Cell Cycle :

Life cycle of the cell and Gametogenesis

Using this book: This book is designed to be used in both introductory and advanced cell biology courses. The primary text is generally on the left side of the vertical divider, and printed in black. Details that are usually left to an advanced course are printed in blue and found on the right side of the divider. Finally, additional biomedically relevant information can be found in red print on either side of the divider.

The Prokaryotic Cell Cycle

Cells, whether prokaryotic or eukaryotic, eventually reproduce or die. For prokaryotes, the mechanism of reproduction is relatively simple, since there are no internal organelles. The process consists of three distinct but short phases: first, a growth phase in which the mass of the cell is increased, then the chromosomal replication phase, and finally the chromosomes are separated and the cells are physically split into two independent new cells. In bacteria, these are referred to as the B, C, and D periods, respectively. Initiation of the reproductive process appears to be primarily a function of cell size. The length of the overall cell cycle is determined by the B period, as the C and D periods have relatively fixed time constraints. The length of B is determined, in part, by environmental conditions and the gain in cell mass. Generation times for bacteria can vary from under half an hour to several days, although most bacterial cultures in laboratory settings and nutrient-rich media have generation times under a day.

DNA replication has already been covered in detail in chapter 7. In bacteria, the process is initiated at the origin of replication by DnaA. However, in archaea, synchronous initiation of replication at multiple sites on the chromosome as well as recognition proteins homologous to eukaryotic ORC proteins suggests that there are similarities between archaebacterial and eukaryotic DNA replication to be explored.

Once the DNA is replicated and moved to opposite sides of the cell, the midcell septum forms to split the cell. At least 9 gene products are involved in this process including FtsZ, the prokaryotic tubulin homologue that forms a circumferential ring, FtsI, a peptidoglycan synthetase involved in septum formation, FtsL, whose function is unclear but is involved in ingrowth of the cell wall at the septum, and ZipA, which anchors the FtsZ ring. The ring contracts, pulling the membrane in with it. Eventually the membrane is pinched in enough to fuse and generate two completely separate cytoplasmic compartments. Other septation enzymes make cell wall components that fill in as the septum forms simultaneously with membrane/FtsZ contraction, and the cells separate.

The Eukaryotic Cell Cycle

Most eukaryotic cells undergo a reproductive cycle to generate either another copy of themselves or to generate gametes (sex cells), and in doing so require a complex mechanism to govern the safe and accurate replication of their much larger (than prokaryote) genomes. Immediately following mitosis, the newly created cells are in the G_1 phase. This is largely a growth phase, during which there is a lot of biosynthesis of proteins, lipids, and carbohydrates. However, there is no synthesis of new DNA at this time. G_1 is the longest of the cell cycle phases in many cell types, and most of the physiological activity of a cell happens during G_1. Following G_1, the next phase of the cell cycle is the S phase, during which synthesis of new DNA occurs. In other words, the genome is being replicated during this phase; thus at the end of S phase, the cell has twice the normal amount of DNA. After S phase, the cell proceeds into G_2, which provides an opportunity for the cell to perform a self-assessment and make final preparations (such as more cell growth, repairs of DNA) as necessary before it finally heads into mitosis. Mitosis, or M phase, is primarily (1) the breakdown of the nucleus, (2) re-distribution of the DNA to opposite sides of the cell, and (3) formation of two new nuclei around that DNA, and cytokinesis, the final splitting of the cell itself.

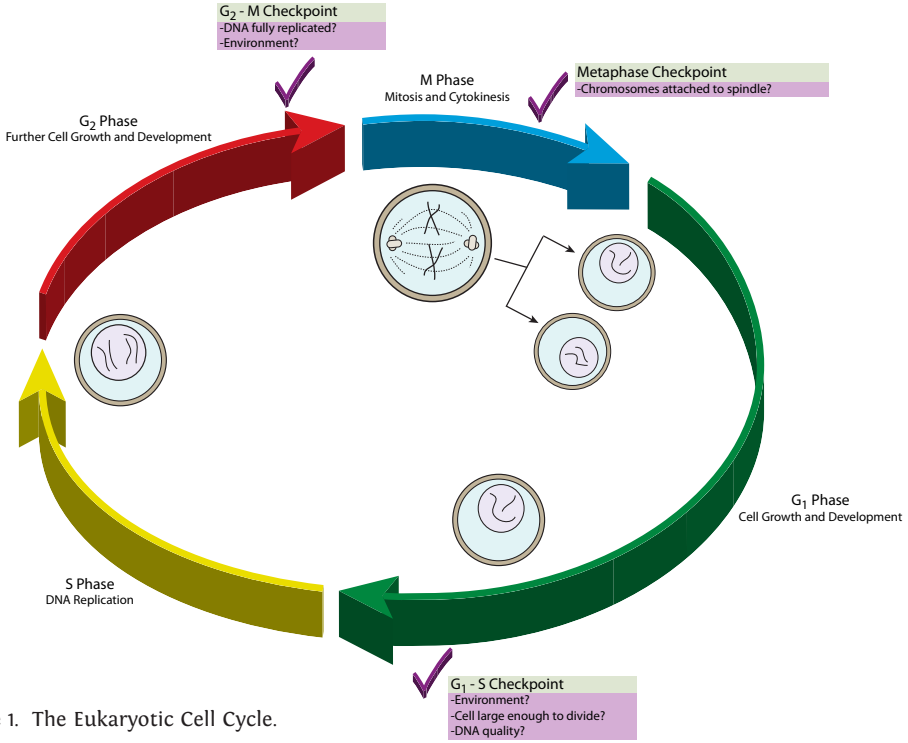

Figure 1. The Eukaryotic Cell Cycle.

As the cell progresses through the various phases of mitosis, and for that matter, the phases of the cell cycle overall, it does so in a specific and controlled manner, with checkpoints that "ask" if the cell is ready for the next step: is it big enough, is the DNA healthy, etc. so that the cell has the best chance of generating healthy daughter cells. For example, if the cell cycle runs too rapidly through each phase, then there is not enough time for the cell to build up its mass in preparation for reproduction, and that leads to abnormally small daughter cells, and potentially even daughter cells that are too small to survive. If a cell undergoes mitosis with damaged or mutated DNA, then that may increase the likelihood of a pathological mutation surviving and harming the organism by turning into a cancerous tumor.

Controlling the Cell Cycle

There are three major checkpoints for cell cycle control (fig. 1). The first regulates the transition from G_1 to S phase. Recall that G_1 can be a very long phase, even (in the case of G_0) as long as the lifespan of the cell. However, once the cell reaches S phase, it is committed to going through S, G_2, and M phases to reproduce. This is because once S phase has begun, there is more than the normal diploid complement of DNA inside the cell. Over time this would confuse the cell (e.g., by overexpression of duplicated genes) as it tried to use the DNA to direct RNA and protein synthesis, and it could become sick and die. The second major checkpoint regulates entry into mitosis. Once mitosis begins, most of the metabolic activity of the cell is shut down, and the cell concentrates its resources on dividing the nuclear and cellular material equally to support the life of both resulting daughter cells. If the cell needs more time to make final repairs on the DNA or even to bulk up a little, this checkpoint can hold the cell in G_2 a little longer for those things to happen. Finally, the third major checkpoint occurs during mitosis, and regulates the transition from metaphase into anaphase. Since the sister chromatids are being split apart and moved to opposite poles to form the new nuclei, it is important that all of them are perfectly lined up at metaphase and the proteins holding them together have dropped off. If they do not split evenly, the daughter cells will have abnormal numbers of chromosomes (aneuploidy) usually leading to deleterious consequences.

What is the molecular mechanism that regulates the progress of the cell cycle? While many of the checkpoint sensing mechanisms are still unclear, they seem to converge on two sets of proteins that act together to trigger cell cycle advancement. These proteins are known as the cyclins and the cyclin-dependent kinases (cdk). As the names suggest, the cyclins are proteins that regulate progression through the cell cycle, and

must be present in sufficient concentration to help activate the appropriate cdk. The cyclin-dependent kinase is the active, enzymatic, half of the partnership, and activates other enzymes by phosphorylation. Although the cyclins appear to be necessary for cdk activation, they are not sufficient, as there are intermediate phosphorylation and dephosphorylation steps required to activate the cdk after cyclin binding. Both the cyclins and the cdk's are families of related proteins, and they can combine in different ways to govern particular points in the cell cycle (fig. 2). Interestingly, the intracellular level of cdks is fairly constant. The level of cyclins, on the other hand, fluctuates dramatically depending on the state of the cell with respect to the cell cycle.

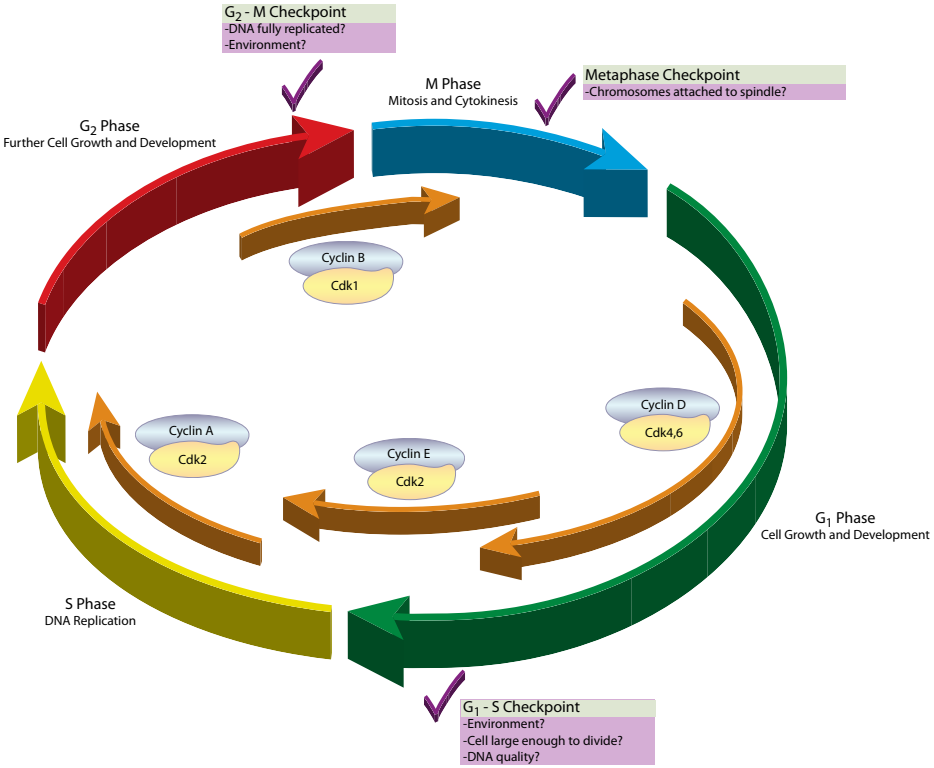

Figure 2. Cyclins are involved in control of the cell cycle.

The methodology of some of the early experiments is perfectly suited to explaining how this works. The seminal paper in this field was a 1971 paper in J. Exp. Zool. by Masui and Markert. In it, they examined frog (Xenopus laevis) eggs that were arrested at G_2. The oocytes arrest for about 8 months naturally in order to build up the mass needed to start a new organism once it has been fertilized. The basic question being asked is what is causing the eggs to come out of G_2 and into M phase? It was already known

that the hormone progesterone can trigger this transition, but what are the intracellular players in the change in cell state? Masui and Markert decided to test whether there was a cytoplasmic molecule that was responsible. They took a small amount of cytoplasm from an M-phase egg and injected it into a G_2-arrested egg. This triggered the maturation of the G_2-arrested egg and pushed it into M phase, even without progesterone. The activity was called maturation promoting factor (MPF), and was hypothesized to be a soluble, cytosolic protein.

In later experiments, other investigators attempted to find the specific protein trigger, and from there, presumably, the rest of the mechanism. Fractionating the M-phase oocyte cytoplasm by column chromatography, a protein, named cyclin B, was found to rise and fall in concentration in direct synchronization with MPF activity. Furthermore, addition of cyclin B alone was sufficient to rescue MPF activity from M-phase cytoplasmic extract that had been depleted by RNase treatment (preventing synthesis of any new proteins, including cyclin B, and abolishing MPF activity). This clearly places cyclin B in the forefront of the maturation mechanism, but there was one major issue: cyclin B had no enzymatic activity. How was it effecting the changes needed for progress from G_2 to M phase?

This problem was answered by experiments on a very different organism, the fission yeast, *Schizosaccharomyces pombe*. Because they have a very short cycle time, a relatively small genome, and they can be given random mutations *en masse* by irradiation or chemical treatment, yeast are excellent model organisms for many types of biological study. After random mutation of a population of yeast, they can be screened for mutations of particular types, such as cell division cycle (cdc). When the mutations are sequenced and identified, they are often named by the type of mutation and order of discovery. Cdc2, it turns out, showed two interesting phenotypes when mutated in opposite directions. Mutations that knocked out function of cdc2 caused the formation of extremely large yeast that do not undergo cell division, while mutations that made cdc2 overactive caused the formation of rapidly dividing very small cells. The interpretation was that when cdc2 is missing or inactive, the cells cannot progress to mitosis, so they stay in G_2 accumulating bulk material in preparation for a cell split that never comes. Conversely, when cdc2 is overactive, it drives the cell quickly into mitosis, even if it has not been in G_2 long enough to synthesize enough mass to form two normal-sized cells. This ties cdc2 nicely to cell cycle regulation, and it even has an enzymatic activity: it is a kinase. This made it a perfect candidate as a first-order coordinator of cellular events because phosphorylation is fast, phosphorylation usually activates some other enzyme, and kinases usually act on an array of targets, not just one. So we now have a cyclin (identified as cdc13 in S. pombe) and a cyclin-dependent kinase that work together to promote cell cycle progression into M phase.

Activation and inactivation of the cyclin-cdk complex

As more mutant yeast were being screened for changes to their cell cycle, two other genes were found in which mutations gave rise to similar phenotypes. Nonfunctional cdc25 or overactive wee1 mutants generated the overly large cells with a single nucleus, and conversely, overactive cdc25 or inactive wee1 generated many severely undersized cells. Both cdc25 and wee1 gene products interact with cdk, and in fact, they are positive and negative regulators of cdk, respectively. Acting together with one more enzyme, CAK (cdk-activating kinase), they activate the cdk (fig. 3). Using the mitotic cyclin/cdk complex as an example, the cyclin (cdc13) and cdk (cdc2) come together to form an inactive complex. The cdk is then phosphorylated by wee1, a kinase. The phosphate it puts on tyrosine-15 is needed for the rest of the activation sequence, but it is inhibitory: it actually *prevents* final activation. But once Tyr-15 is phosphorylated, CAK can phosphorylate a neighboring threonine (Thr-161), which is required for activation. Finally, cdc25, a protein phosphatase, removes the phosphate on Tyr-15, allowing activation of the cdk by the phosphorylated Thr-161, and the MPF is finally on its way. There is self-amplification of the activation as well, because one of the targets of MPF is cdc25, so there is a positive feedback loop in which the activity of cdc25 is upregulated by phosphorylation.

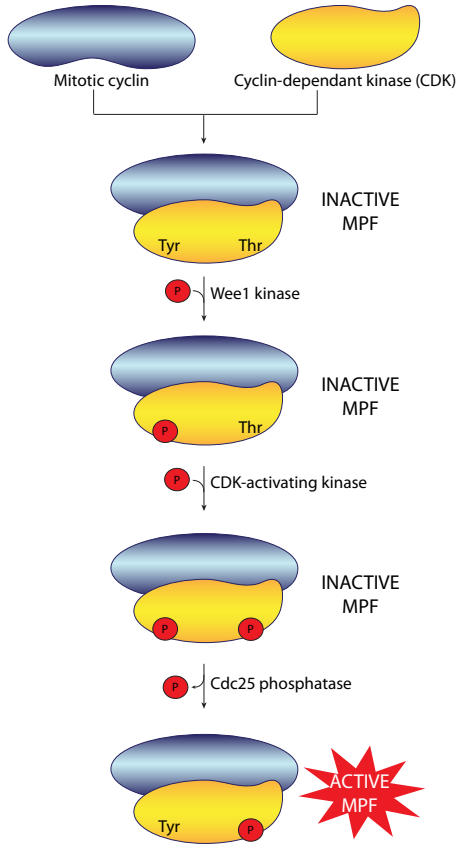

Figure 3. Activation of mitotic cyclin/cdk complex.

As you will see in a later section of this chapter, MPF performs many functions, some of which prevent progress of mitosis past anaphase. Therefore, there must be a way to turn off MPF (and for that matter, any cyclin/cdk complex) quickly and completely when the cell reaches the appropriate stage of the cell cycle. This is borne out by time-course studies of MPF activity, which show a precipitous drop in activity in anaphase. This coincides with a depletion of the cyclin B (cdc13 in S. pombe) due to a combina-

tion of turning off transcription of the gene, and specific proteolytic degradation. The degradation pathway is now well understood, and is an interesting example of a sort of feedback regulation.

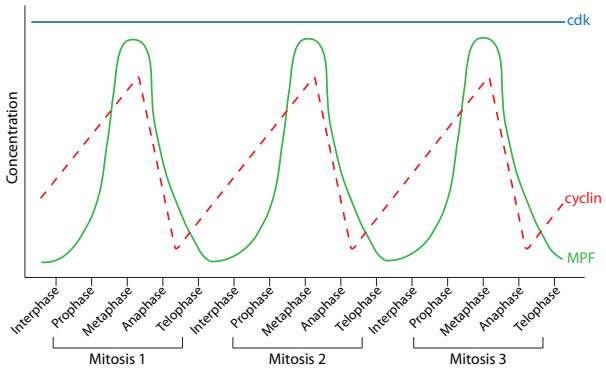

Figure 4. MPF activity and cyclin B protein expression rise as the cell enters mitosis but drop just before anaphase. However, cdk levels remain steady throughout the cell cycle.

Essentially, MPF ensures its own destruction: one of its phosphorylation targets is cdc20. Upon phosphorylation, cdc20 is activated and then activates anaphase promoting complex (APC). APC is a ubiquitin ligase (type E3) that polyubiquitinates the cyclin of the MPF complex, making it a target for proteolytic degradation by a proteosome. Note that only the cyclin is destroyed, while the kinase is left alone. Without the cyclin, the kinase is inactive and must wait for cyclin levels to rise again before it can be reactivated by a fresh round of phosphorylation and dephosphorylation.

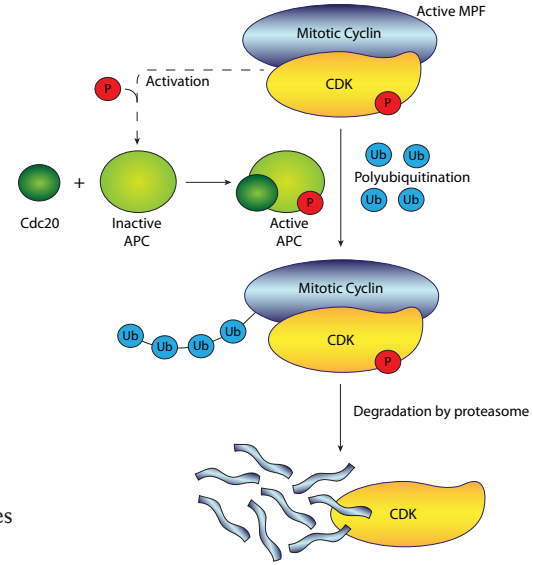

Figure 5. MPF and other cyclin/cdk complexes are inactivated by destroying the cyclin.

G_1/G_0 phase

The G_1 phase is the state a cell is in immediately following cytokinesis. At that point, the cells will be somewhat undersized, and need to take up materials and energy sources, and convert them to cellular components in order to support the eventual cell division. During this time, the cell goes about doing its "normal" business - an endocrine cell makes and secretes hormones, an intestinal epithelial cell absorbs nutrients from the gut and passes them on to the bloodstream, a neuron conducts signals, etc. Most types of cells spend the majority of their cycle in G_1, although there are exceptions, such as the frog oocytes mentioned earlier. The length of G_1 is generally constant for a given cell type under normal conditions, but can vary greatly between different cell types. Post-mitotic cells, which have left the cell cycle and will no longer divide, are in G_1 until they die, barring reactivation of the cell cycle by stress conditions. This continuous G_1-like state is referred to as G_0.

For those cells preparing to move from G_1 into S, cyclins D and E, and cdk 2, 4, and 6 predominate, with activation of cyclin D complexes preceding activation of cyclin E complexes. Two major questions are asked by the cell: is the DNA undamaged and complete, and is the extracellular environment favorable for cell division? The cellular sensors for these conditions then link to cyclin complexes effect restriction points on cell cycle progression. The extracellular environment questions can be a tricky one, because this can include more than just assessment of nutrient availability or predatory threats; it can also be a requirement for an external trigger such as a mitogenic hormone or paracrine signal. In fact, nearly all normal animal cells require an extracellular signal to progress through the G_1/S checkpoint. The cyclin E/ cdk2 combination is the principal regulator of entry into S phase and DNA replication.

The active cyclin E/cdk2 complex phosphorylates the tumor suppressor protein Rb (retinoblastoma), which causes E2F to translocate to the nucleus and turn on genes needed for entry into S phase.

S phase

The mechanisms of DNA replication were discussed in chapter 7. It is important to note that once a cell has entered S phase, it has essentially committed to going through cell division. Cells do not cope well with extra copies of chromosomes, and a cell that went through S phase without going through mitosis would likely have major malfunctions in gene regulation. For similar reasons, the cell must only undergo DNA replication once per cell division. The cyclinA/ cdk2 complex plays a key role in initiation of replication by activating the pre-replicative complex. It also phosphorylates cdc6, causing it to dissociate from the ORC, and consequently the rest of the pre-RC. This prevents immediate re-use of this origin of replication, and since the phosphorylation of cdc6 allows it to be recognized by a ubiquitin ligase complex, it is tagged for proteolysis.

In addition to DNA replication, S phase is also the cell cycle stage in which centrosomes are duplicated in animal cells. The cyclin E/cdk2 combination licenses the duplication of centrosome, phosphorylating nucleophosmin, which then dissociates from the centrosome. This helps to trigger the centrosome duplication. Nucleophosmin does not reassociate with centrosomes until telophase, when it is no longer phosphorylated. Plk4 (Polo-family kinase 4) activity is necessary for centriole duplication, and appears to initiate the centriole assembly mechanism.

G_2 phase

The G_2 phase begins when DNA replication has completed. Having said that, before the cell is allowed out of G_2 and on to M phase, it must pass a DNA fidelity checkpoint, ensuring that not only has replication been fully completed, but that there are no major errors. G_2 is a relatively short phase (compared to G_1) in most cell types, and it is spent building up energy and material stores for cell division and checking the DNA. If everything is ok, and the cyclin B/ cdk1 complex has been activated, the cell proceeds to M phase.

Mitosis

Mitosis consists of prophase, metaphase, anaphase, and telophase, with distinct cellular activities characterizing each phase. This completes the duplication of the nucleus, and is followed by cytokinesis, in which the cell divides to produce two daughter cells.

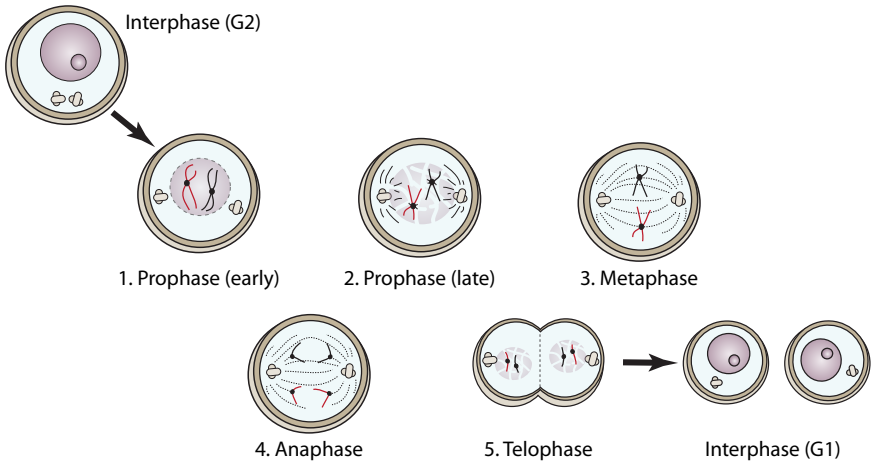

Figure 6. Mitosis. During mitosis, the nuclear envelope breaks apart to allow the spindle access to the chromosomes. Once they have been moved to opposite ends, the nuclear membrane reforms around each set. Finally, cytokinesis divides the cell into two new daughter cells.

The ubiquitin ligase complex, SCF, is made up of three major proteins and several minor species. Skp1 (S-phase kinase-associated protein 1) can be an RNA polymerase elongation factor, but in this complex links the other two proteins together. Cul1 (Cullin 1) is an E3 type ubiquitin ligase. Finally, an F-box family protein like Rbx1 (Ring-box 1), that heterodimerizes with cullin-1 and may also recruit E2 ubiquitinating enzyme.. In addition to cdc6, it also recognizes and ubiquitinates CKIs (cyclin complex kinase inhibitors) such as p27, which is involved in processes such as DNA repair and error-checking.

Prophase is the preparation of each component for this complex cellular dance. The DNA condenses (it is wrapped around itself tightly to make it a smaller and stronger package) so that it is less susceptible to breakage during movement across the cell. In doing so, most of the DNA becomes transcriptionally inactive. The Golgi bodies and the endoplasmic reticulum begin to break apart into membranous vesicles that can be more easily and evenly distributed across the cell so that both daughter cells receive about the same. The centrosomes (in animal cells) move from their original position near the nucleus toward opposite sides of the cell, to establish the poles of the mitotic spindle.

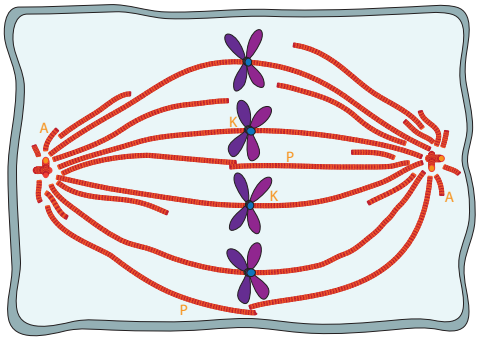

Figure 7. The mitotic spindle. The spindle is made of microtubules that originate from the centrosomes, which have migrated to opposite sides of the cell. There are three types of spindle microtubules: the kinetochore microtubules (K), polar microtubules (P), and astral microtubules (A).

MPF phosphorylates microtubule motor proteins and microtubule associated proteins (MAPs) to alter the normal microtubule dynamics and allow the massive reorganization into a mitotic spindle to occur. For example, one target of MPF is PRC1, a bundling protein that is inactivated by phosphorylation, thus allowing individual microtubules to move to new locations more easily than a large bundle could. Other effects are inactivation of stabilizing MAPs, which leads to greater lability of microtubules due to increased incidences of catastrophe. The motor protein targets of MPF are in the kinesin family and the phosphorylation is necessary for some of them to bind to the mitotic spindle.

Prometaphase is sometimes considered a separate phase but is also referred to as late prophase, and is primarily defined by the breakup of the nuclear envelope. This process is induced by MPF phosphorylation of the nuclear lamins. Adorned with negative charges from the phosphates, the lamins refuse to associate with one another any longer, leading to the breakdown of the nuclear lamina. As the lamins dissociate, the nuclear envelope remains bound to them, and fragments. This nuclear fragmentation must happen so that the mitotic spindle can reach inside and attach to the chromosomes.

Some of the microtubules of the mitotic spindle attach to the chromosomes via the kinetochore proteins, which link the spindle microtubules to the centromere region of each chromosome. These are known as kinetochore microtubules (fig. 8). There are two other types of microtubules in the mitotic spindle (fig. 7): the polar microtubules that reach across the cell and interact with one another to help maintain the separation of the centrosomes and defining the overall length of the spindle, and the aster microtubules that are generally short, radiating out from, and stabilizing the centrosome. Remember that the DNA replicated earlier in S phase, and thus sister chromatids are still partially attached. Visually, the centromere region appears narrower or more compressed than the rest of the chromosome, and generally lies near the middle. The centromere contains repeated sequences that are involved in kinetochore binding and assembly.

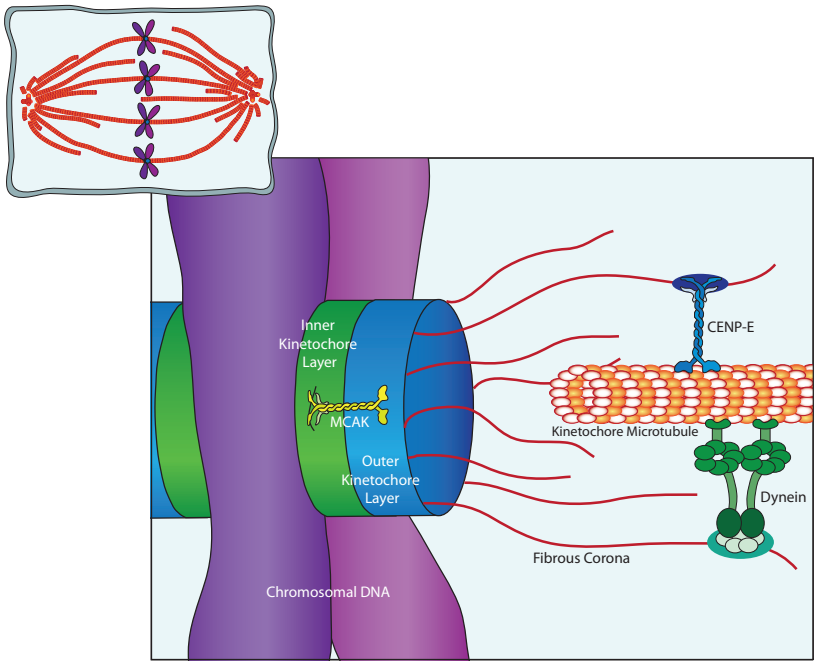

Figure 8. The kinetochore assembles on the centromere of the chromosome. Spindle microtubules attach to the fibrous corona of the kinetochore through kinesins and dyneins.

The kinetochores attaching to the centromere DNA are trilaminar protein structures consisting of an inner layer, an outer layer, and a fibrous corona. The kinetochore microtubules of the mitotic spindle are primarily attached to the fibrous corona. As depicted in the figure, it is attached through CENP-E, a kinesin, and dynein motor proteins that bind along the barrel of the microtubule. In fact, sometimes the first contact between a chromosome (via the kinetochore) and a spindle microtubule is somewhere

In primates, the repeating motif is known as alpha satellite DNA, which is made of multiple instances of tandem repeats of a core ~170bp sequence over a centromeric DNA span over a megabase in length. Similar repeats are found in various other vertebrates as well. In other eukaryotes, the size and sequence may vary; for example, much shorter repeats of ~5bp are found in centromeric DNA measuring 200-600kb in Drosophila chromosomes, and S. pombe has centromeric DNA well under 10kb.

in the middle of the microtubule, and a combination of microtubule dynamics and motor protein activity move the chromosome to the distal end of the microtubule. This is facilitated by MCAK (mitotic centromere-associated kinesin), which is associated with the kinetochore core proteins and plays a role in depolymerizing microtubules near the (+) end.

As the nuclear envelope is breaking apart, the mitotic spindle microtubules are undergoing increased dynamic instability, cycling between periods of growth spurts (polymerization) and rapid shortening (catastrophic disassembly), searching for chromosomes to connect to. Once the kinetochore microtubules connect to the chromosomes, the microtubule dynamics shift. The microtubule will primarily undergo shortening if it is beyond the center of the spindle and primarily lengthening if it is short of center. Since eventually each set of sister chromatids is connected to microtubules on both kinetochores, each chromatid is connected to one shortening and one lengthening microtubule. As the chromosomes approach the center of the mitotic spindle, the rate of microtubule shortening/lengthening slows. The sister chromatids are pushed and pulled by the spindle microtubules until they are all lined up along the midline of the mitotic spindle, which in most (but not all) cases is also the midline of the cell. Once they are all lined up, the cell is considered to have reached metaphase. Unlike the other phases, metaphase is a relatively static phase - it is a checkpoint for lining up the chromosomes.

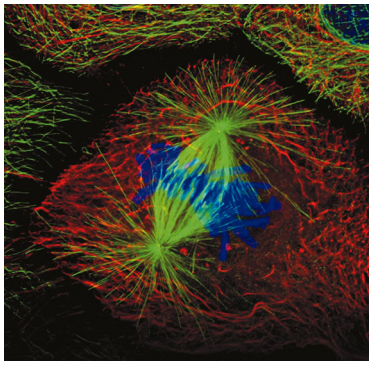

Figure 10. A cell at metaphase. Microtubules are stained green, f-actin is stained red, and chromosomes, with centromeres lined up along the midline, are stained blue. Note the surrounding cells, which are not in mitosis, with their MT and MF cytoskeletons more overlapped. This photo released to public domain by the US goverment.

The chromosomes must be properly aligned to ensure that both daughter cells receive the proper complement of chromosomes. How does the cell know when the chromosomes have reached the center of the spindle? An elegantly simple experiment demonstrated that the general mechanism is a tension check - if the two microtubules connecting to the pair of sister chromatids from each side are of the same length, they should be exerting equal tension on the chromosomes. If the microtubule-kinetochore connection is severed at metaphase, the cell will be prevented from progressing (Nicklas, R.B., et al, *J. Cell Biol.* **130**: 929-39, 1995). However, if an equivalent tension is applied by tugging on the chromosome with a glass microneedle, progression of mitosis is restored!

The transition from the interphase microtubule cytoskeleton to a mitotic spindle require a number of molecular motors to move the centrosomes, align the microtubules, and expand the spindle. These are depicted in fig. 9. Initially, as the duplicated centrosomes move away from each other along with some of the cytoskeletal microtubules, the microtubules will interact at various angles. Because the polar microtubules that help to expand or maintain the spindle width must ineract in parallel, cytoplasmic dyneins bind to the eventual polar microtubules and by moving one along the other, bring them into parallel (9a). Once in that position, BimC and other kinesins take over as the primary motors along polar microtubules. They create an outward pushing force by holding onto a microtubule facing one direction, and driving along a parallel MT facing the opposite direction towards the (+) end (9b). Finally, cytosolic dyneins attached to cortical cytoskeleton pull on the astral microtubules, which pulls the spindle ends further from center (9c).

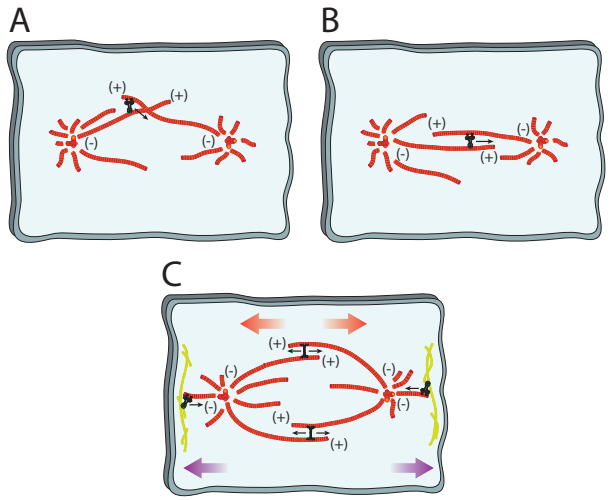

Figure 9. Molecular motors set up the mitotic spindle.

In fact, there appear to be two mechanisms at work: the bub1/bub2 system works in the tension sensing pathway, while another metaphase protein, mad2 appears to be important in suspending mitosis upon disconnection of the kinetochore with the spindle microtubule.

In addition to the tension check, there is another condition that must be met for continuation of mitosis: the MPF must be inactivated. As outlined earlier, MPF in part leads to its own inactivation by activating the anaphase-promoting complex (APC), which polyubiquitinates the cyclin, leading to its destruction and thus MPF-cdk inactivation. APC also tags securin for destruction. Securin is a protein that binds and inhibits the proteolytic enzyme, separase, the activation of which is needed to allow the sister chromatids to separate, which in turn, is necessary for anaphase to proceed.

Barring pathological situations, if and only if the chromosomes all line up at the metaphase plate will the cell proceed to the next stage of mitosis: anaphase. The sister chromatids separate and are pulled toward opposite poles of the mitotic spindle. Somewhat perversely, even as the chromosomes move towards the spindle poles, the poles themselves move outward slightly. Separation of the sister chromatids requires the dissociation of the molecular "glue" holding them together: the cohesin proteins. The cohesins bind to both molecules of DNA and hold them together shortly after replication back in S phase. As anaphase approaches, the enzyme separase is activated, which then cuts the cohesin molecules. Once all of the cohesin molecules are cut, the sister chromatids can finally be separated. The removal of the cohesins proceeds roughly inwards from the distal points of the chromosomes to the centromere, which is generally the last region of attachment.

A cohesin is a multimer of four subunits, Scc1, Scc3, Smc1, and Smc3 in yeast. An additional protein has also been observed in Xenopus. The SCC1 protein is cleaved by separin in yeast, but in metazoans, SCC1 may be removed from chromosomes by another method as well. It is phosphorylated, which decreases its affinity for DNA, and may expose a site for separase-catalyzed hydrolysis.

Anaphase can actually be divided into two stages, sometimes referred to as early and late or A and B. At first, the kinetochore microtubules are shortening from both ends, and kinesin-family motors pull the microtubules back toward the spindle poles. As late anaphase starts, polar microtubules elongate, and an additional chromatid-separating force is applied by kinesin-family motor proteins [kinesin-5] that push the polar microtubules against one another to increase the separation between the poles. Dynein-family motors help to direct movement of the poles as well, through their attachment to the aster microtubules and the cortical (peripheral) cytoskeleton.

Separase also promotes anaphase by activating Cdc14, a phosphatase needed to dephosphorylate the cdk substrates that had been phosphorylated by the cyclin-cdk complexes of early mitosis. In addition, Cdc14 is also required for cytokinesis in the yeast S. cerevisiae and nematode C. elegans.

When both sets of chromosomes arrive at their respective poles, telophase begins. Technically, it was slowly building up since anaphase: when MPF was inactivated by APC, its ability to phosphorylate nuclear lamins was ended. Protein phosphatases in the cell remove the phosphate groups, allowing the lamins to once again interact with one another, and by telophase they are reconstituting the nuclear lamina and the nuclear envelope. Since the lamins and other nuclear membrane proteins also interact with DNA, the nuclear membrane fragments dispersed back in late prophase now coalesce around each set of DNA to form the new nuclear envelopes. The other fragmented membranous organelles (ER, golgi) also start to re-form. By the end of telophase, the product is a single large cell with two complete nuclei on opposite sides. The next and

last step, cytokinesis, splits the cell into two separate and independent daughter cells. In animal cells, cytokinesis is similar to the tightening of a drawstring in the middle of the cell, pulling the "waist" in until all edges meet, and two separate cells result. This contractile ring is composed of actin (structural) and myosin (motive) subunits. These proteins, using ATP for energy, ratchet themselves closer and closer together similar to the actin-myosin "power stroke" described for muscle cell sarcomeres, also primarily made from actin and myosin. This mechanism is universal for animal cells, but the placement of the ring is not always in the center of the cell. The ring often coincides with the center of the cell, but is in fact positioned by the metaphase plate (i.e. the center of mitotic spindle). The most obvious example of a metaphase plate that does not coincide with the center of the cell is found in the formation of egg cells. Because the purpose of an egg cell is to provide all of the material necessary to make a viable new organism upon fertilization (the sperm contributes negligible biomass beyond the genetic material), it divides asymmetrically, with the mitotic spindle located far to one side of the cell (fig. 19). When cytokinesis occurs, one daughter cell, the presumed oocyte, is very large, while the other cell, called a polar body, has minimal cytoplasmic material surrounding the nucleus. The contractile ring works in animal cells because the cell membrane is flexible. In plant cells, the cell membrane is firmly attached to a rigid cell wall, and thus cannot be pulled in. So, the plant cell ingeniously builds a wall down the middle of the cell using specialized vesicles that originate from part of the Golgi, and which contain the materials necessary to form a cell wall. The vesicles travel along the *phragmoplast*, a structure built from the mitotic spindle microtubules, and as the vesicles line up along the middle of the cell, they begin to fuse to form bigger vesicles and then a large disk-like vesicle, the cell plate. Eventually they reach the cell membrane itself, and fusing with that leads to formation of a new cell wall, and two complete and independent cells.

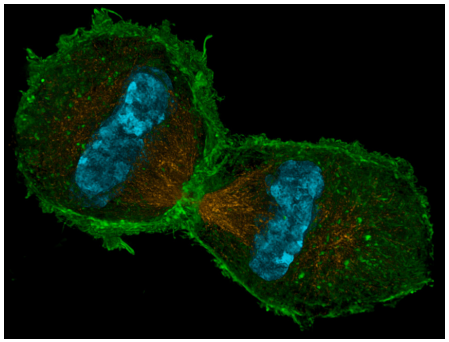

Figure 11. Telophase/ Cytokinesis. The contractile ring and other actin structures are stained green, the microtubules are orange, and the chromosomes are blue. Photo released to public domain by US government.

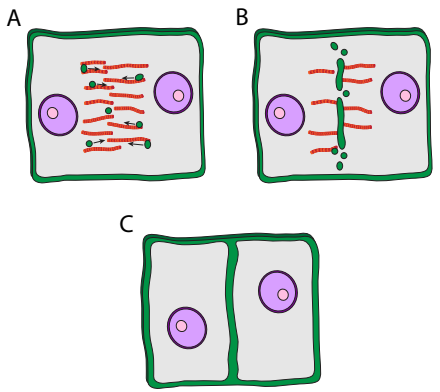

Figure 12. Cytokinesis in plant cells. Golgi-derived vesicles filled with cell wall material travel along the phragmoplast and fuse in the center to form a new cell wall.

The contents of the vesicles traveling along the phragmoplast are not well described. Callose, a glucose polysaccharide with β1-3 linkages is known to be present in the developing cell plate, but has not been found in the Golgi or vesicles. Interestingly, once the cell plate has fused completely with the existing cell walls, callose gradually disappears. It is thought that the same enzyme system that synthesized callose may switch to synthesizing cellulose as the cell plate matures.

Cell Death

A cell may die either intentionally (usually referred to as apoptosis or programmed cell death, though also once known also as "cellular suicide"), or unintentionally (necrosis). The microscopic observation of these two processes shows strikingly different mechanisms at work. In apoptosis, the cell begins to shrink and lose shape as the cytoskeleton is degraded, then the organelles appear to pack together, except for the nucleus. Inside the nucleus, the chromatin condenses and attaches to the nuclear envelope, which then loses its integrity and starts to break apart. The cell membrane begins to show irregularities, descriptively known as blebs, and eventually, the cell breaks apart into vesicles that are neatly cleaned up by phagocytes drawn to the site by apoptotic signals emitted by the dying cell. Necrosis, on the other hand, is quite literally a mess. The cell appears to swell and the plasma membrane begins to lose its integrity. It is soon catastrophically leaking cytoplasm, and leaves behind cell debris that can accumulate and trigger necrotic death of adjacent cells.

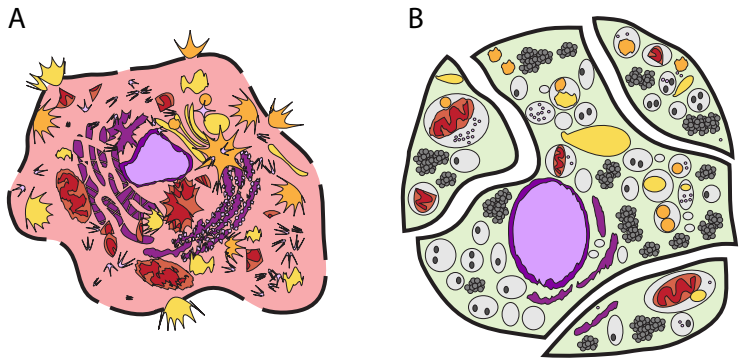

Figure 13. (A) A cell underdying by necrosis is disorganized, generally bursts and leaks its contents. (B) A cell undergoing apoptosis first subdivides itself, digesting itself in an orderly fashion and compartmentalizing everything for scavenging by phagocytes.

Apoptosis is ultimately put into action by a cascade of caspases, a family of proteolytic enzymes. This family of enzymes is generally produced as proenzymes that are activated by other members of the caspase family. Thus a cascade effect occurs, after the initial trigger activating one set of caspases, they can then cleave a variety of proteins including procaspases that are thereby activated and can hydrolyze even more proteins, including yet another type of procaspase, and so on. Of course, other enzymes are also activated and participate by widening the response, activating other groups of proteases and apoptotic enzymes. Triggering the apoptotic cascade is usually one of two general pathways: an internal trigger, arising from damage to the mitochondria, and an external trigger, started by binding an extracellular signal molecule to activate

a “death receptor”. Although there are many variations on both triggers, they follow similar paths to the examples we will use here.

If you recall the section on electron transport in oxidative phosphorylation, then you may also recall the soluble electron carrier, cytochrome c. This protein is exclusively found in the mitochondrial matrix under normal circumstances, so its presence in the cytoplasm can be taken to indicate mitochondria in distress. Given the importance of mitochondria in providing the energy for most aerobic cells to carry out their normal life, such distress is an early indicator that the cell will die soon. The diagram at right shows a sample pathway that can cause cytochrome c leakage from the mitochondria, but mitochondria can also just “get old”, and if the cell is “programmed” (by transcription factors) not to replace failing components, then as the mitochondrial membranes lose integrity and allow cytochrome c out, it is a clear signal to initiate termination protocols, to use the parlance of science fiction novels. The cytochrome c is bound by APAF-1 (apoptotic protease activating factor 1) which oligomerizes to form an apoptosome made of 7 APAF-1 moecules and 7 cytochrome c molecules. The apoptosome binds and activates procaspase-9 to initiate a caspase cascade that continues with activation of procaspase-3. When the mitochondria leaks cytochrome c, it also leaks another apoptotic protein, SMAC/Diablo. This protein, among other functions, inhibits IAP (inhibitor of apoptosis) -family proteins. The IAP proteins normally inhibit caspase activation both directly and indirectly to prevent cell death, and SMAC/Diablo blocks that inhibition.

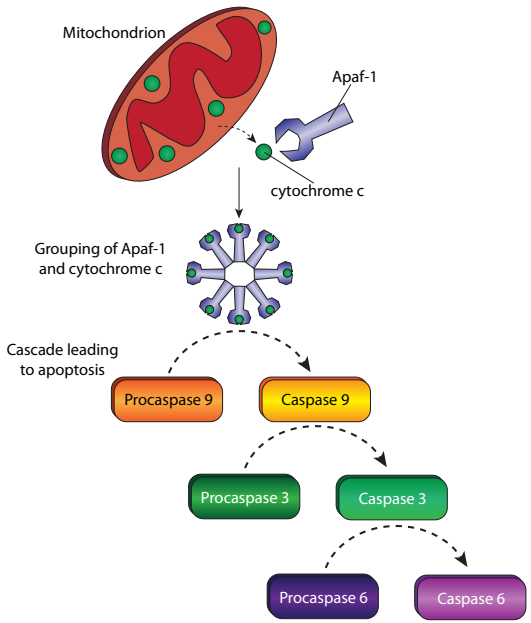

Figure 14. Apoptotic signaling cascades may be initiated by leakage of cytochrome c into cytoplasm.

When death receptors are activated, the subsequent caspase cascade does not involve the mitochondria or APAF-1. The best studied case, FasR (Fas receptor) activates caspases 2, 8, and 10 by clipping procaspases and by releasing caspases from inhibiting complexes. These activate caspases 3, 6, and 7, which leads to the final stages of apoptosis. In both internally and externally triggered apoptosis, the final steps are the same: some of the final targets of the caspases are the nuclear lamins and ICAD (inhibitor of cas-

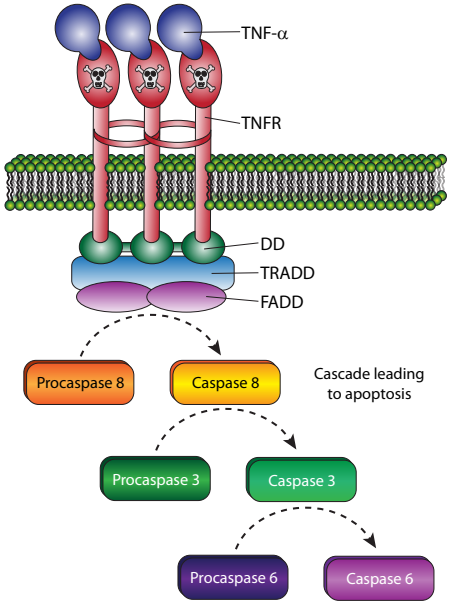

Figure 15. Apoptotic signaling cascades may be started by external activation of a "death receptor" such as FasR.

pase-activated DNase). Destroying the nuclear lamins leads to fragmentation of the nuclear envelope, while removing ICAD activated the caspase-activated DNase (CAD) which then begins to digest the DNA.

Why does the apoptosis mechanism exist? There are two major (and many other) reasons for apoptosis. The first is developmental. In the development of an organism, the most effective strategy is often to have overgrowth of cells that are then pruned back to the proper formations. Examples of this are the apoptotic death of tissue between initially connected fingers and toes (we humans start with webbed fingers and toes embryonically), and death of unconnected or improperly connected neurons. The latter case also illustrates a fundamental principle in mammalian cell biology, and most other vertebrates as well: cells require signals (trophic factors) to stay alive. In this example, the neurons that do not make proper connections to a target cell do not receive the necessary trophic factor (secreted by the target). This leads to apoptotic death of the unconnected neuron. In fact, if apoptosis is blocked due to mutation to a gene in the pathway, there is severe overgrowth of the brain and spinal cord, causing serious malfunction and craniofacial deformities. Thus in development, apoptosis is necessary to control the growth of different parts of a metazoan organism.

The other major function for apoptosis is to kill dangerous cells. In some cases, these may be cells infected by a pathogen. In others, the cells have accumulated mutations that do have affected the DNA error-correction system or cell-cycle checkpoints. When the former occurs, each generation has an increased likelihood of even more mutations. It is important to activate apoptosis in such cells before they have a chance to acquire errors that removes all cell cycle checkpoints, allowing unchecked cell proliferation. This could lead to tumor formation and potentially cancer (see next chapter). When such cells need to be killed for the benefit of the organism, it may happen by the triggering of an internal sensor such as mitochondrial damage, or by external means, such as an immune system cell recognizing an infected cell.

Meiosis

In metazoa, there are two situations in which a cell gives rise to daughter cells. The first, and by far most common, is mitosis. The second is meiosis. Meiosis is the process by which gametes (sex cells) are generated. Animals and plants are generated by sexual reproduction (if this is news to you, please consider majoring in something other than biology). These organisms start life through the fusion of two cells: a sperm and an egg. Both contribute genetic material to the new organism. In order to maintain the proper number of chromosomes in each generation, the gametes each contribute one set of chromosomes, so that the fertilized egg and all other cells in the organism have two sets of chromosomes — one from each parent. The purpose of meiosis, and its primary difference with mitosis, is not generating daughter cells that are exact replicates, but generating daughter cells that only have half the amount of genetic material as the original cell.

Let us take a look at this situation selfishly: meiosis in human beings. Almost every cell in your body has a nucleus containing 46 chromosomes, a set of 23 from your father, and a set of 23 from your mother. The only exceptions are the gametes: the spermatocytes in men and the oocytes in women. The somatic cells are said to be 2n or diploid, that is having 2 sets of chromosomes, and the gametes are 1n or haploid, having only one set of chromosomes. Sometimes, meiosis can be a little confusing to students because it occurs in the same part of the cell cycle as mitosis, which is to say after G_2. Because of this, the cell entering meiosis actually has 4 sets of chromosomes, since the DNA has already undergone replication in S phase.

Meiosis consists of two consecutive meiotic divisions each of which has phases similar to mitosis: prophase, metaphase, anaphase, telophase, and each of which finishes with complete cytokinesis. Note that immediately following meiotic telophase I, the cell divides, and both daughter cells are immediately in prophase II. There is no intervening G_1, S, or G_2 phase.

Prophase I of meiosis begins very similarly to prophase of mitosis: MPF (mitotic-cdk) activation, chromosome condensation, spindle formation and nuclear envelope breakdown. However, compared to mitosis, meiotic prophase I lasts for a very long time and can be subdivided into five stages: leptotene, zygotene, pachytene, diplotene, and diakinesis. During leptotene, the two sets (maternal and paternal) of sister chromatids for each chromosome condense, align and form a structure known as a bivalent. To clarify, this bivalent consists of four copies of a given chromosome: two copies each of the maternal chromosome and of the paternal chromosome. Because the maternal and

Mature red blood cells contain no nucleus, and some muscle cells, while multinucleated because they form from the fusion of several myoblasts, nevertheless have 46 chromosomes in each of the nuclei.

Polyploidy, while uncommon in humans, is a normal state for many organisms. The frog, *Xenopus laevis*, a common research animal, is tetraploid.

paternal versions of a given chromosome are kept in extremely close proximity for an extended period of time, there is a greater chance of a *recombination*, or crossing over and exchange of homologous pieces of each chromosome.

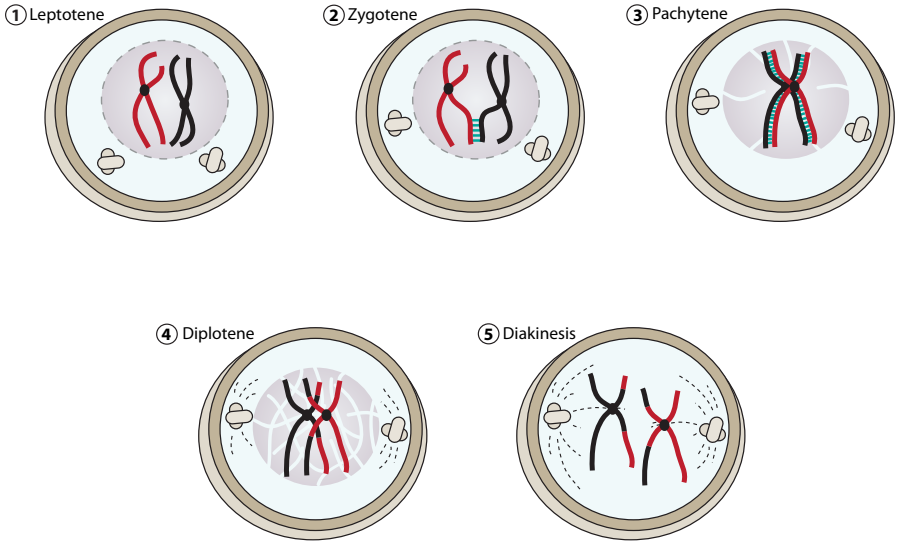

Figure 16. The five stages of Meiotic Prophase I.

Recombination occurs when a piece of the paternal chromosome is swapped for the homologous piece of DNA on the matching maternal chromosome (or vice versa). Note that sister chromatids (i.e. exact copies) do not recombine - only homologous non-sister chromatids can recombine. Obviously, this kind of a DNA swap must be done carefully and with equivalence, so that the resultant DNA on each side contains all the genetic information it is supposed to, and no more information than it is supposed to. In order to ensure this precision in recombination, the non-sister homologous chromatids are held together in a synaptonemal complex (SC). This ladder-like complex begins to form in the zygotene stage of prophase I and completes in pachytene. The complete SC consists of proteinaceous lateral elements (aka axial elements) that run along the length of the chromatids and a short central element composed of fibrous proteins forming the rungs of the ladder perpendicular to the two lateral elements. The central element is formed of transverse filament dimers that interact with one another in offset fashion, as well as with the lateral elements. These filament proteins (e.g. SCP1 (mouse), Zip1p (yeast)) have central coiled-coil regions that function as protein interaction domains. Although SCP3 and therefore complete lateral element formation are unnecessary for a functional synaptonemal complex, condensin and cohesin do appear to be necessary for proper transverse filament attachment of the lateral elements.

Lateral elements are composed several proteins, including condensins and cohesins. The cohesins are meiosis-specific variants, with substitutions for the Scc1 and Scc3. Likewise, condensin subunits also have meiosis-specific alleles. In addition to the condensins and cohesins, which other than their meiotic-specific variants, are common chromosomal proteins, there are SC-specific proteins, including SCP2 and SCP3. Both are localized to condensed chromosomes in early meiosis, and SCP3 has been show by knockout analysis to be necessary for lateral element formation. However, it is not necessary for recombination.

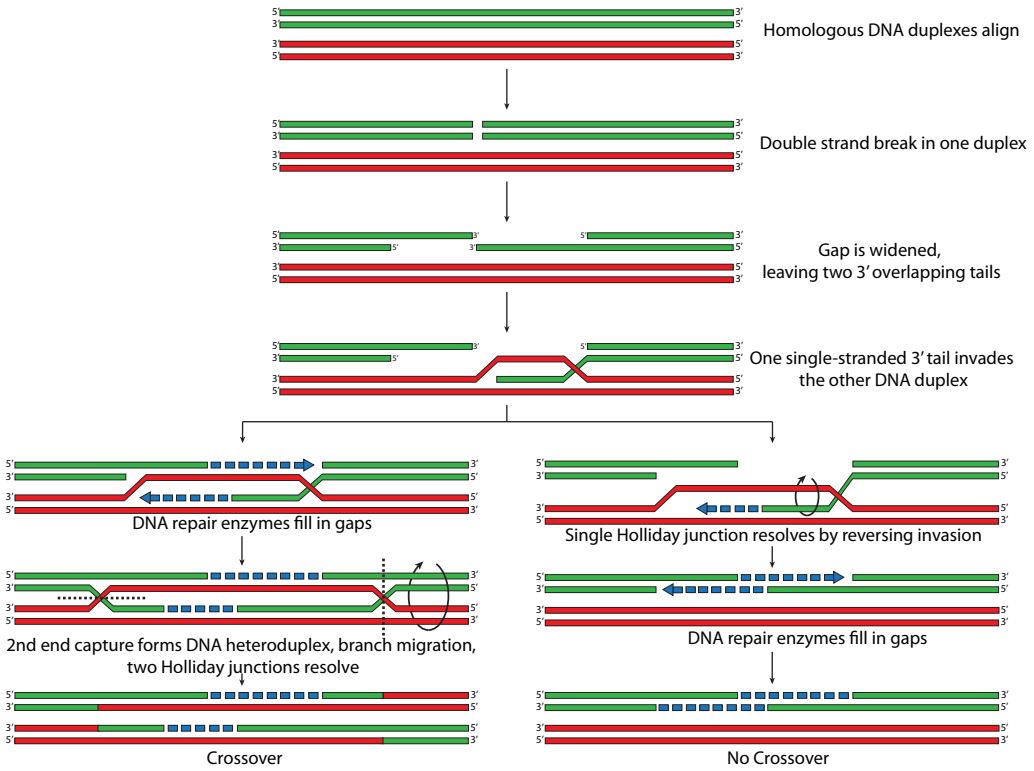

Figure 17. Recombination of homologous chromosomes.

Recombination may occur with or without the formation of double-strand breaks, and in fact, can occur without the formation of the synaptonemal complex, although the SC probably enhances the efficiency of recombination. In *S. pombe*, meiosis occurs without the formation of a synaptonemal complex, but there are small discontinuous structures somewhat similar to parts of the SC. In the fruit fly, *Drosophila melanogaster*, females undergo meiosis using a synaptonemal complex, but males do not undergo meiotic recombination, and their chromosomes do not form synaptonemal complexes. In most cases, recombination is preceded by the formation of recombination nodules, which are protein complexes that form at potential points for recombination. The best studied mechanism for meiotic recombination involves a double-stranded break of one of the chromosomes initiated by the meiosis-specific endonuclease, Spo11. The 5' ends (one in each direction) of this cut are degraded slightly to form 3' single-stranded overhangs. This leads to the formation of Holliday junctions with a strand from one chromosome acting as a template for a missing portion of the homologous cut chromosome. This may be resolved one of two ways, with or without a crossover, as illustrated (fig. 17).

The recombination is initiated in pachytene and completes in diplotene, at which time the synaptonemal complex breaks down. As the chromatids begin to separate, chiasmata become apparent at some of the recombination sites. As prophase completes, the chiasmata resolve from the center of the chromosomes to the ends.

As the cell goes from meiotic prophase I to meiotic metaphase I, another difference between mitosis and meiosis is revealed: the chromosomes line up at the metaphase plate as tetrads rather than as pairs. Because of this, when they pull apart in anaphase, sets of sister chromatids segregate to opposite poles. Of course, due to recombination, the sister chromatids are unlikely to still be identical.

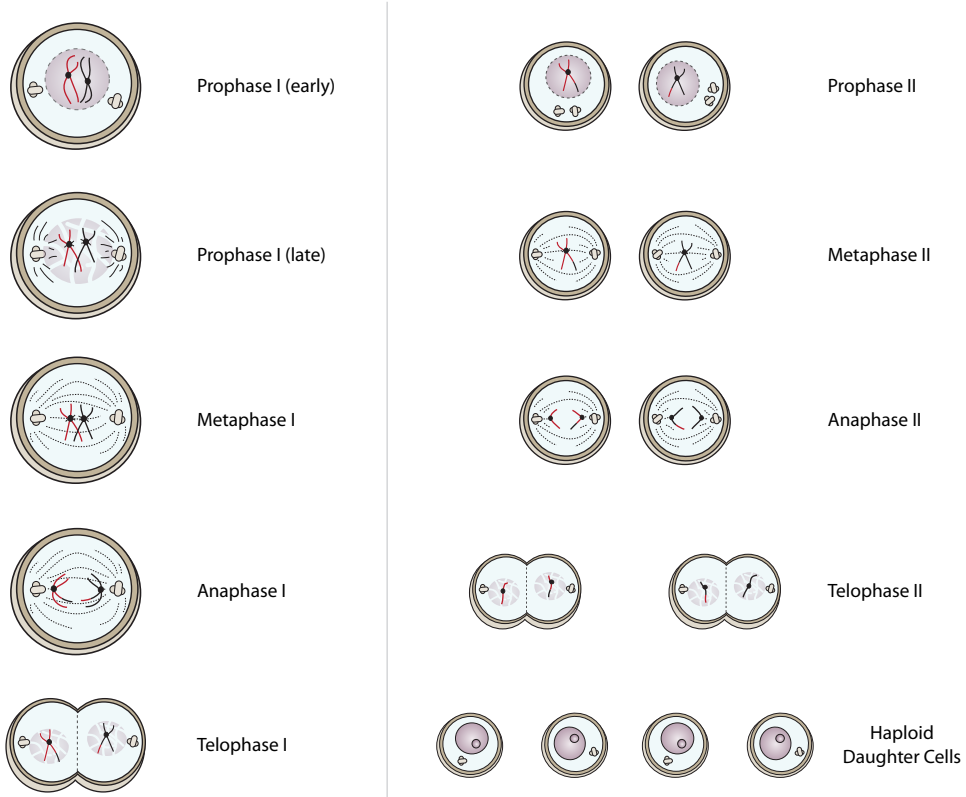

Figure 18. Meiosis generates 4 haploid daughter cells from one diploid precursor. To do so, it undergoes a two rounds of meiotic nuclear and cell division,

After a conventional anaphase and telophase, the cell splits, and immediately the daughter cells begin the second meiotic division (fig. 18, right side). In some cell types, chromosomes do not decondense in meiotic telophase I, but if they have, they re-condense

in meiotic prophase II. Prophase II proceeds similarly to *mitotic* prophase, in that there is no formation of synaptonemal complexes or recombination. At metaphase II, the sister chromatids line up along the metaphase plate just as in mitosis, although now there are only 2n chromosomes in the cell, while in mitosis there would have been 4n (because the DNA has replicated). Again, finishing the rest of the division almost exactly like mitosis, the sister chromatids pull apart in anaphase II, the nucleus reforms in telophase II, and the final cytokinesis generates a total of four cells from the original one that entered into meiosis, each containing 1n chromosomes.

Egg cells, as genetic and bulk material donors, need to be large but sperm cells, as genetic donors only, do not. The diagram below depicts the generation of the egg cells. Only one oocyte is generated from a meiotic event; the other three daughter cells are termed polar bodies, and contain so little cytoplasmic material that they are only viable for a short time. The asymmetric distribution of cytoplasm in the first meiotic division for oocytes is due to the position of the meiotic spindle in the periphery of the cell rather than centered. Since the center of the spindle determines the position of the contractile ring for cytokinesis, this leads to unevenly sized daughter cells.

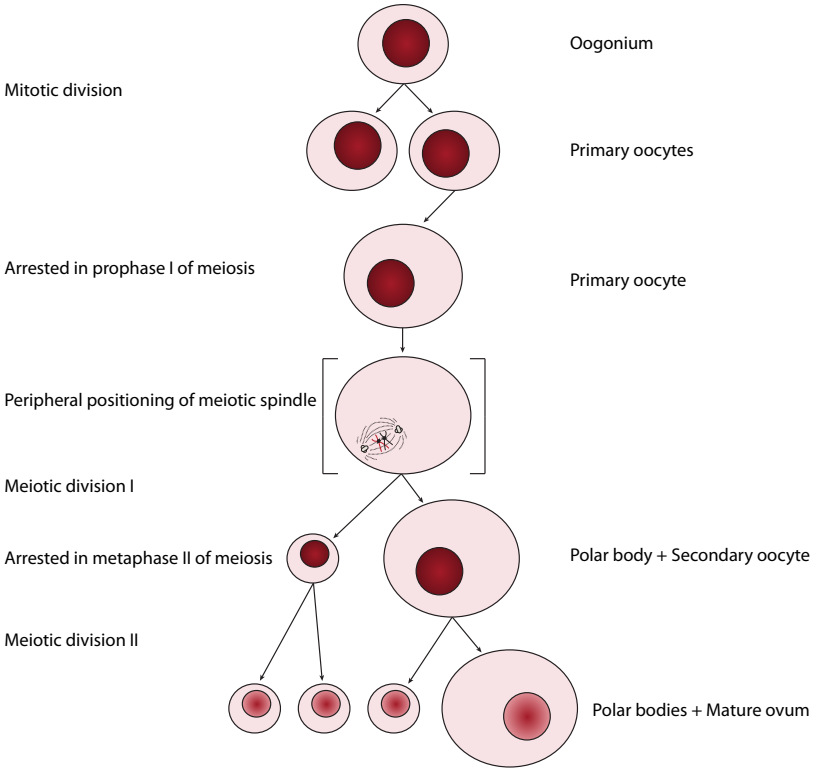

Figure 19. Oogenesis.

The generation of the very small sperm is a different mechanism altogether. In the meiotic steps of spermatogenesis, the cell divisions are equal, with the meiotic spindle aligned with the center of the cell, and the cells have equal amounts of cytoplasm, much like an average cell that has undergone mitosis. The streamlined, minimal-cytoplasm mature sperm is a product of post-meiotic differentiation, in which it gains the flagellar tail, and ejects most of its cytoplasmic material, keeping only some mitochondria to power the flagella, and an acrosomal vesicle, that contains the enzymes and other molecules needed to reach and fuse with (i.e. fertilize) an egg.

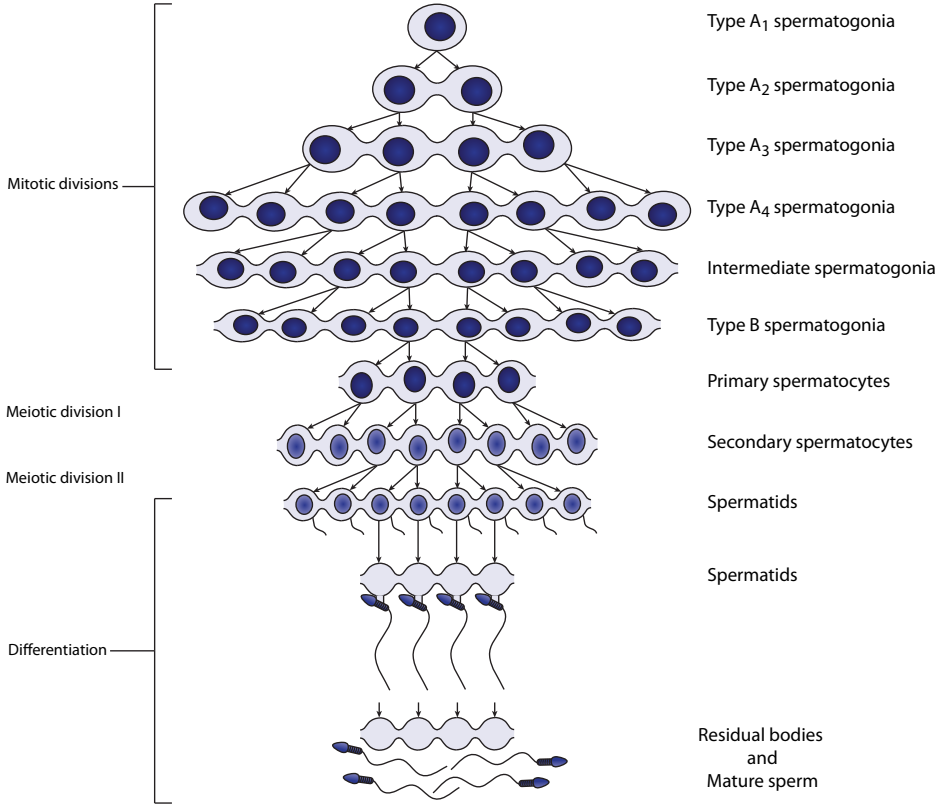

Figure 20. Spermatogenesis

Not all organisms reproduce with the human-like egg and sperm mechanism, i.e. gametic meiosis. As just described, in a gametic meiosis life cycle, meiosis generates haploid gametes, which then fuse/fertilize to become a diploid zygote. The zygote becomes a multicellular diploid organism, and once it reaches sexual maturity can make more haploid gametes via meiosis. The only multicellular state is diploid, and the gametes are haploid.

A common variation is sporic meiosis, used in all plants and many types of algae. In this usage, "spore" refers to eukaryotic spores, and not to bacterial endospores, which are simply dormant bacteria. Sporic meiosis does not directly produce gametes. Instead, meiosis produces haploid spores, which can develop by mitosis in haploid multicellular organisms. These organisms (termed gametophytes) can produce (still haploid) gametes by mitosis, that when fused/fertilized form a diploid zygote. This zygote can then develop into a diploid multicellular form called the sporophyte. Finally, the sporophyte is able to generate more spores by meiosis.

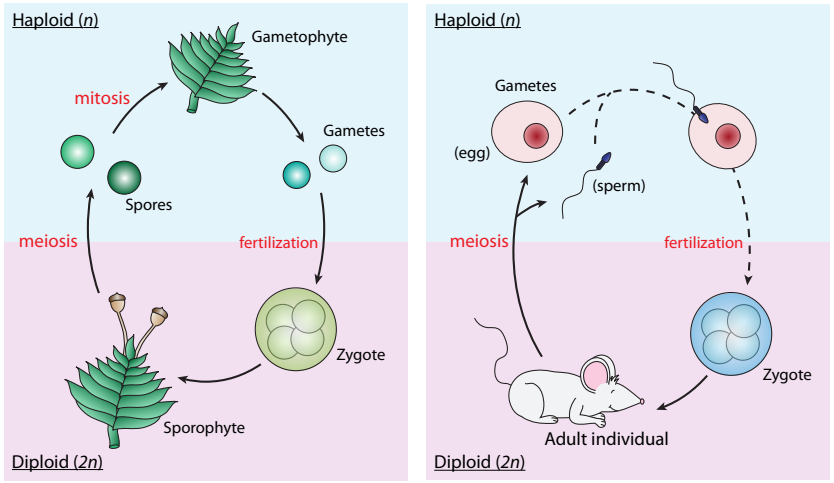

Figure 21. Gametic meiosis (left) and Sporic meiosis (right).

An example of this type of life-cycle and the role of meiosis is found in moss. What we think of as the body of the moss is actually a gametophyte, made up of haploid cells generated by mitotic division of a haploid spore. These gametophytes generate either sperm or eggs in specialized structures in their distal tips, and under the right conditions (e.g. rain) the sperm is carried to the eggs and fertilization occurs. The fertilized (diploid) egg now develops by mitotic division and differentiation into a sporophyte. In this case, the sporophyte is a specialized reproductive structure on the tip of the moss, and is also diploid. On the tip of the sporophyte is the sporangium, which is where meiosis takes place to generate haploid spores. The spores may then be dispersed (by wind or rain) and begin the cycle again by dividing and forming a new gametophyte.