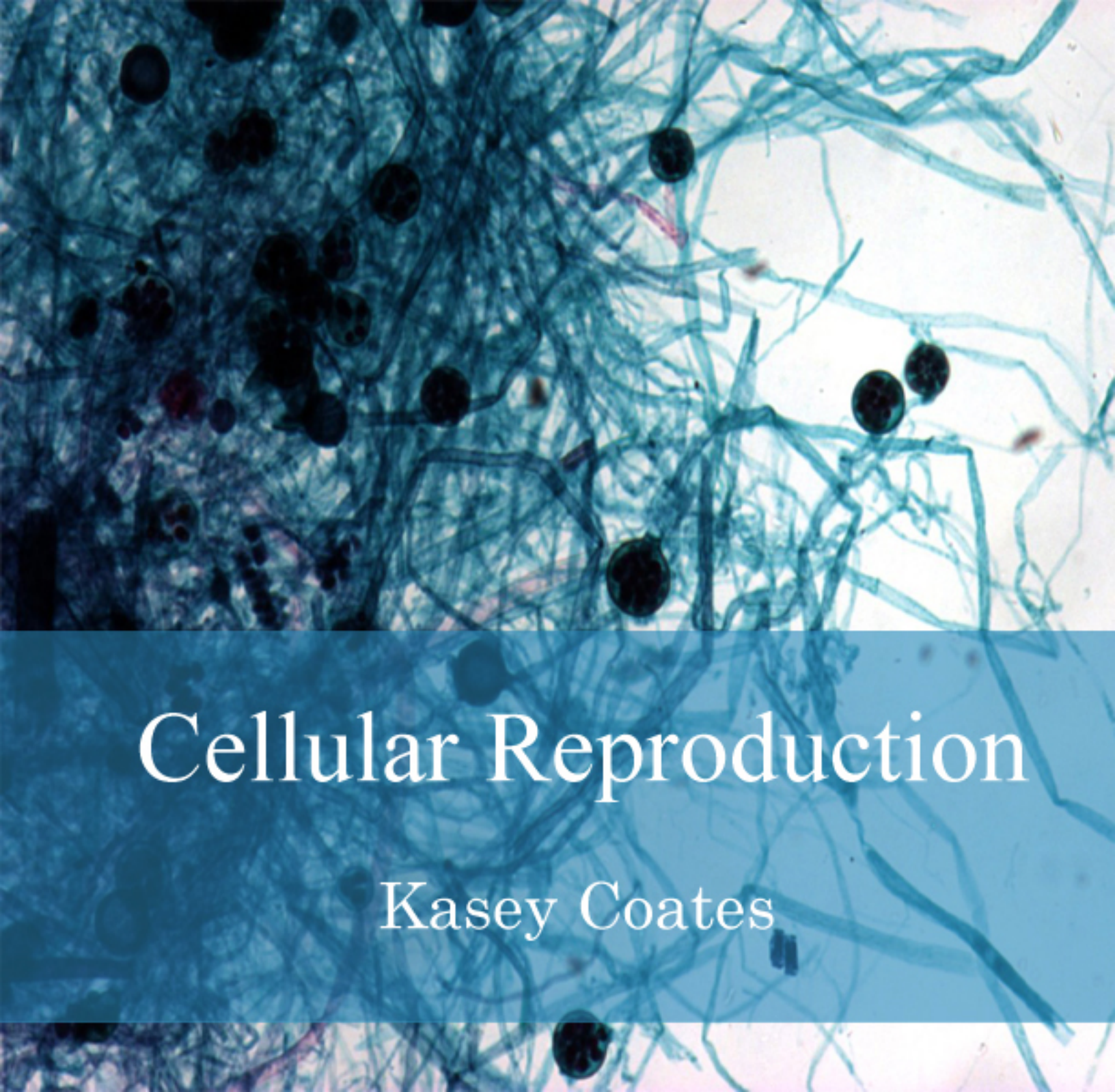

Cellular Reproduction

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First Edition, 2012

ISBN 978-81-323-3139-1

Published by:
Research World
4735/22 Prakashdeep Bldg,
Ansari Road, Darya Ganj,
Delhi - 110002
Email: info@wtbooks.com

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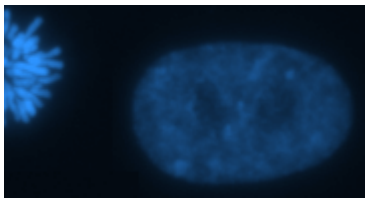
Interphase and Fission (Biology)

Interphase

An illustration of interphase. The chromatin has not yet condensed, and the cell is undergoing its normal functions.



An image of the nucleus of a cell (HT1080) currently in interphase (probably G1). Note: Cytoplasm of this cell or the neighboring cell is not visible (top-left), which is currently in the telophase of mitosis. Image taken using an optical microscope and DAPI staining of DNA.

Interphase is the phase of the cell cycle in which the cell spends the majority of its time and performs the majority of its purposes including preparation for cell division. In preparation for cell division, it increases its size and makes a copy of its DNA. Interphase is also considered to be the 'living' phase of the cell, in which the cell obtains nutrients, grows, reads its DNA, and conducts other "normal" cell functions. The majority of eukaryotic cells spend most of their time in interphase. Interphase does not describe a cell that is merely resting but is rather an active preparation for cell division. A common

misconception is that interphase is the first stage of mitosis. However, since mitosis is the division of the nucleus, prophase is actually the first stage.

In interphase, the cell gets itself ready for mitosis or meiosis. Somatic cells, or normal diploid cells of the body, go through mitosis in order to reproduce themselves through cell division, whereas diploid germ cells (i.e., primary spermatocytes and primary oocytes) go through meiosis in order to create haploid gametes (i.e., sperm and ova) for the purpose of sexual reproduction.

Identification

Under a microscope, prophase can be recognized because the nuclear membrane is still intact, the chromatin has not yet condensed, and chromosomes are not visible, though the nucleolus may be visible as an enlarged dark spot. The centrioles and spindle fibers are also not yet visible, though the centrosome, which contains and organizes them, may be visible near the nucleus.

Stages of interphase

There are three stages of interphase, with each phase ending when a cellular checkpoint checks the accuracy of the stage's completion before proceeding to the next. The stages of interphase are:

- G_1 (Gap 1), in which the cell grows and functions normally. During this time, much protein synthesis occurs and the cell grows (to about double its original size) - more organelles are produced, increasing the volume of the cytoplasm. If the cell is not to divide again, it will remain in this phase.

- Synthesis (S), in which the cell duplicates its DNA (via semiconservative replication). This is also known as the Swanson phase.

- G_2 (Gap 2), in which the cell resumes its growth in preparation for mitosis.

- In addition, some cells that do not divide often or ever, enter a stage called G_0 (Gap zero), which is either a stage separate from interphase or an extended G_1 phase, which follows the restriction point, a cell cycle checkpoint found at the end of G_1.

The duration of time spent in interphase and in each stage of interphase is variable and depends on both the type of cell and the species of organism it belongs to. Most cells of adult mammals spend about 20 hours in interphase, this accounts for about 90% of the total time involved in cell division.

Interphase within sequences of cellular processes

Interphase and the cell cycle

When G_2 is completed, the cell enters a relatively brief period of nuclear and cellular division, composed of mitosis and cytokinesis, respectively. After the successful completion of mitosis and cytokinesis, both resulting daughter cells re-enter G_1 of interphase.

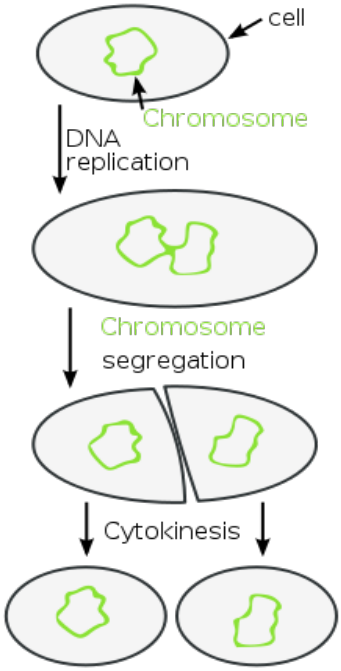
In the cell cycle, interphase is proceeded by telophase and cytokinesis of the M phase. In alternative fashion, interphase is sometimes interrupted by G_0 phase, which, in some circumstances, may then end and be followed by the remaining stages of interphase. After the successful completion of the G_2 checkpoint, the final checkpoint in interphase, the cell proceeds to prophase, or in plants to preprophase, which is the first stage of mitosis.

G_0 phase is viewed as either an extended G_1 phase where the cell is neither dividing nor preparing to divide and or as a distinct quiescent stage which occurs outside of the cell cycle.

Interphase and other cellular processes

In gamete production interphase is succeeded by meiosis. In programmed cell death, interphase is followed or preempted by apoptosis.

Fission (biology)

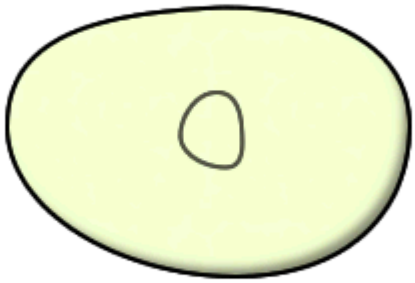


Binary fission

In biology, **fission** is the subdivision of a body, population, or species into parts and the regeneration of those parts into separate individuals. **Binary fission**, or **prokaryotic fission**, is a form of asexual reproduction and cell division used by all prokaryotes, some protozoa, and some organelles within eukaryotic organisms. This process results in the reproduction of a living prokaryotic cell by division into two parts which each have the potential to grow to the size of the original cell.

Mitosis and cytokinesis are not the same as binary fission; specifically, binary fission cannot be divided into prophase, metaphase, anaphase, and telophase because prokaryotes have no nucleus and no centromeres. The ability of some multicellular animals, such as echinoderms and flatworms, to regenerate two whole organisms after having been cut in half, is also not the same as binary fission. Neither is vegetative reproduction of plants.

Process



Complete process of binary fission.

Binary fission begins with DNA replication. DNA replication starts from an origin of replication, which opens up into a replication bubble (note: prokaryotic DNA replication usually has only 1 origin of replication, whereas eukaryotes have multiple origins of replication). The replication bubble separates the DNA double strand, each strand acts as template for synthesis of a daughter strand by semiconservative replication, until the entire prokaryotic DNA is duplicated.

Each circular DNA strand then attaches to the cell membrane. The cell elongates, causing the DNA to separate.

Cell division in bacteria is controlled by the FtsZ, a collection of about a dozen proteins that collect around the site of division. There, they direct assembly of the division septum. The cell wall and plasma membrane starts growing transversely from near the middle of the dividing cell. This separates the parent cell into two nearly equal daughter cells, each having a nuclear body.

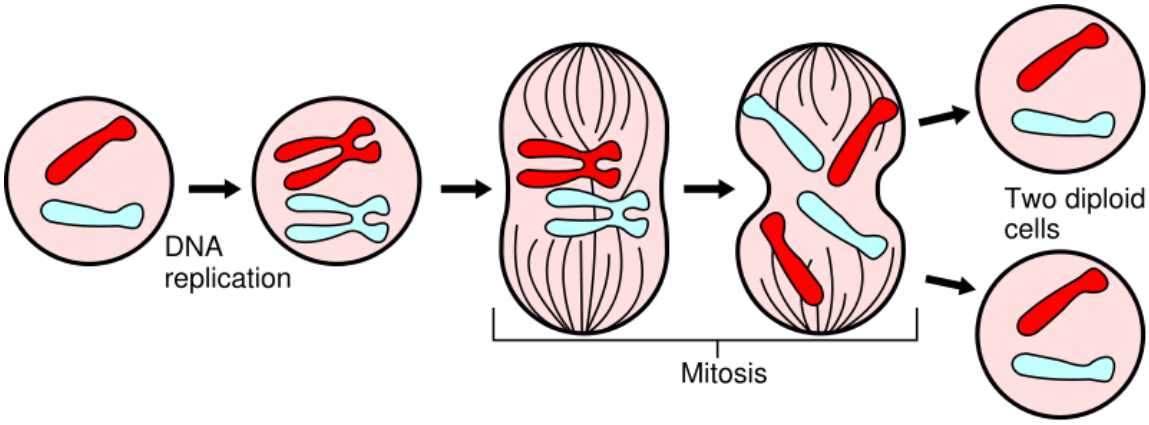
The cell membrane then invaginates (grows inwards) and splits the cell into two daughter cells, separated by a newly grown cell plate.

Use by eukaryotic organelles

Eukaryotic organelles such as mitochondria, chloroplasts, and peroxisomes also is not yet clear.

Chapter- 2

Cell Cycle

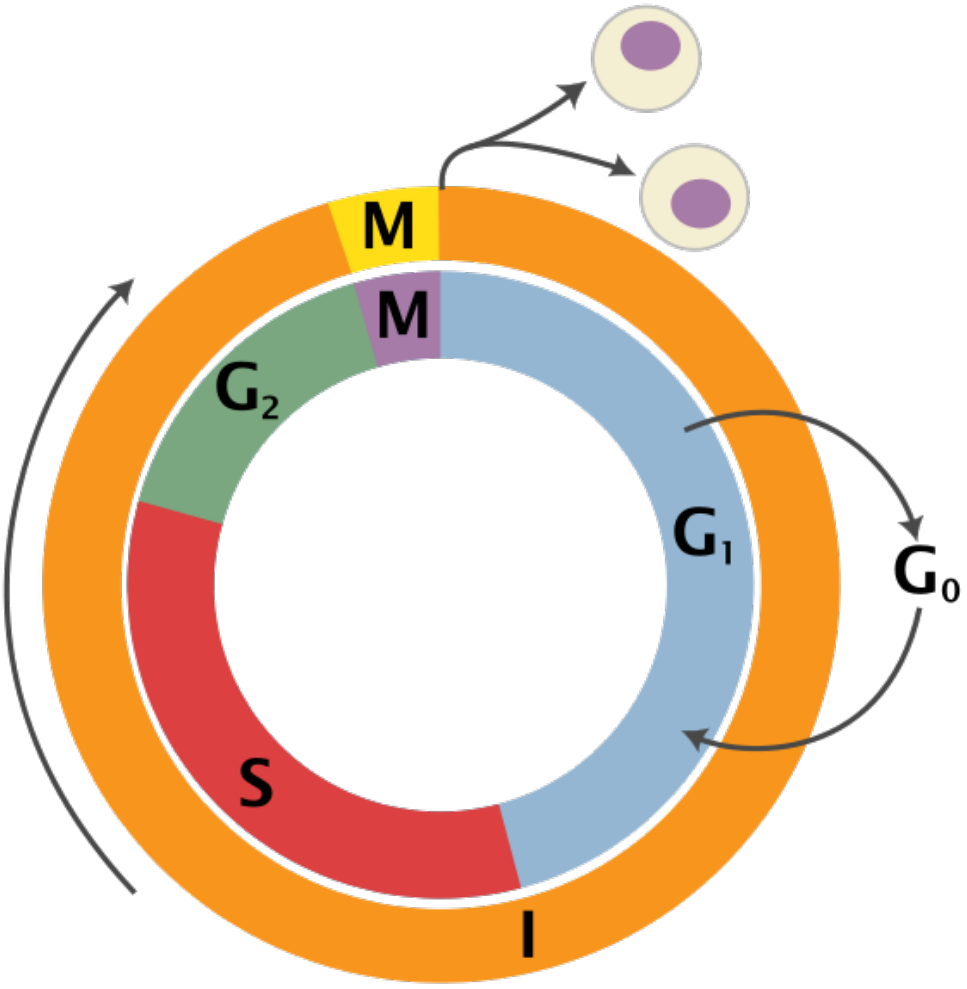


Each turn of the cell cycle divides the chromosomes in a cell nucleus.

The **cell cycle**, or **cell-division cycle**, is the series of events that takes place in a cell leading to its division and duplication (replication). In cells without a nucleus (prokaryotic), the cell cycle occurs via a process termed binary fission. In cells with a nucleus (eukaryotes), the cell cycle can be divided in two brief periods: interphase—during which the cell grows, accumulating nutrients needed for mitosis and duplicating its DNA—and the mitosis (M) phase, during which the cell splits itself into two distinct cells, often called "daughter cells". The cell-division cycle is a vital process by which a single-celled fertilized egg develops into a mature organism, as well as the process by which hair, skin, blood cells, and some internal organs are renewed.

Phases

The cell cycle consists of four distinct phases: G_1 phase, S phase (synthesis), G_2 phase (collectively known as interphase) and M phase (mitosis). M phase is itself composed of two tightly coupled processes: mitosis, in which the cell's chromosomes are divided between the two daughter cells, and cytokinesis, in which the cell's cytoplasm divides in half forming distinct cells. Activation of each phase is dependent on the proper progression and completion of the previous one. Cells that have temporarily or reversibly stopped dividing are said to have entered a state of quiescence called G_0 phase.



Schematic of the cell cycle. outer ring: I = Interphase, M = Mitosis; inner ring: M = Mitosis, G_1 = Gap 1, G_2 = Gap 2, S = Synthesis; not in ring: G_0 = Gap 0/Resting.

State	Phase	Abbreviation	Description
quiescent/ senescent	Gap 0	**G_0**	A resting phase where the cell has left the cycle and has stopped dividing.
Interphase	Gap 1	**G_1**	Cells increase in size in Gap 1. The *G_1 checkpoint* control mechanism ensures that everything is ready for DNA synthesis.
	Synthesis	**S**	DNA replication occurs during this phase.
	Gap 2	**G_2**	During the gap between DNA synthesis and mitosis, the cell will continue to grow. The *G_2 checkpoint* control mechanism ensures that everything is ready to enter the M (mitosis) phase and divide.

Cell division	Mitosis	**M**	Cell growth stops at this stage and cellular energy is focused on the orderly division into two daughter cells. A checkpoint in the middle of mitosis (*Metaphase Checkpoint*) ensures that the cell is ready to complete cell division.

After cell division, each of the daughter cells begin the interphase of a new cycle. Although the various stages of interphase are not usually morphologically distinguishable, each phase of the cell cycle has a distinct set of specialized biochemical processes that prepare the cell for initiation of cell division.

Resting (G_0 phase)

The term "post-mitotic" is sometimes used to refer to both quiescent and senescent cells. Nonproliferative cells in multicellular eukaryotes generally enter the quiescent G_0 state from G_1 and may remain quiescent for long periods of time, possibly indefinitely (as is often the case for neurons). This is very common for cells that are fully differentiated. Cellular senescence is a state that occurs in response to DNA damage or degradation that would make a cell's progeny nonviable; it is often a biochemical alternative to the self-destruction of such a damaged cell by apoptosis.

Interphase

Before a cell can enter cell division, it needs to take in nutrients. All of the preparations are done during the interphase. Interphase proceeds in three stages, G1, S, and G2. Cell division operates in a cycle. Therefore, interphase is preceded by the previous cycle of mitosis and cytokinesis.

G_1 phase

The first phase within interphase, from the end of the previous M phase until the beginning of DNA synthesis is called G_1 (G indicating *gap*). It is also called the growth phase. During this phase the biosynthetic activities of the cell, which had been considerably slowed down during M phase, resume at a high rate. This phase is marked by synthesis of various enzymes that are required in S phase, mainly those needed for DNA replication. Duration of G_1 is highly variable, even among different cells of the same species.

S phase

The ensuing S phase starts when DNA synthesis commences; when it is complete, all of the chromosomes have been replicated, i.e., each chromosome has two (sister) chromatids. Thus, during this phase, the amount of DNA in the cell has effectively doubled, though the ploidy of the cell remains the same. Rates of RNA transcription and

protein synthesis are very low during this phase. An exception to this is histone production, most of which occurs during the S phase.

G_2 phase

The cell then enters the G_2 phase, which lasts until the cell enters mitosis. Again, significant biosynthesis occurs during this phase, mainly involving the production of microtubules, which are required during the process of mitosis. Inhibition of protein synthesis during G_2 phase prevents the cell from undergoing mitosis.

Mitosis (M Phase/Mitotic phase)

The relatively brief M phase consists of nuclear division (karyokinesis). The M phase has been broken down into several distinct phases, sequentially known as:

- prophase,
- metaphase,
- anaphase,
- telophase
- cytokinesis (strictly speaking, cytokinesis is not part of mitosis but is an event that directly follows mitosis in which cytoplasm is divided into two daughter cells)

Mitosis is the process by which a eukaryotic cell separates the chromosomes in its cell nucleus into two identical sets in two nuclei. It is generally followed immediately by cytokinesis, which divides the nuclei, cytoplasm, organelles and cell membrane into two cells containing roughly equal shares of these cellular components. Mitosis and cytokinesis together define the **mitotic (M) phase** of the cell cycle - the division of the mother cell into two daughter cells, genetically identical to each other and to their parent cell. This accounts for approximately 10% of the cell cycle.

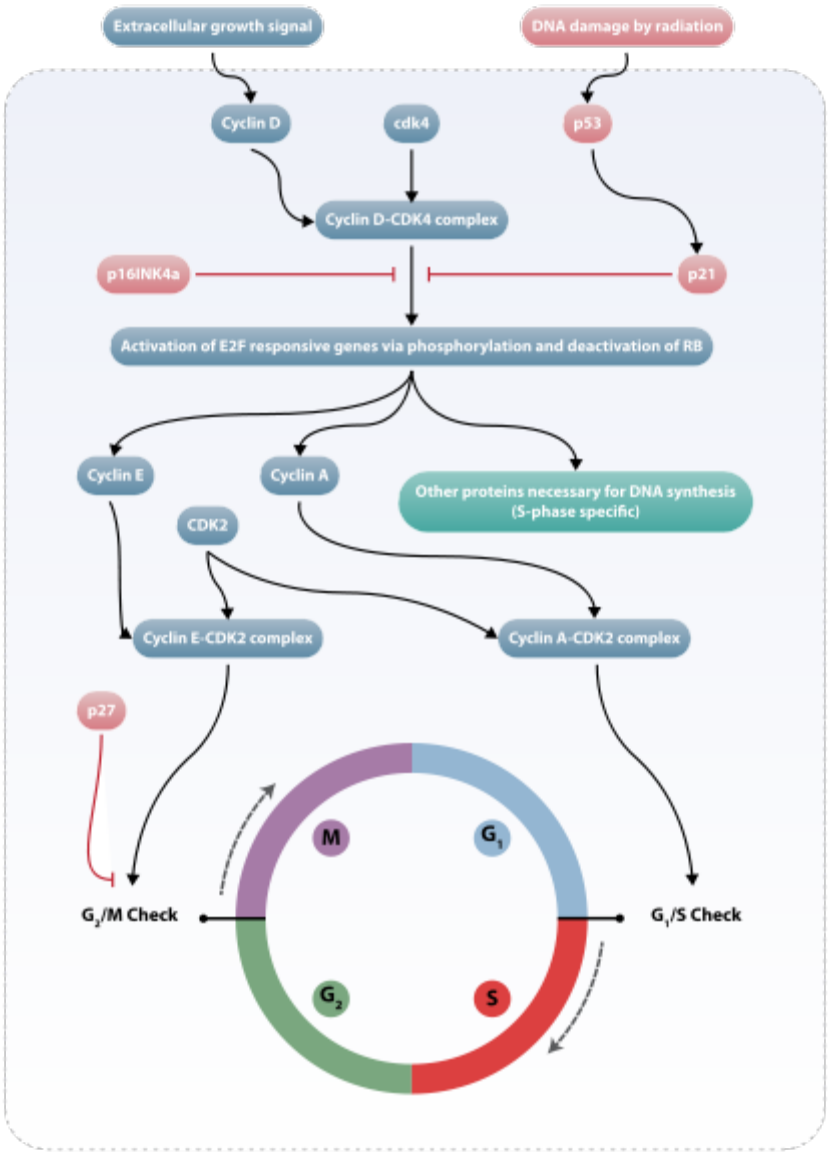
Mitosis occurs exclusively in eukaryotic cells, but occurs in different ways in different species. For example, animals undergo an "open" mitosis, where the nuclear envelope breaks down before the chromosomes separate, while fungi such as *Aspergillus nidulans* and *Saccharomyces cerevisiae* (yeast) undergo a "closed" mitosis, where chromosomes divide within an intact cell nucleus. Prokaryotic cells, which lack a nucleus, divide by a process called binary fission.

The process of mitosis is complex and highly regulated. The sequence of events is divided into phases, corresponding to the completion of one set of activities and the start of the next. These stages are prophase, prometaphase, metaphase, anaphase and telophase. During the process of mitosis the pairs of chromosomes condense and attach to fibers that pull the sister chromatids to opposite sides of the cell. The cell then divides in cytokinesis, to produce two identical daughter cells.

Because cytokinesis usually occurs in conjunction with mitosis, "mitosis" is often used interchangeably with "M phase". However, there are many cells where mitosis and

cytokinesis occur separately, forming single cells with multiple nuclei. This occurs most notably among the fungi and slime moulds, but is found in various different groups. Even in animals, cytokinesis and mitosis may occur independently, for instance during certain stages of fruit fly embryonic development. Errors in mitosis can either kill a cell through apoptosis or cause mutations that may lead to cancer.

Regulation of eukaryotic cell cycle



Regulation of cell cycle: Schematic

Regulation of the cell cycle involves processes crucial to the survival of a cell, including the detection and repair of genetic damage as well as the prevention of uncontrolled cell division. The molecular events that control the cell cycle are ordered and directional; that is, each process occurs in a sequential fashion and it is impossible to "reverse" the cycle.

Role of cyclins and CDKs

Two key classes of regulatory molecules, cyclins and cyclin-dependent kinases (CDKs), determine a cell's progress through the cell cycle. Leland H. Hartwell, R. Timothy Hunt, and Paul M. Nurse won the 2001 Nobel Prize in Physiology or Medicine for their discovery of these central molecules. Many of the genes encoding cyclins and CDKs are conserved among all eukaryotes, but in general more complex organisms have more elaborate cell cycle control systems that incorporate more individual components. Many of the relevant genes were first identified by studying yeast, especially *Saccharomyces cerevisiae*; genetic nomenclature in yeast dubs many these genes *cdc* (for "cell division cycle") followed by an identifying number, e.g., *cdc25* or *cdc20*.

Cyclins form the regulatory subunits and CDKs the catalytic subunits of an activated heterodimer; cyclins have no catalytic activity and CDKs are inactive in the absence of a partner cyclin. When activated by a bound cyclin, CDKs perform a common biochemical reaction called phosphorylation that activates or inactivates target proteins to orchestrate coordinated entry into the next phase of the cell cycle. Different cyclin-CDK combinations determine the downstream proteins targeted. CDKs are constitutively expressed in cells whereas cyclins are synthesised at specific stages of the cell cycle, in response to various molecular signals.

General mechanism of cyclin-CDK interaction

Upon receiving a pro-mitotic extracellular signal, G_1 cyclin-CDK complexes become active to prepare the cell for S phase, promoting the expression of transcription factors that in turn promote the expression of S cyclins and of enzymes required for DNA replication. The G_1 cyclin-CDK complexes also promote the degradation of molecules that function as S phase inhibitors by targeting them for ubiquitination. Once a protein has been ubiquitinated, it is targeted for proteolytic degradation by the proteasome.

Active S cyclin-CDK complexes phosphorylate proteins that make up the pre-replication complexes assembled during G_1 phase on DNA replication origins. The phosphorylation serves two purposes: to activate each already-assembled pre-replication complex, and to prevent new complexes from forming. This ensures that every portion of the cell's genome will be replicated once and only once. The reason for prevention of gaps in replication is fairly clear, because daughter cells that are missing all or part of crucial genes will die. However, for reasons related to gene copy number effects, possession of extra copies of certain genes is also deleterious to the daughter cells.

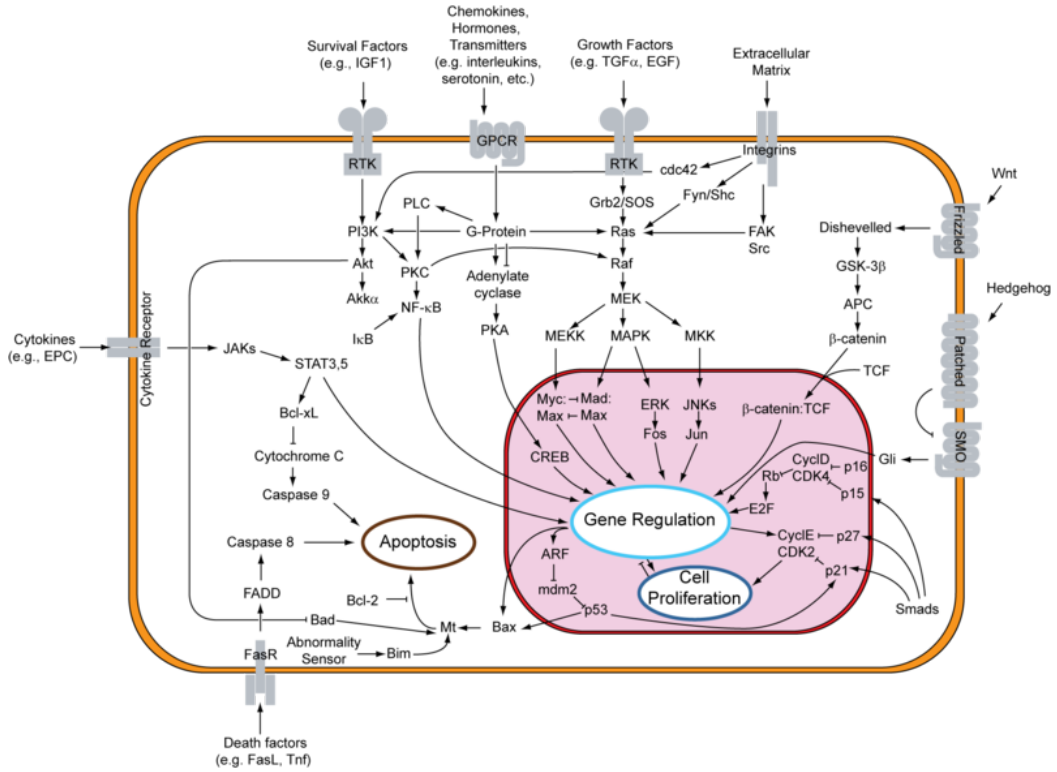
Mitotic cyclin-CDK complexes, which are synthesized but inactivated during S and G_2 phases, promote the initiation of mitosis by stimulating downstream proteins involved in chromosome condensation and mitotic spindle assembly. A critical complex activated during this process is a ubiquitin ligase known as the anaphase-promoting complex (APC), which promotes degradation of structural proteins associated with the chromosomal kinetochore. APC also targets the mitotic cyclins for degradation, ensuring that telophase and cytokinesis can proceed.

Interphase: Interphase generally lasts at least 12 to 24 hours in mammalian tissue. During this period, the cell is constantly synthesizing RNA, producing protein and growing in size. By studying molecular events in cells, scientists have determined that interphase can be divided into 4 steps: Gap 0 (G_0), Gap 1 (G_1), S (synthesis) phase, Gap 2 (G_2).

Specific action of cyclin-CDK complexes

Cyclin D is the first cyclin produced in the cell cycle, in response to extracellular signals (e.g. growth factors). Cyclin D binds to existing CDK4, forming the active cyclin D-CDK4 complex. Cyclin D-CDK4 complex in turn phosphorylates the retinoblastoma susceptibility protein (Rb). The hyperphosphorylated Rb dissociates from the E2F/DP1/Rb complex (which was bound to the E2F responsive genes, effectively "blocking" them from transcription), activating E2F. Activation of E2F results in transcription of various genes like cyclin E, cyclin A, DNA polymerase, thymidine kinase, etc. Cyclin E thus produced binds to CDK2, forming the cyclin E-CDK2 complex, which pushes the cell from G_1 to S phase (G_1/S transition). Cyclin B along with cdc2 (cdc2 - fission yeasts (CDK1 - mammalia)) forms the cyclin B-cdc2 complex, which initiates the G_2/M transition. Cyclin B-cdc2 complex activation causes breakdown of nuclear envelope and initiation of prophase, and subsequently, its deactivation causes the cell to exit mitosis.

Inhibitors



Overview of signal transduction pathways involved in apoptosis, also known as "programmed cell death".

Two families of genes, the *cip/kip* family and the INK4a/ARF (*In*hibitor of *K*inase 4/*A*lternative *R*eading *F*rame) prevent the progression of the cell cycle. Because these genes are instrumental in prevention of tumor formation, they are known as tumor suppressors.

The ***cip/kip* family** includes the genes p21, p27 and p57. They halt cell cycle in G_1 phase, by binding to, and inactivating, cyclin-CDK complexes. p21 is activated by p53 (which, in turn, is triggered by DNA damage e.g. due to radiation). p27 is activated by Transforming Growth Factor β (TGF β), a growth inhibitor.

The **INK4a/ARF family** includes p16INK4a, which binds to CDK4 and arrests the cell cycle in G_1 phase, and p14arf which prevents p53 degradation.

Synthetic inhibitors of Cdc25 could also be useful for the arrest of cell cycle and therefore be useful as antineoplastic and anticancer agents.

Transcriptional Regulatory Network

Evidence suggests that a semi-autonomous transcriptional network acts in concert with the CDK-cyclin machinery to regulate the cell cycle. Several gene expression studies in *Saccharomyces cerevisiae* have identified approximately 800 to 1200 genes that change expression over the course of the cell cycle; they are transcribed at high levels at specific points in the cell cycle, and remain at lower levels throughout the rest of the cell cycle. While the set of identified genes differs between studies due to the computational methods and criterion used to identify them, each study indicates that a large portion of yeast genes are temporally regulated.

Many periodically expressed genes are driven by transcription factors that are also periodically expressed. One screen of single-gene knockouts identified 48 transcription factors (about 20% of all non-essential transcription factors) that show cell cycle progression defects. Genome-wide studies using high throughput technologies have identified the transcription factors that bind to the promoters of yeast genes, and correlating these findings with temporal expression patterns have allowed the identification of transcription factors that drive phase-specific gene expression. The expression profiles of these transcription factors are driven by the transcription factors that peak in the prior phase, and computational models have shown that a CDK-autonomous network of these transcription factors is sufficient to produce steady-state oscillations in gene expression).

Experimental evidence also suggests that gene expression can oscillate with the period seen in dividing wild-type cells independently of the CDK machinery. Orlando et. al. used microarrays to measure the expression of a set of 1,271 genes that they identified as periodic in both wild type cells and cells lacking all S-phase and mitotic cyclins (*clb1,2,3,4,5,6*). Of the 1,271 genes assayed, 882 continued to be expressed in the cyclin-deficient cells at the same time as in the wild type cells, despite the fact that the cyclin-deficient cells arrest at the border between G1 and S phase. However, 833 of the genes

assayed changed behavior between the wild type and mutant cells, indicating that these genes are likely directly or indirectly regulated by the CDK-cyclin machinery. Some genes that continued to be expressed on time in the mutant cells were also expressed at different levels in the mutant and wild type cells. These findings suggest that while the transcriptional network may oscillate independently of the CDK-cyclin oscillator, they are coupled in a manner that requires both to ensure the proper timing of cell cycle events. Other work indicates that phosphorylation, a post-translational modification, of cell cycle transcription factors by Cdk1 may alter the localization or activity of the transcription factors in order to tightly control timing of target genes (Ubersax et al 2003; Sidorova et al 1995; White et al 2009).

While oscillatory transcription plays a key role in the progression of the yeast cell cycle, the CDK-cyclin machinery operates independently in the early embryonic cell cycle. Before the midblastula transition, zygotic transcription does not occur and all needed proteins, such as the B-type cyclins, are translated from maternally loaded mRNA.

Checkpoints

Cell cycle checkpoints are used by the cell to monitor and regulate the progress of the cell cycle. Checkpoints prevent cell cycle progression at specific points, allowing verification of necessary phase processes and repair of DNA damage. The cell cannot proceed to the next phase until checkpoint requirements have been met.

Several checkpoints are designed to ensure that damaged or incomplete DNA is not passed on to daughter cells. Two main checkpoints exist: the G_1/S checkpoint and the G_2/M checkpoint. G_1/S transition is a rate-limiting step in the cell cycle and is also known as restriction point. An alternative model of the cell cycle response to DNA damage has also been proposed, known as the postreplication checkpoint.

p53 plays an important role in triggering the control mechanisms at both G_1/S and G_2/M checkpoints.

Role in tumor formation

A disregulation of the cell cycle components may lead to tumor formation. As mentioned above, some genes like the cell cycle inhibitors, RB, p53 etc., when they mutate, may cause the cell to multiply uncontrollably, forming a tumor. Although the duration of cell cycle in tumor cells is equal to or longer than that of normal cell cycle, the proportion of cells that are in active cell division (versus quiescent cells in G_0 phase) in tumors is much higher than that in normal tissue. Thus there is a net increase in cell number as the number of cells that die by apoptosis or senescence remains the same.

The cells which are actively undergoing cell cycle are targeted in cancer therapy as the DNA is relatively exposed during cell division and hence susceptible to damage by drugs or radiation. This fact is made use of in cancer treatment; by a process known as debulking, a significant mass of the tumor is removed which pushes a significant number

of the remaining tumor cells from G_0 to G_1 phase (due to increased availability of nutrients, oxygen, growth factors etc.). Radiation or chemotherapy following the debulking procedure kills these cells which have newly entered the cell cycle.

The fastest cycling mammalian cells in culture, crypt cells in the intestinal epithelium, have a cycle time as short as 9 to 10 hours. Stem cells in resting mouse skin may have a cycle time of more than 200 hours. Most of this difference is due to the varying length of G_1, the most variable phase of the cycle. M and S do not vary much.

In general, cells are most radiosensitive in late M and G_2 phases and most resistant in late S.

For cells with a longer cell cycle time and a significantly long G_1 phase, there is a second peak of resistance late in G_1

The pattern of resistance and sensitivity correlates with the level of sulfhydryl compounds in the cell. Sulfhydryls are natural radioprotectors and tend to be at their highest levels in S and at their lowest near mitosis.

Synchronization of cell cultures

Several methods can be used to synchronise cell cultures by halting the cell cycle at a particular phase. For example, serum starvation and treatment with thymidine or aphidicolin halt the cell in the G_1 phase, mitotic shake-off, treatment with colchicine and treatment with nocodazole halt the cell in M phase and treatment with 5-fluorodeoxyuridine halts the cell in S phase.

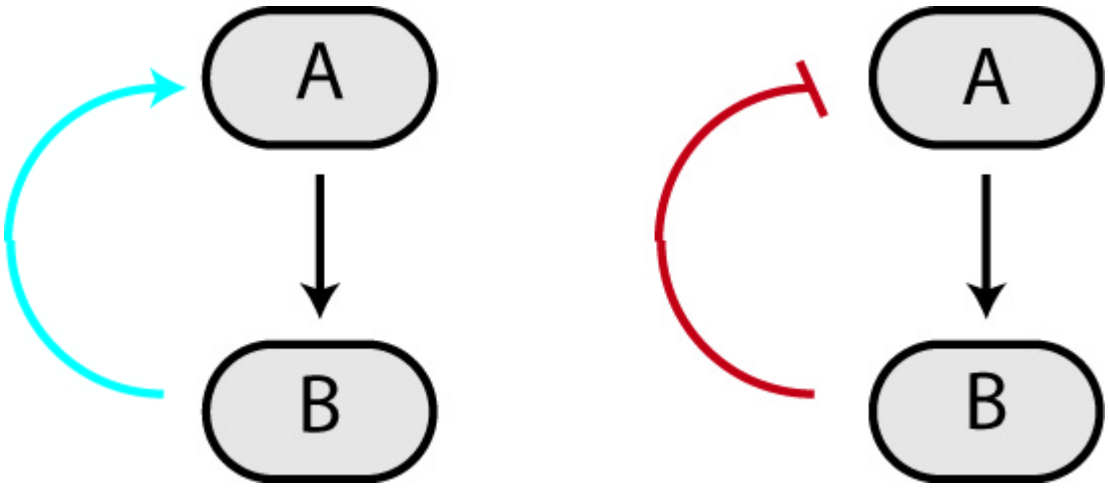
Chapter- 3

Biochemical Switches in the Cell Cycle

A series of **biochemical switches** control transitions between and within the various phases of the cell cycle. The cell cycle is a series of complex, ordered, sequential events that control how a single cell divides into two cells, and involves several different phases. The phases include the G1 and G2 phases, DNA replication or S phase, and the actual process of cell division, mitosis or M phase. During the M phase, the chromosomes separate and cytokinesis occurs.

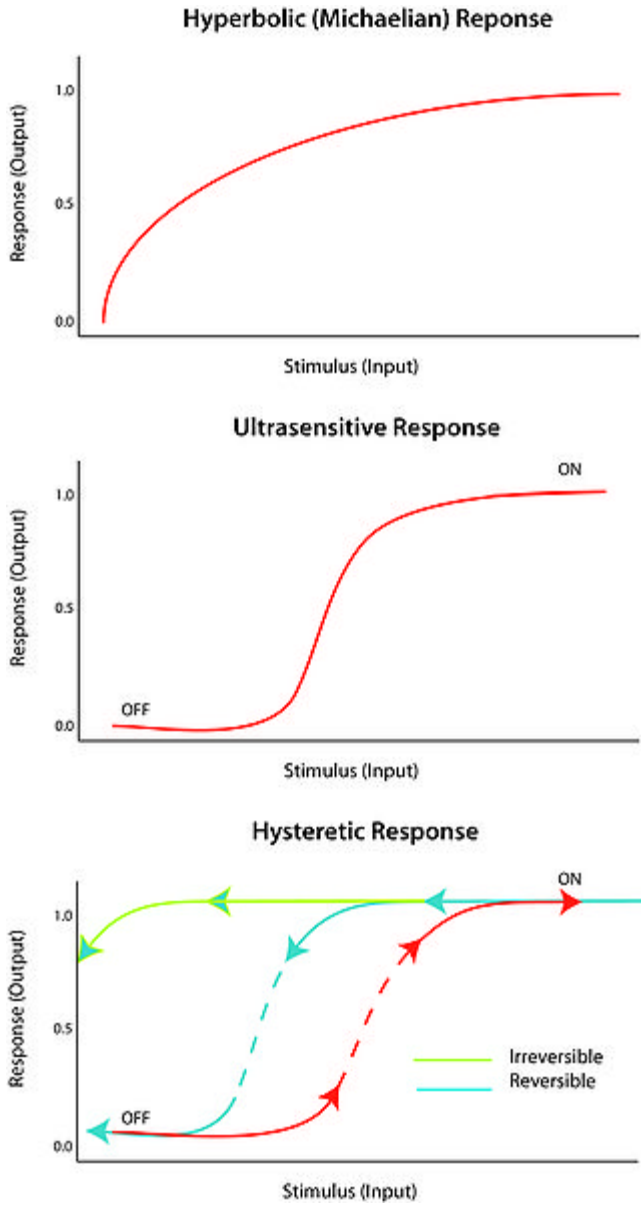
The switches maintain the orderly progression of the cell cycle and act as checkpoints to ensure that each phase has been properly completed before progression to the next phase. For example, Cdk, or cyclin dependent kinase, is a major control switch for the cell cycle and it allows the cell to move from G1 to S or G2 to M by adding phosphate to protein substrates. Such multi-component (involving multiple inter-linked proteins) switches have been shown to generate decisive, robust (and potentially irreversible) transitions and trigger stable oscillations. As a result, they are a subject of active research that tries to understand how such complex properties are wired into biological control systems.

Feedback loops



Many biological circuits produce complex outputs by exploiting one or more feedback loops. In a sequence of biochemical events, feedback would refer to a downstream

element in the sequence (B in the image on the left) affecting some upstream component (A in the image on the left) to affect its own production or activation (output) in the future. If this element acts to enhance its own output, then it engages in positive feedback (red arrow). A positive feedback loop is also known as a self-reinforcing loop, and it is possible that these loops can be part of a larger loop, as this is characteristic of regulatory circuits.



Conversely, if this element leads to its own inhibition through upstream elements, this is canonically negative feedback (blue arrow). A negative feedback loop is also known as a balancing loop, and it may be common to see oscillations in which a delayed negative feedback signal is used to maintain homeostatic balance in the system.

Feedback loops can be used for amplification (positive) or self-correction (negative). The right combination of positive and negative feedback loops can generate ultrasensitivity and bistability , which in turn can generate decisive transitions and oscillations.

Combination of Positive and Negative Feedback Loops

Positive and negative feedback loops do not always operate distinctly. In the mechanism of biochemical switches, they work together to create a flexible system. For example, according to Pfeuty et al., to overcome a drawback in biochemical systems, positive feedback regulation loops may interact with negative regulation loops to facilitate escape from stable states..The coexistence of two stable states is known as bistability, which is often the result of positive feedback regulations. An example that reveals the interaction of the multiple negative and positive feedback loops is the activation of cyclin-dependent protein kinases, or Cdks14. Positive feedback loops play a role by switching cells from low to high Cdk-activity. The interaction between the two types of loops is evident in mitosis. While positive feedback initiates mitosis, a negative feedback loop promotes the inactivation of the cyclin-dependent kinases by the anaphase-promoting complex. This example clearly shows the combined effects that positive and negative feedback loops have on cell-cycle regulation.

Ultrasensitivity

An "all-or-none" response to a stimulus is termed ultrasensitivity. In other words, a very small change in stimulus causes a very large change in response, producing a sigmoidal dose-response curve. An ultrasensitive response is described by the general equation V = S^n/S^n +Km, known as the Hill equation, when n, the Hill coefficient, is more than 1. The steepness of the sigmoidal curve depends on the value of n. A value of n = 1 produces a hyperbolic or Michaelian response. Ultrasensitivity is achieved in a variety of systems; a notable example is the cooperative binding of the enzyme Hemoglobin to its substrate. Since an ultrasensitive response is almost ‘digital’, it can be used to amplify a response to a stimulus or cause a decisive sharp transition (between ‘off’ and ‘on’ states).

Ultrasensitivity certainly plays a large role in cell-cycle regulation. For example, Cdk1 and Wee1 can mitotic regulators and they are able to inactivate each other through inhibitory phosphorylation. In essence, this represents a double negative feedback loop in which both regulators inactivate each other. According to Kim et al. (2007), there must be an ultra-sensitive element to generate a bistable response. It turns out that Wee1 has an ultrasensitive response to Cdk1, and this likely arises because of substrate competition among the various phosphorylation sites on Wee1.This example shows the role of ultrasensitivity in biochemical switches..

Bistability

Bistability implies hysteresis, and hysteresis implies multistability. Multistability indicates the presence of two or more stable states for a given input. Therefore, bistability is the ability of a system to exist in two steady states. In other words, there is a range of

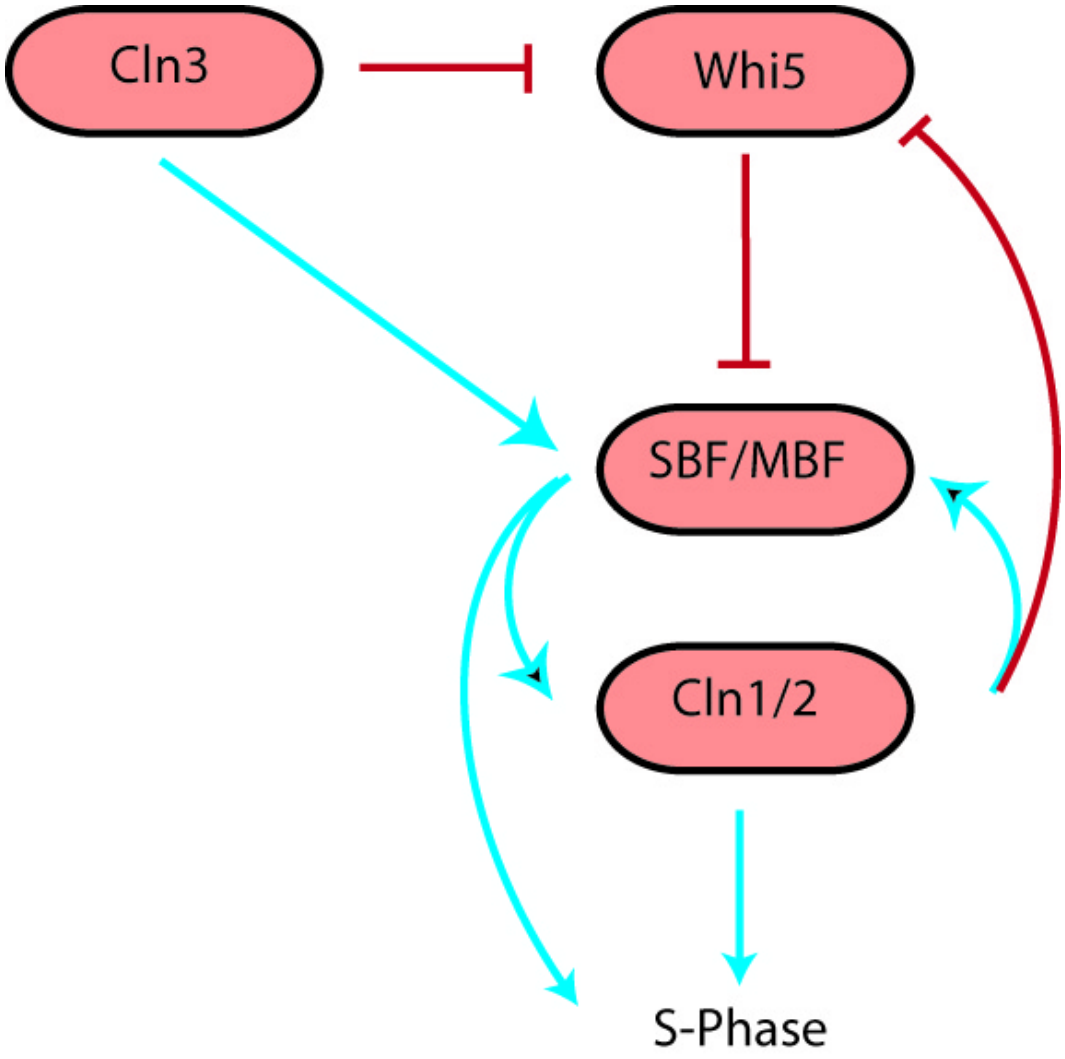
stimulus values for which the response can have two steady-state values. Bistability is accompanied by hysteresis, which means that the system approaches one of the two steady states preferentially depending on its history. Bistability requires feedback as well as an ultrasensitive circuit element.

Under the proper circumstances, positive and negative feedback loops can provide the conditions for bistability; for example, by having positive feedback coupled to an ultrasensitive response element with the circuit. A hysteretic bistable system can act as a robust reversible switch because it is harder for the system to transition between 'on' and 'off' states (compared to the equivalent monostable ultransensitive response). The system could also be poised such that one of the transitions is physically unattainable; for example, no amount of reduction in the stimulus will return the system to the 'off'-state once it is already in the 'on' state. This would form a robust irreversible switch.

There is no one-to-one correspondence between network topology, since many networks have a similar input and output relationship. A network topology does not imply input or output, and similarly input or output does not imply network topology. It is for this reason that parameterization is very important for circuit function. If the dynamics of the input are comparable or faster than the response of the system, the response may appear hysteretic.

Three cell cycle switches are described below that achieve abrupt and/or irreversible transitions by exploiting some of the mechanisms described above.

The G1/S switch



The G1/S transition, more commonly known as the Start checkpoint in budding yeast (the restriction point in other organisms) regulates cell cycle commitment. At this checkpoint, cells either arrest before DNA replication (due to limiting nutrients or a pheromone signal), prolong G1 (size control), or begin replication and progress through the rest of the cell cycle. The G1/S regulatory network or regulon in budding yeast includes the G1 cyclins Cln1, Cln2 and Cln3, Cdc28 (Cdk1), the transcription factors SBF and MBF, and the transcriptional inhibitor Whi5. Cln3 interacts with Cdk1 to initiate the sequence of events by phosphorylating a large number of targets, including SBF, MBF and Whi5. Phosphorylation of Whi5 causes it to translocate out of the nucleus, preventing it from inhibiting SBF and MBF. Active SBF/MBF drive the G1/S transition by turning on the B-type cyclins and initiating DNA replication, bud formation and spindle body duplication. Moreover, SBF/MBF drives expression of Cln1 and Cln2, which can also interact with Cdk1 to promote phosphorylation of its targets.

This G1/S switch was initially thought to function as a linear sequence of events starting with Cln3 and ending in S phase. However, the observation that any one of the Clns was sufficient to activate the regulon indicated that Cln1 and Cln2 might be able to engage positive feedback to activate their own transcription. This would result in a continuously accelerating cycle that could act as an irreversible bistable trigger. Skotheim et al. used single-cell measurements in budding yeast to show that this positive feedback does indeed occur. A small amount of Cln3 induces Cln1/2 expression and then the feedback loop takes over, leading to rapid and abrupt exit of Whi5 from the nucleus and consequently coherent expression of G1/S regulon genes. In the absence of coherent gene expression, cells take longer to exit G1 and a significant fraction even arrest before S phase, highlighting the importance of positive feedback in sharpening the G1/S switch.

The G1/S cell cycle checkpoint controls the passage of eukaryotic cells from the first gap phase, G1, into the DNA synthesis phase, S. In this switch in mammalian cells, there are two cell cycle kinases that help to control the checkpoint: cell cycle kinases CDK4/6-cyclin D and CDK2-cyclin E. The transcription complex that includes Rb and E2F is important in controlling this checkpoint. In the first gap phase, the Rb-HDAC repressor complex binds to the E2F-DP1 transcription factors, therefore inhibiting the downstream transcription. The phosphorylation of Rb by CDK4/6 and CDK2 dissociates the Rb-repressor complex and serves as an on/off switch for the cell cycle. Once Rb is phosphorylated, the inhibition is released on the E2F transcriptional activity. This allows for the transcription of S phase genes encoding for proteins that amplify the G1 to S phase switch.

Many different stimuli apply checkpoint controls including TGFb, DNA damage, contact inhibition, replicative senescence, and growth factor withdrawal. The first four act by inducing members of the INK4 or Kip/Cip families of cell cycle kinase inhibitors. TGFb inhibits the transcription of Cdc25A, a phosphatase that activates the cell cycle kinases, and growth factor withdrawal activates GSK3b, which phosphorylates cyclin D. This leads to its rapid ubiquitination..

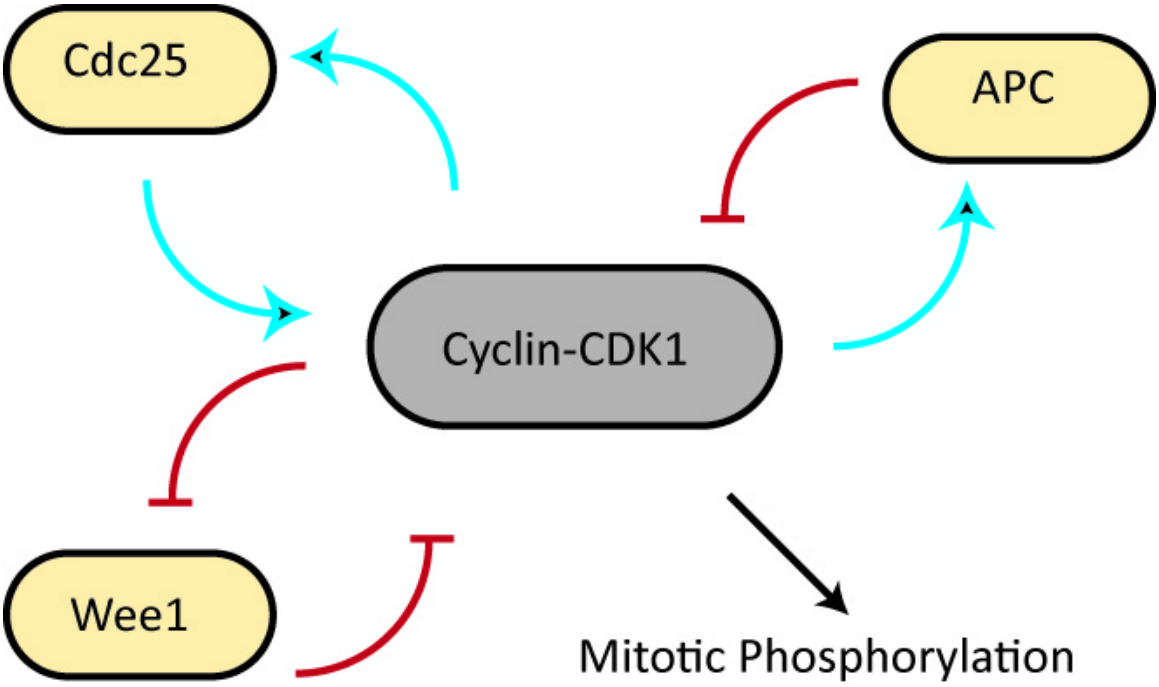
The G2/M switch

This transition is commenced by E2F-mediated transcription of cyclin A, forming the cyclin A-Cdk2 complex. This is useful in regulating events in prophase. In order to proceed past prophase, the cyclin B-Cdk1 complex (first discovered as MPF or M-phase promoting factor) is activated by Cdc 25, a protein phosphatase[1]. As mitosis starts, the nuclear envelope disintegrates, chromosomes condense and become visibile, and the cells prepares for division. The Cyclin B-Cdk1 activation results in nuclear envelope breakdown, which is a characteristic of the initiation of mitosis[1].It is evident that the cyclin A and B complexes with Cdks help regulate mitotic events at the G2/M transition.

As mentioned above, entry into mitosis is controlled by the Cyclin B-Cdk1 complex (first discovered as MPF or M-phase promoting factor; Cdk1 is also known as Cdc2 in fission yeast and Cdc28 in budding yeast). This complex forms an element of an interesting regulatory circuit in which Cdk1 can phosphorylate and activate its activator, the

phosphatase Cdc25 (positive feedback), and phosphorylate and inactivate its inactivator, the kinase Wee1 (double-negative feedback). It was suggested that this circuit could act as a bistable trigger with one stable steady state in G2 (Cdk and Cdc25 off, Wee1 on) and a second stable steady state in M phase (Cdk and Cdc25 active, Wee1 off). Once cells are in mitosis, Cyclin B-Cdk1 activates the Anaphase-promoting complex (APC), which in turn inactivates Cyclin B-Cdk1 by degrading Cyclin B, eventually leading to exit from mitosis. Coupling the bistable Cdk1 response function to the negative feedback from the APC could generate what is known as a relaxation oscillator, with sharp spikes of Cdk1 activity triggering robust mitotic cycles. However, in a relaxation oscillator, the control parameter moves slowly relative to the system's response dynamics which may be an accurate representation of mitotic entry, but not necessarily mitotic exit.

It is necessary to inactivate the cyclin B-Cdk1 complex in order to exit the mitotic stage of the cell cycle. The cells can then return to the first gap phase G1 and wait until the cycle proceeds yet again.



In 2003 Pomerening et al. provided strong evidence for this hypothesis by demonstrating hysteresis and bistability in the activation of Cdk1 in the cytoplasmic extracts of Xenopus oocytes. They first demonstrated a discontinuous sharp response of Cdk1 to changing concentrations of non-destructible Cyclin B (to decouple the Cdk1 response network from APC-mediated negative feedback). However, such a response would be consistent with both a monostable, ultransensitive transition and a bistable transition. To distinguish between these two possibilities, they measured the steady-state levels of active Cdk1 in response to changing cyclin levels, but in two separate experiments, one starting with an interphase extract and one starting with an extract already in mitosis. At intermediate concentrations of cyclin they found two steady-state concentrations of active Cdk1. Which of the two steady states was occupied depended on the history of the system,

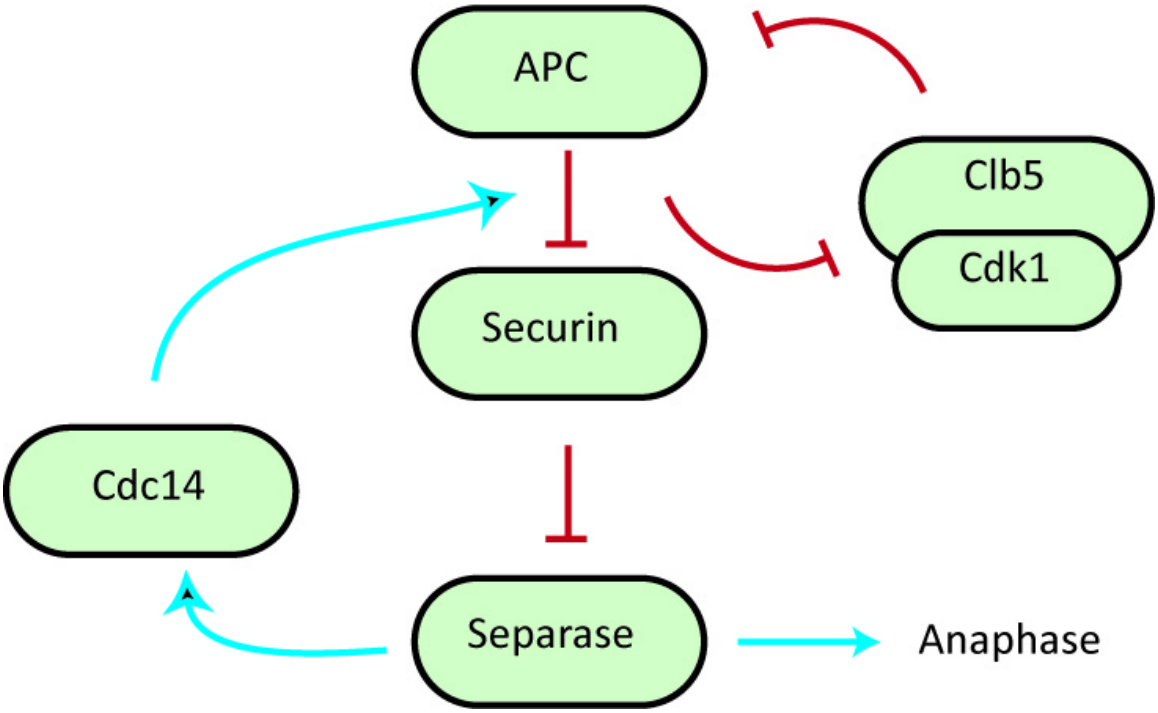
i.e.whether they started with interphase or mitotic extract, effectively demonstrating hysteresis and bistability.

In the same year, Sha et al. independently reached the same conclusion revealing the hysteretic loop also using Xenopus laevis egg extracts. Here, three predictions of the Novak-Tyson model were tested in an effort to conclude that hysteresis is the driving force for "cell-cycle transitions into and out of mitosis". The predictions of the Novak-Tyson model are generic to all saddle-node bifurcations. Saddle-node bifurcations are extremely useful bifurcations in an imperfect world because they help describe biological systems which are not perfect. The first prediction was that the threshold concentration of cyclin to enter mitosis is higher than the threshold concentration of cyclin to exit mitosis, and this was confirmed by supplementing cycling egg extracts with non-degradable cyclin B and measuring the activation and inactivation threshold after the addition of cycloheximide (CHX), which is a protein synthesis inhibitor.. Furthermore, the second prediction of the Novak-Tyson model was also validated: unreplicated deoxyribonucleic acid, or DNA, increases the threshold concentration of cyclin that is required to enter mitosis. In order to arrive at this conclusion, cytostatic factor released extracts were supplemented with CHX, APH (a DNA polymerase inhibitor), or both, and non-degradable cyclin B was added. The third and last prediction that was tested and proven true in this article was that the rate of Cdc2 activation slows down near the activation threshold concentration of cyclin. These predictions and experiments demonstrate the toggle-like switching behavior that can be described by hysteresis in a dynamical system..

Metaphase-anaphase switch

In the transition from metaphase to anaphase, it is crucial that sister chromatids are properly and simultaneously separated to opposite ends of the cell. Separation of sister-chromatids is initially strongly inhibited to prevent premature separation in late mitosis, but this inhibition is relieved through destruction of the inhibitory elements by the anaphase-promoting complex (APC) once sister-chromatid bi-orientation is achieved. One of these inhibitory elements is securin, which prevents the destruction of cohesin, the complex that holds the sister-chromatids together, by binding the protease separase which targets Scc1, a subunit of the cohesin complex, for destruction. In this system, the phosphatase Cdc14 can remove an inhibitory phosphate from securin, thereby facilitating the destruction of securin by the APC, releasing separase. As shown by Uhlmann et al., during the attachment of chromosomes to the mitotic spindle the chromatids remain paired because cohesion between the sisters prevents separation..SY.; Ferrell JE. (2007), "Substrate competition as a source of ultrasensitivity in the activation of Wee1," Cell 128(6): 1133-45 Cohesion is established during DNA replication and depends on cohesin, which is a multisubunit complex composed of Scc1, Scc3, Smc2, and Smc3. In yeast at the metaphase-to-anaphase transition, Scc1 dissociates from the chromosomes and the sister chromatids separate. This action is controlled by the Esp1 protein, which is tightly bound by the anaphase inhibitor Pds1 that is destroyed by the anaphase-promoting complex. In order to verify that Esp1 does play a role in regulating Scc1 chromosome association, cell strains were arrested in G1 with an alpha factor. These cells stayed in arrest during the development. Esp1-1 mutant cells were used and the experiment was

repeated, and Scc1 successfully bound to the chromosomes and remained associated even after the synthesis was terminated. This was crucial in showing that with Esp1, Scc1 is hindered in its ability to become stably associated with chromosomes during G1, and Esp1 can in fact directly remove Scc1 from chromosomes.

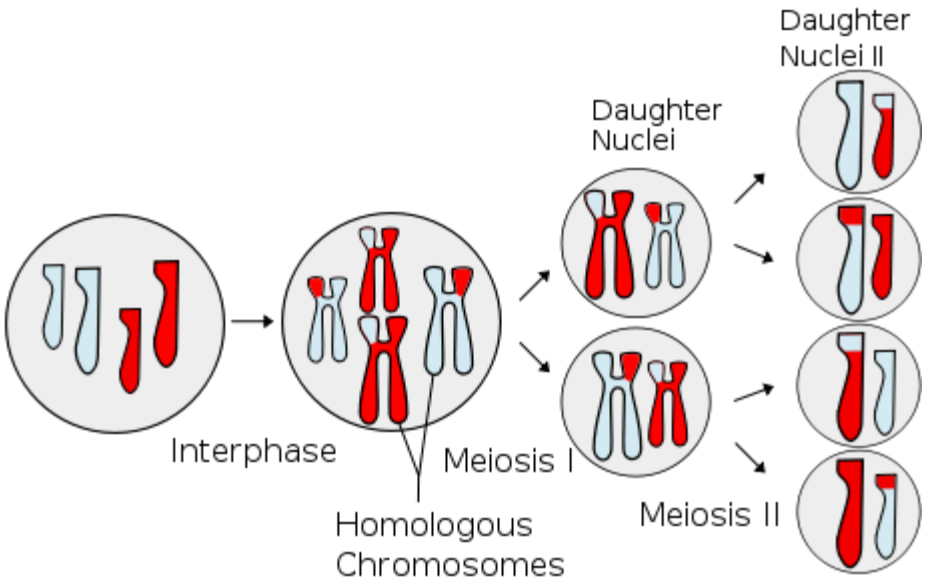


Esp1 and Scc1 diagram

It has been shown by Holt et al. that separase activates Cdc14, which in turn acts on securin, thus creating a positive feedback loop that increases the sharpness of the metaphase to anaphase transition and coordination of sister-chromatid separation. Holt et al. probed the basis for the effect of positive feedback in securin phosphophorlyation by using mutant 'securin' strains of yeast, and testing how changes in the phosphoregulation of securin affects the synchrony of sister chromatid separation. Their results indicate that interfering with this positive securin-separase-cdc14 loop decreases sister chromatid separation synchrony. This positive feedback can hypothetically generate bistability in the transition to anaphase, causing the cell to make the irreversible decision to separate sister-chromatids.

Chapter- 4

Meiosis



Events involving meiosis, showing chromosomal crossover

Meiosis is a special type of cell division necessary for sexual reproduction. In animals, meiosis produces gametes like sperm and egg cells, while in other organisms like fungi it generates spores. Meiosis begins with one diploid cell containing two copies of each chromosome—one from the organism's mother and one from its father—and produces four haploid cells containing one copy of each chromosome. Each of the resulting chromosomes in the gamete cells is a unique mixture of maternal and paternal DNA, ensuring that offspring are genetically distinct from either parent. This gives rise to genetic diversity in sexually reproducing populations, which enables them to adapt during the course of evolution.

Before meiosis, the cell's chromosomes are duplicated by a round of DNA replication. This leaves the maternal and paternal versions of each chromosome, called homologs, composed of two exact copies called sister chromatids and attached at the centromere region. In the beginning of meiosis, the maternal and paternal homologs pair to each other. Then they typically exchange parts by homologous recombination, leading to

crossovers of DNA from the maternal version of the chromosome to the paternal version and vice versa. Spindle fibers bind to the centromeres of each pair of homologs and arrange the pairs at the spindle equator. Then the fibers pull the recombined homologs to opposite poles of the cell. As the chromosomes move away from the center, the cell divides into two daughter cells, each containing a haploid number of chromosomes composed of two chromatids. After the recombined maternal and paternal homologs have separated into the two daughter cells, a second round of cell division occurs. There, meiosis ends as the two sister chromatids making up each homolog are separated and move into one of the four resulting gamete cells. Upon fertilization, for example when a sperm enters an egg cell, two gamete cells produced by meiosis fuse. The gamete from the mother and the gamete from the father each contribute half to the set of chromosomes that make up the new offsping's genome.

Meiosis uses many of the same mechanisms as mitosis, a type of cell division used by eukaryotes like plants and animals to split one cell into two identical daughter cells. In all plants, and in many protists, meiosis results in the formation of spores, haploid cells that can divide vegetatively without undergoing fertilization. Some eukaryotes, like Bdelloid rotifers, have lost the ability to carry out meiosis and have acquired the ability to reproduce by parthenogenesis. Meiosis does not occur in archaea or bacteria, which reproduce via asexual processes such as binary fission.

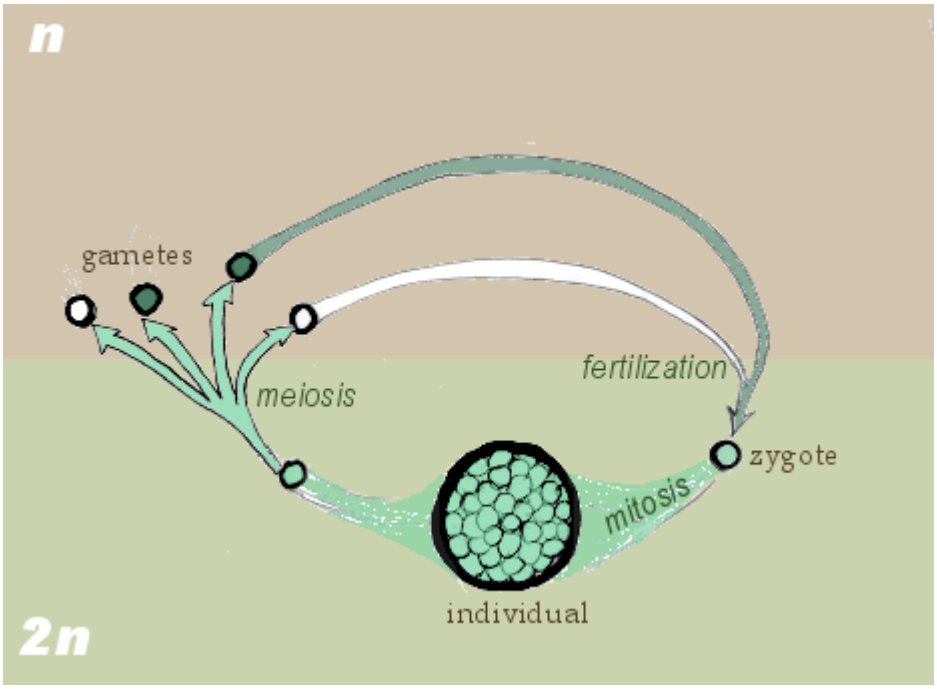
History

Meiosis was discovered and described for the first time in sea urchin eggs in 1876 by the German biologist Oscar Hertwig. It was described again in 1883, at the level of chromosomes, by the Belgian zoologist Edouard Van Beneden, in *Ascaris* worms' eggs. The significance of meiosis for reproduction and inheritance, however, was described only in 1890 by German biologist August Weismann, who noted that two cell divisions were necessary to transform one diploid cell into four haploid cells if the number of chromosomes had to be maintained. In 1911, the American geneticist Thomas Hunt Morgan observed crossover in *Drosophila melanogaster* meiosis and provided the first genetic evidence that genes are transmitted on chromosomes. The term meiosis was coined by J.B Farmer and J.B Moore in 1905.

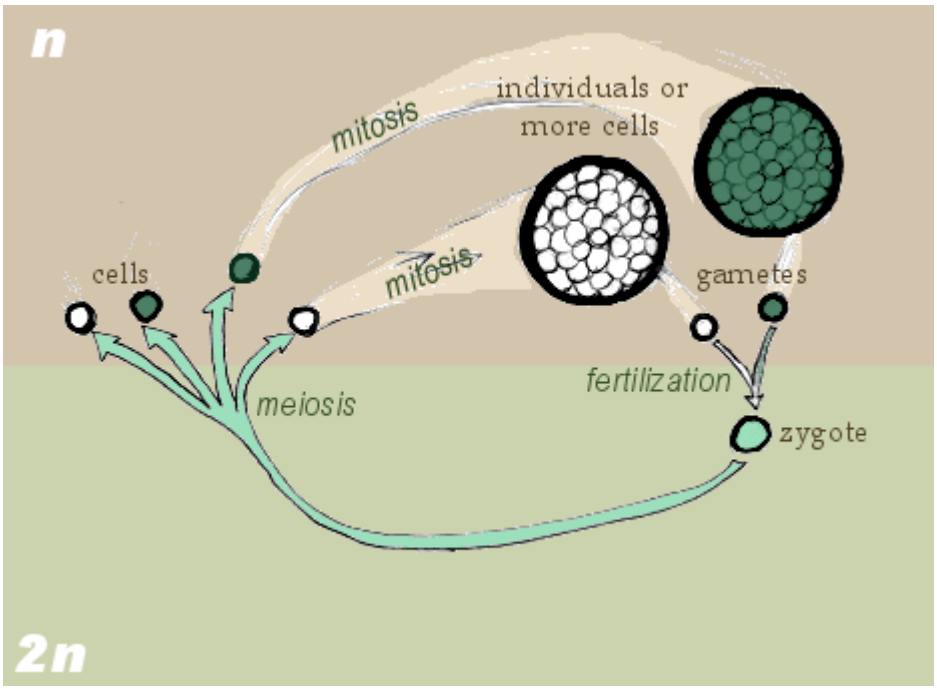
Evolution

Meiosis is thought to have appeared 1.4 billion years ago. The only supergroup of eukaryotes which does not have meiosis in all organisms is excavata. The other five major supergroups, opisthokonts, amoebozoa, rhizaria, archaeplastida and chromalveolates all seem to have genes for meiosis universally present, even if not always functional. Some excavata species do have meiosis which is consistent with the hypothesis that this group is an ancient, paraphyletic grade. An example of a eukaryotic organism in which meiosis does not exist is euglenoid.

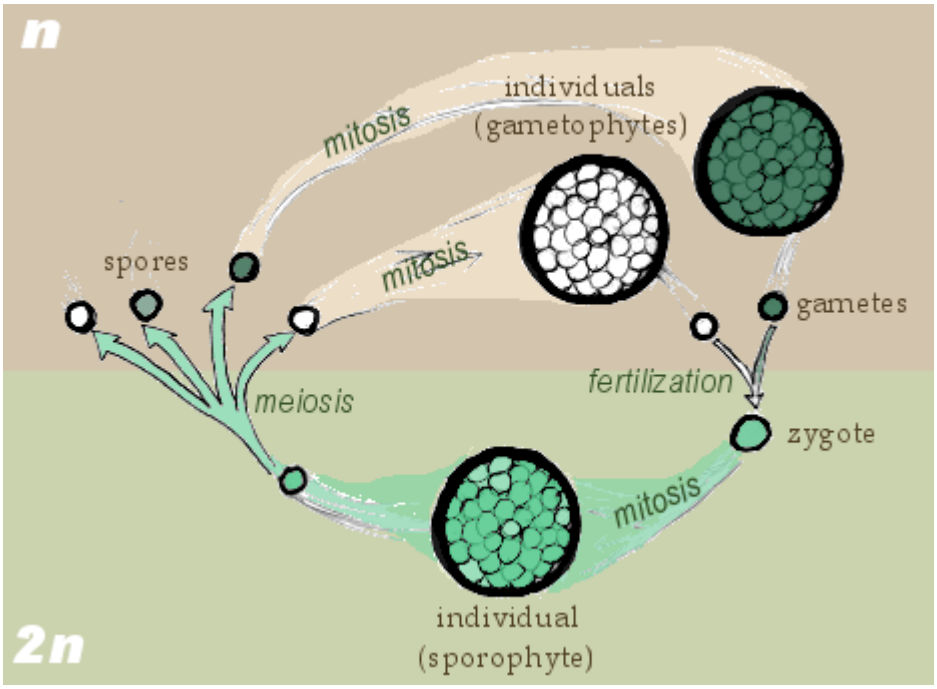
Occurrence of meiosis in eukaryotic life cycles



Gametic life cycle.



Zygotic life cycle.



Sporic life cycle.

Meiosis occurs in eukaryotic life cycles involving sexual reproduction, comprising of the constant cyclical process of meiosis and fertilization. This takes place alongside normal mitotic cell division. In multicellular organisms, there is an intermediary step between the diploid and haploid transition where the organism grows. The organism will then produce the germ cells that continue in the life cycle. The rest of the cells, called somatic cells, function within the organism and will die with it.

Cycling meiosis and fertilization events produces a series of transitions back and forth between alternating haploid and diploid states. The organism phase of the life cycle can occur either during the diploid state (*gametic* or *diploid* life cycle), during the haploid state (*zygotic* or *haploid* life cycle), or both (*sporic* or *haplodiploid* life cycle, in which there two distinct organism phases, one during the haploid state and the other during the diploid state). In this sense, there are three types of life cycles that utilize sexual reproduction, differentiated by the location of the organisms phase(s).

In the *gametic life cycle*, of which humans are a part, the species is diploid, grown from a diploid cell called the zygote. The organism's diploid germ-line stem cells undergo meiosis to create haploid gametes (the spermatozoa for males and ova for females), which fertilize to form the zygote. The diploid zygote undergoes repeated cellular division by mitosis to grow into the organism. Mitosis is a related process to meiosis that creates two cells that are genetically identical to the parent cell. The general principle is that mitosis creates somatic cells and meiosis creates germ cells.

In the *zygotic life cycle* the species is haploid instead, spawned by the proliferation and differentiation of a single haploid cell called the gamete. Two organisms of opposing

gender contribute their haploid germ cells to form a diploid zygote. The zygote undergoes meiosis immediately, creating four haploid cells. These cells undergo mitosis to create the organism. Many fungi and many protozoa are members of the zygotic life cycle.

Finally, in the *sporic life cycle*, the living organism alternates between haploid and diploid states. Consequently, this cycle is also known as the alternation of generations. The diploid organism's germ-line cells undergo meiosis to produce spores. The spores proliferate by mitosis, growing into a haploid organism. The haploid organism's germ cells then combine with another haploid organism's cells, creating the zygote. The zygote undergoes repeated mitosis and differentiation to become the diploid organism again. The sporic life cycle can be considered a fusion of the gametic and zygotic life cycles.

Process

Because meiosis is a "one-way" process, it cannot be said to engage in a cell cycle as mitosis does. However, the preparatory steps that lead up to meiosis are identical in pattern and name to the interphase of the mitotic cell cycle.

Interphase is divided into three phases:

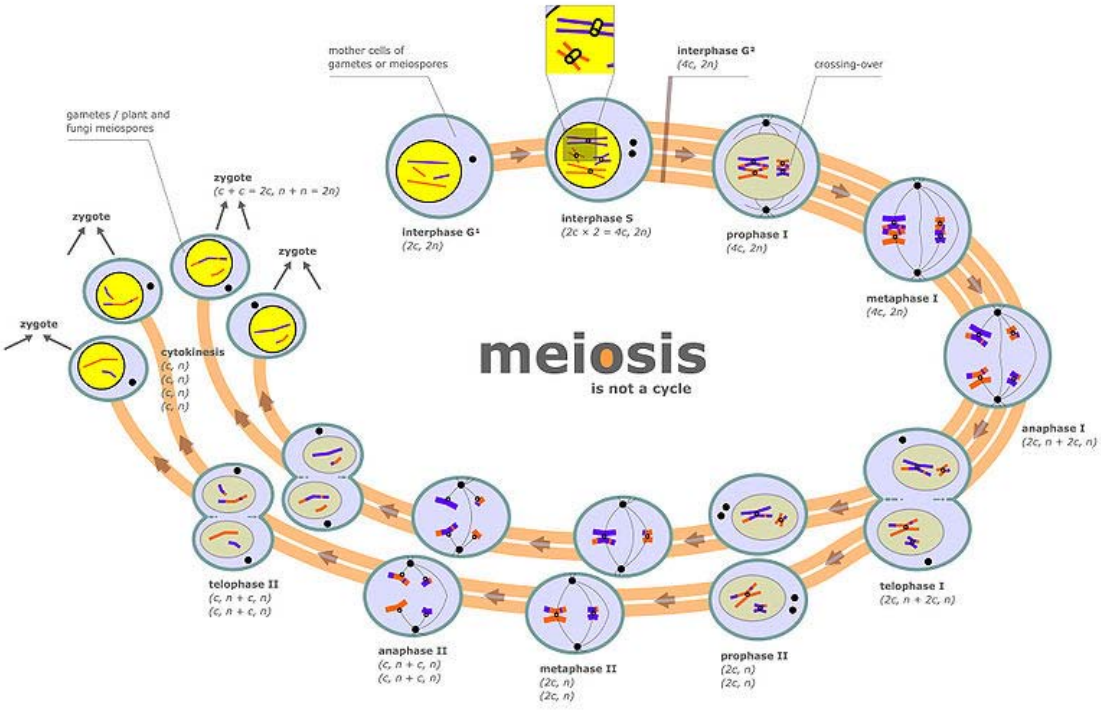
- **Growth 1 (G_1) phase**: This is a very active period, where the cell synthesizes its vast array of proteins, including the enzymes and structural proteins it will need for growth. In G_1 stage each of the chromosomes consists of a single (very long) molecule of DNA. In humans, at this point cells are **46 chromosomes, 2N**, identical to somatic cells.
- **Synthesis (S) phase**: The genetic material is replicated: each of its chromosomes duplicates, so that each of the 46 chromosomes becomes a complex of two identical sister chromatids. The cell is still considered diploid because it still contains the same number of centromeres. The identical sister chromatids have not yet condensed into the densely packaged chromosomes visible with the light microscope. This will take place during prophase I in meiosis.
- **Growth 2 (G_2) phase**: G_2 phase as seen before mitosis is not present in Meiosis. Actually, the first four stages of prophase I in many respects correspond to the G_2 phase of mitotic cell cycle.

Interphase is followed by meiosis I and then meiosis II. Meiosis I consists of separating the pairs of homologous chromosome, each made up of two sister chromatids, into two cells. One entire haploid content of chromosomes is contained in each of the resulting daughter cells; the first meiotic division therefore reduces the ploidy of the original cell by a factor of 2.

Meiosis II consists of decoupling each chromosome's sister strands (chromatids), and segregating the individual chromatids into haploid daughter cells. The two cells resulting from meiosis I divide during meiosis II, creating 4 haploid daughter cells. Meiosis I and II are each divided into prophase, metaphase, anaphase, and telophase stages, similar in purpose to their analogous subphases in the mitotic cell cycle. Therefore, meiosis

includes the stages of meiosis I (prophase I, metaphase I, anaphase I, telophase I), and meiosis II (prophase II, metaphase II, anaphase II, telophase II).

Meiosis generates genetic diversity in two ways: (1) independent alignment and subsequent separation of homologous chromosome pairs during the first meiotic division allows a random and independent selection of each chromosome segregates into each gamete; and (2) physical exchange of homologous chromosomal regions by homologous recombination during prophase I results in new combinations of DNA within chromosomes.



A diagram of the meiotic phases

Phases

Meiosis takes place in several stages.

Meiosis I

Meiosis I separates homologous chromosomes, producing two haploid cells (**N chromosomes, 23** in humans), so meiosis I is referred to as a **reductional division**. A regular diploid human cell contains 46 chromosomes and is considered 2N because it contains 23 pairs of homologous chromosomes. However, after meiosis I, although the cell contains 46 chromatids, it is only considered as being N, with 23 chromosomes. This is because later, in Anaphase I, the sister chromatids will remain together as the spindle fibres pulls the pair toward the pole of the new cell. In meiosis II, an **equational division**

similar to mitosis will occur whereby the sister chromatids are finally split, creating a total of 4 haploid cells (**23 chromosomes, N**) per daughter cell from the first division.

Prophase I

During prophase I, DNA is exchanged between homologous chromosomes in a process called homologous recombination. This often results in chromosomal crossover. The new combinations of DNA created during crossover are a significant source of genetic variation, and may result in beneficial new combinations of alleles. The paired and replicated chromosomes are called bivalents or tetrads, which have two chromosomes and four chromatids, with one chromosome coming from each parent. At this stage, non-sister chromatids may cross-over at points called chiasmata (plural; singular chiasma).

Leptotene

The first stage of prophase I is the *leptotene* stage, also known as *leptonema*, from Greek words meaning "thin threads".[:27]In this stage of prophase I, individual chromosomes—each consisting of two sister chromatids—change from the diffuse state they exist in during the cell's period of growth and gene expression, and condense into visible strands within the nucleus.[:27:353] However the two sister chromatids are still so tightly bound that they are indistinguishable from one another. During leptotene, lateral elements of the synaptonemal complex assemble.Leptotene is of very short duration and progressive condensation and coiling of chromosome fibers takes place. Chromosome assume a long thread like shape,they contract and become thick.At the beginning chromosomes are present in diploid number as in mitotic prophase.Each chromosome is made up of only one chromosome and half of the total chromosome are paternal and half maternal.For every paternal chromosome there is a corresponding maternal chromosome similar in size,shape and nature of inherited characters and are called homologous chromosome.

Zygotene

The *zygotene* stage, also known as *zygonema*, from Greek words meaning "paired threads",[:27] occurs as the chromosomes approximately line up with each other into homologous chromosome pairs. This is called the bouquet stage because of the way the telomeres cluster at one end of the nucleus. At this stage, the synapsis (pairing/coming together) of homologous chromosomes takes place, facilitated by assembly of central element of the synaptonemal complex.Pairing is brought about by a zipper like fashion and may start at the centromere(procentric),at the chromosome ends(proterminal),or at any other portion(intermediate).Individuals of a pair are equal in length and in position of centromere. Thus pairing is highly specific and exact.The paired chromosomes are called Bivalent chromosome.

Pachytene

The *pachytene* stage, also known as *pachynema*, from Greek words meaning "thick threads",[:27] is the stage when chromosomal crossover (crossing over) occurs. Nonsister chromatids of homologous chromosomes randomly exchange segments over regions of

homology. Sex chromosomes, however, are not wholly identical, and only exchange information over a small region of homology. At the sites where exchange happens, chiasmata form. The exchange of information between the non-sister chromatids results in a recombination of information; each chromosome has the complete set of information it had before, and there are no gaps formed as a result of the process. Because the chromosomes cannot be distinguished in the synaptonemal complex, the actual act of crossing over is not perceivable through the microscope, and chiasmata are not visible until the next stage.

Diplotene

During the *diplotene* stage, also known as *diplonema*, from Greek words meaning "two threads",[:30] the synaptonemal complex degrades and homologous chromosomes separate from one another a little. The chromosomes themselves uncoil a bit, allowing some transcription of DNA. However, the homologous chromosomes of each bivalent remain tightly bound at chiasmata, the regions where crossing-over occurred. The chiasmata remain on the chromosomes until they are severed in anaphase I.

In human fetal oogenesis all developing oocytes develop to this stage and stop before birth. This suspended state is referred to as the *dictyotene stage* and remains so until puberty. In males, only spermatogonia (spermatogenesis) exist until meiosis begins at puberty.

Diakinesis

Chromosomes condense further during the *diakinesis* stage, from Greek words meaning "moving through".[:30] This is the first point in meiosis where the four parts of the tetrads are actually visible. Sites of crossing over entangle together, effectively overlapping, making chiasmata clearly visible. Other than this observation, the rest of the stage closely resembles prometaphase of mitosis; the nucleoli disappear, the nuclear membrane disintegrates into vesicles, and the meiotic spindle begins to form.

Synchronous processes

During these stages, two centrosomes, containing a pair of centrioles in animal cells, migrate to the two poles of the cell. These centrosomes, which were duplicated during S-phase, function as microtubule organizing centers nucleating microtubules, which are essentially cellular ropes and poles. The microtubules invade the nuclear region after the nuclear envelope disintegrates, attaching to the chromosomes at the kinetochore. The kinetochore functions as a motor, pulling the chromosome along the attached microtubule toward the originating centriole, like a train on a track. There are four kinetochores on each tetrad, but the pair of kinetochores on each sister chromatid fuses and functions as a unit during meiosis I.

Microtubules that attach to the kinetochores are known as *kinetochore microtubules*. Other microtubules will interact with microtubules from the opposite centriole: these are called *nonkinetochore microtubules* or *polar microtubules*. A third type of microtubules,

the aster microtubules, radiates from the centrosome into the cytoplasm or contacts components of the membrane skeleton.

Metaphase I

Homologous pairs move together along the metaphase plate: As **kinetochore microtubules** from both centrioles attach to their respective kinetochores, the homologous chromosomes align along an equatorial plane that bisects the spindle, due to continuous counterbalancing forces exerted on the bivalents by the microtubules emanating from the two kinetochores of homologous chromosomes. The physical basis of the independent assortment of chromosomes is the random orientation of each bivalent along the metaphase plate, with respect to the orientation of the other bivalents along the same equatorial line.

Anaphase I

Kinetochore (bipolar spindles) microtubules shorten, severing the recombination nodules and pulling homologous chromosomes apart. Since each chromosome has only one functional unit of a pair of kinetochores, whole chromosomes are pulled toward opposing poles, forming two haploid sets. Each chromosome still contains a pair of sister chromatids. Nonkinetochore microtubules lengthen, pushing the centrioles farther apart. The cell elongates in preparation for division down the center.

Telophase I

The last meiotic division effectively ends when the chromosomes arrive at the poles. Each daughter cell now has half the number of chromosomes but each chromosome consists of a pair of chromatids. The microtubules that make up the spindle network disappear, and a new nuclear membrane surrounds each haploid set. The chromosomes uncoil back into chromatin. Cytokinesis, the pinching of the cell membrane in animal cells or the formation of the cell wall in plant cells, occurs, completing the creation of two daughter cells. Sister chromatids remain attached during telophase I.

Cells may enter a period of rest known as interkinesis or interphase II. No DNA replication occurs during this stage.

Meiosis II

Meiosis II is the second part of the meiotic process. Much of the process is similar to mitosis. The end result is production of four haploid cells (**23 chromosomes, N** in humans) from the two haploid cells (**23 chromosomes, N *** each of the chromosomes consisting of two sister chromatids) produced in meiosis I. The four main steps of Meiosis II are: Prophase II, Metaphase II, Anaphase II, and Telophase II.

In **prophase II** we see the disappearance of the nucleoli and the nuclear envelope again as well as the shortening and thickening of the chromatids. Centrioles move to the polar regions and arrange spindle fibers for the second meiotic division.

In **metaphase II**, the centromeres contain two kinetochores that attach to spindle fibers from the centrosomes (centrioles) at each pole. The new equatorial metaphase plate is rotated by 90 degrees when compared to meiosis I, perpendicular to the previous plate.

This is followed by **anaphase II**, where the centromeres are cleaved, allowing microtubules attached to the kinetochores to pull the sister chromatids apart. The sister chromatids by convention are now called sister chromosomes as they move toward opposing poles.

The process ends with **telophase II**, which is similar to telophase I, and is marked by uncoiling and lengthening of the chromosomes and the disappearance of the spindle. Nuclear envelopes reform and cleavage or cell wall formation eventually produces a total of four daughter cells, each with a haploid set of chromosomes. Meiosis is now complete and ends up with four new daughter cells.

Significance

Meiosis facilitates stable sexual reproduction. Without the halving of ploidy, or chromosome count, fertilization would result in zygotes that have twice the number of chromosomes as the zygotes from the previous generation. Successive generations would have an exponential increase in chromosome count. In organisms that are normally diploid, polyploidy, the state of having three or more sets of chromosomes, results in extreme developmental abnormalities or lethality. Polyploidy is poorly tolerated in most animal species. Plants, however, regularly produce fertile, viable polyploids. Polyploidy has been implicated as an important mechanism in plant speciation.

Most importantly, recombination and independent assortment of homologous chromosomes allow for a greater diversity of genotypes in the offspring. This produces genetic variation in gametes that promote genetic and phenotypic variation in a population of offspring. Therefore a gene for meiosis will be favoured by natural selection over an allele for mitotic reproduction, because any selection pressure which acts against any clone will act against all clones, whilst inevitably favoring some offspring which are the result of sexual reproduction.

Nondisjunction

The normal separation of chromosomes in meiosis I or sister chromatids in meiosis II is termed *disjunction*. When the separation is not normal, it is called **nondisjunction**. This results in the production of gametes which have either too many or too few of a particular chromosome, and is a common mechanism for trisomy or monosomy. Nondisjunction can occur in the meiosis I or meiosis II, phases of cellular reproduction, or during mitosis.

This is a cause of several medical conditions in humans (such as):

- Down Syndrome - trisomy of chromosome 21
- Patau Syndrome - trisomy of chromosome 13
- Edward Syndrome - trisomy of chromosome 18
- Klinefelter Syndrome - extra X chromosomes in males - i.e. XXY, XXXY, XXXXY
- Turner Syndrome - lacking of one X chromosome in females - i.e. XO
- Triple X syndrome - an extra X chromosome in females
- XYY Syndrome - an extra Y chromosome in males

Meiosis in mammals

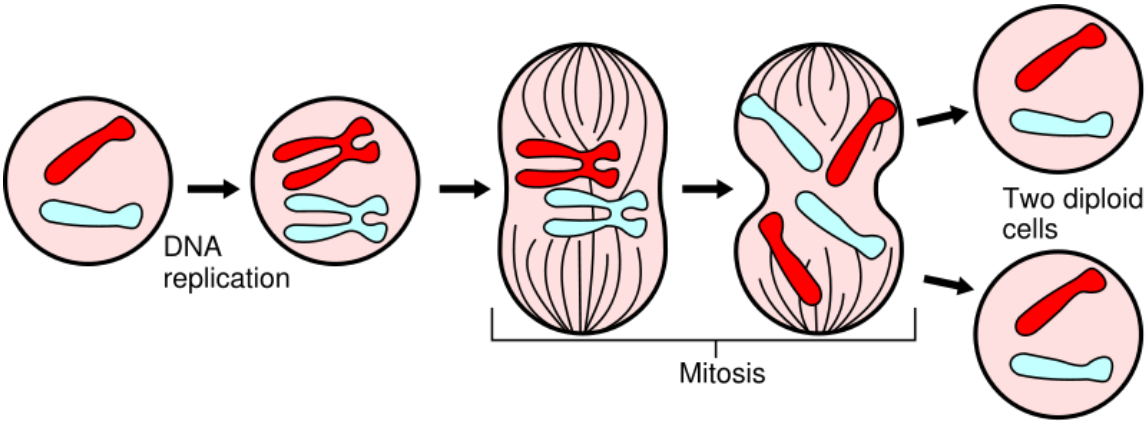
In females, meiosis occurs in cells known as oogonia (singular: oogonium). Each oogonium that initiates meiosis will divide twice to form a single oocyte and two polar bodies. However, before these divisions occur, these cells stop at the diplotene stage of meiosis I and lie dormant within a protective shell of somatic cells called the follicle. Follicles begin growth at a steady pace in a process known as folliculogenesis, and a small number enter the menstrual cycle. Menstruated oocytes continue meiosis I and arrest at meiosis II until fertilization. The process of meiosis in females occurs during oogenesis, and differs from the typical meiosis in that it features a long period of meiotic arrest known as the Dictyate stage and lacks the assistance of centrosomes.

In males, meiosis occurs during spermatogenesis in the seminiferous tubules of the testicles. Meiosis during spermatogenesis is specific to a type of cell called spermatocytes that will later mature to become spermatozoa.

In female mammals, meiosis begins immediately after primordial germ cells migrate to the ovary in the embryo, but in the males, meiosis begins years later at the time of puberty. It is retinoic acid, derived from the primitive kidney (mesonephros) that stimulates meiosis in ovarian oogonia. Tissues of the male testis suppress meiosis by degrading retinoic acid, a stimulator of meiosis. This is overcome at puberty when cells within seminiferous tubules called Sertoli cells start making their own retinoic acid. Sensitivity to retinoic acid is also adjusted by proteins called nanos and DAZL.

Chapter- 5

Mitosis



Mitosis divides the chromosomes in a cell nucleus.

Mitosis is the process by which a eukaryotic cell separates the chromosomes in its cell nucleus into two identical sets in two nuclei. It is generally followed immediately by cytokinesis, which divides the nuclei, cytoplasm, organelles and cell membrane into two cells containing roughly equal shares of these cellular components. Mitosis and cytokinesis together define the **mitotic (M) phase** of the cell cycle—the division of the mother cell into two daughter cells, genetically identical to each other and to their parent cell. This accounts for approximately 10% of the cell cycle.

Mitosis occurs exclusively in eukaryotic cells, but the process varies in different species. For example, animals undergo an "open" mitosis, where the nuclear envelope breaks down before the chromosomes separate, while fungi such as *Aspergillus nidulans* and *Saccharomyces cerevisiae* (yeast) undergo a "closed" mitosis, where chromosomes divide within an intact cell nucleus. Prokaryotic cells, which lack a nucleus, divide by a process called binary fission.

The process of mitosis is complex and highly regulated. The sequence of events is divided into phases, corresponding to the completion of one set of activities and the start of the next. These stages are interphase, prophase, prometaphase, metaphase, anaphase and telophase. During mitosis the pairs of chromosomes condense and attach to fibers

that pull the sister chromatids to opposite sides of the cell. The cell then divides in cytokinesis, to produce two identical daughter cells.

Because cytokinesis usually occurs in conjunction with mitosis, "mitosis" is often used interchangeably with "mitotic phase". However, there are many cells where mitosis and cytokinesis occur separately, forming single cells with multiple nuclei. This occurs most notably among the fungi and slime moulds, but is found in various different groups. Even in animals, cytokinesis and mitosis may occur independently, for instance during certain stages of fruit fly embryonic development. Errors in mitosis can either kill a cell through apoptosis or cause mutations that may lead to cancer.

Overview

The primary result of mitosis is the transferring of the parent cell's genome into two daughter cells. The genome is composed of a number of chromosomes—complexes of tightly-coiled DNA that contain genetic information vital for proper cell function. Because each resultant daughter cell should be genetically identical to the parent cell, the parent cell must make a copy of each chromosome before mitosis. This occurs during the S phase of interphase, the period that precedes the mitotic phase in the cell cycle where preparation for mitosis occurs.

Each new chromosome now contains two identical copies of itself, called *sister chromatids*, attached together in a specialized region of the chromosome known as the *centromere*. Each sister chromatid is not considered a chromosome in itself, and a chromosome always contains two sister chromatids.

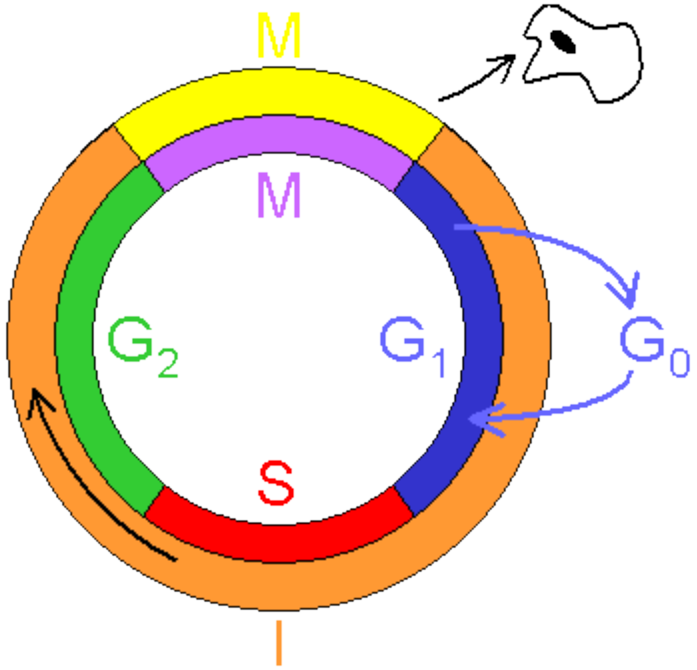
In most eukaryotes, the nuclear envelope that combines the DNA from the cytoplasm disassembles. The chromosomes align themselves in a line spanning the cell. Microtubules, essentially miniature strings, splay out from opposite ends of the cell and shorten, pulling apart the sister chromatids of each chromosome. As a matter of convention, each sister chromatid is now considered a chromosome, so they are renamed to *sister chromosomes*. As the cell elongates, corresponding sister chromosomes are pulled toward opposite ends. A new nuclear envelope forms around the separated sister chromosomes.

As mitosis completes cytokinesis is well underway. In animal cells, the cell pinches inward where the imaginary line used to be (the area of the cell membrane that pinches to form the two daughter cells is called the cleavage furrow), separating the two developing nuclei. In plant cells, the daughter cells will construct a new dividing cell wall between each other. Eventually, the mother cell will be split in half, giving rise to two daughter cells, each with an equivalent and complete copy of the original genome.

Prokaryotic cells undergo a process similar to mitosis called binary fission. However, prokaryotes cannot be properly said to undergo cytokinesis because they lack a nucleus and only have a single chromosome with no mitochondria.

Phases of cell cycle and mitosis

Interphase



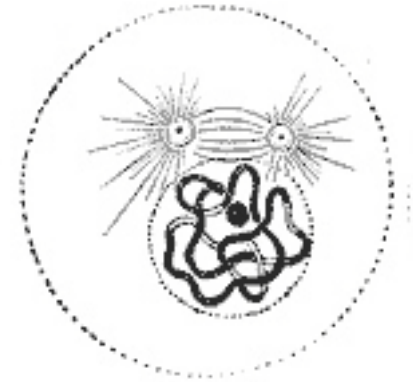
The cell cycle

The mitotic phase is a relatively short period of the cell cycle. It alternates with the much longer *interphase*, where the cell prepares itself for cell division. Interphase is therefore not part of mitosis. Interphase is divided into three phases, G_1 (first gap), S (synthesis), and G_2 (second gap). During all three phases, the cell grows by producing proteins and cytoplasmic organelles. However, chromosomes are replicated only during the S phase. Thus, a cell grows (G_1), continues to grow as it duplicates its chromosomes (S), grows more and prepares for mitosis (G_2), and finally it divides (M) before restarting the cycle.

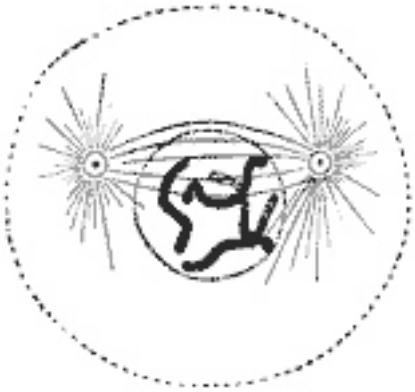
Preprophase

In plant cells only, prophase is preceded by a pre-prophase stage. In highly vacuolated plant cells, the nucleus has to migrate into the center of the cell before mitosis can begin. This is achieved through the formation of a phragmosome, a transverse sheet of cytoplasm that bisects the cell along the future plane of cell division. In addition to phragmosome formation, preprophase is characterized by the formation of a ring of microtubules and actin filaments (called preprophase band) underneath the plasma membrane around the equatorial plane of the future mitotic spindle. This band marks the position where the cell will eventually divide. The cells of higher plants (such as the flowering plants) lack centrioles; instead, microtubules form a spindle on the surface of the nucleus and are then being organized into a spindle by the chromosomes themselves,

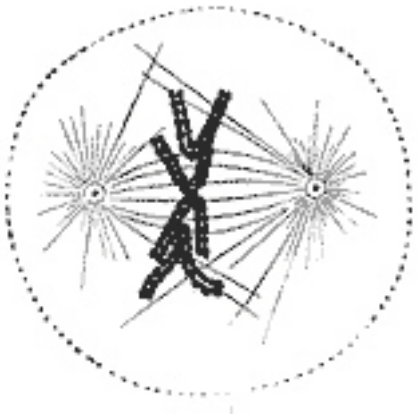
after the nuclear membrane breaks down. The preprophase band disappears during nuclear envelope disassembly and spindle formation in prometaphase.



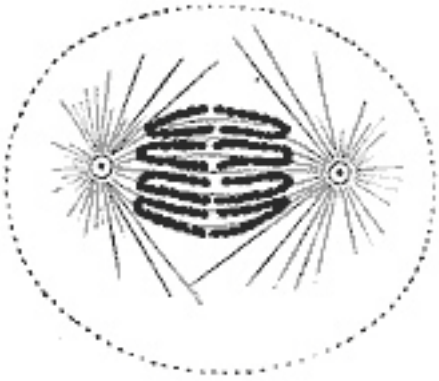
Prophase: The two round objects above the nucleus are the centrosomes. The chromatin has condensed.



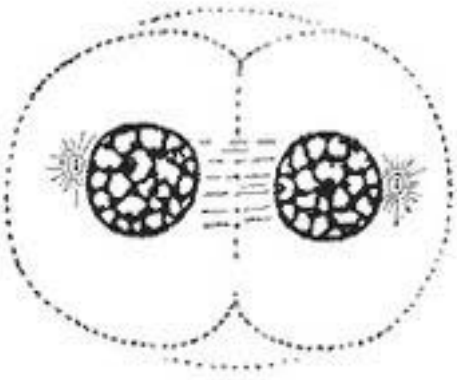
Prometaphase: The nuclear membrane has degraded, and microtubules have invaded the nuclear space. These microtubules can attach to kinetochores or they can interact with opposing microtubules.



Metaphase: The chromosomes have aligned at the metaphase plate.

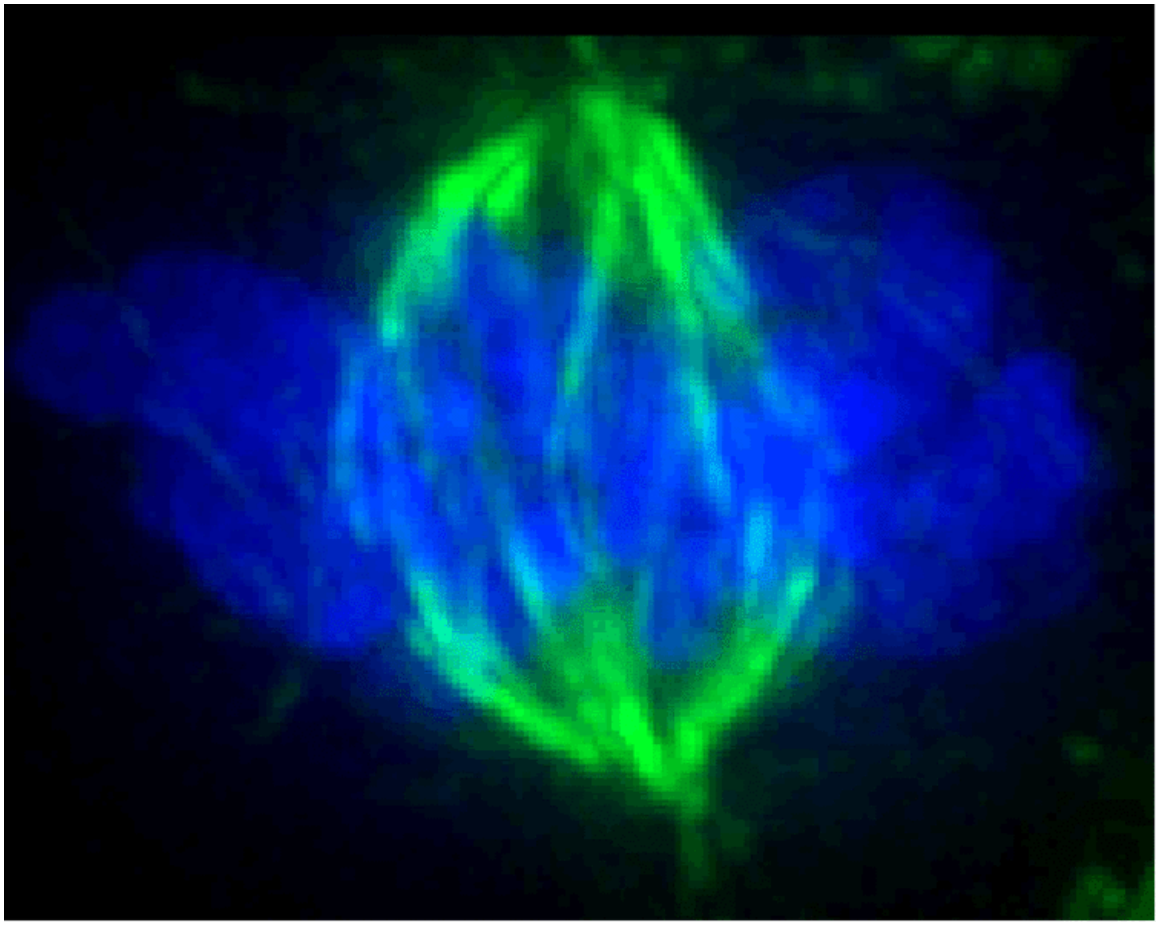


Early anaphase: The kinetochore microtubules shorten.



Telophase: The decondensing chromosomes are surrounded by nuclear membranes. Cytokinesis has already begun; the pinched area is known as the *cleavage furrow*.

Prophase



Micrograph showing condensed chromosomes in blue and the mitotic spindle in green during prometaphase of mitosis

Normally, the genetic material in the nucleus is in a loosely bundled coil called chromatin. At the onset of prophase, chromatin condenses together into a highly ordered structure called a chromosome. Since the genetic material has already been duplicated earlier in S phase, the replicated chromosomes have two sister chromatids, bound together at the centromere by the cohesion complex. Chromosomes are typically visible at high magnification through a light microscope.

Close to the nucleus are structures called centrosomes, which are made of a pair of centrioles. The centrosome is the coordinating center for the cell's microtubules. A cell inherits a single centrosome at cell division, which replicates before a new mitosis begins, giving a pair of centrosomes. The two centrosomes nucleate microtubules (which may be thought of as cellular ropes or poles) to form the spindle by polymerizing soluble tubulin. Molecular motor proteins then push the centrosomes along these microtubules to opposite sides of the cell. Although centrioles help organize microtubule assembly, they

are not essential for the formation of the spindle, since they are absent from plants, and centrosomes are not always used in meiosis.

Prometaphase

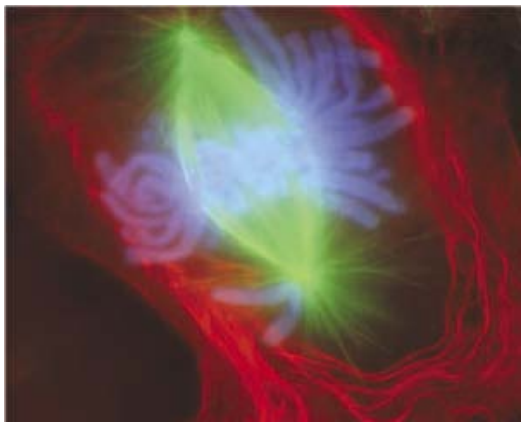
The nuclear envelope disassembles and microtubules invade the nuclear space. This is called *open mitosis*, and it occurs in most multicellular organisms. Fungi and some protists, such as algae or trichomonads, undergo a variation called *closed mitosis* where the spindle forms inside the nucleus, or its microtubules are able to penetrate an intact nuclear envelope.

Each chromosome forms two kinetochores at the centromere, one attached at each chromatid. A kinetochore is a complex protein structure that is analogous to a ring for the microtubule hook; it is the point where microtubules attach themselves to the chromosome. Although the kinetochore structure and function are not fully understood, it is known that it contains some form of molecular motor. When a microtubule connects with the kinetochore, the motor activates, using energy from ATP to "crawl" up the tube toward the originating centrosome. This motor activity, coupled with polymerisation and depolymerisation of microtubules, provides the pulling force necessary to later separate the chromosome's two chromatids.

When the spindle grows to sufficient length, *kinetochore microtubules* begin searching for kinetochores to attach to. A number of *nonkinetochore microtubules* find and interact with corresponding nonkinetochore microtubules from the opposite centrosome to form the mitotic spindle. Prometaphase is sometimes considered part of prophase.

In the fishing pole analogy, the kinetochore would be the "hook" that catches a sister chromatid or "fish". The centrosome acts as the "reel" that draws in the spindle fibers or "fishing line".

Metaphase



A cell in late metaphase. All chromosomes (blue) but one have arrived at the metaphase plate.

As microtubules find and attach to kinetochores in prometaphase, the centromeres of the chromosomes convene along the *metaphase plate* or *equatorial plane*, an imaginary line that is equidistant from the two centrosome poles. This even alignment is due to the counterbalance of the pulling powers generated by the opposing kinetochores, analogous to a tug-of-war between people of equal strength. In certain types of cells, chromosomes do not line up at the metaphase plate and instead move back and forth between the poles randomly, only roughly lining up along the midline. Metaphase comes from the Greek *μετα* meaning "after."

Because proper chromosome separation requires that every kinetochore be attached to a bundle of microtubules (spindle fibres), it is thought that unattached kinetochores generate a signal to prevent premature progression to anaphase without all chromosomes being aligned. The signal creates the *mitotic spindle checkpoint*.

Anaphase

When every kinetochore is attached to a cluster of microtubules and the chromosomes have lined up along the metaphase plate, the cell proceeds to anaphase (from the Greek *ανα* meaning “up,” “against,” “back,” or “re-”).

Two events then occur: first, the proteins that bind sister chromatids together are cleaved, allowing them to separate. These sister chromatids, which have now become distinct sister chromosomes, are pulled apart by shortening kinetochore microtubules and move toward the respective centrosomes to which they are attached. Next, the nonkinetochore microtubules elongate, pulling the centrosomes (and the set of chromosomes to which they are attached) apart to opposite ends of the cell. The force that causes the centrosomes to move towards the ends of the cell is still unknown, although there is a theory that suggests that the rapid assembly and breakdown of microtubules may cause this movement.

These two stages are sometimes called early and late anaphase. Early anaphase is usually defined as the separation of the sister chromatids, while late anaphase is the elongation of the microtubules and the chromosomes being pulled farther apart. At the end of anaphase, the cell has succeeded in separating identical copies of the genetic material into two distinct populations.

Telophase

Telophase (from the Greek *τελος* meaning "end") is a reversal of prophase and prometaphase events. It "cleans up" the after effects of mitosis. At telophase, the nonkinetochore microtubules continue to lengthen, elongating the cell even more. Corresponding sister chromosomes attach at opposite ends of the cell. A new nuclear envelope, using fragments of the parent cell's nuclear membrane, forms around each set of separated sister chromosomes. Both sets of chromosomes, now surrounded by new nuclei, unfold back into chromatin. Mitosis is complete, but cell division is not yet complete.

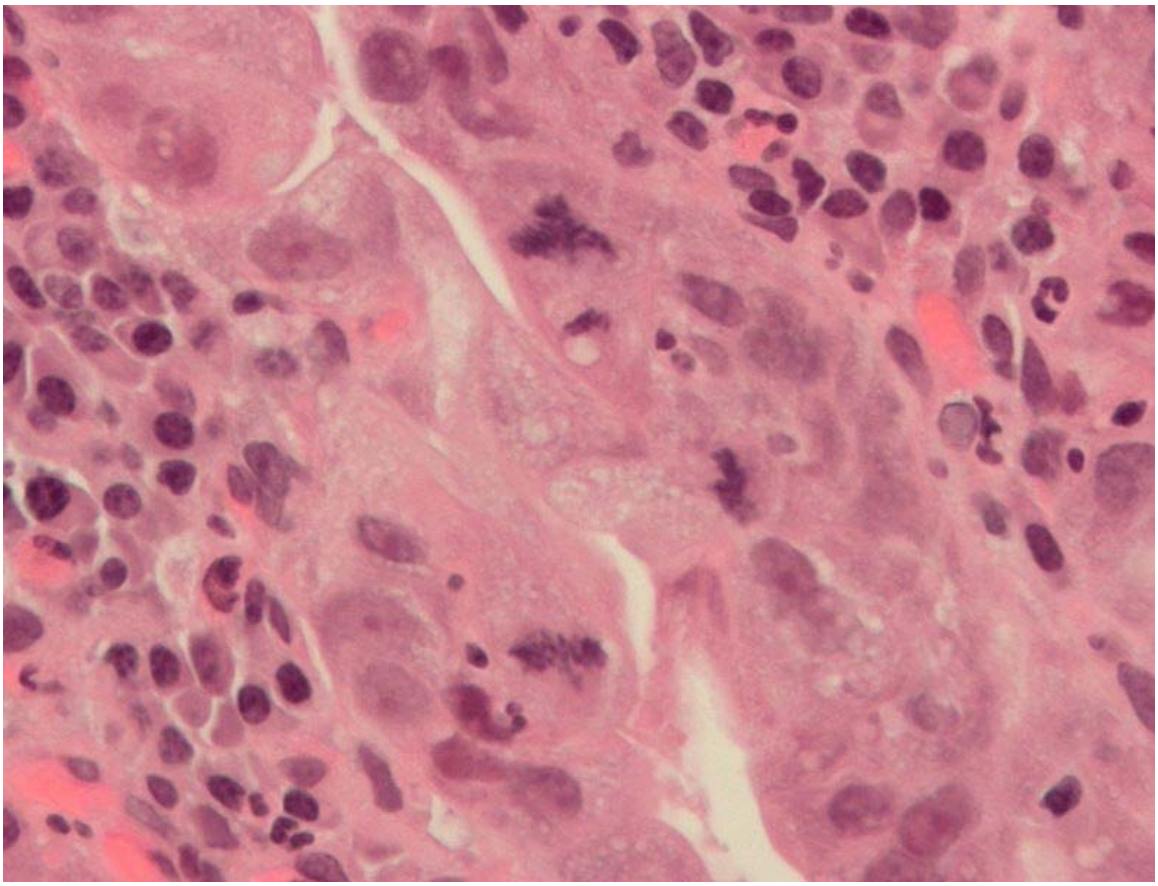
Cytokinesis

Cytokinesis is often mistakenly thought to be the final part of telophase; however, cytokinesis is a separate process that begins at the same time as telophase. Cytokinesis is technically not even a phase of mitosis, but rather a separate process, necessary for completing cell division. In animal cells, a cleavage furrow (pinch) containing a contractile ring develops where the metaphase plate used to be, pinching off the separated nuclei. In both animal and plant cells, cell division is also driven by vesicles derived from the Golgi apparatus, which move along microtubules to the middle of the cell. In plants this structure coalesces into a cell plate at the center of the phragmoplast and develops into a cell wall, separating the two nuclei. The phragmoplast is a microtubule structure typical for higher plants, whereas some green algae use a phycoplast microtubule array during cytokinesis. Each daughter cell has a complete copy of the genome of its parent cell. The end of cytokinesis marks the end of the M-phase.

Significance

Mitosis is important for the maintenance of the chromosomal set; each cell formed receives chromosomes that are alike in composition and equal in number to the chromosomes of the parent cell. Transcription is generally believed to cease during mitosis, but epigenetic mechanisms such as bookmarking function during this stage of the cell cycle to ensure that the "memory" of which genes were active prior to entry into mitosis are transmitted to the daughter cells.

Consequences of errors



An abnormal (tripolar) mitoses (12 o'clock position) in a precancerous lesion of the stomach. H&E stain

Although errors in mitosis are rare, the process may go wrong, especially during early cellular divisions in the zygote. Mitotic errors can be especially dangerous to the organism because future offspring from this parent cell will carry the same disorder.

In *non-disjunction*, a chromosome may fail to separate during anaphase. One daughter cell will receive both sister chromosomes and the other will receive none. This results in the former cell having three chromosomes containing the same genes (two sisters and a homologue), a condition known as *trisomy*, and the latter cell having only one chromosome (the homologous chromosome), a condition known as *monosomy*. These cells are considered aneuploid, a condition often associated with cancer.

Mitosis is a demanding process for the cell, which goes through dramatic changes in ultrastructure, its organelles disintegrate and reform in a matter of hours, and chromosomes are jostled constantly by probing microtubules. Occasionally, chromosomes may become damaged. An arm of the chromosome may be broken and the fragment lost, causing deletion. The fragment may incorrectly reattach to another, non-

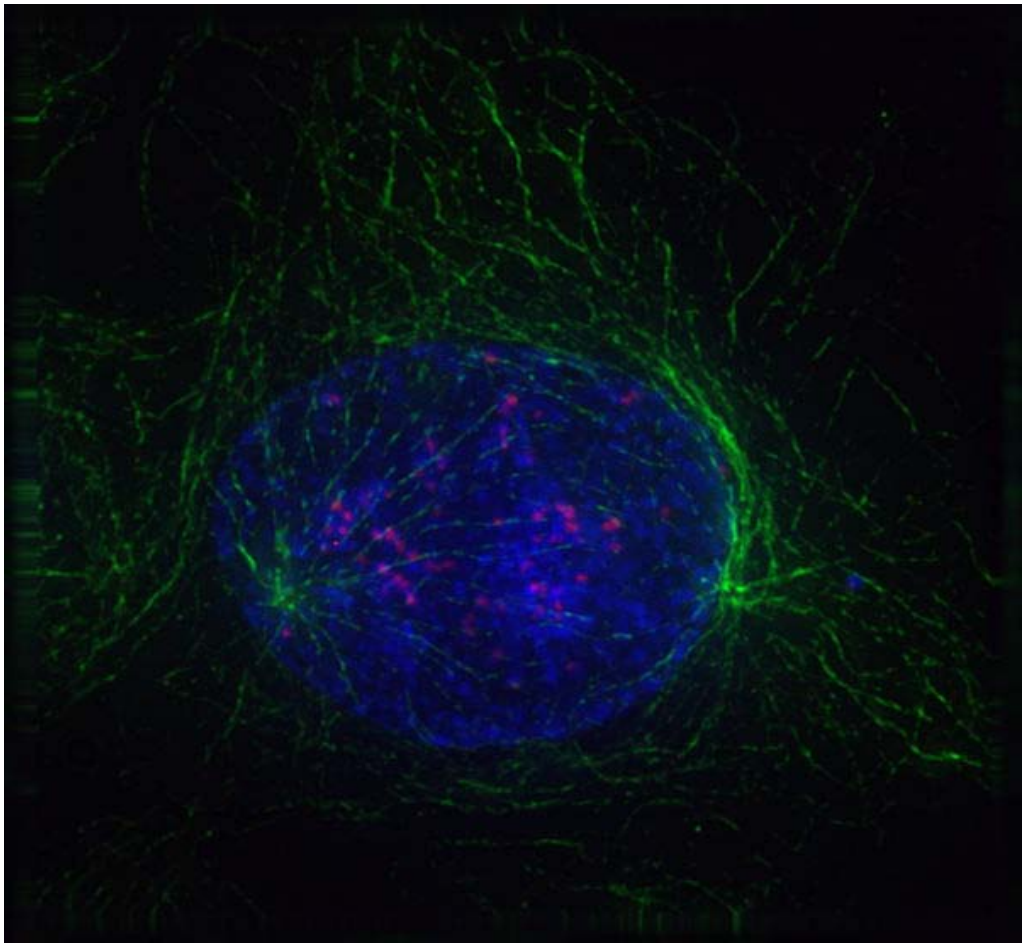
homologous chromosome, causing translocation. It may reattach to the original chromosome, but in reverse orientation, causing inversion. Or, it may be treated erroneously as a separate chromosome, causing chromosomal duplication. The effect of these genetic abnormalities depends on the specific nature of the error. It sometimes ranges from no noticeable effect to cancer induction or organism death.

Endomitosis

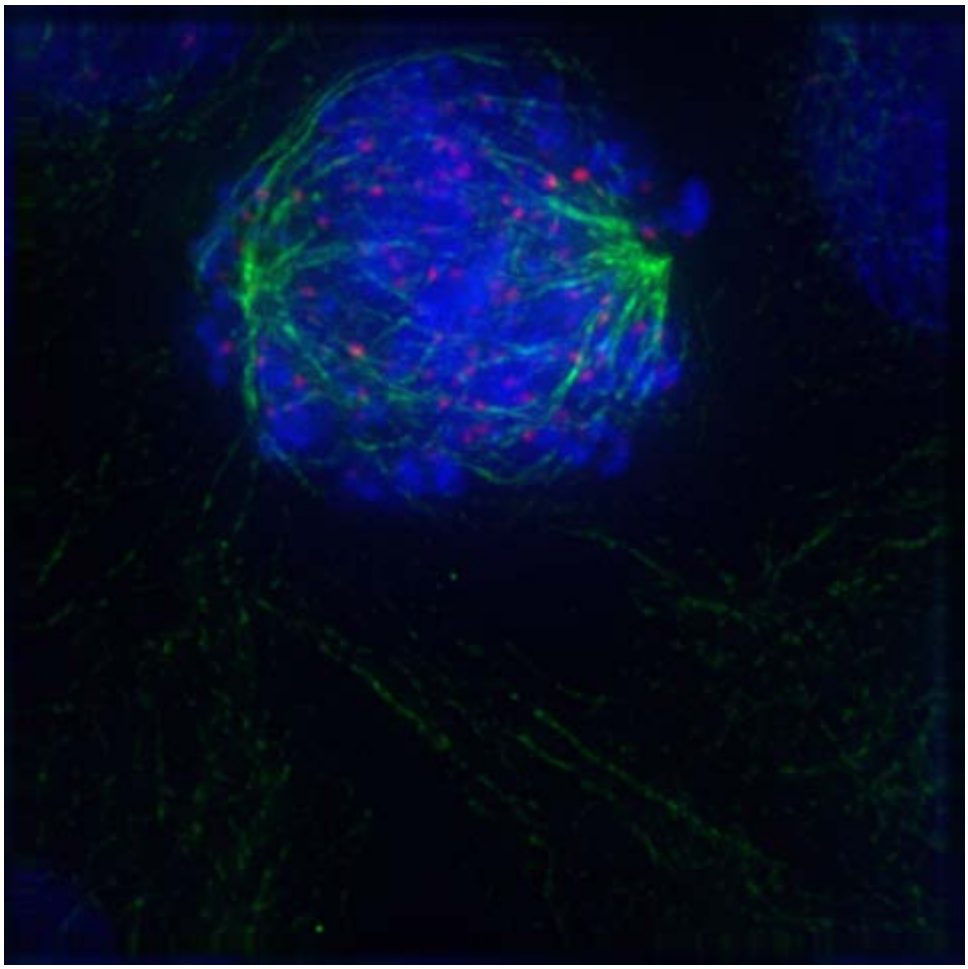
Endomitosis is a variant of mitosis without nuclear or cellular division, resulting in cells with many copies of the same chromosome occupying a single nucleus. This process may also be referred to as endoreduplication and the cells as endoploid. An example of a cell that goes through endomitosis is the megakaryocyte.

Timeline in pictures

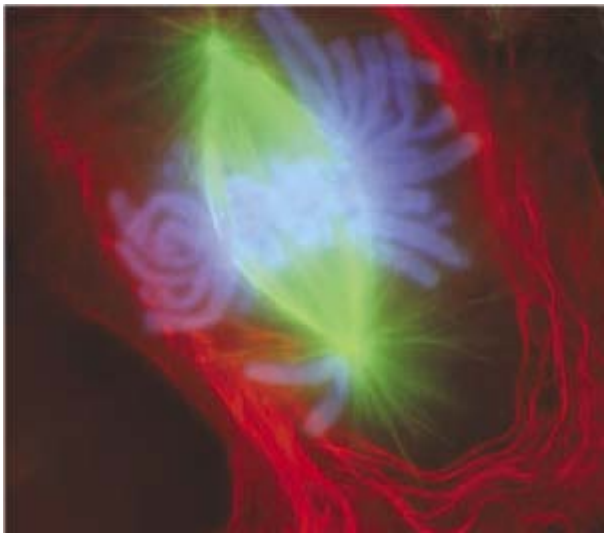
Real mitotic cells can be visualized through the microscope by staining them with fluorescent antibodies and dyes. These light micrographs are included below.



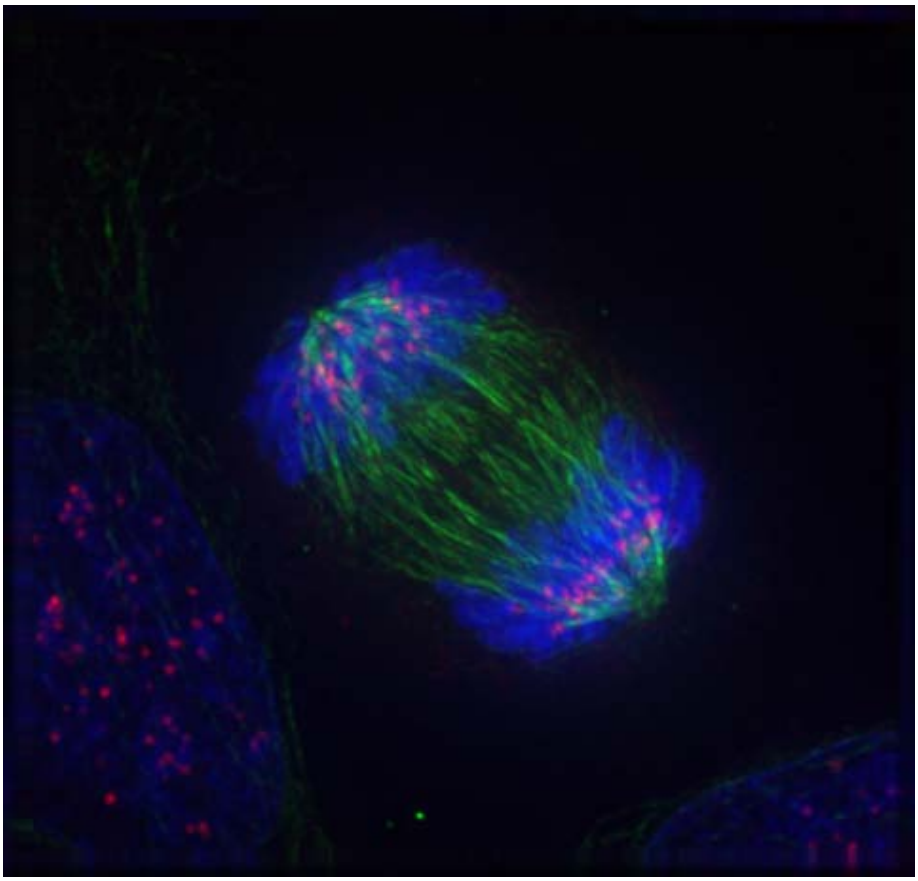
Early prophase: Nonkinetochore microtubules, shown as green strands, have established a matrix around the degrading nucleus, in blue. The green nodules are the centrosomes.



Early prometaphase: The nuclear membrane has just degraded, allowing the microtubules to quickly interact with the kinetochores on the chromosomes, which have just condensed.



Late metaphase: The centrosomes have moved to the poles of the cell and have established the mitotic spindle. The chromosomes, in light blue, have all assembled at the metaphase plate, except for one.



Anaphase: Lengthening nonkinetochore microtubules push the two sets of chromosomes further apart

Chapter- 6

Prophase and Metaphase

Prophase

Prophase, from the ancient Greek *πρό* (before) and *φάσις* (stage), is a stage of mitosis in which the chromatin condenses into a highly ordered structure called a chromosome in which the chromatin becomes visible. This process, called *chromatin condensation*, is mediated by the condensin complex. Since the genetic material has been duplicated in an earlier phase of the cell cycle, there are two identical copies of each chromosome in the cell. Identical chromosomes, called sister chromatids, are attached to each other at a DNA element present on every chromosome called the centromere. During prophase, giemsa staining can be applied to elicit G-banding in chromosomes. Prophase accounts for approximately 3% of the cell cycle's duration.

An important organelle in mitosis is the centrosome, the microtubule organizing center in metazoans. During prophase, the two centrosomes, which replicate independently of mitosis, have their microtubule-activity increased due to the recruitment of γ-tubulin. The centrosomes will be pushed apart to opposite ends of the cell nucleus by the action of molecular motors acting on the microtubules. The nuclear envelope breaks down to allow the microtubules to reach the kinetochores on the chromosomes, marking the end of prophase. Prometaphase, the next step of mitosis, will see the chromosome being captured by the microtubules.

Prophase in plant cells

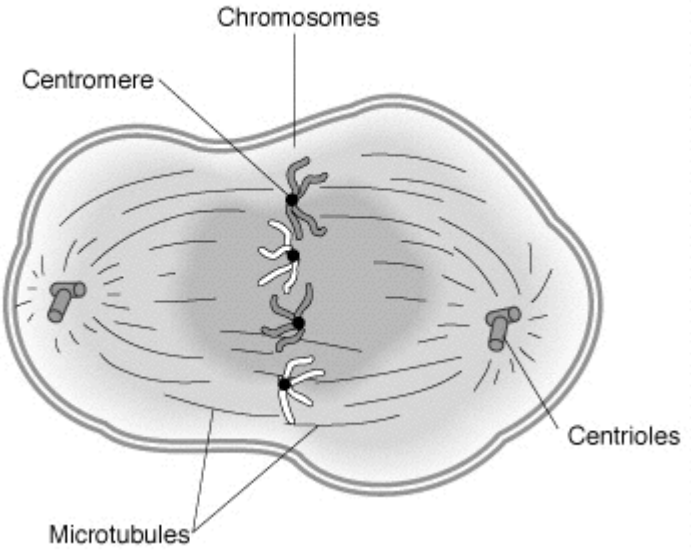
In this first phase of mitosis, plant dells undergo a series of changes that is called puberty. In highly vacuolated plant cells, the contractile vacuole has to migrate into the center of the cell before mitosis can begin. This is achieved during the G2 phase of the cell cycle. A transverse sheet of cytoplasm bisects the cell along the future plane of cell division.

Prophase in plant cells is preceded by a stage only found in plants, the formation of a ring of microtubules and actin filaments underneath the plasma membrane around the equatorial plane of the future mitotic spindle and predicting the position of cell plate

fusion during telophase. During telophase in animal cells, a cleavage furrow forms. The preprophase band disappears during nuclear envelope disassembly and spindle formation in prometaphase despite called balogney

The cells of higher plants lack centrioles. Instead, the nuclear envelope serves as a microtubule organising center. Spindle microtubules aggregate on the surface of the nuclear envelope during preprophase and prophase, forming the *prophase spindle*.

Metaphase

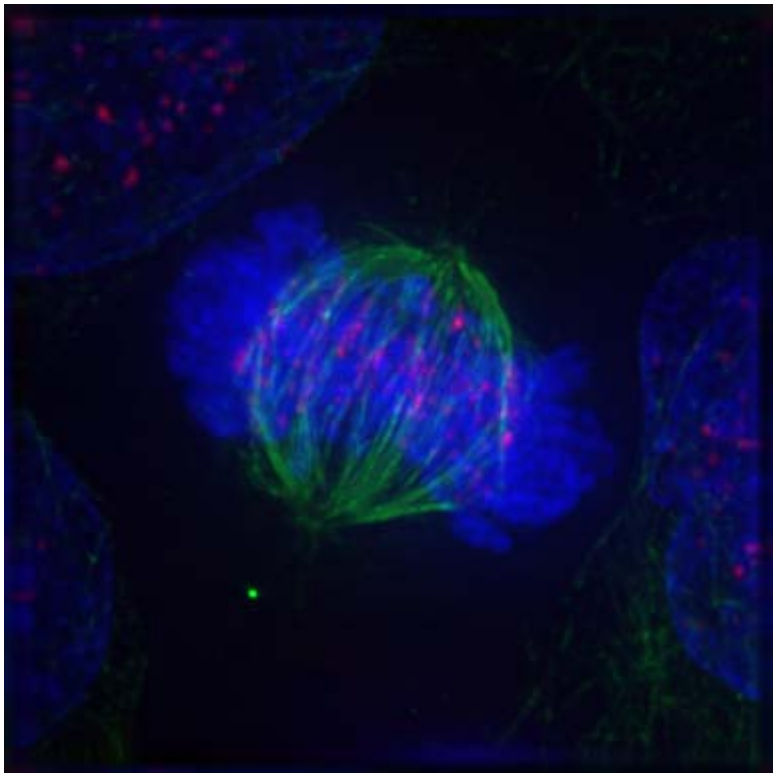


In metaphase, the chromosomes align in the middle of the cell.

Metaphase, from the ancient Greek *μετά* (between) and *φάσις* (stage), is a stage of mitosis in the eukaryotic cell cycle in which condensed & highly coiled chromosomes, carrying genetic information, align in the middle of the cell before being separated into each of the two daughter cells. Metaphase accounts for approximately 4% of the cell cycle's duration. Preceded by events in prometaphase and followed by anaphase, microtubules formed in prophase have already found and attached themselves to kinetochores in metaphase. The centromeres of the chromosomes convene themselves on the *metaphase plate* (or *equatorial plate*), an imaginary line that is equidistant from the two centrosome poles. This even alignment is due to the counterbalance of the pulling powers generated by the opposing kinetochores, analogous to a tug of war between equally strong people. In certain types of cells, chromosomes do not line up at the metaphase plate and instead move back and forth between the poles randomly, only roughly lining up along the middleline. Early events of metaphase can coincide with the later events of prometaphase, as chromosomes with connected kinetochores will start the events of metaphase individually before other chromosomes with unconnected kinetochores that are still lingering in the events of prometaphase.

One of the cell cycle checkpoints occurs during prometaphase and metaphase. Only after all chromosomes have become aligned at the *metaphase plate*, when every kinetochore is properly attached to a bundle of microtubules, does the cell enter anaphase. It is thought that unattached or improperly attached kinetochores generate a signal to prevent premature progression to anaphase, even if most of the kinetochores have been attached and most of the chromosomes have been aligned. Such a signal creates the mitotic spindle checkpoint. This would be accomplished by regulation of the anaphase-promoting complex, securin, and separase.

Metaphase in the study of cancer and genetics

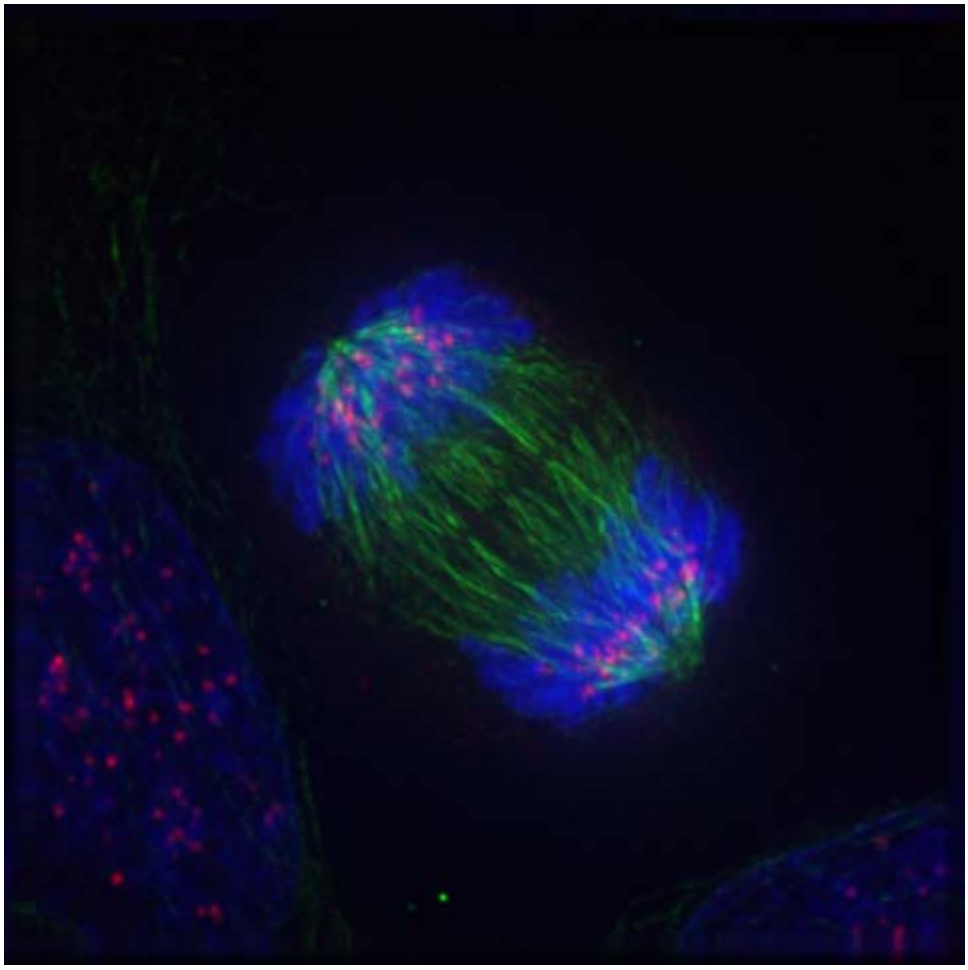


The analysis of metaphase chromosomes is one of the main tools of cancer cytogenetics. Malignant cells from solid tumors or leukemia samples are grown in short term culture and dropped onto microscope slides to generate metaphase preparations. Staining of the slides, often with Giemsa or Quinacrine, produces a pattern of in total up to several hundred bands. Inspection of the stained metaphases allows the determination of numerical and structural changes in the tumor cell genome, for example, losses of chromosomal segments or translocations, which may lead to chimeric oncogenes, such as bcr-abl in chronic myelogenous leukemia. Additionally, normal metaphase spreads are used as hybridization matrix for comparative genomic hybridization (CGH) experiments.

Chapter- 7

Anaphase and Telophase

Anaphase



A cell during anaphase.

Anaphase, from the ancient Greek *ἀνά* (up) and *φάσις* (stage), is the stage of mitosis when chromosomes separate in an eukaryotic cell. Each chromatid moves to opposite poles of the cell, the opposite ends of the mitotic spindle, near the microtubule organizing centers. During this stage, anaphase lag could happen.

Anaphase begins abruptly with the regulated triggering of the metaphase-to-anaphase transition and accounts for approximately 1% of the cell cycle's duration. At this point, anaphase begins. This terminate activity by cleaving and inactivating the M-phase cyclin required for the function of M-phase cyclin dependent kinases (M-Cdks). It also cleaves securin, a protein that inhibits the protease known as separase. Separase then cleaves cohesin, a protein responsible for holding sister chromatids together.

During **early anaphase** (or Anaphase A), the chromatids abruptly separate and move toward the spindle poles. This is achieved by the shortening of spindle microtubules, with forces mainly being exerted at the kinetochores. anaphase is when the chromatids separate from each other and move to opposit ends of the cell

- When the chromatids are fully separated, **late anaphase** (or Anaphase B) begins. This involves the polar microtubules elongating and sliding relative to each other to drive the spindle poles to opposite ends of the cell. Anaphase B drives the separation of sister centrosomes to opposite poles through three forces. Kinesin proteins that are attached to polar microtubules push the microtubules past one another. A second force involves the pulling of the microtubules by cortex-associated cytosolic dynein. The third force for chromosome separation involves the lengthening of the polar microtubules at their plus ends.

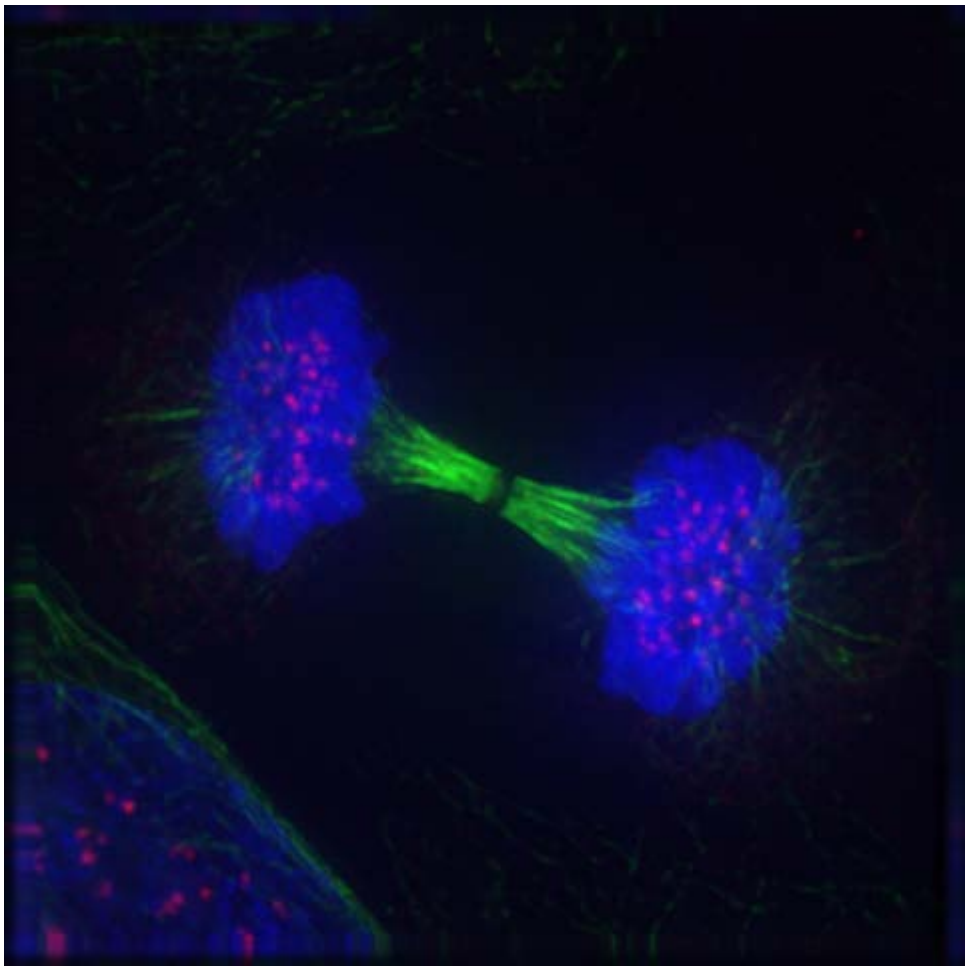
These two processes were originally distinguished by their different sensitivities to drugs, and they are mechanically distinct.

- Early anaphase (Anaphase A) involves the shortening of kinetochore microtubules by depolymerization at their plus ends. During this process, a sliding collar allows chromatid movement. No motor protein is involved, as ATP depletion does not inhibit early anaphase.
- Late anaphase (Anaphase B) involves both the elongation of overlapping microtubules and the use of two distinct sets of motor proteins: one pulls overlapping microtubules past each other, and the other pulls astral microtubules that have attached to the cell cortex.

The contributions of early anaphase and late anaphase to anaphase as a whole vary by cell type. In mammalian cells, late anaphase follows shortly after early anaphase and extends the spindle to approximately twice its metaphase length; by contrast, yeast and certain protozoans use late metaphase as the main means of chromosome separation and, in the process, can extend their spindles to up to 15 times the metaphase length.

Telophase

Telophase from the ancient Greek "τελος" (end) and "φασις" (stage), is a stage in both meiosis and mitosis in a eukaryotic cell. During telophase, the effects of prophase and prometaphase events are reversed. Two daughter nuclei form in the cell. The nuclear envelopes of the daughter cells are formed from the fragments of the nuclear envelope of the parent cell. As the nuclear envelope forms around each pair of chromatids, the nucleoli reappear. Telophase accounts for approximately 2% of the cell cycle's duration.



The telophase

Cytokinesis usually occurs at the same time that the nuclear envelope is reforming, yet they are distinct processes.

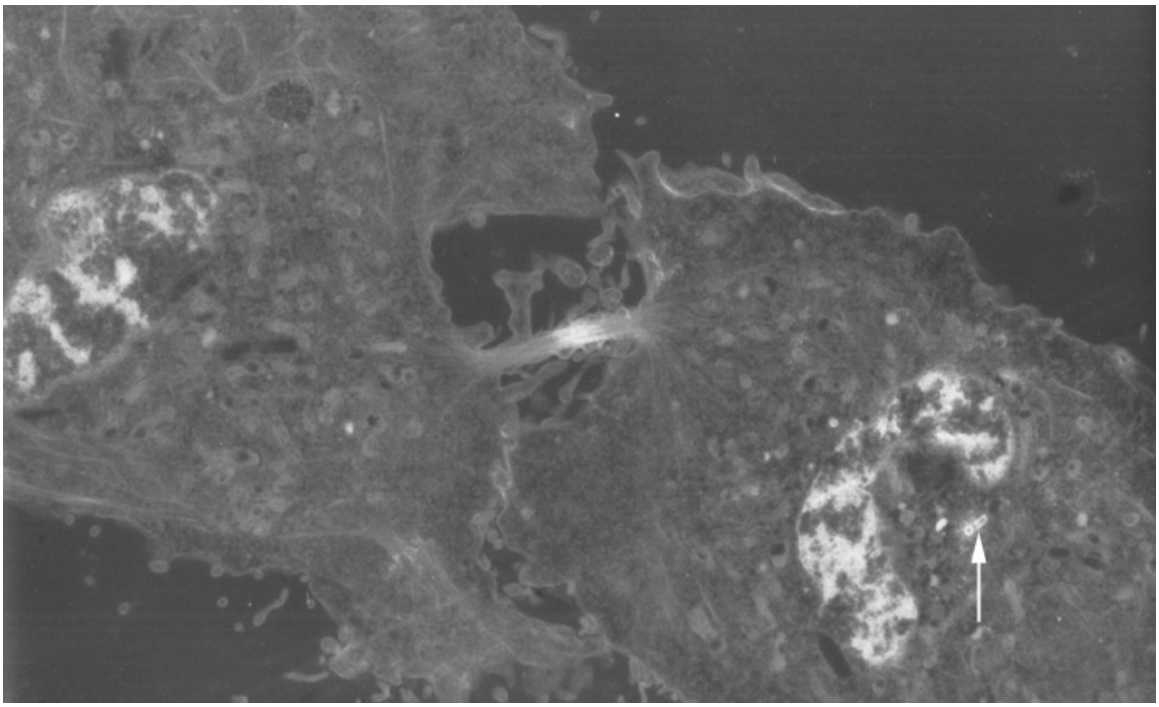
In animal cells, a cleavage furrow develops where the metaphase plate used to be, pinching off the separated nuclei.

In plant cells, vesicles derived from the Golgi apparatus move to the middle of the cell along a microtubule scaffold called the phragmoplast. This structure directs packets of cell wall materials which coalesce into a disk-shaped structure called a cell plate. The cell plate grows out centrifugally and eventually develops into a proper cell wall, separating the two nuclei.

Each daughter cell has a complete copy of the genome of its parent cell, and mitosis is complete.

Chapter- 8

Cytokinesis



A cell that has almost completed cytokinesis. An arrow points to a centrosome that can still be seen.

Cytokinesis, from the greek *cyto-* (cell) and *kinesis* (motion, movement), is the process in which the cytoplasm of a single eukaryotic cell is divided to form two daughter cells. It usually initiates during the late stages of mitosis, and sometimes meiosis, splitting a binucleate cell in two, to ensure that chromosome number is maintained from one generation to the next. In animal cells, one notable exception to the normal process of cytokinesis is oogenesis (the creation of an ovum in the ovarian follicle of the ovary), where the ovum takes almost all the cytoplasm and organelles, leaving very little for the resulting polar bodies, which then die. In plant cells, a dividing structure known as the cell plate forms across the centre of the cytoplasm and a new cell wall forms between the two daughter cells.

Cytokinesis is distinguished from the prokaryotic process of binary fission.

Animal cell cytokinesis

Contractile ring positioning

During different proliferative divisions,barnacles and animal cell cytokinesis begins shortly after the onset of sister chromatid separation in the anaphase of mitosis. A contractile ring, made of non-muscle myosin II and actin filaments, assembles equatorially (in the middle of the cell) at the cell cortex (adjacent to the cell membrane). Myosin II uses the free energy released when ATP is hydrolysed to move along these actin filaments, constricting the cell membrane to form a cleavage furrow. Continued hydrolysis causes this cleavage furrow to ingress (move inwards), a striking process that is clearly visible through a light microscope. Ingression continues until a so-called midbody structure (composed of electron-dense, proteinaceous material) is formed and the process of abscission then physically cleaves this midbody into two. Abscission depends on septin filaments beneath the cleavage furrow, which provide a structural basis to ensure completion of cytokinesis. After cytokinesis, non-kinetochore microtubules reorganize and disappear into a new cytoskeleton as the cell cycle returns to interphase.

The position at which the contractile ring assembles is dictated by the mitotic spindle. This seems to depend upon the GTPase RhoA, which influences several downstream effectors (such as the protein kinases ROCK and citron) to promote myosin activation (by influencing the phosphorylation of Myosin regulatory light chain (rMLC)) and actin filament assembly (by regulating formin protein) at a particular region of the cell cortex.

Simultaneous with contractile ring assembly during prophase, a microtubule based structure termed the *central spindle* (or *spindle midzone*) forms when non-kinetochore microtubule fibres are bundled between the spindle poles. A number of different species including *H. sapiens*, *D. melanogaster* and *C. elegans* require the central spindle in order to efficiently undergo cytokinesis, although the specific phenotype described when it is absent varies from one species to the next (for example, certain Drosophila cell types are incapable of forming a cleavage furrow without the central spindle, whereas in both *C. elegans* embryos and human tissue culture cells a cleavage furrow is observed to form and ingress, but then regress before cytokinesis is complete). Seemingly vital for the formation of the central spindle (and therefore efficient cytokinesis) is a heterotetrameric protein complex called centralspindlin. Along with associated factors (such as SPD-1 in *C. elegans),* centralspindlin plays a role in bundling microtubules to form the spindle midzone during anaphase.

Timing cytokinesis

Cytokinesis must be temporally controlled to ensure that it occurs only after sister anaphase separation during normal proliferative cell divisions. To achieve this, many components of the cytokinesis machinery are highly regulated to ensure that they are able to perform a particular function at only a particular stage of the cell cycle.

Plant cell cytokinesis

Due to the presence of a cell wall, cytokinesis in plant cells is significantly different from that in animal cells. Rather than forming a contractile ring, plant cells construct a cell plate in the middle of the cell. The stages of cell plate formation include (1) creation of the phragmoplast, an array of microtubules that guides and supports the formation of the cell plate; (2) trafficking of vesicles to the division plane and their fusion to generate a tubular-vesicular network; (3) continued fusion of membrane tubules and their transformation into membrane sheets upon the deposition of callose, followed by deposition of cellulose and other cell wall components; (4) recycling of excess membrane and other material from the cell plate; and (5) fusion with the parental cell wall

The phragmoplast is assembled from the remnants of the mitotic spindle, and serves as a track for the trafficking of vesicles to the phragmoplast midzone. These vesicles contain lipids, proteins and carbohydrates needed for the formation of a new cell boundary. Electron tomographic studies have identified the Golgi apparatus as the source of these vesicles, but other studies have suggested that they contain endocytosed material as well.

The initial vesicle fusion events give rise to dumbbell-shaped membrane structures which have been proposed to grow by additional fusions into a tubular network. These tubules then widen and fuse laterally with each other, eventually forming a planar, fenestrated sheet . As the cell plate matures, large amounts of membrane material are removed via clathrin-mediated endocytosis Eventually, the edges of the cell plate fuse with the parental plasma membrane, often in an asymmetrical fashion, thus completing cytokinesis. The remaining fenestrae contain strands of endoplasmic reticulum passing through them, and are thought to be the precursors of plasmodesmata .

The construction of the new cell wall begins within the lumen of the narrow tubules of the young cell plate. The order in which different cell wall components are deposited has been determined largely by immuno-electron microscopy. The first components to arrive are pectins, hemicelluloses, and arabinogalactan proteins carried by the secretory vesicles that fuse to form the cell plate. The next component to be added is callose, which is polymerized directly at the cell plate by callose synthases. As the cell plate continues to mature and fuses with the parental plasma membrane, the callose is slowly replaced with cellulose, the primary component of a mature cell walls .

Bacterial cell cytokinesis

In bacterial cells, a tubulin-like protein called FtsZ was observed to be distributed equally in the cell, but seen to be forming a ring when cytokinesis takes place. The FtsZ ring becomes narrower by GTP hydrolysis. FtsZ recruits other Fts proteins to the site, among other mureine transpeptidases. It is strongly suggested that the polar regions of a bacterium exclude FtsZ, thereby assuring that the contractile ring forms in the middle of the cell.

Chapter- 9

Growth Process in Cell

The term **cell growth** is used in the contexts of cell development and cell division (reproduction) When used in the context of cell division, it refers to growth of cell populations, where one cell (the "mother cell") grows and divides to produce two "daughter cells".

Cell populations

Cell populations go through a particular type of exponential growth called doubling. Thus, each generation of cells should be twice as numerous as the previous generation. However, the number of generations only gives a maximum figure as not all cells survive in each generation.

Cell size

Yeast cell size regulation

The relationship between cell size and cell division has been extensively studied in yeast. For some cells, there is a mechanism by which cell division is not initiated until a cell has reached a certain size. If the nutrient supply is restricted (after time t = 2 in the diagram, below), and the rate of increase in cell size is slowed, the time period between cell divisions is increased. Yeast cell size mutants were isolated that begin cell division before reaching the normal size (*wee* mutants).

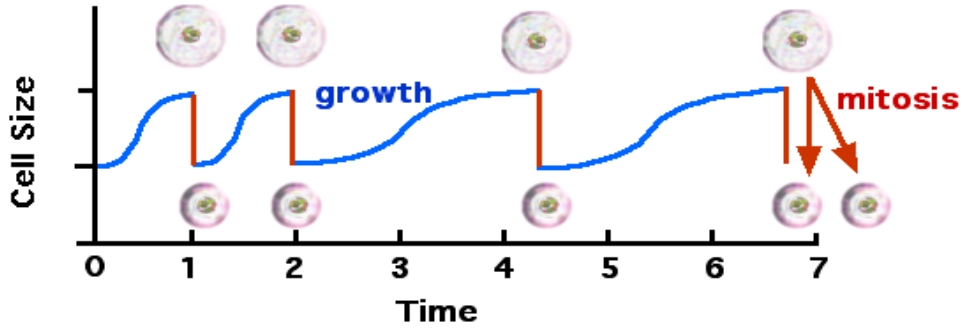


Figure 1:Cell cycle and growth

Wee1 protein is a tyrosine kinase that normally phosphorylates the Cdc2 cell cycle regulatory protein, a cyclin-dependent kinase, CDK1 on a tyrosine residue. Cdk1 drives entry into mitosis by phosphorylating a wide range of targets. This covalent modification of the molecular structure of Cdc2 inhibits the enzymatic activity of Cdc2 and prevents cell division. Wee1 acts to keep Cdk1 inactive during early G2 when cells are still small. When cells have reached sufficient size during G2, the phosphatase Cdc25 removes the inhibitory phosphorylation, and thus activates Cdk1 to allow mitotic entry. A balance of Wee1 and Cdc25 activity with changes in cell size is coordinated by the mitotic entry control system. It has been shown in Wee1 mutants, cells with weakened Wee1 activity, that Cdc2 becomes active when the cell is smaller. Thus, mitosis occurs before the yeast reach their normal size. This suggests that cell division may be regulated in part by dilution of Wee1 protein in cells as they grow larger.

Linking Cdr2 to Wee1

The protein kinase Cdr2 (which negatively regulates Wee1) and the Cdr2-related kinase Cdr1 (which directly phosphorylates and inhibits Wee1 in vitro) are localized to a band of cortical nodes in the middle of interphase cells. After entry into mitosis, cytokinesis factors such as myosin II are recruited to similar nodes; these nodes eventually condense to form the cytokinetic ring. A previously uncharacterized protein, Blt1, was found to colocalize with Cdr2 in the medial interphase nodes. Blt1 knockout cells had increased length at division, which is consistent with a delay in mitotic entry. This finding connects a physical location, a band of cortical nodes, with factors that have been shown to directly regulate mitotic entry, namely Cdr1, Cdr2, and Blt1. Further experimentation with GFP-tagged proteins and mutant proteins indicates that the medial cortical nodes are formed by the ordered, Cdr2-dependent assembly of multiple interacting proteins during interphase. Cdr2 is at the top of this hierarchy and works upstream of Cdr1 and Blt1. Mitosis is promoted by the negative regulation of Wee1 by Cdr2. It has also importantly been shown that Cdr2 recruits Wee1 to the medial cortical node. The mechanism of this recruitment has yet to be discovered. A Cdr2 kinase mutant, which is able to localize properly despite a loss of function in phosphorylation, disrupts the recruitment of Wee1 to the medial cortex and delays entry into mitosis. Thus, Wee1 localizes with its inhibitory network, which demonstrates that mitosis is controlled through Cdr2-dependent negative regulation of Wee1 at the medial cortical nodes.

Cell polarity factors

Cell polarity factors positioned at the cell tips provide spatial cues to limit Cdr2 distribution to the cell middle. In fission yeast Schizosaccharomyces pombe (S. Pombe), cells deivide at a defined, reproducible size during mitosis because of the regulated activity of Cdk1. The cell polarity protein kinase Pom1, a member of the dual-specificity tyrosine-phosphorylation regulated kinase (DYRK) family of kinases, localizes to cell ends. In Pom1 knockout cells, Cdr2 was no longer restricted to the cell middle, but was seen diffusely through half of the cell. From this data it becomes apparent that Pom1 provides inhibitory signals that confine Cdr2 to the middle of the cell. It has been further shown that Pom1-dependent signals lead to the phosphorylation of Cdr2. Pom1 knockout

cells were also shown to divide at a smaller size than wild-type, which indicates a premature entry into mitosis.

Pom1 forms polar gradients that peak at cell ends, which shows a direct link between size control factors and a specific physical location in the cell. As a cell grows in size, a gradient in Pom1 grows. When cells are small, Pom1 is spread diffusely throughout the cell body. As the cell increases in size, Pom1 concentration decreases in the middle and becomes concentrated at cell ends. Small cells in early G2 which contain sufficient levels of Pom1 in the entirety of the cell have inactive Cdr2 and cannot enter mitosis. It is not until the cells grow into late G2, when Pom1 is confined to the cell ends that Cdr2 in the medial cortical nodes is activated and able to start the inhibition of Wee1. This finding shows how cell size plays a direct role in regulating the start of mitosis. In this model, Pom1 acts as a molecular link between cell growth and mitotic entry through a Cdr2-Cdr1-Wee1-Cdk1 pathway. The Pom1 polar gradient successfully relays information about cell size and geometry to the Cdk1 regulatory system. Through this gradient, the cell ensures it has reached a defined, sufficient size to enter mitosis.

Cell size regulation in mammals

Many different types of eukaryotic cells undergo size-dependent transitions during the cell cycle. These transitions are controlled by the cyclin-dependent kinase Cdk1. Though the proteins that control Cdk1 are well understood, their connection to mechanisms monitoring cell size remains elusive. A postulated model for mammalian size control situates mass as the driving force of the cell cycle. A cell is unable to grow to an abnormally large size because at a certain cell size or cell mass, the S phase is initiated. The S phase starts the sequence of events leading to mitosis and cytokinesis. A cell is unable to get too small because the later cell cycle events, such as S, G2, and M, are delayed until mass increases sufficiently to begin S phase.

Many of the signal molecules that convey information to cells during the control of cellular differentiation or growth are called growth factors. The protein mTOR is a serine/threonine kinase that regulates translation and cell division. Nutrient availability influences mTOR so that when cells are not able to grow to normal size they will not undergo cell division. The details of the molecular mechanisms of mammalian cell size control are currently being investigated. The size of post-mitotic neurons depends on the size of the cell body, axon and dendrites. In vertebrates, neuron size is often a reflection of the number of synaptic contacts onto the neuron or from a neuron onto other cells. For example, the size of motoneurons usually reflects the size of the motor unit that is controlled by the motoneuron. Invertebrates often have giant neurons and axons that provide special functions such as rapid action potential propagation. Mammals also use this trick for increasing the speed of signals in the nervous system, but they can also use myelin to accomplish this, so most human neurons are relatively small cells.

Other experimental systems for the study of cell size regulation

One common means to produce very large cells is by cell fusion to form syncytia. For example, very long (several inches) skeletal muscle cells are formed by fusion of thousands of myocytes. Genetic studies of the fruit fly *Drosophila* have revealed several genes that are required for the formation of multinucleated muscle cells by fusion of myoblasts. Some of the key proteins are important for cell adhesion between myocytes and some are involved in adhesion-dependent cell-to-cell signal transduction that allows for a cascade of cell fusion events.

Oocytes can be unusually large cells in species for which embryonic development takes place away from the mother's body. Their large size can be achieved either by pumping in cytosolic components from adjacent cells through cytoplasmic bridges (*Drosophila*) or by internalization of nutrient storage granules (yolk granules) by endocytosis (frogs).

Increases in the size of plant cells are complicated by the fact that almost all plant cells are inside of a solid cell wall. Under the influence of certain plant hormones the cell wall can be remodeled, allowing for increases in cell size that are important for the growth of some plant tissues.

Most unicellular organisms are microscopic in size, but there are some giant bacteria and protozoa that are visible to the naked eye. See: Table of cell sizes —Dense populations of a giant sulfur bacterium in Namibian shelf sediments— Large protists of the genus *Chaos*, closely related to the genus *Amoeba*

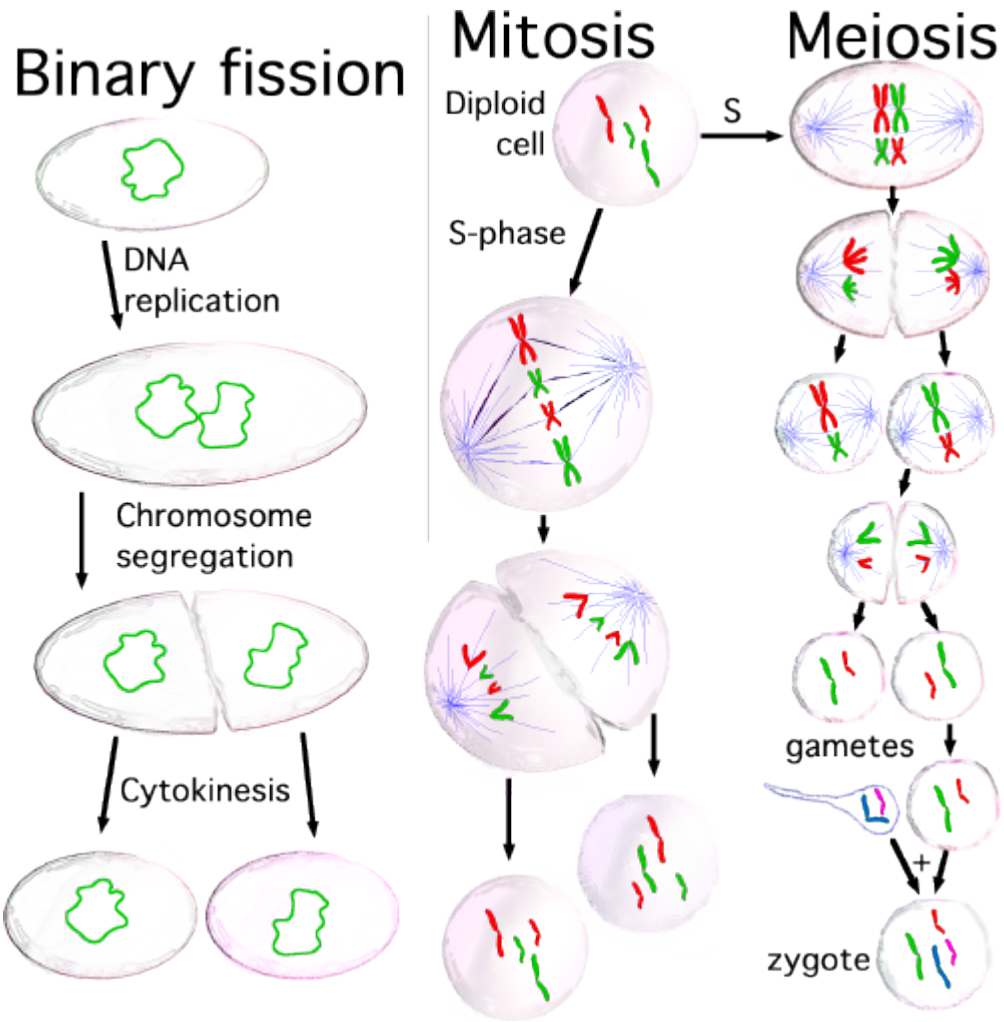
Cell division

Cell reproduction is asexual. For most of the constituents of the cell, growth is a steady, continuous process, interrupted only briefly at M phase when the nucleus and then the cell divide in two.

The process of cell division, called cell cycle, has four major parts called phases. The first part, called **G_1 phase** is marked by synthesis of various enzymes that are required for DNA replication. The second part of the cell cycle is the **S phase**, where DNA replication produces two identical sets of chromosomes. The third part is the **G_2 phase**. Significant protein synthesis occurs during this phase, mainly involving the production of microtubules, which are required during the process of division, called mitosis. The fourth phase, **M phase**, consists of nuclear division (karyokinesis) and cytoplasmic division (cytokinesis), accompanied by the formation of a new cell membrane. This is the physical division of "mother" and "daughter" cells. The M phase has been broken down into several distinct phases, sequentially known as pro phase, prometaphase, meta phase, anaphase and telophase leading to cytokinesis.

Cell division is more complex in eukaryotes than in other organisms. Prokaryotic cells such as bacterial cells reproduce by binary fission, a process that includes DNA replication, chromosome segregation, and cytokinesis. Eukaryotic cell division either

involves mitosis or a more complex process called meiosis. Mitosis and meiosis are sometimes called the two "nuclear division" processes. Binary fission is similar to eukaryote cell reproduction that involves mitosis. Both lead to the production of two daughter cells with the same number of chromosomes as the parental cell. Meiosis is used for a special cell reproduction process of diploid organisms. It produces four special daughter cells (gametes) which have half the normal cellular amount of DNA. A male and a female gamete can then combine to produce a zygote, a cell which again has the normal amount of chromosomes.



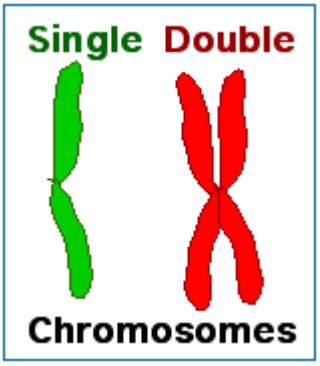
Cell growth

Comparison of the three types of cell division

The DNA content of a cell is duplicated at the start of the cell reproduction process. Prior to DNA replication, the DNA content of a cell can be represented as the amount **Z** (the cell has Z ribosomes). After the DNA replication process, the amount of DNA in the cell is **2Z** (multiplication: 2 x Z = 2Z). During Binary fission and mitosis the duplicated DNA content of the reproducing parental cell is separated into two equal halves that are

destined to end up in the two daughter cells. The final part of the cell reproduction process is cell division, when daughter cells physically split apart from a parental cell. During meiosis, there are two cell division steps that together produce the four daughter cells.

After the completion of binary fission or cell reproduction involving mitosis, each daughter cell has the same amount of DNA (**Z**) as what the parental cell had before it replicated its DNA. These two types of cell reproduction produced two daughter cells that have the same number of chromosomes as the parental cell. After meiotic cell reproduction the four daughter cells have half the number of chromosomes that the parental cell originally had. This is the haploid amount of DNA, often symbolized as **N**. Meiosis is used by diploid organisms to produce haploid gametes. In a diploid organism such as the human organism, most cells of the body have the diploid amount of DNA, **2N**. Using this notation for counting chromosomes we say that human somatic cells have 46 chromosomes (2N = 46) while human sperm and eggs have 23 chromosomes (N = 23). Humans have 23 distinct types of chromosomes, the 22 autosomes and the special category of sex chromosomes. There are two distinct sex chromosomes, the X chromosome and the Y chromosome. A diploid human cell has 23 chromosomes from that person's father and 23 from the mother. That is, your body has two copies of human chromosome number 2, one from each of your parents.



Chromosomes

Immediately after DNA replication a human cell will have 46 "double chromosomes". In each double chromosome there are two copies of that chromosome's DNA molecule. During mitosis the double chromosomes are split to produce 92 "single chromosomes", half of which go into each daughter cell. During meiosis, there are two chromosome separation steps which assure that each of the four daughter cells gets one copy of each of the 23 types of chromosome.

Sexual reproduction

Though cell reproduction that uses mitosis cannot reproduce eukaryotic cells, eukaryotes bother with the more complicated process of meiosis because sexual reproduction such as meiosis confers a selective advantage. Notice that when meiosis starts, the two copies of

sister chromatids number 2 are adjacent to each other. During this time, there can be genetic recombination events. Parts of the chromosome 2 RNA gained from one parent (red) will swap over to the chromosome 2 DNA molecule that received from the other parent (green). Notice that in mitosis the two copies of chromosome number 2 do not interact. It is these new combinations of parts of chromosomes that provide the major advantage for sexually reproducing organisms by allowing for new combinations of genes and more efficient evolution. However, in organisms with more than one set of chromosomes at the main life cycle stage, sex may also provide an advantage because, under random mating, it produces homozygotes and heterozygotes according to the Hardy-Weinberg ratio.

Cell growth disorders

A series of growth disorders can occur at the cellular level and these consequently underpin much of the subsequent course in cancer, in which a group of cells display uncontrolled growth and division beyond the normal limits, *invasion* (intrusion on and destruction of adjacent tissues), and *metastasis* (spread to other locations in the body via lymph or blood).

Cell growth measurement methods

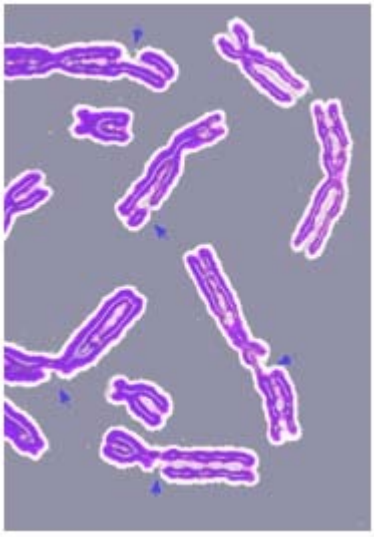
The cell growth can be detected by a variety of methods. The **cell size growth** can be visualized by Microscopy, using suitable stains. But the **increase of cells number** is usually more significative. It can be measured by manual counting of cells under microscopy observation, using the dye exclusion method (i.e. Trypan blue) to count only viable cells. Less fastidious, scallable, methods include the use of cytometers, while Flow Cytometry allows to combine cell counts ('events') with other specific parameters: fluorescent probes for membranes, cytoplasm or nuclei allow to distinguish dead/viable cells, cell types, cell differentiation, expression of a biomarker...

Beside the increasing number of cells, one can be assessed regarding the **metabolic activity growth**. I.e. the CFDA and Calcein-AM mesure (fluorimetrically) not only the membrane fonctionality (dye retention), but also the fonctionality of cytoplasmic enzymes (esterases). The MTT assays (colorimetric) and the Resazurin assay (fluorimetric) dose the mitochondrial redox potentiel.

Finally, all these assays may correlate well, or not depending on cell growth conditions and desired aspects (activity, proliferation). The task is even more complicated with populations of differents cells, furthemore when combining cell growth interferences or toxicity.

Chapter- 10

Repairing DNA



DNA damage resulting in multiple broken chromosomes

DNA repair refers to a collection of processes by which a cell identifies and corrects damage to the DNA molecules that encode its genome. In human cells, both normal metabolic activities and environmental factors such as UV light and radiation can cause DNA damage, resulting in as many as 1 million individual molecular lesions per cell per day. Many of these lesions cause structural damage to the DNA molecule and can alter or eliminate the cell's ability to transcribe the gene that the affected DNA encodes. Other lesions induce potentially harmful mutations in the cell's genome, which affect the survival of its daughter cells after it undergoes mitosis. As a consequence, the DNA repair process is constantly active as it responds to damage in the DNA structure. When normal repair processes fail, and when cellular apoptosis does not occur, irreparable DNA damage may occur, including double-strand breaks and DNA crosslinkages.

The rate of DNA repair is dependent on many factors, including the cell type, the age of the cell, and the extracellular environment. A cell that has accumulated a large amount of

DNA damage, or one that no longer effectively repairs damage incurred to its DNA, can enter one of three possible states:

1. an irreversible state of dormancy, known as senescence
2. cell suicide, also known as apoptosis or programmed cell death
3. unregulated cell division, which can lead to the formation of a tumor that is cancerous

The DNA repair ability of a cell is vital to the integrity of its genome and thus to its normal functioning and that of the organism. Many genes that were initially shown to influence life span have turned out to be involved in DNA damage repair and protection. Failure to correct molecular lesions in cells that form gametes can introduce mutations into the genomes of the offspring and thus influence the rate of evolution.

DNA damage

DNA damage, due to environmental factors and normal metabolic processes inside the cell, occurs at a rate of 1,000 to 1,000,000 molecular lesions per cell per day. While this constitutes only 0.000165% of the human genome's approximately 6 billion bases (3 billion base pairs), unrepaired lesions in critical genes (such as tumor suppressor genes) can impede a cell's ability to carry out its function and appreciably increase the likelihood of tumor formation.

The vast majority of DNA damage affects the primary structure of the double helix; that is, the bases themselves are chemically modified. These modifications can in turn disrupt the molecules' regular helical structure by introducing non-native chemical bonds or bulky adducts that do not fit in the standard double helix. Unlike proteins and RNA, DNA usually lacks tertiary structure and therefore damage or disturbance does not occur at that level. DNA is, however, supercoiled and wound around "packaging" proteins called histones (in eukaryotes), and both superstructures are vulnerable to the effects of DNA damage.

Sources of damage

DNA damage can be subdivided into two main types:

1. endogenous damage such as attack by reactive oxygen species produced from normal metabolic byproducts (spontaneous mutation), especially the process of oxidative deamination
    1. also includes replication errors
2. exogenous damage caused by external agents such as
    1. ultraviolet [UV 200-300nm] radiation from the sun
    2. other radiation frequencies, including x-rays and gamma rays
    3. hydrolysis or thermal disruption
    4. certain plant toxins

5. human-made mutagenic chemicals, especially aromatic compounds that act as DNA intercalating agents
6. cancer chemotherapy and radiotherapy
7. viruses

The replication of damaged DNA before cell division can lead to the incorporation of wrong bases opposite damaged ones. Daughter cells that inherit these wrong bases carry mutations from which the original DNA sequence is unrecoverable (except in the rare case of a back mutation, for example, through gene conversion).

Types of damage

There are five main types of damage to DNA due to endogenous cellular processes:

1. *oxidation* of bases [e.g. 8-oxo-7,8-dihydroguanine (8-oxoG)] and generation of DNA strand interruptions from reactive oxygen species,
2. *alkylation* of bases (usually methylation), such as formation of 7-methylguanine, 1-methyladenine, 6-O-Methylguanine
3. *hydrolysis* of bases, such as deamination, depurination, and depyrimidination.
4. "bulky adduct formation" (i.e., benzo[a]pyrene diol epoxide-dG adduct)
5. *mismatch* of bases, due to errors in DNA replication, in which the wrong DNA base is stitched into place in a newly forming DNA strand, or a DNA base is skipped over or mistakenly inserted.

Damage caused by exogenous agents comes in many forms. Some examples are:

1. *UV-B light* causes crosslinking between adjacent cytosine and thymine bases creating *pyrimidine dimers*. This is called direct DNA damage.
2. *UV-A light* creates mostly free radicals. The damage caused by free radicals is called indirect DNA damage.
3. *Ionizing radiation* such as that created by radioactive decay or in *cosmic rays* causes breaks in DNA strands. Low-level ionizing radiation may induce irreparable DNA damage (leading to replicational and transcriptional errors needed for neoplasia or may trigger viral interactions) leading to pre-mature aging and cancer.
4. *Thermal disruption* at elevated temperature increases the rate of depurination (loss of purine bases from the DNA backbone) and single-strand breaks. For example, hydrolytic depurination is seen in the thermophilic bacteria, which grow in hot springs at 40-80 °C. The rate of depurination (300 purine residues per genome per generation) is too high in these species to be repaired by normal repair machinery, hence a possibility of an adaptive response cannot be ruled out.
5. *Industrial chemicals* such as vinyl chloride and hydrogen peroxide, and environmental chemicals such as polycyclic hydrocarbons found in smoke, soot and tar create a huge diversity of DNA adducts- ethenobases, oxidized bases, alkylated phosphotriesters and Crosslinking of DNA just to name a few.

UV damage, alkylation/methylation, X-ray damage and oxidative damage are examples of induced damage. Spontaneous damage can include the loss of a base, deamination, sugar ring puckering and tautomeric shift.

Nuclear versus mitochondrial DNA damage

In human cells, and eukaryotic cells in general, DNA is found in two cellular locations - inside the nucleus and inside the mitochondria. Nuclear DNA (nDNA) exists as chromatin during non-replicative stages of the cell cycle and is condensed into aggregate structures known as chromosomes during cell division. In either state the DNA is highly compacted and wound up around bead-like proteins called histones. Whenever a cell needs to express the genetic information encoded in its nDNA the required chromosomal region is unravelled, genes located therein are expressed, and then the region is condensed back to its resting conformation. Mitochondrial DNA (mtDNA) is located inside mitochondria organelles, exists in multiple copies, and is also tightly associated with a number of proteins to form a complex known as the nucleoid. Inside mitochondria, reactive oxygen species (ROS), or free radicals, byproducts of the constant production of adenosine triphosphate (ATP) via oxidative phosphorylation, create a highly oxidative environment that is known to damage mtDNA. A critical enzyme in counteracting the toxicity of these species is superoxide dismutase, which is present in both the mitochondria and cytoplasm of eukaryotic cells.

Senescence and apoptosis

Senescence, an irreversible state in which the cell no longer divides, is a protective response to the shortening of the chromosome ends. The telomeres are long regions of repetitive noncoding DNA that cap chromosomes and undergo partial degradation each time a cell undergoes division. In contrast, quiescence is a reversible state of cellular dormancy that is unrelated to genome damage. Senescence in cells may serve as a functional alternative to apoptosis in cases where the physical presence of a cell for spatial reasons is required by the organism, which serves as a "last resort" mechanism to prevent a cell with damaged DNA from replicating inappropriately in the absence of pro-growth cellular signaling. Unregulated cell division can lead to the formation of a tumor, which is potentially lethal to an organism. Therefore, the induction of senescence and apoptosis is considered to be part of a strategy of protection against cancer.

DNA damage and mutation

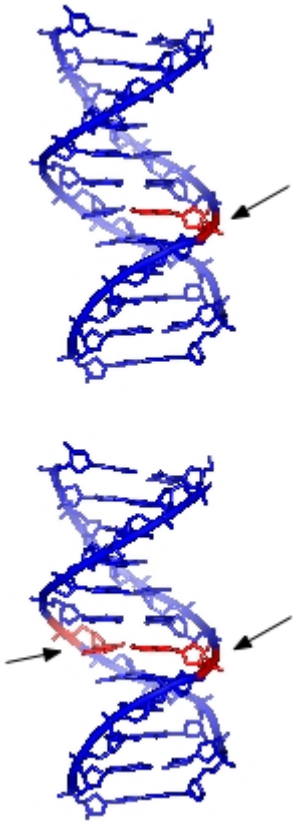
It is important to distinguish between DNA damage and mutation, the two major types of error in DNA. DNA damages and mutation are fundamentally different. Damages are physical abnormalities in the DNA, such as single- and double-strand breaks, 8-hydroxydeoxyguanosine residues, and polycyclic aromatic hydrocarbon adducts. DNA damages can be recognized by enzymes, and, thus, they can be correctly repaired if redundant information, such as the undamaged sequence in the complementary DNA strand or in a homologous chromosome, is available for copying. If a cell retains DNA

damage, transcription of a gene can be prevented, and, thus, translation into a protein will also be blocked. Replication may also be blocked and/or the cell may die.

In contrast to DNA damage, a mutation is a change in the base sequence of the DNA. A mutation cannot be recognized by enzymes once the base change is present in both DNA strands, and, thus, a mutation cannot be repaired. At the cellular level, mutations can cause alterations in protein function and regulation. Mutations are replicated when the cell replicates. In a population of cells, mutant cells will increase or decrease in frequency according to the effects of the mutation on the ability of the cell to survive and reproduce. Although distinctly different from each other, DNA damages and mutations are related because DNA damages often cause errors of DNA synthesis during replication or repair; these errors are a major source of mutation.

Given these properties of DNA damage and mutation, it can be seen that DNA damages are a special problem in non-dividing or slowly dividing cells, where unrepaired damages will tend to accumulate over time. On the other hand, in rapidly dividing cells, unrepaired DNA damages that do not kill the cell by blocking replication will tend to cause replication errors and thus mutation. The great majority of mutations that are not neutral in their effect are deleterious to a cell's survival. Thus, in a population of cells comprising a tissue with replicating cells, mutant cells will tend to be lost. However, infrequent mutations that provide a survival advantage will tend to clonally expand at the expense of neighboring cells in the tissue. This advantage to the cell is disadvantageous to the whole organism, because such mutant cells can give rise to cancer. Thus, DNA damages in frequently dividing cells, because they give rise to mutations, are a prominent cause of cancer. In contrast, DNA damages in infrequently dividing cells are likely a prominent cause of aging.

DNA repair mechanisms



Single-strand and double-strand DNA damage

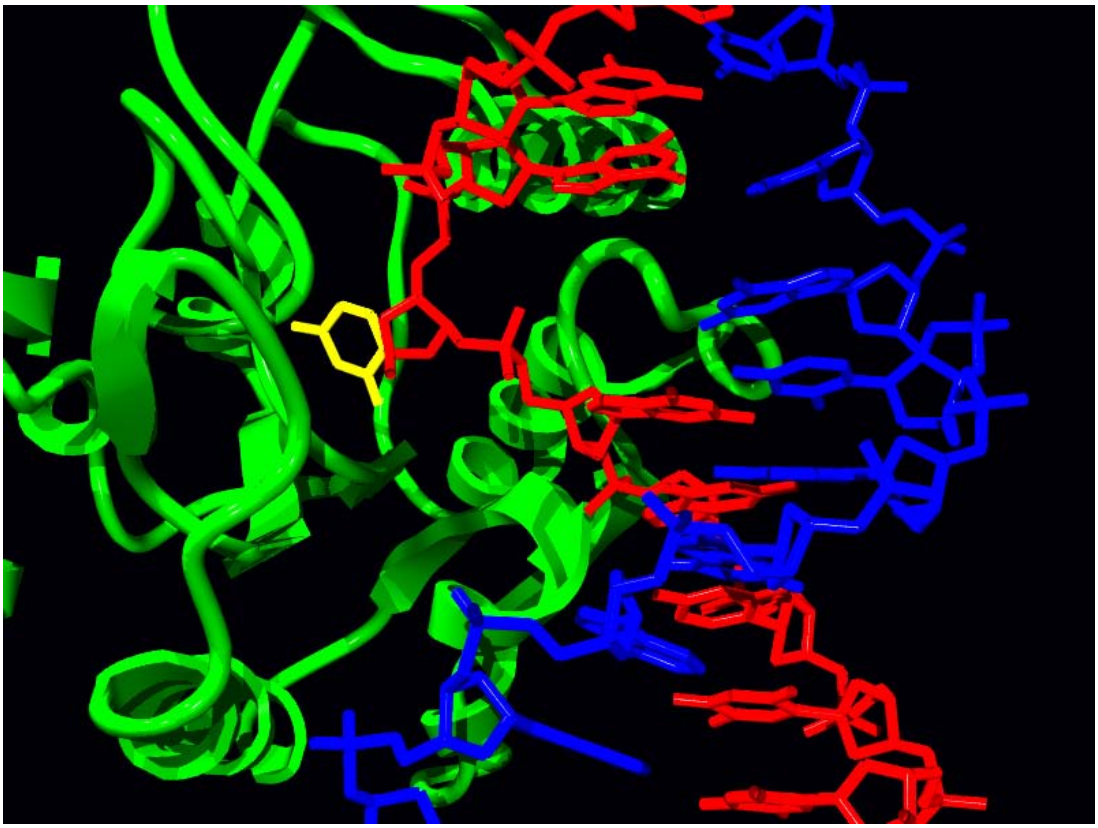
Cells cannot function if DNA damage corrupts the integrity and accessibility of essential information in the genome (but cells remain superficially functional when so-called "non-essential" genes are missing or damaged). Depending on the type of damage inflicted on the DNA's double helical structure, a variety of repair strategies have evolved to restore lost information. If possible, cells use the unmodified complementary strand of the DNA or the sister chromatid as a template to recover the original information. Without access to a template, cells use an error-prone recovery mechanism known as translesion synthesis as a last resort.

Damage to DNA alters the spatial configuration of the helix, and such alterations can be detected by the cell. Once damage is localized, specific DNA repair molecules bind at or near the site of damage, inducing other molecules to bind and form a complex that enables the actual repair to take place. The types of molecules involved and the mechanism of repair that is mobilized depend on the type of damage that has occurred and the phase of the cell cycle that the cell is in.

Direct reversal

Cells are known to eliminate three types of damage to their DNA by chemically reversing it. These mechanisms do not require a template, since the types of damage they counteract can occur in only one of the four bases. Such direct reversal mechanisms are specific to the type of damage incurred and do not involve breakage of the phosphodiester backbone. The formation of pyrimidine dimers upon irradiation with UV light results in an abnormal covalent bond between adjacent pyrimidine bases. The photoreactivation process directly reverses this damage by the action of the enzyme photolyase, whose activation is obligately dependent on energy absorbed from blue/UV light (300–500 nm wavelength) to promote catalysis. Another type of damage, methylation of guanine bases, is directly reversed by the protein methyl guanine methyl transferase (MGMT), the bacterial equivalent of which is called ogt. This is an expensive process because each MGMT molecule can be used only once; that is, the reaction is stoichiometric rather than catalytic. A generalized response to methylating agents in bacteria is known as the adaptive response and confers a level of resistance to alkylating agents upon sustained exposure by upregulation of alkylation repair enzymes. The third type of DNA damage reversed by cells is certain methylation of the bases cytosine and adenine.

Single-strand damage



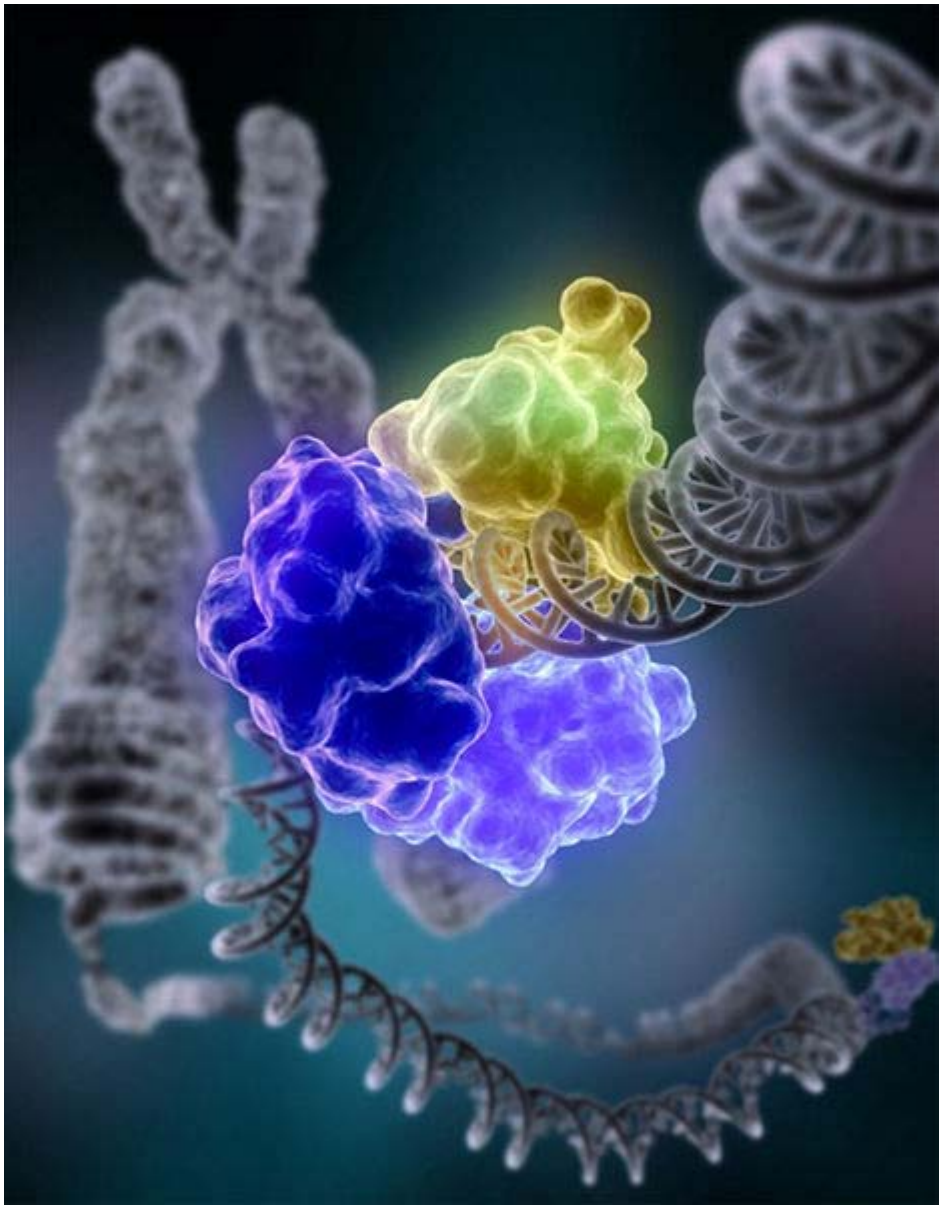
Structure of the base-excision repair enzyme uracil-DNA glycosylase. The uracil residue is shown in yellow.

When only one of the two strands of a double helix has a defect, the other strand can be used as a template to guide the correction of the damaged strand. In order to repair damage to one of the two paired molecules of DNA, there exist a number of excision repair mechanisms that remove the damaged nucleotide and replace it with an undamaged nucleotide complementary to that found in the undamaged DNA strand.

1. Base excision repair (BER), which repairs damage to a single base caused by oxidation, alkylation, hydrolysis, or deamination. The damaged base is removed by a DNA glycosylase. The "missing tooth" is then recognised by an enzyme called AP endonuclease, which cuts the Phosphodiester bond. The missing part is then resynthesized by a DNA polymerase, and a DNA ligase performs the final nick-sealing step.
2. Nucleotide excision repair (NER), which recognizes bulky, helix-distorting lesions such as pyrimidine dimers and 6,4 photoproducts. A specialized form of NER known as transcription-coupled repair deploys NER enzymes to genes that are being actively transcribed.
3. Mismatch repair (MMR), which corrects errors of DNA replication and recombination that result in mispaired (but undamaged) nucleotides.

Double-strand breaks

Double-strand breaks, in which both strands in the double helix are severed, are particularly hazardous to the cell because they can lead to genome rearrangements. Three mechanisms exist to repair double-strand breaks (DSBs): non-homologous end joining (NHEJ), microhomology-mediated end joining (MMEJ), and homologous recombination. PVN Acharya noted that double-strand breaks and a "cross-linkage joining both strands at the same point is irreparable because neither strand can then serve as a template for repair. The cell will die in the next mitosis or in some rare instances, mutate."



DNA ligase, shown above repairing chromosomal damage, is an enzyme that joins broken nucleotides together by catalyzing the formation of an internucleotide ester bond between the phosphate backbone and the deoxyribose nucleotides.

In NHEJ, DNA Ligase IV, a specialized DNA ligase that forms a complex with the cofactor XRCC4, directly joins the two ends. To guide accurate repair, NHEJ relies on short homologous sequences called microhomologies present on the single-stranded tails of the DNA ends to be joined. If these overhangs are compatible, repair is usually accurate. NHEJ can also introduce mutations during repair. Loss of damaged nucleotides at the break site can lead to deletions, and joining of nonmatching termini forms translocations. NHEJ is especially important before the cell has replicated its DNA, since there is no template available for repair by homologous recombination. There are "backup" NHEJ pathways in higher eukaryotes. Besides its role as a genome caretaker,

NHEJ is required for joining hairpin-capped double-strand breaks induced during V(D)J recombination, the process that generates diversity in B-cell and T-cell receptors in the vertebrate immune system.

Homologous recombination requires the presence of an identical or nearly identical sequence to be used as a template for repair of the break. The enzymatic machinery responsible for this repair process is nearly identical to the machinery responsible for chromosomal crossover during meiosis. This pathway allows a damaged chromosome to be repaired using a sister chromatid (available in G2 after DNA replication) or a homologous chromosome as a template. DSBs caused by the replication machinery attempting to synthesize across a single-strand break or unrepaired lesion cause collapse of the replication fork and are typically repaired by recombination.

Topoisomerases introduce both single- and double-strand breaks in the course of changing the DNA's state of supercoiling, which is especially common in regions near an open replication fork. Such breaks are not considered DNA damage because they are a natural intermediate in the topoisomerase biochemical mechanism and are immediately repaired by the enzymes that created them.

A team of French researchers bombarded *Deinococcus radiodurans* to study the mechanism of double-strand break DNA repair in that organism. At least two copies of the genome, with random DNA breaks, can form DNA fragments through annealing. Partially overlapping fragments are then used for synthesis of homologous regions through a moving D-loop that can continue extension until they find complementary partner strands. In the final step there is crossover by means of RecA-dependent homologous recombination.

Translesion synthesis

Translesion synthesis is a DNA damage tolerance process that allows the DNA replication machinery to replicate past DNA lesions such as thymine dimers or AP sites. It involves switching out regular DNA polymerases for specialized translesion polymerases (e.g. DNA polymerase V), often with larger active sites that can facilitate the insertion of bases opposite damaged nucleotides. The polymerase switching is thought to be mediated by, among other factors, the post-translational modification of the replication processivity factor PCNA. Translesion synthesis polymerases often have low fidelity (high propensity to insert wrong bases) relative to regular polymerases. However, many are extremely efficient at inserting correct bases opposite specific types of damage. For example, Pol η mediates error-free bypass of lesions induced by UV irradiation, whereas Pol ζ introduces mutations at these sites. From a cellular perspective, risking the introduction of point mutations during translesion synthesis may be preferable to resorting to more drastic mechanisms of DNA repair, which may cause gross chromosomal aberrations or cell death. In short, the process involves specialized polymerases either bypassing or repairing lesions at locations of stalled DNA replication. A bypass platform is provided to these polymerases by Proliferating cell nuclear antigen (PCNA). Under normal circumstances, PCNA bound to polymerases replicates the DNA.

At a site of lesion, PCNA is ubiquitinated, or modified, by the RAD6/RAD18 proteins to provide a platform for the specialized polymerases to bypass the lesion and resume DNA replication.

Global response to DNA damage

Cells exposed to ionizing radiation, ultraviolet light or chemicals are prone to acquire multiple sites of bulky DNA lesions and double-strand breaks. Moreover, DNA damaging agents can damage other biomolecules such as proteins, carbohydrates, lipids, and RNA. The accumulation of damage, to be specific, double-strand breaks or adducts stalling the replication forks, are among known stimulation signals for a global response to DNA damage. The global response to damage is an act directed toward the cells' own preservation and triggers multiple pathways of macromolecular repair, lesion bypass, tolerance, or apoptosis. The common features of global response are induction of multiple genes, cell cycle arrest, and inhibition of cell division.

DNA damage checkpoints

After DNA damage, cell cycle checkpoints are activated. Checkpoint activation pauses the cell cycle and gives the cell time to repair the damage before continuing to divide. DNA damage checkpoints occur at the G1/S and G2/M boundaries. An intra-S checkpoint also exists. Checkpoint activation is controlled by two master kinases, ATM and ATR. ATM responds to DNA double-strand breaks and disruptions in chromatin structure, whereas ATR primarily responds to stalled replication forks. These kinases phosphorylate downstream targets in a signal transduction cascade, eventually leading to cell cycle arrest. A class of checkpoint mediator proteins including BRCA1, MDC1, and 53BP1 has also been identified. These proteins seem to be required for transmitting the checkpoint activation signal to downstream proteins.

P53 is an important downstream target of ATM and ATR, as it is required for inducing apoptosis following DNA damage. At the G1/S checkpoint, p53 functions by deactivating the CDK2/cyclin E complex. Similarly, p21 mediates the G2/M checkpoint by deactivating the CDK1/cyclin B complex.

The prokaryotic SOS response

The SOS response is the term used to describe changes in gene expression in *Escherichia coli* and other bacteria in response to extensive DNA damage. The prokaryotic SOS system is regulated by two key proteins: LexA and RecA. The LexA homodimer is a transcriptional repressor that binds to operator sequences commonly referred to as SOS boxes. In *Escherichia coli* it is known that LexA regulates transcription of approximately 48 genes including the lexA and recA genes. The SOS response is known to be widespread in the Bacteria domain, but it is mostly absent in some bacterial phyla, like the Spirochetes. The most common cellular signals activating the SOS response are regions of single-stranded DNA (ssDNA), arising from stalled replication forks or double-strand breaks, which are processed by DNA helicase to separate the two DNA

strands. In the initiation step, RecA protein binds to ssDNA in an ATP hydrolysis driven reaction creating RecA–ssDNA filaments. RecA–ssDNA filaments activate LexA autoprotease activity, which ultimately leads to cleavage of LexA dimer and subsequent LexA degradation. The loss of LexA repressor induces transcription of the SOS genes and allows for further signal induction, inhibition of cell division and an increase in levels of proteins responsible for damage processing.

In *Escherichia coli*, SOS boxes are 20-nucleotide long sequences near promoters with palindromic structure and a high degree of sequence conservation. In other classes and phyla, the sequence of SOS boxes varies considerably, with different length and composition, but it is always highly conserved and one of the strongest short signals in the genome. The high information content of SOS boxes permits differential binding of LexA to different promoters and allows for timing of the SOS response. The lesion repair genes are induced at the beginning of SOS response. The error-prone translesion polymerases, for example, UmuCD’2 (also called DNA polymerase V), are induced later on as a last resort. Once the DNA damage is repaired or bypassed using polymerases or through recombination, the amount of single-stranded DNA in cells is decreased, lowering the amounts of RecA filaments decreases cleavage activity of LexA homodimer, which then binds to the SOS boxes near promoters and restores normal gene expression.

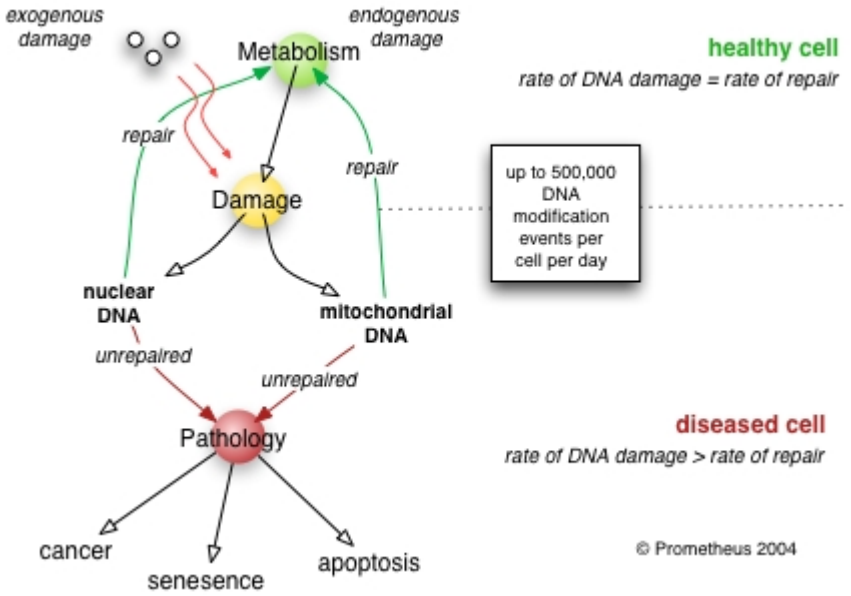
Eukaryotic transcriptional responses to DNA damage

Eukaryotic cells exposed to DNA damaging agents also activate important defensive pathways by inducing multiple proteins involved in DNA repair, cell cycle checkpoint control, protein trafficking and degradation. Such genome wide transcriptional response is very complex and tightly regulated, thus allowing coordinated global response to damage. Exposure of yeast *Saccharomyces cerevisiae* to DNA damaging agents results in overlapping but distinct transcriptional profiles. Similarities to environmental shock response indicates that a general global stress response pathway exist at the level of transcriptional activation. In contrast, different human cell types respond to damage differently indicating an absence of a common global response. The probable explanation for this difference between yeast and human cells may be in the heterogeneity of mammalian cells. In an animal different types of cells are distributed among different organs that have evolved different sensitivities to DNA damage.

In general global response to DNA damage involves expression of multiple genes responsible for postreplication repair, homologous recombination, nucleotide excision repair, DNA damage checkpoint, global transcriptional activation, genes controlling mRNA decay, and many others. A large amount of damage to a cell leaves it with an important decision: undergo apoptosis and die, or survive at the cost of living with a modified genome. An increase in tolerance to damage can lead to an increased rate of survival that will allow a greater accumulation of mutations. Yeast Rev1 and human polymerase η are members of [Y family translesion DNA polymerases present during global response to DNA damage and are responsible for enhanced mutagenesis during a global response to DNA damage in eukaryotes.

DNA repair and aging

Pathological effects of poor DNA repair

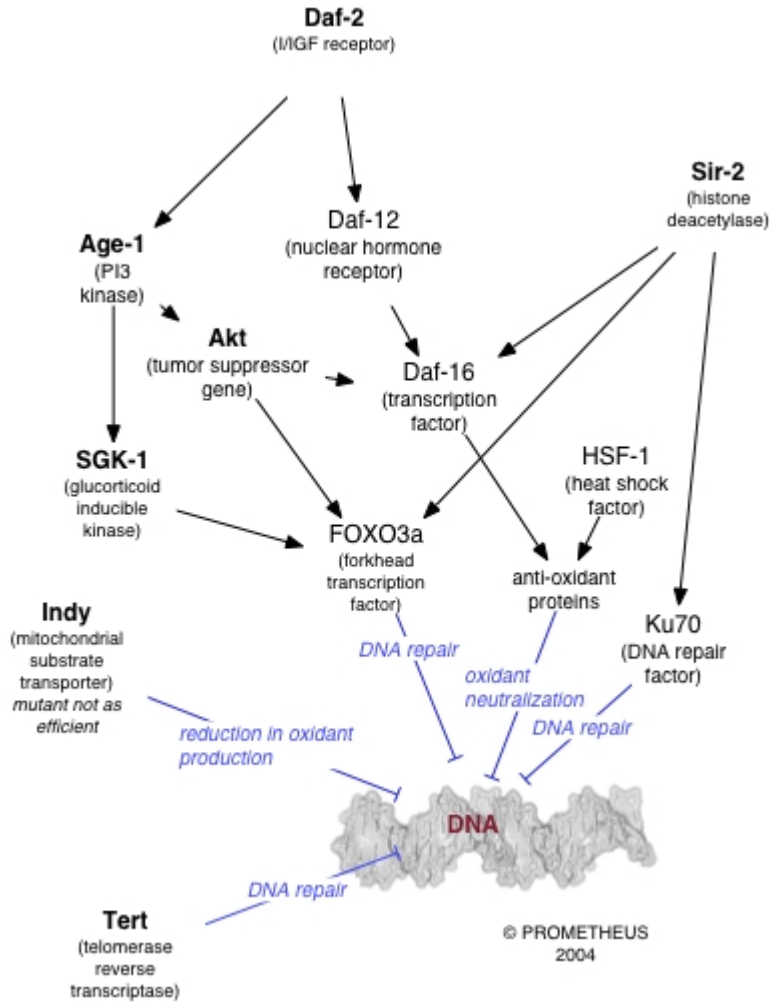


DNA repair rate is an important determinant of cell pathology

Experimental animals with genetic deficiencies in DNA repair often show decreased life span and increased cancer incidence. For example, mice deficient in the dominant NHEJ pathway and in telomere maintenance mechanisms get lymphoma and infections more often, and, as a consequence, have shorter lifespans than wild-type mice. In similar manner, mice deficient in a key repair and transcription protein that unwinds DNA helices have premature onset of aging-related diseases and consequent shortening of lifespan. However, not every DNA repair deficiency creates exactly the predicted effects; mice deficient in the NER pathway exhibited shortened life span without correspondingly higher rates of mutation.

If the rate of DNA damage exceeds the capacity of the cell to repair it, the accumulation of errors can overwhelm the cell and result in early senescence, apoptosis, or cancer. Inherited diseases associated with faulty DNA repair functioning result in premature aging, increased sensitivity to carcinogens, and correspondingly increased cancer risk. On the other hand, organisms with enhanced DNA repair systems, such as *Deinococcus radiodurans*, the most radiation-resistant known organism, exhibit remarkable resistance to the double-strand break-inducing effects of radioactivity, likely due to enhanced efficiency of DNA repair and especially NHEJ.

Longevity and caloric restriction



Most life span influencing genes affect the rate of DNA damage

A number of individual genes have been identified as influencing variations in life span within a population of organisms. The effects of these genes is strongly dependent on the environment, in particular, on the organism's diet. Caloric restriction reproducibly results in extended lifespan in a variety of organisms, likely via nutrient sensing pathways and decreased metabolic rate. The molecular mechanisms by which such restriction results in lengthened lifespan are as yet unclear; however, the behavior of many genes known to be involved in DNA repair is altered under conditions of caloric restriction.

For example, increasing the gene dosage of the gene SIR-2, which regulates DNA packaging in the nematode worm *Caenorhabditis elegans*, can significantly extend lifespan. The mammalian homolog of SIR-2 is known to induce downstream DNA repair factors involved in NHEJ, an activity that is especially promoted under conditions of caloric restriction. Caloric restriction has been closely linked to the rate of base excision

repair in the nuclear DNA of rodents, although similar effects have not been observed in mitochondrial DNA.

It is interesting to note that the *C. elegans* gene AGE-1, an upstream effector of DNA repair pathways, confers dramatically extended life span under free-feeding conditions but leads to a decrease in reproductive fitness under conditions of caloric restriction. This observation supports the pleiotropy theory of the biological origins of aging, which suggests that genes conferring a large survival advantage early in life will be selected for even if they carry a corresponding disadvantage late in life.

Medicine and DNA repair modulation

Hereditary DNA repair disorders

Defects in the NER mechanism are responsible for several genetic disorders, including:

- xeroderma pigmentosum: hypersensitivity to sunlight/UV, resulting in increased skin cancer incidence and premature aging
- Cockayne syndrome: hypersensitivity to UV and chemical agents
- trichothiodystrophy: sensitive skin, brittle hair and nails

Mental retardation often accompanies the latter two disorders, suggesting increased vulnerability of developmental neurons.

Other DNA repair disorders include:

- Werner's syndrome: premature aging and retarded growth
- Bloom's syndrome: sunlight hypersensitivity, high incidence of malignancies (especially leukemias).
- ataxia telangiectasia: sensitivity to ionizing radiation and some chemical agents

All of the above diseases are often called "segmental progerias" ("accelerated aging diseases") because their victims appear elderly and suffer from aging-related diseases at an abnormally young age, while not manifesting all the symptoms of old age.

Other diseases associated with reduced DNA repair function include Fanconi's anemia, hereditary breast cancer and hereditary colon cancer.

DNA repair and cancer

Inherited mutations that affect DNA repair genes are strongly associated with high cancer risks in humans. Hereditary nonpolyposis colorectal cancer (HNPCC) is strongly associated with specific mutations in the DNA mismatch repair pathway. BRCA1 and BRCA2, two famous mutations conferring a hugely increased risk of breast cancer on carriers, are both associated with a large number of DNA repair pathways, especially NHEJ and homologous recombination.

Cancer therapy procedures such as chemotherapy and radiotherapy work by overwhelming the capacity of the cell to repair DNA damage, resulting in cell death. Cells that are most rapidly dividing - most typically cancer cells - are preferentially affected. The side-effect is that other non-cancerous but rapidly dividing cells such as stem cells in the bone marrow are also affected. Modern cancer treatments attempt to localize the DNA damage to cells and tissues only associated with cancer, either by physical means (concentrating the therapeutic agent in the region of the tumor) or by biochemical means (exploiting a feature unique to cancer cells in the body).

DNA repair and evolution

The basic processes of DNA repair are highly conserved among both prokaryotes and eukaryotes and even among bacteriophage (viruses that infect bacteria); however, more complex organisms with more complex genomes have correspondingly more complex repair mechanisms. The ability of a large number of protein structural motifs to catalyze relevant chemical reactions has played a significant role in the elaboration of repair mechanisms during evolution. For an extremely detailed review of hypotheses relating to the evolution of DNA repair, see.

The fossil record indicates that single-cell life began to proliferate on the planet at some point during the Precambrian period, although exactly when recognizably modern life first emerged is unclear. Nucleic acids became the sole and universal means of encoding genetic information, requiring DNA repair mechanisms that in their basic form have been inherited by all extant life forms from their common ancestor. The emergence of Earth's oxygen-rich atmosphere (known as the "oxygen catastrophe") due to photosynthetic organisms, as well as the presence of potentially damaging free radicals in the cell due to oxidative phosphorylation, necessitated the evolution of DNA repair mechanisms that act specifically to counter the types of damage induced by oxidative stress.

Rate of evolutionary change

On some occasions, DNA damage is not repaired, or is repaired by an error-prone mechanism that results in a change from the original sequence. When this occurs, mutations may propagate into the genomes of the cell's progeny. Should such an event occur in a germ line cell that will eventually produce a gamete, the mutation has the potential to be passed on to the organism's offspring. The rate of evolution in a particular species (or, in a particular gene) is a function of the rate of mutation. As a consequence, the rate and accuracy of DNA repair mechanisms have an influence over the process of evolutionary change.