BIO1140 StudyFest 2011
Answer Key

1. Name the five functions of the cell membrane.

1. Define boundaries; selectively permeable barrier
2. Localisation and organisation
    - Scaffold for biochemical activities (enzymes)
    - e.g. mitochondria
3. Regulation of solute transport
    - In and out of cell and/or organelles
    - Uphill or downhill
    - e.g. Na^+,K^+-ATPase
4. Responses to external signals
    - Receptors and signal transduction
    - e.g. ß-adrenoreceptor
5. Cell-to-cell communication
    - Recognition, adhesion, exchange of materials
    - Gap junctions, plasmodesmata

2. Describe what is meant by the "Fluid Mosaic Model", and why this is applicable to the cell membrane.

Fluid mosaic model:

- Lipid-protein assembly in which components are held together in a thin sheet by non-covalent bonds
- Two fluid lipid layers – structural backbone, permeability barrier
- Mosaic of proteins – unique complement responsible for specific functions

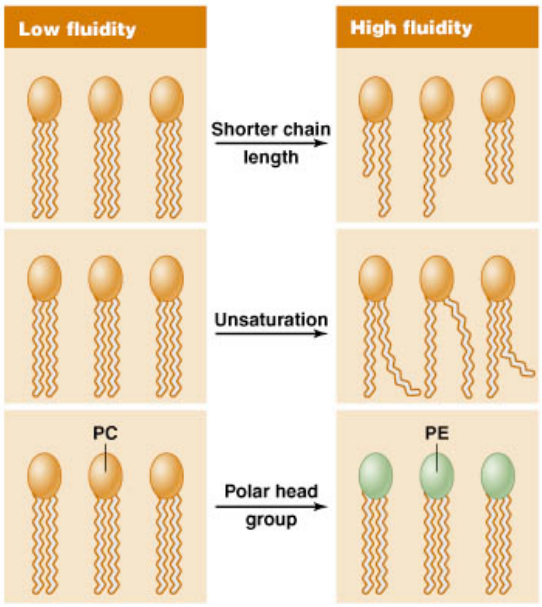
3. What are some of the key components of the "unit membrane" (Gorter and Grendel, 1925)?

Composition

- Phosphoglycerides
    - serine, choline, ethanolamine, inositol
    - 16-18 C
    - saturated & unsaturated
- Glycolipids
    - single sugars or oligosaccharides
    - marker (e.g. ABO blood groups)
- Sterols
    - Cholesterol, phytosterols
    - Absent from prokaryotes

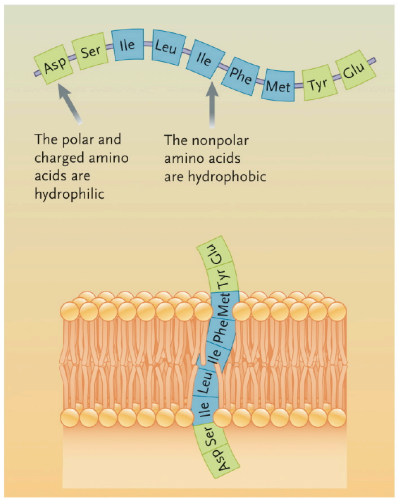
Variable

4. What features of the cell membrane allow for increased fluidity (even when temperatures are decreased)?



Membrane fluidity
- Fluid nature of membrane essential for function
- As temperature increases, fluidity increases
  - C chain length and saturation
  - Head group polarity
  - Buffering effect of sterols
- Homeoviscous adaptation
  - Alterations in lipid composition to maintain membrane fluidity at different environmental temperatures

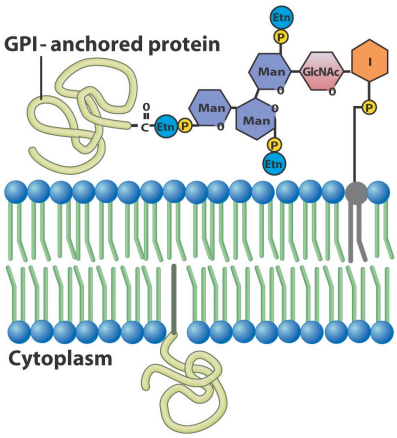
5. What are the three types of membrane proteins and how are they attached to the cell membrane?

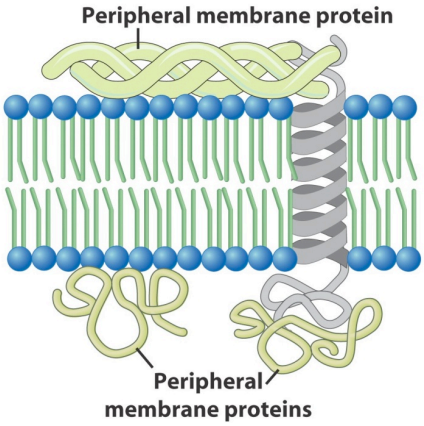


i. Integral membrane proteins
- Amphipathic with one or more hydrophobic regions
- Usually transmembrane (single or multipass)
- Transmembrane regions typically a-helix of 20-30 hydrophobic aa residues

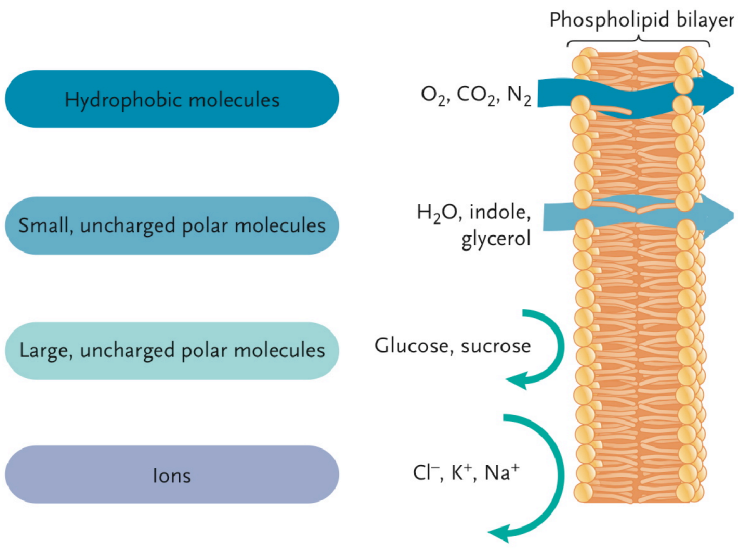
ii. Peripheral membrane proteins
- Membrane-associated through non-covalent interactions
- Dynamic relationship with membrane

iii. Lipid-anchored membrane proteins
- Membrane-associated through covalent linkages to phospholipids
- Fatty-acid anchored
  - Intracellular orientation
- GPI anchor (glycosylphosphatidylinositol)
  - extracellular orientation
  - can be cleaved with phospholipase C
    - e.g. type IV carbonic anhydrase

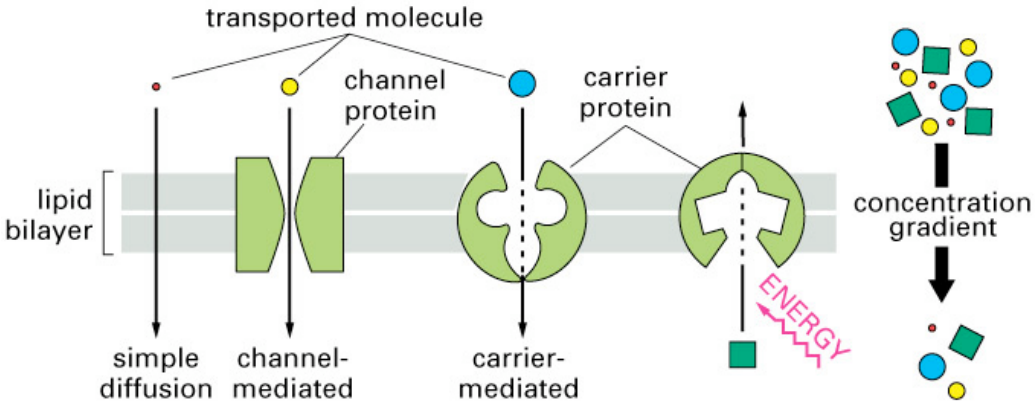




6. What kinds of molecules can get across the cell membrane easily? What kinds cannot? What are the different types of transportation that allow movement across the cell membrane?



How?
1. Passive transport
- Simple diffusion
- Facilitated diffusion
2. Active transport
- Primary
-Secondary



7. Describe how passive transport works. For simple diffusion, what gradients regulate the transport of:

i. gases
ii. ions
iii. water

Diffusion

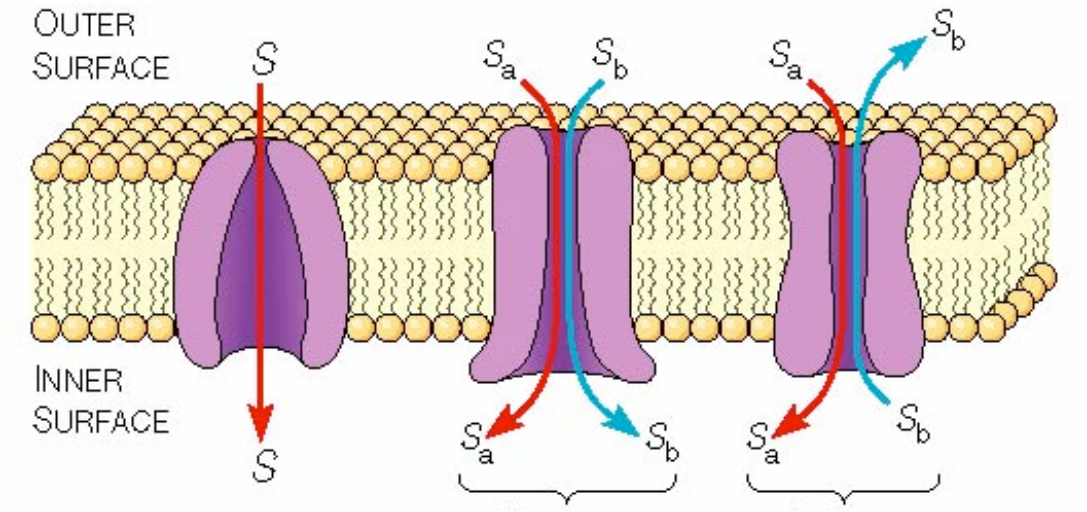
Spontaneous; based on random movements
Driven by diffusion gradient (downhill)
For solutes without a charge concentration gradient
For gases partial pressure gradient
For ions electrochemical gradient

For water osmotic gradient

Facilitated diffusion
- Channel proteins
  - Ions, water (aquaporins)
  - Highly selective
  - Leak vs gated (e.g. voltage-gated, ligand-gated)

Facilitated diffusion
- Carrier proteins
  - Ions, larger and/or polar molecules
  - Highly selective
  - Types

8. What is the difference between: an antiporter, a symporter, and a uniporter?



9. What is the difference between primary and secondary active transport? What is the difference between p-type and v-type primary transport?

Primary active transport
- ATP hydrolysis directly coupled to transport of solute
- Several types
  - P-type
    - Move cations (Na^+, K^+, Ca^{2+}, H^+)
    - found in plasma membrane
    - Reversibly phosphorylated
    - e.g. Na^+,K^+-ATPase

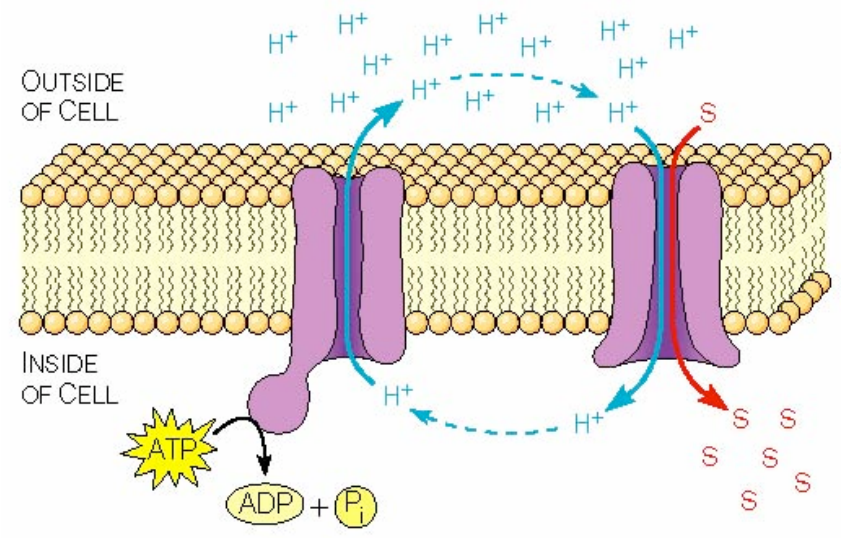
V-type – move H^+ into organelles

Secondary active transport

Simultaneous transport of two solutes. Downhill transport along gradient established by 1° active transport allows uphill transport of second solute.

Na^+ gradient in animal cells

H^+ gradient in plant cells



The Cytoskeleton

1. What are some of the important functions of the cytoskeleton?

Functions include:

The provision of structure and support
Intracellular transport
The positioning of organelles within the cell
The generation of force for cell movement
Contributing to cell division

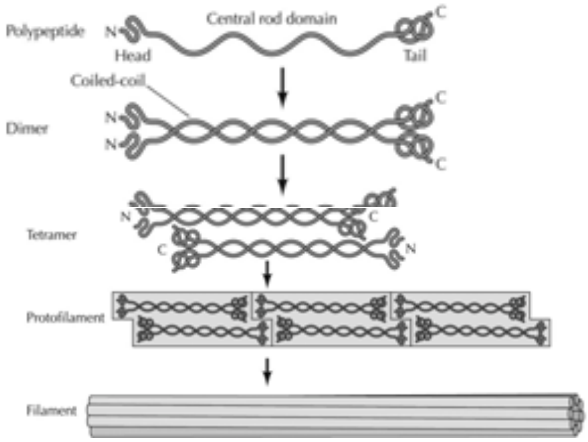
Types: Intermediate Filaments, Microfilaments, and Microtubules.

IF: Mechanical strength in animal cells
MF: Shape/support and motility (of cell and within cell)
All eukaryotic cells

Shape/support
Cell cortex networks
Microvilli bundles
Adherens junctions

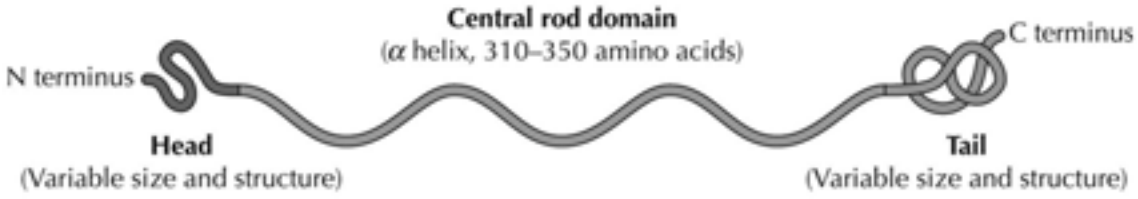
MT: mitosis (aid in orientation of spindles during cell division); also, help move things like vesicles (conveyor belts)

2. Describe the structure of an IF. What is the difference between:
i. Dimer
ii. Tetramer
iii. Protofilament
iv. Filament



Structure:
- *α*-helical rod domain of about 310-350 aa
- Globular Head and Tail domains, variable size
- Head and tail domains determine the specific functions

Assembly
- Dimer: central rod form coiled coil
- Tetramer: staggered tetramers assemble end to end
- Filament: 8 interwound protofilament



3. What is the name of the proteins that control the assembly/dissembly of actin filaments? Give some examples, and their specific roles.

Tertiary structures of Actin:
-globular (**G-actin**), 375 aa (43 kD), barbed and pointed ends, binds head-tail to nucleate a trimer
- Filamentous (**F-actin**): monomers added to both end

Actin is “polar” because:
- Polymerization is reversible

• The rate at which monomers are added to filaments is proportional to their concentration
• ATP bound actin binds to barbed end with high affinity
• ADP-actin has low affinity to the pointed ends
• when ATP hydrolyses to ADP
• ADP-actin dissociates from filaments more readily than ATP-actin
• Therefore, the critical concentration of actin monomers is higher for addition to the pointed end than to the barbed end of actin filaments

Treadmilling:
• At cellular actin concentrations
• Barbed end of a filament grows 5–10 times faster than the pointed end
• ADP-actin dissociates from pointed end
• Exchange of ATP for ADP added to barbed-end
• Process is called **Treadmilling**
• Dynamic growth
• Direction?
Pointed to Barbed

4. What is the name of the proteins that control the assembly/dissembly of actin filaments? Give some examples, and their specific roles.

Actin Binding Proteins (ABP): modulate the Assembly and disassembly of actin filaments

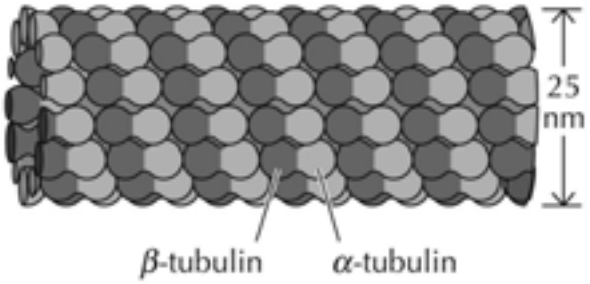
Function	Protein Name
Filament initiation and polymerization	Arp2/3, formin
Filament stabilization	Nebulin, tropomyosin
Filament cross-linking	α-actinin, filamin, fimbrin, villin
End capping	CapZ, tropomodulin
Filament severing/depolymerization	ADF/cofilin, gelsolin, thymosin
Monomer binding	Profilin, twinfilin
Actin filament linkage to other proteins	Dystrophin, spectrin, talin, vinculin

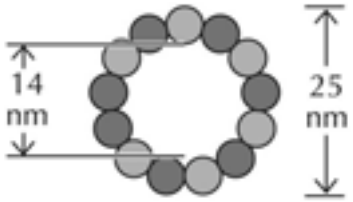
• Some actin-binding proteins bind along the length of actin filaments, stabilizing them or cross-linking them to one another
• Others stablize by capping the ends and preventing dissociation
• Others promote dissociation, while others regulate the exchange of ATP for ADP.

5. **Myosin** is a **molecular motor:** converts chemical energy (ATP) to mechanical energy to force and movement.
Myosin II; Involved with muscle contraction and cell division.
• **Myosin I:** much smaller than myosin II,contains a globular head group, acts as a molecular motor. INVOLVED WITH VESICULAR TRANSPORT.
• Short tails bind to other structures
• Movement of myosin I along actin filament
• transport cargo, such as a vesicle

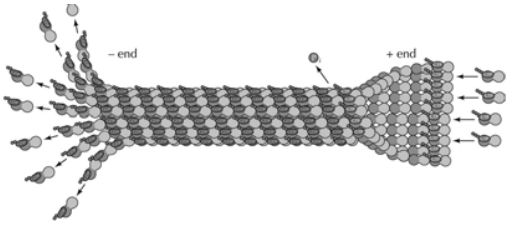
6. • **Intermediate filaments** (8-11 nm)
• not directly involved in cell movements
• provide mechanical strength
• Supporting scaffold: for organelles and cytoskeleton
• Diverse fibrous proteins
• Size: 50-200 kD
• Not dynamic
• Not polar
• Regulated by phosphorylation
• Six types I-VI

7. • **Microtubules** are rigid hollow rods (25 nm)
• Dynamic structures, undergo continual assembly and disassembly
• Function: cell movements and determining cell shape, organelle transport, mitosis
• Tubulin, globular protein is the monomer
• *α*-tubulin and *β*-tubulin dimers make up microtubules
• *γ*-tubulin in the centrosome plays a critical role in initiating microtubule assembly





8. • Tubulin dimers polymerize to form microtubules
• consisting of 13 protofilaments assembled around a hollow core
• Protofilaments composed of head-to-tail arrays of tubulin dimers arranged in parallel
• two distinct ends: a fastgrowing + end and a slowgrowing minus end



Treadmilling:

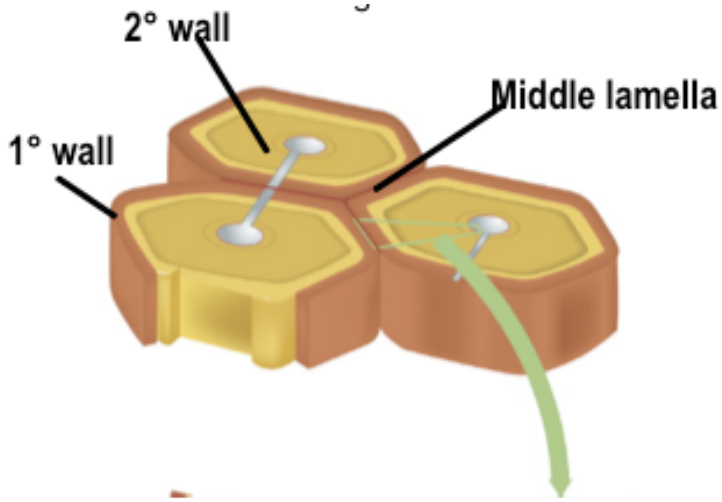
• Microtubules can undergo **treadmilling**

• Tubulin dimers with GTP bound to β-tubulin associate with the growing end
• GTP is hydrolyzed , tubulin gets less stable, minus end
dimers disassociate

Extracellular Interactions

1. What is the purpose of the plant cell wall and what are the three classes of molecules that are found within it?

Purpose:
Provides rigidity and protection, regulates permeability
Structure involves 3 classes of molecules
- Structural fibres – cellulose microfibrils
- Matrix – polysaccharides (e.g. hemicellulose, pectins)
- Adhesive molecules – pectins



2. What is the difference between primary and secondary plant cell walls?

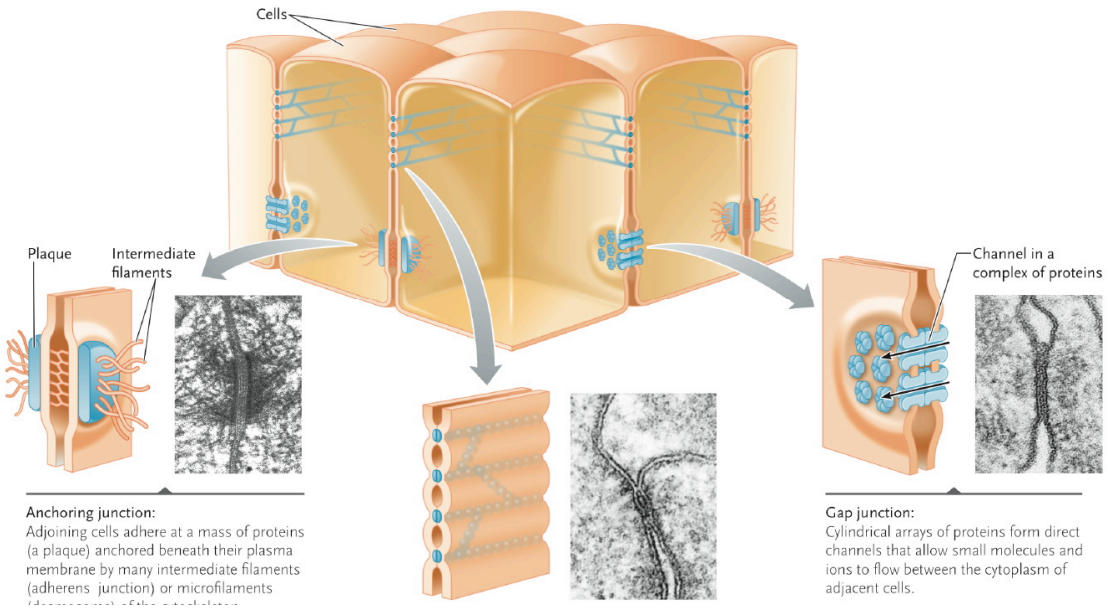
Primary vs secondary cell wall
Middle lamella (pectins)
Primary cell wall – loose network of cellulose fibrils
Secondary cell wall – cellulose fibrils highly organized
- Multi-layered
- Lignin

3. There are three main types of methods for cell-to-cell communication in animal cells. What are they called?

Adhesive (anchoring) junctions: adherens junction, desmosome

Tight junctions

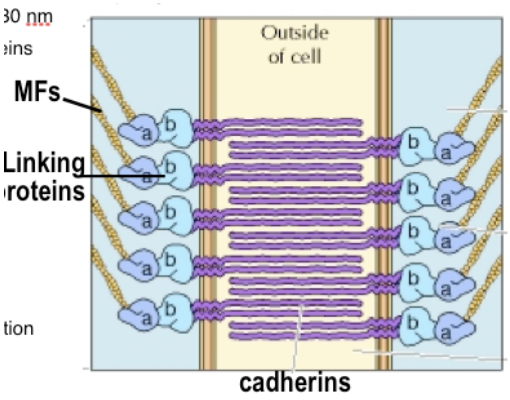
Gap junctions



4. Describe the structure of an adhesive junction, and draw a picture.

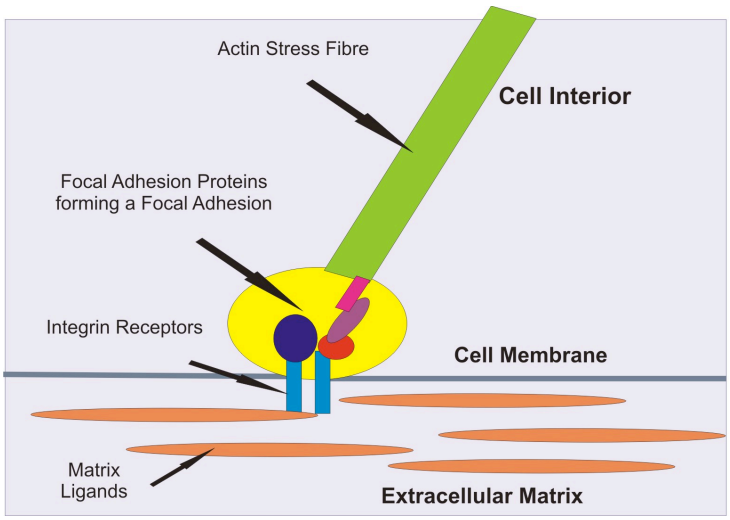
Adhesive (anchoring) junctions

- Adherens junctions
  - Connect to MFs
  - Small areas or continuous zones of attachment in epithelia
- Desmosomes
  - Connect to IFs
  - Protein plaque for strong adhesion (skin, cardiac muscle, cervix of uterus)
- Cadherins
  - Transmembrane glycoprotein
  - Repeats in extracellular domain “zip” together
  - Cell-cell separation ~30 nm
- Intracellular linking proteins



Two types of cell-matrix junction

• **Focal adhesions**: bundles of actin filaments are anchored to *β* subunits of integrins via
– ***α*-actinin**
– **Vinculin via talin**
• Assembly of focal adhesions
• Focal complex: small group of integrins
• Recruite Talin, Vinculin, *α*-actinin and Formin
• Formin initiates actin bundles
• FOCAL ADHESIONS ARE REVERSIBLE
• Integrins can reversibly bind matrix components
• change conformation between active and inactive states
• Inactive state: integrin heads turned close to cell surface
• Cell signaling extends heads to matrix
• Migrating cells: focal adhesions form at the leading edge



• **Hemidesmosomes** anchor epithelial cells to the basal lamina
• α6β4 integrins bind to lamins
• long cytoplasmic tail of β subunit binds to intermediate filaments via
• Plectin and BP230 and BP180 (similar to transmembrane collagens)

5. Describe the functions of a tight junction, and draw a picture.

Tight junctions
Permeability barrier in epithelia
e.g. intestine, bladder, skin, gill
Barrier to lateral diffusion in plasma membrane
Polarized cell

Structure
Ridges formed of junctional proteins claudins, occludin

6. What tissues are gap junctions predominantly found in, and why? What are connexins/connexons?

Gap junctions
Cytoplasmic contact
Electrical and chemical communication
Muscle and nerve tissue
e.g. Coordination of heart beat

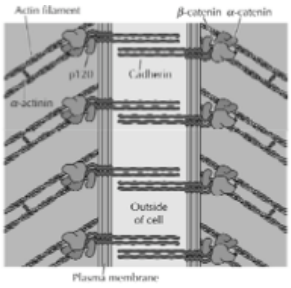
Structure
- Cell-cell separation 3 nm
- Connexin – integral membrane protein
  - 6 form linking structure

7. What are integrins?

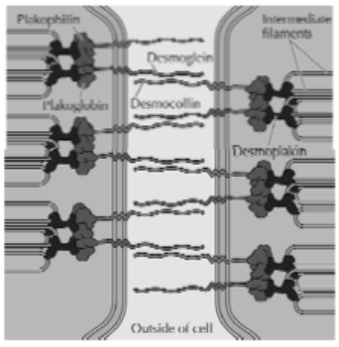
Integrins
- Heterodimer (a and ß chains)
- Integrate ECM with cytoskeleton
  - Extracellular binding site for adhesive glycoproteins
  - Intracellular binding sites for linking proteins
- Focal adhesions and hemidesmosomes

- Integrins are receptors that mediate attachment between a cell and the tissues surrounding it, which may be other cells or the extracellular matrix (ECM). They also play a role in cell signaling and thereby regulate cellular shape, motility, and the cell cycle.

- Typically, receptors inform a cell of the molecules in its environment and the cell responds. Not only do integrins perform this outside-in signalling, but they also operate an inside-out mode. Thus, they transduce information from the ECM to the cell as well as reveal the status of the cell to the outside, allowing rapid and flexible responses to changes in the environment, for example to allow blood coagulation by platelets.



- Integrins work alongside other proteins such as cadherins, Immunoglobulin superfamily cell adhesion molecules, selectins and syndecans to mediate cell-cell and cell-matrix interaction and communication. Integrins bind cell surface and ECM components such as fibronectin, vitronectin, collagen, and laminin.

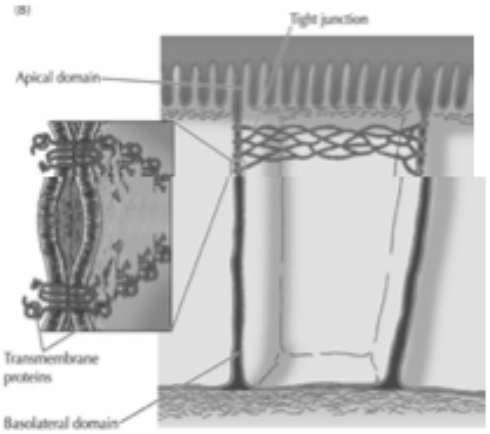


ADDITIONAL INFO:

Two types of cell-matrix junction
• **Focal adhesions**: bundles of actin filaments are anchored to β subunits of integrins via

– ***α*-actinin**
– **Vinculin via talin**

Focal adhesions serve as the mechanical linkages to the ECM, and as a biochemical signaling hub to concentrate and direct numerous signaling proteins at sites of integrin binding and clustering.



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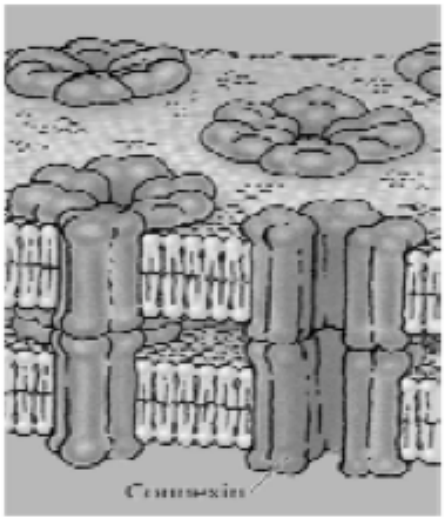

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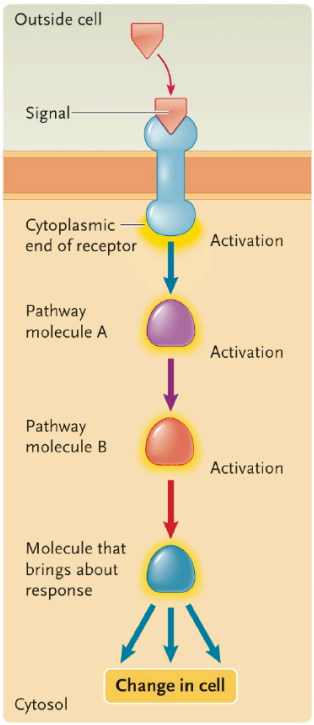
i. Adherens Junctions:
• **Cadherin** form stable cell-cell connections involving actin filaments
• Also include *β*-catenin, p120, and *α*-catenin,
• *β*-catenin and p120 bind to **cadherin** and help maintain stability
• *β*-catenin binds *α*-catenin that interacts with actin filament of cytoskeleton

ii. Desmosomes:
• link the intermediate filament of adjacent cells
• **Desmoglein** and **desmocollin** (transmembrane cadherins) bind by heterophilic interactions across the junction

• Plakoglobin and plakophilin bind to the cadherins and link to the intermediate filament binding protein, desmoplakin

iii. Tight Junctions:
Tight junctions provide minimal adhesive strength between the cells, usually associated with adherens junctions and desmosomes in a **junctional complex**
• **Tight junctions** in epithelial cell form a seal that prevents free passage of molecules and ions between cells
• separate apical and basolateral domains of the plasma membrane
• prevent free diffusion of lipids and membrane proteins
• transmembrane proteins, occludin, claudin, and junctional adhesion molecule (JAM), anchored on F-actin
• Bind similar proteins on the adjacent cell
• Sealing the space between cells



iv. Gap Junctions:
• open channels through the plasma membrane
• allowing ions and small molecules to diffuse freely
• Proteins and nucleic acids can not pass through• heart muscle cells, passage of ions through gap junctions synchronizes the contractions of neighboring cells
• allow passage of some signaling molecules, such as cAMP and Ca2+, coordinating responses of cells in tissues
• Gap junctions are made of transmembrane proteins in the **connexin** family.
• 6 connexins form a cylinder with an open aqueous pore in its center, called a **connexon**
• Connexons in the plasma membrane adjacent cells align
• form open channels between the two cytoplasms
• Specialized gap junctions occur on specific nerve cells and form an **electrical synapse**
• Individual connexons can be opened or closed. When open, they allow rapid passage of ions between the two nerve cells

Cell-to-cell Signalling

1. Describe the various elements of the cell-signaling system.

i. Signaling molecule (first messenger)

ii. Reception
Receptor – membrane, cytosolic or nuclear

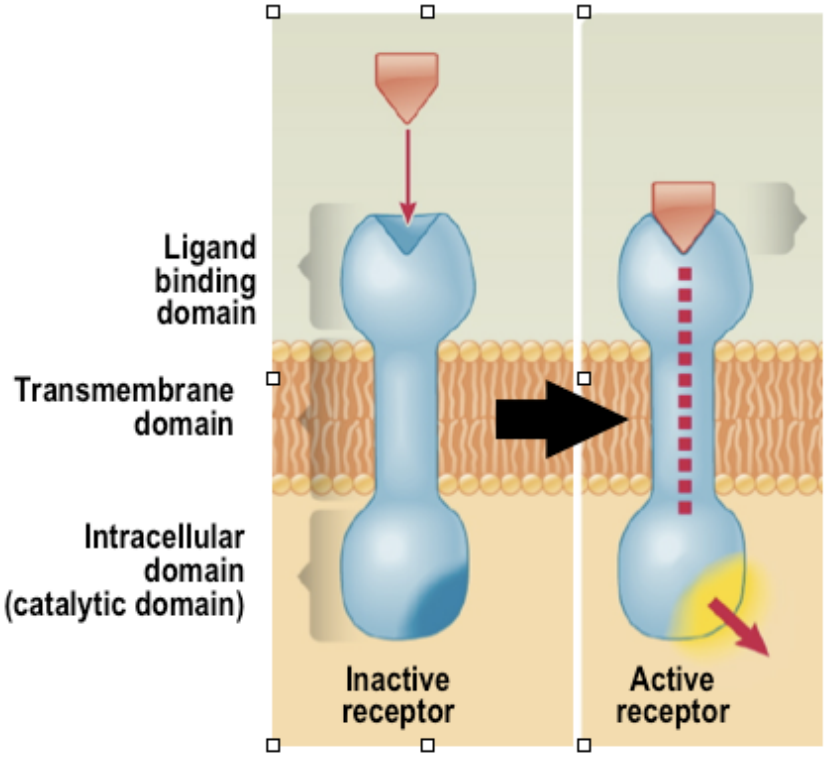
iii. Transduction

Signalling cascades and second messengers

iv. Response
Changes in gene expression
Changes in activities of enzymes, transcription factors or other regulatory proteins, transporters, cytoskeleton etc

v. Termination

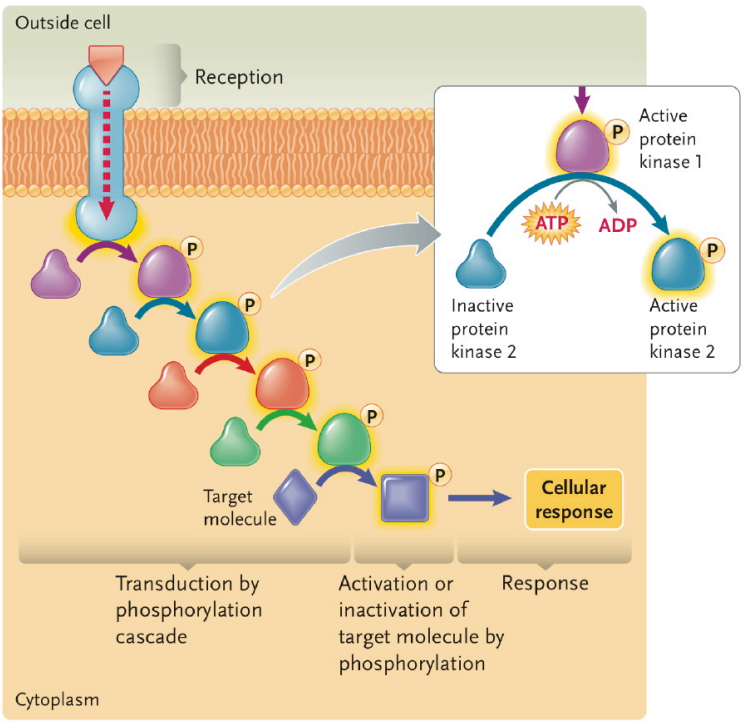
2. Describe the structure of membrane receptor signaling systems (i.e. the different domains, the different types of membrane receptors, etc.)



Signals are water-soluble chemicals
Signalling molecule does not enter cell
Signal removal
e.g. environmental chemicals, neurotransmitters, hormones

Membrane receptors are integral membrane glycoproteins
Ligand-gated channels
Receptor tyrosine kinases
G protein-coupled receptors

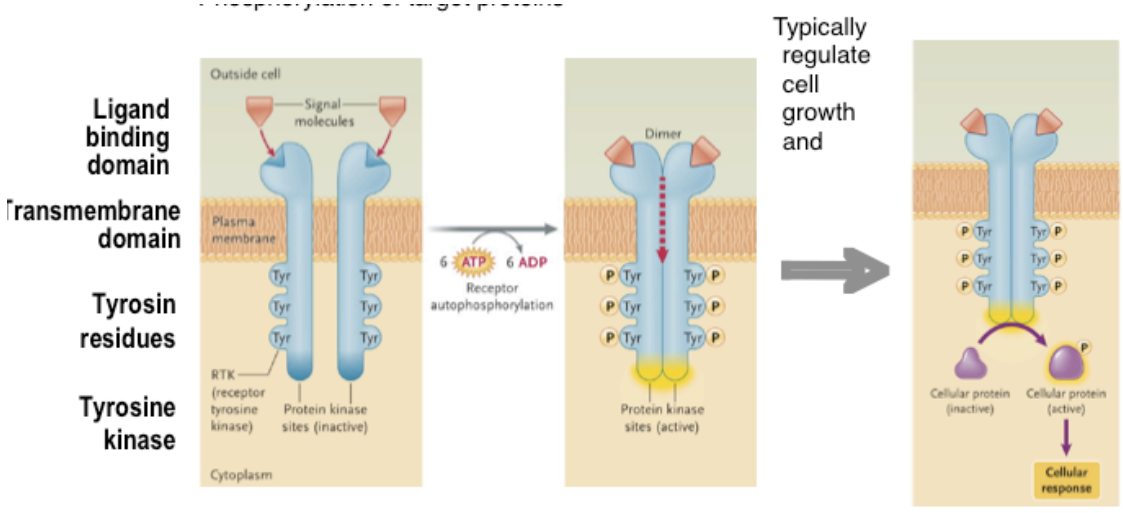
3. What happens in signaling cascades?



Signalling cascades
- Role of phosphorylation: kinases vs phosphatases (Kinase phosphorylates, phosphatase dephosphorylates)
- Amplification
- Termination
  - Removal of ligand-receptor complex
  - Inactivation of receptor
  - Inactivation of signalling cascade

4. Describe what happens in receptor tyrosine kinases, and draw a picture.

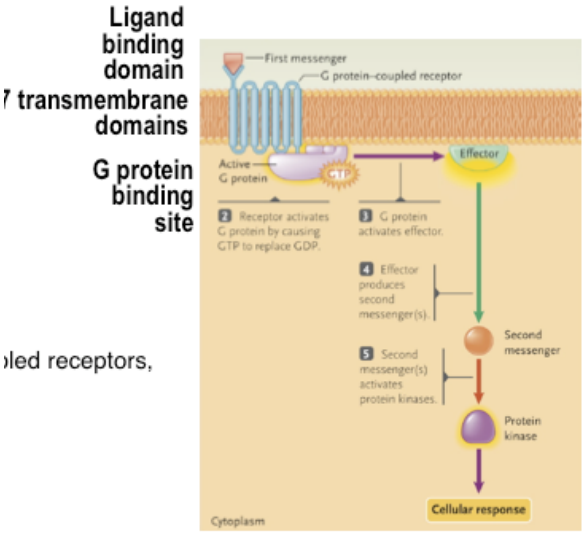
Receptor tyrosine kinases
- Dimer formation
- Activation of cytoplasmic protein kinase domain
- Autophosphorylation (tyrosine residues)
- Phosphorylation of target proteins
- Typically regulate cell growth and proliferation



5. Describe what happens in G-protein coupled receptors, and draw a picture.

G protein-coupled receptors
- Activate guanine nucleotide-binding proteins (G protein)
- G protein activates enzyme to produce 2nd messenger

2nd messenger activates
protein kinases



6. Fill in the blanks:

i. G proteins are molecular switches; when GTP is bound, they are on, when it is not bound, it is off.

ii. G_α binds GDP and GTP.

iii. G_β and G_γ form a unit.

DNA, RNA Structure and Function

1. Fill in the blanks:

i. A base is a purine or a pyridimine.
ii. A base + a pentose sugar = a nucleoside.
iii. A nucleoside + a phosphate = deoxynucleoside.

2. What is the role of histones in DNA organization? How does DNA packaging differ between bacteria, viruses and eukaryotes?

Histones pack eukaryotic DNA at successive levels of organization i.e. hierarchical organization

Viruses:

Use basic proteins that may be part of the virus particle (capsid).

Bacteria:

Use basic proteins such as “HU”, “IHF”, “H-NS” to form a series of loops called a nucleoid.

Eukaryotes:

Use basic proteins called histones AND a hierarchical organization

3. Describe the bacterial chromosome and the eukaryotic chromosome, and how they differ.

Bacterial Chromosome:

Closed, circular molecule of DNA packed into nucleoid

Replication begins from a single origin, proceeds in both directions

Plasmids (in many bacteria) replicate independently of the host chromosome

Eukaryotic Chromosomes:

Consist of DNA complexed with histone and nonhistone proteins, called chromatin.

DNA wraps around a nucleosome (two molecules each of histones H2A, H2B, H3, H4)
Linker DNA connects adjacent nucleosomes
Binding of histone H1 causes nucleosomes to package into a coiled structure (solenoid)

Nonhistone proteins help control the expression of individual genes

4. What are the two types of chromatin? How are they different? During nuclear division, what do they fold and pack to form?

Distributed between:

Euchromatin (loosely packed region, genes active in RNA transcription)

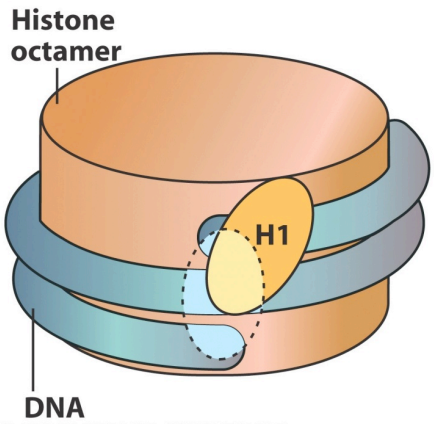
Heterochromatin (densely packed masses, genes are inactive)

Folds and packs to form thick, rodlike chromosomes during nuclear division

5. What are nucleosomes?

Nucleosomes are the basic unit of DNA packaging in eukaryotes (cells with a nucleus), consisting of a segment of DNA wound around a histone protein core. This structure is often compared to thread wrapped around a spool.

Nucleosomes form the fundamental repeating units of eukaryotic chromatin, which is used to pack the large eukaryotic genomes into the nucleus while still ensuring appropriate access to it.



DNA wraps around a nucleosome (two molecules each of histones H2A, H2B, H3, H4)
Linker DNA connects adjacent nucleosomes
Binding of histone H1 causes nucleosomes to package into a coiled structure (solenoid)

Nucleosome parameters
2 each of 4 histones named H2A, H2B, H3 and H4 (the “octomer”)
DNA (~147bp) is wrapped around the outside
adding histone H1 brings nucleosomes together to form the 10nm fibre

sequences that are distant can now be closer together!!!

6. Describe the 'Histone Code' and its' consequences.

Euchromatin:

Higher histone acetylation
Lower histone methlylation
Lower DNA methylation
Active genes

Heterochromatin:

Lower histone acetylation
Higher histone methlylation
Higher DNA methylation
Inactive genes

7. What is the purpose of chromatin remodelling?

Eukaryotic DNA wraps around histones, to form nucleosomes
Promoters inaccessible

Chromatin remodeling makes gene promoters more accessible
Activators recruit remodeling complexes that displace nucleosomes
Activators recruit enzymes that acetylate and loosen histone assocation with DNA
Other modifications can include methylation of Lys, methylation of Arg and His, phosphorylation of Ser and His.

These modifications affect the "tails" (amino termini)

Chromatin remodeling can convert accessible to inaccessible
Remodeling in influenced by alterations of the cell physiology
Remodeling influences the expression of many genes, thus the "transcriptome"

Replication

1. i. The DNA structure is antiparallel. DNA strands go from 5' to 3'.
ii. Synthesis follows the base-pairing rules, A-T, G-C.
iii. One new DNA strand is synthesized continuously; the other, discontinuously
iv. DNA polymerases are the primary enzymes of DNA replication.
v. DNA replication begins at replication origins
vi.RNA primers provide the starting point for DNA polymerase to begin synthesizing a new DNA chain

2. Helicase unwinds the DNA
- Primase synthesizes RNA primer (starting point for nucleotide assembly by DNA polymerases)
- DNA polymerases assemble nucleotides into a chain, remove primers, and fill resulting gaps
- DNA ligase closes remaining single-chain nicks

3. Leading strand=continuous
Lagging strand=discontinuous

-As DNA helix unwinds, one template strand runs in a direction allowing new DNA strand to be made continuously in the direction of unwinding
- Other template strand is copied in short lengths that run in the direction opposite to unwinding
- Discontinuous replication produces short lengths, then linked into a continuous strand

4. Q? Ends of eukaryotic chromosomes. What happens to them?
- Short sequences repeated hundreds to thousands of times (Humans have (TTAGGG)n)
- Repeats protect against chromosome shortening during replication
- Chromosome shortening is prevented in some cell types which have a telomerase enzyme (adds telomere repeats to chromosome ends)

5. Mechanisms That Correct Errors

- Errors inevitably occur, during replication or caused by DNA damage
- Proofreading depends on the ability of DNA polymerases to reverse and remove mismatched bases
- DNA repair corrects errors that escape proofreading or caused by DNA damaging agents

Proofreading by DNA Polymerase:
-If a replication error causes a base to be mispaired, DNA polymerase reverses and removes the most recently added bases.
-The enzyme then resumes DNA synthesis in the forward direction

DNA polymerase enzymes
- Recognize distorted regions caused by mispaired base pairs
- Remove DNA section with mispaired base from the newly synthesized nucleotide chain
- Resynthesize the section correctly, using original template chain as a guide

Transcription

6. Transcription:

-sequence in DNA is copied into a complementary RNA
-template strand of DNA is used to create mRNA or rRNA, etc

Translation:
-sequence mRNA specifies amino acid sequence in polypeptide according to a code
-ribosome assembles the amino acid sequence

7. Why are there multiple RNA Polymerases?
Different RNA polymerases function in different locations. Within the nucleus different RNApol transcribe different sets of genes. In order to recognize the correct gene to transcribe, RNApols must recognize different signals in the DNA.
- RNA polymerase I transcribes rRNA in the nucleolus (part of the nucleus)
- RNA polymerase II transcribes mRNA and most snRNAs
- RNA polymerase III transcribes tRNA, 5S rRNA, some snRNAs and scRNAs

8. Promoter: Control sequence initiates transcription
- Transcription unit: Portion of gene that is copied into RNA
- Terminator: Signals the end of transcription of a gene

9. Overview of transcription:
- Begins as RNA polymerase binds to DNA
- DNA double helix begins to unwind
- RNA polymerase adds RNA nucleotides sequentially according to DNA template
- Enzyme and completed RNA transcript release from DNA template

RNA polymerase:
- catalyses the polymerization of ribonucleoside 5'-triphosphates(rNTP or NTP)
- does not require a primer, transcription is initiated de novo.
- initiates synthesis at 'promoter' sequences on DNA, upstream (5') to the transcription start site. Other sequences such as enhancers can affect transcription.

- adds NTPs at 3' end of new polynucleotide until it encounters a termination signal at defined sequence and/or structure on the DNA template.
- the "pre-RNA" is "processed" into the mature RNA. In eucaryotes mRNA is transported to the cytoplasm.

10. 1. RNA cleavage:
In many species rRNA genes are transcribed as one long precursor containing several genes and intergenic regions. This precursor is is cleaved into the mature rRNAs while the intergenic regions are discarded
- 45S rRNA precursor 28S rRNA + 18S rRNA +5.8S rRNA
- 5' end of some tRNAs is removed by RNase P

2. RNA addition:
After synthesis, the non-templated addition of extra
ribonucleotides (CCA) to some tRNAs.
3. RNA Splicing:
Many mRNAs in eukaryotes (and a few in procaryotes) have an
intron/exon structure. Removal of introns is necessary prior to translation.
(Actually some non-coding RNAs have introns too!)
Introns: Non-protein-coding sequences in the pre-mRNA
Exons: Amino acid coding sequences in pre-mRNA
A complex, the "Spliceosome", carries out the splicing reactions.

3. RNA Splicing:
If a gene has several introns, in some cases "alternative splicing" will
results in different versions of mRNA can be produced.
- In these mRNAs exons are joined in different combinations that can
be translated into different proteins with different functions.
- More information can be stored in the DNA!

4. mRNA Capping:
A cap attached to the 5´ end of the
mRNA results in more efficient translation.
(don't memorize the structure, just call it
a 7MeG cap).

5. Polyadenylation:
3' to the signal AAUAAA, polyA polymerase adds ~50-250 adenine
nucleotides to form the "Poly(A) tail"
Proteins bind to the "tail" and protect mRNA from RNA-digesting enzymes
6. Nucleoside modification

7. RNA Editing:
Specific bases in the RNA are changed. Some examples are
- a C in the RNA becomes a uracil (U) (cytidine deaminase)
this can change a codon if in the open reading frame (ORF)
- an A in the RNA becomes an inosine (I): (adenosine deaminase)
the ribosome translates I as a G thus changing a codon in the ORF.
These changes can lead to changes in the protein and its function, or splicing
or may have no none function at all!

Translation

11. i. tRNAs are small RNAs of a highly distinctive structure that
<u>bring amino acids to the ribosome.</u>
ii. <u>Ribosomes</u> are rRNA-protein complexes that work as automated protein assembly
machines

iii. Translation initiation brings the ribosomal subunits, an mRNA, and the first aminoacyl-tRNA together
iv. Newly synthesized polypeptides are processed and folded into finished form
v. Finished proteins contain sorting signals that direct them to cellular locations

12. DNA, three-letter code: Triplet
RNA, three-letter code: Codon

the codon in DNA is written the same as the codon in RNA, except that T becomes U. 5'GAC3' in DNA is 5'GAC3' in RNA.
But 5'GTC3 in DNA is 5'GUC3' in RNA (molecular types often mess this up and use T when writing RNA).

13. **Start codon or initiator codon**
- First amino acid recognized during translation
Specifies amino acid "methionine"

Sense Codons
- Establishes the reading frame
- Sense codons
- 61 codons specify amino acids
- Most amino acids specified by several codons (degeneracy or redundancy)

Ex: CCU, CCC, CCA, CCG all specify proline

Stop codons or termination codons (sometimes "nonsense")
- End of a polypeptide-encoding mRNA sequence
UAA, UAG, UGA (sometimes referred to as "ochre", "amber" and "opal"; the names relate to their discovery in mutations)

14. Initiation
- Ribosome assembled with mRNA molecule and initiator methioninetRNA

Elongation
- Amino acids linked to tRNAs added one at a time to growing polypeptide chain

Termination
- New polypeptide released from ribosome
- Ribosomal subunits separate from mRNA

INITIATION
- Initiator tRNA (Met-tRNA) binds to small subunit
- Complex binds to 5´ cap of mRNA, scans along mRNA to find AUG start codon
- Large ribosomal subunit binds to complete initiation

ELONGATION
- Aminoacyl-tRNA matching the next codon enters A site

- Peptidyl transferase catalyzes formation of first peptide bond and cleaves tRNA in P site
- Ribosome moves along mRNA to next codon
- Empty tRNA moves from P site to E site, then released
- Newly formed peptidyl-tRNA moves from A site to P site
- A site empty again

TERMINATION
Begins when A site reaches stop codon
- Release factor (RF) or termination factor binds to A site
- Polypeptide chain released from P site
- Remaining parts of complex separated

Information Flow

1. What is gene regulation? Why is it so important to understanding life?

Gene regulation refers to the regulation of activity and may occur at any level. While the main control is at the level of transcription additional controls are at the posttranscriptional, translational and posttranslational levels.

Regulation of gene expression **includes the processes that cells and viruses use to regulate the way that the information in genes is turned into gene products**. Although a functional gene product may be an RNA or a protein, the majority of known mechanisms regulate protein coding genes. Any step of the gene's expression may be modulated, from DNA-RNA transcription to the post-translational modification of a protein.

Gene regulation is essential for viruses, prokaryotes and eukaryotes as it increases the versatility and adaptability of an organism by allowing the cell to express protein when needed. The first discovered example of a gene regulation system was the lac operon, discovered by Jacques Monod, in which protein involved in lactose metabolism are expressed by E. coli only in the presence of lactose and absence of glucose.

Furthermore, gene regulation drives the processes of cellular differentiation and morphogenesis, leading to the creation of different cell types in multicellular organisms where the different types of cells may possess different gene expression profiles though they all possess the same genome sequence.

In summary, important because:

- Changes in environment or changes in nutrients
- Changes in make up of cell is response to signals
- In a multicellular organism, cellular differentiation
- Conserve energy

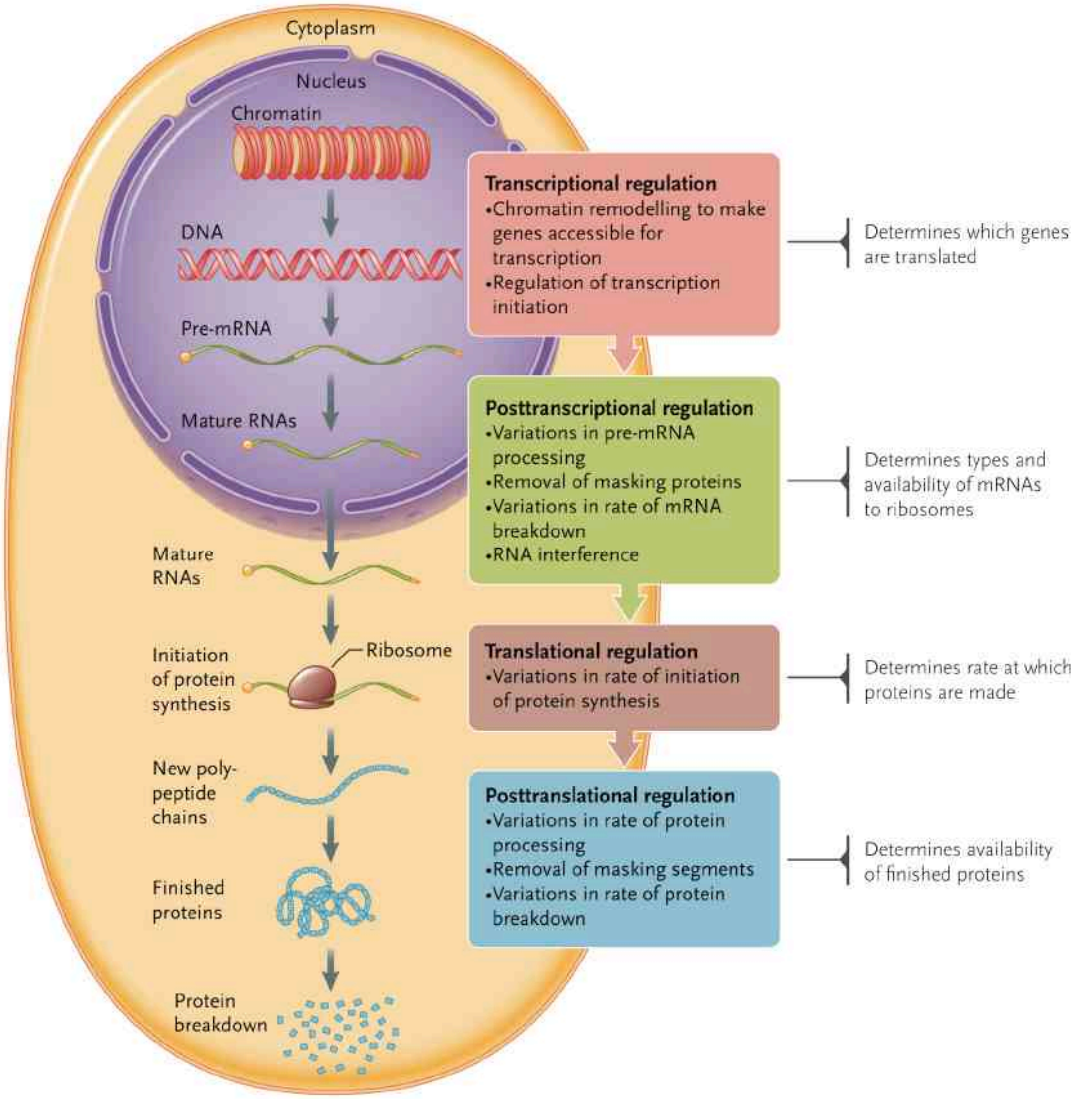
2. Describe gene regulation in prokaryotes; be sure to mention activator and repressor proteins, as well as operons.

Typically RNA polymerase binds to a DNA sequence 5' to the gene called the promoter. Within the promoter may be the consensus sequence 5'-TATAAT-3' called a TATA box.

Repressor proteins binding to other regulatory DNA sequences may prevent the gene from being expressed.

Activator proteins binding to other regulatory DNA sequences may turn on expression of the gene.

Repressors and activators may regulate the same gene.



In prokaryotes many genes are organized into clusters (transcription units) that are implicated in a single function.

At a smaller scale many genes are organized into operons (one or more operons may be found within a transcription unit)

The operon itself can be considered as a unit of transcription with several genes controlled by a single promoter. In effect an operon is a cluster of genes and DNA sequences involved in their regulation. RNA polymerase binds at the promoter and transcribes all the genes in the operon into one mRNA (called polycistronic because it contains several cistrons--an older definition of a gene used in genetics).

3. Name the 4 different levels that the regulation of gene expression occurs at in eukaryotes. What does each level do?
4. What is the transcription complex composed of and what do they do to initiate transcription?

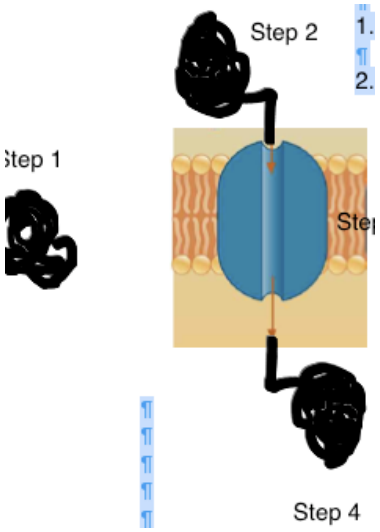
Organization:

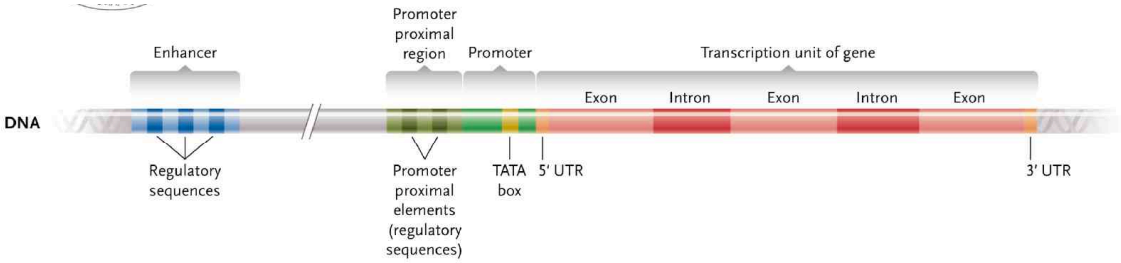
Promoter includes TATA box that binds transcription factors
Promoter proximal region upstream of promoter increases transcription
Enhancer further determine maximum transcription rate
(what are Silencers?)

How do these factors assemble to initiate transcription?

Bind to TATA box area and recruit RNA polymerase II
Transcriptional initiation
complex, low rate ("basal")
Activators bind to promoter proximal elements and
increase transcription rate
Coactivators bridge enhancer and promoter
Interactions between coactivator, proteins at promoter, and RNA polymerase increase transcription

Repressors oppose effect of activators
Transcription rate depends on activation and repression signals
In a multicellular organism these signals may be external (heat, light etc.) or internal (hormones etc.)
May bind to sites on an activator or coactivator or increase association with histones





5. Fill in the blanks:
i. Posttranscriptional regulation controls mRNA availability
ii. Translational regulation controls the rate of protein synthesis
iii. Posttranslational regulation controls the availability of functional proteins

Protein Targetting

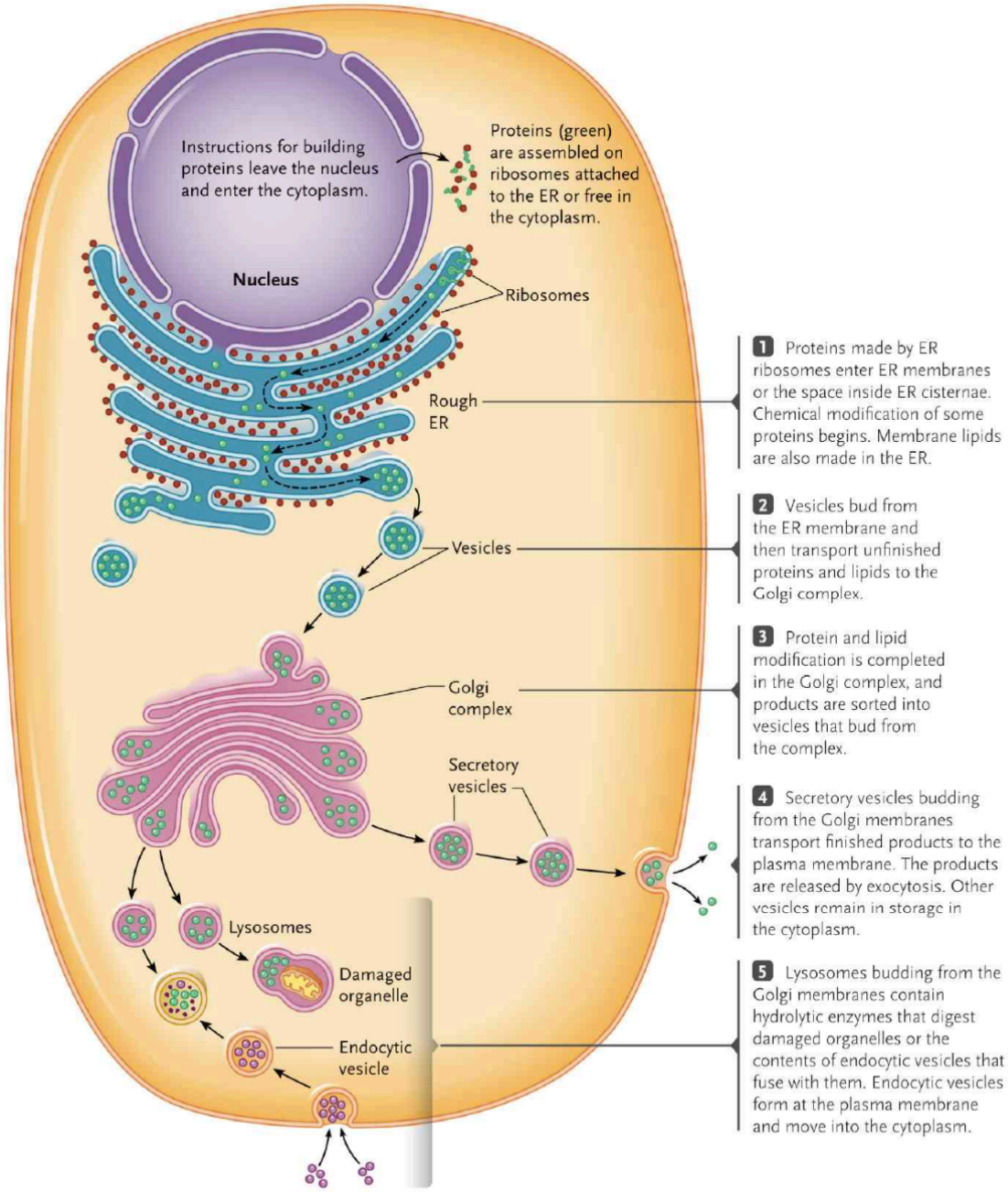
1. Using the accompanying diagram, describe the four steps involved with protein targetting.

1. Translation

2. Interaction with receptor & unfolding
   -signals on protein (a “signal peptide”)
   -proteins aid unfolding (chaperones)

3. Translocation

4. Refolding/processing
   -proteases may remove signals
   -proteins aid unfolding (chaperones)



2. Describe how proteins are transported across the ER. What is the nature of the signal peptide? What is the fate of the signal peptide?

Transport across the membrane into the ER (the lumen) is the first step for targeting to many locations-ER, Golgi, several vesicles and outside of the cell. A signal peptide is required, as is a receptor.
In the absence of further information the protein is exported.
This is referred to as the “default pathway”.

What is the nature of the signal peptide?

What is the fate of the signal peptide?
The signal peptide for ER transport is an length of 20-50 aa's with an hydrophobic core. These are recognized by the signal recognition particle and the complex binds to the SRP receptor.
When translation resumes the signal peptide is cleaved by the signal peptidase. We refer to the original protein as the preprotein.
An example: prelysozyme becomes lysozyme when the SP is removed.

3. Describe the journey of a protein, starting from the inside of the nucleus, and ending outside the cell. - See next page

4. In what kinds of systems are there multiple signals? Also, give an example and describe one.

System: Multiple membrane system.

Example: Mitochondrion

enter matrix (M) via receptor and remove
targeting signal with signal peptidase. e.g. DHFR
enter inter membrane space (IMS) via receptor
and remove spacer sequence. e.g.cytochrome