Chapter 14
Cell Division

14.1. The Cell Cycle

A eukaryotic cell cannot divide into two, the two into four, etc. unless two processes alternate: **doubling** of its **genome** (**DNA**) in **S phase** (synthesis phase) of the cell cycle and **halving** of that genome during **mitosis** (**M phase**). The period between M and S is called $\mathbf{G_1}$; that between S and M is $\mathbf{G_2}$.

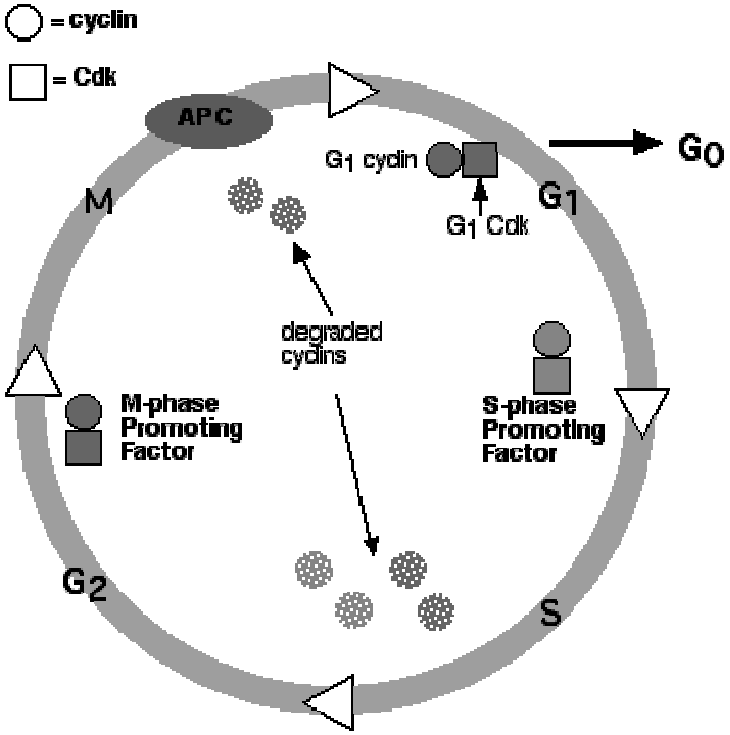

Fig.14.1. The Cell Cycle

So, the cell cycle consists of:

1. $\mathbf{G_1}$ = growth and preparation of the chromosomes for replication
2. **S** = synthesis of DNA (and centrosomes)
3. $\mathbf{G_2}$ = preparation for
4. **M** = mitosis

When a cell is in any phase of the cell cycle other than mitosis, it is often said to be in **interphase**.

(a) Control of the Cell Cycle

The passage of a cell through the cell cycle is controlled by proteins in the cytoplasm. Among the main players in animal cells are **Cyclins,** a $\mathbf{G_1}$ **cyclin** (cyclin D), **S-phase cyclins** (cyclins E and A) and **mitotic cyclins** (cyclins B and A). Their levels in the cell rise and fall with the stages of the cell cycle. **Cyclin-dependent kinases (Cdks),** a $\mathbf{G_1}$ **Cdk** (Cdk4), an **S-phase Cdk** ((Cdk2) and an **M-phase Cdk** (Cdk1). Their levels in the

cell remain fairly stable, but each must bind the appropriate cyclin (whose levels fluctuate) in order to be activated. They add phosphate groups to a variety of protein substrates that control processes in the cell cycle. The **anaphase-promoting complex (APC)**. (The APC is also called the **cyclosome**, and the complex is often designated as the **APC/C**.) The APC/C triggers the events leading to destruction of the **cohesins** thus allowing the sister chromatids to separate; degrades the mitotic cyclin B.

(b) Steps in the Cycle

A rising level of **G_1-cyclins** bind to their Cdks and signal the cell to prepare the chromosomes for replication. A rising level of **S-phase promoting factor** (**SPF**) — which includes cyclin A bound to Cdk2 — enters the nucleus and prepares the cell to duplicate its DNA (and its centrosomes). As DNA replication continues, cyclin E is destroyed, and the level of mitotic cyclins begins to rise (in G_2). **M-phase promoting factor** (the complex of mitotic cyclins with the M-phase Cdk) initiates assembly of the mitotic spindle breakdown of the nuclear envelope and condensation of the chromosomes. These events take the cell to **metaphase** of mitosis. At this point, the M-phase promoting factor activates the **anaphase-promoting complex** (**APC/C**) which allows the sister chromatids at the metaphase plate to separate and move to the poles (= anaphase), completing mitosis; destroys **cyclin B**. It does this by attaching it to the protein **ubiquitin** which targets it for destruction by **proteasomes**. turns on synthesis of G_1 cyclin for the next turn of the cycle and degrades **geminin,** a protein that has kept the freshly-synthesized DNA in S phase from being re-replicated before mitosis. This is only one mechanism by which the cell ensures that every portion of its genome is copied once — and only once — during S phase. Some cells deliberately cut the cell cycle short allowing repeated S phases without completing mitosis and/or cytokinesis. This is called **endoreplication**

(c) Meiosis and the Cell Cycle

The special behaviour of the chromosomes in **meiosis I** requires some special controls. Nonetheless, passage through the cell cycle in meiosis I (as well as meiosis II, which is essentially a mitotic division) uses many of the same players, e.g., **MPF** and **APC**. (In fact, MPF is also called **m**aturation-**p**romoting **f**actor for its role in meiosis I and II of developing **oocytes.**

(d) Checkpoints: Quality Control of the Cell Cycle

The cell has several systems for interrupting the cell cycle if something goes wrong. A check on completion of S phase. The cell seems to monitor the presence of the **Okazaki fragments** on the lagging strand during DNA replication. The cell is not permitted to proceed in the cell cycle until these have disappeared. **DNA damage** checkpoints. These sense DNA damage before the cell enters S phase (a G_1 checkpoint); during S phase, and after DNA replication (a G_2 checkpoint). The **spindle checkpoints**. Some of these that have been discovered detect any failure of spindle fibres to attach to **kinetochores** and arrest the cell in **metaphase** (M checkpoint); detect improper alignment of the spindle itself and block **cytokinesis** and trigger **apoptosis** if the damage is irreparable.

All the checkpoints examined require the services of a complex of proteins. Mutations in the genes encoding some of these have been associated with **cancer**; that is, they are

oncogenes. This should not be surprising since checkpoint failures allow the cell to continue dividing despite damage to its integrity.

(d) Examples

(i) p53

The p53 protein senses DNA damage and can halt progression of the cell cycle in G_1. Both copies of the *p53* gene must be mutated for this to fail so mutations in *p53* are recessive, and *p53* qualifies as a **tumour suppressor** gene. The p53 protein is also a key player in **apoptosis**, forcing "bad" cells to commit suicide. So if the cell has only mutant versions of the protein, it can live on — perhaps developing into a cancer. More than half of all human cancers do, in fact, harbor *p53* mutations and have no functioning p53 protein. In some way, p53 seems to evaluate the extent of damage to DNA, at least for damage by radiation. At low levels of radiation, producing damage that can be repaired, p53 triggers arrest of the cell cycle until the damage is repaired. At high levels of radiation, producing hopelessly damaged DNA, p53 triggers apoptosis. Possible mechanism: Serious damage, e.g., **double-stranded breaks** (DSBs), causes a **linker histone** (H1) to be released from the chromatin. H1 leaves the nucleus and enters the cytosol where it triggers the **release of cytochrome c** from mitochondria leading to **apoptosis**.

(ii) ATM

ATM (="**a**taxia **t**elangiectasia **m**utated") gets its name from a human disease of that name, whose patients — among other things — are at increased risk of cancer. The ATM protein is involved in detecting DNA damage, especially **double-strand breaks;** interrupting (with the aid of p53) the cell cycle when damage is found; and maintaining normal **telomere** length.

(iii) MAD

MAD (="**m**itotic **a**rrest **d**eficient") genes (there are two) encode proteins that bind to each kinetochore **until** a spindle fibre (one microtubule will do) attaches to it. If there is any failure to attach, MAD remains and blocks entry into anaphase. Mutations in *MAD* produce a defective protein and failure of the checkpoint. The cell finishes mitosis but produces daughter cells with too many or too few chromosomes (aneuploidy). **Aneuploidy** is one of the hallmarks of **cancer cells** suggesting that failure of the spindle checkpoint is a major step in the conversion of a normal cell into a cancerous one. Infection with the **human T cell leukemia virus-1** (**HTLV-1**) leads to a cancer (**ATL** = "adult T cell leukemia") in about 5% of its victims. HTLV-1 encodes a protein, called **Tax**, that binds to MAD protein causing failure of the spindle checkpoint. The leukemic cells in these patients show many chromosome abnormalities including aneuploidy. A **kinesin** that moves the kinetochore to the end of the spindle fibre also seems to be involved in the spindle checkpoint.

(e) G_0

Many times a cell will leave the cell cycle, temporarily or permanently. It exits the cycle at G_1 and enters a stage designated G_0 (G zero). A G_0 cell is often called "quiescent", but that is probably more a reflection of the interests of the scientists studying the cell cycle than the cell itself. Many G_0 cells are anything but quiescent. They are busy carrying out their functions in the organism. e.g., secretion, attacking pathogens. Often G_0 cells are

terminally differentiated: they will never re-enter the cell cycle but instead will carry out their function in the organism until they die. For other cells, G_0 can be followed by re-entry into the cell cycle. Most of the **lymphocytes** in human blood are in G_0. However, with proper stimulation, such as encountering the appropriate antigen, they can be stimulated to re-enter the cell cycle (at G_1) and proceed on to new rounds of alternating **S phases** and **mitosis**. G_0 represents not simply the absence of signals for mitosis but an active repression of the genes needed for mitosis. **Cancer cells** cannot enter G_0 and are destined to repeat the cell cycle indefinitely.

14.2. Mitosis

When a **eukaryotic** cell divides into two, each daughter or progeny cell must receive a **complete set of genes** (for diploid cells, this means 2 complete **genomes, 2n**), a pair of **centrioles** (in animal cells), some **mitochondria** and, in plant cells, chloroplasts as well and some **ribosomes**, a portion of the **endoplasmic reticulum**, and perhaps other organelles. There are so many mitochondria and ribosomes in the cell that each daughter cell is usually assured of getting some. But ensuring that each daughter cell gets two (if diploid) of every gene in the cell requires the greatest precision. This involves duplication of each chromosome during the S phase of the cell cycle. This produces **dyads**, each made up of 2 identical **sister chromatids** held together by a ring of proteins called **cohesins**. Condense the chromosomes into a compact form. This requires **ATP** and a protein complex called **condensin**. Separate the sister chromatids and distribute these equally between the two daughter cells.

Steps 3 - 5 are accomplished by **mitosis**. It distributes one of each duplicated chromosome (as well as one centriole) to each daughter cell. It is convenient to consider mitosis in 5 phases.

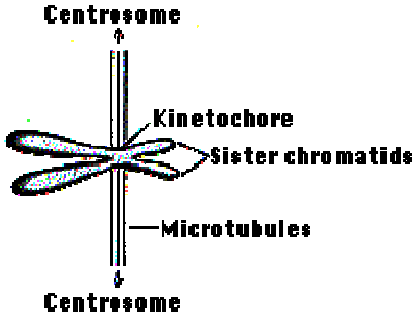

*Fig.14.2. Centrosom*e

When a cell is not engaged in mitosis (which is most of the time), it is said to be in **interphase**.

1. Prophase

The two **centrosomes** of the cell, each with its pair of centrioles, move to opposite "poles" of the cell. The **mitotic spindle** forms. This is an array of spindle fibres, each containing **~20 microtubules**. Microtubules are synthesized from tubulin monomers in the cytoplasm and grow out from each centrosome. The chromosomes become shorter and more compact.

2. Prometaphase
The **nuclear envelope** disintegrates because of the dissolution of the **lamins** that stabilize its inner membrane. A protein structure, the **kinetochore**, appears at the **centromere** of each chromatid. With the breakdown of the nuclear envelope, spindle fibres attach to the kinetochores as well as to the arms of the chromosomes. For each dyad, one of the kinetochores is attached to one pole, the second (or sister) chromatid to the opposite pole. Failure of a kinetochore to become attached to a spindle fibre interrupts the process.

3. Metaphase
At metaphase all the dyads have reached an equilibrium position midway between the poles called the **metaphase plate**. The chromosomes are at their most compact at this time.

4. Anaphase
The sister kinetochores suddenly separate and each moves to its respective pole dragging its attached chromatid (chromosome) behind it. Separation of the sister chromatids depends on the breakdown of the **cohesins** that have been holding them together. It works like this. Cohesin breakdown is caused by a **protease** called **separase** (also known as separin). Separase is kept **inactive** until late metaphase by an inhibitory **chaperone** called **securin**. Anaphase begins when the **anaphase promoting complex** (APC) destroys securin (by tagging it for deposit in a proteasome) thus ending its inhibition of separase and allowing separase to break down the cohesins.

5. Telophase
A nuclear envelope reforms around each cluster of chromosomes and these return to their more extended form.

14.3. Cytokinesis
Mitosis is the process of separating the duplicates of each of the cell's chromosomes. It is usually followed by division of the cell. However, there are cases (cleavage in the insect embryo is an example) where the chromosomes undergo the mitotic process without division of the cell. Thus a special term, cytokinesis, for the separation of a cell into two.

In **animal cells**, a belt of **actin filaments** forms around the perimeter of the cell, midway between the poles. The interaction of actin and a **myosin** (not the one found in skeletal muscle) tightens the belt, and the cell is pinched into two daughter cells.
In **plant cells**, a membrane-bounded **cell plate** forms where the metaphase plate had been. The cell plate, which is synthesized by the Golgi apparatus, supplies the plasma membrane that will separate the two daughter cells. Synthesis of a new **cell wall** between the daughter cells also occurs at the cell plate.

14.4. Meiosis
Mitosis produces two cells with the same number of **chromosomes** as the parent cell. Mitosis of a **diploid** cell (**2n**) produces two diploid daughter cells. If two diploid cells went on to participate in sexual reproduction, their fusion would produce a tetraploid (**4n**) **zygote**. Meiosis is a process of cell division in **eukaryotes** characterized by two consecutive divisions: **meiosis I** and **meiosis II,** no DNA synthesis (no S phase) between the two divisions, the result: 4 cells with half the number of chromosomes of the starting cell, e.g., **2n → n.** Fusion of two such cells produces a **2n** zygote. Meiosis in Animals is

used to produced the gametes: sperm and eggs. Meiosis in Plants is used to produce spores. Spores are the start of the **gametophyte generation** which, in time, will produce gametes (by mitosis because the starting cells are already haploid).

14.4. 1. Meiosis I

Prophase of meiosis I (**prophase I**) is a more elaborate process than prophase of mitosis (and usually takes much longer). Here is a brief overview of the process. When the chromosomes first become visible they are already doubled, each homologue having been duplicated during the preceding S phase. Result: pairs of **homologous dyads** each dyad consisting of two sister chromatids held together by proteins called **cohesins**. **Pairing**: Each pair of homologous dyads align lengthwise with each other. Result: a **tetrad**. (These structures are sometimes referred to as **bivalents** because at this stage you cannot distinguish the individual sister chromatids under the microscope.) The two homologous dyads are held together by one or more **chiasmata** (sing. = chiasma) which form between two **nonsister** chromatids at points where they have **crossed over**. The **synaptonemal complex** (SC), a complex assembly of proteins (including cohesins). At **metaphase I**, microtubules of the spindle fibres attach to the sister kinetochores of one homologue, pulling both sister chromatids toward one pole of the cell and sister kinetochores of the other homologue pulling those sisters toward the opposite pole.
Result: one homologue is pulled above the metaphase plate, the other below. The chiasmata keep the homologues attached to each other, and the cohesins keep the sister chromatids together. At **anaphase I**, the cohesins between the chromosome **arms** break down allowing, the chiasmata to slip apart. Result: the homologous dyads separate and migrate toward their respective poles.

14.4. 2. Meiosis II

Chromosome behaviour in meiosis II is like that of mitosis. At metaphase II, spindle fibres attach one kinetochore of the dyad to one pole, the other to the opposite pole. At anaphase II, the chromatids separate and (each now an independent chromosome) move to their respective poles.

(a) Genetic Recombination

Meiosis not only preserves the genome size of sexually reproducing eukaryotes but also provides three mechanisms to diversify the genomes of the offspring.

1. Crossing Over

Chiasmata represent points where earlier (and unseen) nonsister chromatids had swapped sections. The process is called crossing over. It is reciprocal; the segments exchanged by each nonsister chromatid are identical (but may carry different alleles). Each chromatid contains a single molecule of DNA. So the problem of crossing over is really a problem of swapping portions of adjacent DNA molecules. It must be done with great precision so that neither chromatid gains or loses any genes. In fact, crossing over has to be sufficiently precise that not a single nucleotide is lost or added at the crossover point if it occurs within a gene. Otherwise a **frameshift** would result and the resulting gene would produce a defective product or, more likely, no product at all.

2. Random Assortment

In meiosis I, the orientation of paternal and maternal homologues at the metaphase plate is random. Therefore, although each cell produced by meiosis contains only one of each homologue, the number of possible combinations of maternal and paternal homologues is 2^n, where **n** = the haploid number of chromosomes. In this diagram, the haploid number is 3, and 8 (2^3) different combinations are produced. Random assortment of homologues in humans produces 2^{23} (8,388,608) different combinations of chromosomes. Furthermore, because of crossing over, none of these chromosomes is "pure" maternal or paternal. The distribution of recombinant and non-recombinant sister chromatids into the daughter cells at anaphase II is also random. All the billions of sperm produced by a man during his lifetime (and the hundreds of eggs that mature over the life of a woman), no two have exactly the same gene content.

3. Fertilization

By reducing the number of chromosomes from **2n** to **n**, the stage is set for the union of two genomes. If the parents differ genetically, new combinations of genes can occur in their offspring. Taking these three mechanisms together, I think that it is safe to conclude that no two human beings have ever shared an identical genome **unless** they had an **identical** sibling; that is a sibling produced from the same fertilized egg. The behaviour of chromosomes during meiosis ($2n \rightarrow n$) and fertilization ($n + n \rightarrow 2n$) provide the structural basis for Mendel's rules of inheritance.

(b) Prophase I — A detailed View

The lengthy and complex events of prophase I can be broken down into 5 stages.

1. Leptotene

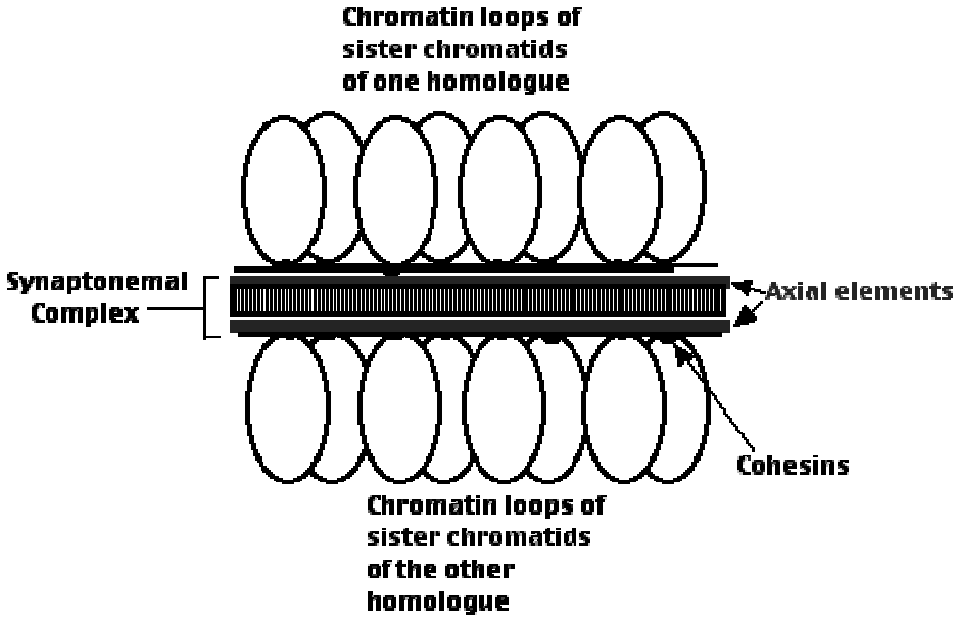

Fig.14.3. Prophase

All the chromosomes condense. Pairing. Homologous dyads (pairs of sister chromatids) find each other and align themselves from end to end with the aid of an **axial element** (that contain cohesins). In **budding yeast** (and perhaps other eukaryotes) the process

follows a period of trial-and-error. Any two dyads pair at their centromeres. If they are not homologs, they separate and try again. How the nonsisters recognize their shared regions of DNA homology is uncertain. **Double-stranded breaks** (DSBs) often occur in the DNA of the chromatids, and these may be necessary for the homologs to recognize each other.

2. Zygotene

Synapsis. The **synaptonemal complex** begins to form. DNA strands of nonsister chromatids begin the process of **recombination**. How they are able to do so across the synaptonemal complex, which is over 100 nm thick, is unknown.

3. Pachytene

Synapsis is now complete. **Recombination nodules** appear (at least in some organisms, including humans). They are named for the idea that they represent points where DNA recombination is occurring. There must be at least one for each bivalent if meiosis is to succeed. There are often more, each one presumably representing the point of a crossover. They contain enzymes known to be needed for DNA recombination and repair. The steps in recombining DNA continue to the end of pachytene.

4. Diplotene

DNA recombination is complete. The synaptonemal complex begins to break down. The chromatids begin to pull apart revealing **chiasmata**. At first the chiasmata are located at the sites of the recombination nodules, but later they migrate towards the ends of the chromatids.

5. Diakinesis

In some organisms, the chromosomes de-condense and begin to be transcribed for a time. This is followed by the chromosomes re-condensing in preparation for metaphase I. In creatures where this does not occur, the chromosomes condense further in preparation for metaphase I.

(c) Checkpoints: Quality Control of Meiosis

It shouldn't be surprising that things can go wrong in such a complicated process. However, cells going through meiosis have **checkpoints** that monitor each pair of homologues for proper recombination of their DNA and correct formation of the synaptonemal complex. Any failure that is detected stops the process and usually causes the cell to self-destruct by **apoptosis.** However, despite these checkpoints, errors occasionally do go uncorrected.

(d) Errors in Meiosis

It is estimated that from 10–20% of all human fertilized eggs contain chromosome abnormalities, and these are the most common cause of pregnancy failure (35% of the cases). These chromosome abnormalities arise from errors in meiosis, usually meiosis I; occur more often (90%) during **egg formation** than during sperm formation and become more frequent as a woman ages. **Aneuploidy** — the gain or loss of whole chromosomes — is the most common chromosome abnormality. It is caused by **nondisjunction**, the failure of chromosomes to correctly separate, homologues during meiosis I or sister chromatids during meiosis II. Zygotes missing one chromosome ("monosomy") cannot develop to birth (except for females with a **single X chromosome**). Three of the same

chromosome ("trisomy") is also lethal except for chromosomes 13, 18, and 21 (trisomy 21 is the cause of **Down syndrome**). Three or more X chromosomes are viable because all but one of them are inactivated.