

Cell biology for the masses

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with reference to notes from BIOM 600, Tom, and Ira

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1 Protein processing mechanisms and pathways

1.1 Translation

Amino acids are not incorporated into a protein in free-floating form; rather, they are covalently bound to specific tRNAs via their carboxy terminus as aminoacyl tRNAs. This reaction is catalyzed by aminoacyl tRNA synthetase and requires an ATP molecule to first adenylate the amino acid. A nascent polypeptide consists of a chain of amino acids with a tRNA still bound to the last amino acid on the C-term end. The formation of a peptide bond between this last amino acid and a new tRNA-bound amino acid causes the previous tRNA to be released.

Translation occurs on ribosomes. Ribosomes consist of two riboprotein subunits; the large subunit catalyzes the peptidyl transfer, while the small subunit provides the framework for matching appropriate tRNAs to the next mRNA codon. The ribosome can accommodate two tRNAs at any given time in one of three sequential binding sites: the A (acceptor) site, the P (peptidyl-tRNA) site, and the E (exit) site. Initiation occurs when an initiator tRNA bound to Methionine and in complex with eIF-2, other initiation factors, and a small ribosomal subunit associates with a mature mRNA. The complex slides along the mRNA until an appropriate start (AUG) codon is reached, at which point the initiation factors dissociate and the large ribosomal subunit binds, such that the tRNA is positioned in the P site. The first peptide bond is formed when the next aminoacyl tRNA binds at the A-site, and the tRNAs slide down such that they are now occupying the E and P sites. The tRNA in the E site dissociates, and another aminoacyl tRNA can now bind in the the A site. Subsequent rounds of this constitute the elongation phase. Aminoacyl tRNAs with appropriate anticodon regions complementary to the mRNA will preferentially bind, while incorrect tRNAs may bind but will rapidly dissociate.

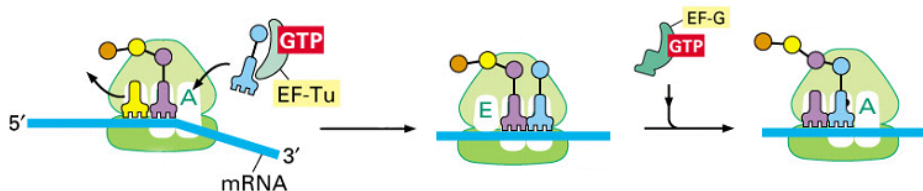


Figure 1: Translation (modified from Alberts)

Termination occurs when a stop codon is encountered in the mRNA. At this point, a release factor (eRF1 in eukaryotes) will bind in the A site, which catalyzes the hydrolysis of the polypeptide chain at the C-terminal end, causing the protein to be released. At this point the ribosomal subunits dissociate.

Ribosomes can be free floating in the cytosol, in which case the proteins that are synthesized remain there. Ribosomes are also associated with the rough endoplasmic reticulum membrane; proteins translated in this context are fed into the ER and are destined for transport.

1.2 Protein folding

Protein 3-dimensional structure is determined by primary sequence and is realized primarily by interactions among the side chains of the individual amino acids. However, in a cellular context there is high potential for “wrong” interactions. For example, nascent proteins will tend to aggregate due to constant collisions during folding, say due to hydrophobic regions being exposed and wanting to associate with other exposed hydrophobic regions due to energetic constraints. And because folding is a dynamic process that occurs cotranslationally, the “correct” interactions between regions on either side of the protein chain may not be able to form if the N-terminal side is already folded up. Thus, protein folding *in vivo* is aided by a family of chaperone proteins.

Most chaperones are heat shock proteins, so named because they are upregulated during cell stress, e.g. heat. The Hsp70 family (e.g., BiP) has homologs in the nucleus, ER, and mitochondria, and works by binding to the hydrophobic regions of polypeptides in a cotranslational fashion. This process is ATP-dependent: ADP-bound Hsp70 binds to its substrate and releases the substrate when the ADP is exchanged for an ATP, which is then hydrolyzed, causing Hsp70 to bind the substrate again. The Hsp60 family is primarily cytosolic in eukaryotes. It “traps” and “stretches” the folding intermediate inside its 8-member ring structure, allowing nonproductive interactions to break. There are several other families.

1.3 Protein translocation into the endoplasmic reticulum

1.3.1 ER

The rough ER is so named because it is studded with ribosomes (smooth ER lacks these). The smooth ER has a role in lipid biosynthesis, detoxification, and calcium sequestration and controlled release (the rough ER does this also). The rough ER serves as the entry point to the protein secretory pathway; all membrane-bound and secreted proteins must pass through the rough ER. The ER is a membrane-bound compartment that provides an oxidizing environment for protein folding, in particular facilitating the formation of disulfide bonds (this is in contrast to the cytosol, which is a reducing environment).

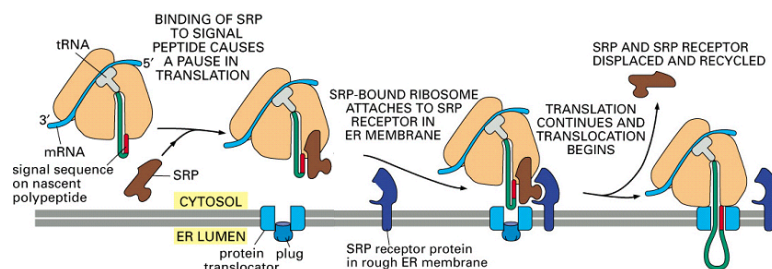


Figure 2: Translocation (Lodish)

1.3.2 Translocation

Entry into the secretory pathway is determined by a particular amino acid signal sequence (13-36 a.a.) on the N-terminal end of the polypeptide. There is no consensus sequence, but this region tends to be largely hydrophobic. Translation of any protein will begin on free floating ribosomes. After approximately 80 residues have been added, the signal recognition particle (SRP) binds the signal sequence if present, and causes translation to stall. The complex will bind the SRP receptor in the ER membrane, and translation will continue; however, the protein is now being threaded through an aqueous translocation channel (Sec61) into the ER lumen, tethered to the channel by the signal sequence. Secretory proteins will continue to be translated into the lumen, after which a signal peptidase cleaves the signal sequence, and the protein is processed and folded. Membrane proteins will contain an internal stop-transfer sequence further downstream, which will prevent further translocation, so translation simply continues in the cytosol; eventually a lateral release event will push the protein from the channel, such that it is still embedded in the membrane and has distinct cytosolic and lumen sides. This is a much more complex process for multi trans-membrane proteins, though.

The translocon can also facilitate translocation of proteins post-translation, i.e., not associated with a ribosome. Conversely, it can also be the site of retro-translocation, by which misfolded proteins are exported out of the ER, to be degraded in the cytosol.

1.3.3 Chaperone activity

The ER contains many chaperone proteins to facilitate proper folding of the translocated proteins. Besides BiP, there are two lectin chaperones, calnexin and calreticulin, that facilitate binding in a manner that depends on the glycosylation of the polypeptide chain. During translocation, the membrane lipid dolichol phosphate transfers an N-linked oligosaccharide to the growing polypeptide in a motif-specific fashion in the ER lumen. This oligosaccharide is important in proper folding of the protein. Once added, the oligosaccharide is successively trimmed by glucosidases and manosidases. Calnexin and calreticulin will bind the proteins at the point where there is one glucose monomer remaining, and facilitate proper folding. This final glucose is cleaved after this point. If the protein is misfolded, UDP-glucosyltransferase will reattach a glucose, which induces additional cycles of binding by the chaperone proteins. This binding depends on the fact that only in a misfolded protein will the interior-most part of the oligosaccharide be accessible.

Retro-translocation of a misfolded protein also depends on the action of a glycosidase, ER mannosidase I. ER mannosidase I is “slow acting,” meaning that it will only be able to remove a mannose residue if the protein is still in a misfolded state, despite the better efforts of the chaperones. EDEM, a protein that interacts with calnexin, recognizes such proteins with mannose removed, and facilitates their unfolding and retro-translocation. Excessive levels of misfolded proteins will trigger the Unfolded Protein Response (UPR), which in eukaryotes simultaneously increases protein degradation activity, reduces translation, and induces chaperones.

1.4 Vesicular trafficking

Transport between organelles in the cell is mediated by vesicles, which are small, mobile organelles that can bud from one compartment’s membrane, bringing with it cargo and fluid, and fuse with the membrane of a destination compartment, delivering the contents to the interior. Both budding and fusing are specific events. Specificity is largely mediated by the coat proteins that line the vesicle membrane. The coat is also responsible both for aggregating the correct contents into the vesicle and forming the curved architecture of the vesicle itself.

The three rough classes of coated vesicles are the clathrin-coated (Golgi to plasma membrane transport), COPI-coated (Golgi to ER and other Golgi compartments), and COPII-coated (ER to Golgi). Clathrin consists of hexamers (triskelion) that assemble into a basket shape, and one of several types of

adaptin complexes, which provide structural support and specificity for cargo. Clathrin-coated buds grow off of the Golgi membrane and are pinched off by dynamin. This process is mediated by GTPases, the ARF proteins. The clathrin coat is lost once the vesicle is budded off. COPI and COPII vesicles form in a similar fashion.

Surface markers on the vesicles dictate where the vesicle may dock. SNAREs are transmembrane proteins that exist as a complementary set of v-SNAREs (on the vesicle membrane) and t-SNAREs (on the target). Complementary SNAREs bind to form a trans-SNARE complex, causing the two membrane surfaces to dock. Fusion of the membranes is also believed to be due to SNARE activity; following docking, the SNAREs tighten, squeezing the water molecules from the interface between the two membranes and allowing them to come in contact. Rab proteins on the vesicle surface are recognized by Rab effectors on the target, and serve to facilitate specific SNARE binding. NSF catalyzes disassembly of the SNAREs so they can be reused.

1.5 Protein transport and the Golgi complex

The next destination for most secreted proteins is the Golgi apparatus, where they undergo further processing and modification such as glycosylation, sulfation, phosphorylation, and proteolytic cleavage, before they are sent via vesicles to the plasma membrane. Proteins destined for other organelles also pass through the Golgi.

1.5.1 The Golgi apparatus

The Golgi consists of a stack of self-contained membranes called cisternae, such that each cisterna has a distinct luminal environment, as well as associated tubes and vesicles. The Golgi are roughly positioned next to the ER and face outward toward the plasma membrane, though position as well as size and structure are variable. Those cisternae nearest the ER are the cis compartments, those farthest are the trans, those in the middle are medial. Between the ER and the Golgi are the vesicular tubular clusters (VTC). Past the trans cisternae are sometimes a collection of membranes known as the trans Golgi network. Different cisternae contain different resident proteins, e.g., different flavors of glycosyl transferases for carbohydrate modifications.

The stacked appearance is thought to be mediated by a Golgi “matrix” consisting of structural template proteins called Golgins and GRASPs. The entire apparatus is tethered near the microtubule organizing center (MTOC, or centriole), though it turns out that microtubule support is not strictly required for protein secretion, since secretion is not completely inhibited by nocadazole (a microtubule depolymerizer). The Golgi disperses into small vesicles and tubes during mitosis, thus pausing secretion, and is reassembled after cytokinesis.

1.5.2 Protein transport mechanisms

Proteins pass from the ER to the Golgi via COPII coated vesicles via the “forward pathway,” where they fuse with the VTC. Generally, two types of proteins are contained in such vesicles – those that are destined for secretion or transport, and those that are ER resident proteins that should not be sent to the Golgi. ER resident proteins contain a protein localization signal (KDEL) that is recognized by a receptor in membrane derived from ER. Thus, any ER resident proteins that make their way to the VTC are bound by the KDEL receptors; the surrounding membrane buds into COPI coated vesicles and are returned to the ER via the retrieval pathway.

Contents of the VTC end up passing through subsequent cisternae of the Golgi; however, the mechanism by which this occurs is not known for sure. In the vesicular transport model, COPI vesicles carry their cargo between cisternae in either an anterograde or retrograde fashion, depending on where the proteins need to go. Secreted proteins are passed forward through the stacks. Golgi cisterna-specific enzymes that are mistakenly caught in the anterograde vesicles are passed backward. This implies that

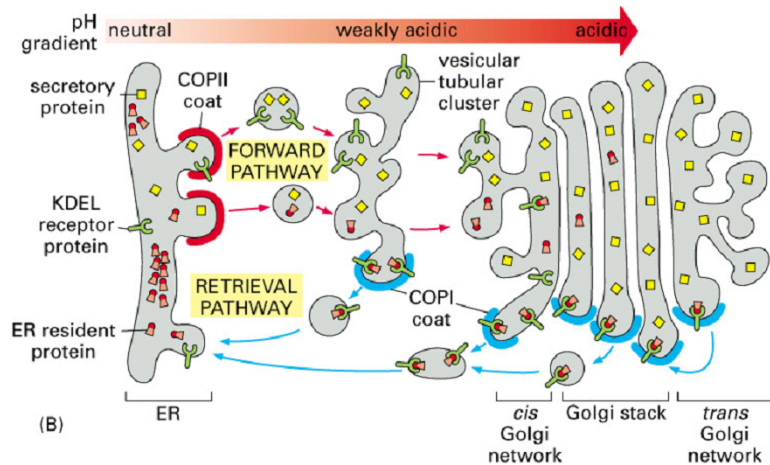


Figure 13-21 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Figure 3: Protein transport through the Golgi (Alberts)

resident Golgi enzymes have fixed, discrete locations (i.e., cis, medial, or trans), and also that there is potential for different cargos to be carried forward at different rates, depending on the vesicles. So far there is experimental evidence for both anterograde and retrograde vesicular movement.

In contrast, the cisternal maturation model posits that cisternae themselves carry the cargo forward through the Golgi, such that the cis cisternae “mature” into medial cisternae, which in turn mature into trans cisternae. Vesicular transport is thus limited to retrograde transport. This suggests a more fluid system where enzymes don’t necessarily have fixed locations, but since cargo is carried en masse by the cisternae, all cargo will generally be secreted at the same rate. One piece of evidence in favor of this model is that fact that some cargo molecules are too large to fit into a COPI vesicle.

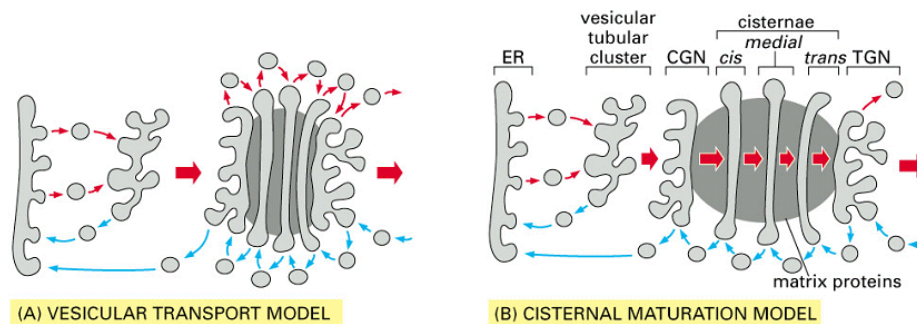


Figure 13-30. Molecular Biology of the Cell, 4th Edition.

Figure 4: Golgi transport models (Alberts)

As proteins pass through the various cisternae, they are subject to modification. In particular, there is a rigidly ordered process of addition and removal of oligosaccharides that takes place on cargo proteins passing through the compartments. The end product will depend on the particular protein being secreted. Glycoproteins have a role in cell-cell interactions and the extracellular matrix.

The final destination for secreted proteins is the plasma membrane. Vesicles bud from the TGN and fuse with the plasma membrane carrying their particular cargo. However, not all proteins passing through the Golgi are immediately secreted.

1.6 Regulated secretion

Secreted proteins are roughly divided into two classes – those that are continuously secreted (i.e., secreted once produced, such as extracellular matrix proteins) and those that undergo regulated secretion (such as hormones, neurotransmitters, digestive enzymes). Such proteins aggregate in the TGN and eventually bud off into storage vesicles called granules. Granules mature as the concentration of secretory product increases.

There are two models for how proteins are sorted into their respective categories. In the sorting-for-entry hypothesis, this process is controlled by the TGN in a self-aggregation manner. Receptors exist in the nascent granule membrane for particular classes of secretory proteins. Proteins will either bind these receptors, or associate with self proteins bound to the receptors; these will eventually be contained within a specific granule. Proteins that fail to associate follow a default route to the constitutive secretory pathway.

In the sorting-by-retention model, immature secretory granules will contain a mixture of regulated and unregulated proteins, though the target regulated protein will be in high concentration. Within the granule, the same sort of high-order associations between same proteins observed in the other model serve to aggregate and retain the regulated secretory proteins in the granule. For the remaining proteins, there exists an exit route by which vesicles bud off the granule and return to the TGN; by virtue of the aggregation, the regulated protein does not tend to populate these vesicles, and is thus retained in the granule.

Secretion occurs upon stimulation, either by internal or external factors. At this point the secretory vesicle is directed toward the plasma membrane via the actin cytoskeleton and allowed to dock, releasing its contents to the extracellular matrix. There is evidence that rather than completely fusing with the plasma membrane, these vesicles can perform a “kiss-and-run” form of exocytosis, such that the contents are released via a fusion pore that forms between the intact vesicle and the plasma membrane, which is subsequently pinched off. These vesicles are then recycled in a process largely not understood, such that Golgi membrane is replenished.

Regulated secretory proteins are often processed in the mature secretory vesicle. Insulin, for example, exists in proinsulin form prior to the secretion signal, and only after stimulus is the proinsulin proteolytically cleaved to form mature insulin.

1.7 Endocytosis

Cells internalize material from outside for a variety of reasons, such as for nutrient uptake or as part of signalling pathways. Because of this heterogeneity, there are several dynamic organelles that make up the endocytotic pathway, each containing a particular complement of enzymes and environment specific to their function. Transport between compartments is vesicle-mediated.

1.7.1 Organelles

In the classic endocytotic pathway, the following organelles are found:

Endocytic vesicles are small vesicles derived from endocytosis events.

Early (sorting) endosomes provide a mildly acidic environment and are the first stop for endocytic vesicles. In particular, ligands uncouple from receptors here.

Late endosomes have lower pH and thus higher proteolytic activity. They are an entry site for lysosomal proteases, so they are also known as pre-lysosomes.

Lysosomes are the site of protein and carbohydrate degradation.

The Golgi apparatus is also involved in the endocytic pathway, since enzymes destined for use in the various organelles pass through the Golgi after they are translated.

1.7.2 Sorting

Like the ER localization signal sequence, proteins are directed to various locations based on short amino acid motifs. Many such signals can be present in the same protein. These sequences are recognized by specific receptors, many of which are so-called traditional clathrin adapters – that is, they are associated with the clathrin coat of the vesicle; these include the AP family of adapter proteins. Some of the common signals are:

NPXY – internalization from the plasma membrane, recognized primarily by AP2 adapter; LDL receptor is the prototypical example

YXX Φ – both an endocytosis signal and a TGN-to-endosome/lysosome signal, recognized by AP1 and AP2; Φ is any large hydrophobic amino acid, such as F

DXLL – recognized by Golgi GGA proteins for TGN-to-endosome; particularly found in the mannose-6-phosphate receptor

mono-Ubiquitin – not intrinsic to the protein itself, rather a modification bound to an arginine; endocytosis, bound by many classes of proteins, such as Epsins.

1.7.3 Trafficking

Delivery of enzymes from the Golgi to the endosomes/lysosomes is primarily mediated by the mannose-6-phosphate receptor (M6PR). Most lysosomal enzymes' mannose residues are phosphorylated early in the cis Golgi. M6PR binds these enzymes in the TGN and packages them into clathrin-coated vesicles that fuse with late endosomes. Since late endosomes are acidic, M6PR dissociates from its ligand, and the M6PR is recycled back to the Golgi by virtue of its DXLL sorting signal. It turns out the the M6PR can also make it to the plasma membrane, and thus also carries a YXX Φ signal.

Trafficking through the endosomal system is mediated by SNAREs and Rabs.

1.8 Protein degradation

Degradation is important in turnover of damaged or denatured proteins. The amino acid monomers resulting from breakdown are recycled.

Nonregulated degradation occurs inside the late endosome or lysosome. The lysosome is a membrane-bound organelle containing several hydrolytic enzymes that function at low pH and are specific to a particular biomolecule (i.e., not just proteins), and can also be sequence specific. This is the major site for degradation of foreign proteins, but also old and tired proteins from organelles scheduled for destruction.

A form of regulated degradation occurs in the cytosol via the ubiquitin/proteasome pathway. Ubiquitin is a covalently-bound protein modification, which is conjugated to substrate proteins by the enzymes E1, E2 (the UBC), and E3 in an ATP-dependent process. Typically, once the first ubiquitin is attached to a substrate protein, it serves as the site for a second ubiquitylation event, eventually resulting in a ubiquitin chain. Polyubiquitylation is normally required to target the substrate for degradation. Substrate proteins are recognized by particular amino acid patterns at their N-terminus, for example, the

presence of an “unstable” residue, such as any of the charged amino acids, in the N-terminal position due to some prior processing event. There is also substrate specificity conferred by the particular E2/E3 in the complex, such that a particular species of protein can be the target for degradation. Additionally, chaperone proteins have a role in recruiting the UBC in the event that a protein is misfolded.

The proteasome is a large cytosolic protease and comes in a couple of different flavors. The 20S proteasome is largely nonspecific and produces short (8-15 a.a.) cleavage products as a denatured protein is threaded through its core. The 19S proteasome confers ubiquitin dependence, and when associated with the 20S proteasome forms the 26S proteasome complex. The 19S proteasome has intrinsic protein unfolding activity, allowing it to thread the denatured protein through the 20S proteasome. It additionally has isopeptidase activity, which causes release of the polyubiquitin chains from the substrate. All of this is ATP-dependent.

2 The cell cycle

Replicating cells have roughly two tasks – replicate their chromosomes, and divide into two daughter cells. Thus, the cell cycle is defined around these two events, along with intervening periods of growth and verification of proper conditions to continue replication:

G1 phase : growth and preparation for DNA synthesis (“gap 1”)

S phase : DNA synthesis (“synthesis”)

G2 phase : growth and preparation for cell division (“gap 2”)

M phase : mitosis or meiosis

Collectively, the G1, S, and G2 phases are known as interphase. The timing of each of these phases is variable and largely depends on the cell type and situation. For example, embryonic cells have nonexistent G1 and G2 phases, so division occurs rapidly and with no increase in overall cell mass size. Cells that are not actively replicating are said to occupy G0 phase. Such cells can be stimulated to enter G1 under the right conditions, though; once they pass a particular “restriction point,” they are committed to one round of cell division.

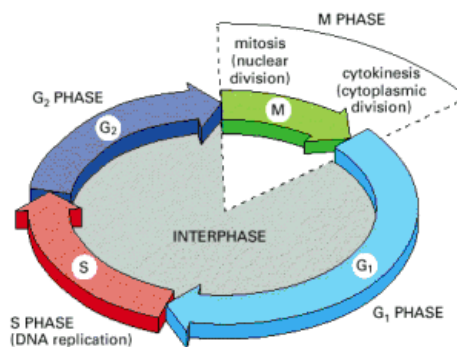


Figure 5: Cell cycle (Alberts)

2.1 Control and checkpoints

Entry into any particular phase is controlled by the activity of a family of cyclically activated protein kinases called Cdks. The activity of these kinases over the course of the cell cycle is regulated by cyclins, which form specific complexes with the cdks. There are four classes of cyclins, each binding a specific

class of cdks at appropriate points during the cell cycle – G₁, G₁/S, S, and M. Cyclin concentration rises and falls throughout the cell cycle, thus determining the level of Cdk activity. Additional inhibition of the activity of a cyclin-Cdk complex is achieved through phosphorylation or binding by Cdk inhibitor proteins (CDKIs). Cyclins are destroyed at particular points by a ubiquitin-dependent process, thus releasing the bound Cdks. Ubiquitylation is catalyzed by ubiquitin ligases, two of which are SCF and APC.

S cyclins bind Cdk2 during S phase, allowing DNA replication to be initiated. The S-Cdk initiates origin of replication firing by causing the phosphorylation of the origin recognition complex (ORC) protein responsible for initiating DNA replication. In addition, S-Cdk activity remains high throughout G₂ and early mitosis in order to prevent re-replication, by phosphorylating Cdc6 (causing degradation) and Mcm (causing export out of the nucleus), both of which are required for pre-replicative complex (pre-RC) assembly on the DNA.

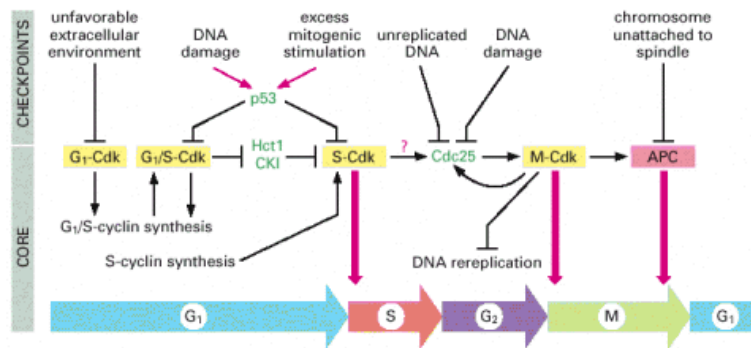


Figure 6: Overview of cell cycle control(Alberts)

M-cyclin concentration increases at the end of G₂, allowing it to bind Cdk1 to form M-Cdk complexes. These are dominant until the protein phosphatase Cdc25 removes the inhibitory phosphates on the M-Cdk. M-Cdk and Cdc25 operate in a positive feedback loop, such that increased levels of active M-Cdk in turn activate more Cdc25. A DNA replication checkpoint, which ensures that the entire genome has been duplicated, controls activation of M-Cdk, and if the checkpoint is triggered, M-Cdk remains inactive. M-Cdk has roles in assembling the mitotic spindle and chromosome condensation, as described below.

APC in complex with Cdc20 is required for the metaphase to anaphase transition (below) and constitutes the point of regulation for the spindle-attachment checkpoint; if a chromosome is not attached to the spindle, mitosis is stalled. Cdc20-APC also causes the inactivation of M-Cdk during late M-phase by destruction of the M-cyclins. Because M-Cdk also promotes Cdc20-APC activity, Cdc20-APC is also inactivated following M-phase.

In G₁-phase, Cdk activity is suppressed to allow for growth. This is accomplished by increased concentrations of CDKIs, decreased cyclin production, and the formation of Hct1-APC complexes, which continue to target M-Cdks for destruction (recall that Cdc20-APC complexes are inactivated in parallel with the M-Cdks, so the Hct1-APC can still be active to ensure all the M-Cdks are destroyed; Hct1-APC is inhibited by M-Cdk, so inactive during mitosis).

G₁-cyclins accumulate in G₁-phase cells, to the point where the high concentration of G₁-Cdk triggers the restriction point to commit the cell to enter into S phase. This is accomplished by removing

the blocks enabled at the beginning of G1. In animal cells, the transcription factor E2F activates transcription of G1/S-cyclins, S-cyclins, and other proteins required for S-phase entry; E2F is inhibited by retinoblastoma protein (Rb) during G1, but G1-Cdk phosphorylates Rb and removes the block.

DNA damage checkpoints are triggered either in late G1, preventing entry into S phase; or in late G2, preventing entry into mitosis. The G2 checkpoint blocks M-Cdk activity via phosphorylation of Cdc25. For the G1 checkpoint, p53 is activated when DNA damage is detected. p53 stimulates transcription of several genes including a CKI that inactivates G1/S-Cdk and S-Cdk complexes.

2.2 Mitosis and cytokinesis

M-phase is traditionally divided into 6 parts, 5 of which constitute mitosis: prophase, prometaphase, metaphase, anaphase, and telophase. Cytokinesis is the sixth part, though technically cytokinesis begins in anaphase. Chromosomes condense into compact chromatids during prophase; by metaphase, they've aligned at the center of the mitotic spindle, the cytoskeletal apparatus that mediates chromosome segregation; in anaphase, the sister chromatids are segregated to opposite poles.

2.2.1 Prophase

Duplicated chromosomes at the end of S phase are tightly bound to one another by cohesin proteins. Condensins, triggered by M-Cdk, cause the chromosomes to condense by inducing supercoiling. The sister chromatids are held together at the centromere by the kinetochore. Also prior to M-phase, the centrosome duplicates but the daughters remain together in a complex; the centrosome is the principal microtubule-organizing center in animal cells. During prophase, the centrosomes each nucleate a set of astral microtubules and migrate to opposite ends of the nucleus. In addition, overlap microtubules from each centrosome meet in the middle and associate. Kinesin motor proteins attach to a pair of overlap microtubules, and are able to push the microtubules and their associated centrosomes in opposite directions. Microtubule-associated proteins (MAPs) promote stability of microtubules during M-phase.

2.2.2 Prometaphase

This stage is marked by the breakdown of the nuclear envelope, which allows the microtubules to come in contact with the chromosomes. In a dynamic "search and capture" process, microtubules attach themselves to the kinetochores of the chromosome pairs, such that one microtubule from each pole ends up being attached to the kinetochore (bipolar attachment). The microtubules then expand or contract until all the chromosomes are aligned in a position equidistant from the two poles; this is called the metaphase plate.

2.2.3 Metaphase

Metaphase is largely a stasis state, awaiting the signal to separate the sister chromatids. This is the point where the spindle-attachment checkpoint is triggered if necessary.

2.2.4 Anaphase

Anaphase is triggered by activation of APC. APC cleaves M-cyclin, which is no longer necessary, and activates a protease called separase by cleaving the inactivating protein securin. Separase is responsible for cleaving the cohesin complex, allowing the sister chromatids to separate. In a process called anaphase A, kinetochore microtubules shorten, thus pulling the chromatids to their respective poles. Oddly, this is accomplished by depolymerization of the microtubule at the (+) end, which is the same end that is attached to the kinetochore; it is possible that motor proteins are involved in this. A second overlapping process called anaphase B pulls the poles themselves apart. This is accomplished by kinesin motors pushing the overlap microtubules apart, as well as motors pulling from the astral microtubule end against the cell cortex.

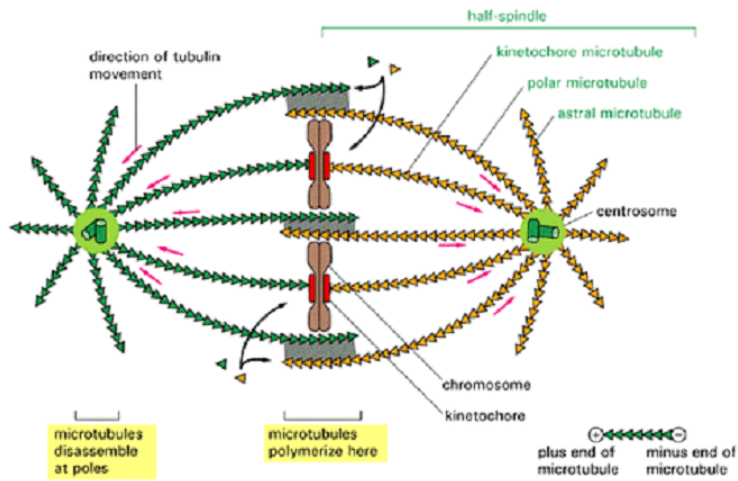


Figure 7: The mitotic spindle (Alberts)

2.2.5 Telophase

As the chromosomes begin to decondense, a new nuclear envelope in each daughter cell is formed around them from the fragments of the original nuclear envelope.

2.2.6 Cytokinesis

By telophase, a contractile ring forms at the midsection of the plasma membrane, as defined by the mitotic spindle. In cells that divide asymmetrically (e.g., during development), the spindle is repositioned. The contractile ring consists of actin and myosin filaments as well as several regulatory and structural proteins, and serves to pinch off two daughter cells. Organelles are roughly equally distributed, either by chance or by associating with the spindle. In particular, the ER and Golgi break down during mitosis along with the nuclear envelope, and the resulting fragments seem to bind to the spindle microtubules via motor proteins.

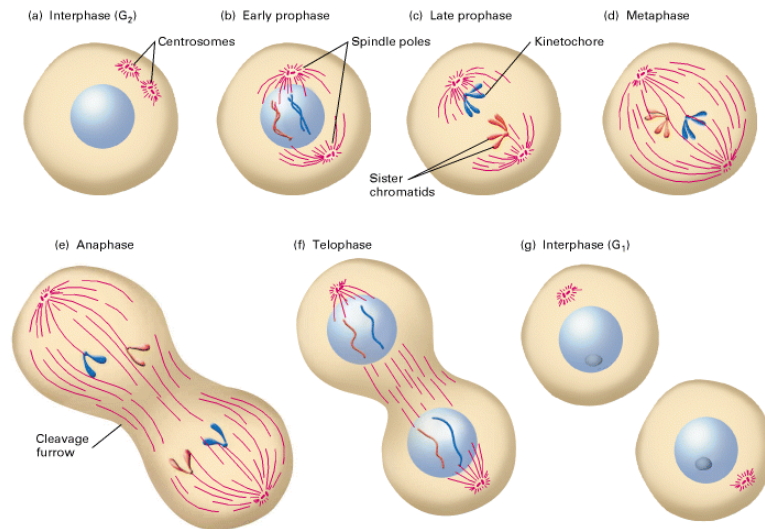


Figure 8: Overview of mitosis (Lodish)

2.3 Meiosis

In organisms that reproduce sexually, progeny are created that have chromosomes from both parents. In most multicellular organisms, the majority of cells are diploid, meaning that they carry two versions of each chromosome, one derived from each parent; the pair of chromosomes is known as a homologous pair. Haploid cells, which have only one version of each chromosome, are produced for the purposes of sexual reproduction, such that two haploid cells fuse to form a diploid progeny cell. The haploid cells are typically called germ cells or gametes, while the diploid cells are somatic cells. The goal of meiosis is to produce gametes.

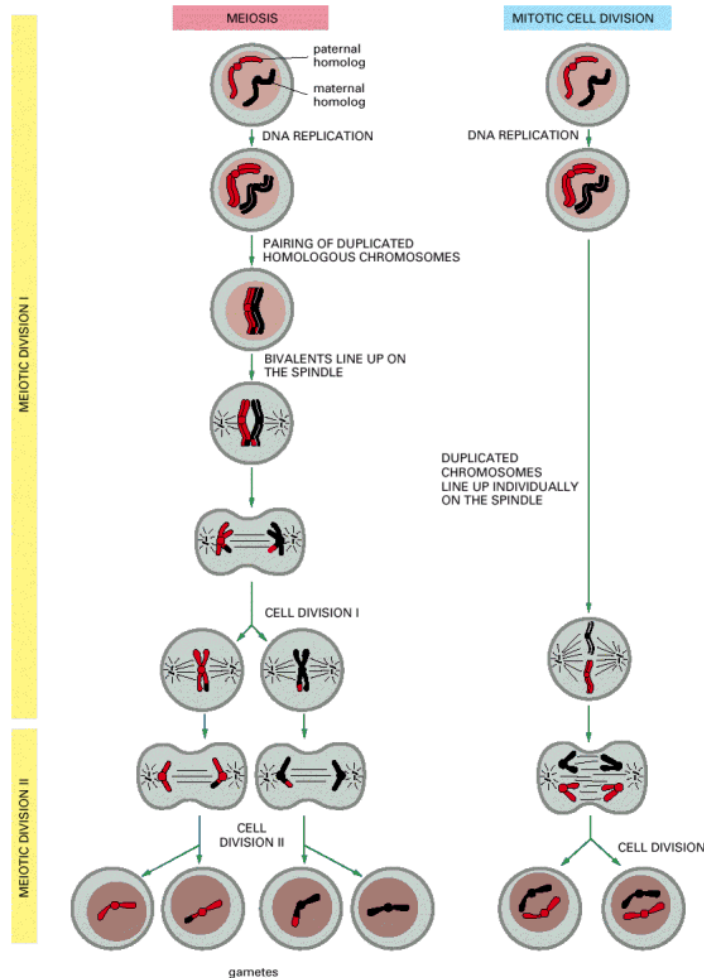


Figure 9: Comparison of meiosis and mitosis (Alberts)

Meiosis is divided into two stages: meiosis I, followed by meiosis II. Like in mitosis, meiosis is preceded by a stage of complete genome duplication. Meiosis I creates two daughter cells that each contain one and only one homolog of each chromosome. It consists of the same stages as mitosis, except that during prophase I, homologous chromosomes must pair together on the meiotic spindle. Each chromosome has been replicated, so there are two identical sister chromatids for each chromosome; each duplicated chromosome pairs with its duplicated homolog (usually not identical), for a total of four chromatids in one structure, called a bivalent. The chromosomes in a bivalent form a synaptonemal complex around a long protein core, a structure that promotes crossover. Based on the morphological changes that occur to the complex, five sequential stages of prophase I are defined: leptotene, zygotene, pachytene,

diplotene, and diakinesis. Pachytene refers to the state of complete synapsis, during which crossover is completed, and before the synapse is disassembled.

The bivalents line up on the spindle in metaphase I. In anaphase I, separation is between the homologs, not the sister chromatids. The sister chromatids remain attached at the centromere, though their arms become unglued. Thus each daughter cell contains half the number of chromosomes, where each chromosome is still a duplicated pair. These daughter cells are still diploid, since there are two copies of each chromosome. The destination for any given chromosome in the parent cell is randomly determined.

Meiosis II is basically a mitotic division, with no further DNA replication. The net result is thus four haploid daughter cells, containing half the number of chromosomes as the original diploid cell. Occasionally chromosomes will fail to segregate properly in either meiosis I or II, creating gametes with either 0 or 2 copies of some chromosome; this phenomenon is called nondisjunction. When such gametes fuse, the resulting embryos are called aneuploidic, since they will have the wrong number of chromosomes. Nondisjunction is the cause of several disorders such as Down syndrome (trisomy of chromosome 21), but is usually lethal.

2.3.1 Crossover

During prophase I, homologous chromosomes exchange portions of themselves with each other; this event is called crossover. Between two and three crossover events happen per chromosome pair during meiosis in humans. A crossover is classified as a “general recombination” event (as opposed to a site-specific event), a phenomenon that can also occur in mitotic cells and is in fact a mechanism for repairing some forms of DNA damage; in meiosis, the site of general recombination for a crossover is called the chiasma. There is evidence that recombination is catalyzed by recombination nodules, which are large protein complexes containing Rad51 (a RecA homolog) that sit at intervals on the synaptonemal complex. These nodules appear to be somewhat non-randomly distributed.

In general recombination, an endonuclease cleaves both strands of the DNA double helix of one chromatid (out of the 4). An exonuclease cleaves back the 5' ends to form 3' overhangs of single-stranded DNA. One of these overhangs finds the homologous region on one of the other chromatids aided by Rad51, to form a three-stranded intermediate. Some of the base pairs from the invaded double helix flip out to pair with the single strand to form synapses, where two strands are essentially competing for the same complementary strand. The single strand displaces the other strand and extends its base pairing interactions in a process called branch migration. This is accompanied by DNA replication using the complementary strand as a template and further displacement of the original duplexed strand. Meanwhile, the other overhang not involved in the invasion base pairs with the newly displaced strand and extends via further DNA synthesis.

The result of this strand displacement is a DNA structure called a Holliday junction, consisting of two crossing strands and two noncrossing strands. Which strands are crossing and which are not can change in a process called isomerization. This junction must be resolved by cutting the two crossing strands, and then ligating the appropriate ends. Depending on which two strands are crossing, resolution results in either two original chromatids (one of each homolog) plus two recombined chromatids, or four recombined chromatids.

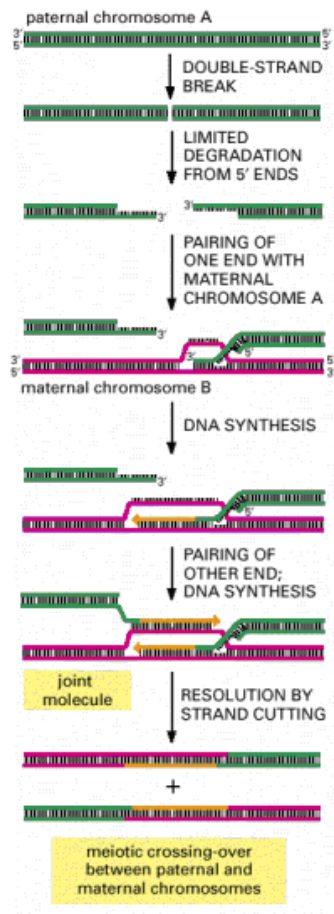


Figure 10: Overview of crossover (Alberts)

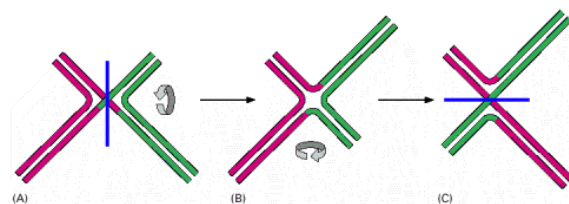


Figure 11: Isomerization of a holliday junction, with resolution cleavage sites marked in blue (modified from Alberts)