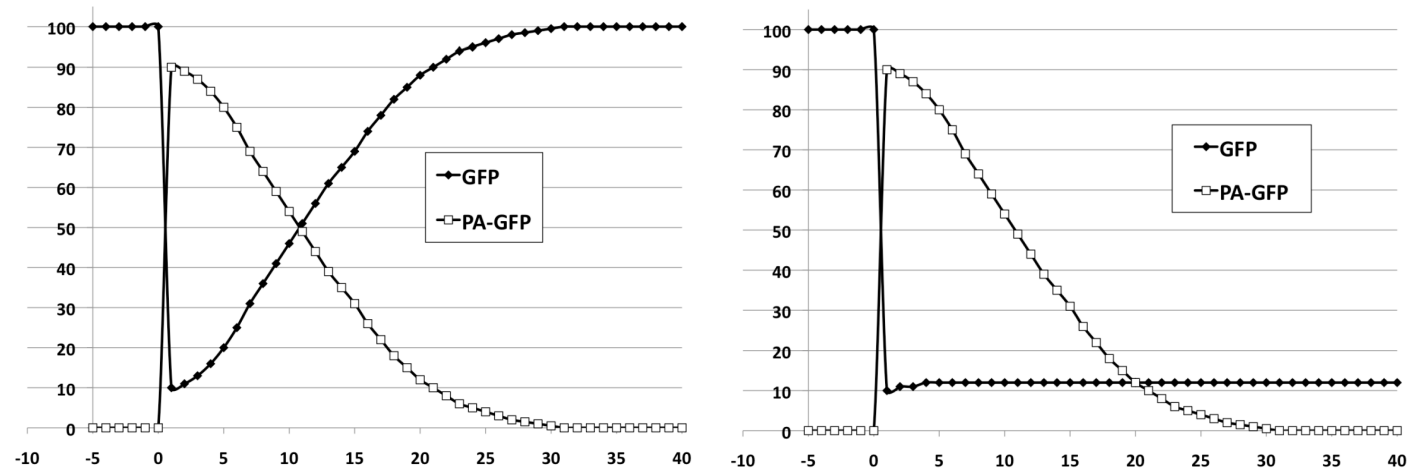
Problems

If there is any confusion about the questions, shoot me an email or talk to me after class. Remember, draw out what a pathway, interaction, or what-have-you looks like if you get confused.

1. You want to test protein turnover of nuclear pore proteins using two strategies. For the first, you use standard GFP and conduct FRAP. For the second, you use photoactivatable GFP (PA-GFP) which is invisible until you pulse with a laser. In each case the fluorescent protein is fused to the same nuclear pore proteins, with a laser pulse at 5 minutes of imaging (although these two experiments are obviously conducted separately). See Fig. 22.
   - What is protein A doing? Explain findings in terms of the GFP and PA-GFP response.
   - Protein A is bound to the nuclear pore and leaves to make space for new protein.
   - Explain the similarities and differences between protein A and B.
   - The protein B is leaving the nuclear pore, like protein A, but new protein is not being delivered there. This could be because protein B needs to have a signal to continually localize to the nucleus (e.g. extracellular).
2. This is a two part question investigating signal transduction. You are trying to dissect what a particular pathway does. To do this, you test each protein in the pathway with the following techniques: SDS-PAGE gel from whole cell extracts (Western blot), for association with protein 4 (co-immunoprecipitation), phosphorylation state (using radioactive ATP or P^{32}-ATP), and localization (GFP-tag). You test pathway response both without and with a known ligand being added.
   - For each protein (1-4) in Fig. 23, say what happens to it upon ligand stimulation of the pathway. Note, removal of a phosphate group causes a protein to run slower on a gel. **Protein 1:** This protein is normally degraded, but pathway ligand treatment allows this protein to be stabilized and to translocate to the nucleus. **Protein 2:** This is a plasma-membrane associated protein. Ligand addition allows it to recruit protein 4. **Protein 3:** In the absence of ligand, this protein is phosphorylated and associates with protein 4. Ligand treatment prevents phosphorylation and releases protein 4. **Protein 4:** Ligand addition causes protein 4 to be targeted to the plasma membrane, and changes its associations from binding to protein 3 to instead associating with protein 2.
   - Next, you isolate a gain-of-function mutant in a fifth protein (protein 5) that results in the pathway being constitutively active regardless of whether the ligand is present. This is illustrated in Fig. 23. The MUT column in the gel indicates this was a mutant sample, ligand addition doesn't matter. Propose what this mutant does and how it affects the pathway (e.g. what does protein 5 activate/inhibit). This neomorphic mutant causes protein 4 to be cleaved. Although this prevents it from associating with either protein 3 or protein 2, this cleavage allows protein 1 to be stabilized and translocate to the nucleus, where it can activate transcription.
   - Based on the previous two experiments, propose a pathway (e.g. protein 1 inhibits protein 4, etc.). Ligand$\rightarrow$ Protein 2$\dashv$ Protein 4$\rightarrow$ Protein 3$\dashv$ Protein 1$\rightarrow$ Gene expression. Protein 6$\dashv$ Protein 3.
3. You prepare extracts from Xenopus eggs that will undergo replication and mitosis after addition of Ca^{2+}. See Fig. 24.
   - You take out samples from the extract after inducing with Ca^{2+} at t=0 minutes. You then run a gel and blot for cycle E and B. Explain why the two cyclins appear at distinct times using knowledge of when these particular cyclins act. Cyclin E drives G1-S and appears in G1. Cyclin B drives G2-M and appears in G2. In the activated extracts, G1 happens first, then G2 later.
   - Cyclin E and B are immunoprecipitated and a CDK kinase activity assay is done at each time-point indicated. Based on the figure, explain the kinase activity relative to the cyclin protein levels and why there is a sharp peak in the activity (as opposed to a gradual rise like the cyclins)? Accumulation of cyclin alone does not activate cyclin/CDK kinase activity. Removal of the inhibitory Tyr15 phosphorylation by cdc25 is required to activate the kinase. Cyclin levels gradually accumulate, but there is a switch to activate the kinase, driven by cdc25.
4. Why are both anaphase A and anaphase B important for accurate chromosome segregation? Anaphase A needed to separate sister chromatids. Anaphase B needed to move spindle poles apart–ensures that chromosomes get far enough away from the midzone so not caught by cytokinesis and correctly incorporated into 2 separate daughter cells.
5. Is the cell cycle rapidly reversible? No, once past `start' or the restriction point, the cell cycle will progress through S, G_2, and M phase before reaching G_1 again.
6. Would a truncated Raf lacking its Ras-associated regulatory domain be constitutively active? What is the molecular consequence of this mutation? This domain normally inhibits Raf activity. Removal of this domain would cause Raf to be active even if it did not associate with Ras. Raf would be constitutively active.

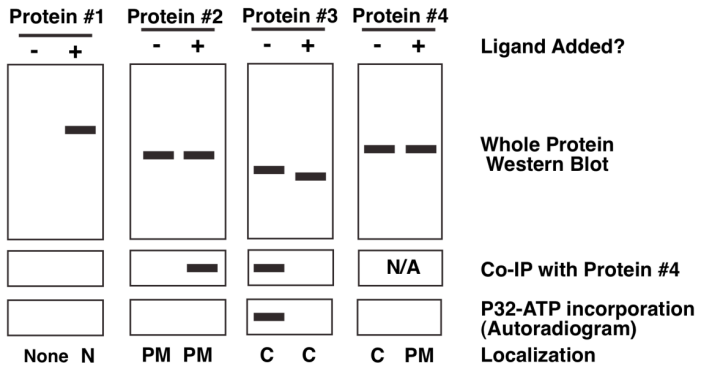
7. A MAP kinase kinase mutant changes the Raf phosphorylation site from serine to aspartate. What happens and is the protein now constitutively active? Aspartate mimics constitutive phosphorylation. This site is used to activate MAP kinase kinase during MAP kinase signaling, and thus would result in this constitutive activation.
8. What is the benefit of having multiple downstream molecules activated by a receptor as opposed to the receptor doing all the work itself? Allows different levels of regulation. If the pathway is turned on, but you want to regulate its response via another pathway, much easier to do if there are downstream molecules rather than turning off one receptor. In addition, a cascade allows amplification of the signal.
9. A mutation causes MAPK (ERK) to gain a cleavable signal sequence. How will this affect EGFR signaling? EGFR signaling would be disrupted since MAPK will be secreted and no longer present in the cytoplasm to respond to EGFR activation.
10. You have cells that contains a mutant CDK1 that is always active (Thr-14/Tyr-15 gone and Thr-160 always phosphorylated).
    - Your cells are growing too rapidly and as a quick solution, you express normal CDK1 in the cells. Nothing happens. Why not? The mutant CDK1 is **dominant** to the wild-type CDK1, the wild-type CDK1 will be normally regulated and thus won't prevent the mutant CDK1 from continuously initiating cell cycle events.
    - You decide to add CKIs and notice that the cell proliferation slows. Why might this be? The CKIs inhibit the CDK/cyclin complex and thus overexpressing them in cells with mutant CDK1 will cause the complex to be inhibited more than normal, leading to a slowing of cell cycle as less proteins are phosphorylated and a reduced number of cell cycle events occur.
11. Why do Cdc2 (CDK1) null mutants become large? Cdc2 activated CDK1 by dephosphorylating Thr-14/Tyr-15. Because they can't progress through G_2 and thus continue to grow but cannot divide.
12. Why do Wee1 null mutants become small? Wee1 no longer inhibits CDK1 activity, leading to premature entry into mitosis sans necessary growth in G_2. This leads to cells dividing before they can grow, so the daughter cells continuously become smaller each generation. Look at division after fertilization for an example in mammals.
13. Curious as to why some Wee1 mutants seem to be arresting in G_2, you sequence them and find that CAK is also inactive. Explain this finding. CAK phosphorylates Thr-161, leading to activation of CDK1. Without this, the cells will progress to G_2 and then become arrested as CDK1/cyclin complex is left inactive.
14. What is the key difference between the kinase activity of CDK1 and the EGF receptor? CDK1 is a Ser/Thr kinase while EGFR contains a tyrosine kinase domain.
15. What experiment could you do to show CDK1 and cyclin form a complex? Immunoprecipitation. Antibody against CDK1 or cyclin that is attached to a bead. Centrifuge and wash away other proteins then run protein attached to beads on a gel and image via western blot (anti-CDK1 or anti-cyclin) and total protein. If you see two bands, they are
16. Why should activation of CDK1/cyclin-A trigger cyclin A degradation? Negative feedback allows the system to be self-regulated and cause its response to be narrowly defined, as is the case with MPF activity.

Figures and Tables

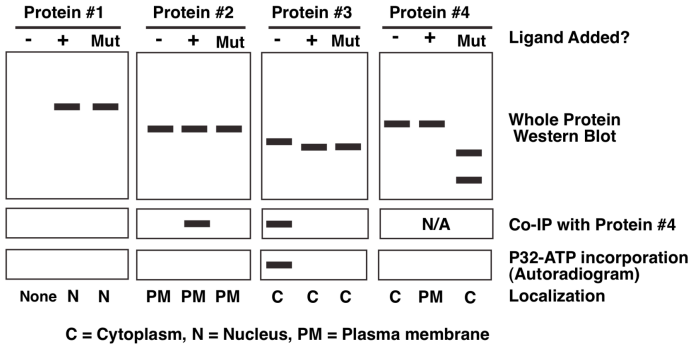


(a) Protein A (b) Protein B

Figure 22 | Problem: FRAP
Describe what protein A is doing that protein B is not. See text for details.

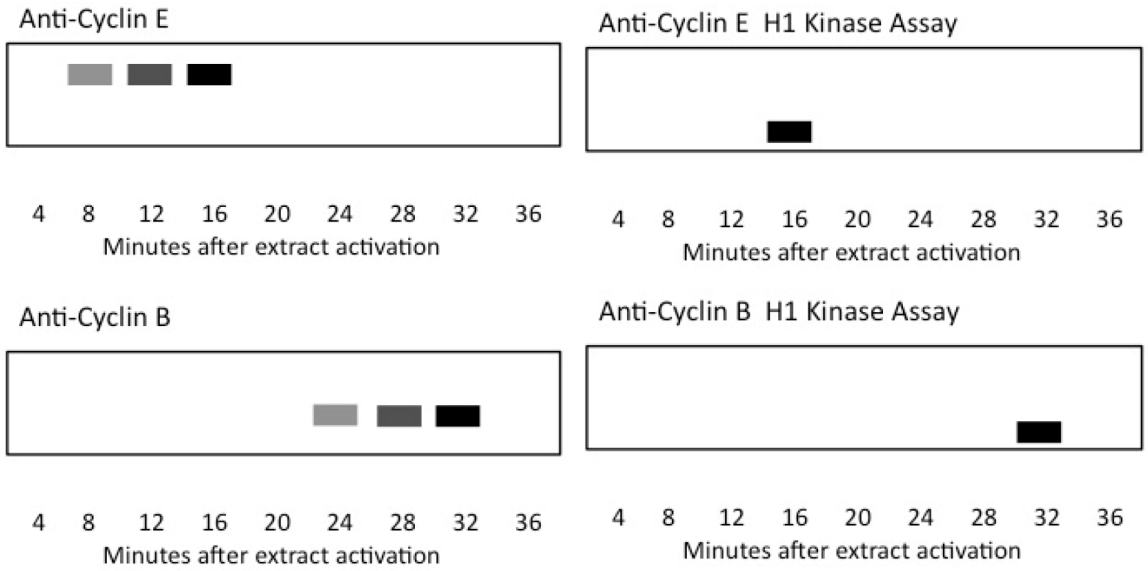


(a) Protein A



(b) Mutant

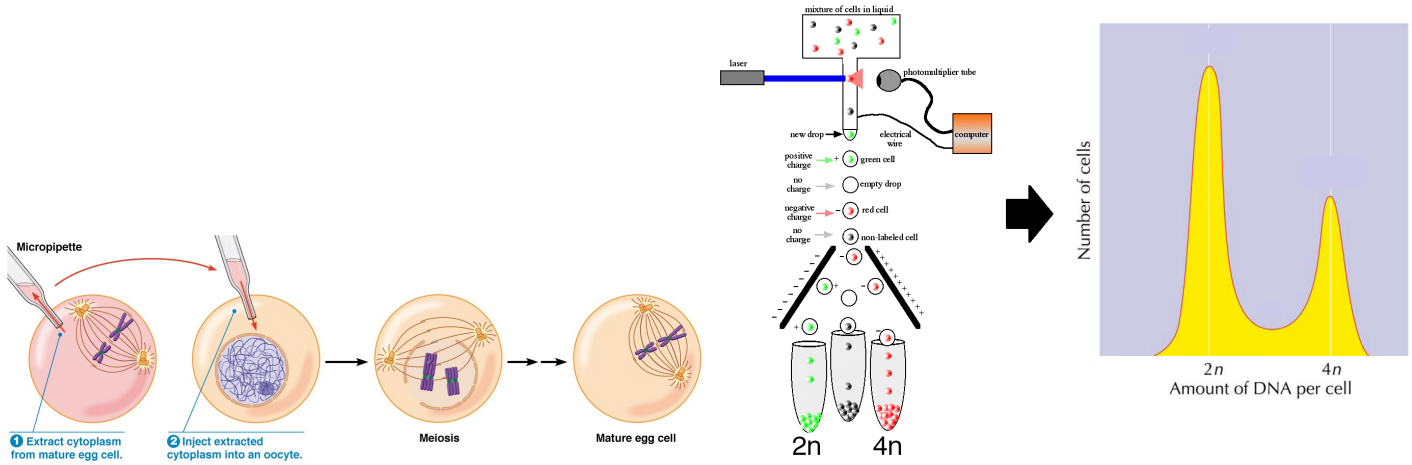
Figure 23 | Problem: Signaling Cascade
You test several proteins in a single cascade with a variety of techniques and wish to reconstruct the order of events and interactions within the pathway. See text for further details.



(a) Cyclin western blots (b) CDK kinase activity assay

Figure 24 | Problem: cell cycle in xenopus

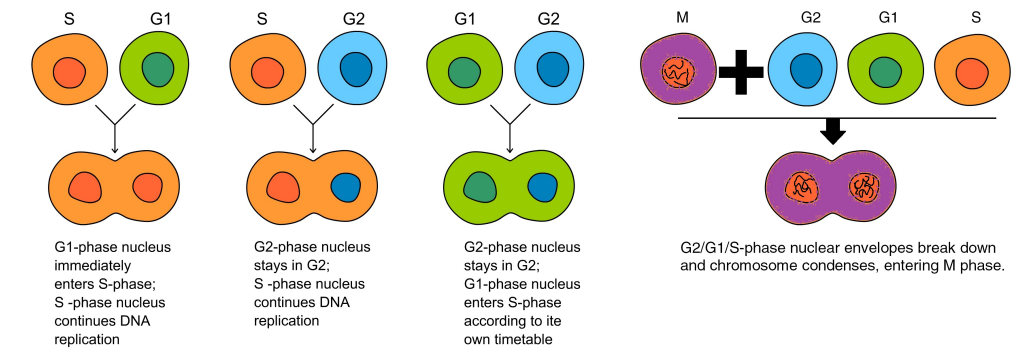
You obtain extracts from xenopus eggs that can be induced to enter the cell cycle and undergo mitosis after addition of Ca^{2+}. You then check levels of cyclin E and B in addition to CDK1 activity. Explain the data seen, particularly why cyclin levels fall (specific to these cyclins) and why CDK's activity isn't 1-1 correlated with cyclin levels.



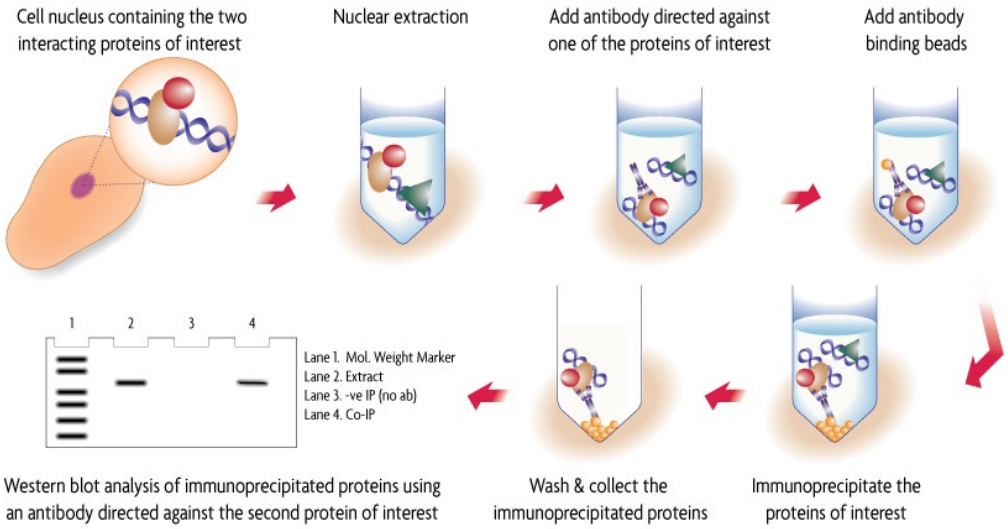
(a) Oocyte microinjection (b) Flow cytometry

Figure 25 | Experiments

Injection of cytoplasm or purified protein into oocytes was used to identify particular proteins involved in cell cycle. Flow cytometry can be used to separate cells based on a variety of factors, in this case DNA content.



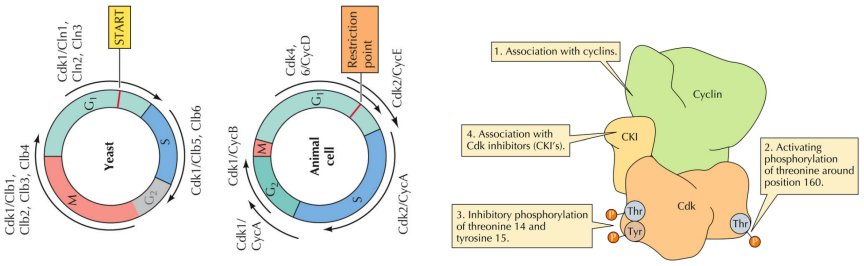
(a) Cell fusion assay



(b) Immunoprecipitation

Figure 26 | Experiments

The cell fusion assay can give you an idea of cell cycle inducers and inhibitors. Immunoprecipitation helps you determine what proteins associate at particular times or after certain stimuli (e.g. growth factors) are applied.



(a) CDKs and cyclins in cell cycle (b) CDK/cyclin complex

Figure 27 | CDKs and cyclins

A variety of CDKs and cyclins control cell cycle. CDK is regulated at multiple levels, both by kinases (Wee1 and Cdc25C) and proteins (cyclin and CKIs).

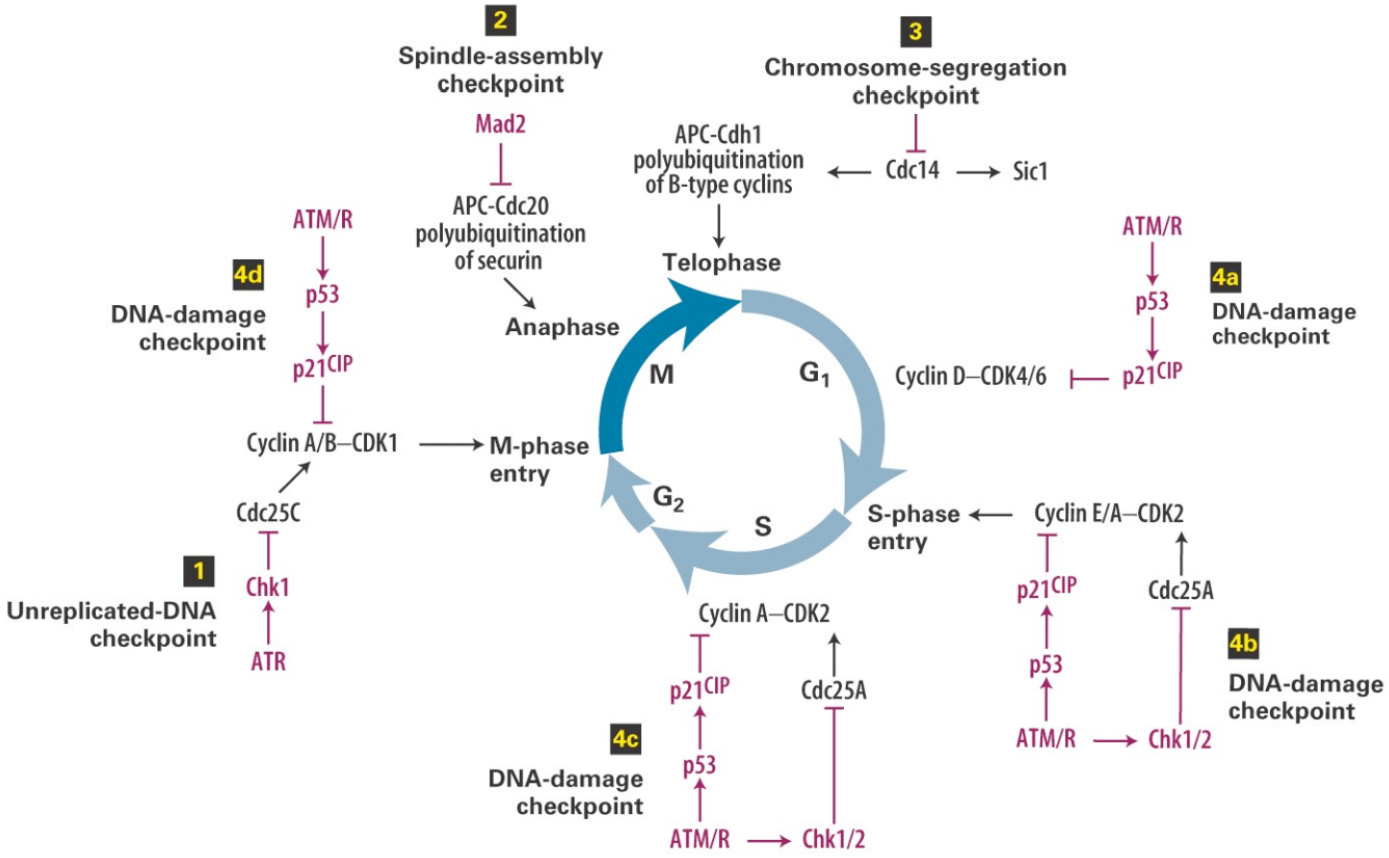
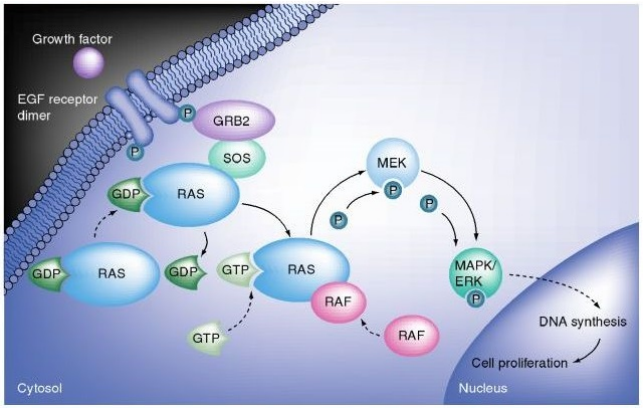
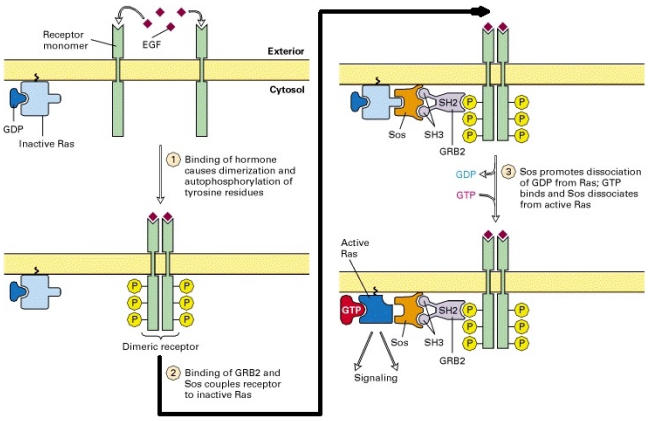


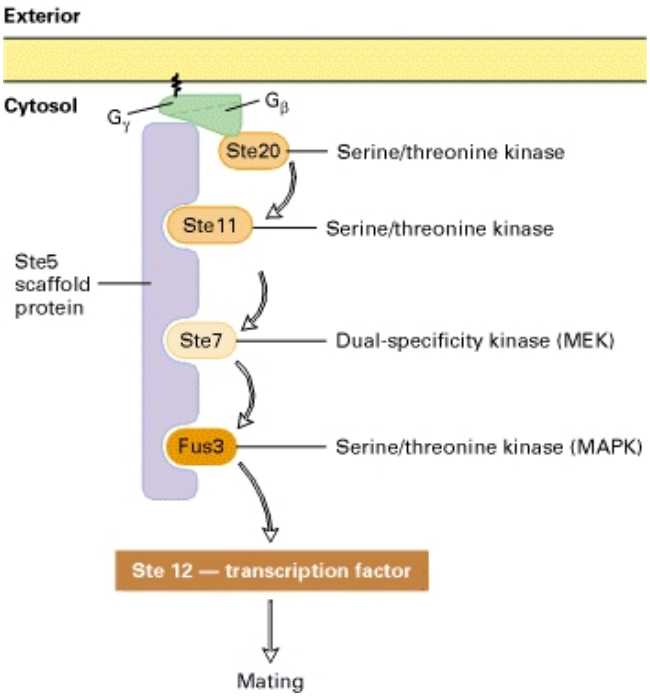
Figure 28 | Cell cycle checkpoints
Different checkpoints that the cell uses to determine if the cell cycle should proceed.



(a) Overview of EGF/MAPK pathways



(b) Activation of EGFR/Sevenless



(c) MAPK kinase cascade

Figure 29 | EGF pathway
Hello world.

Week 4 Development

Readings

LIFE 8th: Ch. 19 20 43.1 43.2 18 | LIFE 9th: Ch. 19 20 44.1 44.2 42

Overview

This week Prof. Shen gave an overview of development. Prof. Jones went over the immune system and the general functions of the adaptive and innate immune system. We will focus on development and cover the immune system in its entirety next week.

Concepts

General frameworks in which to think about this week's material.

- Necessary vs. sufficient
- Stages of development, gene expression patterns, spatial organization and differentiation
- Cell fate and signaling pathways
- Morphogen gradients and differentiation
- Understand determination, differentiation, morphogenesis, and growth in the context of development.
- Loss of function vs. gain of function
- Modularity in development

Terms

development stages

- fertilization
- cleavage
- gastrulation
- neurulation
- organogenesis
- gametogenesis

development stages

- zygote
- blastocyte
- eight cell
- animal pole
- vegetal pole
- blastrula
- gastrula
- blastocel
- blastopore
- notocord
- mesoderm
- ectoderm
- endoderm
- neurula
- neural folds
- anterior
- posterior
- dorsal
- ventral
- somites

development

- expansion
- division
- movement
- apoptosis
- cell fate
- calli
- enucleated egg
- genomic equivalence
- stem cells

fertilization

- zona pellucida
- ovum
- acrosomal vesicle
- midpiece
- flagellum
- haploid
- meiosis I
- meiosis II
- symmetric division
- asymmetric division
- polar body
- oocyte
- sperm entry point

embryo organization

- inner cytoplasm
- animal pole
- vegetal pole
- cortical cytoplasm
- gray crescent
- zygote
- gradient
- blastopore lip
- blastocoel
- blastomere
- blastula
- discoidal cleavage
- blastodisc
- archenteron
- yolk plug
- involution
- neural tube
- neural crest
- gut cavity
- tail bud
- hypoblast
- epiblast
- dorsal lip

cell fate

- totipotent
- pluripotent
- multipotent
- unipotent
- ectoderm precursor
- mesoderm precursor
- endoderm precursor
- germline
- neuron
- glia
- keratinocyte (skin cell)
- muscle cell
- lymphocyte (white blood cell)
- erythrocyte (red blood cell)
- platelet
- osteocyte (bone cell)
- hepatocyte (liver cell)
- intestinal epithelium
- sperm or egg
- neural/glia progenitor
- epidermal basal cell
- myoblast
- hematopoietic stem cell
- osteoblast
- hepatocyte
- intestinal epithelium
- stem cell

- spermatocyte
- oocyte

differentiation

- stem cell
- self renewal
- differentiation
- proliferation
- embryonic stem cells
- induced pluripotent stem cells
- cellularization
- precursor cells
- anchor cell
- secondary inducer
- primary inducer
- segmentation
- Hox genes
- transcription factors
- egg-polarity genes
- GAP genes
- pair-rule genes
- segment polarity genes
- homeotic selector genes
- parasegments
- homeodomain
- transcriptional regulation
- cytoplasmic segregation
- induction
- polarity
- cytoplasmic determinants
- inducer
- hermaphroditic
- EGF
- pattern formation
- organ identity genes
- homeotic mutation
- homeosis
- combinatorial gene expression
- positional information
- zone of polarizing activity
- transcriptional cascade
- homeobox

drosphila development

- fertilized egg
- synctial blastoderm
- embryo
- instar
- larva
- pupa

more development

- homologous genes
- developmental modules
- genetic switches
- cervical
- thoracic
- lumbar
- sacral
- caudal
- developmental plasticity
- diapause
- parallel phenotypic evolution
- mosaic development
- regulative development
- mesenchyme
- epiboly
- Hensen's node

molecules/genes

- β-catenin
- GSK-3
- Tcf-3
- Siamois
- goosecoid
- GSK-3 inhibitor
- morphogen
- primary organizer
- spemann organizer
- TGF-β
- Lin-3
- bicoid
- Krüppel
- Hunchback
- Eve
- Ftz
- Engrailed
- Nanos
- knirps
- tailless
- ced genes
- Bcl-2
- Apaf1
- caspase genes
- sonic hedgehog
- eyeless
- Pax6
- heterochrony
- BMP

model organisms

- c. elegans

people

- Hans Spemann
- Hilde Mangold
- John Gurdon
- Ian Wilmut
- Shinya Yamanaka
- Frederick Steward
- Robert Briggs
- Thomas King
- Ernest McCulloch
- James Till
- Lewis Wolpert
- Edward Lewis
- Rebecca Quiring
- Walter Gehring

Techniques

For each technique, you should know when is appropriate to use it, what its limitations are, and a particular cellular process that was used to study it.

- Transplantation experiments Move sections of a developing embryo from one region to another, test for determination.
- Bisection experiments Done by the skilled Hans Spemann to show that the gray crescent was needed for proper embryonic development.
- Fate map Inject dye into cells during development and see where they end up.
- Nuclear transplantation Transfer nucleus from differentiated cell to embryo to see if it can still develop normally.
- Cloning Take nucleus from one animal and transplant into enucleated egg.
- Microarray Can be used to identify differentially regulated genes during development.
- Laser ablation Use a laser to destroy specific cells, see the effect on development. e.g. think ablation of anchor cell means no vulva in *C. elegans*.
- Immunohistochemistry Probe with an antibody for a specific protein to determine location during development.
- In situ hybridization Probe with complementary DNA or RNA to see transcription levels of particular DNA sequence.
- Dissociation experiments Dissociate cells in culture to see if cell-cell contact contributes to cell fate.
- Co-culture experiments Place cells from two different tissues in the same dish to see if they influence each others development.

Sperm and Eggs

Development can be a soup of words

- **Spermatogenesis** primordial germ cell enters the gonad and becomes a spermatogonium. Mitotic cell division helps spermatogonium proliferate. They become primary spermatocytes during meiosis I and secondary spermatocytes during meiosis II. During this time homologous recombination causes each spermatocytes to have different genetic composition. After meiosis they become spermatids which differentiate into mature sperm.
- **Oogenesis** primordial germ cell enters the gonad and becomes a oogonium. Diploid oogonia proliferate via mitotic division (symmetric). They become primary oocytes during meiosis I, during which they grow in prophase I developing a egg coat (zona pellucida). Meiosis I is completed with asymmetric division and the first polar body is produced. After meiosis II the final egg and second polar body are produced. The egg then halts the cell cycle until fertilization.

Development Stages

Development can vary wildly between different species, but many of the concepts are the same: fertilization, movement of cells, and use of morphogen gradients and gene induction to specify fate. Note: transcription occurs in placental mammals but not other species during initial stages of development. They also contain no yolk.

Development

- Morphogen genes (egg-polarity genes) bicoid and nanos define the anterior and posterior regions.
- Bicoid at anterior pole diffusing away and turns on hunchback gene.
- Nanos at posterior pole diffusing away (mRNA transported along filaments) and turns off hunchback gene.
- Establishes gradient in egg of transcriptional regulators prior to embryo development.
- Fertilization occurs upon sperm entry. The cell undergoes a variety of events: becomes diploid, blocks sperm entry, ion flux, changes pH, increases metabolism and protein synthesis, and restarts cell divison. Sperm contributes a centriole that helps reorganize the eggs re-organizing development.
- Synctial blastoderm
- Hunchback gene stimulates GAP genes
- Gap genes define broad areas along anterior-posterior axis and stimulate pair-rule genes.
- Pair rule genes divide embryo into units of 2 segments each (14 parasegments) and stimulate segment polarity genes
- Segment polarity genes determine boundaries and anterior-posterior organization.
- Hox (homeobox) genes define what each segment should have anatomically.

Frog: initial development

- Animal and vegetal hemispheres
- β-catenin located evenly throughout cytoplasm.
- GSK-3 located evenly throughout cytoplasm, phosphorylating beta-catenin, causing β-catenin degradation.
- GSK-3 Inhibitor located in vesicles at the vegetal pole.
- Sperm entry via animal hemisphere and deposition of sperm centriole.
- Cortical rotation of the animal hemisphere toward the site of sperm entry (due to centriole reoganization of filament network).
- Gray crescent evident opposite site of sperm entry.
- Microtubules organized by sperm centriole transport the GSK-3 inhibitor vesicles to the gray crescent.
- Release of GSK-3 Inhibitor.
- Gray crescent regional protection of β-Catenin that defines dorsal and ventral cell fates.
- β-catenin present on dorsal side and degraded on ventral side.
- Cell cleavage with differential distribution of cytoplasmic determinants, β-catenin nutrients

Frog: blastula formation

- Formation of the primary organizer due to goosecoid gene expression.
- β-catenin at gray crescent inhibits Tcf-3, an inhibitor of siamois gene transcription.
- Siamois gene expressed at gray crescent.

- Maternal mRNA for TGF-β family promotes goosecoid gene expression.
- Siamois gene expression and TGF-β family promote goosecoid gene expression.

Frog: gastrulation

- Gastrulation begins when cells of the gray crescent begin to elongate and bulge inward.
- Dorsal lip of the blastopore forms as bottle cells move inward
- Involution - sheet of cells moves over the dorsal lip of the blastopore and into the blastocoel
- Innermost sheet will become the endoderm
- Middle sheet between the endoderm and ectoderm will become the mesoderm
- Cells of animal hemisphere expand around vegetal pole toward site of involution
- Archenteron expands, displacing the blastocoel
- First cells to move over the dorsal lip will become the head region and the last the tail

C. elegans: development

- Anchor cell secreted primary inducer (encoded by Lin-3)
- Cell closest to anchor cell received highest `dose' of primary inducer and generate primary precursor cells.
- Same cell as in 2 also secretes secondary inducer.
- Cells closest to cell in 2 receive smaller dose of primary inducer and higher dose of secondary inducer and generate secondary precursor cells.
- Cells that receive no primary inducer (farthest away) become epidermal precursors.

Tissues

Some tissues you should know.

- **Ectoderm** Epidermal layer of the skin.
- **Neural ectoderm** Form the nervous system.
- **Mesoderm** Muscle, bone, kidney, blood, gonads and connective tissue.
- **Endoderm** Forms the lining of the gut, the liver, and the lungs.

Gene Expression

Types

- Egg-polarity are factors received from the mother than help determine the initial polarity.
- GAP genes define broad areas along anterior-posterior axis and stimulate pair-rule genes.
- Pair-rule divide embryo into units of 2 segments each (14 parasegments) and stimulate segment polarity genes.
- Segment polarity genes determine boundaries and anterior-posterior organization.
- Homeotic selector genes (Homeobox genes) define what each segment should have anatomically.

Specific genes

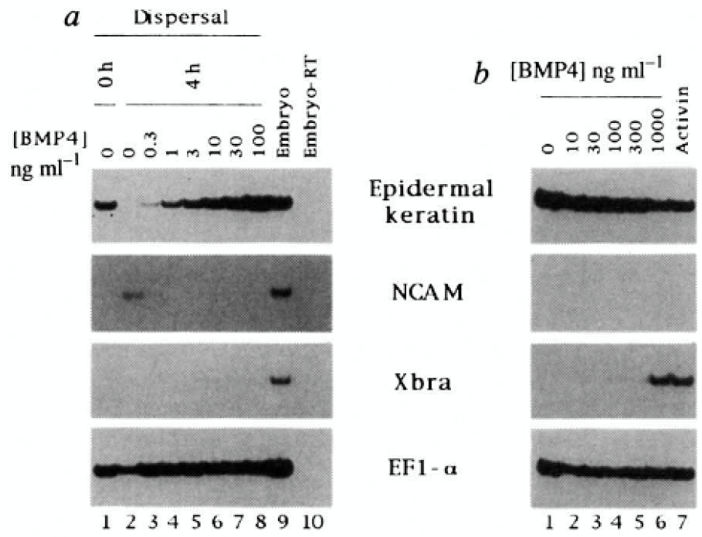
- Bicoid turns on hunchback.
- Nanos works with bicoid to setup hunchback gradient.
- Hunchback works to both repress and activate Kruppel.
- Kruppel regulated by hunchback.
- Eve regulates segment patterns.
- Ftz regulates segment patterns.

Problems

If there is any confusion about the questions, shoot me an email or talk to me after class. Remember, draw out what a pathway, interaction, or what-have-you looks like if you get confused.

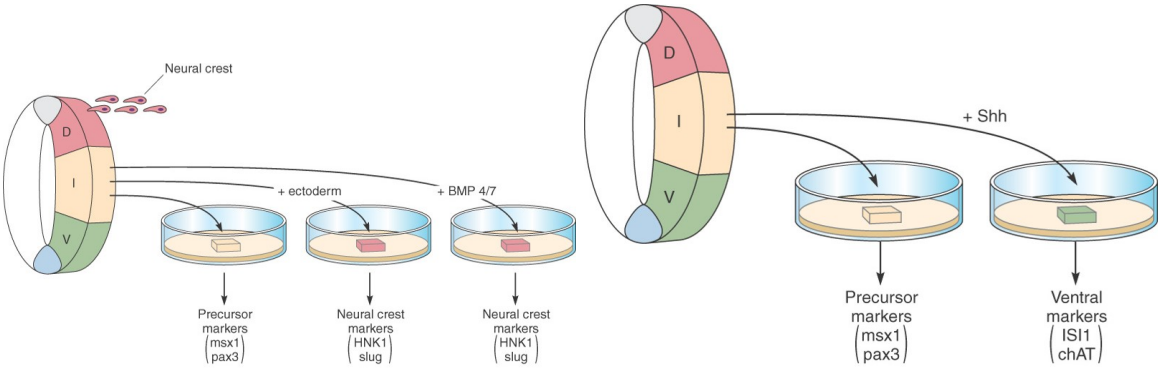
1. In this experiment, animal cap cells were taken from a developing embryo. They were added to a petri dish whole or after dissociating the cells. Then, BMP-4 was added. Different markers help test for epidermal (keratin) or neuronal (NCAM) fate.
   - What is the default state of animal cap cells, based on the figure? Neural tissue
   - What is the endogenous source of BMP-4? Animal cap
2. If an herd of animals spontaneously all developed a mutation in proteins required for acrosome development, what would happen to the herd? It would die off because the males would be considered sterile since their sperm would be unable to penetrate the egg's zona pellucida.
3. What would you expect to see if you added a DNA binding agent to a recently fertilized embryo and separated cells based on DNA fluorescence? Synchronous, so would only see 2n, 4n or S phase.
4. You want to test if the movement of the mesoderm past the animal pole induces neural tube development. How might you test this? Do a co-culture experiment: isolate animal cap with and without mesoderm and see the fate.
5. If you took a neuron and place it inside a newly dividing embryo, what would you expect to happen? It would stay a neuron or die, already differentiated.
6. If you took cells at the blastula stage and transplanted them into a brain, what would you expect to happen? The should differentiate into neurons while neurons would just stay the same, regardless of environment.
7. If you knocked out bicoid, which of the following gene's expression pattern would be affected? Nanos, Kruppel, Ftz or Hox gene. Kruppel, Ftz or Hox gene, everything downstream.
8. Suppose you were replicating the Wilmut cloning experiments. The sheep you chose for the enucleated eggs have defective mitocondria. The donor nuclei sheep are normal. What phenotype will the cloned animal have? Suppose these sheep also had a genetic mutation that caused small lungs, what phenotype would the cloned animal have? The clone will have respiratory problems in the first case but no problems in the second case.
9. You wish to clone your favorite pet and do so. Would it share the same personality and phenotype as your original pet? No, environmental factors would cause the actual phenotype to be slightly different.
10. When is the earliest we can determine the dorsal-ventral patterning of a fruit fly? In the unfertilized egg due to unequal distribution of Dorsal (a protein that promote dorsal formation) and other factors. See this site for more detail.

Figures and Tables

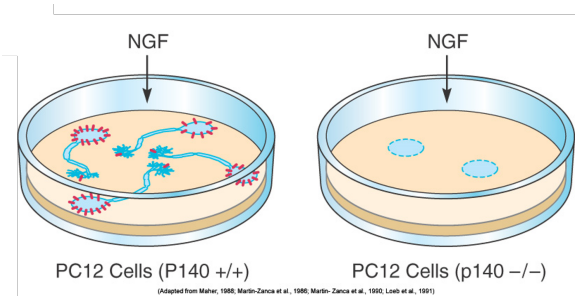


(a) Oogenesis

Figure 30 | Problem: Animal Cap fate

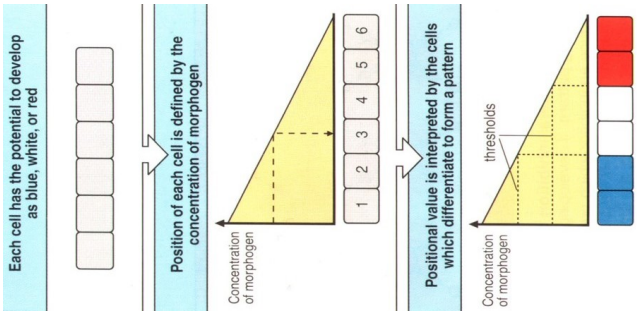


(a) co-culture (b) morphogen

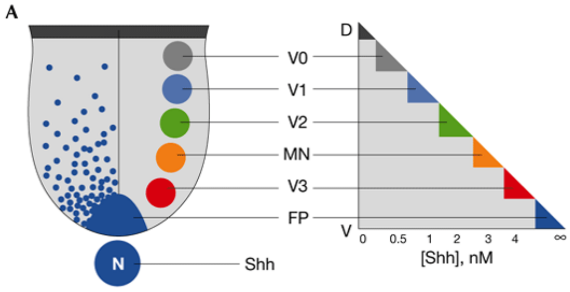


(c) Growth Factors

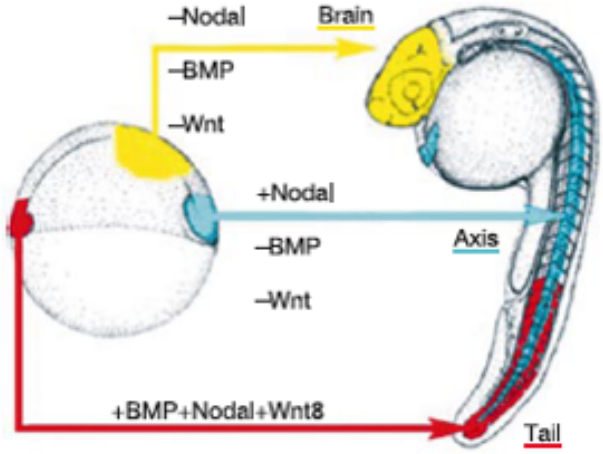
Figure 31 | Some experiments



(a) morphogen

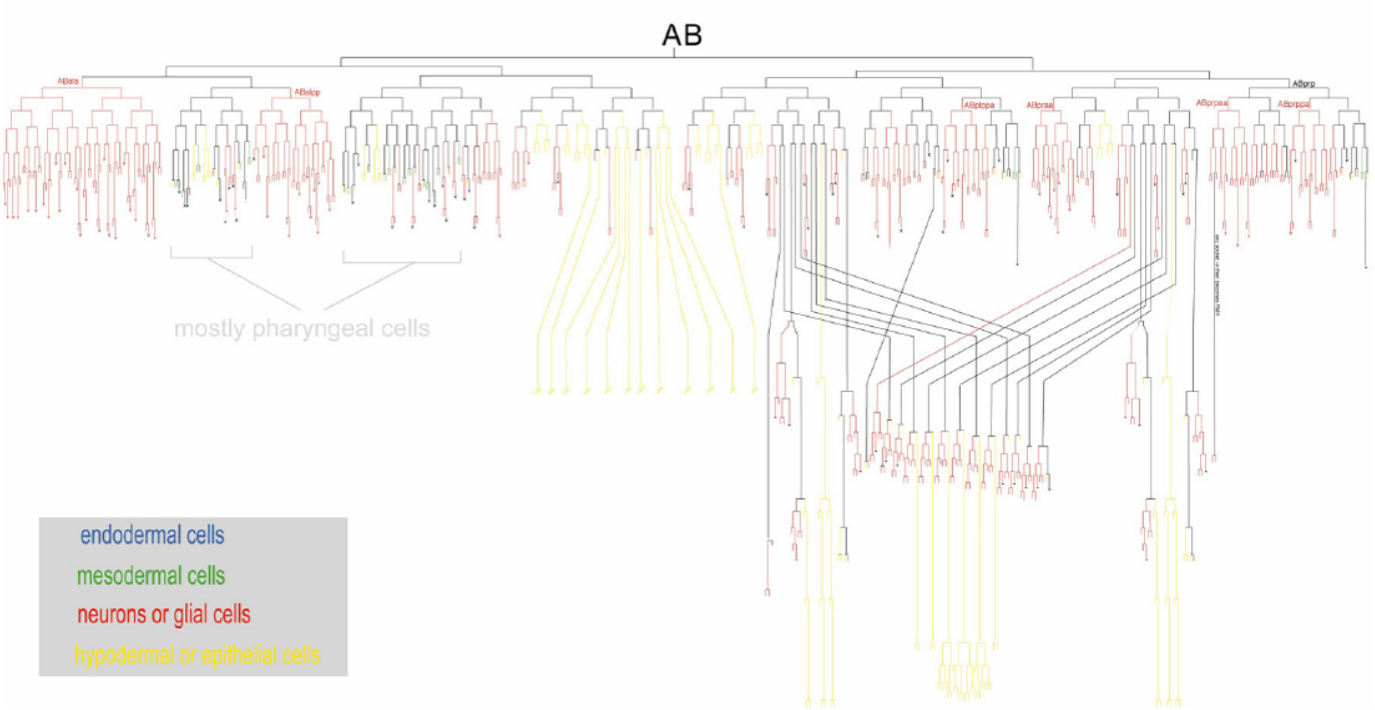


(b) morphogen shh



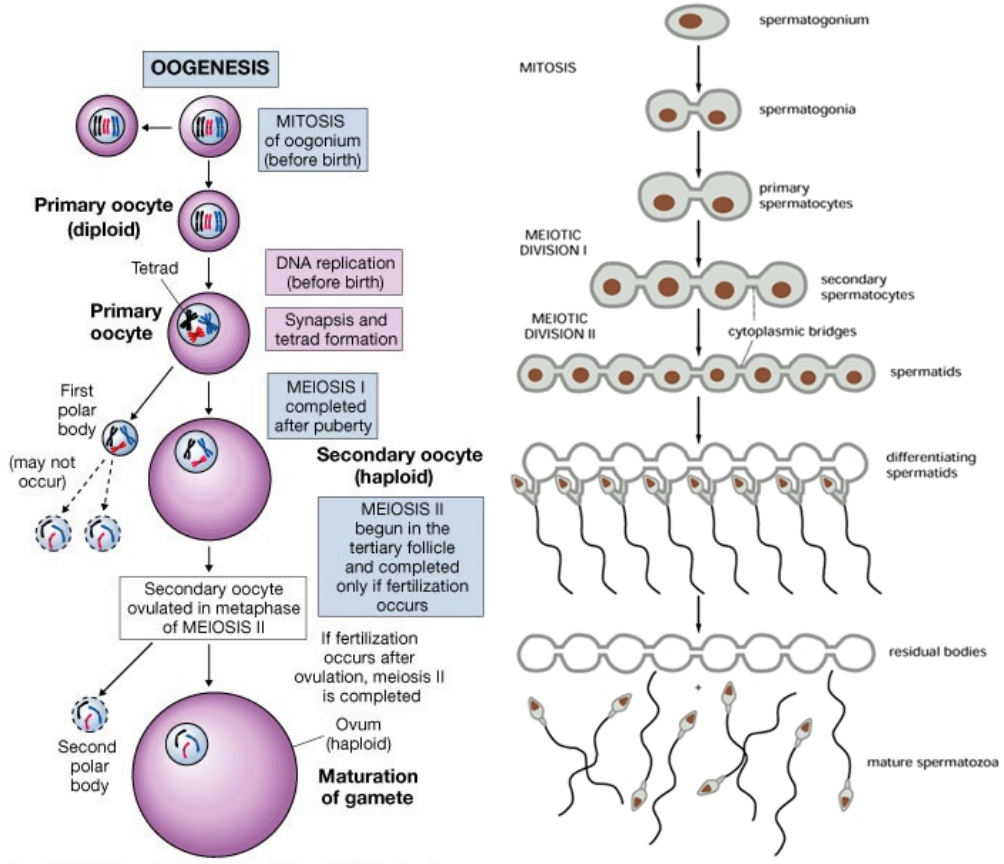
(c) morphogens and structure

Figure 32 | Morphogens and structures



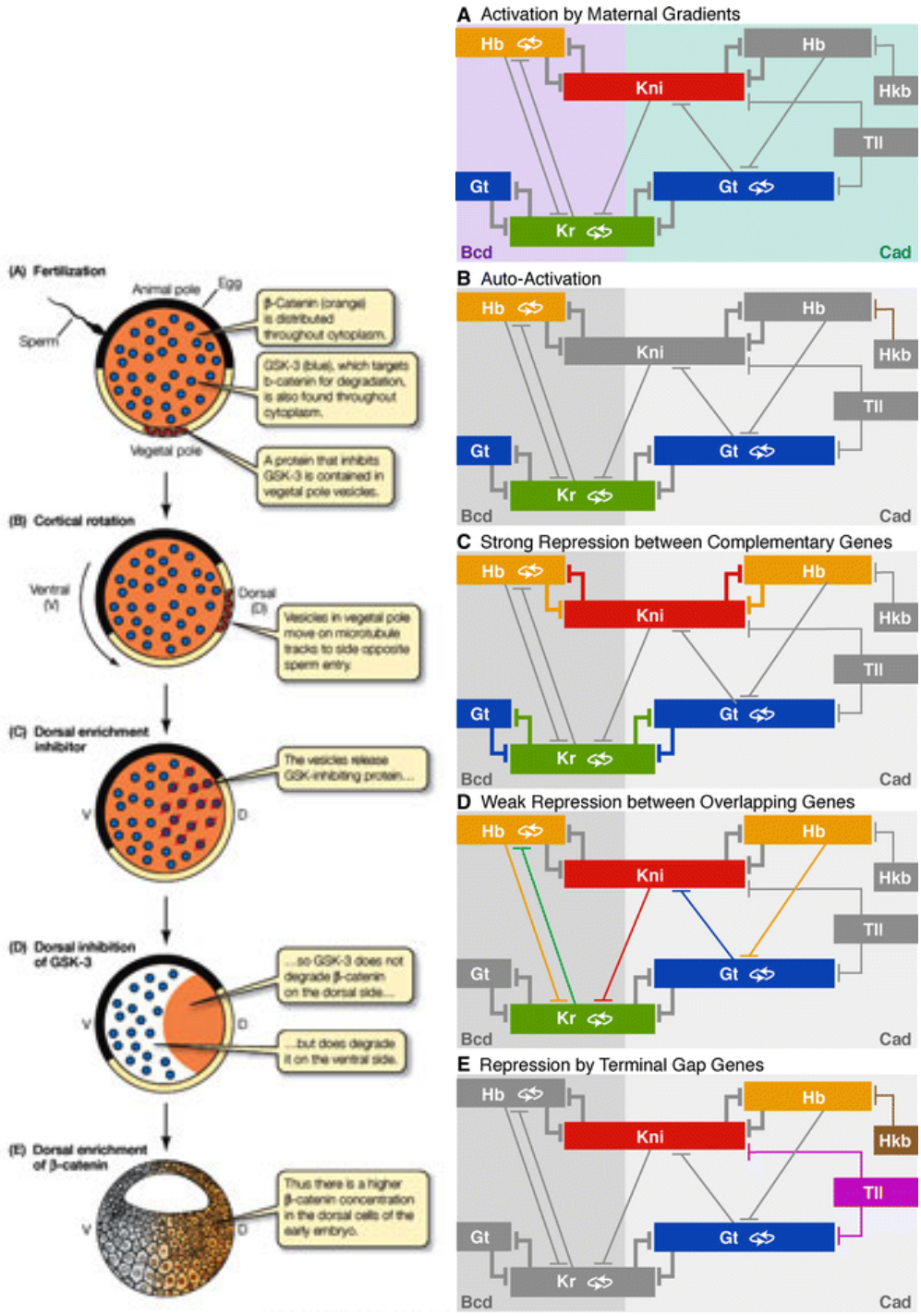
(a) c. elegans fate map

Figure 33 | Fate maps



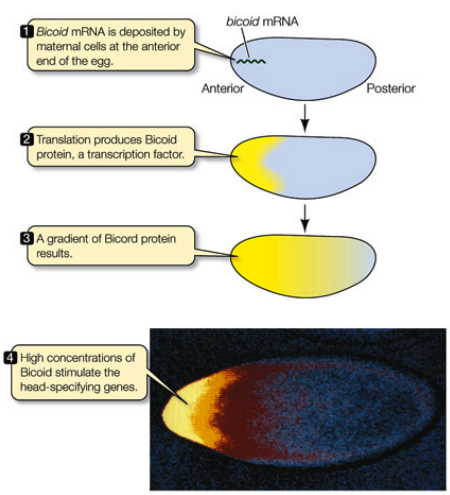
(a) Oogenesis (b) Spermatogenesis

Figure 34 | Some experiments

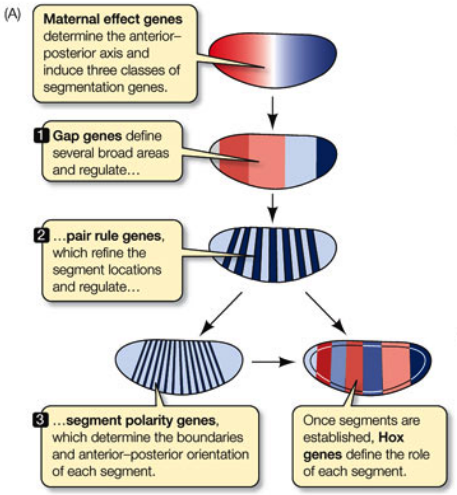


(a) β-catenin dorsal specification (b) Gap genes

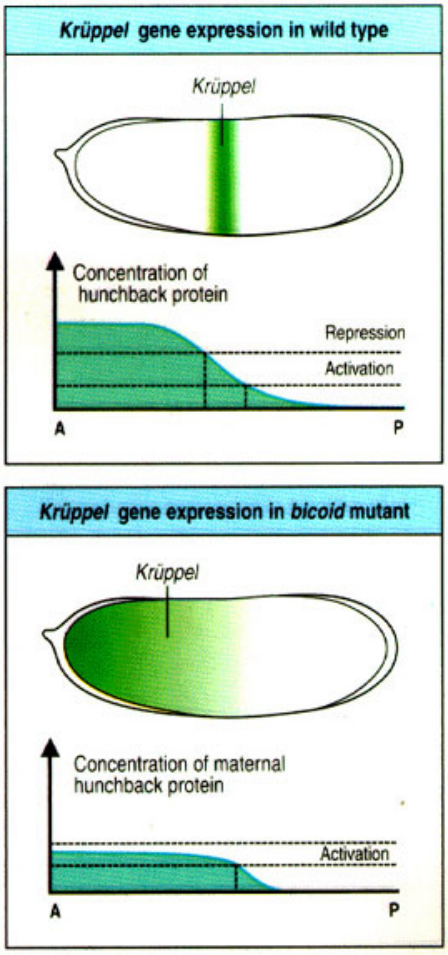
Figure 35 | Protein/Gene interactions



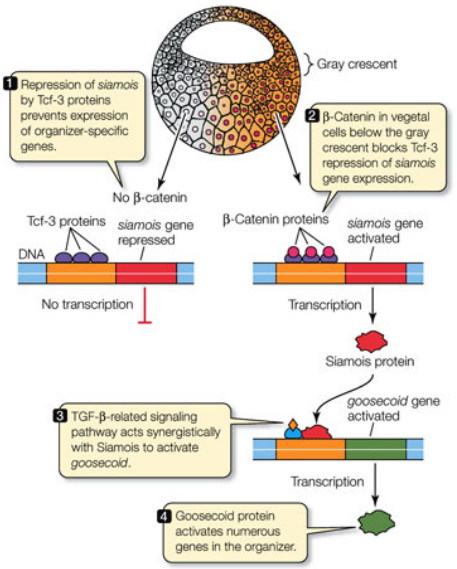
(a) Bicoid



(b) Development genes

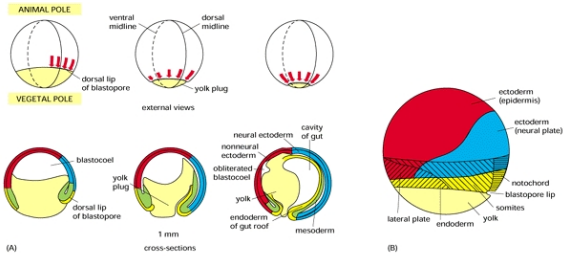


(c) kruppel

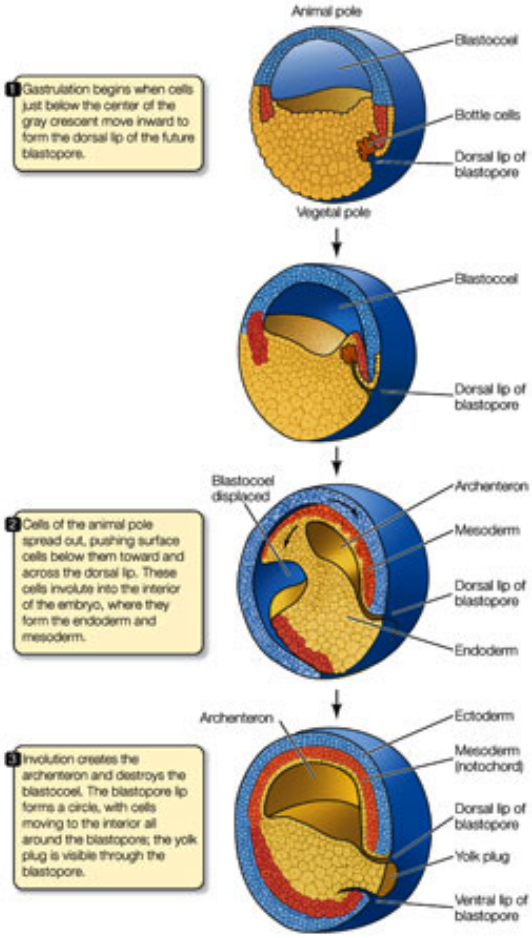


(d) transcription

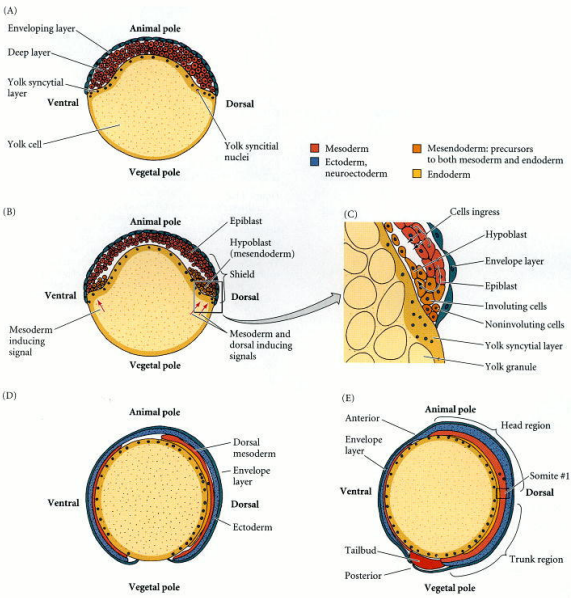
Figure 36 | Development and its genes



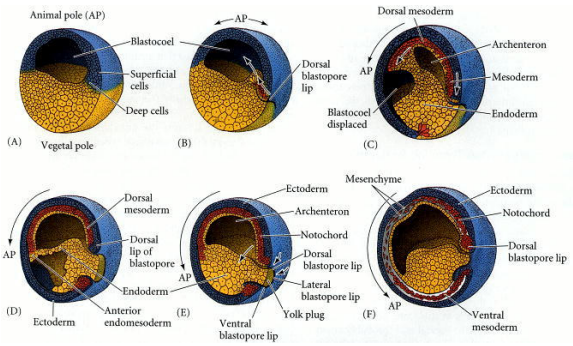
(a) Two view of gastrulation



(b) Frog gastrulation

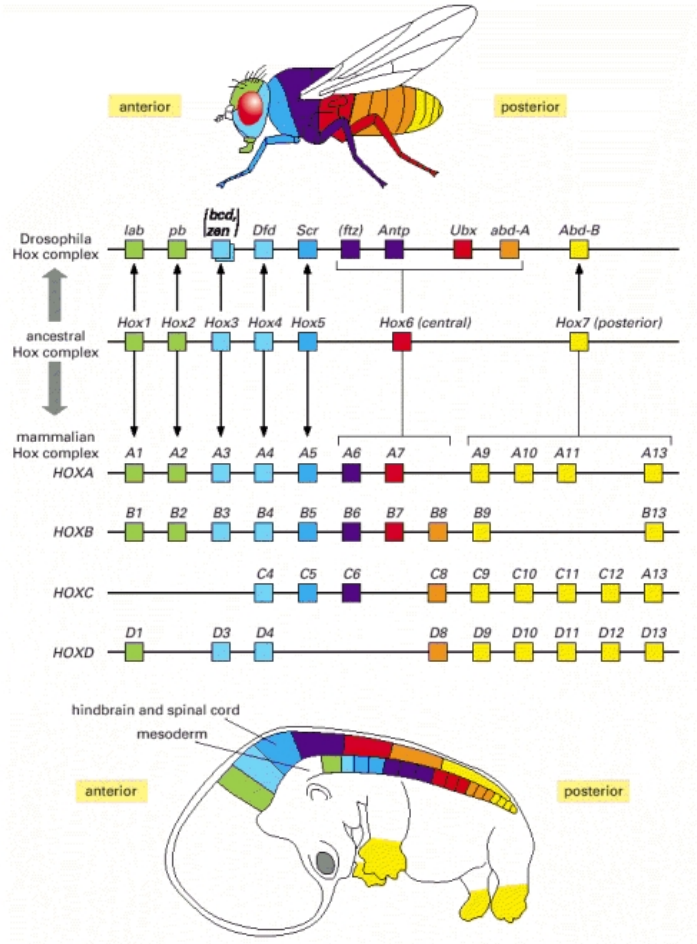


(c) zebrafish gastrulation

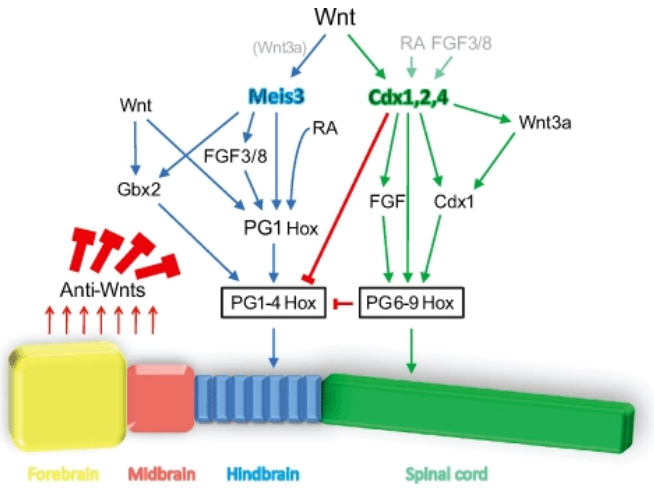


(d) frog gastrulation

Figure 37 | Gastrulation

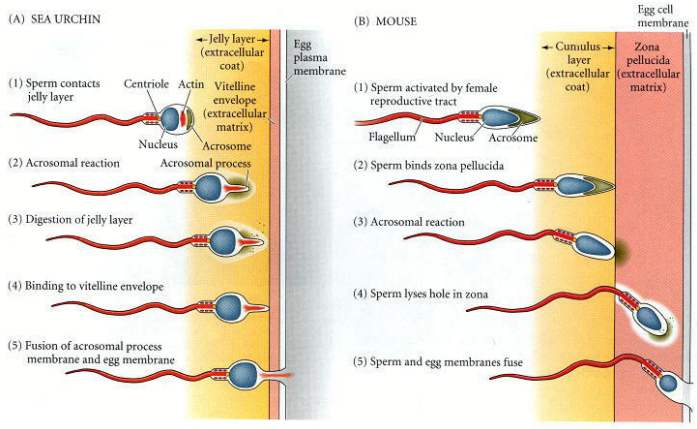


(a) Hox genes

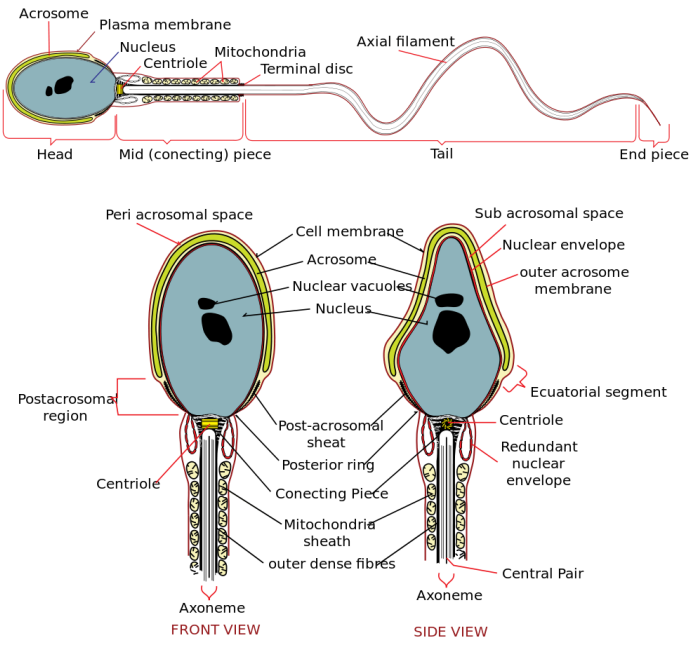


(b) Regulatory networks

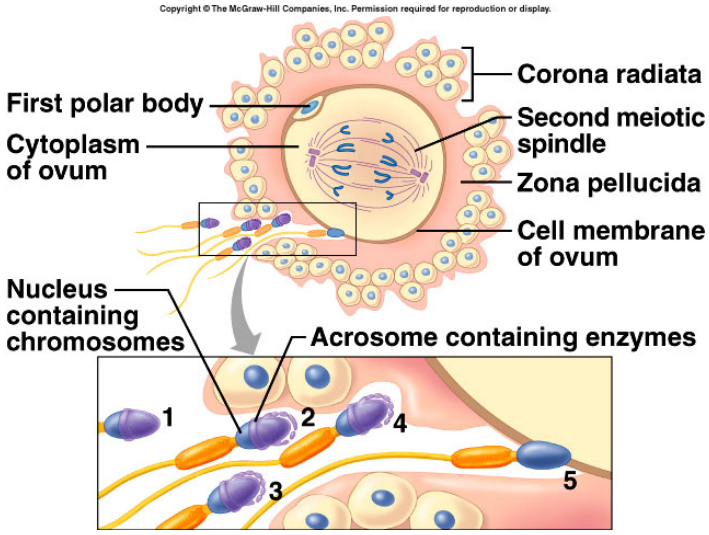
Figure 38 | Hox genes and networks



(a) Sperm entry

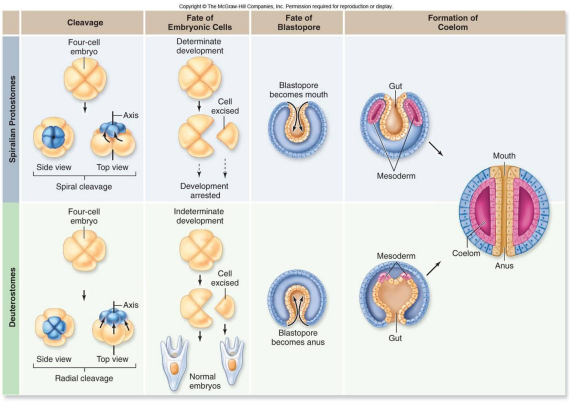


(b) sperm structure

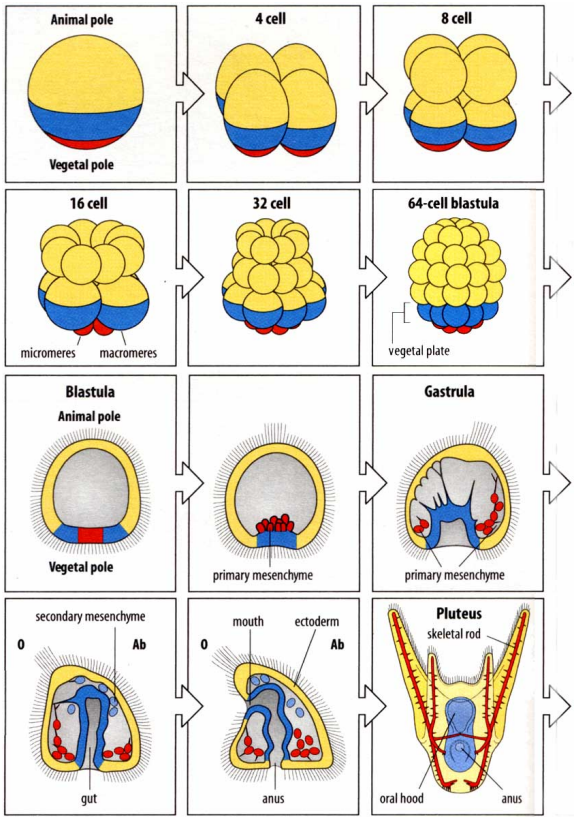


(c) oocyte

Figure 39 | Sperm Dynamics



(a) Deuterosome and Protostomes



(b) Sea urchin gastrulation

Figure 40 | Other developmental processes

Week 5 Immune System

Readings

LIFE 8th: Ch. 18 44 | LIFE 9th: Ch. 42 45 **Overview**

Prof. Jones went over the immune system and the general functions of the adaptive and innate immune system. We will focus on problems that cover key areas of the immune system. **Concepts**

General frameworks in which to think about this week's material.

- Primary and secondary lymphoid tissues, functions and location.
- Differences between innate, humoral, and cell-mediated immune responses and which types of animals have each.
- Specificity, diversity, memory and self discrimination.

Terms

lymph system

- primary lymphoid tissue
- bone marrow
- thymus
- secondary lymphoid tissue
- spleen
- lymph nodes
- tonsils
- mucosal tissues
- secretory glands
- lymph ducts
- vein
- artery

immune cells

- multipotent hematopoietic cell
- myeloid progenitor cell
- lymphoid progenitor cell
- red blood cells
- erythrocytes
- white blood cells
- leukocytes
- granular cells
- basophils
- eosinophils
- neutrophils
- mast cells
- agranular cells
- monocytes
- macrophages
- dendritic cells
- B lymphocytes
- T lymphocytes
- Natural killer cells
- phagocytes
- natural killer T cells

other

- NF-κB
- monocytes
- macrophages
- monokines
- inflammation
- Toll-like receptors
- PAMPs
- phagocytosis
- interleukins
- interferons
- growth factors
- complement system
- pathogens
- skin
- passive immunity
- clonal deletion
- clonal anergy
- allergen

humoral

- B cell
- plasma cell
- clonal expansion
- cytokines
- IgD
- IgG
- IgM
- memory cells
- clonal selection
- clonal proliferation
- BCR

cell-mediated

- Helper T cell
- Cytotoxic T cell
- MHCII
- CD4
- MHCI
- CD8
- lysosome
- T cell receptors
- activation phase
- effector phase

immunoglobins

- IgM
- IgG
- IgA
- IgE
- IgD
- VDJ
- epitope
- heavy chain
- light chain
- variable region
- constant region
- V (variable)
- D (diversity)
- J (joining)
- C (constant)
- recombinases
- splicing errors
- deoxyribonucleotidyltransferase
- Fab
- Fc
- hinge region
- VL
- CL
- VH
- CH1
- CH2
- CH3

disease

- myeloma
- diabetes
- AIDS
- immune dificiency disorder
- severe combined immune deficiency disease
- graft rejection
- graft-versus-host disease
- viral fusion

experimental

- serum
- antiserum
- hybridoma
- monoclonal antibody
- gene therapy
- papain

Techniques

For each technique, you should know when is appropriate to use it, what its limitations are, and a particular cellular process

that was used to study it.

- Antibodies Inject the desired protein antigen into a mouse. A few days later, isolate the B cells from the spleen and fuse them with myeloma cells. Culture the cells in medium that allows only the growth of hybridoma cells that will proliferate continuously (a property that is derived from the myeloma cells used for fusion) and will secrete antibody directed against a *specific* epitope of the antigen (a property derived from the antibody producing B cells isolated from the spleen). You allow each hybrid cell to clonally expand in a 96 well plate and produce monoclonal antibody against a unique epitope of the antigen.

Lymph and Immune Cells

Lymph System

- Bone marrow: main site of blood cell formation, including B cells).
- Thymus: where T cells complete their differentiation.
- Primary tissues: site of formation of blood cells.
- Secondary tissues: sites of B and T cell activation by antigen and initiation of responses.
- Spleen: filters antigens from the blood.
- Lymph nodes: filters antigen from the lymph.
- Tonsils: sites of responses to mouth throat infections.
- Mucosal tissues: e.g., intestinal tract, lungs, urogenital tract.
- Secretory glands: e.g., salivary, lacrimal, mammary.

Immune cells

- Red blood cell (erthrocytes): transport oxygen and carbon dioxide.
- Platelets: initiate blood clotting, contain granules.
- White blood cell (leukocytes): see below.
- Basophils: Release histamine, promote T cell development.
- Eosinophils: Kill antibody-coated parasites.
- Neutrophils: Stimulate inflammation.
- Mast cells: Release histamine when damaged, bound by IgE.
- Monocytes: Develop into macrophages and dendritic cells.
- Macrophages: Engulf and digest microorganisms, activate T cells
- Dendritic cells: Present antigens to T cells.
- B lymphocytes: Differentiate to form antibody-producing cells and memory cells.
- T lymphocytes: Kill virus-infected cells, regulate activity of other white blood cells.
- Natural killer cells: Attack and lyse virus-infected or cancerous body cells, less discriminate than T cells.

Innate

Pathogen-associated molecular patterns (PAMPs) are recognized by patttern recognition receptors known as Toll-like receptors. Binding causes expression of proteins:

- anti-microbial substances.
- chemoattractants (e.g., chemokines) to bring macrophages and other cells to site of infection.
- cytokines (protein hormones of the immune system) that induce inflammation.
- cell surface proteins, such as receptors and adhesion proteins.

General infection response

1. Example: inhaled influenza virus infects airway endothelial cells lung macrophages.
2. Macrophages phagocytose viruses.
3. Macrophages travel via lymph vessels to regional lymph node.

4. Within node, macrophages come into contact with naïve helper T cells whose TCR can bind MHC II and can bind the presented influenza viral antigen.
5. TCR engagement with MHC II antigen, CD4 engagement with MHC II and cytokines released by the macrophage stimulate the naïve helper T cell to divide, producing clones (maintain their antigen specificity).
6. Some clones develop into memory T_H cells while others develop into effector T_H cells.
7. Naïve B cells within the node whose membrane immunoglobulin can bind the virus, phagocytose the virus and present antigens on their MHC II.
8. Effector T_H cells that are specific for the antigen being presented by the B cell will bind the antigen complexed to MHC II, their CD4 will bind the MHC II and they will release cytokines that will activate the B cells.
9. The B cells will be stimulated to clonally divide class switch forming memory cells or plasma cells.
10. Plasma cells will secrete antibodies that can then neutralize the virus, increase phagocytosis, and/or activate complement pathway.
11. Also, within the node, a macrophage (or any other infected cell) presenting antigen on its MHC I comes into contact with a naïve cytotoxic T cell that can both bind MHC I and bind the antigen being presented.
12. TCR engagement with MHC I antigen, CD8 engagement with MHC I and cytokines released by other stimulated T_H cells causes the naïve cytotoxic T cell to clonally divide producing memory cells and effector cells.
13. Effector cytotoxic T cells travel via the blood to sites of infection where they recognize infected cells by recognizing the MHC I, the specific antigen being presented on the MHC I, and CD8 can bind MHC I.
14. Effector cytotoxic T cells then release toxic proteins (including perforin) from secretory vesicles at the infected cell surface to cause infected cell death.

Adaptive

- B cell: Activated B cells proliferate and differentiate into memory B cells and plasma B cells. They also serve as antigen presenting cells in the humoral immune response.
- T-helper: Activated T_H cells secrete cytokines to activate B cells in the humoral immune response.
- T-cytotoxic: Activated T_C cells release toxins/enzymes (i.e. granzymes or perforins) to kill invading pathogens.
- Macrophages: Present antigens to T_H and activate them. They also phagocytose the antibodies coated antigens during opsonization.
- Plasma B cells: Secrete antibodies that bind to the circulating antigens that either results in their neutralization or opsonization.

Humoral

B cell activation

1. Mature B cell displaying a surface bound antibody binds antigen.
2. Antigen internalized and displayed on MHCII.
3. T helper cell recognizes MHCII + antigen and activates B cell.
4. B cell divides (clonal expansion) to produce two types of cells: memory B cells and plasma cells.

Cell-mediated

Helper T cells

1. The helper T cells are involved in the antigen recognition and clonal expansion of the B cells.
2. When a B cell ingests pathogen, it uses its specific antibody to bind to and internalize the pathogen.
3. When a B cell processes and presents the same antigen to the primed T_H cell, the T cell secrete cytokines that activate the B cell.
4. These cytokines trigger B cell proliferation and differentiation into plasma cells that produce the IgG (and IgM in the primary response) which acts against the antigen.
5. Simultaneously these antibodies can also bind to specific proteins on the surface of antigen thereby coating and neutralizing the antigen.

6. The antibody coated antigen can then be engulfed and digested/ phagocytosed by macrophages.

Cytotoxic T cell

1. The cytotoxic T cells react with the foreign peptides that are presented by MHC-I complex which are present on the surface of all cells of the body.
2. Virally infected cells can be eliminated by the T_C cells by presenting a fragment of the virus on the cell surface.
3. Once the T_C cells encounter the antigen they kill the target cells by secreting a protein called perforin (which creates holes in the membrane of the infected cells and leads to their lysis) and releasing granzymes that stimulate apoptosis.

Immunoglobins

- IgG: is a kind of antibody that works efficiently to coat microbes, speeding their uptake by other cells in the immune system.
- IgM: is very effective at killing bacteria.
- IgA: concentrates in body fluids-tears, saliva, and the secretions of the respiratory and digestive tracts-guarding the entrances to the body.
- IgE: is responsible for the symptoms of allergy. Its natural job probably is to protect against parasitic infections.
- IgD: remains attached to B cells and plays a key role in initiating early B cell responses.

Antibody creation

Light chain

1. Germ-line V and J segments are joined
2. Transcription of VJ gene segments along with C segment.
3. RNA splicing joins the VJ and C regions
4. Translation of antibody with unique VJ (variable) region

Heavy chain

1. V, D, J and C genes in different clusters.
2. D-J joining occurs, leading to D and J clusters to be near eachother.
3. V-DJ joining occurs, a V segment is now adjoined to a DJ segment.
4. Transcription of the DNA leads to an mRNA with multiple constant regions.
5. RNA splicing removes one of the constant regions, giving the antibody its specific sequence.
6. Translation of mRNA leads to IgM, IgG, etc. antibodies depending on constant region type (μ, γ, etc.)

Notes

- Ig gene rearrangements, which occur in the DNA during the formation of B cells in the bone marrow, generate intact heavy and light chain genes that can be transcribed and translated into heavy and light polypeptide chains.
- Multiple gene segments contribute the coding sequence for V regions in the light chains (V's, and J's) and heavy chains (V's, D's, and J's)
- Different combinations of these gene segments generates a high level of diversity from relatively small 's of gene segments.
- The gene segments that are randomly selected to be spliced together in each developing B cell determine its antigen-binding specificity.
- Gene rearrangements are unique to antigen-binding proteins of the immune system: immunoglobulins and T-cell receptors.

Problems

If there is any confusion about the questions, shoot me an email or talk to me after class. Remember, draw out what a pathway, interaction, or what-have-you looks like if you get confused.

1. Describe one experiment proving that stem cells exist in the bone marrow. A bone marrow transplant experiment: Prior to the experiment, some bone marrow cells are removed from a mouse. This mouse is then irradiated with X-rays at a dose that kills all the remaining bone marrow cells, which are more sensitive to X-rays than other stem cells. Subsequent injection of the bone marrow cells taken from the mouse prior to irradiation completely replenishes the supply of all types of differentiated blood cells and the bone marrow stem cell population. Alternatively, you could inject the irradiated mouse with bone marrow from a syngeneic donor.
2. While doing reproductive cloning you can technically implant the nucleus from any somatic cell into a recipient enucleated egg. If a mature B cell is used as the donor cell to provide the nucleus, what potential disadvantage(s) can the clone have? The nucleus of the matured B cells would already show a rearrangement of the immunoglobin gene. Accordingly, the animal generated through somatic cell nuclear transfer using this nucleus will lack the ability to produce the many millions of needed antibodies and hence will be immunocompromised.
3. Could you create a mouse by organismal cloning if the adult cell you began with was a gut epithelial cell? If yes, then predict what the phenotype of the organism would be as it develops from a newborn to an adult mouse. If no, explain why not. Yes. These mice would mostly likely appear phenotypically normal unless the cell from which they were derived had accumulated detrimental somatic mutations during its lifespan in the gut.
4. Could you make a clone from a red blood cell? No, it contains no nucleus.
5. Sometimes, the immune system turns against particular molecules of the body resulting in autoimmune diseases. The examples include lupus, rheumatoid arthritis, diabetic mellitus and multiple sclerosis. Briefly describe how the self-reacting T cells are eliminated during the development of immune system. This can be explained by Clonal Deletion/ negative selection theory according to which self-reactive lymphoid cells are destroyed during the development of the immune system in an individual.
6. Circle all the cell types that are involved in humoral immune response. Briefly describe the role of each cell type that you selected. B cells, T- helper, T-cytotoxic, Macrophages, or Plasma B cells. B cells, T- helper Macrophages, or Plasma B cells. See notes for descriptions.
7. Briefly describe the process that produces millions of unique antibody molecules from only two different genetic loci.
8. Outline the steps involved in the shift of the immune system from producing millions of unique antibody molecules to producing millions of a single type of antibody molecule.
9. Fig. 44 shows two lanes that contain immunoglobins from B cells right after injection of a novel pathogen (primary response).
   - Which two Ig are present and identify which lanes they belong to. Lane 1 is IgG while lane 2 is IgM.
   - Do the two classes of antibodies have identical or different antigen binding sites? Identical.
   - Same number of antigen binding sites? It has different number of antigen binding sites. Since IgG is monomer it has 2-antigen binding sites/IgG molecule in comparison to 10 binding sites/ IgM molecules, which is a pentamer.
10. Towards the end of a primary and/or secondary immune response, you incubate the two cell-types with a fluorescent dye (Annexin V). This dye specifically binds to terminally differentiated cells that are undergoing apoptosis. You then sort the cells using a flow cytometer and observe two distinct populations of the cells: population 1 being negative for annexin staining and population 2 being positive for Annexin staining.
    - Identify the cell-types that most likely make up each population (1 and 2). population 1 is memory B cells while population 2 is plasma B cells
    - Which of the two cell-types shows a higher potency (number of fates a cell can acquire)? Memory B cells are more potent since they can divide to make more of their own kind and also generate plasma B cells. In comparison, plasma B cells produce and secrete the IgG antibodies and then die.
11. In terms of the epitopes being recognized, how do monoclonal antibodies differ from the polyclonal antibodies? Each monoclonal antibody recognizes a single epitope. In comparison, polyclonal antibodies represent a mixture of different antibodies that can recognize different epitopes on the surface of the same antigen.
12. Immunological memory is the basis of vaccination. When an individual is exposed to an infectious agent, the immune

system responds by producing soluble antibodies specific for that infectious agent. Assume that you have three different viruses, Virus A, Virus B and Virus C. You expose two individuals, individual 1 and individual 2 to Virus A and measure the following response, see Fig. 42. **a)** Virus A and Virus C have different capsid proteins, but the surface of each capsid protein has an identical stretch of 40 amino acids. **b)** Virus A and Virus B have completely different capsid proteins, but they have a 1000 base-pair region of DNA that is identical. **c)** Virus B and Virus C have nothing in common.

13. Explain why the response of individual 1 to virus A infection is different from the response of individual 2. Individual 1 was exposed to virus A at some point and therefore has memory B cells specific for this virus. Following a secondary exposure to the same virus, these memory B cells rapidly proliferate to make more memory B cells and antibody secreting plasma cells. This accounts for a rapid and strong immune response. In comparison, individual 2 has not been previously exposed to virus A and therefore takes a longer time to mount a primary immune response.
14. Indicate as Yes/No if the following conditions could result in the increased response seen in individual 1. Explain.
    - Individual 1 has more immature B cells. No, you would not expect a response since the response is due to the presence of memory cells generated after exposure to a specific antigen and does not depend on the number of immature B cells.
    - Individual 1 was previously exposed to virus B. No, you would not expect a response since the response is against the viral protein and not its DNA.
    - Individual 1 was previously exposed to virus C. Some of the memory B cells will also be effective against Virus A because it will share epitopes with Virus C. These cells are more readily activated, so tha$T_H$umoral response is strong and rapid.
15. Immunological response requires the collaboration of $T_H$elper cells and B cells. Briefly describe the steps involved in B cell activation. The helper T cells are involved in the antigen recognition and clonal expansion of the B cells. When a B cell ingests antigen, it attaches epitopes of the pathogen's proteins to a MHC-II protein. This complex is moved to the cell membrane, where it can be recognized by a T Helper cell (T_H) that are activated by the antigen presenting cells (APC cells) displaying the epitope through the MHC-II. When a B cell processes and presents the same antigen to the primed T_H cell, the T_H cell secretes cytokines that activate the B cell. These cytokines trigger B cell proliferation and differentiation into plasma cells that produce the IgG antibodies which act against the antigen.
16. Secondary immune response to an antigen is much stronger and rapid than the primary immune response. You collect the blood sample from an individual following the secondary immune response to a specific antigen. You then discard the red blood cells (RBC) and incubate the remaining sample with a green fluorescent probe that specifically recognizes and binds to the antibodies that are produced in response to this antigen. Under the fluorescent microscope you observe that the serum as a whole and the surface of some cells fluoresce green.
    - Explain why the surface of some cells fluoresces green. The fluorescent probe recognizes and binds to the antibodies that are expressed on the surface of memory B cells that have undergone clonal expansion following an infection.
    - Explain why the serum fluoresces green. The fluorescent probe recognizes and binds to the circulating antibodies that are produced and secreted by the plasma B cells.
17. The immune system provides us with defense against foreign antigens including viruses. Briefly explain how both the humoral and cellular immune systems can counteract the viral infection. See notes.
18. From the choices below, list all the cell types that are involved in the cell mediated immune response. Briefly describe the role of each cell type that you selected. B cells, T- helper, T-cytotoxic, Macrophages, or Plasma B cells. T- helper, T-cytotoxic, Macrophages. See notes for descriptions.
19. See Fig. 41 for picture of receptor.
    - What molecular interactions stabilize the interaction between the α and β chains of the TCR? Disulfide bonds
    - Which amino acid(s) or class of amino acids mediates the interaction in part (i)? Cysteines.
    - What would happen to an animals response to viral infection if the membrane region was mutated to contain arginines? They would be unable to respond because the TCRs would no longer be properly inserted into the membrane.
20. Cytotoxic T lymphocytes will recognize antigen presented by virally infected body cells.
    - What proteins are involved in the presentation of antigen to T lymphocytes? MHCI
    - Explain why cytotoxic T lymphocytes do not recognize this infected cell if translation is inhibited. If the viral proteins

are not being synthesized, then no viral epitomes will be displayed on the MHCI molecules, and the Tc cell will not `see' this cell as infected.

21. When an organ is transplanted, the patient must take immunosuppressant drugs for the rest of his or her life. Explain why. The immune system recognizes the MHC-I proteins on the surface of nucleated cells of transplanted organ as foreign and mounts a response against it which may lead to graft rejection. For the organ and accordingly for the patient to survive, this response must be suppressed.
22. Which surface molecule(s) is critical for the success of organ transplant? MHCI
23. Which cell types express this molecule on their cell surface? MHC-I surface molecules are located on the membrane of all the cells except for mature red blood cells.
24. Could you alleviate this need if the donor and the recipient were fraternal twins? Explain why. Fraternal twins develop simultaneously from two zygotes. They are not genetically identical to each other and likely express different MHC I molecules. Therefore there will always be a chance of graft versus host rejection.
25. Could you alleviate this need if the donor and the recipient were monozygotic twins? Explain why. The monozygotic twins are genetically identical to each other and should express identical MHC I molecules. Therefore the graft cells will be recognized as normal.
26. Fig. 43 is a schematic diagram of a portion of immunoglobin (Ig) heavy chain gene locus in the configuration found in the germ-line cells.
    - How many different heavy chains can be made based on the diagram? 4 x 3 x 4 = 48 Ig heavy chains can be made based on the diagram.
    - You use primers 1 and 2 to PCR amplify the Ig heavy chain locus indicated above from germ-line cells and from mature B cells found in the spleen. Would the PCR product of the heavy chain locus in the spleen be of the same length as the PCR product obtained from the same region in the germ-line cells? Explain. The B cells in the spleen are the mature B cells that have already undergone rearrangement of its heavy chain locus. As a result much of the DNA has been removed from the gene and only one domain has been selected from the V region, D region and the J region. Therefore the PCR product would be shorter than that found in the germ-line cells.
    - Continuing, would you expect the same set of primers to amplify the Ig heavy chain locus from mature B cells in the spleen? Explain. Yes, you can still use the same primers since these are designed based on the sequence of the regions that flank the V, D and J domains present at the germ-line level.
27. Fig. 44 shows the structure of human immunodeficiency virus (HIV). It is a retrovirus and its genome is a single (+) stranded RNA that is packaged with the reverse transcriptase enzyme within a protein capsid. This is further packaged into an envelope that is derived from the plasma membrane of the host cell in which the virus had replicated. The surface of the envelope is covered with the envelope glycoprotein, called gp120.
    - a) HIV specifically infects the T- helper (T H) cells of the human immune system. If HIV enters the host cell by means of host receptor recognizing a viral protein, what would be the most likely ligand and its corresponding receptor during HIV infection? The gp120 protein on the surface of HIV envelop binds to the CD4 receptor on the surface of T helper cells and this ligand-receptor binding event is the first step of infection.
    - Some individuals are resistant to HIV infection even after repeated exposure. Assuming that these individuals express a normal level of the functional receptor that you have recognized above, how can you explain their resistance to HIV? The gp41 protein on the surface of virus binds to a chemokine receptor (CCR) on the surface of T helper cells. If a person shows a homozygous mutation for the CCR gene (CCR-/ CCR-) he/she will not have the chemokine receptor and will not contract AIDS even after repeated exposure to HIV.
28. You decide to generate vaccine against Influenza virus using either a live-attenuated form of the virus or heat–killed viral particles.
    - Which of these two vaccine strategies would produce both humoral and cell mediated immune responses? Explain why you selected this strategy. Live-attenuated form of virus will produce both the responses. The virus is still replicating although at a very slow pace and hence the viral proteins can presented on the surface of infected cells through MHC1 leading to a Tc mediated cell killing or through MHC-II by Antigen presenting cells leading to a T H and B cell mediated humoral immune response.
    - If the vaccine generated results only in a humoral response and production of antibodies, propose a mechanism

through which the secreted antibodies can counteract the viral infection. The secreted antibodies can bind to antigens located at the viral surface thus neutralizing the virus. Thus the virus gets coated by the antibodies and in this form it can be engulfed and digested by macrophage. This process is also called opsonization.

29. Pigs can be infected both by the avian and human forms of the Influenza virus. This allows them to act as virus mixing bowls in which the two viral strains can readily exchange RNA strands. Thus the virus that emerges from such double-infected species can represent a unique assortment of genes, generating new strains of virus. The HIN1 virus that causes Swine flu is one such example. Why is a newly emerged virus considered a threat that is significant enough to cause a global pandemic, compared to a seasonal viral strain? Newly emerged virus may have surface antigens which have never been encountered by the immune system of the human population. This may therefore cause a global pandemic.

Figures and Tables

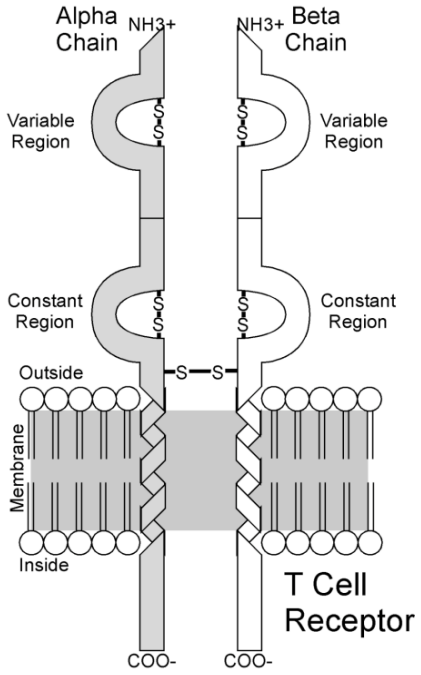


Figure 41 | **T cell receptor**

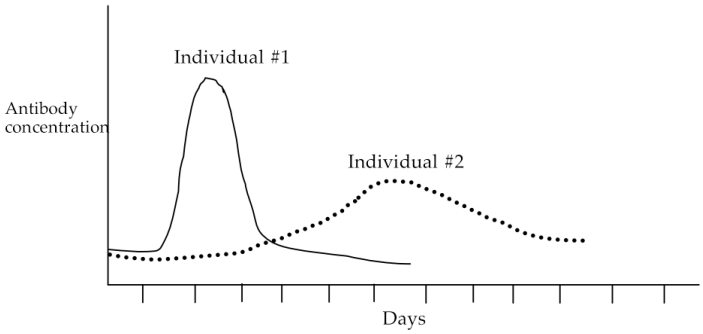


Figure 42 | **B cell memory**

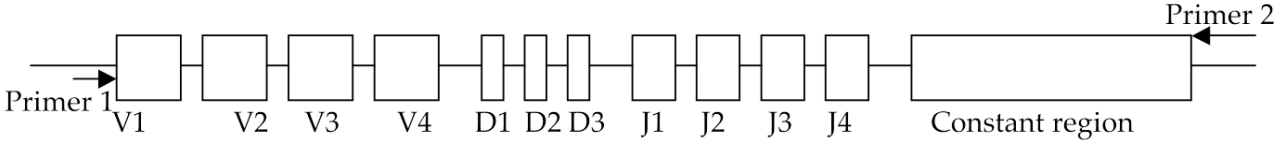
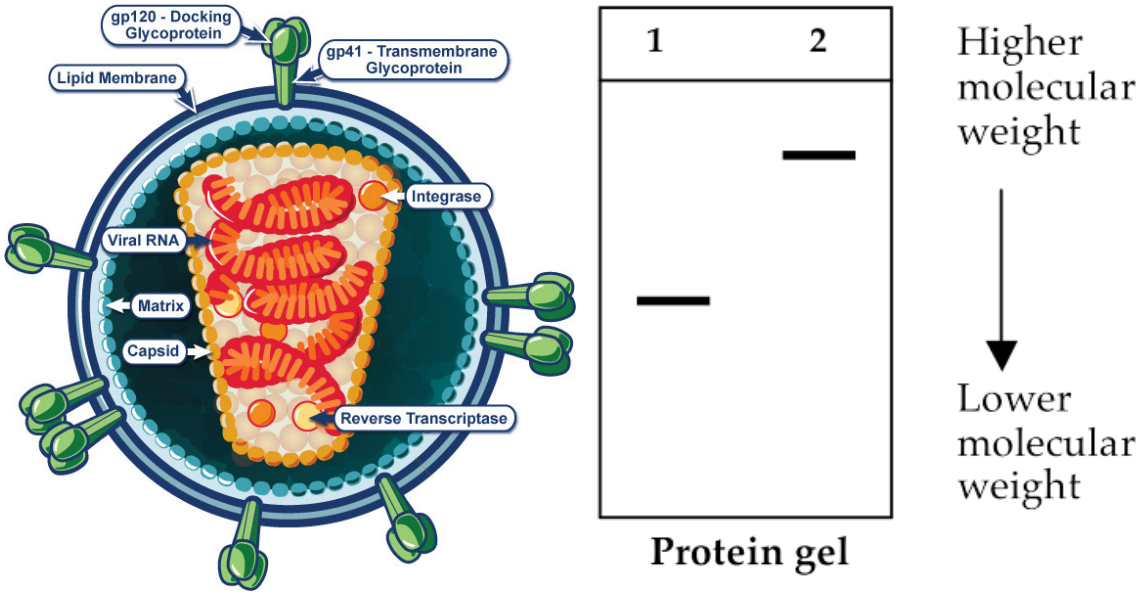
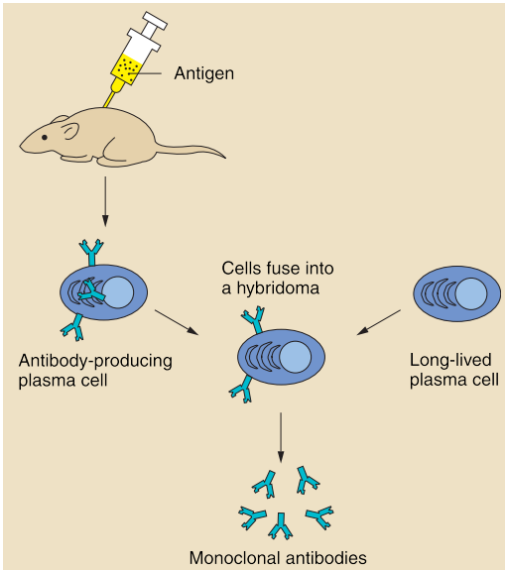


Figure 43 | **VDJ recombination**



(a) HIV particle (b) Immunoglobins

Figure 44 | HIV particle and Immunoglobins gel



(a) Antibody production

Figure 45 | Antibody production

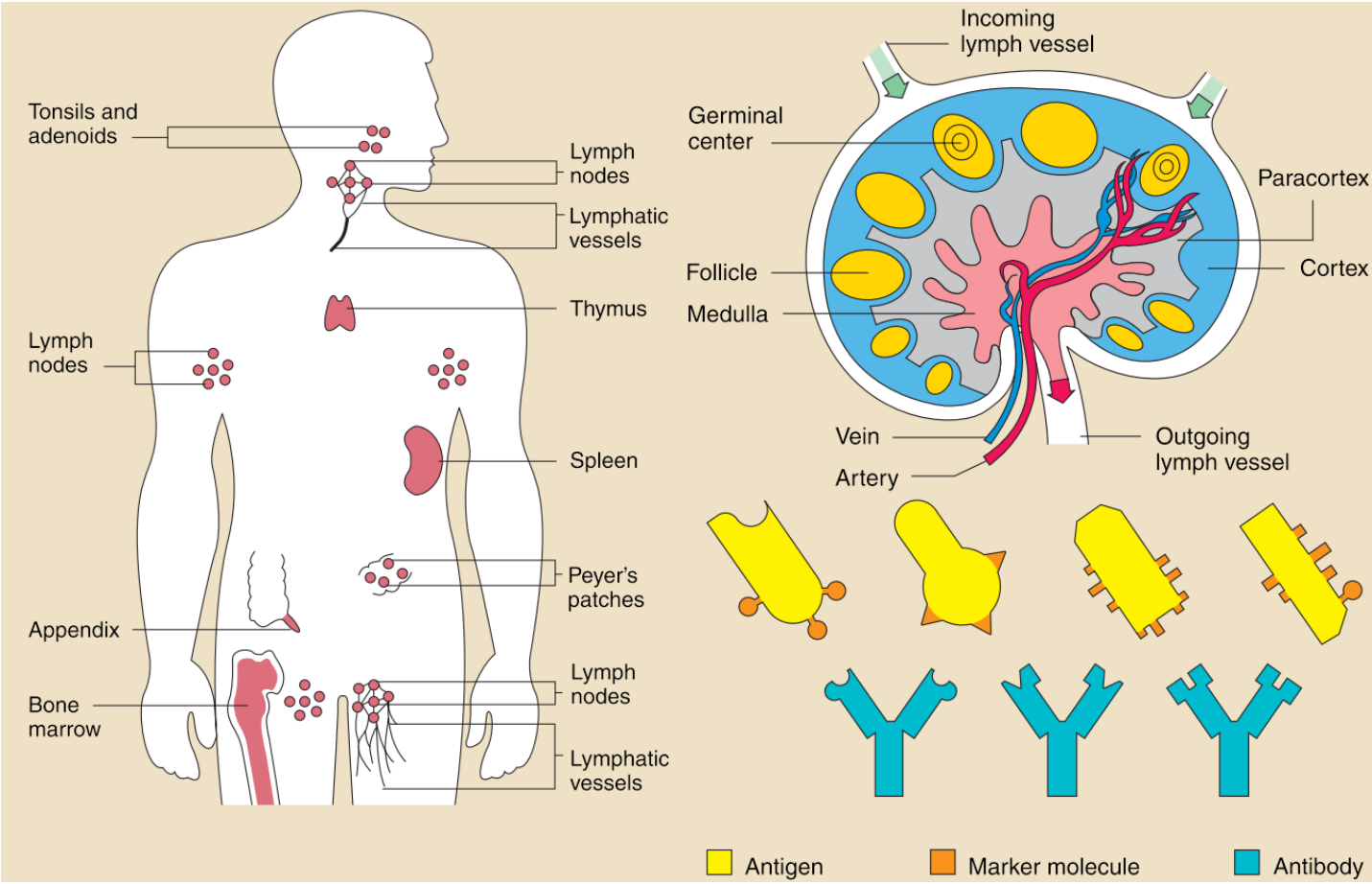


Figure 46 | Lymphatic Tissues

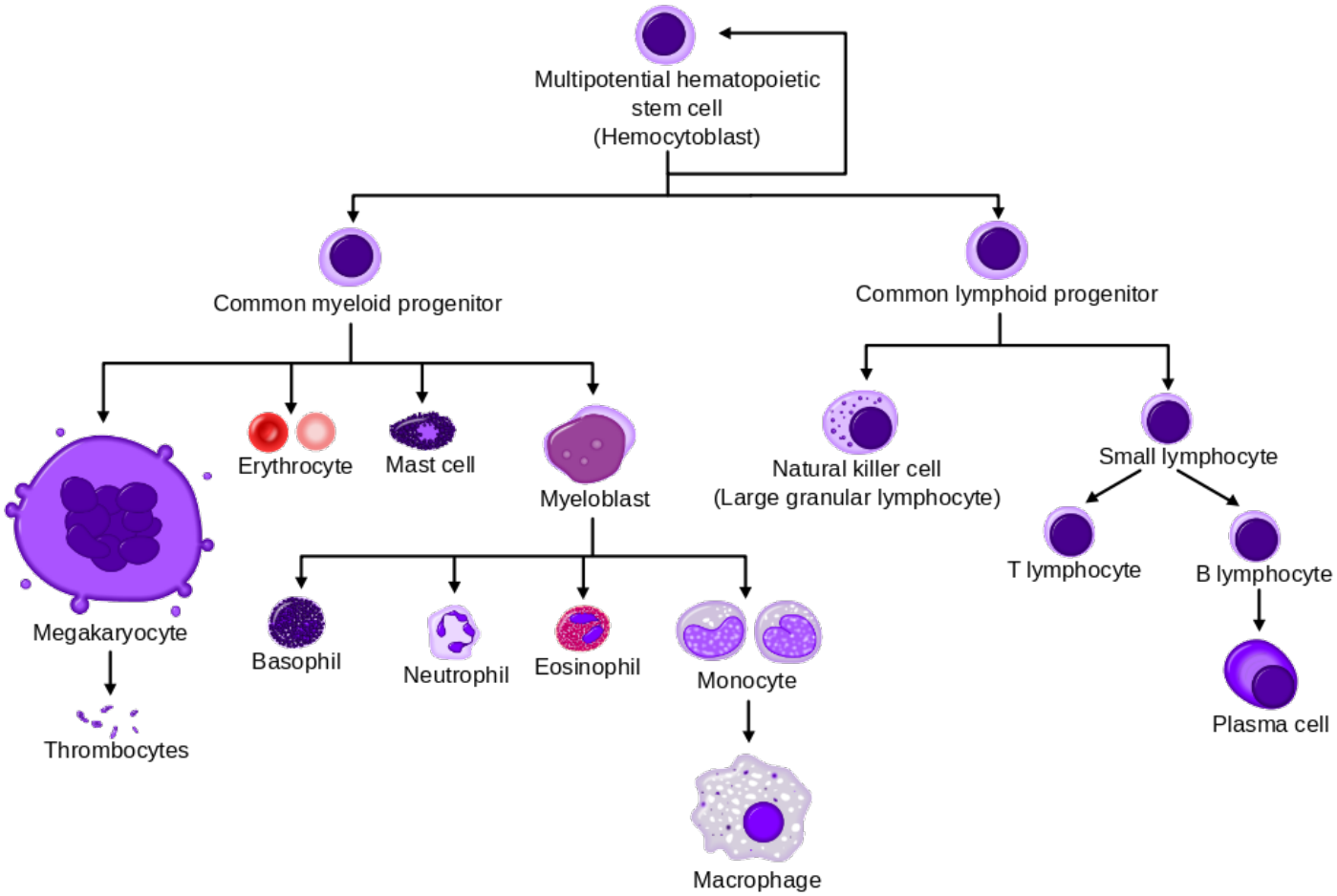


Figure 47 | Hematopoiesis

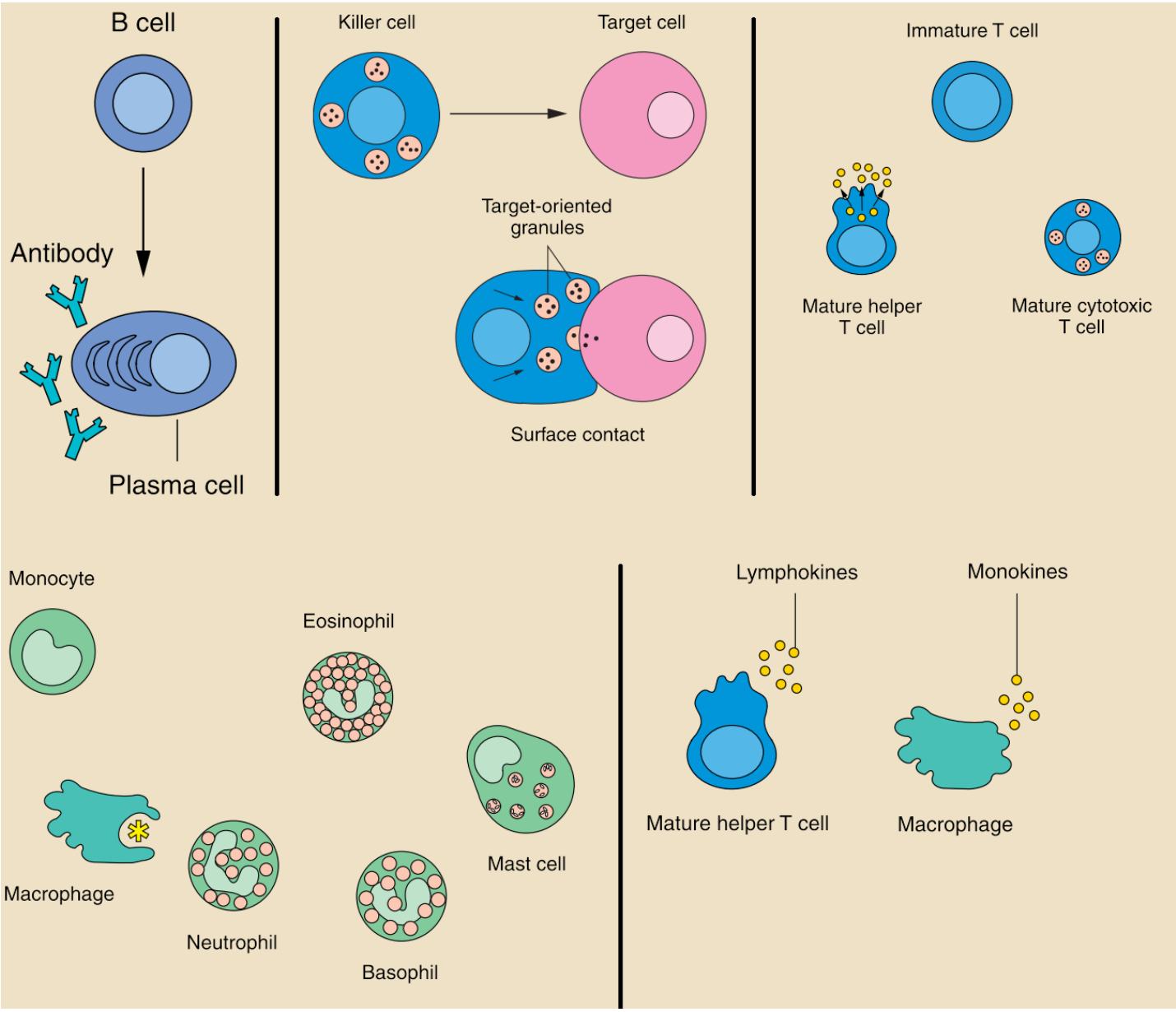


Figure 48 | **Immune Cells**

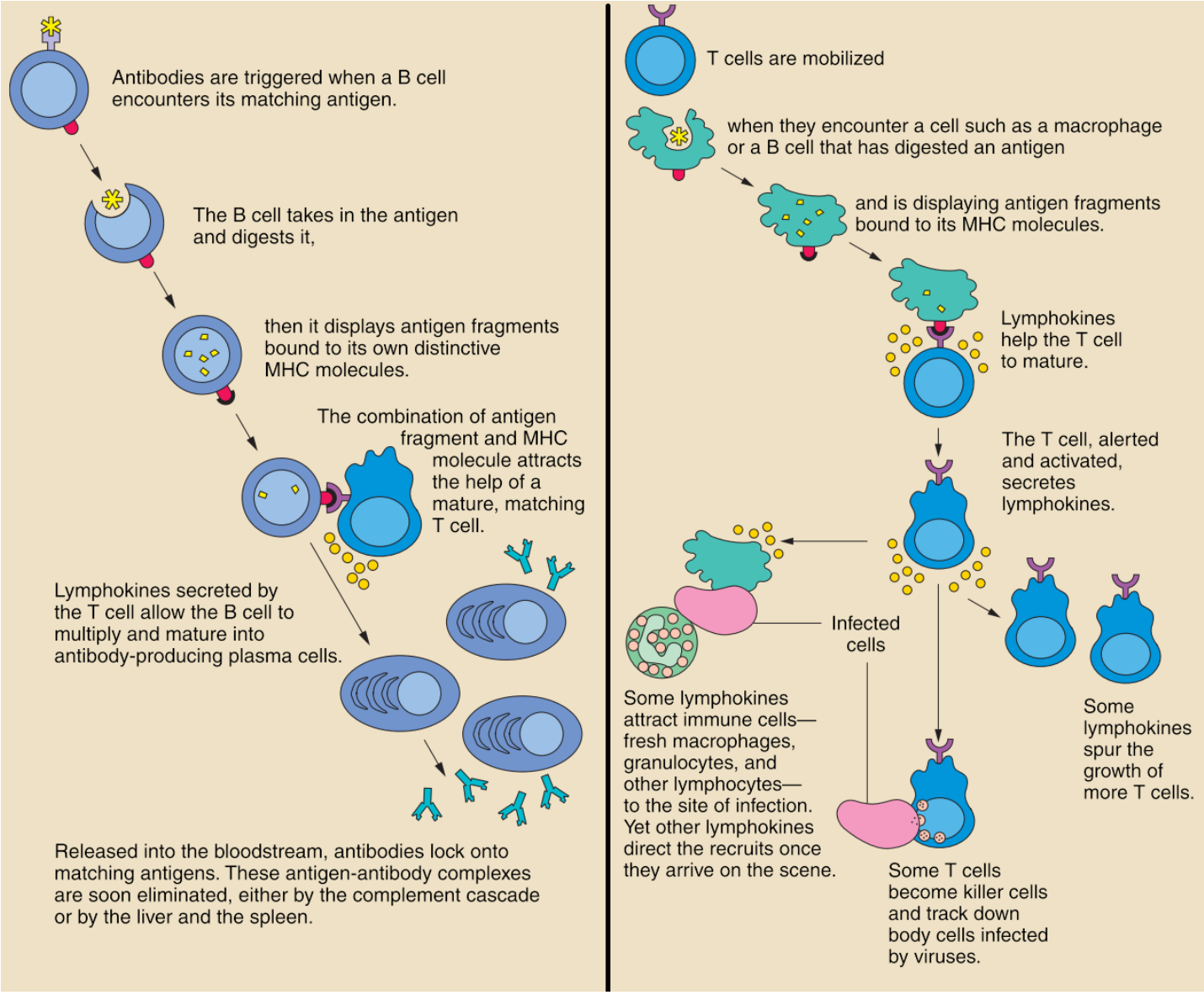


Figure 49 | B and T cell activation

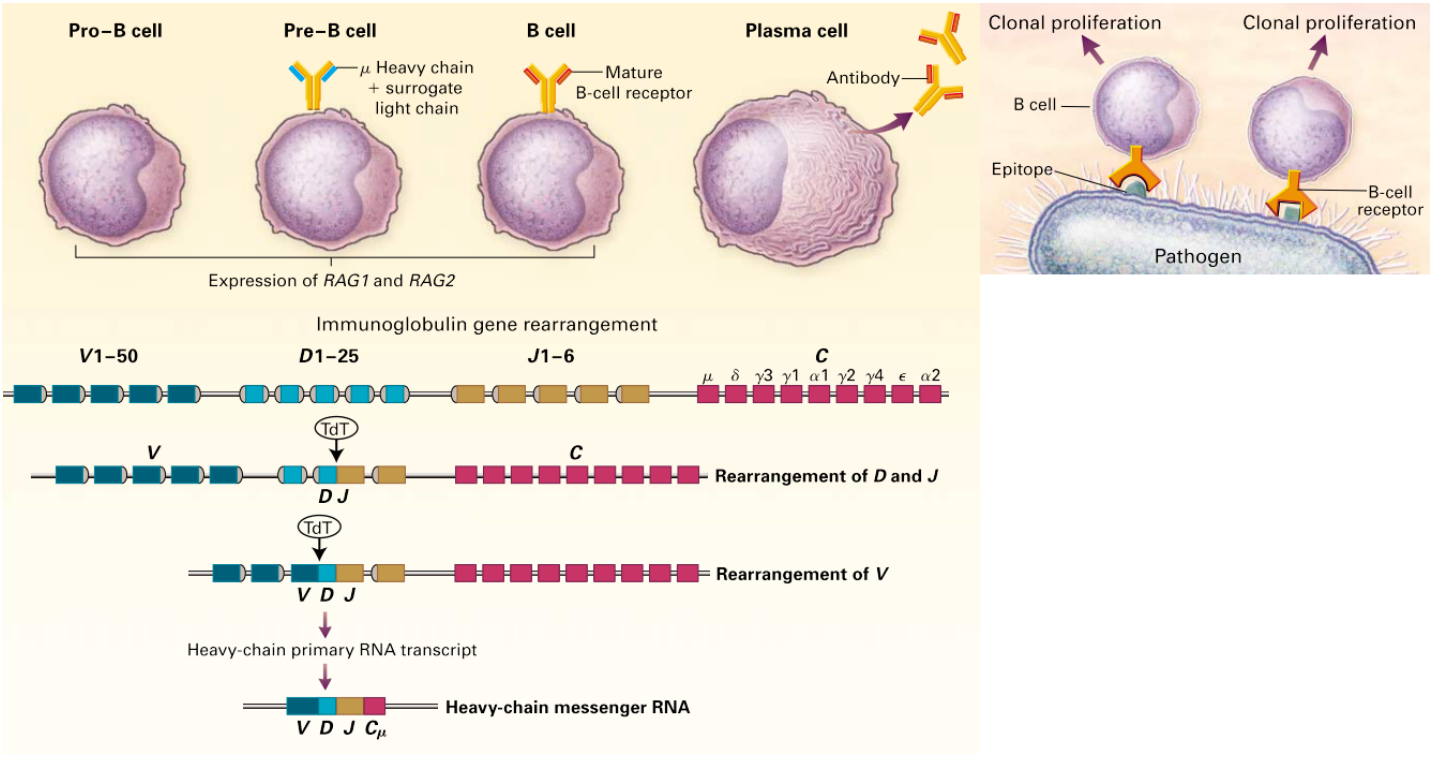


Figure 50 | **B cell maturation and VDJ recombination**

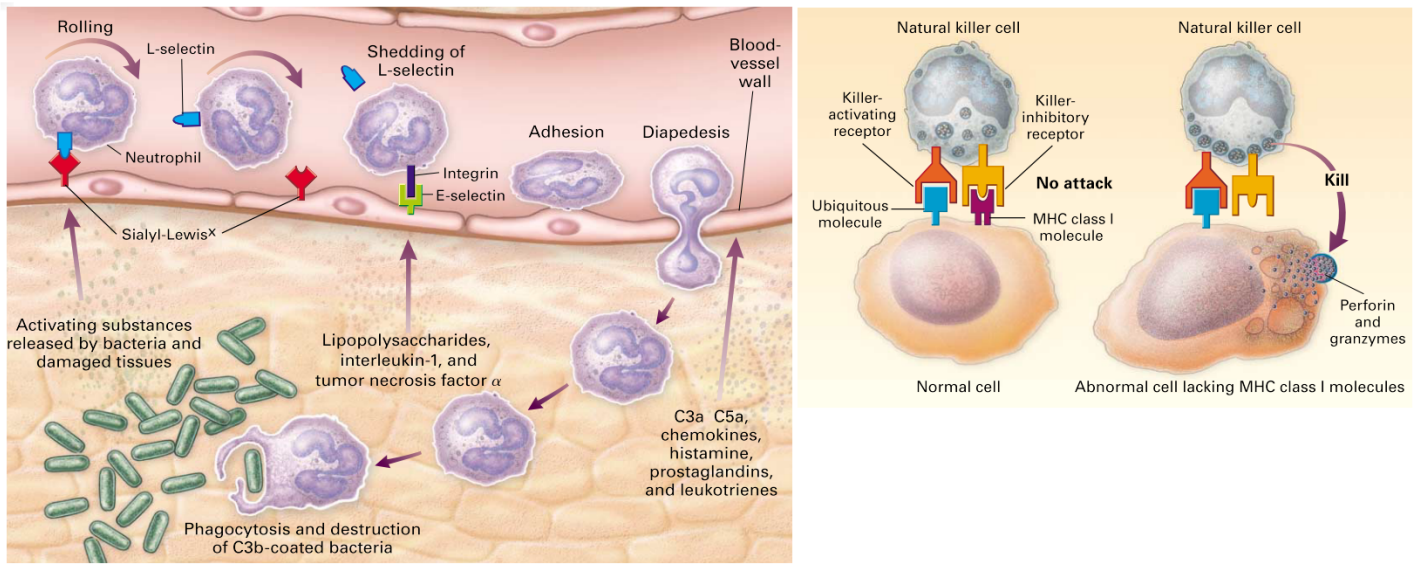


Figure 51 | **Killer cells and Phagocytes**

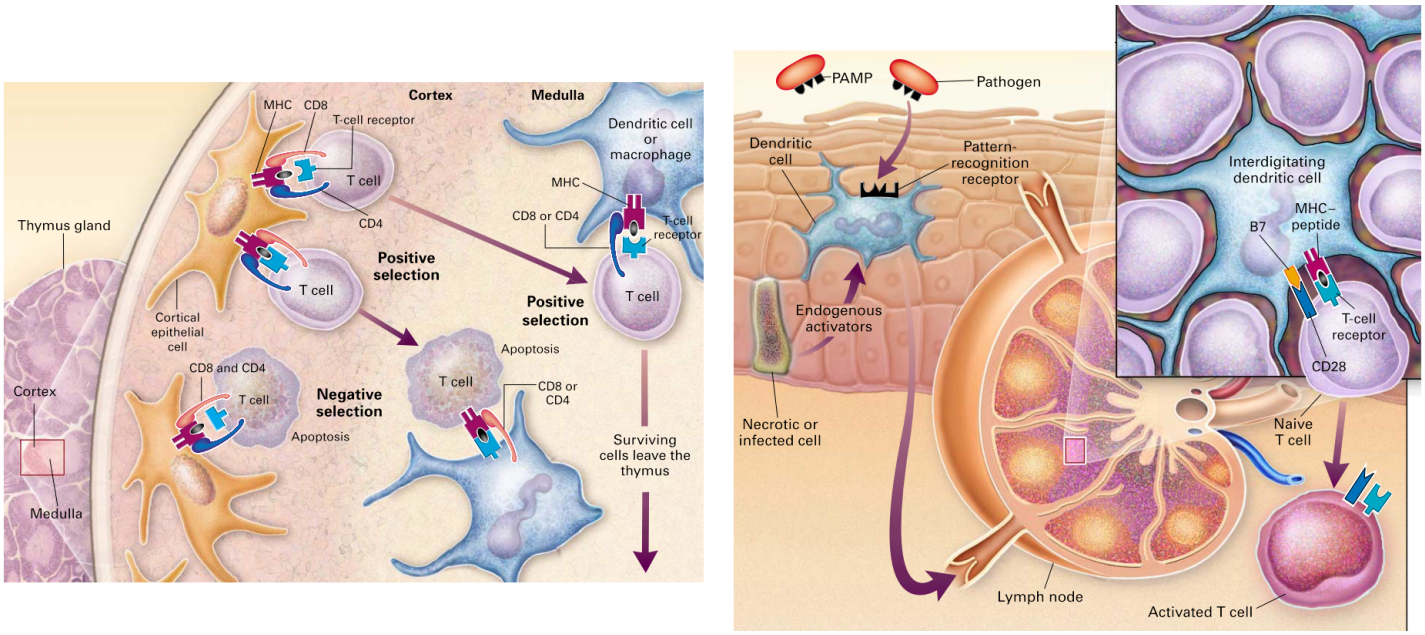
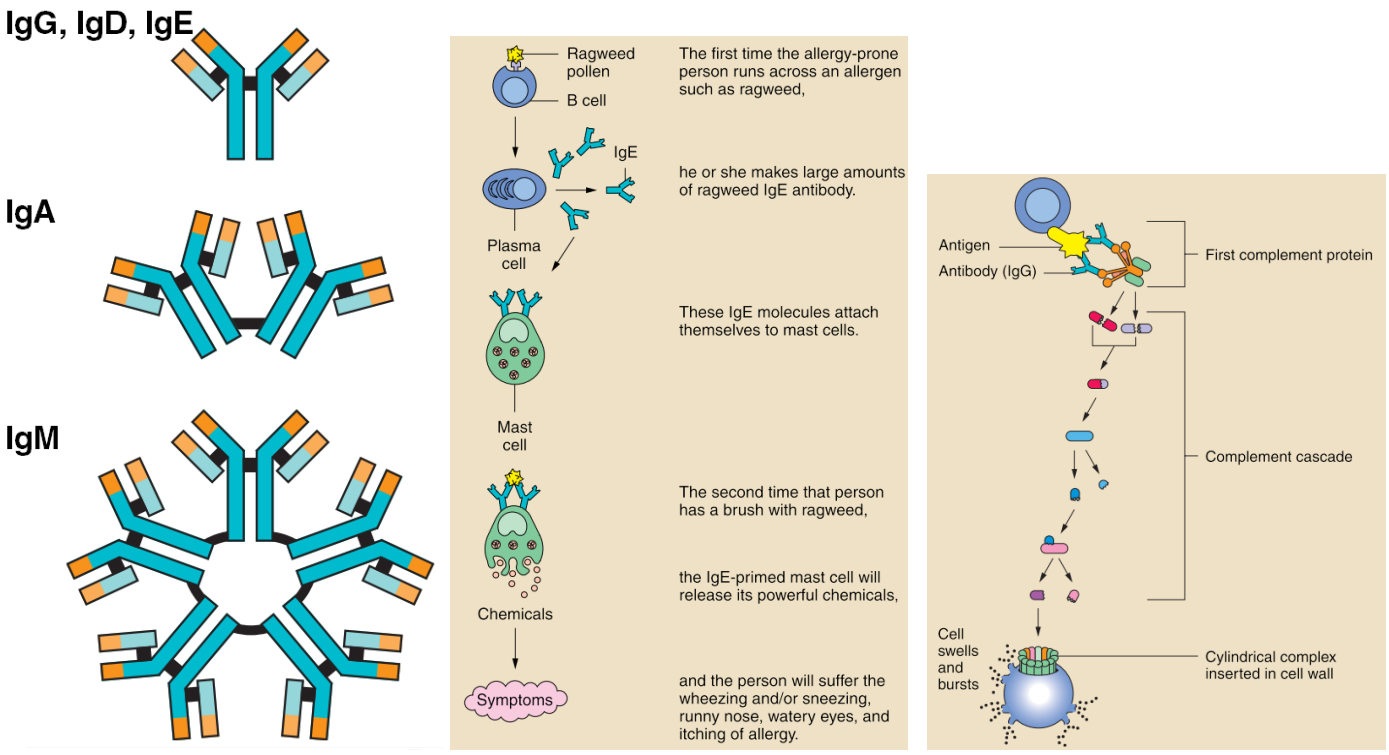


Figure 52 | Maturation/Selection T cells



(a) Immunoglobins (b) IgE mediated allergic response (c) Complement

Figure 53 | Immunoglobins, allergic response and complement system

Week 6 Nervous System

Readings
LIFE 8th: Ch. 44 45 | LIFE 9th: Ch. 45 46

Overview
Prof. Sapolsky went over the nervous system, from the basic components of a synapse to the logic behind neural circuits and long-term potentiation. We will cover each of these, diagrams are at the end.

Concepts
General frameworks in which to think about this week's material.

- Neuron anatomy, receptor types and myelination.
- Know how neurotransmitter release is achieved.
- Concept of signal-to-noise and how the nervous system achieves this in action potentials and networks.
- Know the Nernst and Goldman equations.
- All-or-nothing, threshold, and propagation of action potential.
- Understand how ion gradients are created and utilized during an action potential.
- Neurotransmitter synthesis, release, reuptake and degradation.
- Classical neurotransmitter definition and exceptions (e.g. arachidonic acid).
- Know the molecular mechanisms of long-term potentiation (LTP).
- How temporal and spatial input summation is achieved.
- Understand the networks for visual processing, intestinal peristalsis, and the pain model. Know how center-surround is achieved.

Terms

general

- Nodes of Ranvier
- myelin sheath
- oligodendrocyte
- Axon Hillock
- soma
- dendrites
- Na+/K+ ATPase
- actin
- voltage-gated Na+ receptors
- action potential
- nerve
- afferent
- efferent
- ganglia
- oligodendrocyte
- Schwann cell
- blood-brain barrier
- microglia
- leaky potassium channel
- electrochemical gradient

synapse

- vesicles
- pre-synaptic
- post-synaptic
- axon terminal
- astrocyte
- voltage-gated Ca^{2+} channels
- glutamate reuptake receptors
- cAMP
- acetylcholinesterase
- acetylcholine
- ligand-gated Na+ receptors
- resting membrane potential
- mechanical-gated channels

neurotransmitters

- depolarize
- hyperpolarize
- glutamate
- GABA
- serotonin
- dopamine
- acetycholine
- norepinephrine
- nitric oxide
- arachidonic acid
- ATP
- adenosine

LTP

- NMDA receptor
- AMPA receptor
- non-NMDA receptor
- magnesium
- arachidonic acid
- nitric oxide

neural network

- self-inhibiting collateral
- interneuron
- self-inhibiting collateral
- interneuron
- feed forward inhibition
- feed forward activation
- lateral inhibition
- center-surround
- visual processing
- intestinal peristalsis
- fast pain fiber
- Hubel-Wiesel visual
- Wall-Melzack model

Techniques
For each technique, you should know when is appropriate to use it, what its limitations are, and a particular cellular process that was used to study it.

- Electrode recordings Stick an electrode into a neuron and record the voltage.
- Voltage clamp Clamp the neuron and set it to a particular voltage, helps you measure voltage-dependent receptors and how they change.
- Neuropharmacology addition of drugs to synapses for specific receptors or neurotransmitters can help determine the behavioral and cellular function.

Neurons

Know the basic layout of a neuron and why they are different than most other cells in the body.

- Soma is the neuron cell body that connects to the axon, which sends action potentials, via the axon hillock.
- The dendrites extend from the soma (or axon) and provide input to the neuron. They contain ligand and voltage-gated receptors.
- The axon extends from the axon hillock and terminates at the axon terminal. It contains voltage-gated sodium and potassium channels that allow it to propagate an action potential.
- The axon is covered in myelin that insulates the membrane and increases the resistance, leading to a reduction in drag and increase action potential speed.
- Nodes of Ranvier are regions where myelin isn't present and help replenish the action potential.
- The blood brain barrier provides protection against unwanted chemicals, organisms and other junk from freely entering the brain.
- Microglia provide immune defense and develop from the bone marrow.
- Leaky potassium channels are responsible for creating the low resting membrane potential.

The resting potential of a neuron is defined by the equilibrium between ions inside and outside the cell. The Nernst equation defines the equilibrium potential of a single ion:

$$E = E_0 + \frac{2.303RT}{ZF} \log\left(\frac{[ion^+]_{outside}}{[ion^+]_{inside}}\right) \tag{1}$$

Useful, but flawed in the context of a real neuron. To more accurately describe the resting membrane potential, we use the Goldman equation:

$$V_m = 58 \log\left(\frac{\sum_{i=1}^{n} P_i[i]_{out}}{\sum_{i=1}^{n} P_i[i]_{in}}\right) \tag{2}$$

This gives a more accurate view by taking into account multiple ions and their relative permeabilities.

Synapse

The synapse is the site where neurotransmission takes place.

1. Action potential arrives at axon terminal.
2. Depolarization causes voltage-gated calcium channels to open.
3. Neurotransmitter vesicle fusion triggered by calcium influx.
4. Neurotransmitter diffuses across the membrane and binds to post-synaptic receptors.
5. Ions flow in or out (depending on receptor) or second messenger systems activated.
6. Neurotransmitter is uptaken by reuptake receptors in neurons or glia. They can also be degraded.
7. Vesicles are recycled.

Neurotransmitters

We talked about several neurotransmitters and what they do. In general, remember they key neurotransmitters and how they affect the synapse (inhibition and excitation). Further, we talked about several neurotransmitters that function as neuromodulators, they can initiate second messenger cascades or modify how the synapse responds. Lastly, we talked

Neurotransmitter properties

We talked about classical neurotransmitters and what you need to know to identify ones.

- Localized to the axon terminal.
- Released when an action potential occurs.
- Depolarize and hyperpolarize post-synaptic neuron.
- Can be pharmacologically manipulated.

Synthesis

- The nervous system uses amino acids and other readily available, cheap precursors to synthesize neurotransmitters.
- Nervous system reuses neurotransmitters as precursors for other neurotransmitters, it's lazy.
- Dopamine, norepinephrine, and epinephrine are synthesized from tyrosine.
- Serotonin (5HT) is synthesized from tryptophan.
- Glutamate is an amino acid, so it is everywhere and doesn't need special synthesis.
- GABA is synthesized from glutamate.

Classical

- Glutamate is associated with excitatory synapses and is involved in LTP.
- GABA is associated with inhibitory synapses and can cause chloride channels to open, hyperpolarizing the cell.
- Serotonin is synthesized from tryptophan and can help the body gauge food availability.
- Dopamine is associated with reward and can function as a neuromodulator.
- Acetycholine is found at the neuromuscular junction (NMJ) and its release causes muscle contraction. It is also the major neurotransmitter of the parasympathetic pathway. Acetylcholinesterase degrades acetylcholine, inhibiting its action.
- Norepinephrine modulates neuronal activity, such as causing cardiac muscle cells to depolarize, and is associated with the sympathetic pathway.

Odd-balls

- Nitric oxide can be made during LTP in the post-synaptic neuron and diffuse across the synapse back to the pre-synaptic neuron. It causes an increase in the synthesis and release of glutamate.
- Arachidonic acid same as nitric oxide
- ATP binds to receptors in the pre-synaptic neuron and promotes glutamate release.
- Adenosine can diffuse back to the pre-synaptic neuron and inhibit glutamate release.

Neuropharmacology

- Manipulate circuit by adding antagonist (opposite effect normal ligand) or agonist (same effect as normal ligand).
- Other methods: block receptor, inhibit release of neurotransmitter, and increase neurotransmitter release.
- Inhibit reuptake or degredation of neurotransmitter, increasing signal.
- Alter synthesis rates of neurotransmitter; can also change the number or affinities of receptors.
- **Remember**: there will always be side-effects when adding a neuro-targeted drug. They have to be delivered systemically because of the blood-brain barrier and thus will act in normal parts of the brain. e.g. giving L-DOPA to cure Parkinson's will cause psychotic behavior because dopamine levels throughout the brain will rise.

Action Potential

The action potential is the basic method of information flow in the brain. It involves a series of well-timed steps:

1. Note: **plasma membranes are impermeable to ions**. Lipids and charged ions don't like each other. People seem to think otherwise and try to justify permeability of an ion based on ionic size. This thinking is wrong. Don't do it. Back to the action potential…
2. Ligand binds to receptor in the dendrite.
3. Soma becomes depolarized until the voltage passes a threshold.

4. Then the voltage-gated sodium channels at the axon hillock open. This can either be through spatial or temporal summation of inputs from different axons.
5. Opening of v-gated Na+ channels causes sodium to rush into the axon, depolarizing it further. Sodium has a positive equilibrium potential, so the region of the axon reaches almost that for a brief time.
6. Voltage-gated potassium channels open as the axon becomes depolarized, leading to a efflux of potassium ions, this hyperpolarizes the cell (positive ions leaving).
7. Inactivation gate on the sodium channels causes them to become closed, this is partially responsible for the refractory period after an action potential has occured.
8. Potassium has a equilibrium potential that is below resting potential, thus the brief period when sodium channels are closed, the cell hyperpolarizes past the resting potential.
9. Potassium channels close and the cell slowly returns to resting potential, leaky potassium channels are always open and keep the cell negative.
10. Na+/K+ ATPase restores the concentration gradient by moving Na+ out and K+ in. It uses ATP to do this and thus consumes a majority of the cells energy.
11. The refractory period causes a unidirectional flow of information along with the pull of negative membrane potential in the downstream membrane.
12. Myelin gaps called nodes of Ranvier allow saltatory conduction, whereby action potentials are replenished at notes and appear to `jump'.

You should understand the basic steps involved in generating an action potential and know what would happen if you removed different components:

- Chloride channels can be used to inhibit action potentials by hyperpolarizing the cell further (chloride flows in and is a negative ion).
- Spatial and temporal summation can occur, in which inputs alone aren't enough to initiate an action potential, but several at the same time (spatial) or successively (temporal) can cause the cell to fire.

Long-term Potentiation

Memory is thought to be stored through synaptic strengthening rather than neuronal growth or other mechanisms. We will talk about NMDA receptor mediated long-term potentiation (LTP).

- Glutamate is released from the pre-synaptic neuron, diffuses across the synapse.
- Glutamate binds to AMPA (or non-NMDA) receptors, causing Na+ to rush inside.
- NMDA receptors need to have both glutamate bound and magnesium block removed to be active. Magnesium falls off when the synapse is depolarized.
- NMDA receptor allows calcium and sodium to enter the cell, depolarizing it further.
- Calcium has multiple functions that **do not** involve depolarization:
  - Cause phosphorylation of AMPA receptors, leading to longer activation.
  - Allow insertion of more AMPA receptors, making the synapse more sensitive and easily excitable.
  - Causes arachidonic acid and nitric oxide to be released, diffuse to the pre-synaptic neuron and modify glutamate release.
  - Change shape of dendrite, lowering the input resistance and causing it to be more excitable.
- LTP can be inhibited by ethanol by inhibiting NMDA receptor calcium influx.

Neural Networks

Neural networks consist of afferent and efferent neurons that function as inputs or outputs, respectively. To understand neural networks, extend the core A$\xrightarrow{activates}$B or A$\xrightarrow{inhibits}$B ideas from cell biology. When dealing with problems, label each neuron in terms of how it affects the final output and just walk through the connections.

Network Properties

Remember, a main goal of the nervous system is to maximize signal-to-noise.

- There are different methods of sharpening a signal, both temporally and spatially.
- This allows you to produce signals that turn themselves on then off.
- In addition, you can create center-surround setups where inhibiting connections cause spatial sharpening.
- When looking at traces of neuronal activity, you'll often see random spiking without obvious stimuli. This is noise created by neurons randomly crossing their threshold and firing or other stimuli you aren't directly observing.

Types

- Self-inhibiting collateral
- Interneuron
- Feed forward inhibition
- Feed forward activation
- Lateral inhibition is when a neuron projects to other neurons in the same layer, inhibiting them. This allows spatial contrast enhancement.

Examples

- Center-surround uses lateral inhibition to create groups to cells where the center or surround inhibits the other, causing a sharp distinction between the two parts.
- Hubel-Wiesel visual Visual processing uses parallel projections and converging connections to help distinguish between different objects and movement. The example we use is retinal cells projecting to stellate cells that project to the retina.
- Intestinal peristalsis is controlled by inhibitory axo-dendritic connections.
- Wall-Melzack model for pain involves a fast A fiber that activates pain while a slow C fiber inhibits pain. This network uses an interneuron to achieve control. Known as the Wall Melzack pain model. Also, there is epicritic and protocritic pain, which are acute and chronic, respectively.

Problems

If there is any confusion about the questions, shoot me an email or talk to me after class. Remember, draw out what a pathway, interaction, or what-have-you looks like if you get confused.

1. What experiments did Hodgkin and Huxley perform? Recording from the squid giant axon.
2. How can voltage clamping help study receptors? Can give us the current-voltage relationship.
3. Suppose you inject sodium ions into an axon midway between the axon hillock and terminal. What happens? Action potential propagates both up and down the axon.
4. Where are voltage-gated sodium channels? Ligand-gated? Voltage-gated are at the axon hillock, axon, and axon terminal. Ligand-gated are at the dendrites (mainly).
5. What difficulty in terms of protein synthesis and transport do neurons have that other cells don't? Axons (and dendrites) can be long and thus they need to transport materials along lengths that are order-of-magnitude longer than other synapses.
6. Why do brain and central nervous system injuries normally last longer than other types? Neurons are differentiated and have a limited capacity to regenerate themselves after injury.
7. What would happen if you blocked calcium influx at an axon terminal? Get no neurotransmitter release as vesicles will not fuse.
8. What does adding botox do? Destroys ability for vesicles to fuse with membrane, inhibiting neurotransmission.
9. What is the advantage of using neurochemical synapses as opposed to eletrical junctions to transmit information throughout the nervous system? Neurochemical synapses can be modulated and their effects controlled while electrically connected networks cause rapid propagation, but it is less controlled.
10. Why does adding L-DOPA to compensate for substantia nigra degradation and resulting motor defects cause psychotic behavior? Because dopamine levels throughout the brain will increase.
11. You add drug X to a GABA-ergic synapse and observe an action potential in the post-synaptic neuron. What does the structure of this drug look like? Is this an antagonist or agonist? Similar to GABA, you are tricking GABA receptors into thinking they are binding the right molecule. It is an agonist.
12. What is the difference between peptide and small molecule neurotransmitter synthesis? Small molecule enzymes are transported to axon terminal and synthesis occurs there. Peptides are synthesized in the soma and transported in vesicles to the synapse.
13. Why do action potentials saltate? Using this logic, provide a way to slow or stop action potentials. Myelin prevents leakage of ions to the extracellular environment and nodes of Ranvier provide locations to replenish the action potential. By increasing the distance between nodes of Ranvier (no sites to replenish ions) or reducing myelination (all leaks out), you can slow or stop action potentials.
14. What happens if you block voltage-gated potassium channels? Voltage-gated sodium channels? The inactivation gate on the sodium channel? Blocking potassium channel will lead to a action potential that lasts longer since it won't be hyperpolarized as fast. Blocking sodium channels will prevent an action potential from taking place, cell will become depolarized but threshold won't cause positive feedback to occur. Clipping off the inactivation gate will eliminate the refractory period.
15. What is the charge in the intracellular and extracellular environment normally? During an action potential? Normally negative inside, positive outside. During an action potential, the area where it is propagating has the reverse: positive inside, negative outside.
16. You add a drug to inhibit AMPAR kinase. How will this affect LTP? Phosphorylation helps increase the excitability of AMPARs, so this would reduce LTP by making the synapse less sensitive to inputs.
17. Why does a tetanic stimulation induce LTP as opposed to a single input? Explain the molecular mechanism. Tetanic stimulation causes the post-synaptic cell to be depolarized by binding of glutamate to AMPARs. This removes the magnesium block on NMDA receptors. Then, a pre-synaptic stimulus follows close behind while the post-synaptic neuron is still depolarized, allowing glutamate to bind NMDA receptors and allow sodium and (crucially) calcium to flow in. Calcium mediates LTP by inducing AMPAR insertion, arachidonic acid synthesis, etc.
18. What happens if you increase glucose levels in the post-synaptic neuron? Increase glutamate release at the pre-synaptic neuron due to increase ATP levels in the post-synaptic neuron diffuse back across the synapse to the pre-synaptic neuron and increasing glutamate synthesis/release.

19. See pain model in Fig. 54. How does inhibiting A fibers affect pain? C fibers? Inhibiting A will lead to chronic pain, since C fibers inhibit the interneuron and stimulate B. Inhibiting C fibers will lead to the same sharp pain, but dull throbbing pain won't be registered.
20. See intestinal peristalsis in Fig. 54. How would digestion be affected by inhibition of neuron 5? You would have a much harder time getting food down the esophagus and thus could choke.
21. See lateral inhibition in Fig. 54. How is this system used in the retina? Lateral inhibition allows for center-surround to be created. The parallel and converging projections allow for simple cells to detect edges, shapes or moving objects.

Figures and Tables

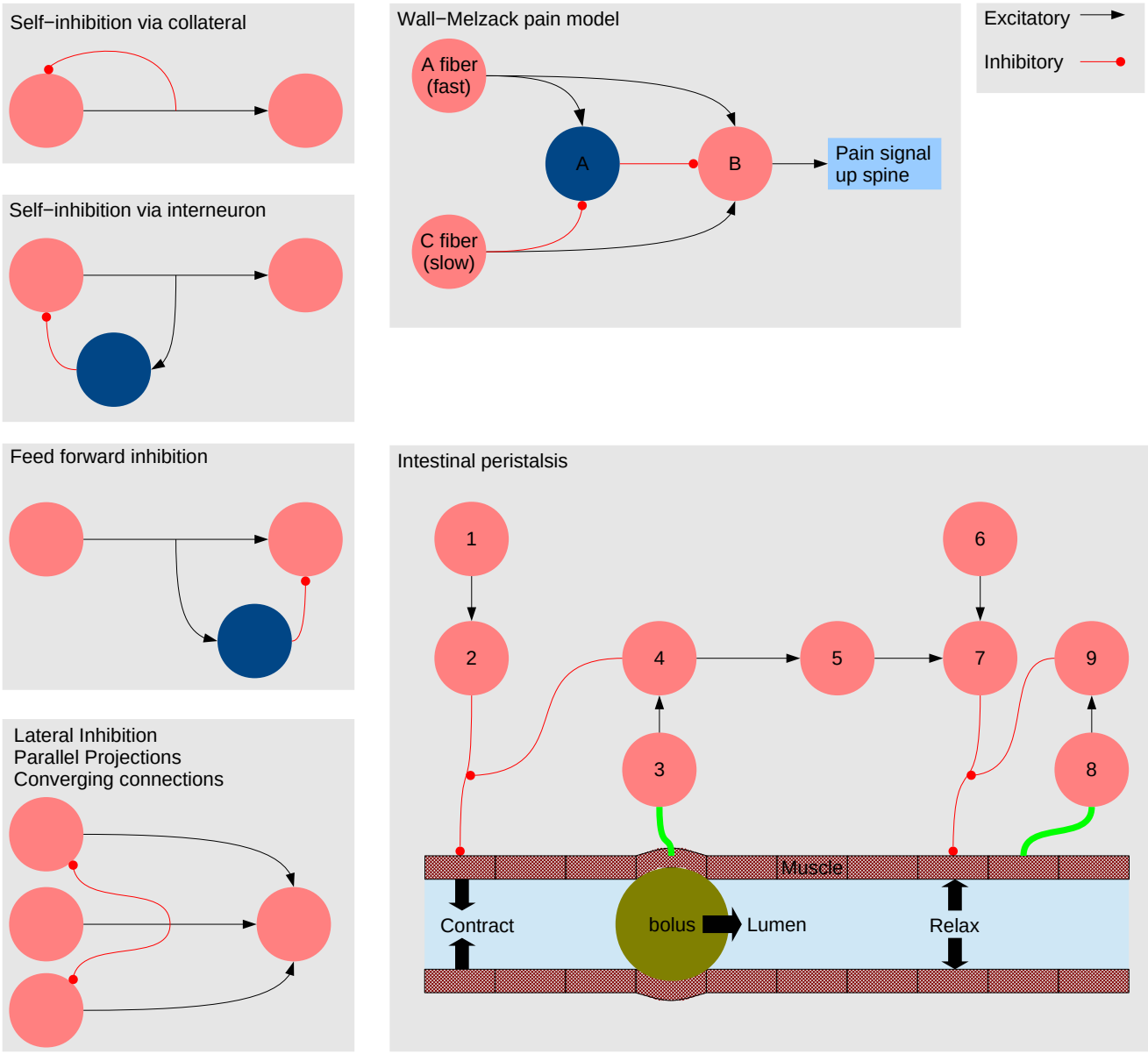


Figure 54 | Neural Networks
Above are different types of neural networks discussed in class. Know the general idea and places where they are applied. For the pain model, know what happens if the A or C fibers undergo apoptosis. Understand for the intestinal peristalsis why the circuit is laid out that way.

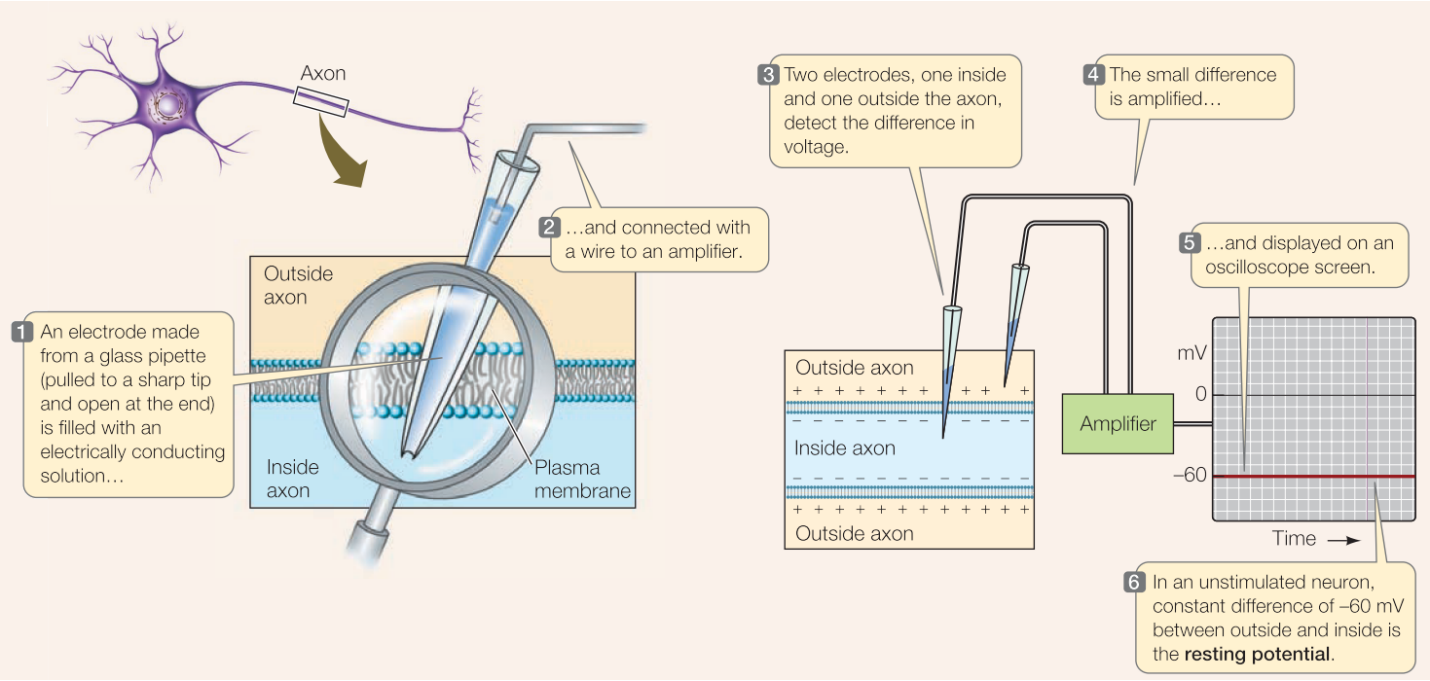


Figure 55 | Electrode Recordings

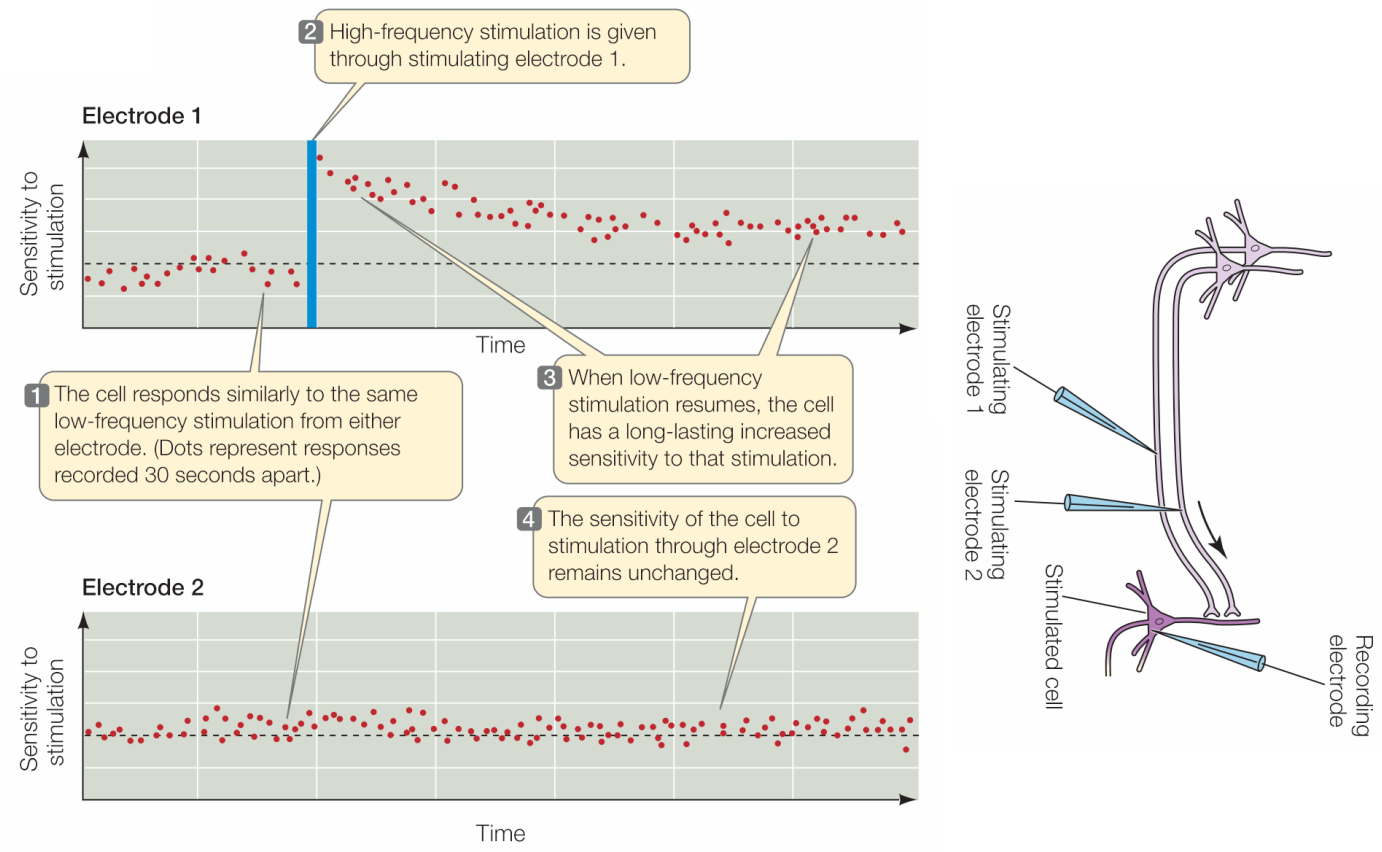


Figure 56 | Inducing long-term potentiation.

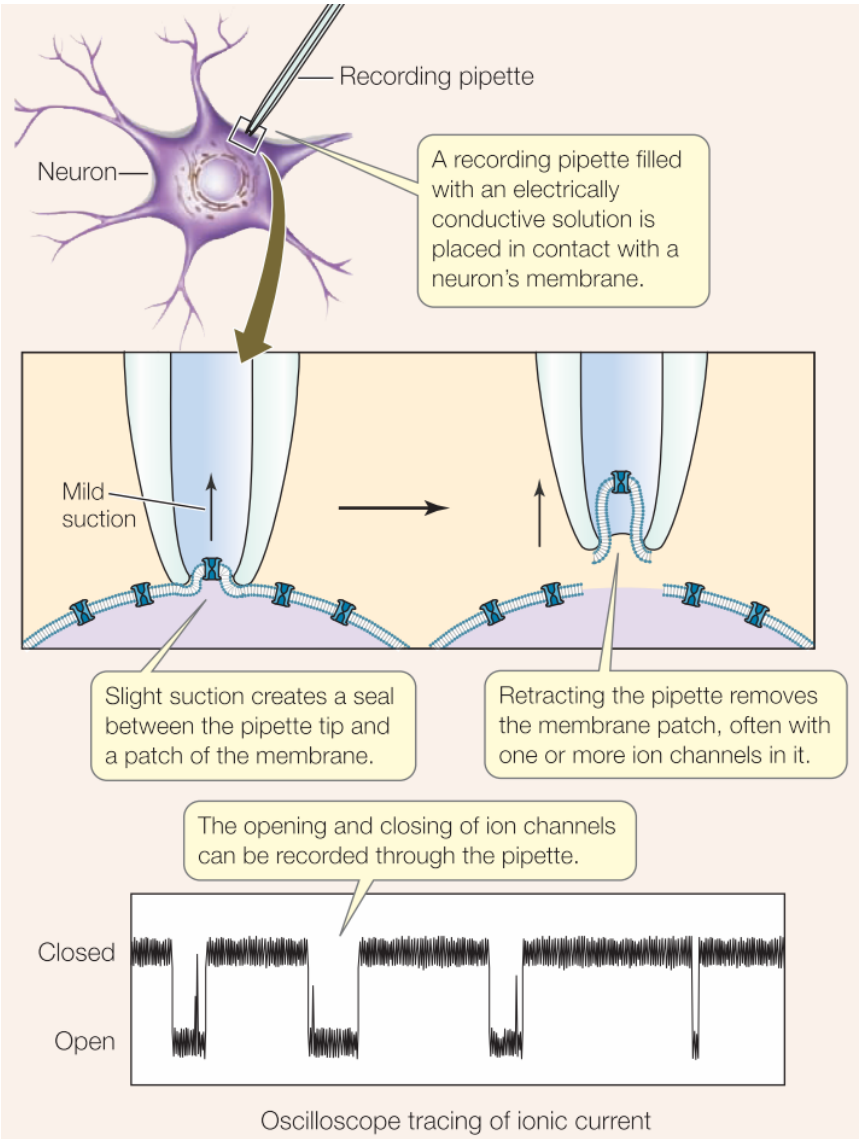


Figure 57 | TITLE

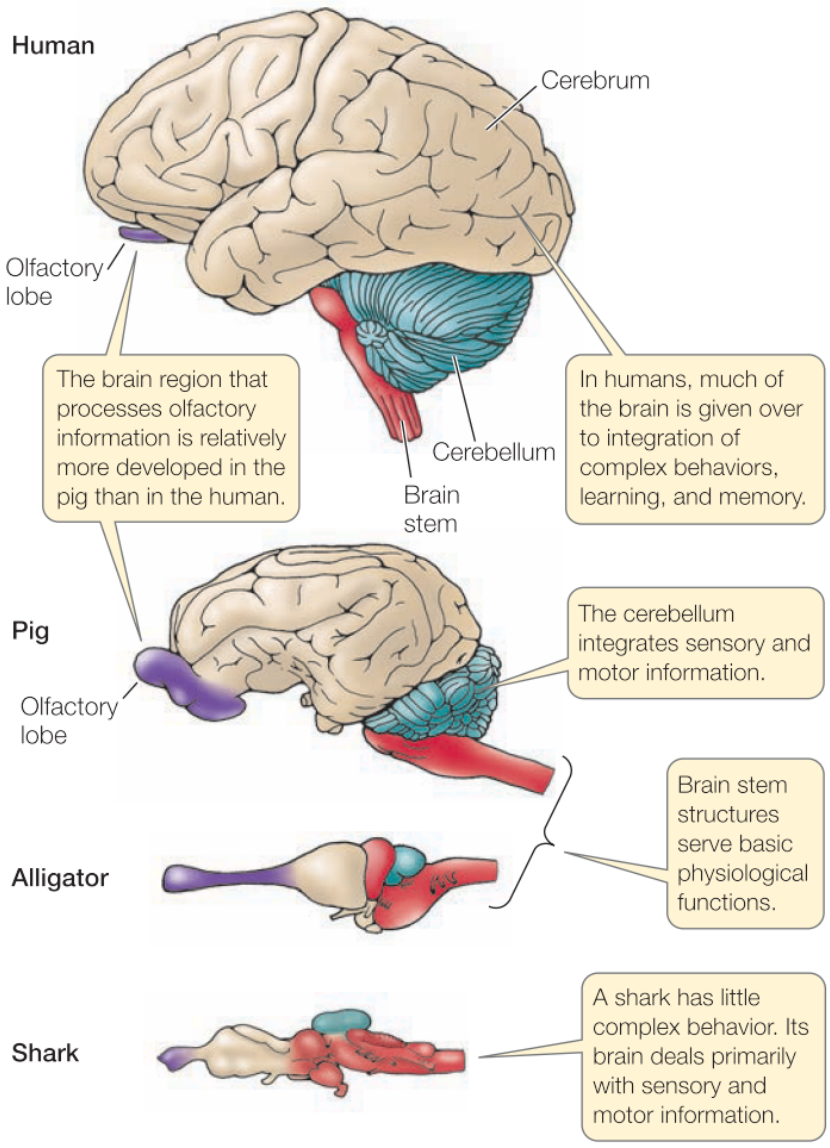


Figure 58 | Brain anatomy of different species.

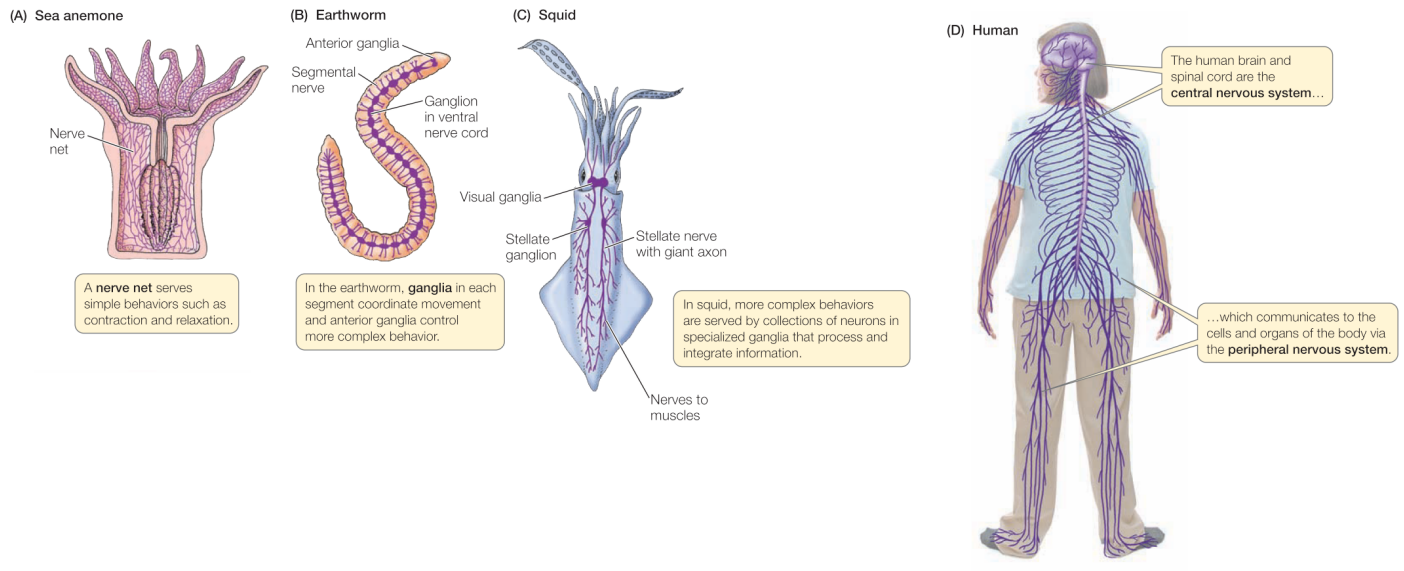


Figure 59 | Nervous system of different species.

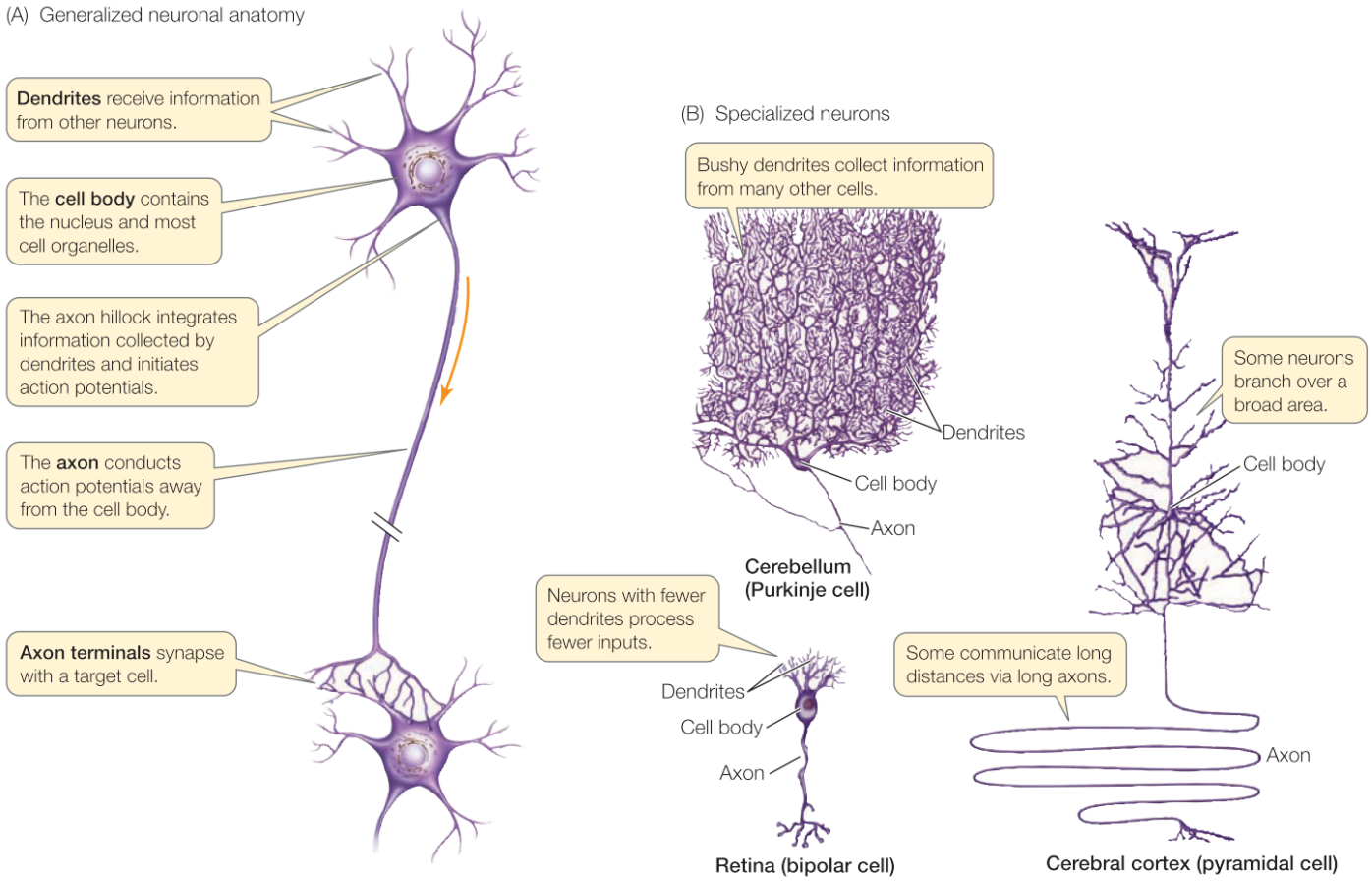


Figure 60 | Neuron anatomy

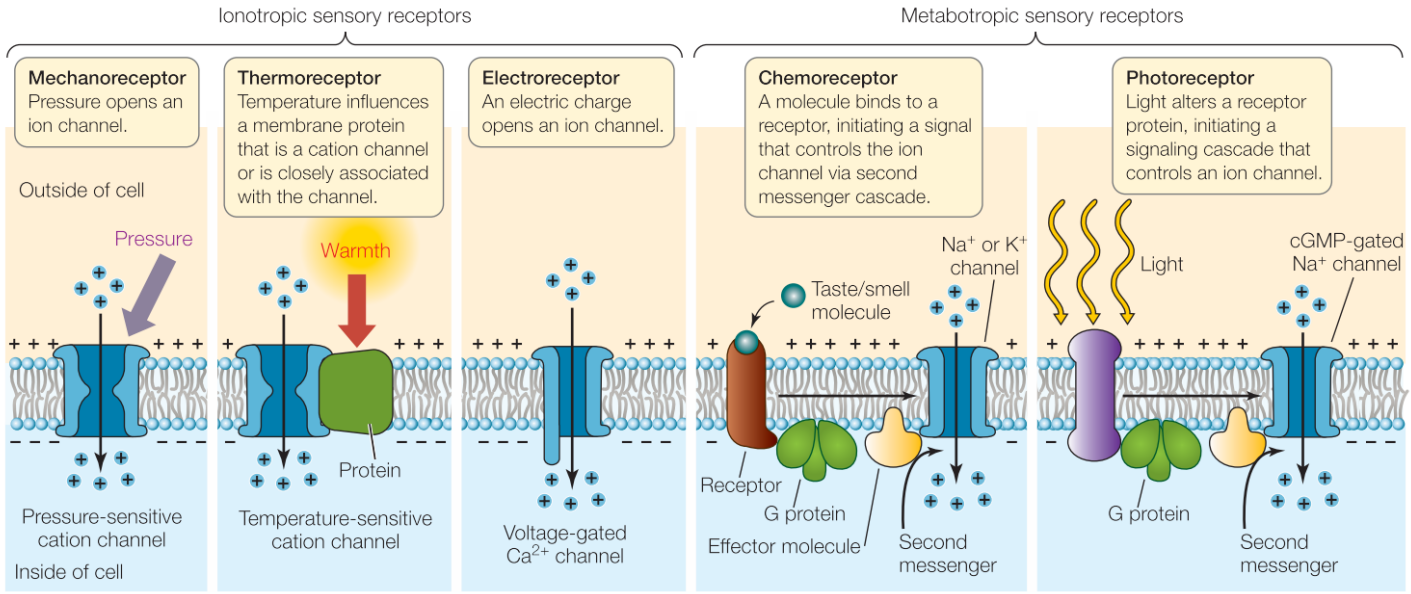


Figure 61 | **Ion channels**

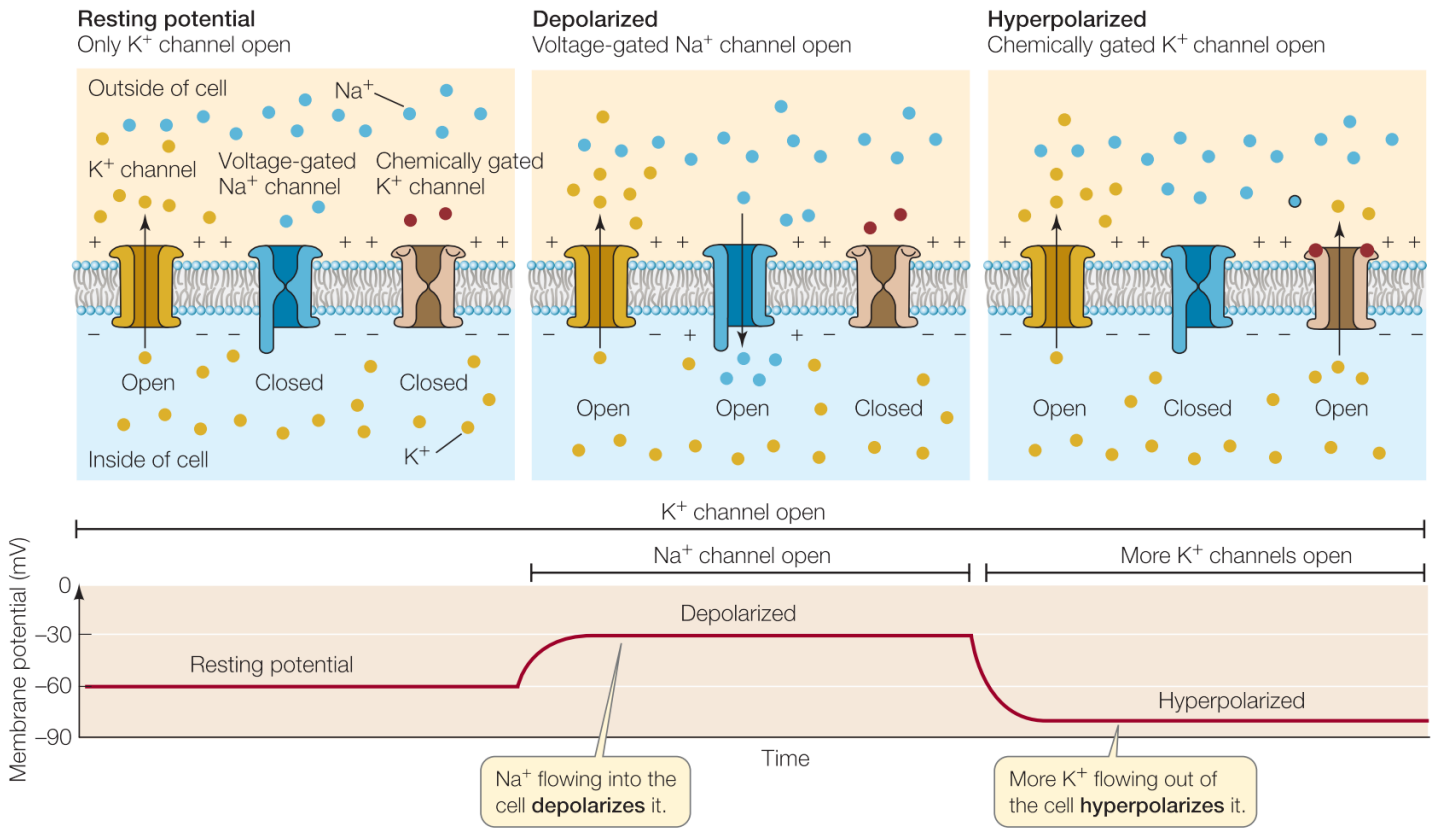
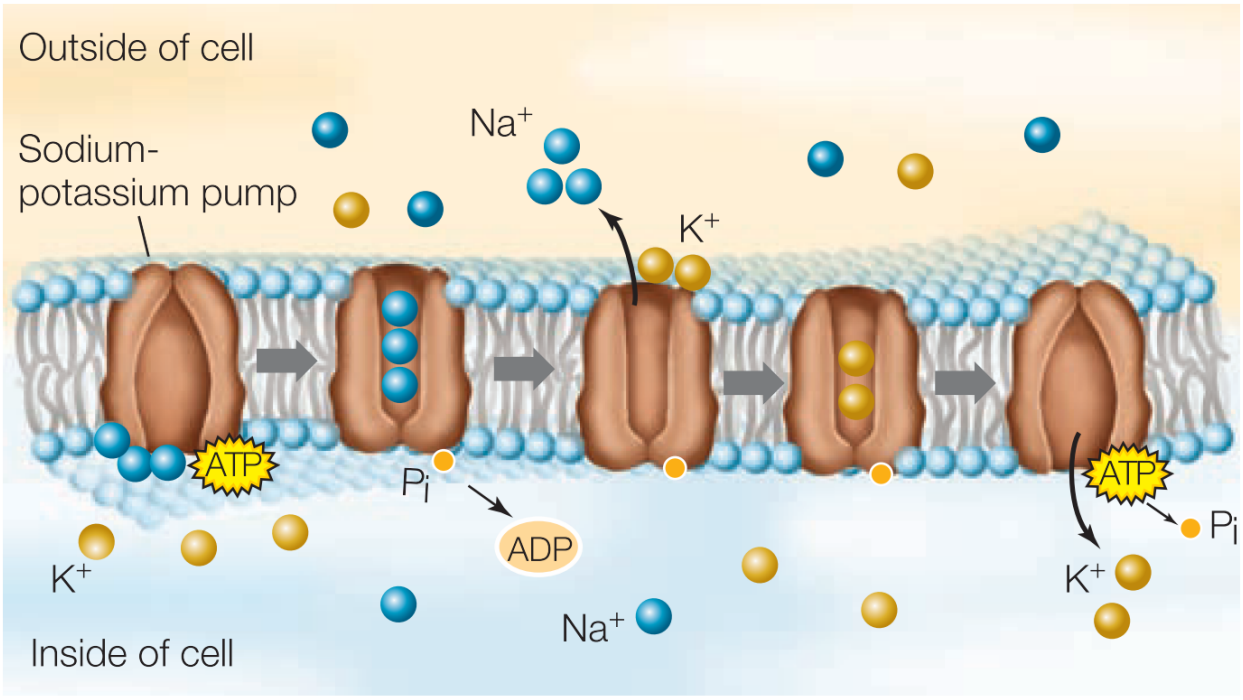


Figure 62 | **Membrane potential**

(A) Na^+–K^+ pump (ATPase)



(B) Na^+–K^+ channels

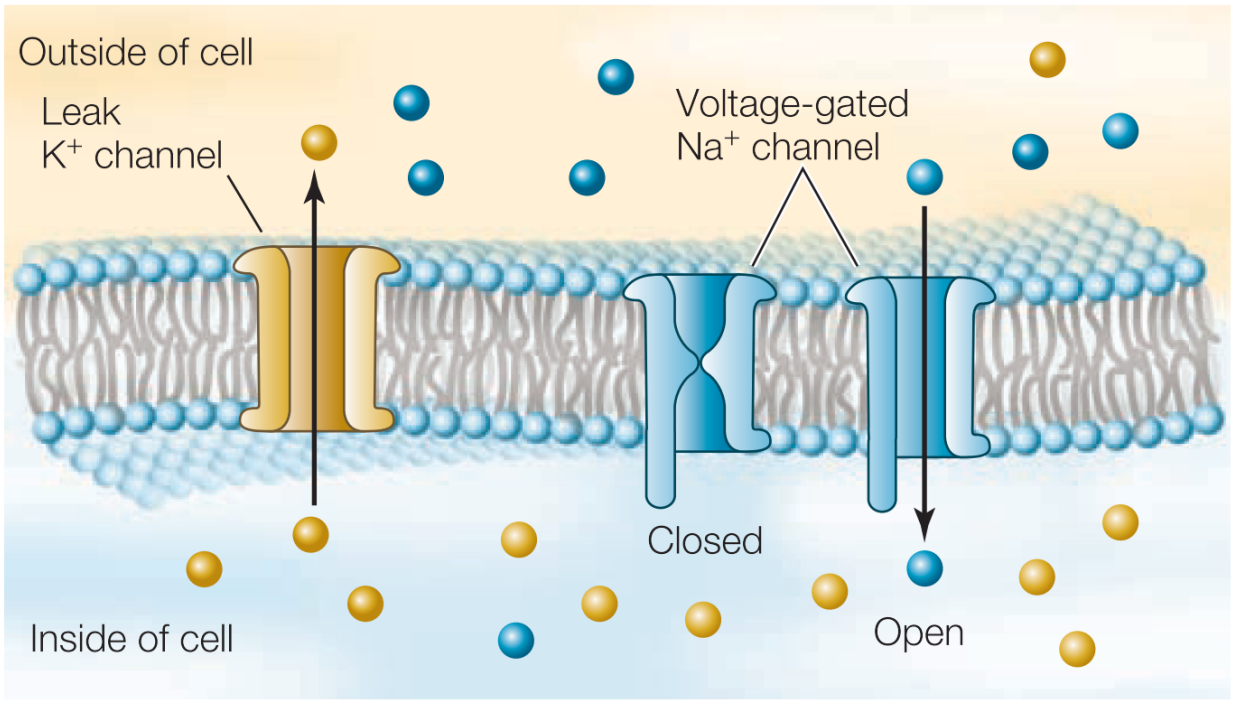


Figure 63 | Creation of the sodium-potassium gradients

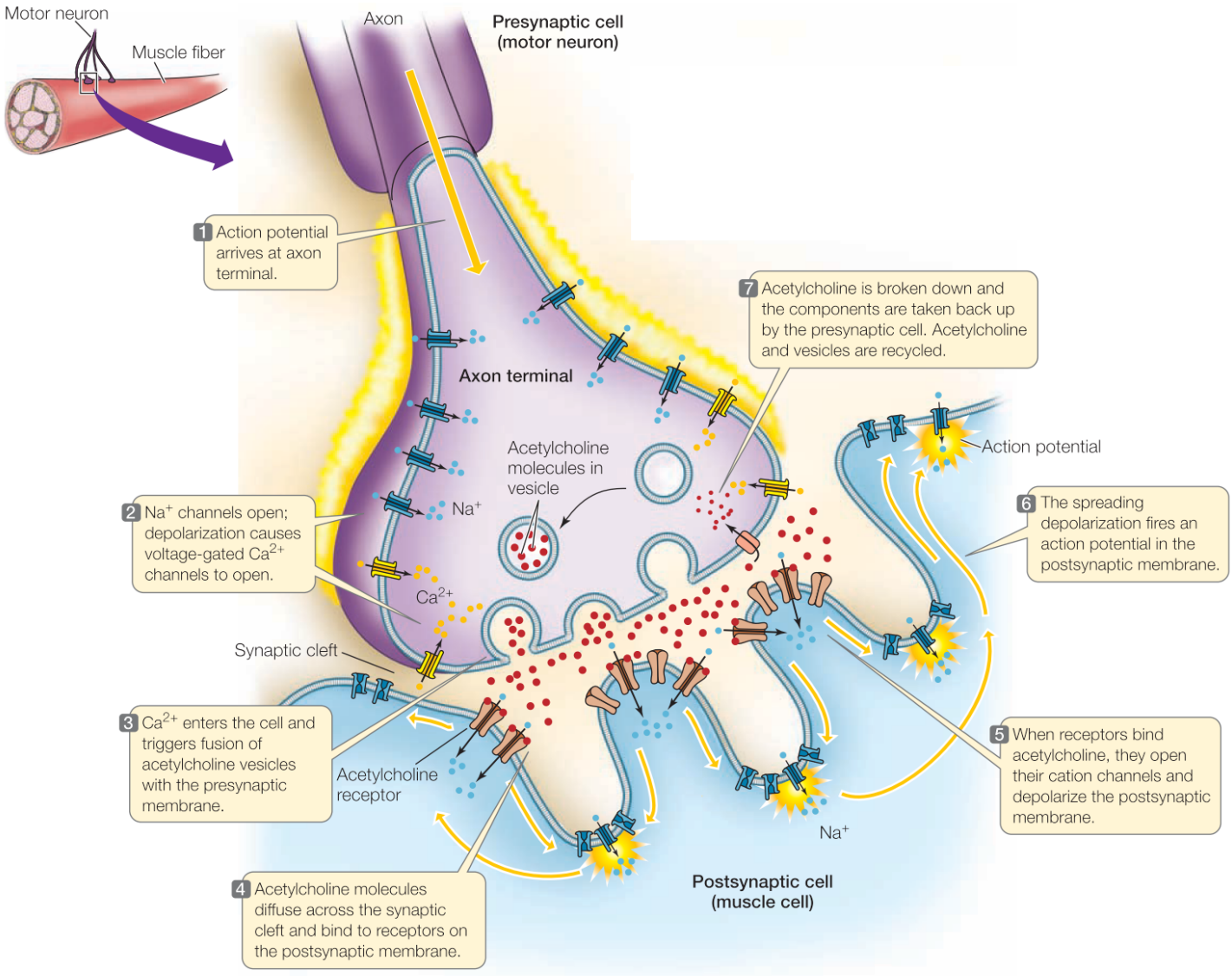


Figure 64 | Neurotransmitter release

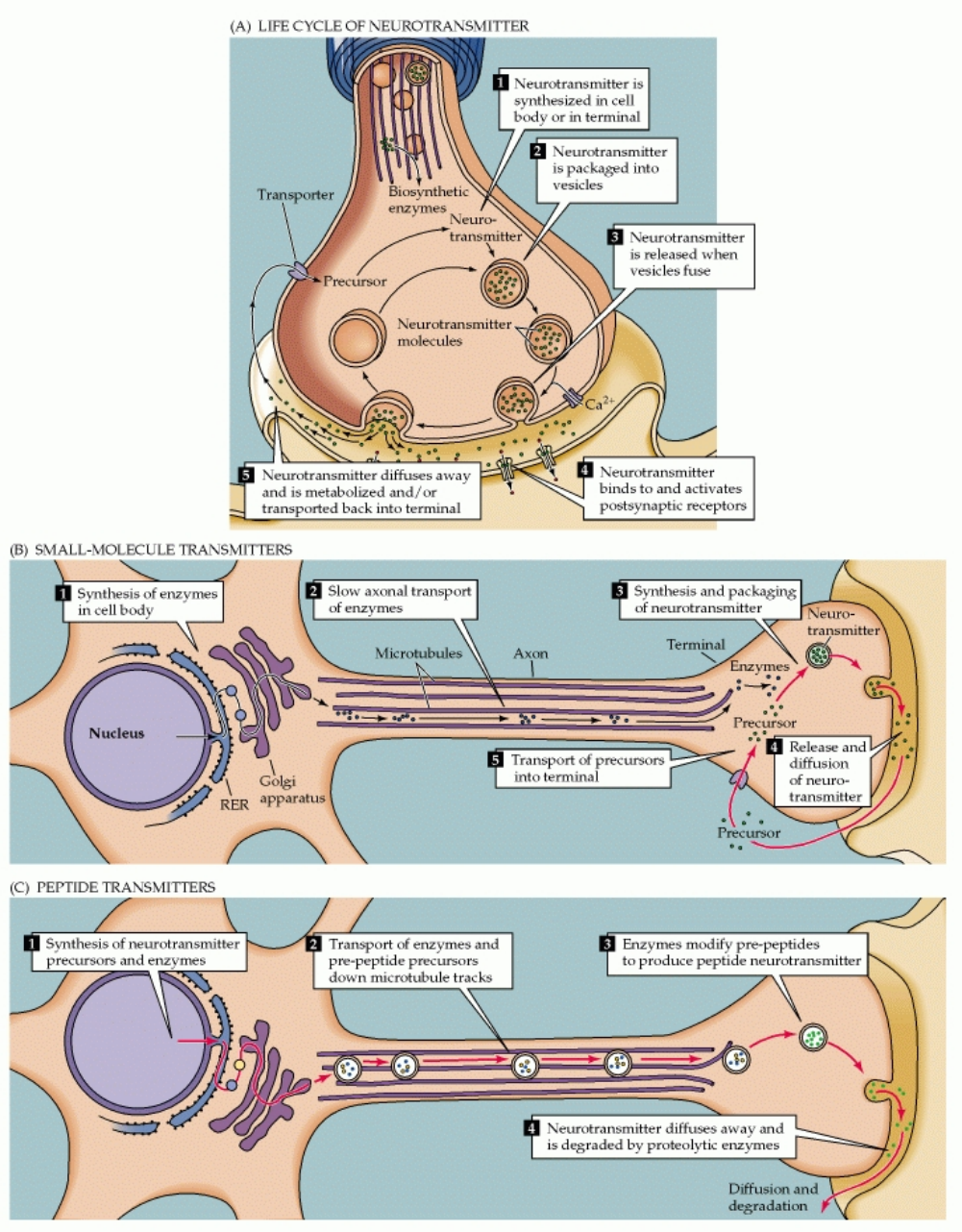


Figure 65 | Neurotransmitter synthesis

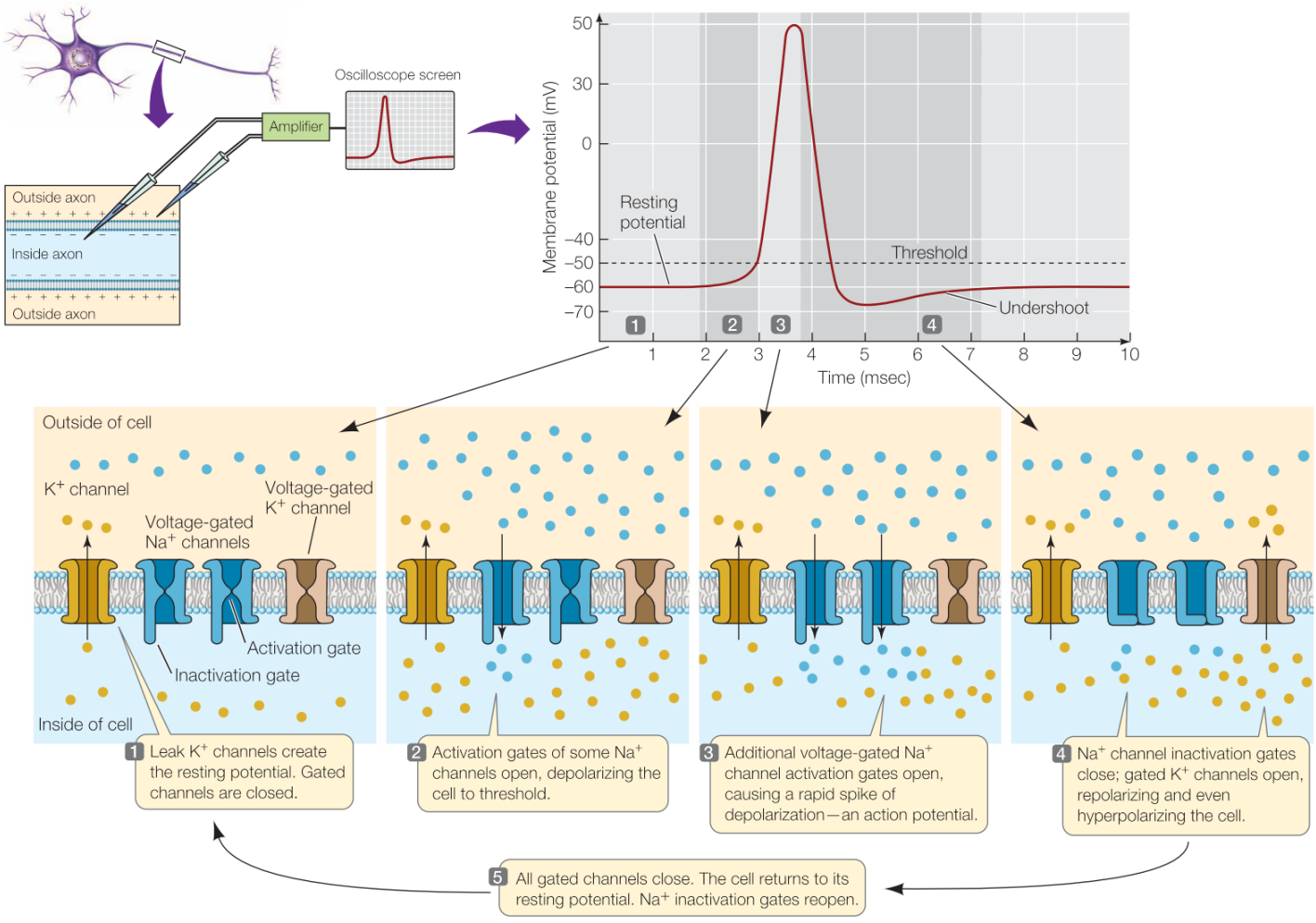


Figure 66 | Time-course of an action potential

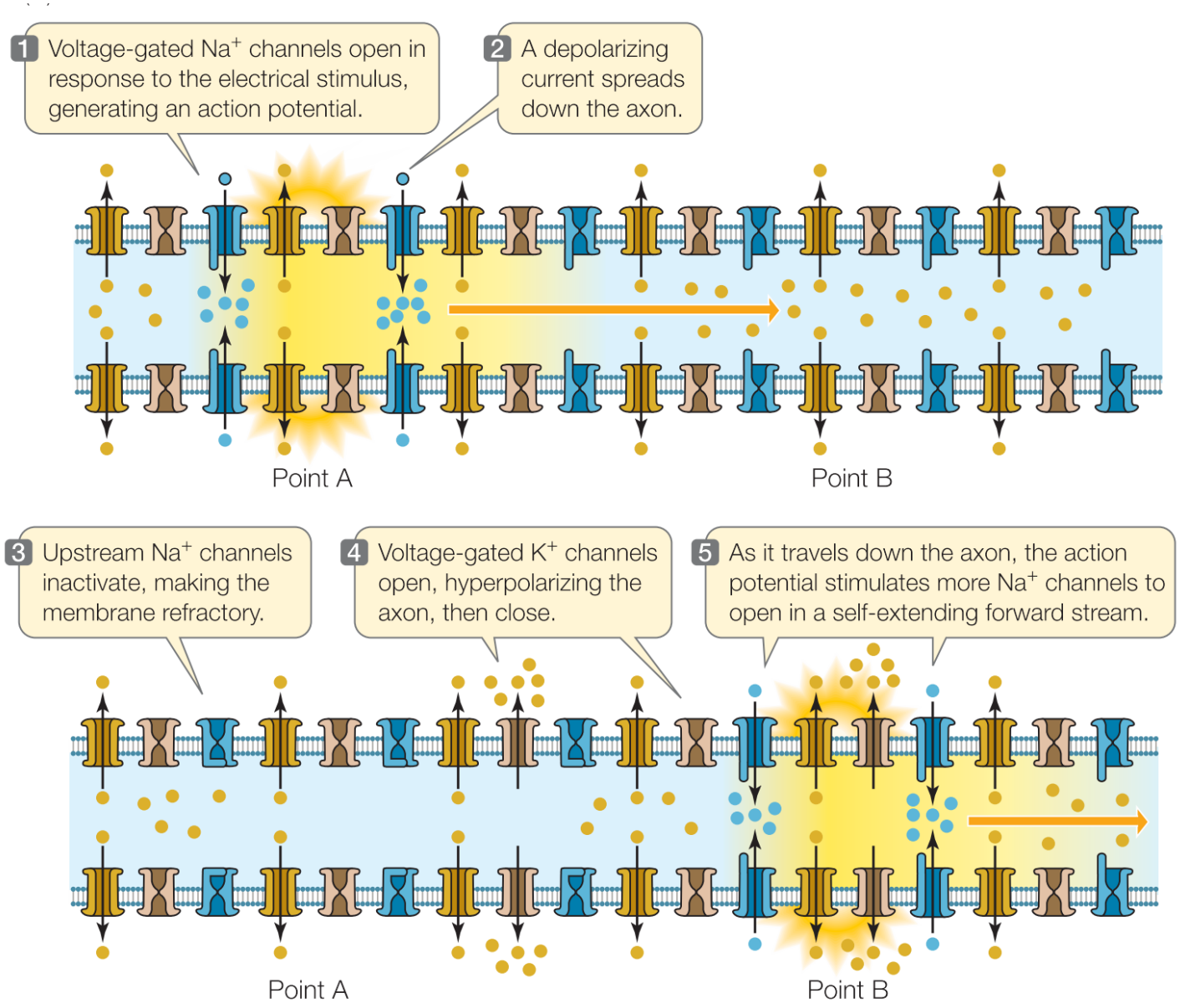


Figure 67 | Action potential propagation

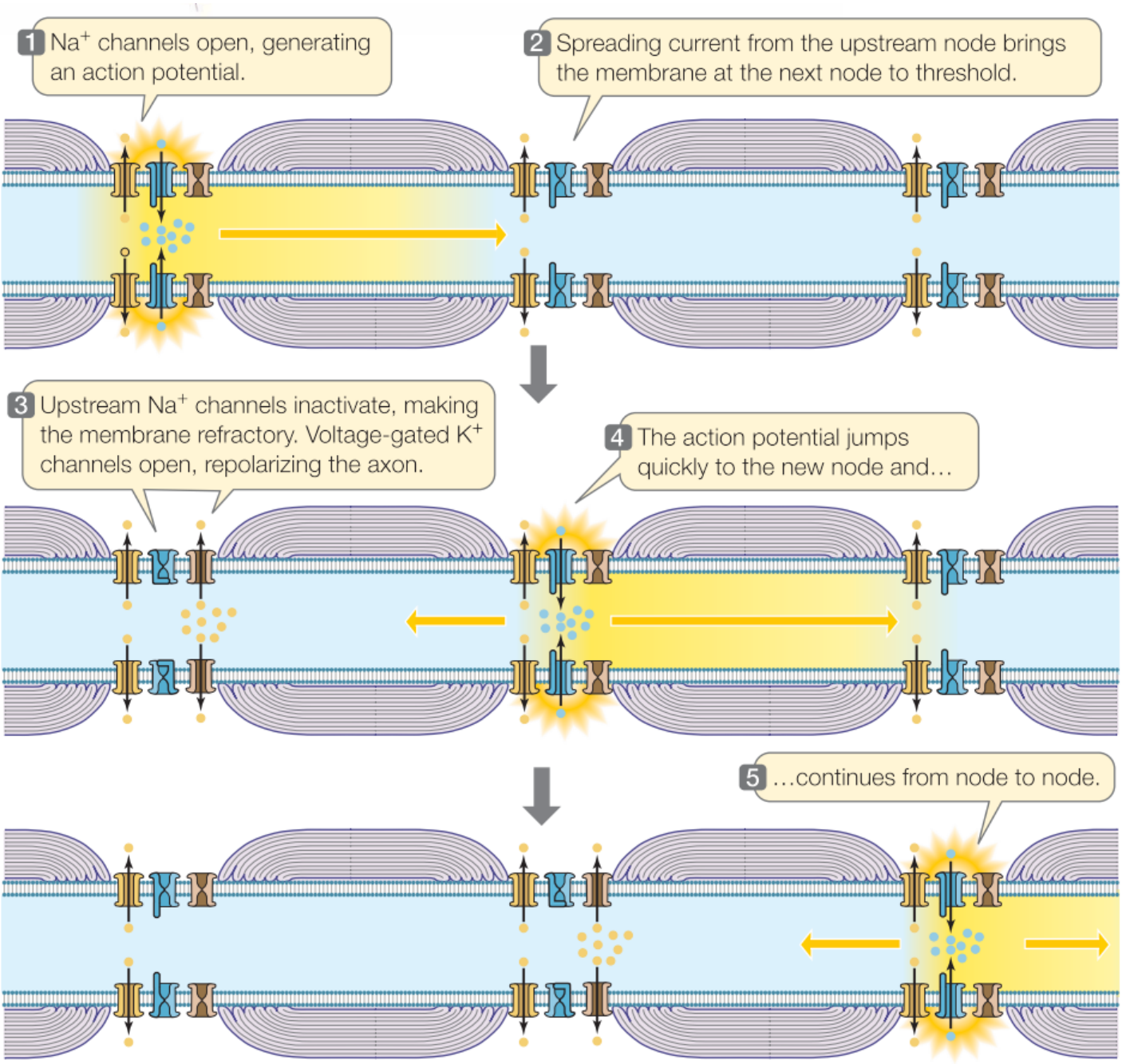


Figure 68 | Saltatory conduction

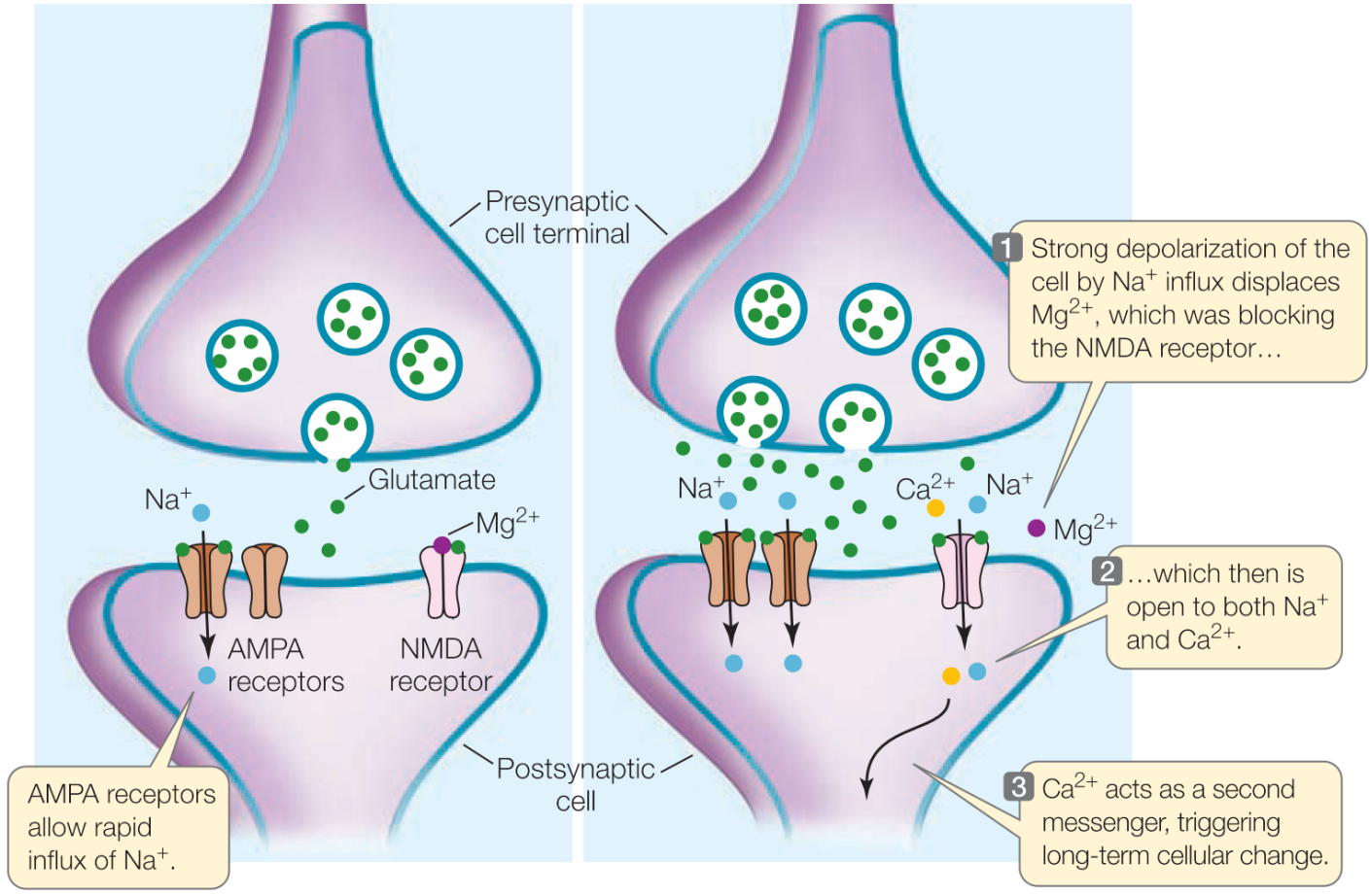


Figure 69 | Mechanism of LTP.
See Fig. 56for more.

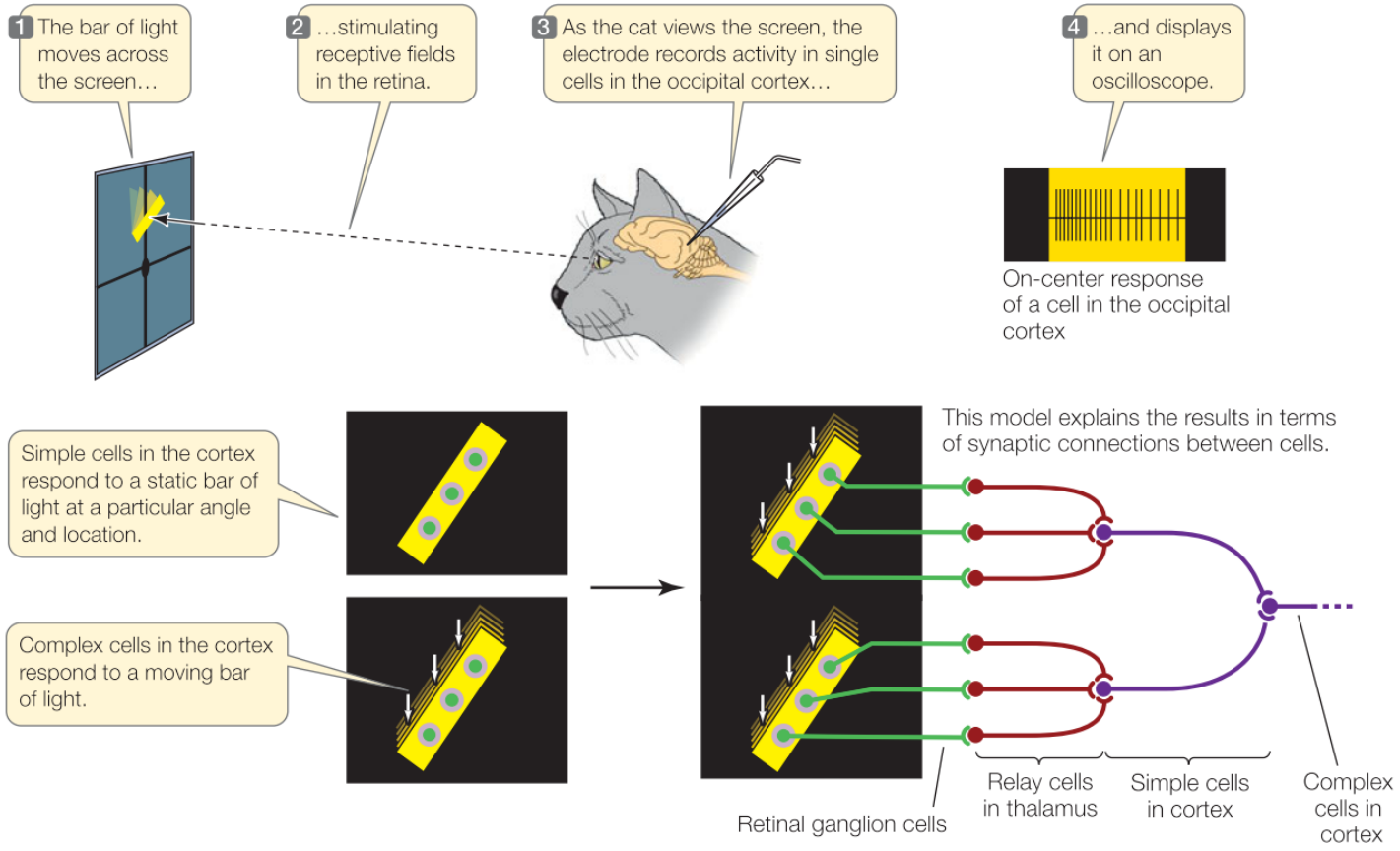


Figure 70 | Hubel and Wiesel model of visual system.
Experiments were originally done on cats.

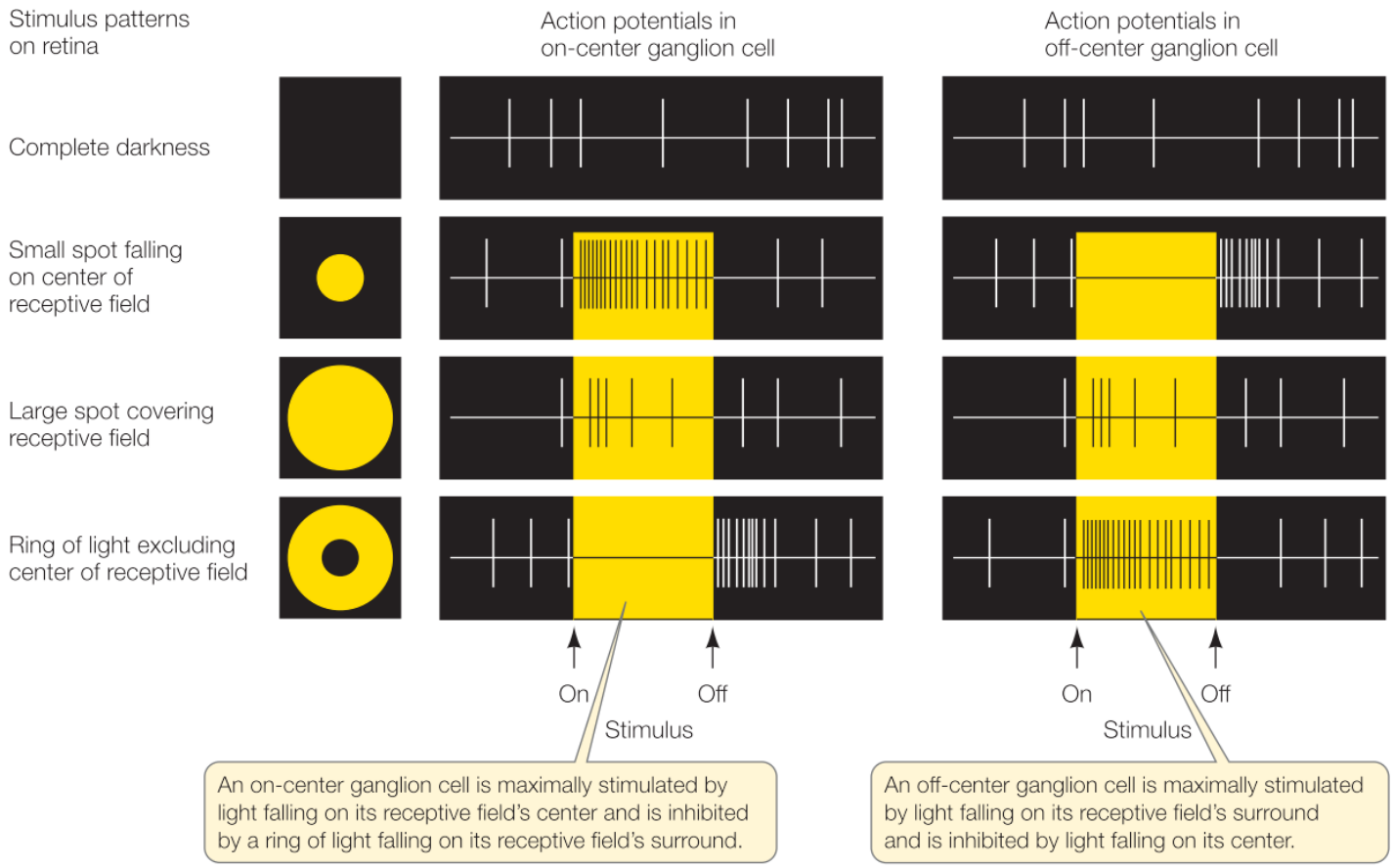


Figure 71 | Receptive fields
Center-surround created by lateral inhibition.

Week 7 ANS and Homeostasis

Readings

LIFE 8th: Ch. 46 41 | LIFE 9th: Ch. 47 40

Overview

Prof. Heller covered the autonomic nervous system, spinal cord organization, and homeostasis (in particular, thermoregulation).

Concepts

General frameworks in which to think about this week's material.

- Autonomic nervous system: sympathetic and parasympathetic
- Information flow between sensory systems, effectors, and CNS
- General organization of the brain (cortical, midbrain and brainstem)
- Systems physiology
- Internal milleau and homeostasis
- Control and regulation
- Effects of temperature on living systems
- Ectotherms and endotherms
- Pathways of thermal energy exchange
- Thermoregulatory adaptations
- Metabolic rate/temperature curve
- Neural control of thermoregulatory adaptations

Terms

CNS

- cerebrum
- diencephalon
- midbrain
- pons
- cerebellum
- medulla
- frontal
- parietal
- temporal
- occipital
- thalamus
- hypothalamus
- midbrain
- pons
- medulla
- cerebellum
- spinal cord
- basal ganglia
- hippocampus
- association cortex
- convolution
- reticular system
- Broca's area
- Wernicke's area
- consciousness
- interoception
- insula

spine

- cervical
- thoracic
- lumbar
- sacral
- ventral root
- root
- column
- horn
- dorsal
- root
- column
- horn
- gray matter
- white matter
- sympathetic chain
- sympathetic chain ganglion
- dorsal root ganglion
- dura mater
- spinal nerve
- vertebra
- lumbar enlargment
- cauda equina
- interneuron
- spinal reflex
- knee-jerk reflex
- flexor
- extensor

sympathetic

- ganglia in spinal cord
- post-ganglionic noradrenergic
- noradrenaline
- norepinephrine
- thoracic cord
- lumbar cord
- fight-or-flight
- adrenal gland

parasympathetic

- ganglia near organs
- pre/post-ganglionic neurons cholinergic
- brainstem
- sacral cord
- rest-and-digest

autonomic

- acetycholine
- adrenalin/noadrenalin
- mAChR
- nAChR
- micturition reflex
- artery
- vein
- gap junctions

limbic system

- amygdala
- hippocampus
- fornix
- corpus callosum
- cingulate gyrus
- midbrain
- parahippocampal gyrus
- temporal lobe
- orbital and medial prefrontal cortex

temperature

- Q_{10}
- Arrhenius equation
- Van't Hoff

metabolic compensation

- endotherms
- ectotherms
- heterotherm
- homeotherms
- poikilotherms
- countercurrent heat exchange
- shivering
- heat rate
- relative to the rate of change

- critical temperature
- thermoregulatory system
- difference from home heating
- hypothalamus
- stressors
- preoptic anterior hypothalamus
- hiberation
- hypothermia
- hyperthermia
- mitocondria
- krebs cycle
- lactic acid

energy exchange

- radiation
- convection
- conduction
- evaporation
- blood flow
- countercurrent heat exchanger
- insulation
- heat exchanger
- retia venosa
- radiator
- arteriovenous anatomosis
- hypodermal layer

homeostasis

- nervous system
- endocrine system
- set point
- feedback information
- error signal
- effectors
- controlled systems
- regulatory systems
- sensors
- negative feedback
- positive feedback
- feedforward
- liver
- heart
- lungs
- kidneys
- epithelial
- muscle
- cardiac
- skeletal
- muscle
- connective
- collagen
- adipose
- blood
- bone
- nervous
- acclimatize
- thermoneutral zone
- basal metabolic rate
- lower critical temperature
- upper critical temperature
- shivering
- contractile machinery
- skeletal muscles
- brown fat
- thermogenin
- insulation
- active heat loss
- passive heat loss
- hypothalamus
- pyrogens

Techniques

For each technique, you should know when is appropriate to use it, what its limitations are, and a particular process that was used to study it.

- Lesion Remove a piece of the brain and see how the animal (or human) is affected. This gives you some first-pass idea of what the brain region does
- Artificial heating Put a device in the hypothalamus and alter its temperature and see the effect on animals.

Central Nervous System

Conscious control of behavior and control of many regulatory processes is mediated by the central nervous system. You should know the basic layout along with the functions of several key structures. The limbic system was covered and its general function should be known.

Structures

- Temporal lobe senses of smell and sound, as well as processing of complex stimuli like faces and scenes.
- Parietal lobe plays important roles in integrating sensory information from various senses, and in the manipulation of objects; portions of the parietal lobe are involved with visuospatial processing.
- Occipital lobe sense of sight; lesions can produce hallucinations
- Frontal lobe conscious thought; damage can result in mood changes, social differences, etc. The frontal lobes are the most uniquely human of all the brain structures.
- Limbic system is important for fear and memory, contains amygdala, hippocampus, hypothalamus and other structures.
- Hypothalamus temperature regulator and loctation of circadian clock (suprachiasmatic nucleus or SCN).
- Pituitary gland secretes hormones (e.g. FSH, LH, oxytocin) that modulate the bodies response to various stimuli or help stimulate sex hormone production.
- Cortex involved in conscious perception, e.g. motor and somatosensory cortex. Also involved in language and various other higher cognitive functions.
- Medulla contains the cardiac, respiratory, vomiting and vasomotor centers and deals with autonomic, involuntary functions, such as breathing, heart rate and blood pressure.
- Thalamus relay station, e.g. LGN relays visual information to visual cortex and amygdala.
- Amygdala involved in fear.
- Hippocampus short term memory and temporary storage for long-term memory.
- Substantia nigra releases dopamine, helps control motor behavior. Loss leads to Parkinson's.

- Pons this region is thought to regulate breathing (see respiration notes).
- Cerebellum involved in various basic nervous system functions and motor control.
- Insula thought to be where consciousness resides; it receives input from various brain areas along with pain input.

Spine

The spinal cord is part of the central nervous system and contains neurons that have outputs to the autonomic nervous system.

- The spine is organized into the cervical, thoracic, lumbar and sacral regions.
- Know the general layout of segments in the spinal cord, such as dorsal, ventral, ganglion (dorsal and sympathetic), and spinal nerves.
- Remember that the spinal cord can help initiate rapid responses to stimuli without involving higher brain areas, such as withdrawal of hand from a fire before conscious awareness.
- Ventral spinal cord contains efferent pathways, or output pathways of the CNS or reflex arcs.
- Dorsal spinal cord contains afferent pathways, these are inputs to the CNS (sensory, etc.). For reflex arcs, these are inputs from stretch, temperature, and other receptors that cause a loop to facilitate rapid response.
- Central part of spinal cord contains gray matter or neuron bodies. White mater are the axon tracks.
- Ganglion are locations with many neuron cell bodies and thus their bulging appearance compared to thinner nerves that contain only axons.

Autonomic Nervous System

Know the general organization of the autonomic nervous system and, especially after later lectures, how it allows the central nervous system to control the body. Have a general idea that the parasympathetic and sympathetic pathways are antagonistic in addition to being associated with particular responses.

Parasympathetic Often associated with rest and digest, it has pre-ganglionic acetycholine neurons and post-ganglionic acetycholine neurons. Mainly leaves from the brain stem and sacral spinal cord. Several functions:

- constricts pupils
- stimulates salivation
- slows heartbeat
- constricts airways
- stimulates digestion
- slight stimulation of glucose uptake and glycogen synthesis
- stimulates activity of intestines
- stimulates urinary bladder to contract
- stimulates penile or clitoral arousal

Sympathetic Often associated with fight-or-flight response, it has pre-ganglionic acetycholine neurons and post-ganglionic norepinephrine neurons. Mainly located in thoracic and lumbar spinal cord. Several functions:

- inhibits salivation
- relaxes airways
- accelerates heartbeat
- inhibits digestion
- stimulates breakdown of glycogen and release of glucose
- stimulates secretion of epinephrine and norepinephrine
- stimulates orgasm
- vaginal contraction
- dilates pupil
- inhibits activity of intestines
- relaxes urinary bladder

Acetylecholine

- Depolarizes smooth muscle of iris, bronchii, gut, and bladder.
- Hyperpolarizes cardiac muscle, smooth muscle in arterioles of sex organs, and others.

Adrenalin/noadrenalin

- Depolarizes cardiac muscle.
- Hyperpolarizes gut.

Homeostasis

Body temperature Body must control temperature to keep enzymes and other body functions working. Eq. 8 gives an idea of why body temperature regulation is crucial. Most biological functions have a Q_{10} of 2 or 3, so they undergo drastic changes

in reaction rate as temperature changes.

$$Q_{10} = \frac{R_T}{R_{T-10}} \tag{3}$$

Thus the body needs to keep temperature in an optimal range. How does it do this? Take the below response after blood temperature has decreased:

1. Blood circulates to hypothalamus, cooling it. Thermoreceptors in the hypothalamus detect this (it also has osmoreceptors to detect ionic balance).
2. Two different outputs
   - Signals to the medulla (parasympathetic vagal nuclei and neurons that project to sympathetic spinal cord neurons)
   - This causes sympathetic nervous system to cause vasoconstriction of skin, induce brown fat to be burned, piloerection (raise hairs, trap air near skin).
   - The sympathetic also activates adrenal medulla, which releases epinephrine that causes increase in BMR.
   - Hypothalamus also sends information to anterior pituitary to release TSH. Thyriod then releases thyroid hormone into the blood stream, increasing basal metabolic rate.
3. Hypothalamus receives other information besides blood temperature:
   - nucleus of the solitary tract - this nucleus collects all of the visceral sensory information from the vagus and relays it to the hypothalamus and other targets. Information includes blood pressure and gut distension.
   - reticular formation - this catchall nucleus in the brainstem receives a variety of inputs from the spinal cord. Among them is information about skin temperature, which is relayed to the hypothalamus.
   - retina - some fibers from the optic nerve go directly to a small nucleus within the hypothalamus called the suprachiasmatic nucleus. This nucleus regulates circadian rhythms, and couples the rhythms to the light/dark cycles.
   - circumventricular organs - these nuclei are located along the ventricles, and are unique in the brain in that they lack a blood-brain barrier. This allows them to monitor substances in the blood that would normally be shielded from neural tissue. Examples are the OVLT, which is sensitive to changes in osmolarity, and the area postrema, which is sensitive to toxins in the blood and can induce vomiting. Both of these project to the hypothalamus.
   - limbic and olfactory systems - structures such as the amygdala, the hippocampus, and the olfactory cortex project to the hypothalamus, and probably help to regulate behaviors such as eating and reproduction.

Metabolic rate The response to temperature differences can be modeled with Eq. 9. This can also give us an indication of the amount of insulation an animal uses.

$$MR = K(T_b – T_a) \tag{4}$$

Heat exchange The heat in and out must be equal for the system to be in equilibrium. There are several ways to do this, see Eq. 10. You can change metabolism and radiation heat (i.e. sun). Or you can lose heat via radiation, convection (think wind), conduction (contact with colder surfaces), or evaporation. Can increase radiation via retia venosa and arteriovenous anatomosis. Fish can reduce conductive lose by use of countercurrent systems.

$$M + Q_{abs} = \epsilon\sigma T_r^4 + h_c(T_r - T_a) + E + C \tag{5}$$

Problems

If there is any confusion about the questions, shoot me an email or talk to me after class.

1. See Fig. 73.
   - What type of synapse is A? Excitatory
   - What type of synapse is B? Excitatory or inhibitory
   - What type of synapse is C? Excitatory
   - What type of synapse is D? Inhibitory or excitatory
   - What occurs to heat withdrawal if neuron D undergoes apoptosis?
2. An athlete comes in after receiving a hard knock in his back. He complains that his heart no longer speeds up during exercise. What likely happened to cause this defect? Damage to sympathetic fibers somewhere in the thoracic region.
3. Why do you need two types of receptors for both the sympathetic and parasympathetic nervous systems? They each mediate different responses.
4. Indicate if the following are true or false.
   - Both the SNS and PNS use acetylcholine at their first synapse. True
   - Beta receptors function in the SNS, and alpha receptors function in the PNS. False
   - Alpha receptors mediate hyperpolarization, and beta receptors mediate depolarization. True
   - SNS and PNS axons project collaterals to inhibit each other. True
   - Acetylcholine causes an increase in heart rate, whereas norepinephrine causes a decrease in heart rate. False
   - Both the SNS and PNS can be regulated by the limbic system. True
5. Indicate which are true about the autonomic nervous system:
   - The hypothalamus is the primary center of ANS (autonomic nervous system) regulation True.
   - Changes in autonomic function require signals from the cortex False.
   - Emotions can cause changes in autonomic function via projections from the limbic system True.
   - Neurons in the hypothalamus secrete norepinephrine to stimulate sympathetic activity False.
   - Sustained sympathetic activity can increase sensory sensitivity True.
   - You cannot consciously change autonomic function False.
6. Say what will happen if you remove or inhibit the following brain areas:
   - Amygdala No response to fear.
   - Visual cortex Cognitively blind, your eyes still receive and send visual input, but can't consciously perceive it. Can still flinch.
   - Substantia nigra Motor defects, see Parkinson's.
7. Someone has a lesion that stops their lateral geniculate nucleus (visual thalamus) from communicating with their amygdala. They are walking in the woods at night and see a shadow shaped like a tiger. How is their reaction different from a normal person? They don't instinctively react to run away, but their response is delayed.
8. These question are regarding Fig. 74.
   - What did Prof. Heller say some scientists concluded from these images? Insula might be the location of consciousness.
   - For what other reason could the insula be consistently lighting up? fMRI measures BOLD signals or blood-oxygen level dependent signals. If the insula had unusual vasculature, released a different level of NO, aracadonic acid, etc. or had neurons with more inefficient mitocondria, this could cause increased signal without indicating increased activation.
9. When is positive feedback used in biological systems? Sex, birth, etc.
10. While an animal is hibernating, you attach a device that can alter the temperature of its hypothalamus.
    - What would likely happen if you lowered the temperature? Animal would think it is colder than it actually is, expending energy. In a hibernating animal, this might lead to death since it is not foraging for food.
    - What would happen if you raised it?
11. These questions are regarding figure Fig. 75.
    - Which line represents an arctic animal? Line 4.
    - The slope of each line might **inversly** correspond to what? Thickness of animal's fur.
    - How does animal 2's body temperature compare between temperatures T2 and T3? They are the same.

- How does the TNZ of animal 4 likely compare to the TNZ of animal 2? Wider.

12. How would acclamation to new temperature (going from a hot water to a cold water environment) occur at the cellular level? Change in transcription, activate particular TFs that cause alternative splicing or production of proteins that have different reaction rates at new temperature.
13. What causes lactic acid build-up? Shut down of the Krebs cycle.
14. Look at Fig. 84. Explain the uptick in metabolic rate of endotherms at around 32C. Why would this trend eventually cause problems? Panting and other active processes to dissapate heat also generate heat, so eventually you are fighting a losing battle as both temperature and metabolic rate increase.
15. In Fig. 77, draw the curve when the ambient air temperature is now warm. Shifts down and to the left.
16. Look at Fig. 87, why is the heart rate of the iguana different at the same temperature? In the first case the body temperature is decreasing while in the second case the iguana raises its heart rate to increase blood flow, which leads to increased body temperature.
17. How does increasing heart rate while in direct sunlight help warm the body? More blood can flow to the skin and be warmed.
18. What does it mean for the Q10 of the metabolic rate of an animal to be 2? To be 1? the animal consumes half as much oxygen per hour at 20C as it does at 30C. In the case of 1, it doesn't change.
19. What method do `hot' fish use to retain body heat? Countercurrent exchange to retain body heat.
20. What would taking aspirin when you have a fever do to your hypothalamic set point? Cause it to decrease due to reduction in pyrogen production.
21. See Fig. 76.
    - Draw an arrow indicating where aspirin would fit in this diagram. Inhibits pyrogens.
    - Indicate a feed-foward process in this system. Skin input to hypothalamus.
    - Indicate where negative feedback can occur in this system. Feedback signal provided by blood circulating through hypothalamus.
    - How is the body's response to the difference between the set point and true temperature different than your home heating? The magnitude of the response changes depending on the error between the set point and temperature.

Figures and Tables

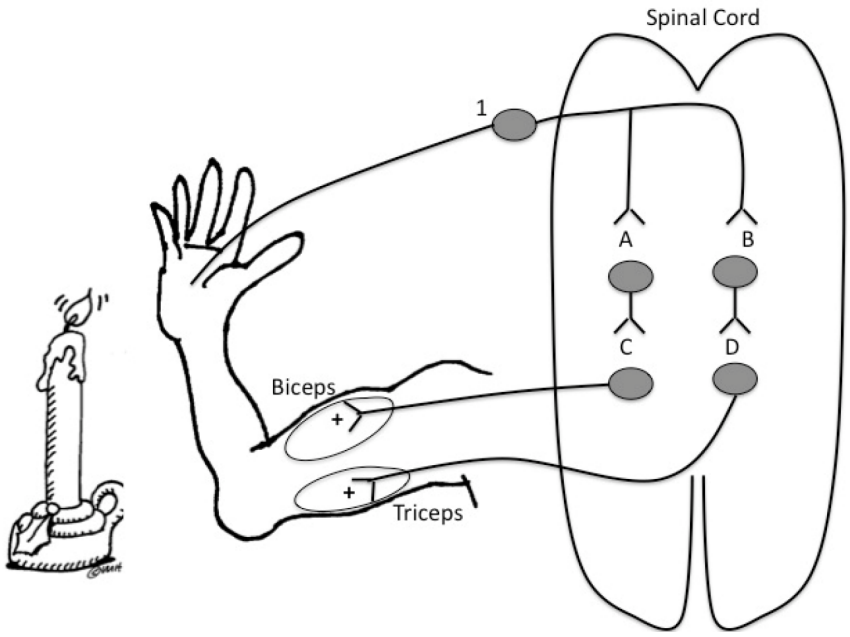


Figure 73 | Reflex arc.
See problem for question, this looks at the neuronal connectivity needed to initiate a reflex to burning sensation in the hand.

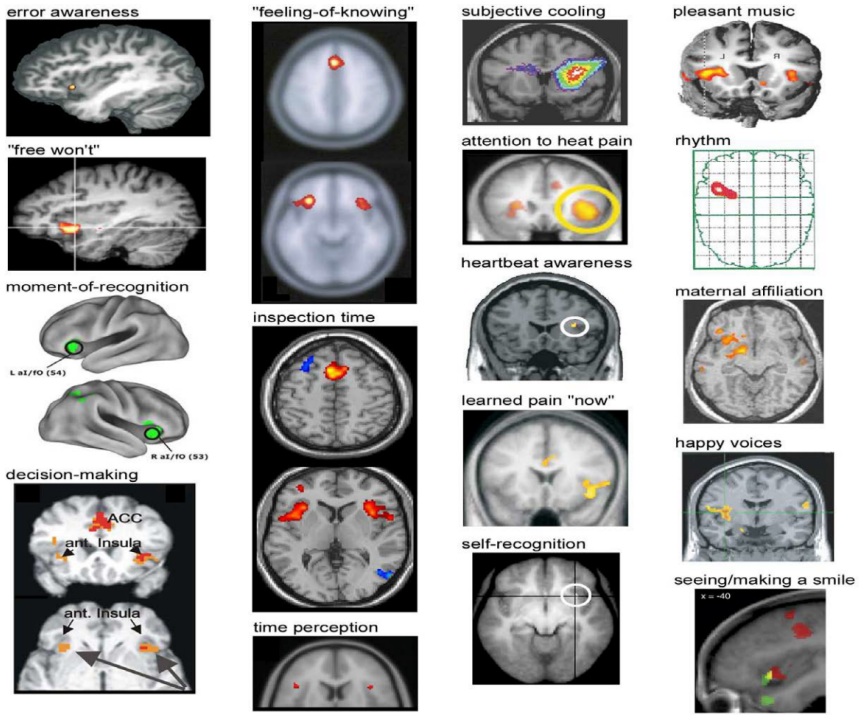


Figure 74 | Insula and consciousness.
Be mindful of the difference between correlations and causation. What else could explain this data?

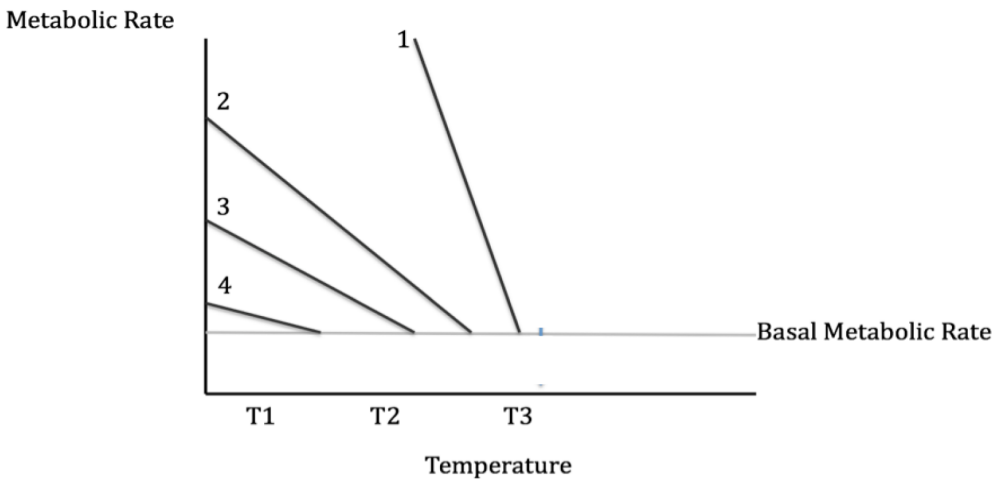


Figure 75 | Metabolic rate regulation in animals.

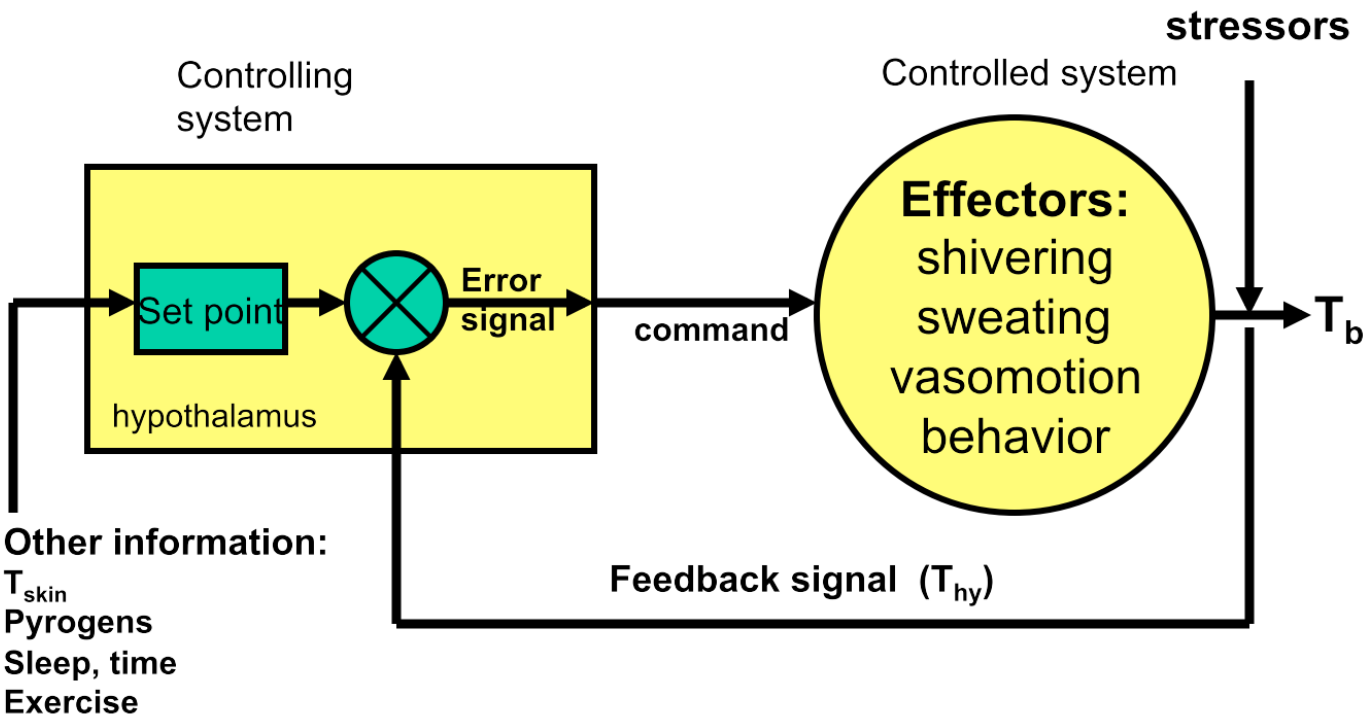


Figure 76 | Temperature control diagram.
Understand the logic of this flow diagram, it gives the main regulatory mechanisms behind thermoregulation. Remember, skin and other sensory input can count as feedforward, e.g. they can drive the hypothalamic set point up or down.

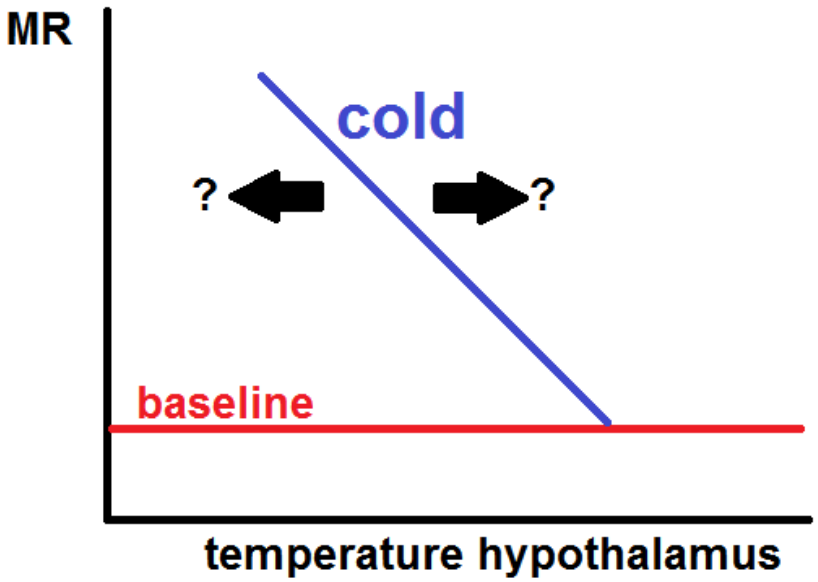


Figure 77 | **See problems for more.**

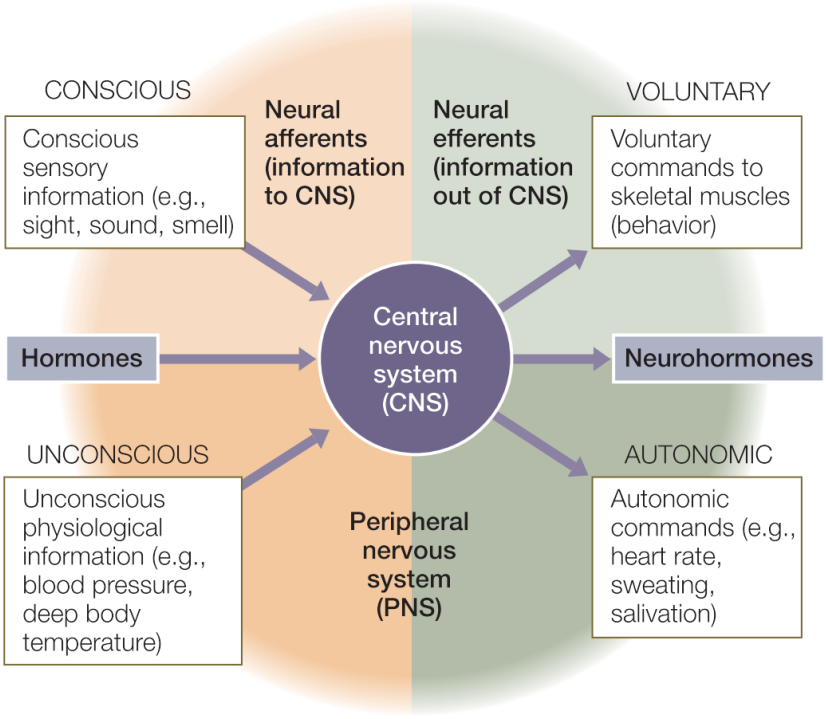
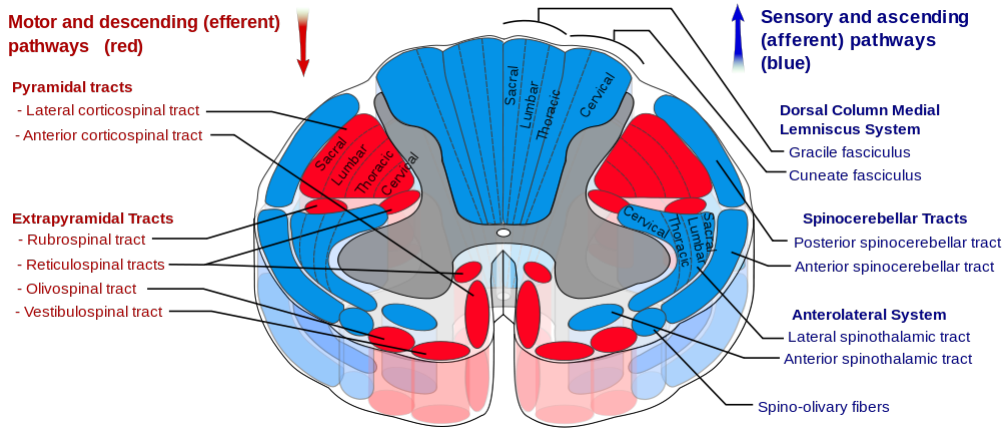
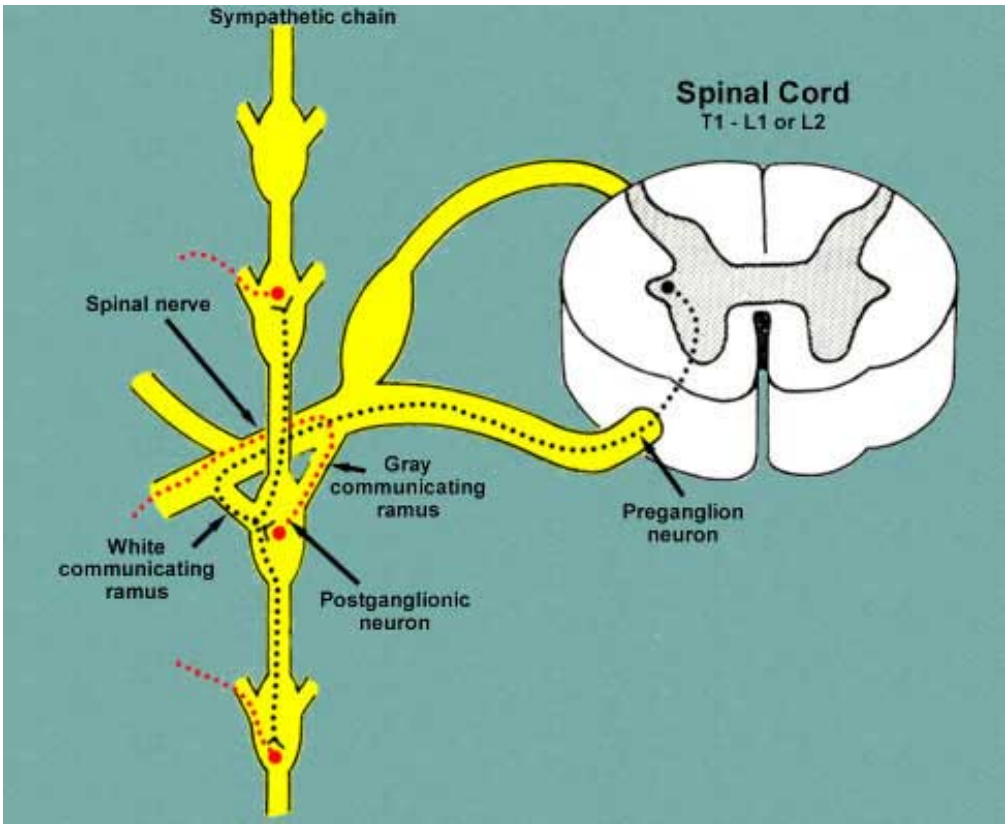


Figure 78 | **Organization of the nervous system**



(a) Spinal cord organization



(b) Sympathetic spinal cord

Figure 79 | Spinal cord

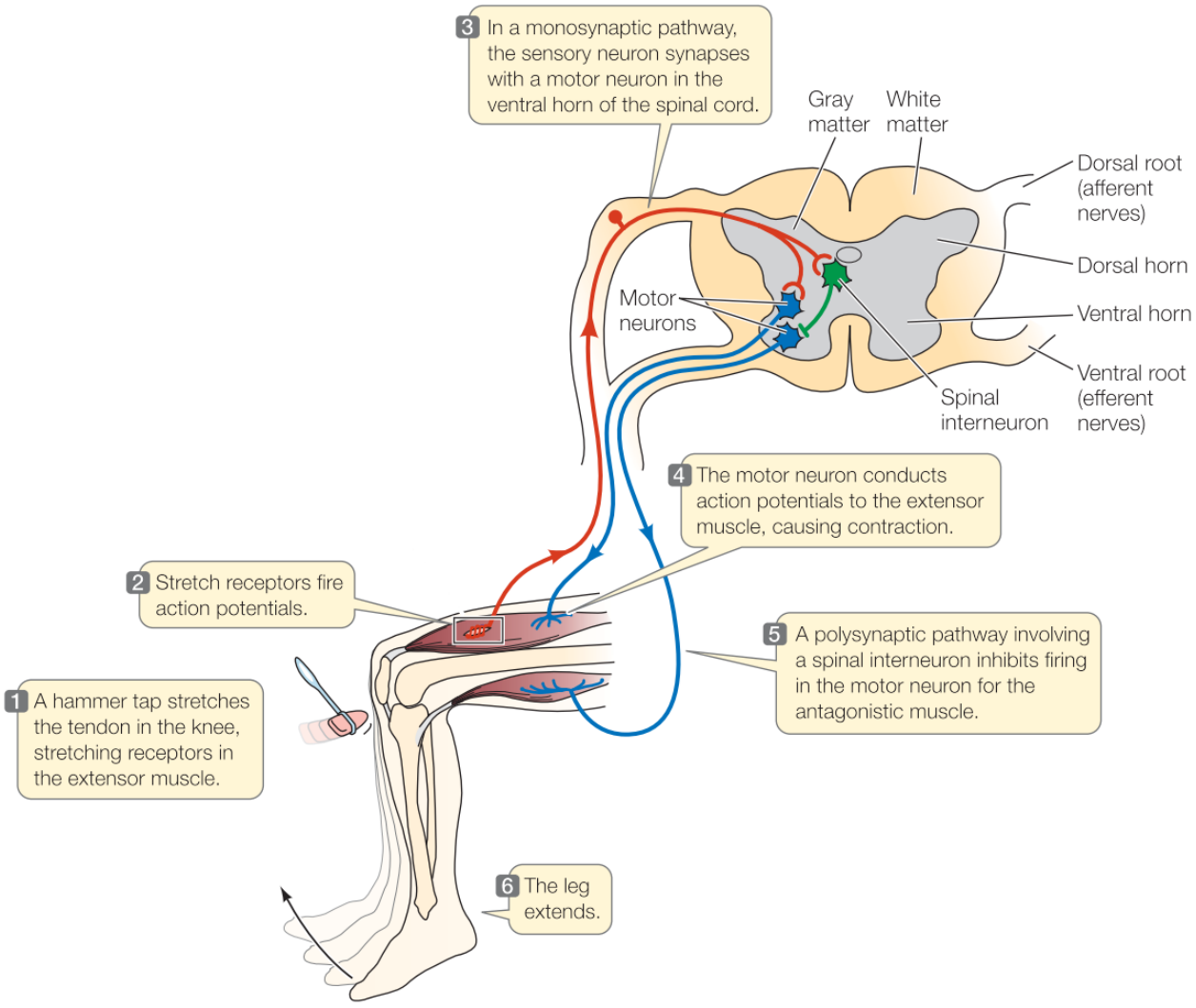


Figure 80 | Knee jerk reflex

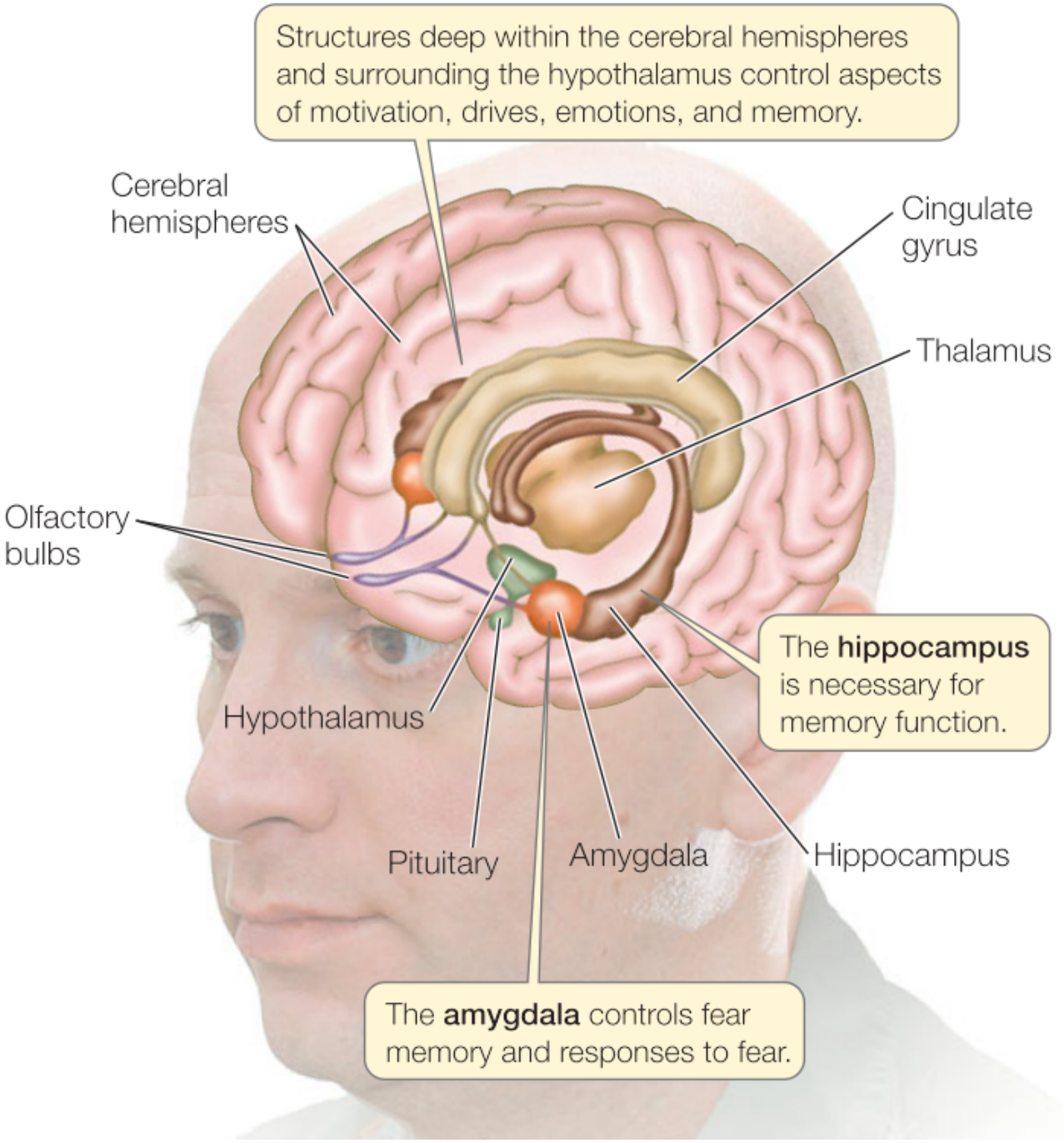


Figure 81 | Limbic system

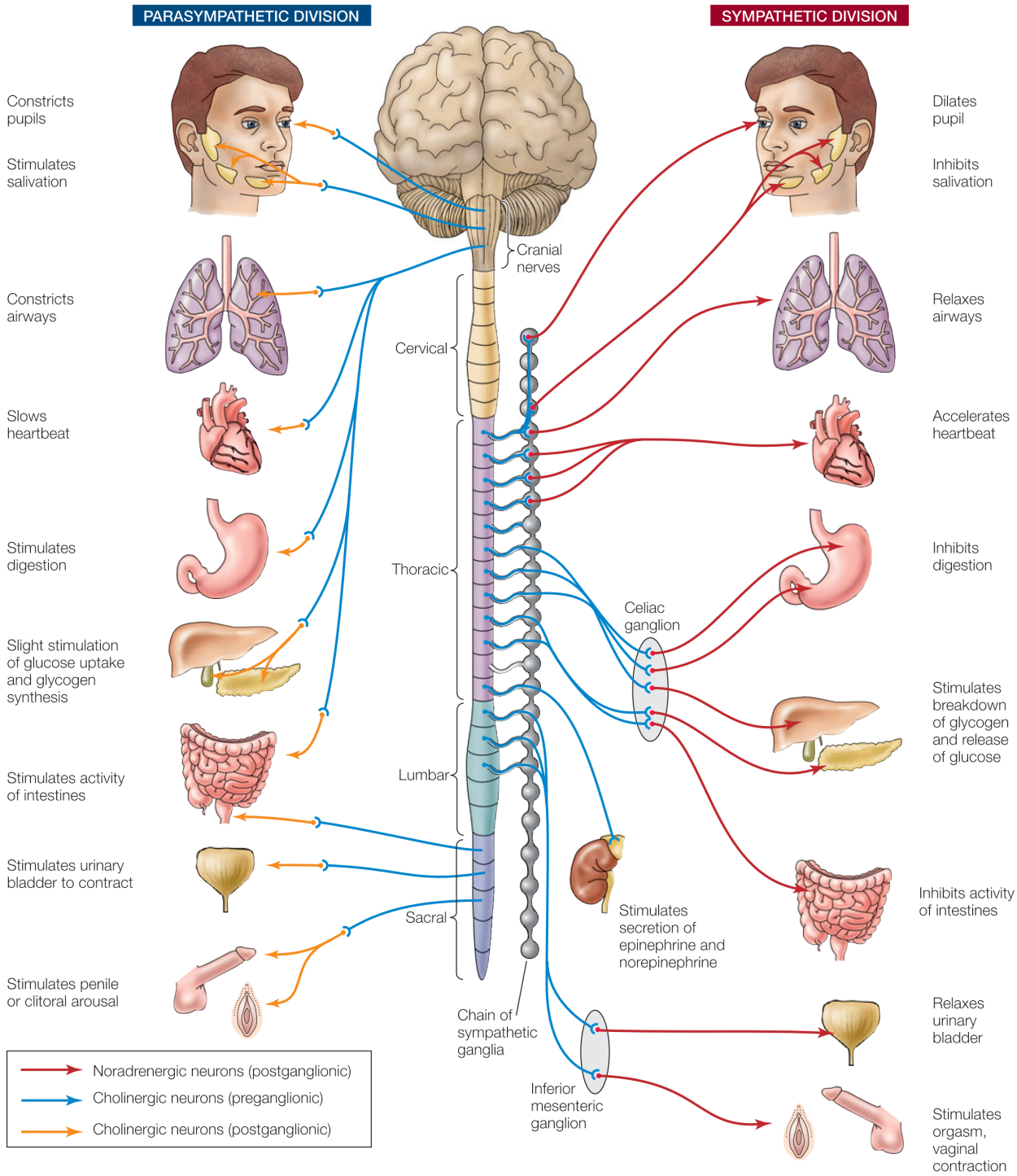


Figure 82 | Autonomic nervous system

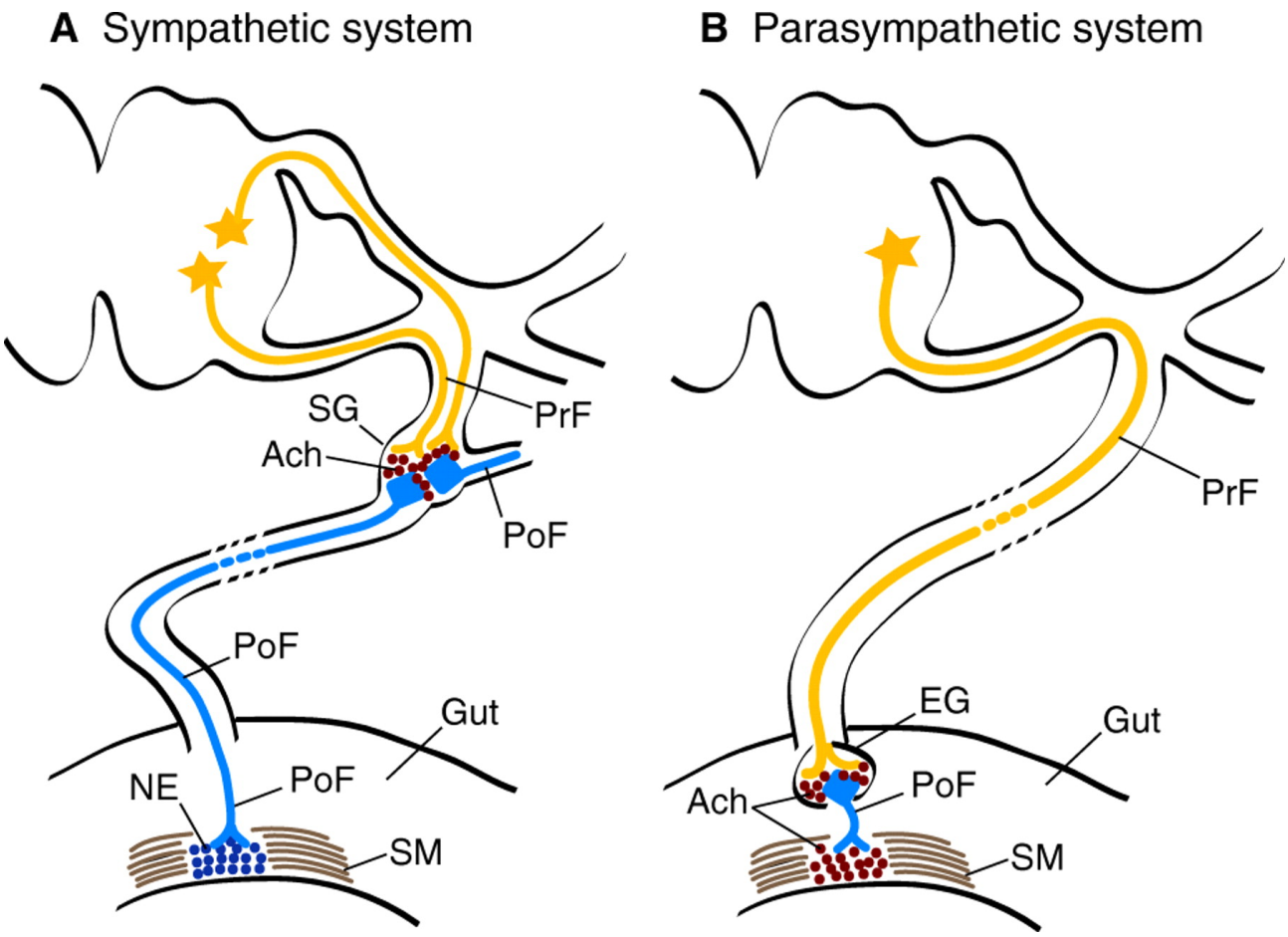


Figure 83 | **Organization of autonomic nervous system at spinal cord.**

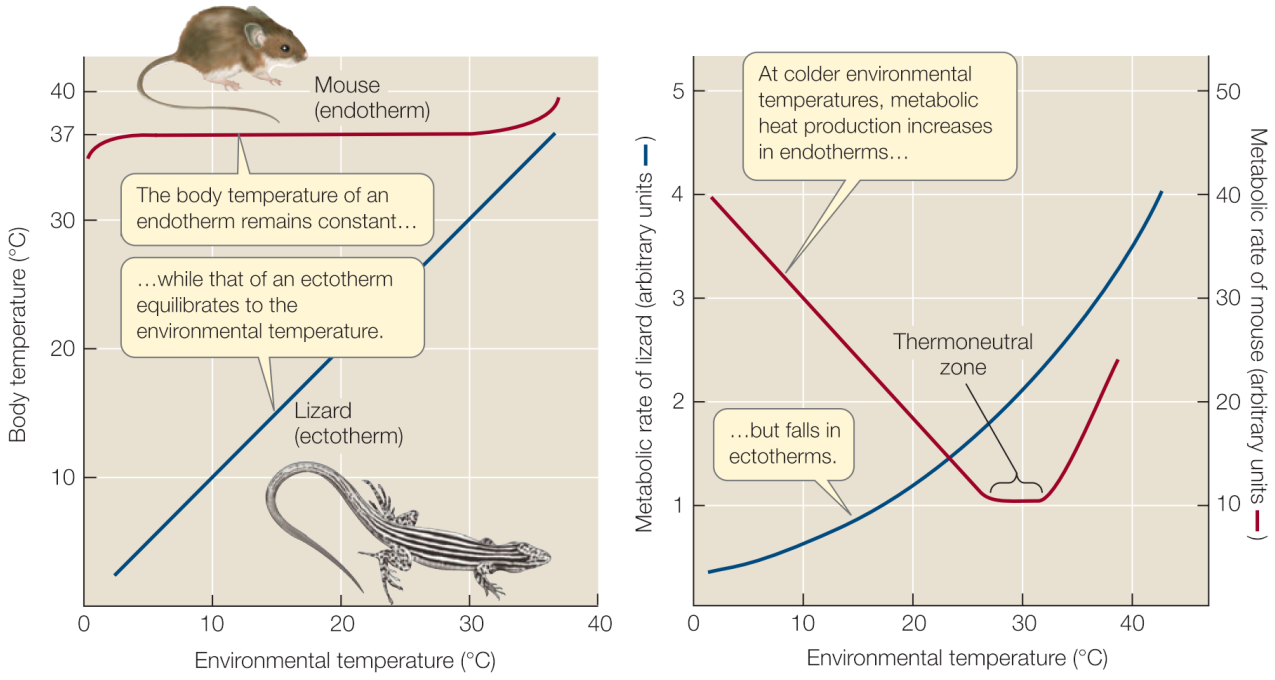


Figure 84 | **Endotherms vs. ectotherms**

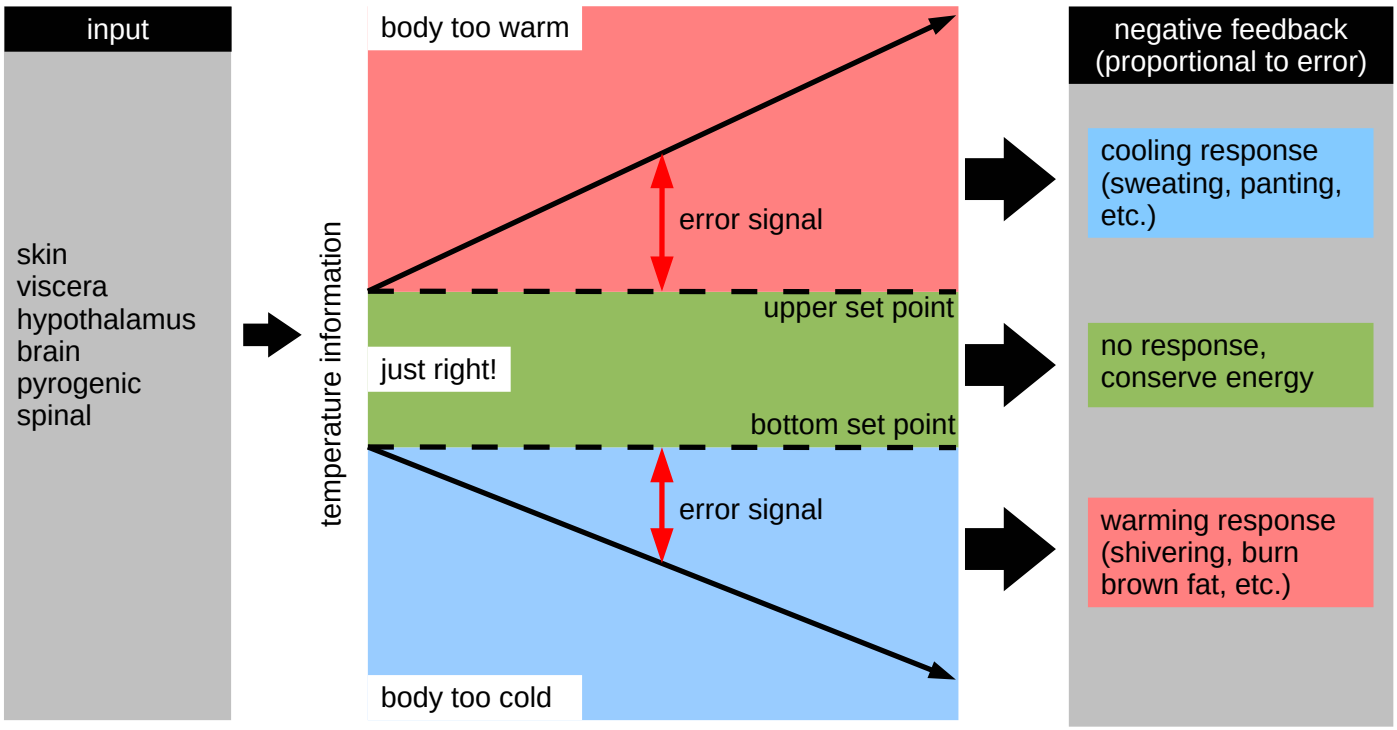


Figure 85 | Response regulation
General concept of integrating temperature information and formulating a response.

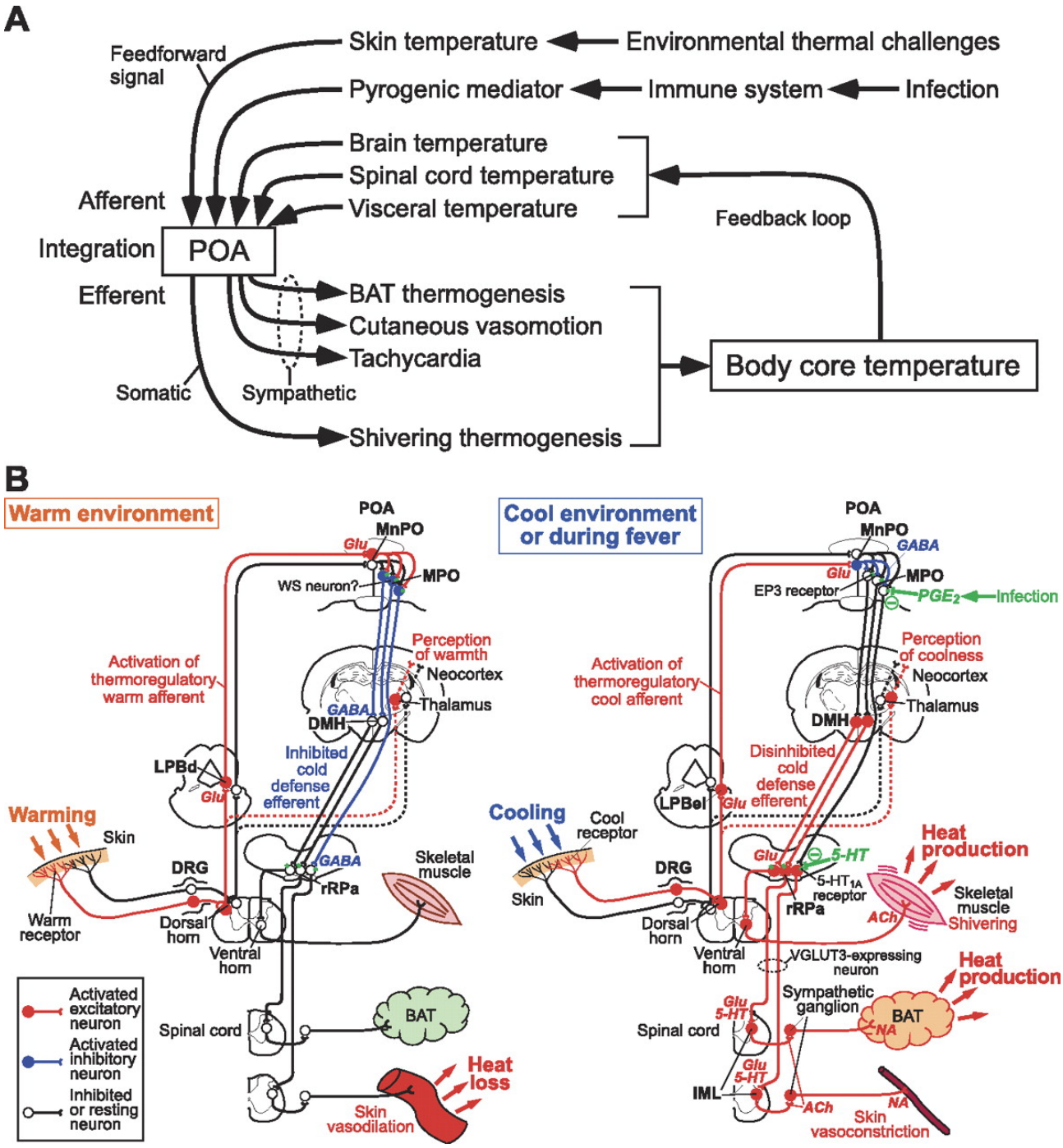


Figure 86 | Set point regulation
Skin temperature can modulate body response.

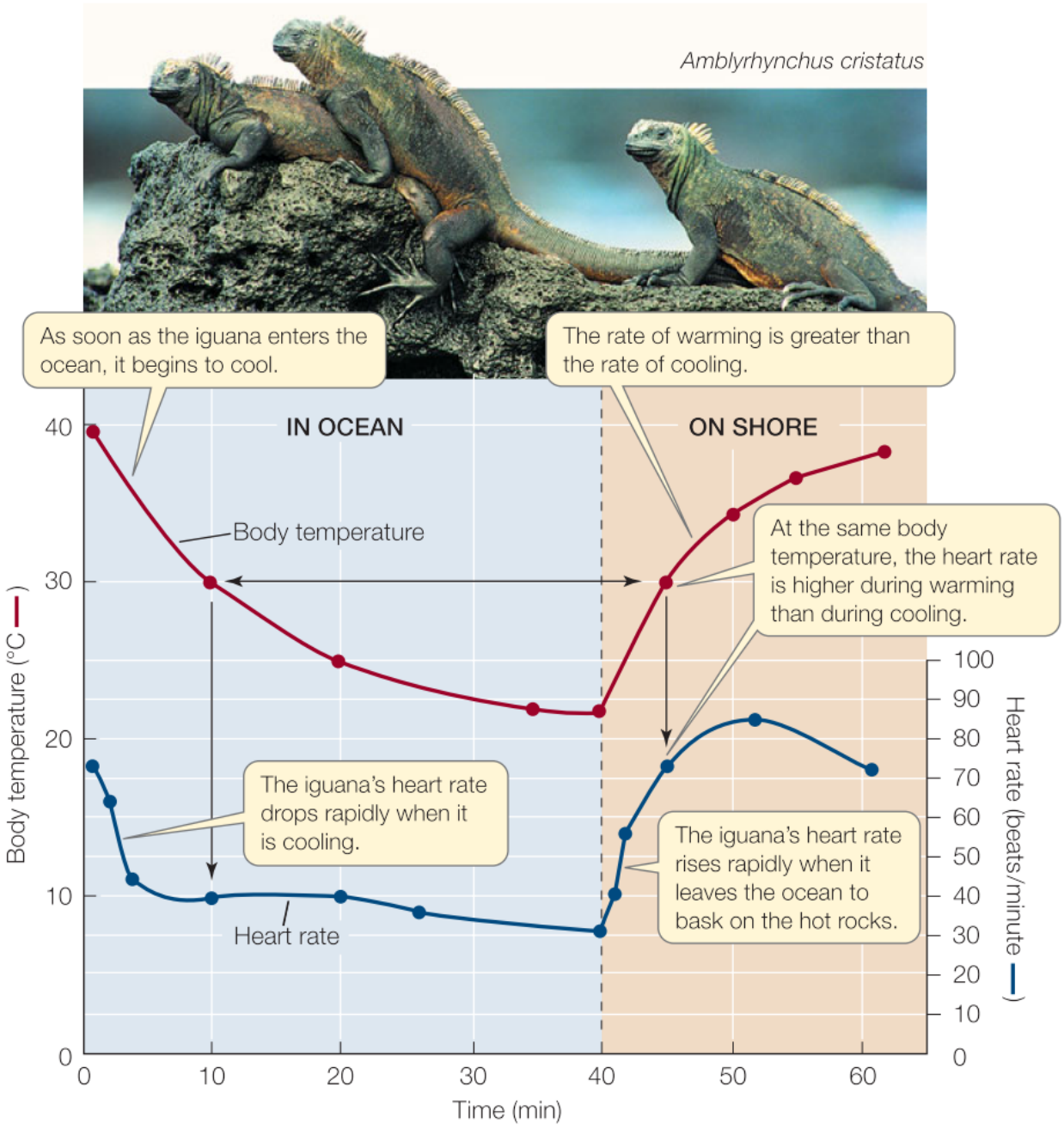


Figure 87 | Body temperature and heart rate
The direction and rate of body temperature change is more important than the absolute temperature.

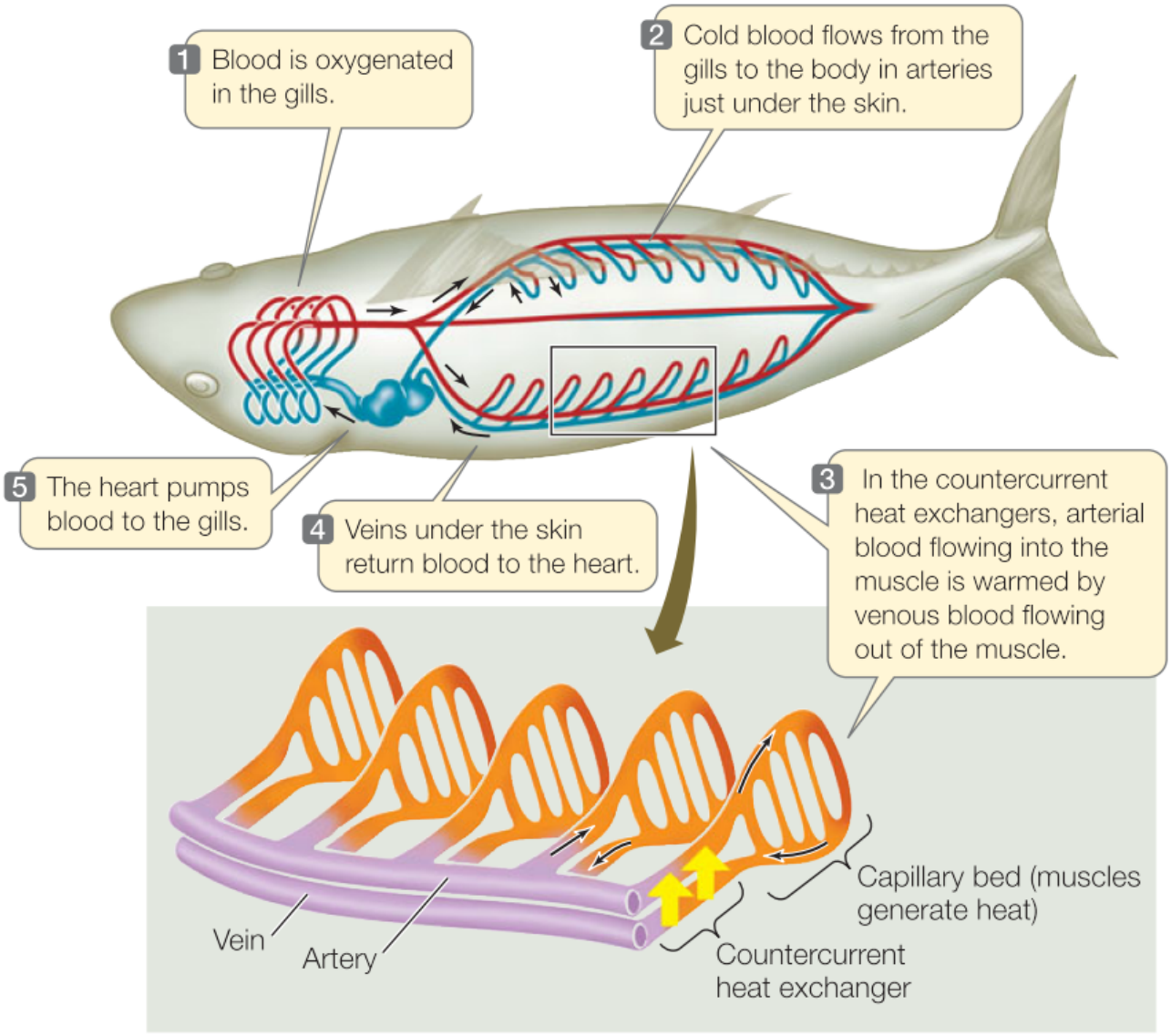


Figure 88 | Fish thermo-regulation
Countercurrents help drive heat exchange.

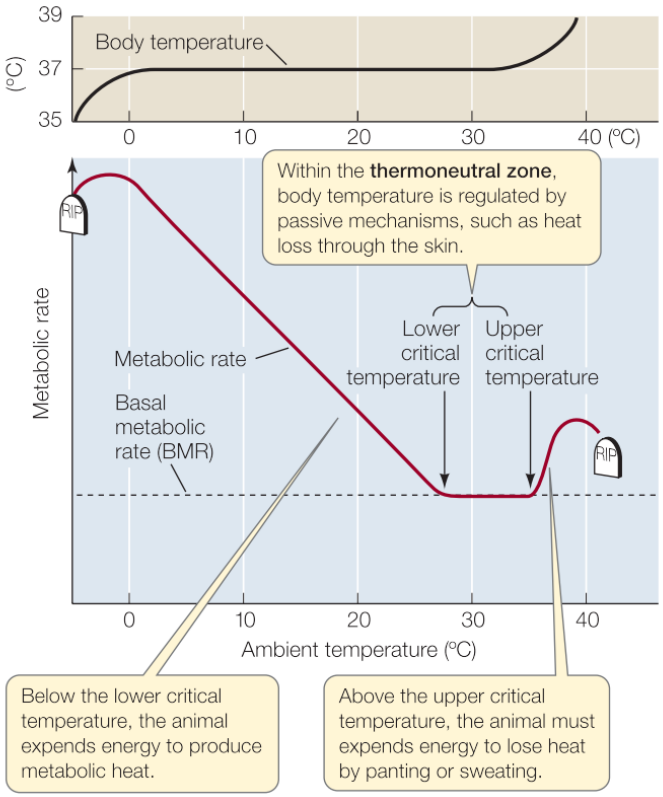


Figure 89 | Mammal metabolic regulation

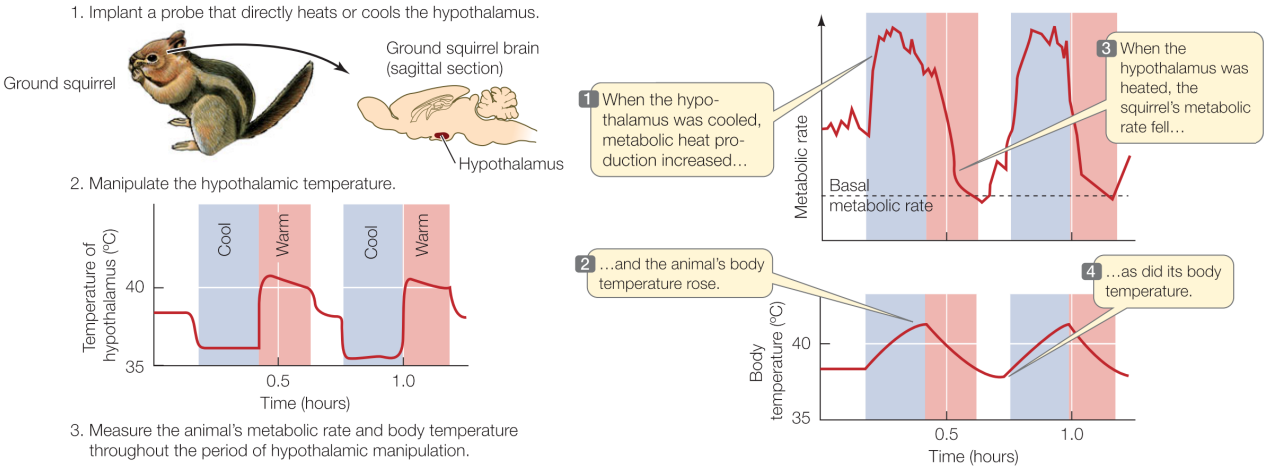


Figure 90 | Hypothalamus and body temperature

Week 8 Endocrine, Gastrointestinal, Reproduction

Readings
LIFE 8th: Ch. 40 42 50 | LIFE 9th: Ch. 41 43 51

Overview
Prof. Sapolsky went over the endocrine system while Prof. Heller went over sexual reproduction and digestion.

Concepts
General frameworks in which to think about this week's material.

Endocrine

- Know the endocrine glands and what they secrete.

Reproduction

- Types of asexual reproduction advantages/disadvantages
- Determinants of genetic, gonadal sex, phenotypic, and behavioral sex.
- Male and female reproductive anatomy, development, and functions (including ovarian and uterine cycles).
- Birth control methods and their basis for action.

Gastrointestinal

- Anatomy of the GI tract, mechanism of food movements (anticipatory relaxation), absorption mechanism and regulation along with secreted substances and digestion.
- Koch's postulate, consistently associate germ with disease, extract from diseased tissue and infect health

Terms

endocrine

- hormone
- paracrine
- autocrine
- endocrine glands
- exocrine glands
- neurohormones
- prolactin
- prothoracicotropic hormone
- ecdysone
- lipid-soluble steroid
- juvenile hormone
- portal blood vessel

endocrine chemicals

- proteins
- steroid hormones
- amine hormones
- testosterone
- insulin
- thyroxine
- epinephrine
- tropic hormones
- thyrotropin
- luteinizing hormone
- follicle-stimulating hormone
- corticotropin
- growth hormone
- prolactin
- melanocyte-stimulating hormone
- enkephalins
- endorphins
- POMC

endocrine organs

- pineal gland
- thyroid gland
- parathyroid glands
- adrenal gland
- gonads
- hypothalamus
- anterior pituitary
- posterior pituitary
- thymus
- pancreas

Reproduction

- budding
- regeneration
- Parthenogenesis
- genetic
- gonadal
- phenotypic
- behavioral
- Sertoli cells
- Leydig cells
- LH
- FSH
- GnRH
- follicular cells
- granulosa cells
- corpus luteum
- theca cells
- seminiferous tubules
- epididymis
- testosterone
- estrogen
- progesterone
- contraception

Gastrointestinal

- insulin
- glucagon
- gastrin
- CCK
- secretin
- esophagus
- epiglottis
- larynx
- bolus
- pharynx
- peristalsis
- anticipatory relaxation
- sphincter
- chyme
- ulcers
- large intestine
- colon
- small intestine
- leptin
- bile

- pancreas
- liver
- zymogens
- parietal cells
- chief cells
- pepsin
- gallbladder

- LDL
- enteric nervous system
- adipose tissue

disease

- gigantism
- pituitary dwarfism

- bleeding ulcer
- pancreatitis. goiter
- Klinefelter's syndrome
- Turner syndrome

drugs

- RU-486

- misoprostol
- viagra

people

- Robbin Warren
- Barry Marshall

Techniques

For each technique, you should know when is appropriate to use it, what its limitations are, and a particular cellular process that was used to study it.

- Immunoassay Detect the interaction between an antigen and an antibody (for that antigen) to measure its concentration.
- Half-life Take measurements after stimulating hormone release to determine amount of time in circulation.
- Affinity chromatography Add sample to column containing beads with antibody to antigen (protein/hormone of interest). After sample flows through, wash out non-specific binding and elute out sample containing only antigen.

Endocrine

The endocrine system is how the body uses hormones to upregulate or downregulate specific processes in the body. Hormones can bind to multiple receptors, leading to different actions in different parts of the body. Know the endocrine organs along with one or two hormones released/synthesized by them.

- Thyroid secretes thyroxine and calcitonin, which increase cell metabolism and stimulate calcium incorporation into the bone. Of the thyroxines, T_3 is the more active than T_4.
- Adrenal gland secretes corticosteroids (cortisol, cortisone, aldosterone, etc.), epinerphrine, and norepinephrine. It helps maintain water balance, mediate response to stress and stimulate flight-or-flight response. It is composed of the adrenal medulla and adrenal cortex.
- Gonads secretes androgens, e.g. testosterone, estrogen, and progesterone. It helps maintain male and female sexual characteristics along with supporting proper pregnancy development. Their effects begin around seven weeks post-fertilization.
- Pineal gland secretes melatonin, which regulates biological rhythms. Melatonin release is inhibited by light.
- Parathyroid glands secretes parathyroid hormone (PTH), stimulates calcium release from bone (acts on osteoblasts/clasts) and absorption of calcium by gut and kidney.
- Hypothalamus is involved in thermoregulation (see last week's notes) and secretes neurohormones that control the anterior pituitary. Sends ADH and oxytocin to posterior pituitary.
- Anterior pituitary actually makes hormones unlike the posterior pituitary.
  - thyrotropin (TSH) activates thyroid
  - follicle-stimulating hormone (FSH) stimulates ovarian follicle maturation and spermatogenesis)
  - luteinizing hormone (LH) stimulates estrogen/progesterone and testosterone production
  - corticotropin (ACTH) stimulates adrenal cortex, releases cortisol
  - growth hormone (GH) stimulates growth (surprise!)
  - prolactin stimulates milk production
  - melanocyte-stimulating hormone (MSH) stimulates melanin release by skin and hair and has effects on appetite and sexual arousal.
  - endorphines and enkephalins help control pain.
- Posterior pituitary secretes oxytocin (stimulates uterus contraction and social bonding) along with antidiuretic hormone (ADH/vasopressin), which promotes water conservation.
- Thymus secretes thymosin, which activates immune system T cells.
- Pancreas secretes insulin, glucagon, and somatostatin (slows release of insulin and glucagon), which help cells utilize glucose (insulin) after kidney release (glucagon). Insulin is produced in islets of Langerhans.
- Adipose tissue secretes leptin.

- Heart secretes atrial natriuretic peptide.
- Kidney secretes erythropoietin.
- Stomach secretes gastrin.
- Intestine secretes secretin, cholecystokinin.
- Skin secretes vitamin D (cholecalciferol).
- Liver secretes somatomedins and insulin-like growth.factors

Reproduction

Asexual and sexual reproduction both have their advantages and disadvantages. Know the anatomical organization of the human reproductive system and the hormones/cells involved.

Asexual

- Budding involves an outgrowth forming on the parent that turns into the child.
- Regeneration the ability of a part of an organism to be separated and form an entirely new organism, think cutting starfish into pieces and watching each piece create a new starfish.
- Parthenogenesis development of offspring from unfertilized eggs, e.g. *Cnemidophorus uniparens*.

Sexual Sexual reproduction is advantageous because it allows genetic diversity to arise through crossing over and indepedent assortment. Gonads help produce motile sperm (testes) and nonmotile ova (overies). Fertilization was covered in earlier lectures, but recall that eggs can be fertilized externally (fish, etc.) or internally (humans, etc.).

- Primary sex organs are the gonads where gametogenesis occurs.
- Secondary sex organs include the penis, vagina and other glands/ducts that help facilitate fertilization.
- Four types of sex: genetic, gonadal, phenotypic, and behavioral

Development We will focus on development in humans.

- Utheral groove, genital turbcle, and urethral folds are present in both male/female genitalia
- Genetic sex: determined at fertilization by presence of X/Y chromosomes.
- Gonadal sex: SRY produced in seventh week of development, induces testes formation via SOX9. DAX1 and Wnt4a promote ovary development.
- Phenotypic sex: determined by androgens around week eight. This includes musculature, pubic hair pattern, body proportions, etc.
- Behavioral sex: Can be determined at different times throughout an organisms life depending on social curs (clown fish), hormonal levels, and others.
- Accessory organs: Wolffian and Mullerian ducts develop around 4-7 weeks.
- Sertoli cells provide nutrients for developing sperm/germ cells. Also secrete inhibin to reduce FSH production by anterior pituitary.
- Leydig cells produce testosterone and are clustered between seminiferous tubules. They are stimulated by luteinizing hormone (LH) and follicle-stimulating hormone (FSH) of the anterior pituitary, which itself is activated by gonadotropin-releasing hormone (GnRH) from the hypothalamus.
- Follicular cells or granulosa cells form the corpus luteum, produces estrogen and progesterone. The corpus luteum degrades if no implantation occurs.
- Theca cells comprise a layer of the ovarian follicles and produce androstenedione.

Anatomy Easiest to just look at the following figures to get a sense of male/female anatomy: Fig. **??** and Fig. **??**.

- Spermatogenesis takes place in the seminiferous tubules and afterward sperm travel to the epididymis.
- Prostate gland secretes alkaline fluid to neutralize male/female acidic reproductive environments and also contributes about a third of sperm volume.
- Bulbourethral glands produce secretions that help facilitate passage of semen during climax.
- Erection results from nitric oxide release into the blood vessels, which stimulates cGMP production that causes vasodilation.

- human chrionic gonadotropin (hCG) is released by cells covering blastocyst.

Digestion

Gastrointestinal tract is the main component of the digestive system. Smooth muscle in the gut are depolarized when stretched, allowing them to detect the presence of food.

See figures near the end of this handout for flow charts on absorptive and postabsorptive state along with metabolism of lipids and effects of insulin and glucagon on the body. **GI components**

- tongue
- pharynx
- trachea (windpipe)
- epiglottis
- larynx
- esophagus
- sphincters
- stomach
- small intestine
- large intestine

Food movement

1. Food contacts soft palate that causes neuronal response, leading to movement of a bolus (chewed food) past the pharynx and down the esophagus. The epiglottis covers the larynx, preventing food from entering the lungs.
2. The upper esophagus is skeletal muscle controlled by the CNS while the lower tract is smooth muscle controlled by ANS and ENS.
3. Stretching of smooth muscle causes them to contract, called peristalsis, pushing food down.
4. Muscle further down the tract relax due to an anticipatory wave of relaxation.
5. Food enters stomach, tonically closed lower esophageal sphincter prevents food returning to esophagus.
6. Peristaltic movement in antrum cause stomach to empty, via a ring of contraction. Pyloric sphincter controls stomach to intestine movement of chyme, the fluid mix of gastric juices and partially digested food.
7. Stomach ulcers, caused by *helicobacter pylori*, is caused by bacteria.
8. Peristalsis moves food down small intestine until it reaches ileocaecal sphincter.
9. In large intestine, or colon, further contraction and relaxation allow absorbing of water. Goes from ascending to transverse colon then reaches the rectum where it is solid.
10. Anal sphincter opens to allow solid waste to exit Parasympathetic reflex, but positive control via
11. Note: gastro enteric reflex is enteric nervous system while cephalic reflex is via autonomic nervous system.

Hormones Below are several hormones involved.

- gastrin, peptide that is released by stomach walls, when comes back to stomach causes increased motility and secretion of digestive enzymes
- small intestine releases cholecystokinin (CCK), secretin, and GIP to inhibit stomach motility
- CCK stimulates bile (liver) and digestive enzyme release (pancreas).
- Secretin stimulates pancreatic bicarbonate production.
- Leptin is produced by fat cells and acts on the hypothalamus to alert it about body fat reserves, inhibiting fat uptake.

Secretions

- Salivary gland secretes saliva into the mouth that contains mucus and extracellular fluid along with amylase to break down carbohydrates.
- sodium is actively reabsorbed while potassium is passively secreted in the mouth.
- gastric pits are stomach lining folds that contain parietal cells (acid, i.e. HCl) and chief cells (secrete pepsin).
- pepsinogen is secreted and inactive but converted to pepsin by HCl secreted by parietal cells.
- carbonic anhydrase catalyzes the reaction $CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow HCO_3^- + H^+$ that allows HCl to be released into the stomach.
- cephalic phase stimuli is relayed from CNS to ENS, which drives acid secretion. HCl inhibits gastrin secretion, a inhibitory feedback loop.

enteric nervous system

Pancreas

- Sends digestive enzymes and bicarbonate solution.
- Exocrine cells secrete externally, digestive enzymes.
- Zymogens, all digestive enzymes secreted are inactive. Enterokinase causes masking amino acids to be cut off, same with trypsinogens.
- Bicarbonate ions are alkaline, it neutralizes acidic environment coming from stomach.

Liver

- Produces bile, allows emulsification (enclosure) and digestion of fat, that flows down the hepatic duct.
- Gallbladder stores bile via a branch called the cystic duct.
- Gallstones caused by precipitation of various components in bile.
- Sphincter of Oddi controls bile entry from common bile duct into small intestine, controlled by anticipatory relaxation.
- nutrients are delivered from gut via the hepatic portal vein. Kupffer cells phagocytose particles that pass through endothelial cells.
- Lipoproteins are used to transport lipids in the blood stream and can be classified according to their lipid-to-protein ratio: high-density (HDL), low-density (LDL), and very low-density (VLDL). Lipids are low density, so higher lipid-to-protein means lower density particles.

Digestion

- Several classes of enzymes break down food and are produced in inactive forms known as zymogens. Hydrolysis is main mechanism used to break bonds.
  - proteases amino acids in proteins.
  - carbohydrases hydrolyze carbohydrates.
  - peptidases, peptides.
  - lipases, fats.
  - nucleases, nucleic acids.
- Once protein broken down into amino acids, sodium co-transport allows sodium and transporter to attach to amino acid and follow concentration gradient down into the cell, where it delivers in contents.
- Osmotic potential increased as you break down carbohydrates, to prevent osmotic shock, break down only right at lining of the gut.
- Fat is digested after being broken down slightly, since enzymes can't operate in hydrophobic environment. Micelles are formed to increase the surface areas over which lipases can operate.

Cell biology

- Muscle layers: longitudinal and circular muscle layer, essential for peristalsis.
- Epithelial lining: where absorption occurs, single layer
- Mucosa and submucosa: sensory neurons that send signals to the nervous system, altering to progress of digestion. Also secrete digestive enzymes
- Enteric neurons: they are an autonomous nervous system for the gut.
- Myenteric plexus: between longitudinal and circular muscle layer, it can integrate information about the gut and turn on/off layers.
- Meissner's plexus: control action of the gut, similar to myenteric plexus except it is associated with the submucosa layer.

Glucose

- Uptaken by carrier molecules, it is facilitated diffusion as opposed to passive diffusion.
- Insulin affects carrier molecules
- Glucose is phosporylated to prevent it from leaving the cell once it has entered.
- Glycogenolysis, breakdown of glycogen to glucose for use as fuel, induced by epinephrine and insulin.
- Gluconeogenesis, synthesize glucose from certain substrates such as some amino acids, glycerol, pyruvate, lactate.

- Cori cycle, lactate from muscle converted to glucose in the liver and returned to the muscle. See Fig. 116
- Adipose tissue stores triglycerides
- Chylomicrons transport fat (triglycerides, cholesterol, etc.) through circulation, bind cell receptors and broken down.

Problems

If there is any confusion about the questions, shoot me an email or talk to me after class. Remember, draw out what a pathway, interaction, or what-have-you looks like if you get confused.

Endocrine

1. What are the developmental origins of the anterior and posterior pituitary? Nervous and gut respectively.
2. Glucagon promotes conversion of glucose to fat in the liver. True or false, explain. Glucagon stimulates the liver to break down glycogen in the liver in response to low glucose levels.

Reproduction

- Order the following contraceptives by effectiveness: vasectomy, coitus interruptus, condom, intrauterine device (iud). vasectomy, intrauterine device (iud), condom, coitus interruptus
- Before puberty the hypothalamus does not secrete much gonadotropin-releasing hormone.
  - the pituitary secretes luteinizing hormone and follicle-stimulating hormone, but the gonads are unresponsive.
  - the hypothalamus does not secrete much gonadotropin-releasing hormone.
  - males can stimulate massive muscle development through a vigorous training program.
  - testosterone plays no role in development of the male sex organs.
  - genetic females will develop male genitals unless estrogen is present.
- Both epinephrine and cortisol are secreted in response to stress. Which of the following statements is also true for both of these hormones? They act to increase blood glucose availability.
  - They act to increase blood glucose availability.
  - Their receptors are on the surfaces of target cells.
  - They are secreted by the adrenal cortex.
  - Their secretion is stimulated by corticotropin.
  - They are secreted into the blood within seconds of the onset of stress.
- Growth hormone stimulates protein synthesis.
  - can cause adults to grow taller.
  - stimulates protein synthesis.
  - is released by the hypothalamus.
  - can be obtained only from cadavers.
  - is a steroid.
- PTH is released when blood calcium levels fall.
  - stimulates osteoblasts to lay down new bone.
  - reduces blood calcium levels.
  - stimulates calcitonin release.
  - is produced by the thyroid gland.
  - is released when blood calcium levels fall.
- Steroid hormones act by altering gene expression in the target cell.
  - are produced only by the adrenal cortex.
  - have only cell surface receptors.
  - are water-soluble.
  - act by altering the activity of proteins in the target cell.
  - act by altering gene expression in the target cell.
- The posterior pituitary secretes neurohormones.
  - synthesizes oxytocin.
  - is under the control of hypothalamic releasing neuro-hormones.
  - secretes tropic hormones.
  - secretes neurohormones.
  - is under feedback control by thyroxine.

- Which of the following contributes to the development of goiter? All of the above.
  - Inadequate iodine in the diet
  - Autoimmune antibodies that stimulate the TSH receptor
  - Lack of feedback from circulating T3 and T4
  - Overproduction of thyroglobulin
  - All of the above
- Which of the following is a likely cause of diabetes? Loss of insulin receptors
  - Overproduction of insulin by □ cells of the pancreas
  - Loss of □ cells of the pancreas
  - Loss of insulin receptors
  - Overproduction of glucagon
  - Loss of receptors for somatostatin
- Which statement is true of all hormones? They may stimulate different responses in different cells.
  - They are secreted by glands.
  - They have receptors on cell surfaces.
  - They may stimulate different responses in different cells.
  - There is a gene that codes for each hormone.
  - When the same hormone occurs in different species, it has the same action.

Gastrointestinal

1. How do the intestinal linings maximize food intake? By having folds that contain villi on which cells containing microvilli reside.
2. What would happen if you inhibited CCK production? Less fat is absorbed.
3. Where are most proteins digested? The small intestine.
4. The large intestine absorbs as many nutrients as the small intestine. True or false, explain your answer. False, most nutrients are uptaken in the small intestine after being broken down, the large intestine largely involves water and ion uptake.
5. Why is it dangerous for the pancreatic duct to become blocked? Digestive enzymes can become activated and degrade the pancreas. This is known as pancreatitis.
6. What is the advantage of allowing fluid to flow into the interstitial space along the gut instead of directly into the cells? Allows fluid to carry nutrients via solvent drag
7. How is peristalsis controlled in the lower esophagus? The upper esophagus? Smooth muscle contraction via circular and longitudinal layers, CNS controls upper while ANS/ENS controls lower.

Figures and Tables

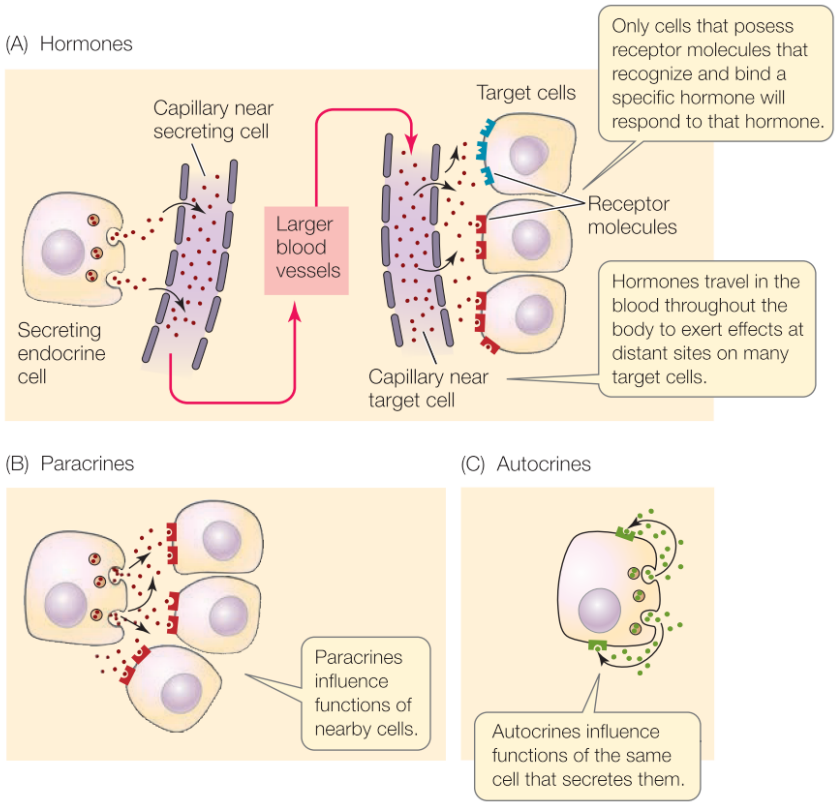


Figure 91 | Cell signaling
Different types of secreted cell signaling

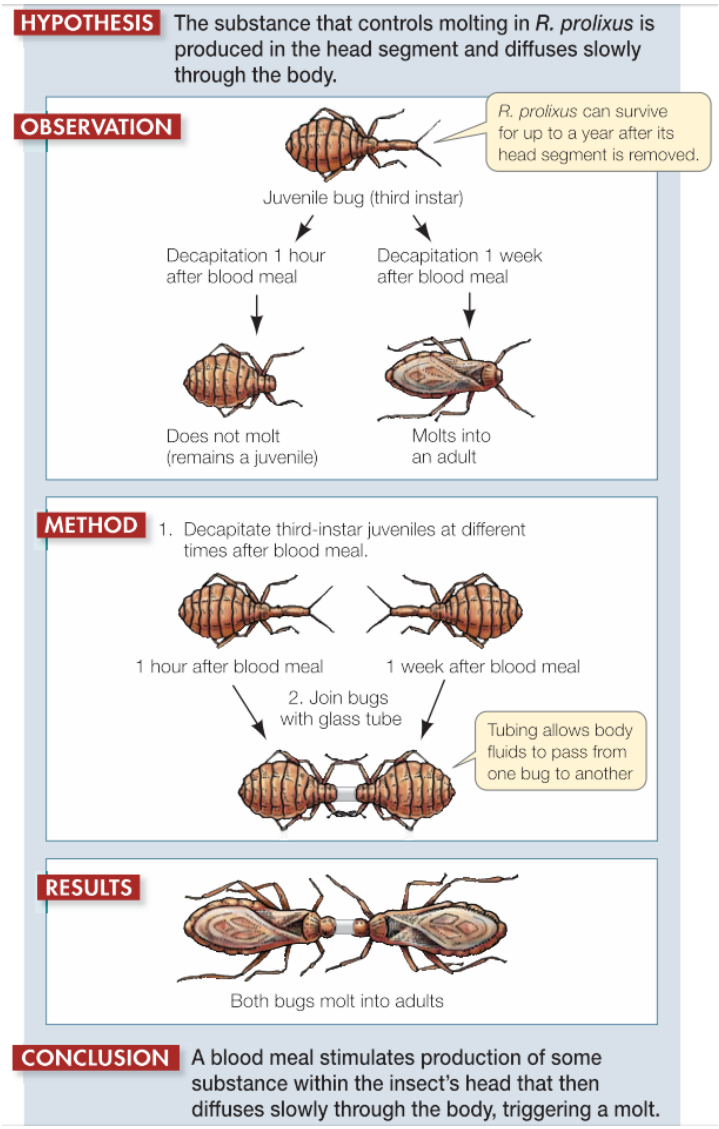


Figure 92 | Necessary and sufficient experiments

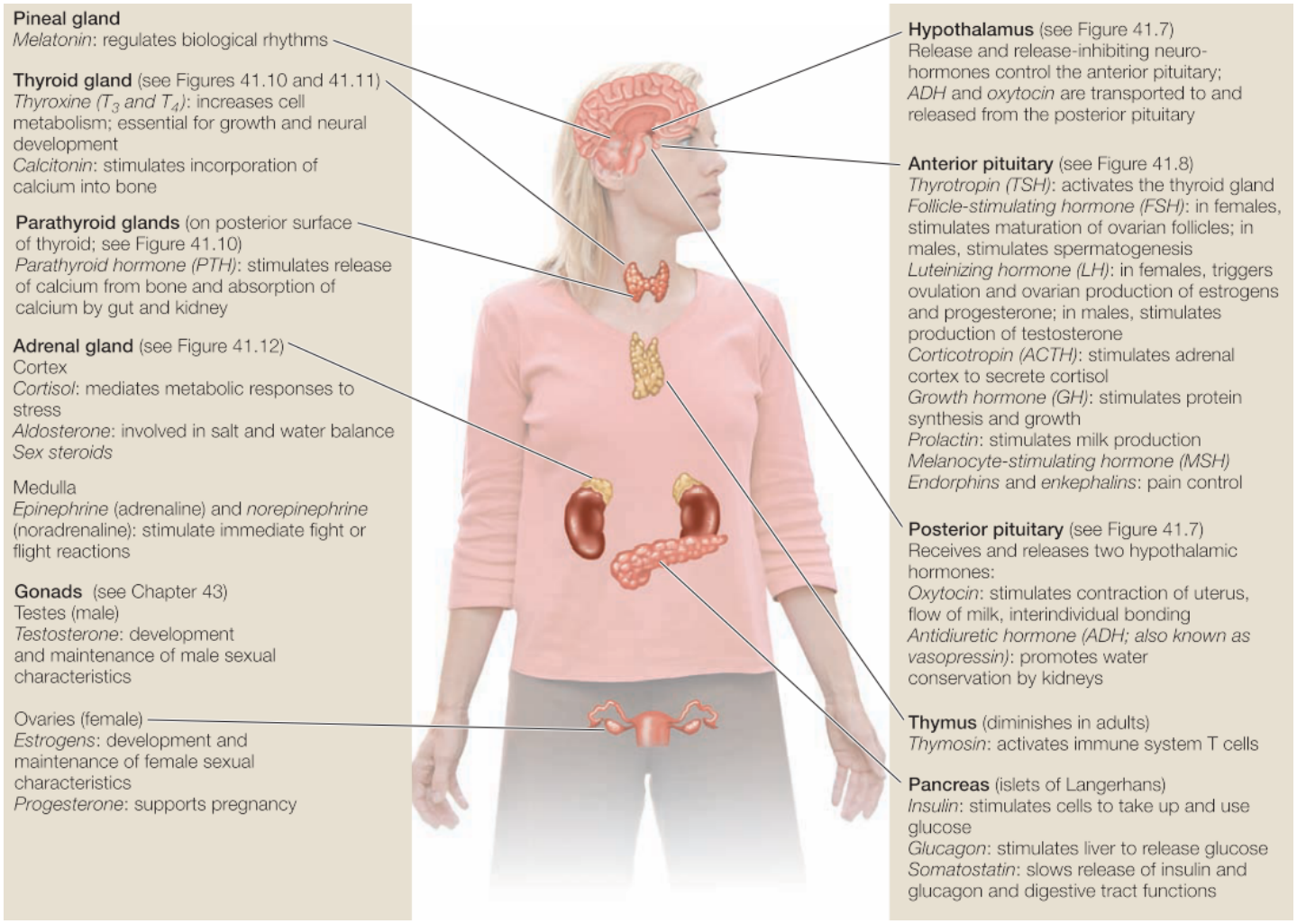


Figure 93 | Endocrine system

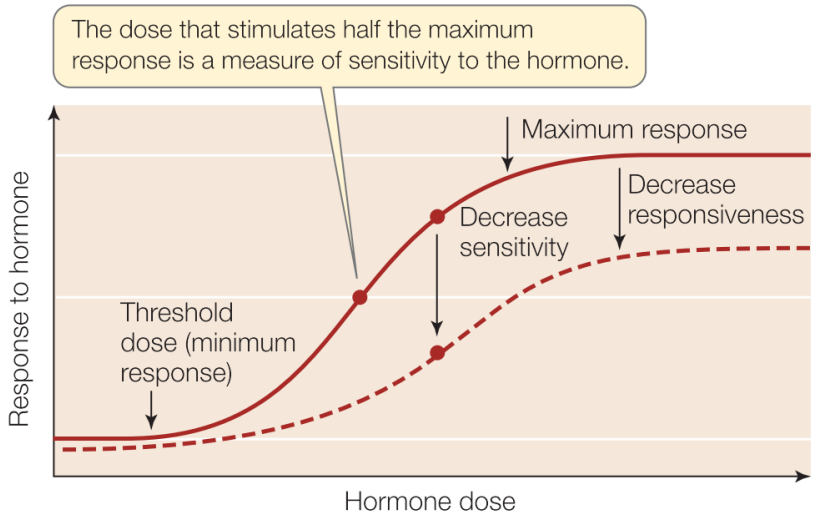


Figure 94 | Dose response curve for hormones

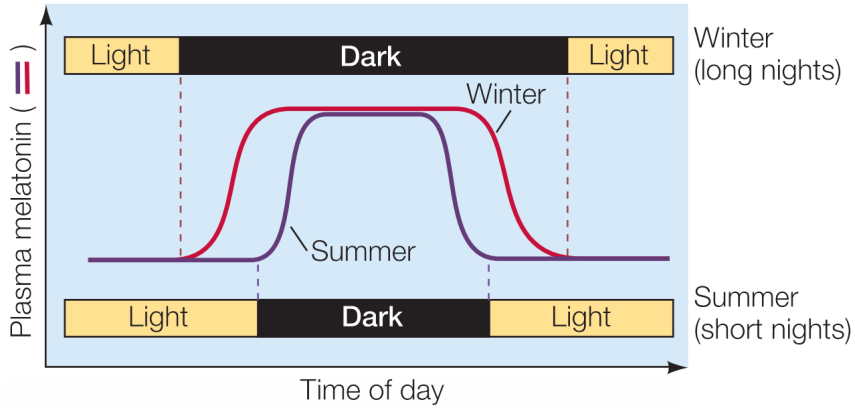


Figure 95 | Melatonin
Regulation of melatonin levels can help alter day/light cycles at different times throughout the year.

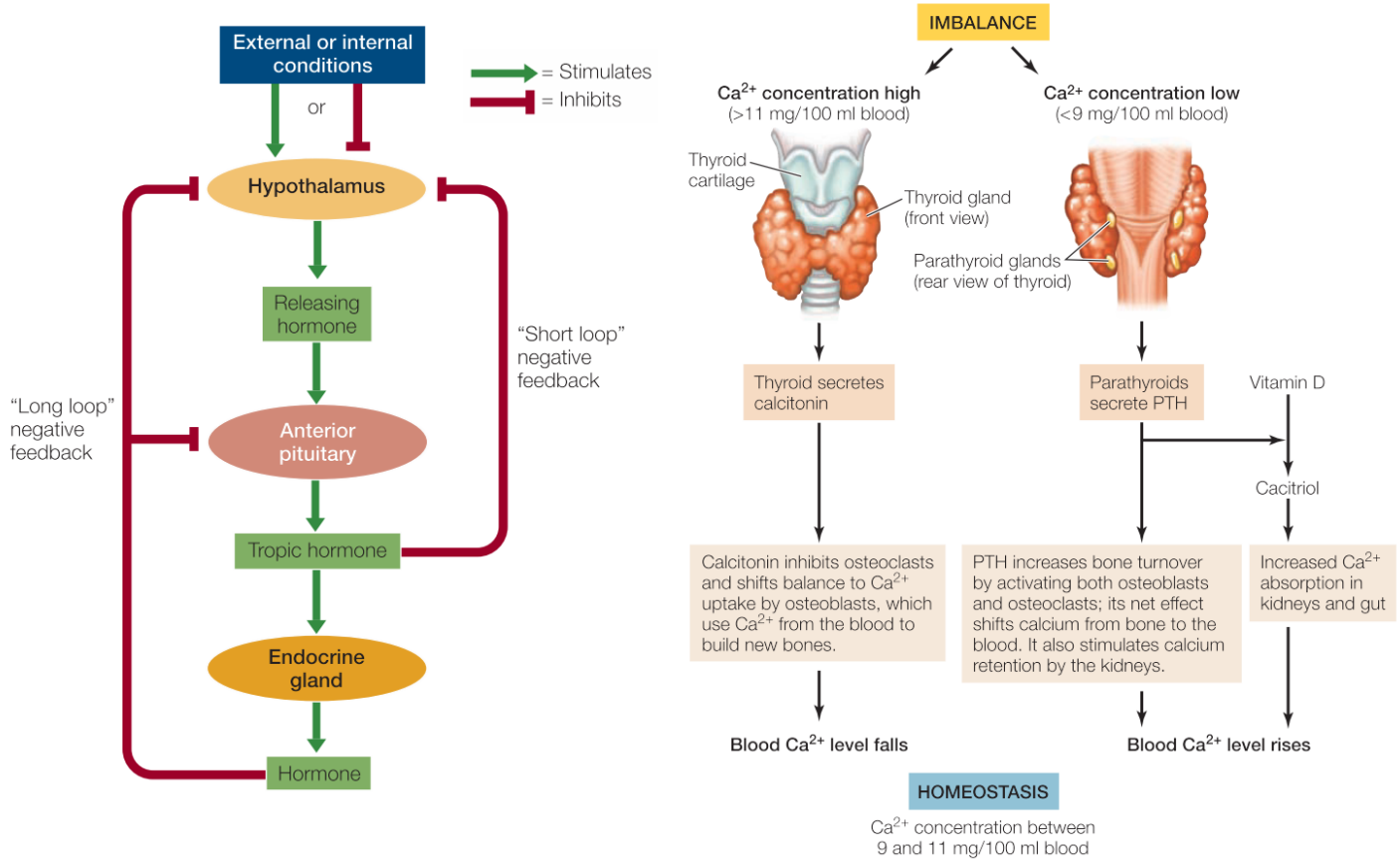


Figure 96 | Hypothalamus and thyroid regulation

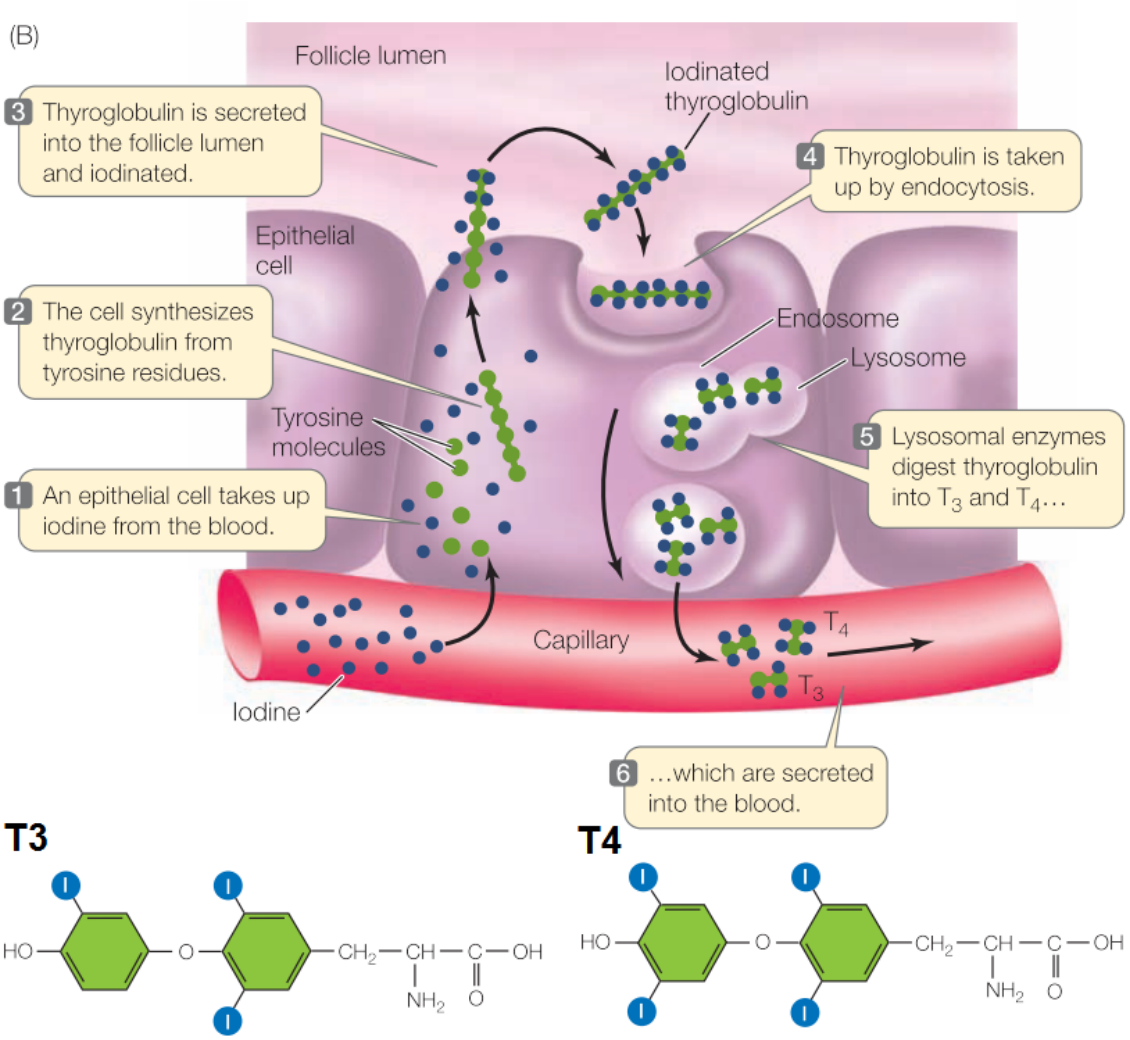


Figure 97 | Thyroid uses thyroglobulin to produce T_3 and T_4.

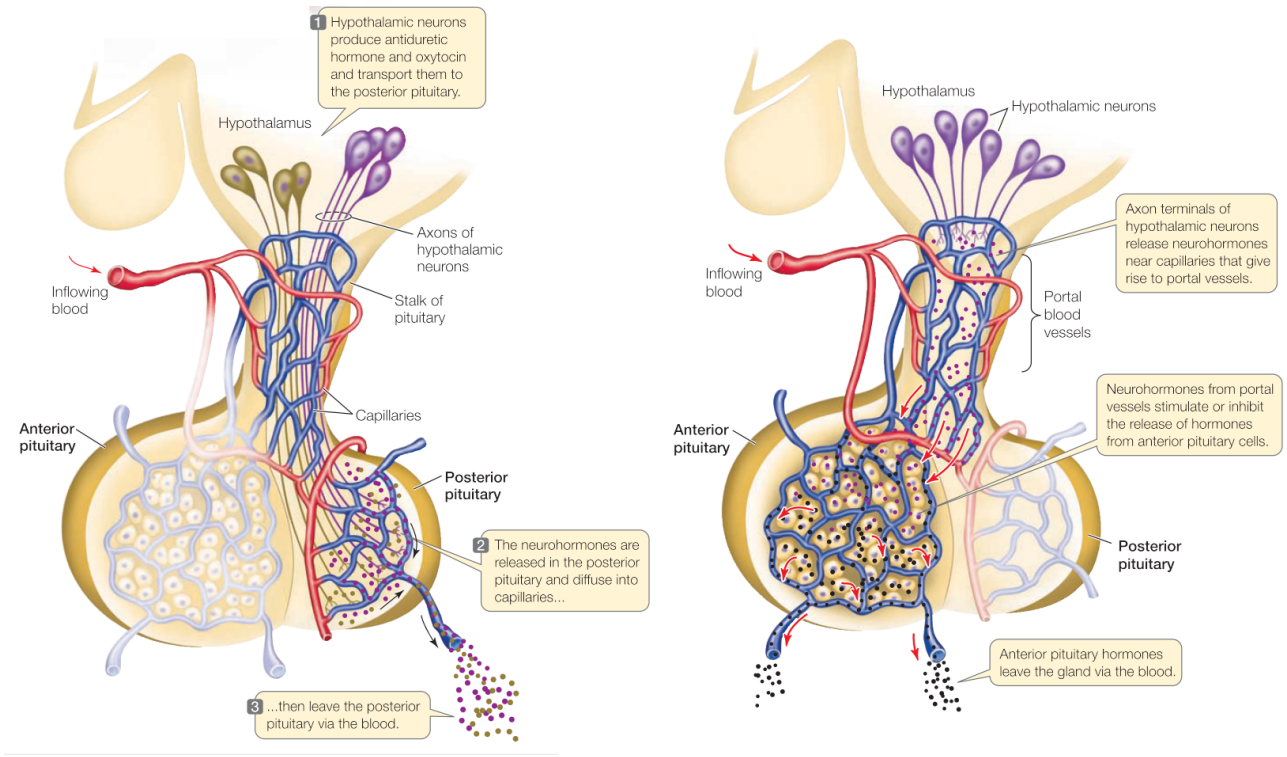


Figure 98 | Pituitary structure and function
Anterior pituitary comes from the gut epithelial tissue and the posterior pituitary from the nervous tissue during development. They serve different roles.

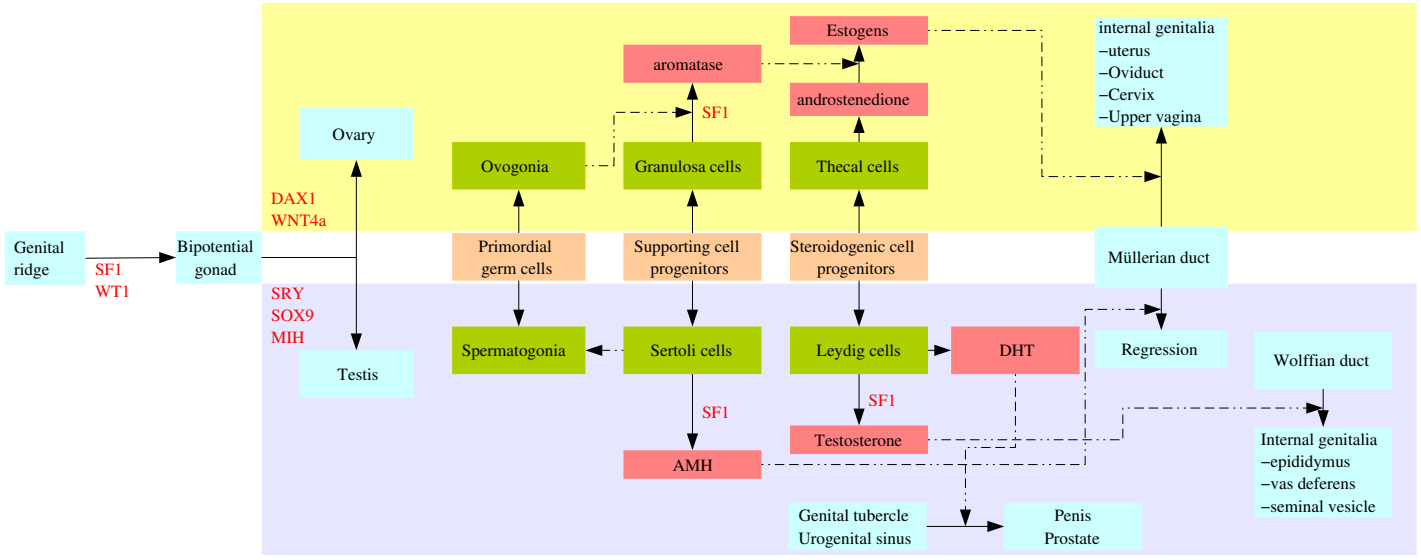


Figure 99 | Sex determination in mammals, flow chart
Know the names of the cells and genes.

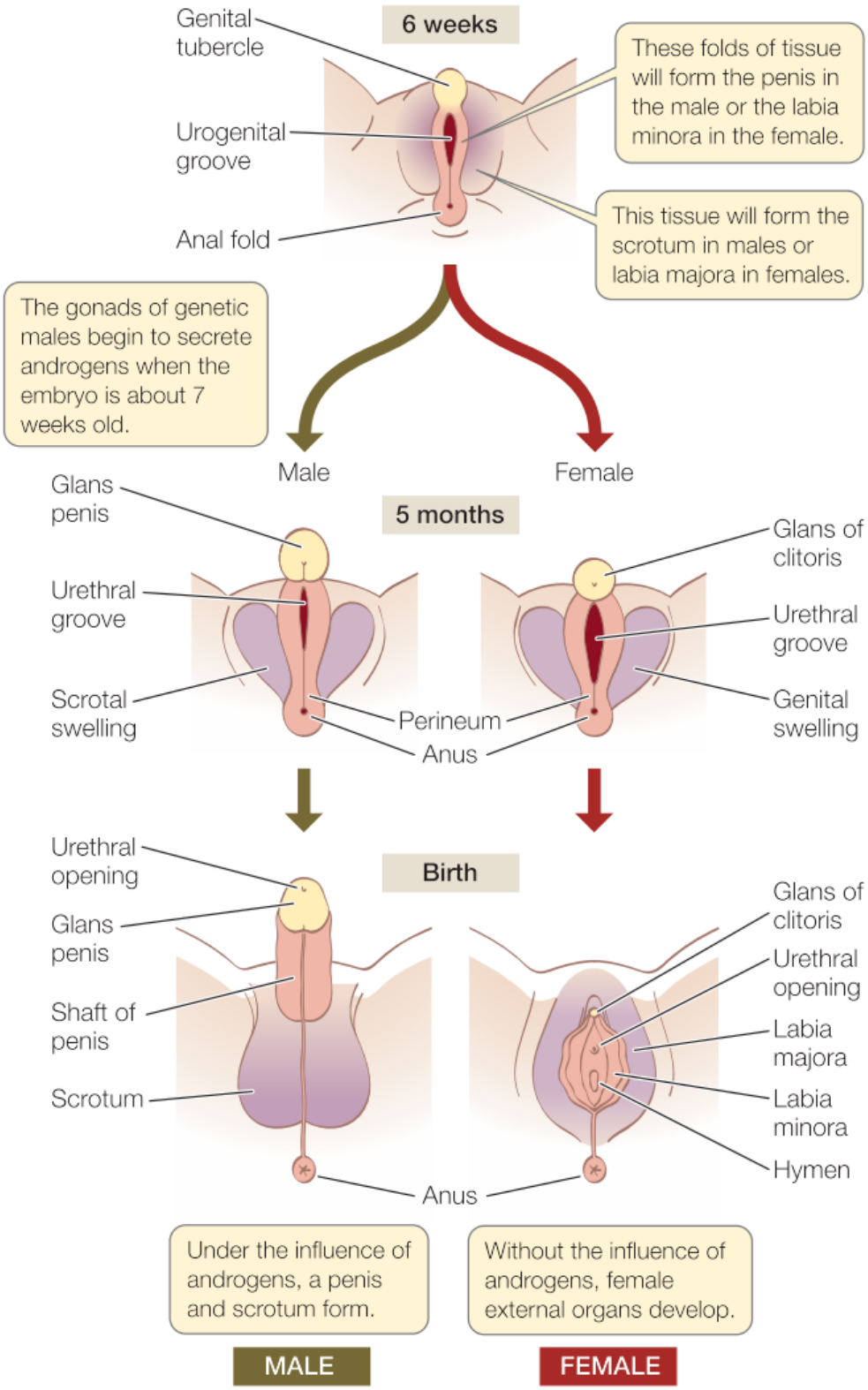


Figure 100 | Sex determination in mammals

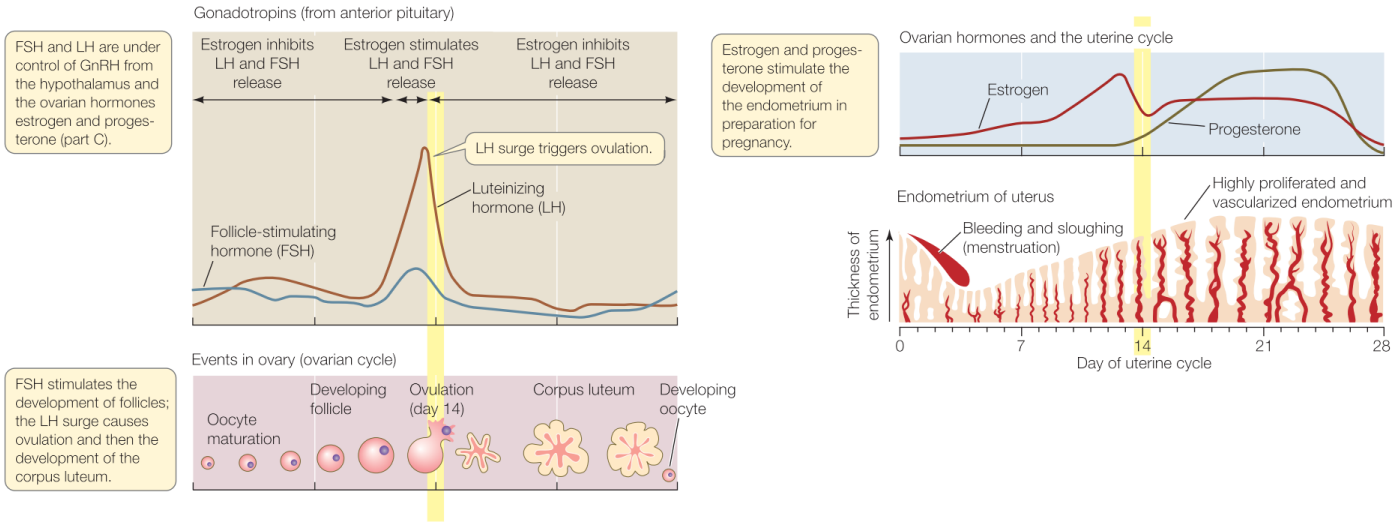


Figure 101 | Female hormones

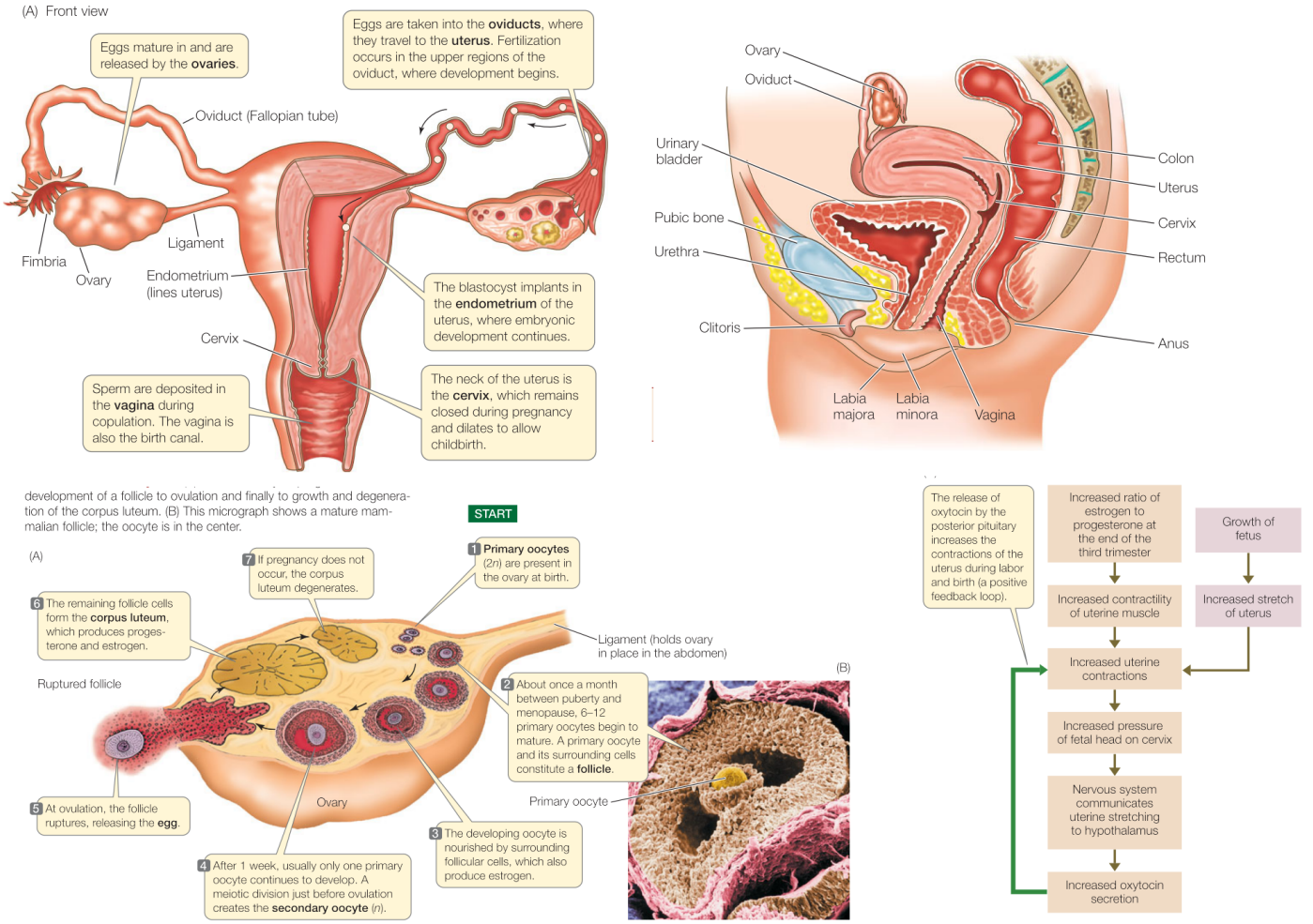


Figure 102 | Female anatomy

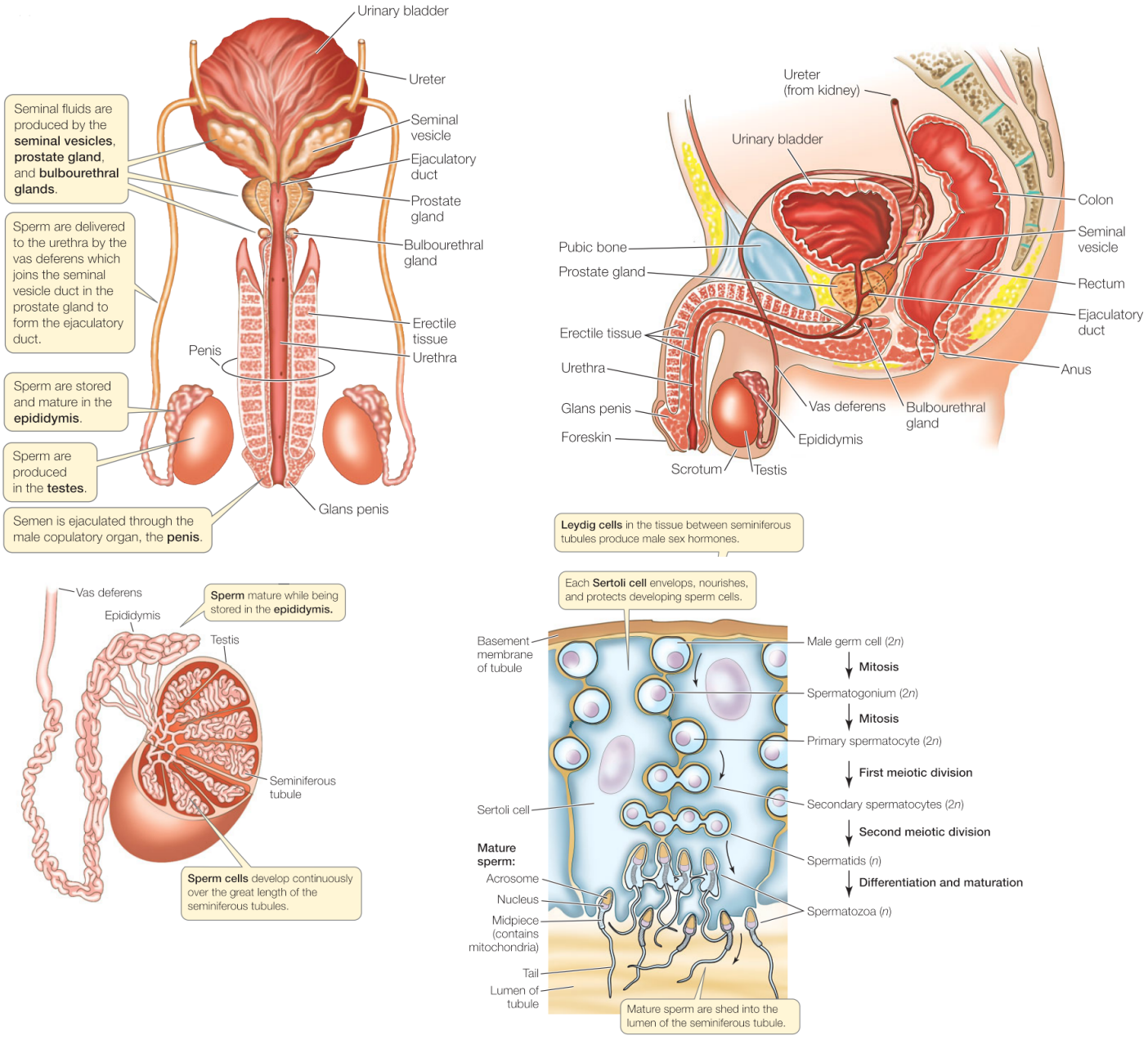


Figure 103 | Male reproductive anatomy

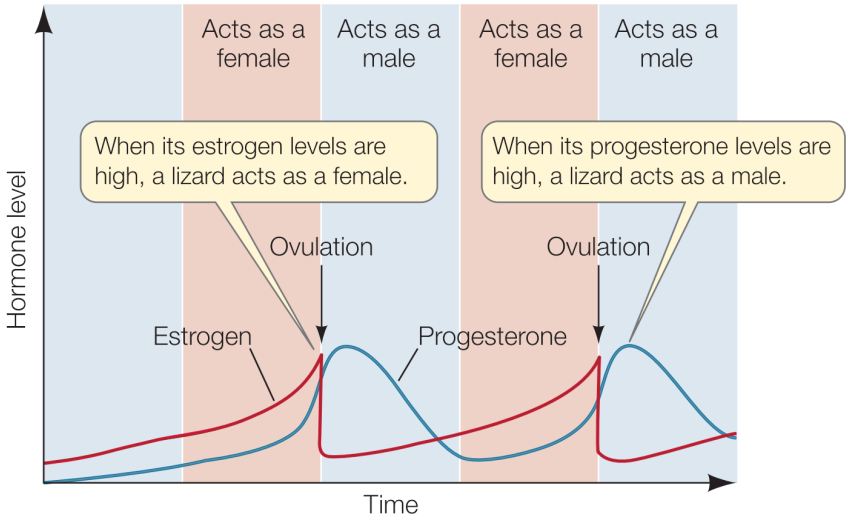


Figure 104 | Hormones and sex

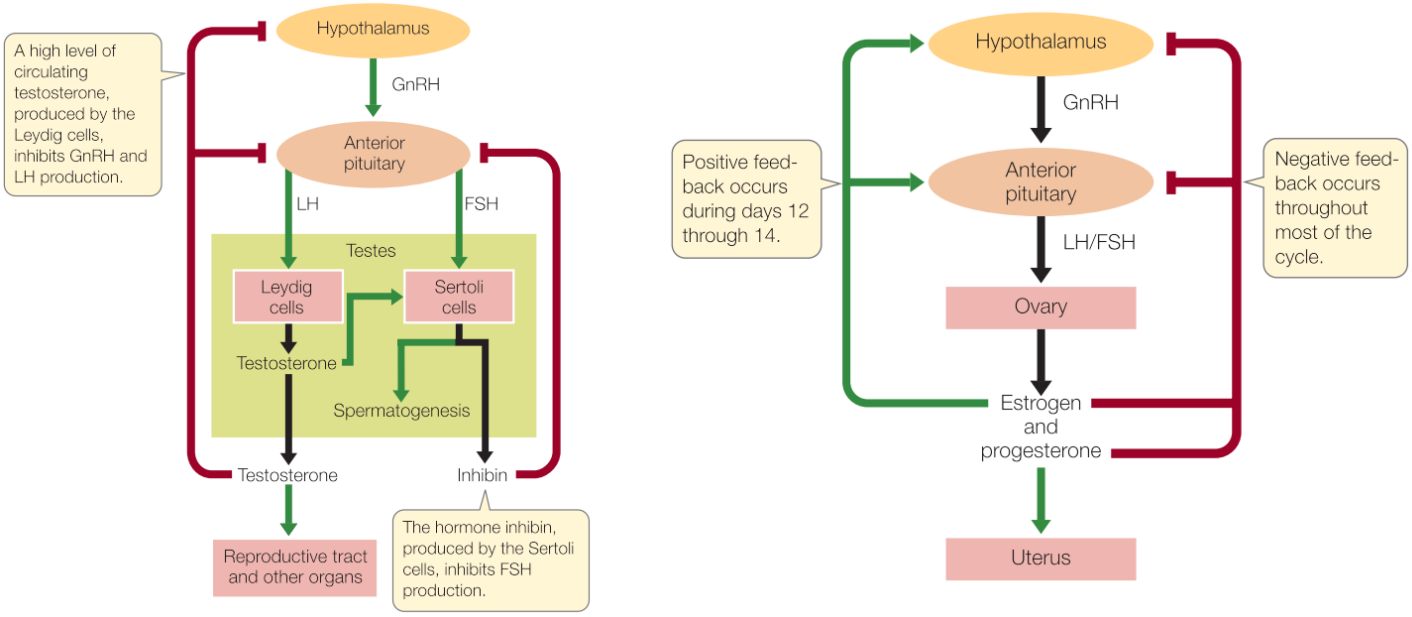


Figure 105 | Interaction networks in human reproduction

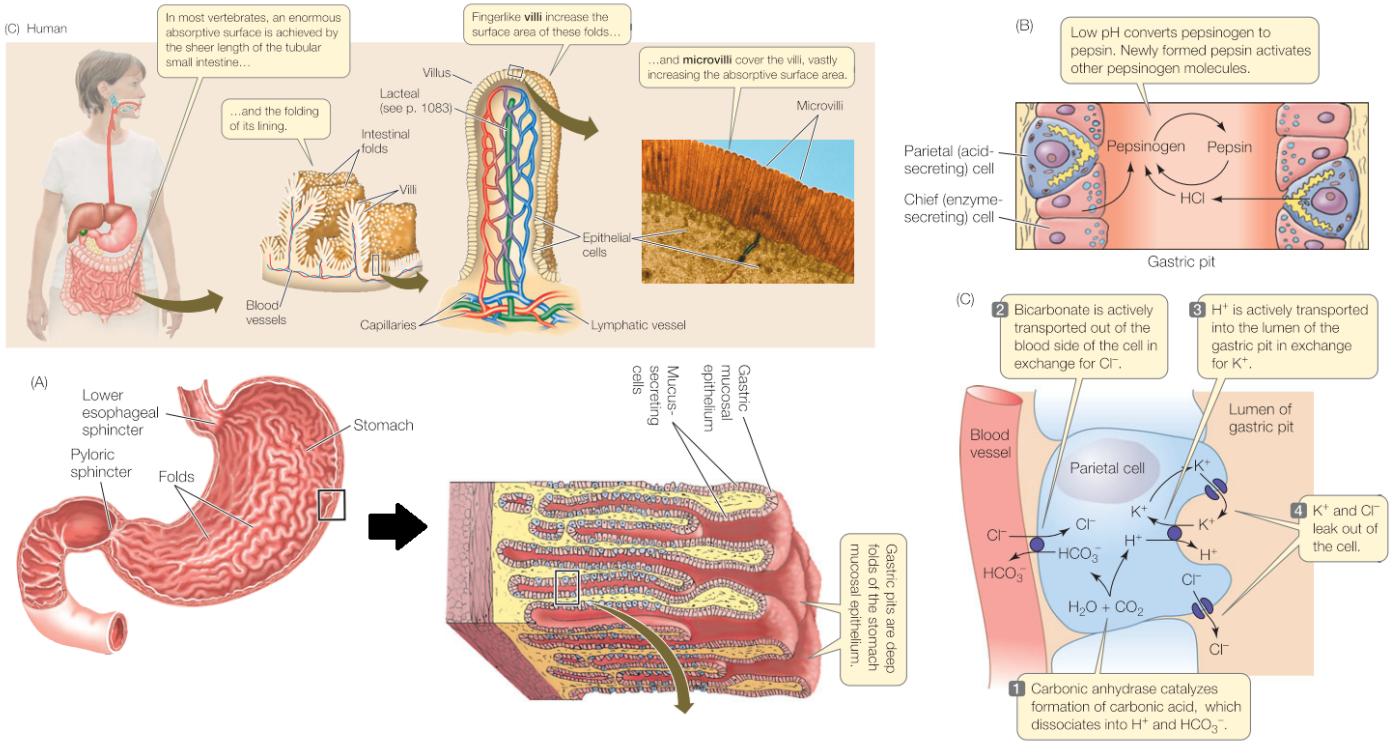


Figure 106 | The gastrointestinal tract
Uses high surface area created by many folds to increase nutrient uptake.

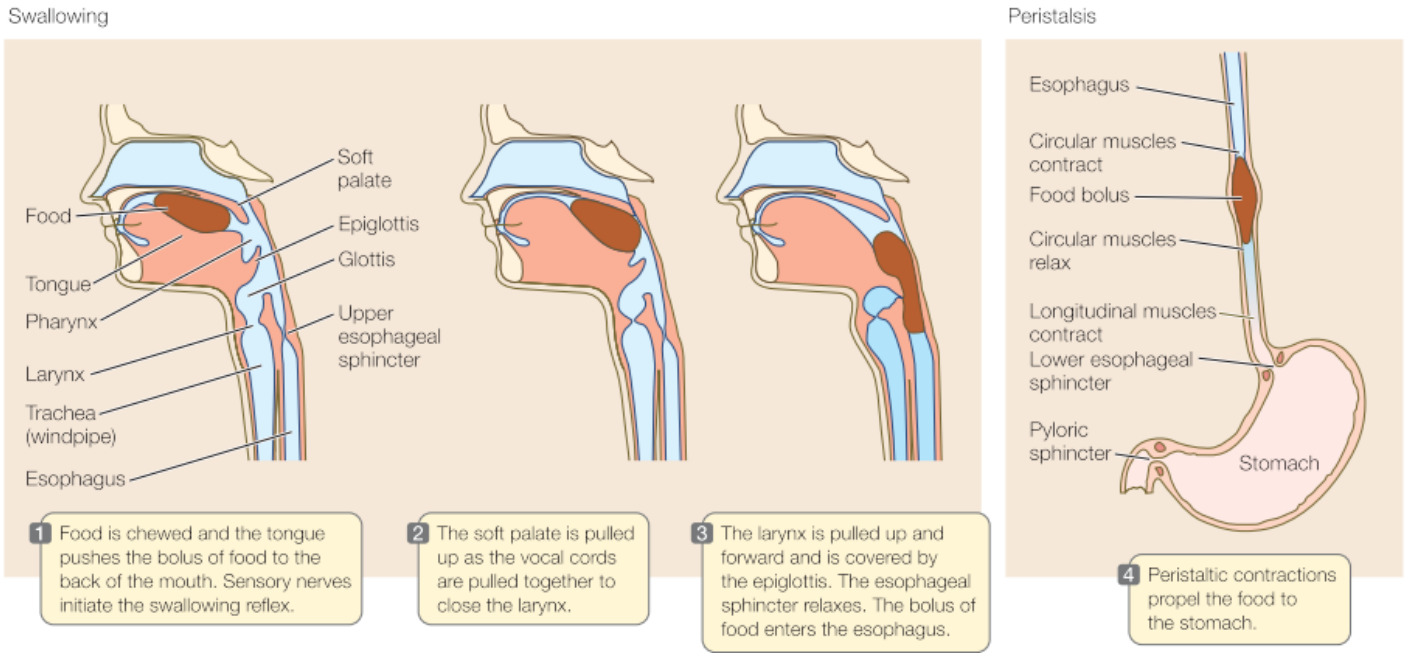


Figure 107 | Swallowing

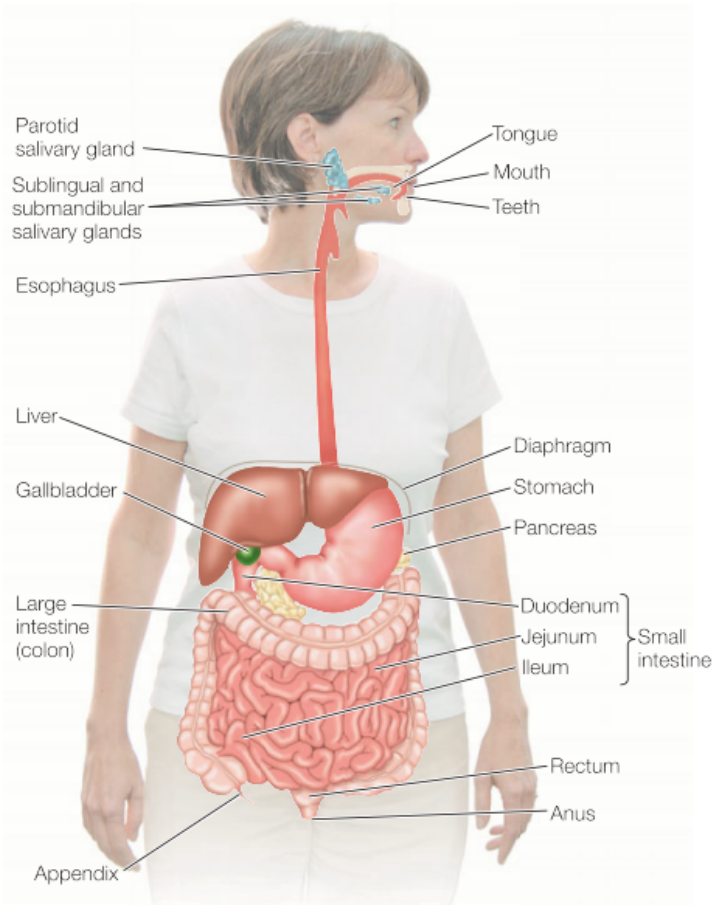


Figure 108 | Gastrointestinal components

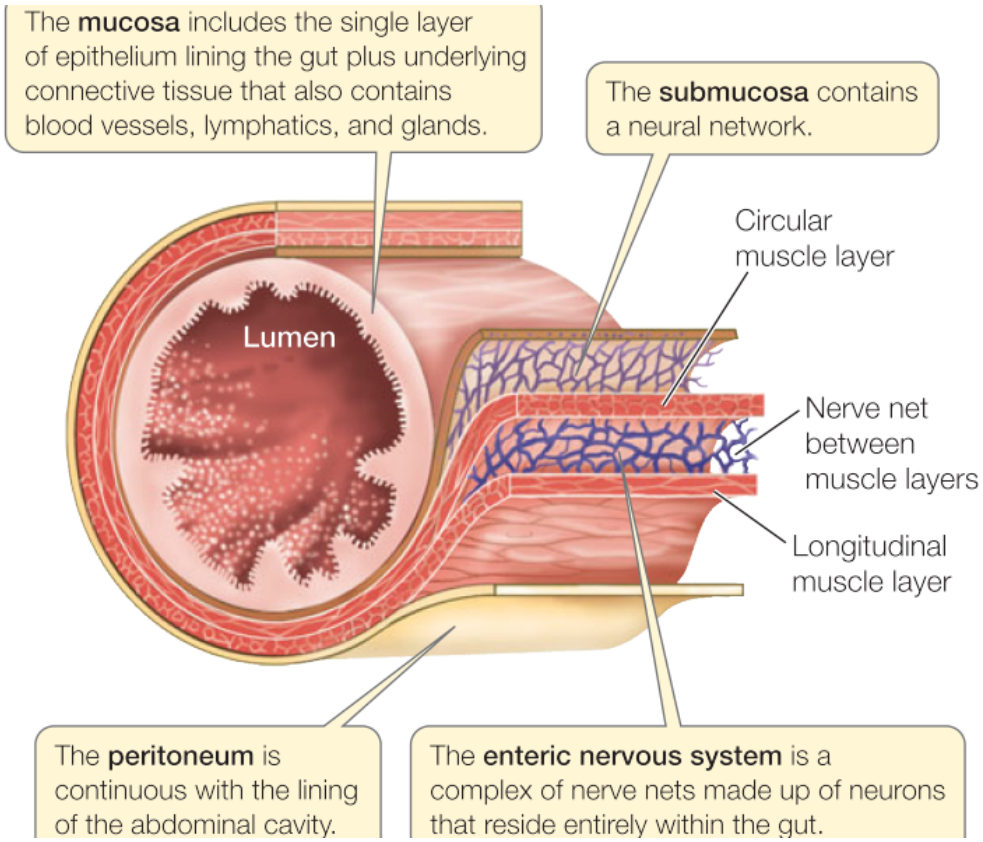


Figure 109 | Gastrointestinal lining

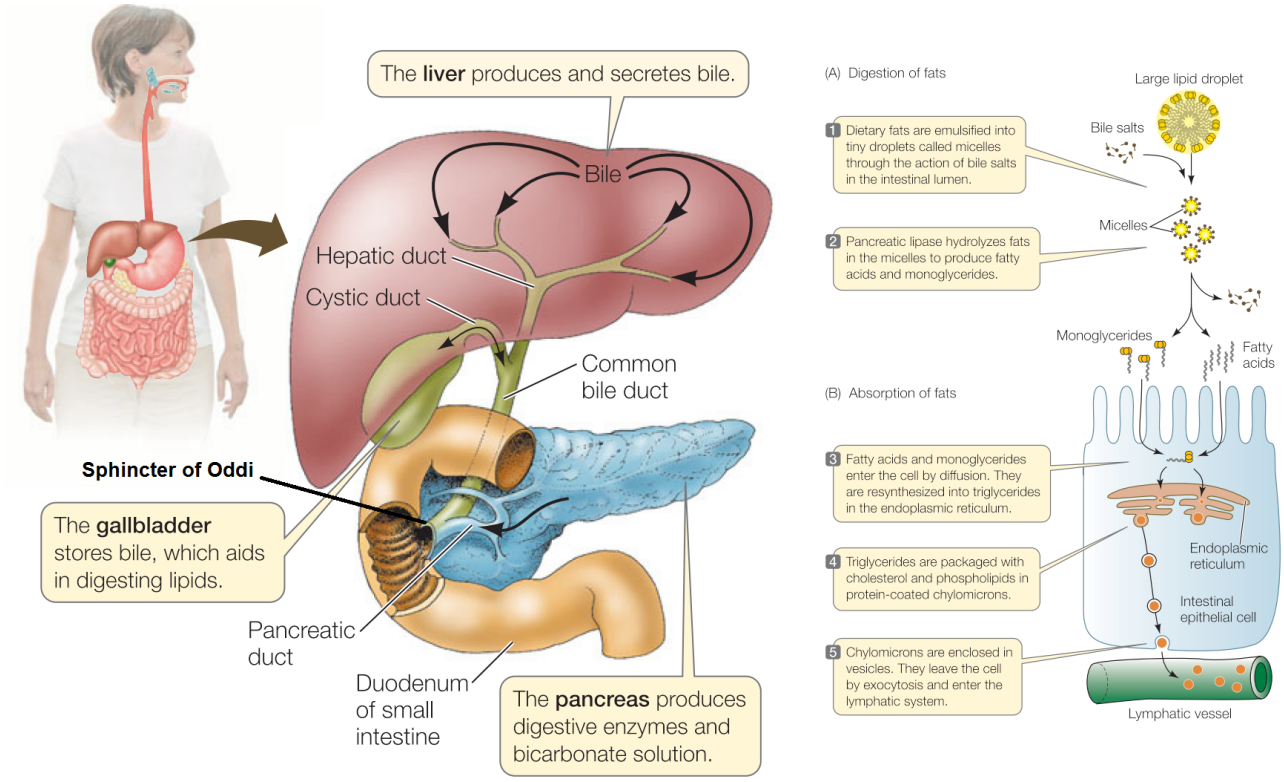


Figure 110 | Layout and function of the liver and pancreas

TABLE 51.3

Major Digestive Enzymes of Humans

SOURCE/ENZYME	ACTION
SALIVARY GLANDS	
Salivary amylase	Starch → Maltose
STOMACH	
Pepsin	Proteins → Peptides; autocatalysis
PANCREAS	
Pancreatic amylase	Starch → Maltose
Lipase	Fats → Fatty acids and glycerol
Nuclease	Nucleic acids → Nucleotides
Trypsin	Proteins → Peptides; zymogen activation
Chymotrypsin	Proteins → Peptides
Carboxypeptidase	Peptides → Shorter peptides and amino acids
SMALL INTESTINE	
Aminopeptidase	Peptides → Shorter peptides and amino acids
Dipeptidase	Dipeptides → Amino acids
Enterokinase	Trypsinogen → Trypsin
Nuclease	Nucleic acids → Nucleotides
Maltase	Maltose → Glucose
Lactase	Lactose → Galactose and glucose
Sucrase	Sucrose → Fructose and glucose

Figure 111 | Enzymes used in the digestive tract

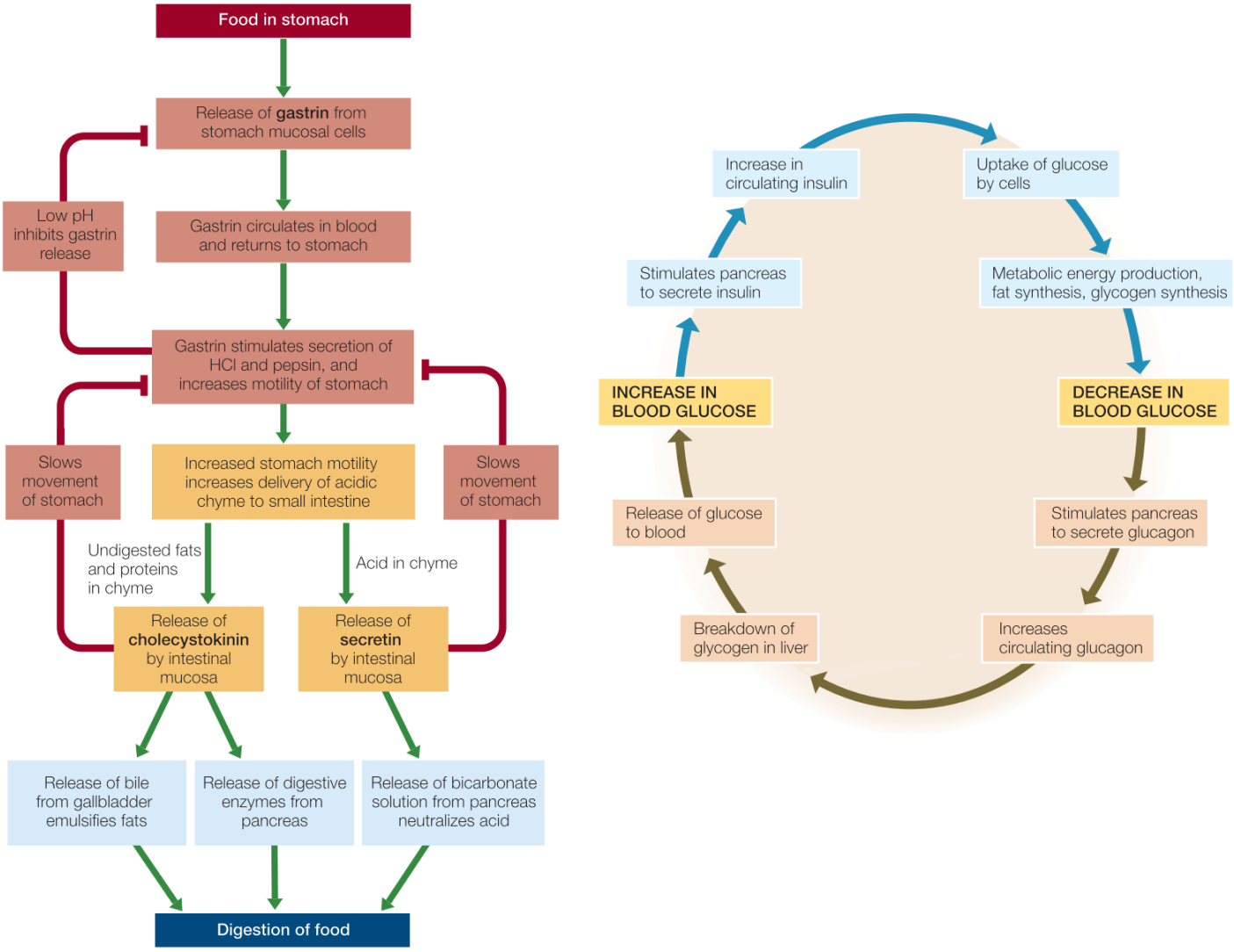


Figure 112 | Hormonal regulation of digestion

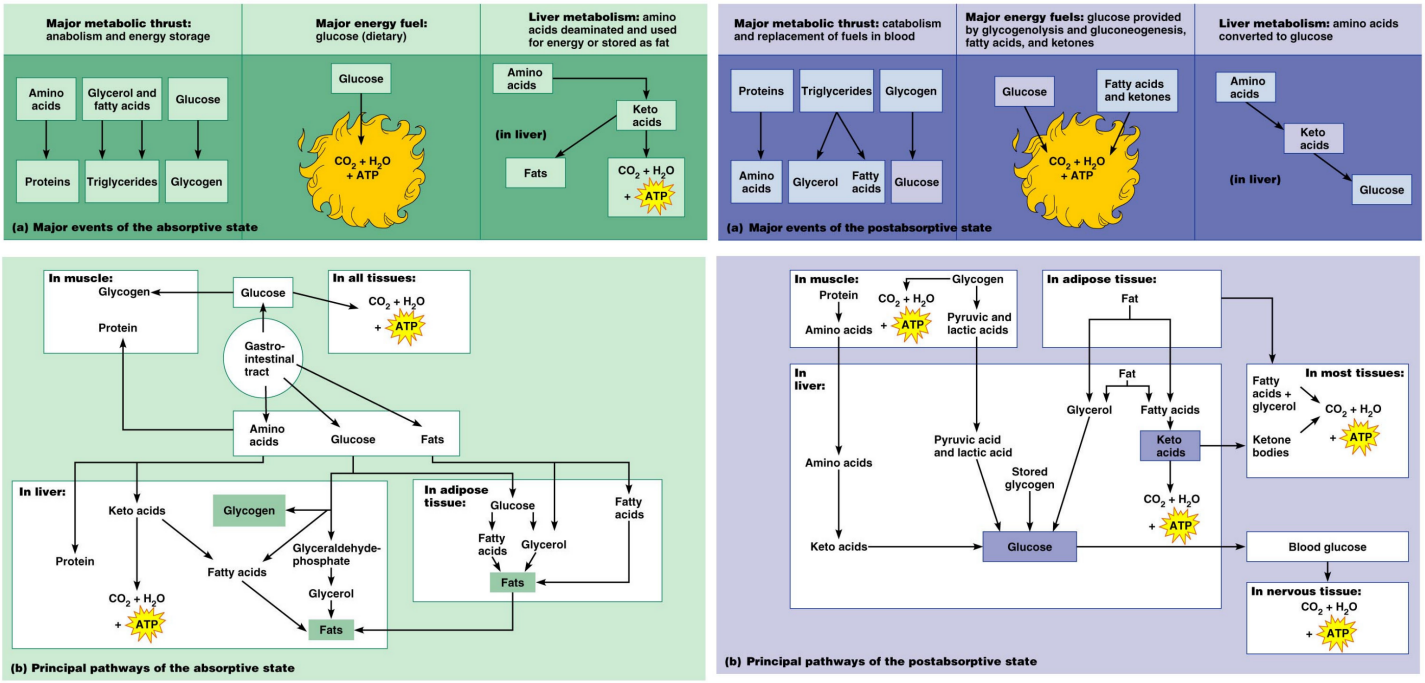


Figure 113 | Change in fuel use before (left) and after (right) eating.
These are also known absorptive and postabsorptive states

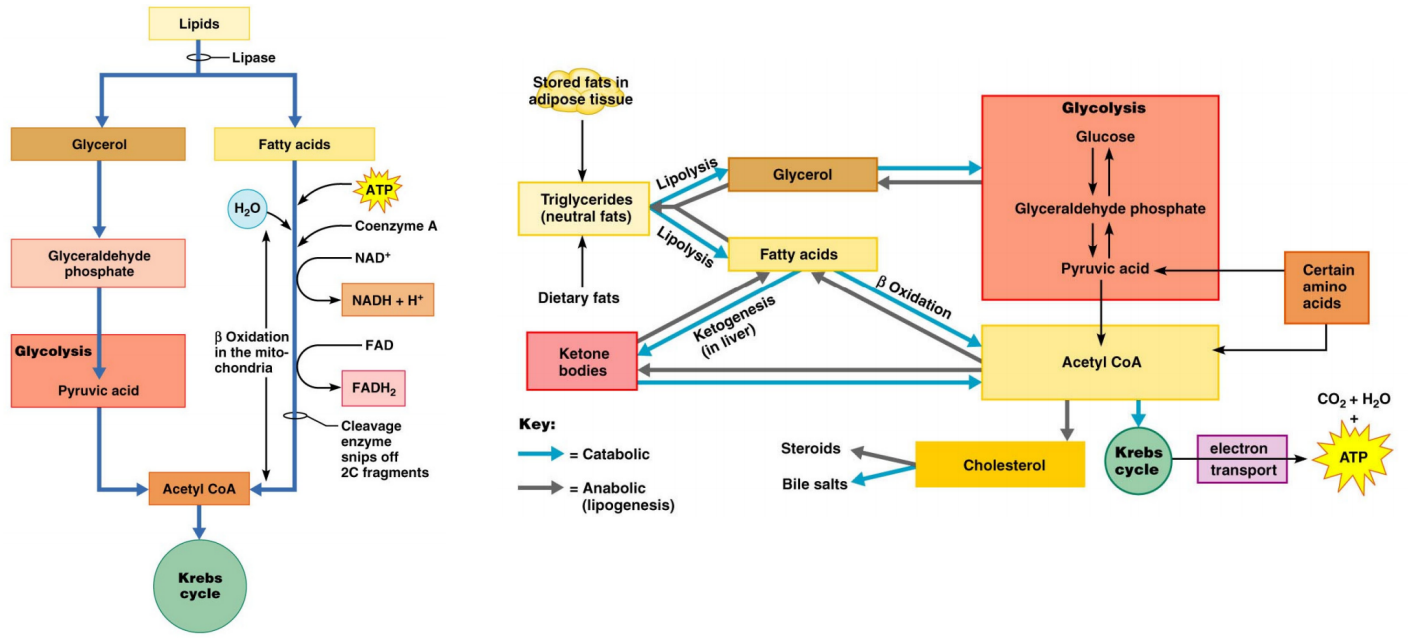


Figure 114 | Metabolism of lipids and glucose in the body.

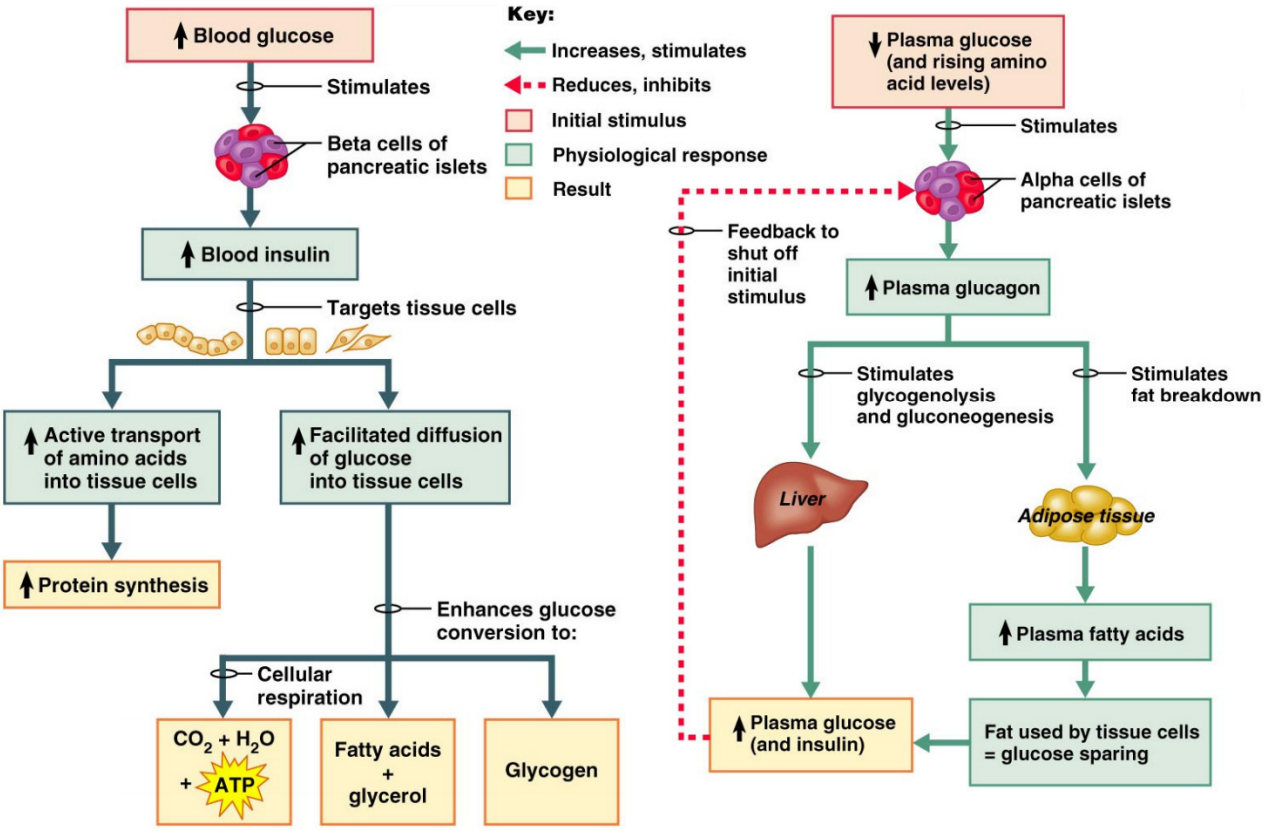


Figure 115 | **Effects of insulin and glucagon on the body.**

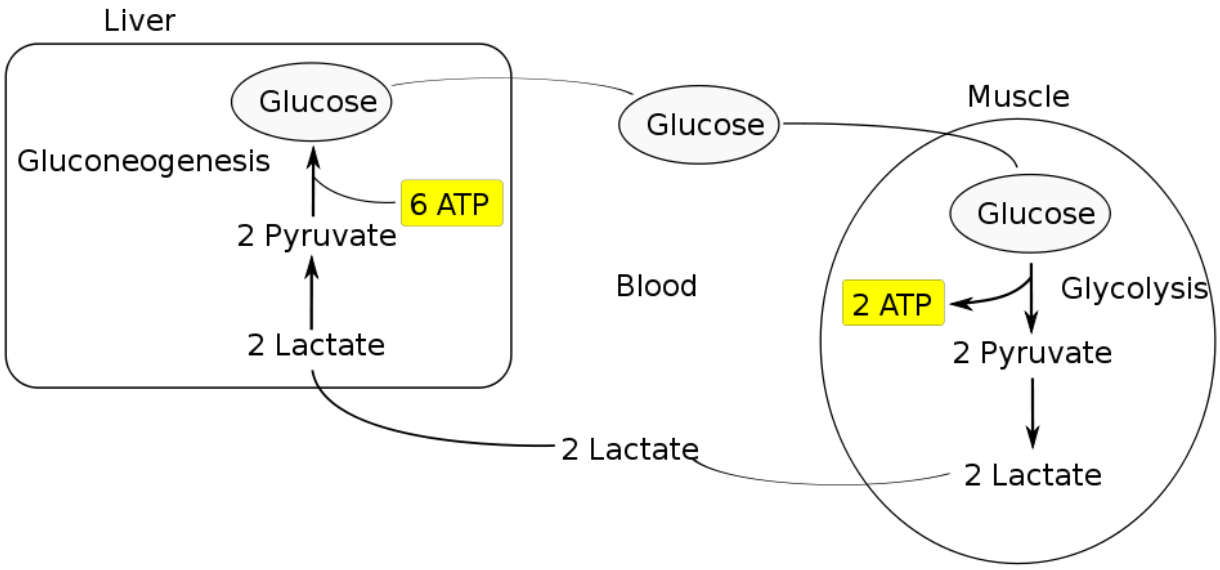


Figure 116 | **Cori cycle.**

Equations

Equation reference sheet:

Neurobiology

$$E = E_0 + \frac{2.303RT}{ZF} \log\left(\frac{[ion^+]_{outside}}{[ion^+]_{inside}}\right) \tag{6}$$

$$V_m = 58 \log\left(\frac{\sum_{i=1}^{n} P_i[i]_{out}}{\sum_{i=1}^{n} P_i[i]_{in}}\right) \tag{7}$$

Homeostasis

$$Q_{10} = \frac{R_T}{R_{T-10}} \tag{8}$$

$$MR = K(T_b – T_a) \tag{9}$$

$$M + Q_{abs} = \epsilon\sigma T_r^4 + h_c(T_r - T_a) + E + C \tag{10}$$

Diagrams

These are some diagrams I have made for specific pathways.

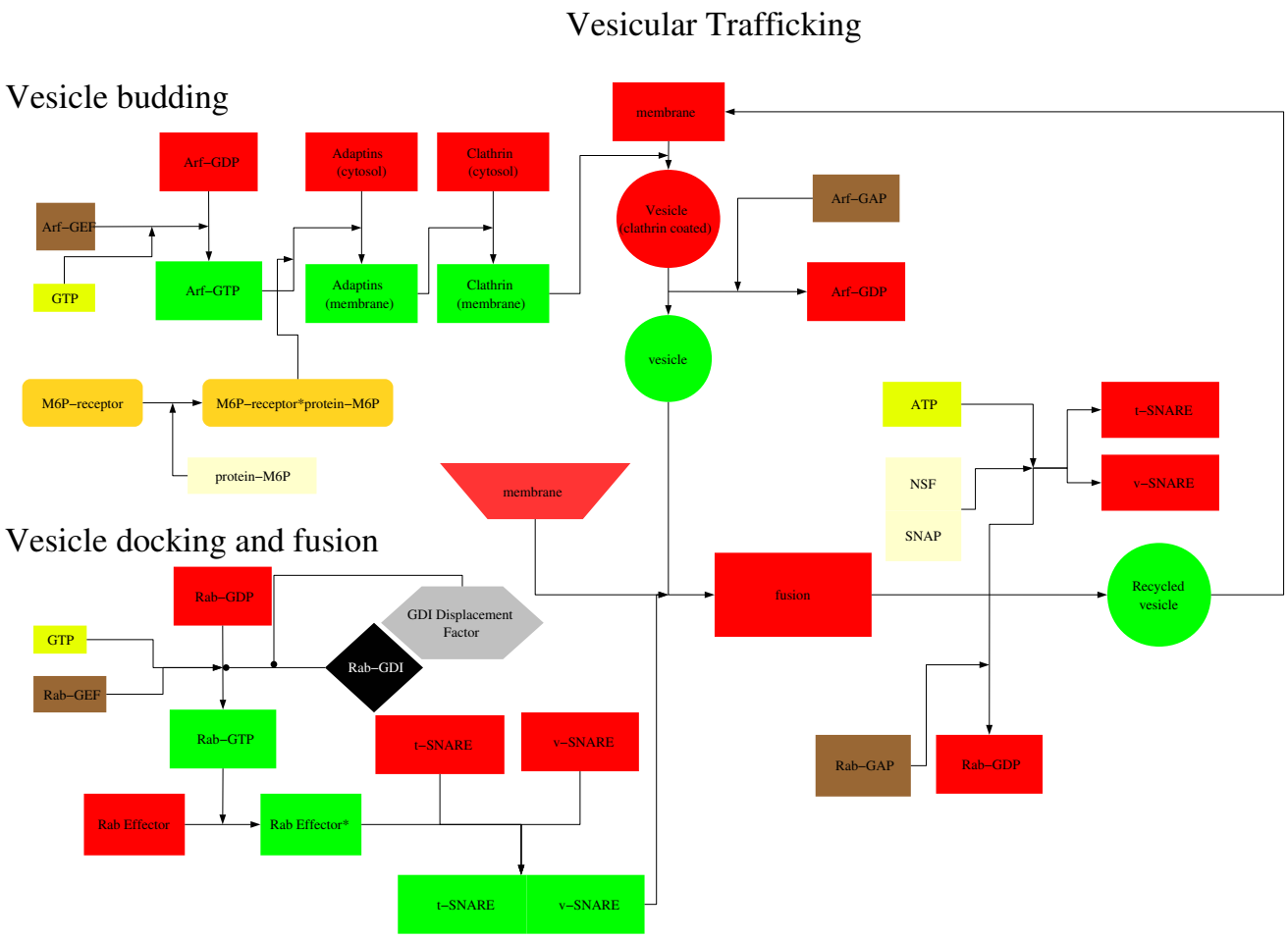
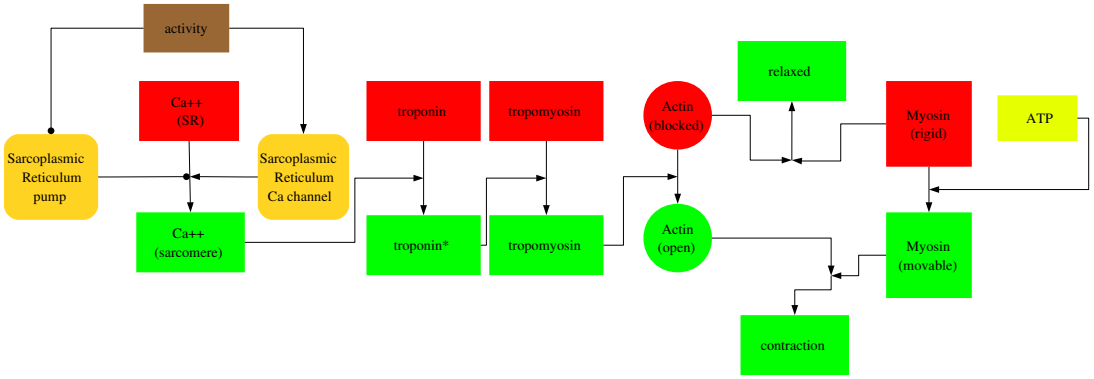


Figure 121 | Diagram: vesicle trafficking
A flow-chart view of vesicle trafficking with major components discussed in class.

Skeletal muscle contraction



Smooth muscle contraction

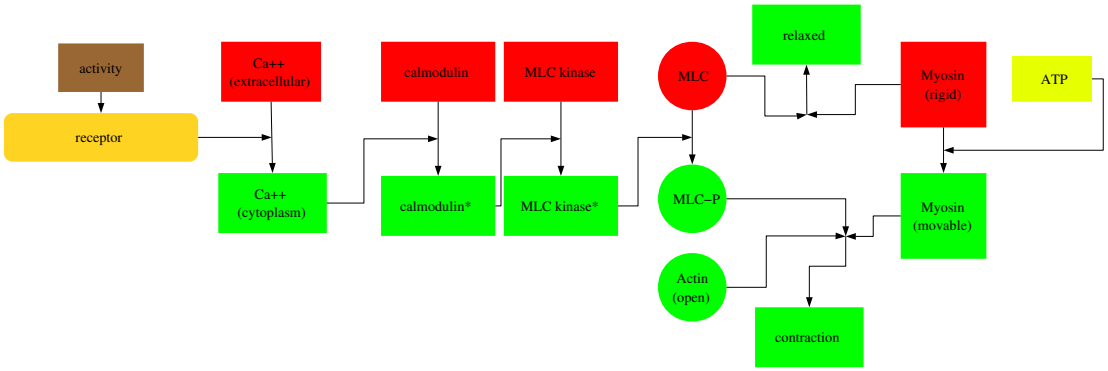


Figure 121 | Diagram: muscle contraction
Overview of muscle contraction in skeletal and smooth muscle.

Appendix

Additional information can be found here, such as amino acid table, pathway diagrams and supplementary material. References and index found at the end. A useful resource is the bio42 TA site.

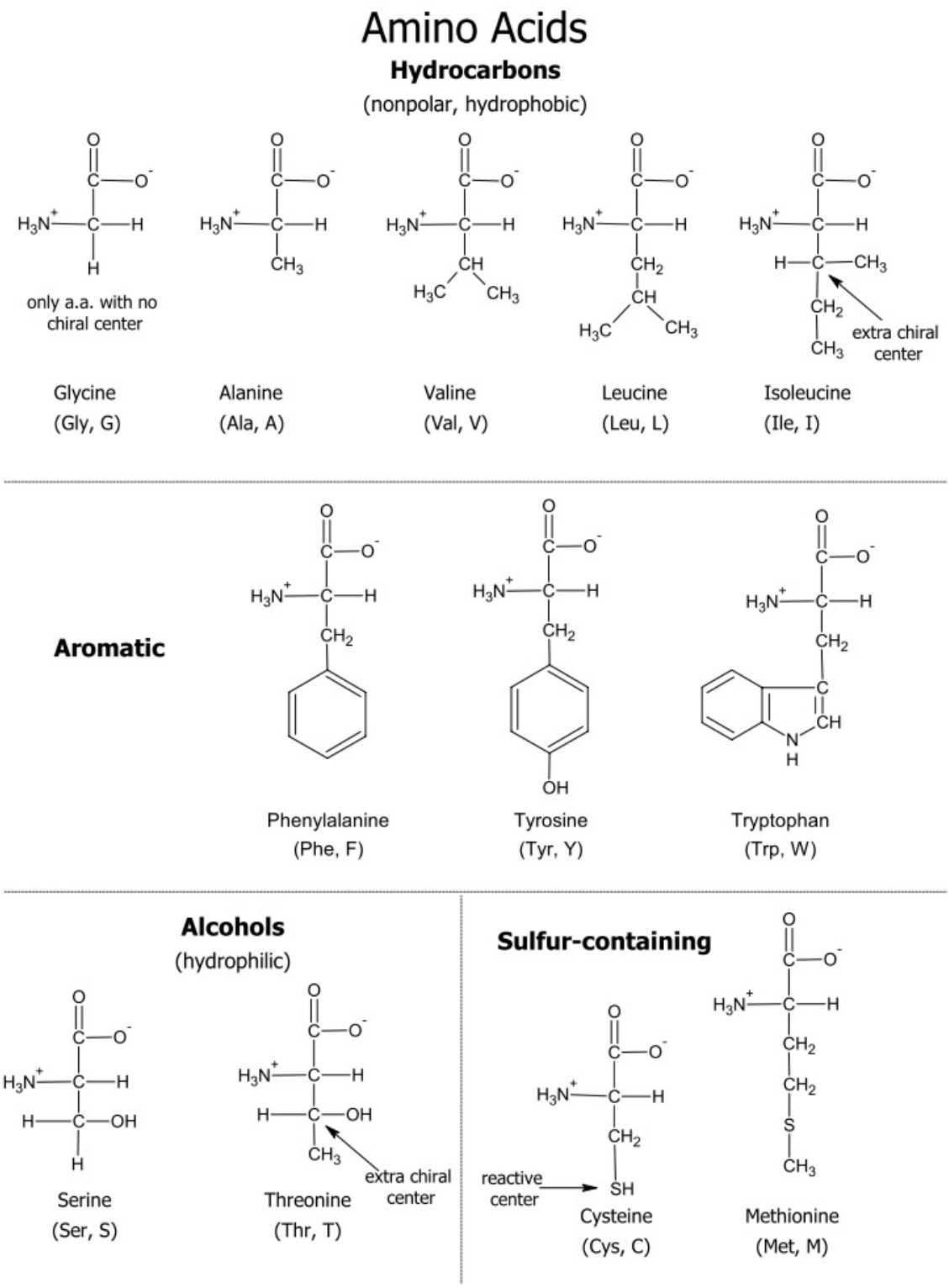


Figure 119 | Amino Acids
Memorize these.[6]

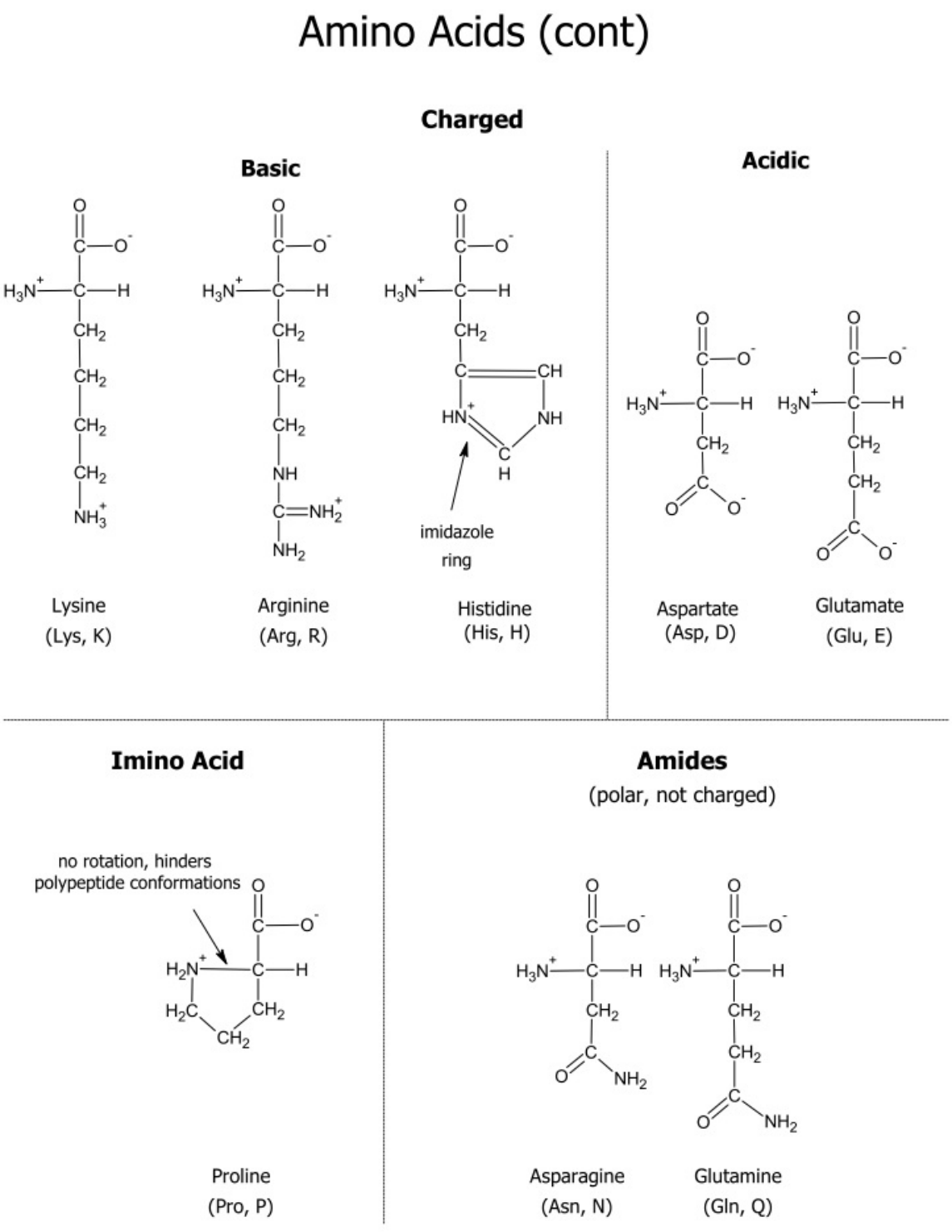


Figure 120 | Amino Acids
Memorize these.[6]

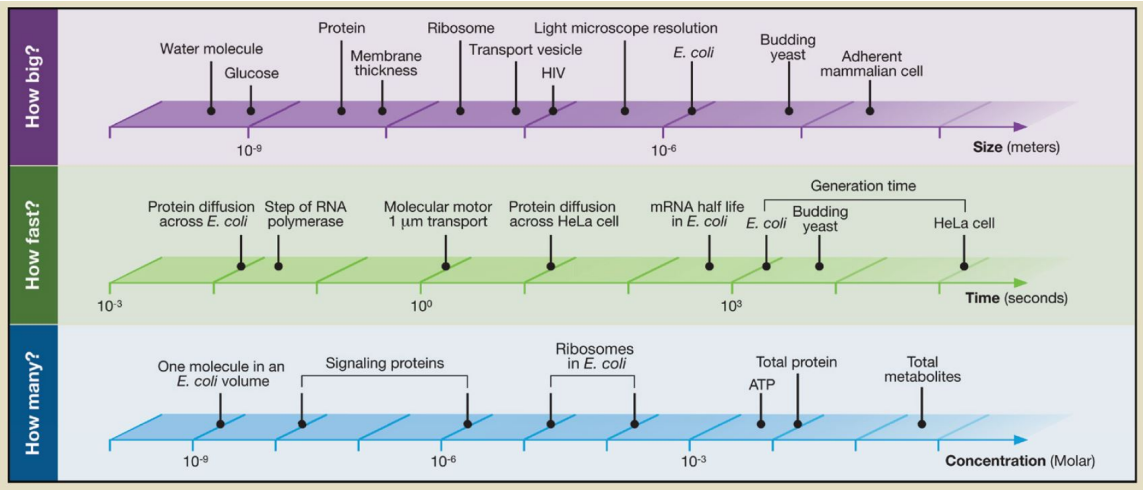


Figure 121 | Scales in biology
Useful to think about the different scales, can help you discern what is feasible.[7]

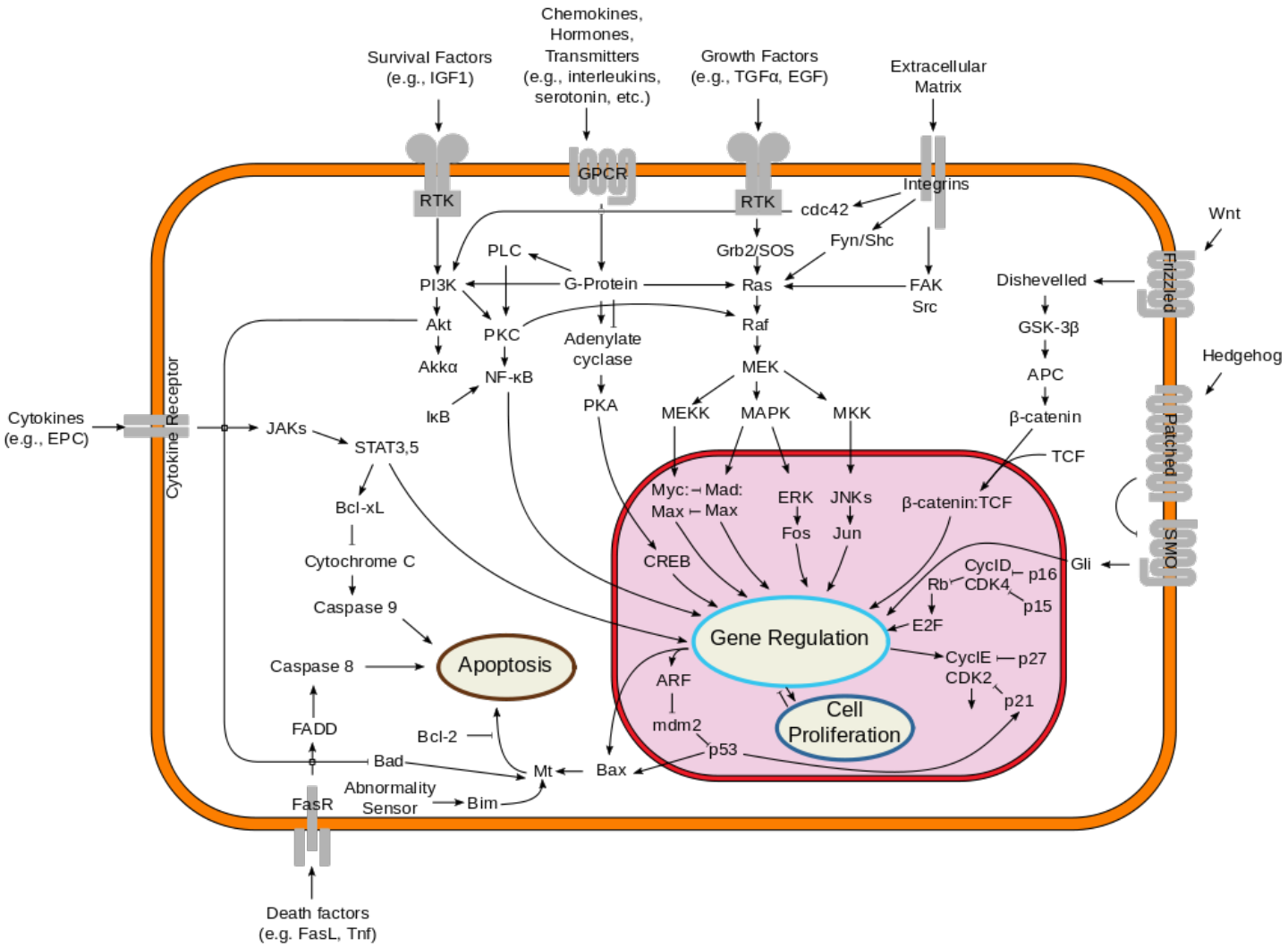


Figure 122 | Canonical signal transduction pathways.
Description forthcoming!

References

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