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Mitosis and its regulation

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ABSTRACT

Cell division by mitosis is essential for the development of organisms and their reproduction; it is also necessary that each new cell is genetically identical to that from which it comes. In eukaryotes this is achieved by the presence of complex mechanisms that ensure the integrity of genomic material and their proper segregation during mitosis. The traditional view of mitosis has been divided into different stages that were characterized by morphological studies in dividing cells; advances in molecular biology have led beyond this characterization, so that we now know a range of participant molecules. This article will discuss the process of mitosis, both at the cellular and molecular level and a brief summary of the molecular players that regulate this process.

Key words: Cell division, Mitosis, Cell cycle.

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In multicellular organisms, like the human, which start life with an egg cell or cygote, mitotic cell division is decisive for the development and maintenance of the various tissues, organs, and systems that make up the organism. The new cells produced by mitosis are usually identical to the stem cell; this is achieved by means of complex regulating mechanisms which ensure the integrity of genomic material and its proper segregation. The series of events that lead to the division of a cell is known as the cell cycle and is made up by two main phases: interphase and cell division, which may be mitosis or meiosis. Mitosis is the nuclear process whereby replicated chromosomes are segregated in two daughter nuclei, and is usually accompanied by cytokinesis, which is the division of the cytoplasm and physical separation of the two daughter cells.

The mitotic process was first described by Flemming, en 1882; its duration is usually less than one hour, in which time the cell is capable of separating its genetic information in two identical groups, which, with the rest of its subcellular components, will be passed on to the daughter cells. During mitosis, the cell is engaged in a primary activity, which is chromosome segregation, and practically suspends metabolism, transcription, and translation.[1]

Before the process of mitosis, in the S-phase of the cell cycle, the chromosomes replicate, so that, when cell division starts in humans, each of the 46 replicated chromosomes will have two chromatid joined by the centromere, each of which represents a functional chromosome. Each sister chromatid of a chromosome will segregate a daughter cell, as will the other 45, and together they will form the diploid "group" of 46 chromosomes of the new cell. Mitosis is characterized by two important events which can be viewed under the light microscope: condensation and chromosome segregation. Chromosome motion is mediated by a structure formed primarily by microtubules: the mitotic spindle, which aligns the replicated and condensed chromosomes at the center of the cell, to position the sister chromatids with the kinetochore of one pointing toward one pole and that of the other pointing toward the opposite pole; at this point the protein that kept the centromeres joined, and in turn kept the sister chromatids joined, splits off. Here the mitotic spindle, by means of its kinetochore microtubules pulls the chromatids toward opposite poles. The last step is the reinstallation of an interphasic nucleus and division of the cytoplasm to form two identical daughter cells. All these events occur in five nuclear stages: prophase, prometaphase, metaphase, anaphase, and telophase, and finally cytokinesis occurs, which is the division of the cytoplasm.[2] (Figure 1).

STAGE OF MITOSIS

Prophase

The transition from interphase to mitosis is prophase. At this stage the following events

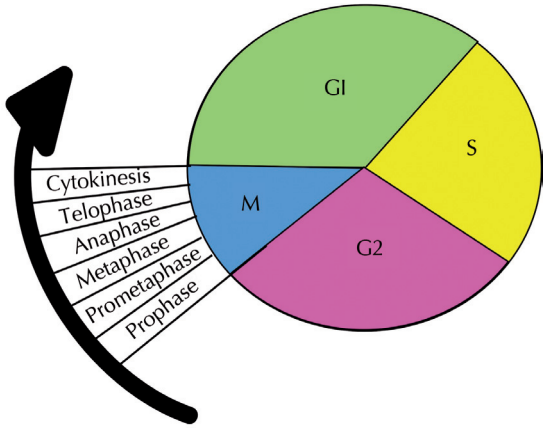

Figure 1. Mitosis in the context of the different phases of the cell cycle. In a mitotic cell cycle with approximate duration of 24 hours, the M phase is completed in approximately one hour, and therefore each of the five nuclear stages and cytokinesis take minutes.

take place: the chromatin condenses to form chromosomes, the mitotic spindle forms, and the nuclear envelope disappears.

1) Chromosome condensation

The first visible manifestation of cell division is the progressive compacting of the nuclear chromatin (Figure 2a), to give rise to chromosome strands; at this point chromosomes are defined as dual strands because they are replicated; this packaging is indispensable to prevent the chromosomes from undergoing alterations caused by the mechanical stress to which they are subjected due to the movements of the mitotic spindle during chromosome segregation.[2]

Chromatin, which is in 30 nm fibers in interphase, starts to condense due to the intervention of a protein complex of condensin and topoisomerase II, which wrap the 30 nm fiber forming DNA loops wrapped along each chromatid. To keep the sister chromatids of the replicated and condensed chromosome joined, another protein complex called cohesin intervenes, which keeps the two chromatids joined from when they finish their replication in S-phase until the mitotic anaphase. Condensin and cohesin are similar structures which can form rings that keep distant segments of chromatin joined (Figure 3). Cohesin is found along the chromosome arms, keeping the sister chromatids longitudinally joined and giving the early mitotic chromosome the appearance of a single strand. It is also firmly bonded to the chromatids by the centromere; the cohesin located along the arms separates from the chromatin in prophase and that of the centromere is retained until anaphase.[3]

The chromatin extended from the nucleus in interphase allows the entry of the transcription machinery; but during mitosis, when the chromatin has the greatest condensation in the cell cycle, transcription is inhibited. Therefore, the nucleolus – a structure formed mainly by products of ribosomal DNA transcription and proteins, disappears, and consequently translation is also halted.

2) Formation of the mitotic spindle

The microtubules of the interphase cytoskeleton are disassembled due to modification of

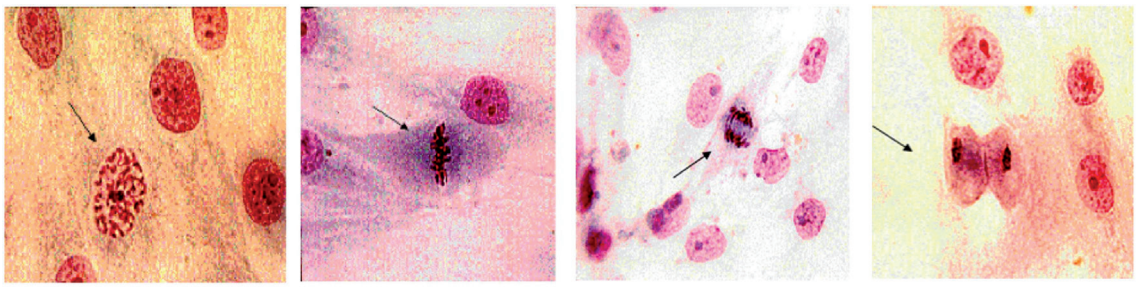

Figure 2. The different phases of mitosis. Culture of fibroblasts in which we can observe: a) Prophase-prometaphase: with chromosomes in the process of condensation and nuclear envelope disintegrating b) Metaphase: chromosomes aligned on the equatorial plate with greater condensation c) Anaphase: separation of sister chromatids in two groups d) Telophase: cytoplasmic division and reintegration of nuclear envelopes to form two daughter cells. (Photograph courtesy of Dr. Joaquin Carrillo, Institute of Hematopathology).

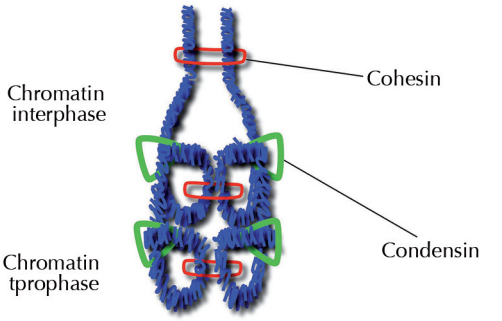

Figure 3. DNA packaging mediated by condensin and cohesin complexes. Condensin is formed by four subunits of SMC 2/4 proteins and associated proteins which close or open the ring that will longitudinally bind chromatin loops to condense each of the sister chromatids. Cohesin is formed by four SMC 1/3 subunits and its ringed formation is regulated by associated proteins of the SCC family; this ring links the chromatin of the two sister chromatids and joins them to form a typical mitotic chromosome; this bond is more durable between the chromatin of the centromere which enters the chromatin along the chromosomal arms.

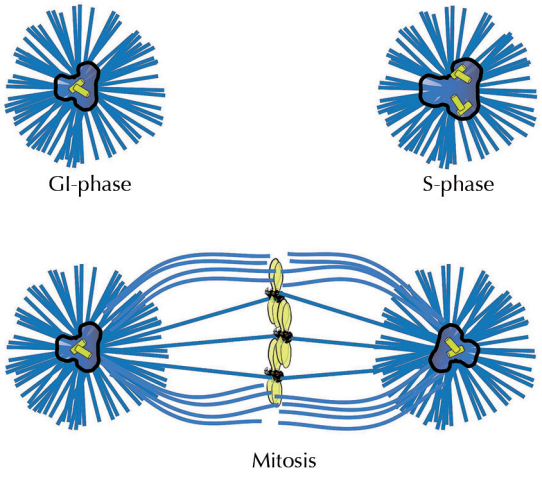

Figure 4. Role of centrosomes in formation of the mitotic spindle. The centrioles which are in pairs duplicate during the interphase, in the S-phase, integrating the centrosomes which during mitosis will establish the poles of the mitotic spindle.

microtubule associated proteins or MAPs; the microtubules reorganize to contribute to the formation of the mitotic spindle. This new organization of microtubules is triggered by the splitting of a duplicate structure in the S-phase of the cell cycle called centrosome; each of the two centrosomes consists of two centrioles positioned at right angles to one another, surrounded by a protein matrix. During prophase, the first step to form the mitotic spindle is the appearance or nucleation of microtubules around the centrosomes forming a kind of star, thereby forming asters, whose microtubules have a negative (-) end associated with the centrosome and a positive (+) end to which tubulin dimers are added more quickly. Each aster migrates to opposite positions in the cell, establishing the cellular poles from which a bipolar mitotic spindle will form (Figure 4). The microtubules of the asters continue growing at their + ends until some of them, called kinetochore microtubules, find the kinetochore of one of the two sister chromatids of a chromosome, where they are anchored to proteins of the kinetochore, specifically of the fibrous crown with the aid of other proteins, such as Ndc80, CENP-E-kinesin, and dynein (Figure 5). The microtubules of the opposite pole will make contact with the other sister chromatid of the same chromosome. The microtubules that do not find a kinetochore, called interpolar microtubules, continue growing on their + end until they meet and are superimposed on the + ends of the microtubules of the opposite pole thereby forming a functional mitotic spindle, with three types of microtubules: astral microtubules, kinetochore microtubules, and interpolar microtubules (Figure 6).[4]

There are two types of mitosis: centrosome dependent and independent; in the latter nucleation of microtubules originates in the kinetochores and it is highly likely that, even in cells with functional centrosomes there is also nucleation of microtubules from the kinetochore of the chromatids.[5]

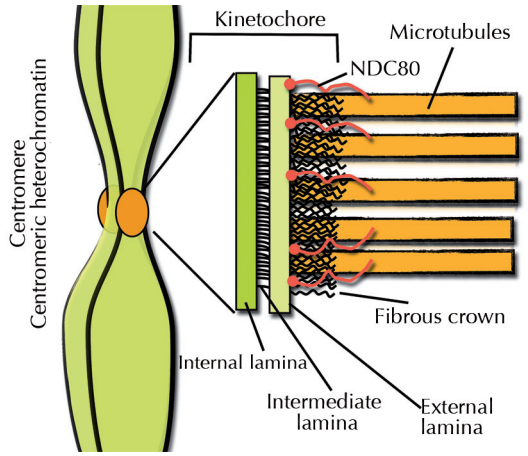

Figure 5. Structure of the kinetochore. Is made up by three protein laminas: one internal, one middle, and one external, after which fibrous proteins extend forming the fibrous crown. The protein Ndc80 keeps the microtubules attached to the outer layer of the kinetochore during depolymerization of tubulin by kinesins.

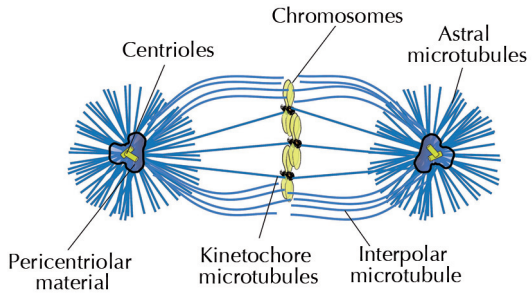

Figure 6. Different types of microtubules in the mitotic spindle. The astral microtubules surround the centrioles; they have their– end on the perimeter of the pericentriolar material and their + end irradiates in all directions. The kinetochore microtubules are microtubules associated by their + end to the kinetochores of the sister chromatids; each of them has a beam of kinetochore microtubules pointing toward opposite ends. The interpolar microtubules grow on their + ends without finding a kinetochore until they are superimposed with another interpolar microtubule.

3) Disappearance of nuclear envelope and fragmentation of Golgi apparatus

For kinetochore microtubules to interact with chromosomes, the nuclear envelope must disappear; this is achieved by fragmentation due to the interaction of the cyclin B-Cdk1 complex with elements of each of the three components of the envelope: *a)* nuclear membranes, *b)* nuclear pores, and *c)* the nuclear lamina.[6] It is probable that the resulting fragments in the form of vesicles will disperse through the mitotic cell or combine with fragments of the endoplasmic reticulum. Similarly, the Golgi apparatus will disintegrate into small vesicles whose fate is similar to that of the nuclear envelope. In either case, the vesicles, independent or associated with the endoplasmic reticulum, will be segregated in the daughter cells, where they will reform their original structures. The endoplasmic reticulum may not fragment into vesicles, and merely segregate in fragments toward the daughter cells. Other membranous components, such as mitochondria, lysosomes, and peroxisomes, do not disaggregate, and merely segregate in generally symmetric form to the daughter cells.[7]

Prometaphase

This second phase is characterized by active movement directing the chromosomes to the cell equator. The start of the prometaphase is recognized by the interaction of the mitotic spindle with the chromosomes duplicated due to the dissolution of the nuclear envelope, after which chromosomal movements begin.[8]

The + ends of the microtubules in the asters move by polymerization toward the center of the cell seeking chromosomes, in what is thought to be a random motion, and when they make contact with a kinetochore they stabilize; eventually, the kinetochore of the other chromatid, sister of the same chromosome, also bonds to a group of

microtubules coming from the opposite pole. It has also been proposed that untrapped kinetochores may, on their own, initiate a nucleation of microtubules. One way or another, the chromosomes eventually have their two kinetochores, each connected to microtubules coming from opposite poles; at that point the microtubules pull and push the chromosome by means of polymerization-depolymerization of tubulin with the aid of kinesin and dynein motor proteins. Because the two poles attract the same chromosome through their two kinetochores — which remain joined by cohesin — a state is attained where the forces of traction from each pole start to balance, more actively polymerizing the + end of the microtubules associated with the kinetochore closest to its pole and shortening the microtubules associated with the farthest pole (Figure 7). The shortening or elongation of microtubules responds to differences in the tension forces on the kinetochores. With these motions, congregation is achieved, which is the convergence of all the chromosomes at the cell equator, positioned there by the balance of traction forces from the opposite poles of the spindle. Chromosome congregation marks the end of the prometaphase and the start of the metaphase.[4,8]

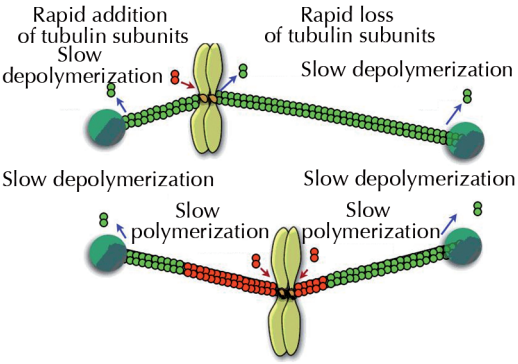

Figure 7. Polymerization-depolymerization of microtubules to form the metaphase plate. The kinetochores of each of the two chromatids of the chromosomes are associated with microtubules which come from opposite poles of the mitotic spindle, usually at an acentric site, and therefore to take them to the center of the spindle and form the metaphase plate the shorter microtubules polymerize rapidly until a balance of length is achieved; when this happens, the chromosomes are on the cell equator and oriented so that when the centromeres split, the traction force takes a complete chromosome group toward one pole and the other set to the opposite pole.

Metaphase

The chromosome condensation initiated in the prophase continues, and therefore metaphasic chromosomes are observed as a perfectly packaged chromatin which allows the genetic material to maintain its integrity under the mechanical stress from anaphase motions.[2] This is the stage at which chromosome studies are usually performed, because their morphology is very clear.

The chromosomes, moved by the mitotic spindle, position themselves at the center, between the two asters, and form the metaphase plate (Figure 2b) in which the chromosomes are positioned in such a way that the kinetochores of each sister chromatid are oriented toward opposite poles.[2] Keeping the chromosomes at the cell equator requires a balance between the forces from the microtubules which tend to move the kinetochores toward opposite poles, with the result that positioning them at the center involves a large quantity of energy. The energy of chromosomal motion comes from the polymerization-depolymerization of microtubules using the conversion of GTP to GDP and from motor proteins using the conversion of ATP to ADP.[4]

Each kinetochore can anchor 20-30 microtubules which apply traction force toward the pole from which they come, as a result of which the metaphase plate is maintained by the balance between the opposing forces from the poles

on the chromosomes, which keep their sister chromatids together with centromeric cohesin, so that when that protein is withdrawn from the centromere, the metaphase ends and the anaphase begins with migration of sister chromatids to opposite poles.[9,10]

Anaphase

When all the chromosomes are on the equator forming the metaphase plate, the centromeres, which keep the sister chromatids joined, split off allowing each chromatid to migrate toward the pole it was facing, thereby initiating the anaphase, which is divided in anaphase A and anaphase B. The motions of anaphase A are made primarily by the kinetochore microtubules which are shortened by depolymerization at both tubulin ends, with the intervention of kinesins (family 13); this allows them to pull the single chromosomes — which we call sister chromatids when they are joined by the centromere — toward the cell poles, dividing the replicated genome into two diploid chromosome groups, with 46 single chromosomes each. In anaphase B interpolar microtubules intervene, separating the two chromosome groups by increasing the length of the mitotic spindle (Figure 2c).[2]

Anaphase A starts when the anaphase promoting complex (APC) bonded to CDC20 induces splitting of the centromere, leaving the sister chromatids free to each migrate toward opposite poles. The active mechanism of APC-CDC20 is to ubiquitinate a protein called securin,[11,12] which it sends for destruction by proteasome and the destruction of securin liberates a protein called separase, which is a protease that acts on cohesin, the complex that keeps sister chromatids joined. When cohesin is removed from the centromere there is a change in tension on the kinetochores, which triggers a depolymerization of tubulin at both + and – ends of the kinetochore microtubules; when shortened, they pull the chromosomes bonded to them toward the centrosomes located at the cell poles (Figure 8).[11-13]

During anaphase B, the two groups of chromosomes already positioned at the poles are separated from one another by the intervention of the polar microtubules, which depolymerize tubulin dimers at their - ends and actively polymerize at their positive poles directed toward the cell center; these microtubules grow so much that the ends cross and continue to grow in opposite directions. As a result of the intervention of motor proteins associated with microtubules, the superimposed segments of the polar microtubules start a process of sliding "backward" toward the poles, which causes the entire mitotic spindle to elongate and thereby separate the two groups of chromosomes, which had reached the poles in anaphase A.[2,12]

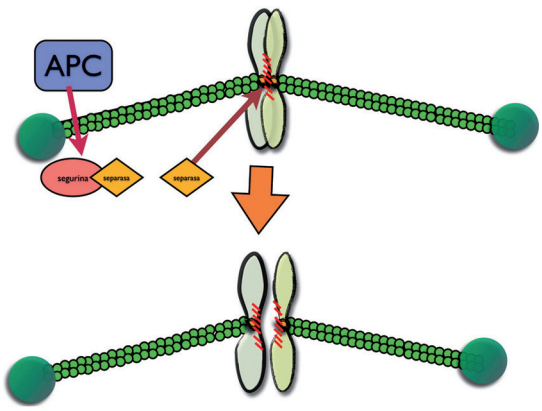

Figure 8. Anaphase A. In anaphase A, the APC complex sends securin for destruction, which in turn releases the protein separase. Separase acts on the cohesin which keeps the two chromatids joined by the centromere, in the chromosome in metaphase positioned on the cell equator. Releasing cohesin involves separating the chromatids which cede to the forces from the kinetochore microtubules which, by depolymerization of both ends, pull the chromosomes toward the poles.

Telophase

In the telophase, the kinetochore microtubules disappear and the chromosomes are left free at both cell ends. At this point, the chromosomes start to decondense and the nuclear envelope is restored. In the late telophase the chromosomes start to transcribe and the nucleolus is restored, marking the end of mitosis.[2] (Figure 2d).

Cytokinesis

Cytokinesis is the last stage of cell division, when the cytoplasm divides to create two fully independent daughter cells. However, cytokinesis starts from the anaphase, when a band of filamentous proteins, mainly actin and myosin forms immediately below the plasma membrane at the level of the cell equator, which contract and form a cleavage furrow, which grows progressively deeper, until the band that forms touches the remaining microtubules of the mitotic spindle; this entire group of proteins is known as a midbody structure. Finally, the cell is strangled and creates two daughter cells (Figure 9).[14]

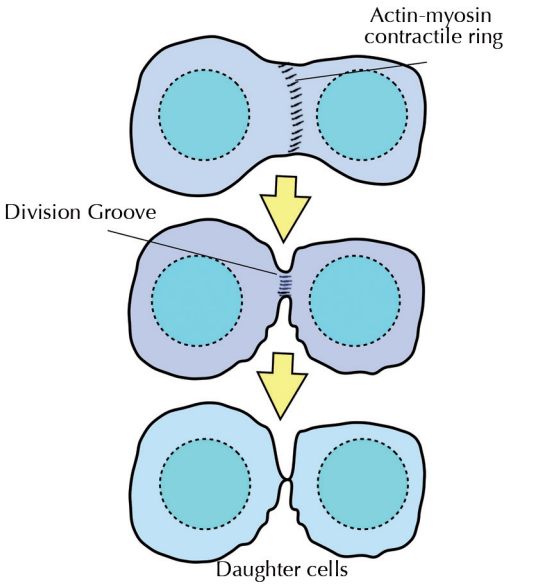

Figure 9. Establishment of the cleavage furrow with the intervention of the proteins actin and myosin II. The actin-myosin association starts immediately below the plasmatic membrane and perpendicular to the center of the mitotic spindle. During cytokinesis this contractile ring strangles the cell which already contains two sister nuclei, between them, until only a midbody formed by remaining microtubules from the mitotic spindle and the last of the acto-myosin ring remains. Finally the two daughter cells separate.

MOLECULAR REGULATION OF MITOSIS

The cell cycle is regulated by a pattern of cyclins and cyclin-dependent kinases (CDKs)

For study, the cell cycle can be divided in two stages: the interphase, made up by the G1, S, and G2 phases, and the M-phase, in when chromosome segregation and cytokinesis take place.

For a cell to advance through the phases of the cell cycle, and finally segregate its genetic material, a very fine regulation is required, which is characterized by the action of CDKs (cyclin-dependent kinases), which act as kinases of other proteins that regulate the cell cycle, and thus mark the progression from one phase of the cycle to another. These kinases take their active form in a heterodimeric complex with a regulating cyclin (Figure 10a). Cyclin expression is a limiting factor for activation of CDKs. Usually, cyclin levels are determined by transcriptional control and their proteolysis through the ubiquitin-proteasome system.[15,16]

The expression of cyclin D1 is necessary in the G1 phase to stimulate cells' entrance into the cell cycle and their concentrations remain high as long as there are mitogenic agents. Cyclin E is required in the G1 phase following the intervention of cyclin D1 and reaches its maximum

expression in the G1/S transition, which makes it necessary for entry to the synthesis phase. Cyclin A is required for the start of S and the G2/M transition, while cyclin B regulates the entry and exit of mitosis (Figure 10b).[15,16]

The G2 control point regulates entry to mitosis

Following DNA synthesis, and to reduce the accumulation of genetic errors, cells monitor that DNA has been replicated correctly, and if any error is detected, the cell cycle stops, and if no errors are detected, the cell will progress into mitosis. This checkpoint is called G2 (or G2/M).

When there is damage in the DNA the kinases ATM (ataxia telangiectasia mutated) and ATR (ATM and Rad3 related) are activated. The former detects dual chain breaks in DNA, while the latter detects single chain breaks.[17] The kinases ATM/ATR propagate the alert signal, assisted by the checkpoint mediating proteins, which include MDC1 (mediator of DNA-damage checkpoint 1, also known as NFBD1), 53BP1 (p53 binding protein 1) and BRCA (breast cancer protein). Also, signal transduction kinases CHK1 and CHK2 are required.[18-21] ATM/ATR phosphorylate histone H2AX (γ-H2AX) and marks the region of the chromatin flanking each break; in turn, γ-H2AX permits grouping of the repairing proteins at the site of damage. The 53BP1 MDC1 and BRCA mediators act as a molecular bridge between histone γ-H2AX and the proteins of the MRN repairing complex (MRE11, RAD50, NBS1). The MRN complex, already at the site of the damage, repairs the dual chain break in the DNA strand by means of homologous recombination.[17,18]

The cells prevent entry into mitosis with chromosome damage, so that, while the DNA is repaired, the G2 checkpoint blocks the action of the mitosis promoting factor (MPF), formed by cyclin B and CDK1. The action of MPF can be inhibited by signaling through ATM/ATR, by means of two primary actions:

1. The kinases of the p38 family can inhibit CDC25 phosphatases, which trigger the MPF complex at the limit of the G2/M phases, and therefore when they are inhibited entry into mitosis does not occur.

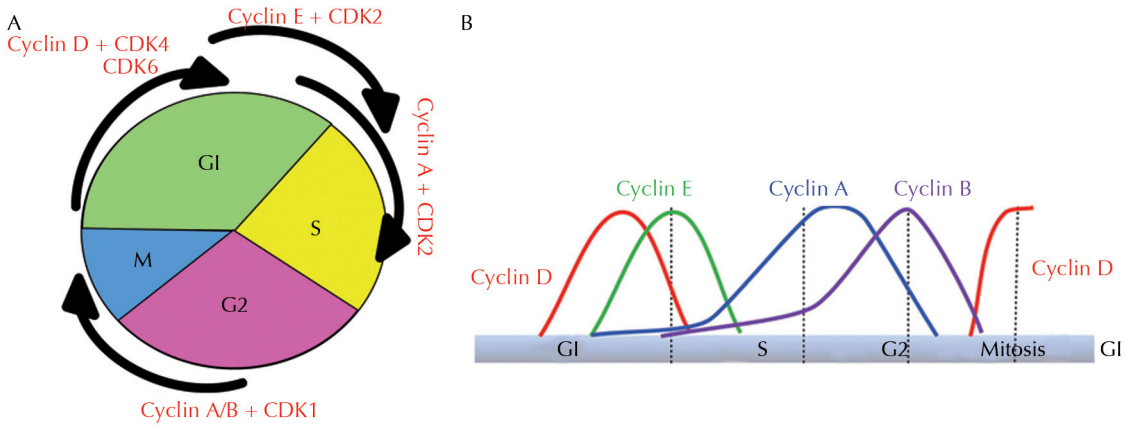

Figure 10. Pattern of activity of CDKs and cyclins through the cell cycle.

2. The CHK1/CHK2 kinases can activate WEE1, which is a negative regulator of the MPF complex, or sequentially activate p53 and GADD45, a cyclin B sequesterer. Thus, cyclin B is prevented from entering the nucleus and the MPF complex is not formed.[15,22]

G2/M transition (entry into mitosis)

Progression from the G2 to M phase is due to activation of the MPF complex, the action of which is maintained from the prophase to the metaphase. The MPF complex phosphorylates approximately 70 mitotic substrata which act in centrosome separation, phosphorylation and activation of chromatin condensation regulating enzymes, phosphorylation of histone H1, rupture of the nuclear membrane, and reorganization of the cytoskeleton of microtubules and actin to achieve mitosis.[23,24]

The action of the MPF complex is regulated at two levels, the first transcriptional by protein p53, which maintains transcription, and therefore keeps the presence of CDK1 stable throughout the cell cycle and that of cyclin B from the end of the S-phase. The second level of regulation of MPF activity is by post-translational modifications, specifically inhibitory and activating phosphorylation; in the G2 phase inhibitory phosphorylations are made on CDK1 by the kinases MYT and WEE1, and then the phosphatase CDC25 releases inhibition to CDK1;[15,25] this, combined with activating phosphorylation on the same CDK1, performed by CAK (CDK activating kinase, a heterodimer of cyclin H and CDK7), leads the cell to enter into mitosis.

The mitosis promoting action of CDK1 may directly inhibit the p21 tumor suppressor gene (Cip1/Waf1). In relation to cyclin B, a phosphorylation is needed to change its subcellular location from cytoplasm to nucleus in the early stages of mitosis. Exit from the metaphase is characterized by a critical drop in concentrations of MPF complex due to its destruction.[15, 22]

When the cell has passed the G2 phase checkpoint, its duplicate chromosomes start the process of condensation, as a result of condensin complexes I and II, which act by packaging interphase chromatin in mitotic chromosomes, so that it can be segregated properly between the daughter cells.[26-29] Condensin complex I contains ATPase active SMC (Structural maintenance of chromosome) proteins, such as SMC2/CAP-E and SMC4/CAP-C, and non-SMC proteins, such as CAP-G and CAP-H.[30,31] Condensin complex II consists of subunits SMC2 and SMC4 and the proteins hCAP-D3, hCAP- G2, and hCAP-H2.[32,33] Apparently, complex I functions in prometaphase and complex II in early prophase.[31] During packaging, the sister chromatids remain joined due to the cohesins, which are loaded in the chromosomes in the late G1/early S phases and remain until bi-orientation of chromatids is achieved in the metaphase.[29]

The process of chromosome condensation and entry into mitosis can be reverted in the presence of stress or non-optimum conditions for cell division, as a result of the activation of antephase checkpoint proteins.[29,32-38] The proteins involved in this process are only starting to be described, but CHFR (*Checkpoint with FHA and RING domains*) and kinase p38 are considered essential parts of the process. CHFR is a ubiquitin ligase which accumulates in response to damaged microtubules and has among its targets proteins important to mitotic progression, such as Aurora A and PLK1, which are marked for destruction by means of the ubiquitin-proteasome system; thus, mitotic progression is delayed.[35,36] On the other hand, kinase p38, which is activated in case of damage by ultraviolet radiation or alteration of the chromatin structure blocks entry into mitosis. It has been speculated that the inhibitory target of the antephase checkpoint is condensin complex II. [29,34,37]

The metaphase checkpoint

Among the checkpoints specialized in blocking mitotic progression, the most studied, and perhaps the principal, is that known as SAC (Spindle Assembly Checkpoint) or mitotic checkpoint. The SAC is activated in each cell cycle immediately after entry into mitosis and functions by delaying entry into anaphase until all the chromosomes are properly anchored on the metaphase plate, bioriented, and with adequate tension.[38]

When the alignment of chromosomes satisfies the SAC, segregation of chromosomes occurs. This process begins when the protein cofactor CDC20 bonds and activates the anaphase-promoting complex/cyclosome (APC/C), which fulfils the function of an ubiquitin ligase. When the APC/C is activated, it transfers three ubiquitins to cyclin B1 and to the protein securin, marking them for degradation by proteasome 26S. Securin is important because it keeps the protease separase sequestered; when it is released, as a result of degradation of securin, degradation of the cohesin which keeps the sister chromatids joined occurs. Cohesin degradation permits chromosome segregation, whereas cyclin B1 degradation results in the inactivation of CDK1; as a result, exit from mitosis is achieved.[38,39]

When the alignment of chromosomes does not satisfy the SAC, the signal from unanchored kinetochores induces recruitment of checkpoint proteins, such as MAD2 (mitotic arrest deficient 2), BUB3 (budding uninhibited by benzimidazoles 3), and BUBR1 (BUB related). This signal leads to the formation of two independent complexes, one made up by BUB3/BUBR1 and the other by C-MAD2/CDC20. The combination of those two complexes will give rise to MCC (mitotic control complex), in which CDC20 is already sequestered, thereby inhibiting activation of the APC/C complex. Thus, securin will not be marked for degradation, separase will not be released, and chromosomes will not segregate until the requisites of SAC are satisfied.[39-41]

Despite its high level of specialization, SAC is incapable of recognizing erroneous attachments between kinetochores and microtubules, known as merotelic attachments; however, they can be repaired as a result of the action of the CPC (Chromosome Passenger Complex). The CPC is formed by the proteins SUR (survivin), BOR (borealin), INCENP (centromere protein), and AurB (aurora kinase B) and to date it is the leading candidate for the kinetochore attachment tension sensor complex. The action of AurB is essential because it detects and destabilizes erroneous attachments between microtubules and kinetochores, such as syntelic and merotelic attachments, thereby producing unanchored kinetochores which are detected by the SAC leading to the blockage of mitosis. On reaching the anaphase, AurB moves out of the centromere region. In the prometaphase, CDC20 and all the SAC proteins concentrate in the kinetochores, as a result of recruitment by the kinases BUB1, MPS1, and AurB, but are activated in response to signals from unanchored kinetochores.[38,40-42]

Several mechanisms have been proposed for silencing of SAC. The first consists of removing proteins from the SAC, with dynein, in properly anchored microtubules; the second is silencing, which occurs when the protein CENP-E bonds to microtubules and inhibits the kinase action of BUBR1; the third silencing process is that mediated by inhibitory binding of p31-COMET to MAD2-CDC20, and even degradation of the components of the SAC by the APC/C mediated ubiquitin-proteasome system.[43,44-46]

Progression by mitosis is not regulated only by checkpoints but is also closely related to the action of APC/C, which in late mitosis is associated with CDH1 and remains active through the next G1 phase. APC/C-CDC20 degrades into cyclin

B and cohesin when all the chromosomes have made contact with the bipolar spindle and move through the metaphase plate, leading to separation of the sister chromatids. For its part, the APC/CDH1 complex marks the kinases polo like kinase 1 (PLK1), aurora A, survivin, NEK2, CDC20, and SKP2 for degradation in the anaphase and in the next G1 phase. CDH1 levels remain relatively constant throughout the cell cycle and their activity is regulated above all through cell cycle dependent phosphorylations. Phosphorylation of CDH1 by CDKs in S, G2, and early M inhibits its binding to APC/C, whereas its dephosphorylation in the late M phase permits binding to APC/C and activation of the complex[15] (Figure 8).

Regulation of cytokinesis

Cytokinesis should not occur until the separation of chromosomes is complete. In general, proper execution of cytokinesis is believed to depend on structural organization of cells and not on diffusible molecules; however, recent studies have found that kinase CHK1 deficiencies may result in errors in chromosome segregation, regression of cytokinesis, and binucleation. Also, such deficiency correlates with an erroneous positioning of the proteins AurB and CHK1, which accumulate in the cleavage furrow to facilitate production of two daughter cells. This makes clear that the proteins that regulate checkpoints in the cell cycle may also have important functions in chromosome segregation and cytokinesis.[47]

Covalent modifications of histones during mitosis

Histones are a group of conserved proteins which play a crucial role in DNA packaging in eukaryotic cells. There are four types of nucleosomal histones, H2A, H2B, H3, and H4, and the histone H1, for binding to spacer DNA. Histones perform regulatory functions as a result of covalent modifications of amino acid residues in their tails.[48] Many kinds of modifications in histones have been described, including phosphorylation, acetylation, methylation, ubiquitination, and sumoylation. Different combinations of histone modifications have been proposed as a code that identifies different functional states of chromatin; such codes play a relevant part in epigenetic regulation of gene expression. These modifications are also relevant to the mitotic process.[48,49]

Phosphorylation of H3S10 (histone 3 serine 10) and H3S28 (istone 3 serine 28) is required for condensation of chromosomes and their segregation. Methylation of H3K9 (histone 3 lysine 9) promotes the formation of heterochromatin and inhibits phosphorylation of H3S10, and vice-versa, which indicates an interaction between those two modifications. Phosphorylation of H3S10 prevents binding of protein HP1 (heterochromatin forming protein) which is disassociated from chromatin during mitosis; when dephosphorylation of H3S10 occurs, HP1 can bind to chromatin.[49,50,51]

Apparently, ubiquitination of histone H2A inhibits the action of aurora A in stages other than mitosis, whereas its deubiquitination is required for H3S10 phosphorylation and chromosome segregation. During cell division, histone acetylation and methylation are antagonistic events, with a drop in acetylation and increase in methylation during the process.[52]

REFERENCES

1. Salaün P, Rannou Y, Prigent C. Cdk1, PLKs, Auroras, and NEKs: the mitotic bodyguards. Adv Exp Med Biol 2008;617:41–56.
2. Walczak CE, Cai S y Khodjakov A. Mechanisms of chromosome behaviour during mitosis. Nat Rev Mol Cell Biol 2010; 11: 91-102.
3. Hirano T. At the heart of the chromosome: SMC proteins in action. Nat Rev Mol Cell Biol 2006;7:311-322.
4. Sharp DJ, Rogers GC, Scholey JM. Microtubule motors in mitosis. Nature 2000;407:41-47.

5. Oegema K, Hyman AA. Cell division (January19, 2006). WormBook, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/ wormbook.1.72.1,http://www.wormbook.org.
6. Güttinger S, Laurell E, Kutay U. Orchestrating nuclear envelope disassembly and reassembly during mitosis. Nat Rev Mol Cell Biol 2009;10:178-191.
7. Pecot MY, Malhotra V. Golgi membranes remain segregated from the endoplasmic reticulum during mitosis in mammalian cells. Cell 2004;116:99–107.
8. Wittmann T, Hyman A, Desai A. The spindle: a dynamic assembly of microtubules and Motors. Nat Cell Biol 2001;3:28-34.
9. Lampert F, Westermann S. A blueprint for kinetochores — new insights into the molecular mechanics of cell division. Nat Rev Mol Cell Biol 2011;12:407-412.
10. Akiyoshi B, Sarangapani KK, Powers AF, Nelson CR, Reichow SL, Arellano-Santoyo H, Gonen T, et al . Tension directly stabilizes reconstituted kinetochore-microtubule attachments Nature 2010;468:576-581.
11. Acquaviva C, Pines J. The anaphase- promoting complex/cyclosome: APC/C. 2006. Journal of Cell Science 2006;119:2401-4.
12. Sullivan M, Morgan DO. Finishing mitosis, one step at a time. Nat Rev Mol Cell Biol 2007;8:894-903.
13. Pines J. Mitosis: a matter of getting rid of the right protein at the right time. Trends Cell Biol 2006;16:55-63.
14. Norden C, Mendoza M, Dobbelaere J. The NoCut Pathway Links Completion of Cytokinesis to Spindle Midzone Function to Prevent Chromosome Breakage. 2006 Cell 125:85–98.
15. van den Heuvel, S. Cell-cycle regulation (September 21, 2005), WormBook, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.28.1, http://www.wormbook.org.
16. Malumbres M, Barbacid M. Cell cycle, CDKs and cancer: a changing paradigm. Nat Rev Cancer 2009;9:153- 166.
17. Branzei D, Foiani M. Regulation of DNA repair throughout the cell cycle. Nat Rev Mol Cell Biol 2008;3:297-308.
18. Reinhardt HC, Yaffe MB. Kinases that control the cell cycle in response to DNA damage: Chk1, Chk2, and MK2. Curr Opin Cell Biol 2009;21:245–255.
19. Kitagawa R, Bakkenist CJ, McKinnon PJ, Kastan MB. Phosphorylation of SMC1 is a critical downstream event in the ATM–NBS1–BRCA1 pathway. Genes Dev 2004;18:1423–38.
20. Shibata A, Barton A, Noon AT, Dahm K, Deckbar D, Goodarzi AA, Löbrich M, Jeggo PA. Role of ATM and the Damage Response Mediator Proteins 53BP1 and MDC1 in the Maintenance of G2/M Checkpoint Arrest. Mol Cell Biol 2010;30:3371–83.
21. Stucki M, Jackson SP. MDC1/NFBD1: a key regulator of the DNA damage response in higher eukaryotes. DNA Repair (Amst) 2004;3:953–7.
22. Kastan MN, Bartek J. Cell-cycle checkpoints and cancer. Nature 2004;432:316-23.
23. Nigg EA. Mitotic kinases as regulators of cell division and its checkpoints. Nat Rev Mol Cell Biol 2001;2:21-32.
24. Löbrich M, Jeggo P. The impact of a negligent G2/M checkpoint on genomic instability and cancer induction Nat Rev Cancer 2007;7:861-869.
25. Malumbres M, Barbacid M. Mammalian cyclin-dependent kinases. Trends Biochem Sci 2005;30,630–41.
26. Belmont AS. Mitotic chromosome structure and condensation. Curr Opin Cell Biol 2006;18:632–8.
27. Hirano T. Condensins: organizing and segregating the genome. Curr Biol 2005;15:265–75.
28. Hirota T, Gerlich D, Koch B, Ellenberg J, Peters JM. Distinct functions of condensin I and II in mitotic chromosome assembly. J Cell Sci 2004;117:6435–45.
29. Chin FC, Yeong MF. Safeguarding entry into Mitosis: the Antephase Checkpoint. Mol Cell Biol 2010;30:22–32.
30. Hagstrom KA, Meyer BJ. Condensin and cohesin: more than chromosome compactor and glue. Nat Rev Genet 2003;4:520–34.
31. Hirano T. At the heart of the chromosome: SMC proteins in action. Nat Rev Mol Cell Biol 2006;7:311-322.
32. Ono T, Fang Y, Spector DL, Hirano T. Spatial and temporal regulation of condensins I and II in mitotic chromosome assembly in human cells. Mol Biol Cell 2004;15:3296–308.
33. Yeong FM, Hombauer H, Wendt KS, Mudrak I, Mechtler K, Loregger T, Marchler-Bauer A, Tanaka K, Peters JM, Ogris E. Identification of a subunit of a novel Kleisin-beta/SMC complex as a potential substrate of protein phosphatase 2A. Curr Biol 2003;13:2058–64.
34. Mikhailov A, Shinohara M, Rieder CL. The p38-mediated stress-activated checkpoint. A rapid response system for delaying progression through antephase and entry into mitosis. Cell Cycle 2005;4:57–62.
35. Kang D, Chen J, Wong J, Fang G. The checkpoint protein CHFR is a ligase that ubiquitinates PLK1 and inhibits CDC2 at the G2 to M transition. J Cell Biol 2002;156:249–259.
36. Kim JS, Park YY, Park SY, Cho H, Kang D, Cho H. The Auto-ubiquitylation of Chfr at G2 Phase Is Required for Accumulation of Plk1 and Mitotic Entry in Mammalian Cells. J Biol Chem 2011.
37. Mikhailov A, Shinohara M, Rieder CL. Topoisomerase II and histone deacetylase inhibitors delay the G2/M transition by triggering the p38 MAPK checkpoint pathway. J Cell Biol 2004;166:517–26.
38. Musacchio A, Salmon ED. The spindle-assembly checkpoint in space and time. Nat Rev Mol Cell Biol 2007;8:379-93.
39. Pines J, Rieder CL. Re-staging mitosis: a contemporary view of mitotic progression. Nat Cell Biol 2001;3:3-6.
40. Perez de Castro I, de Carcer G, Malumbres M. A census of mitotic cancer genes: new insights into tumor cell biology and cancer therapy. Carcinogenesis 2007;28:899-912.

41. Ditchfield C, Johnson VL, Tighe A, Ellston R, Haworths C, Johnson T, Mortlock A, Keen A, Keen N, Taylor SS. Aurora B couples chromosome alignment with anaphase by targeting BubR1, Mad2, and Cenp-E to kinetochores. J Cell Biol 2003;161:267-80.
42. Kops GJ, Weaver BA, Cleveland DW. On the road to cancer: aneuploidy and the mitotic checkpoint. Nat Rev Cancer 2005;5:773-85.
43. Howell BJ, McEwen BF, Canman JC, Hoffman DB, Farrar EM, RIeder CL, Salmon ED. Cytoplasmic dynein/ dynactin drives kinetochore protein transport to the spindle poles and has a role in mitotic spindle checkpoint inactivation. J Cell Biol 2001;155:1159–72.
44. Mao Y, Desai A, Cleveland DW. Microtubule capture by CENP-E silences BubR1-dependent mitotic checkpoint signaling. J Cell Biol 2005; 170:873–80.
45. Xia G, Luo X, Habu T, Rizo J, Matsumoto T, Yu H. Conformation-specific binding of p31(comet) antagonizes the function of Mad2 in the spindle checkpoint. EMBO J 2004;23:3133–43.
46. Palframan WJ, Meehl JB, Jaspersen SL, Winey M, Murray AW. Anaphase inactivation of the spindle checkpoint. Science 2006;313:680–684.
47. Peddibhotla S, Rosen J. Checking and executing cell division to prevent genomic instability. Cell Cycle 2009;8:15:2339-42.
48. Luger K, Richmond TJ. The histone tails of the nucleosome. Curr Opin Genet Dev 1998;8:140-6.
49. Xu D, Bai J, Duan Q, Costa M, Dai W. Covalent modifications of histones during mitosis and meiosis. Cell Cycle 2009;8:3688-94.
50. Eissenberg JC, Elgin SC. The HP1 protein family: getting a grip on chromatin. Curr Opin Genet Dev 2000;10:204-10.
51. Fischle W, Tseng BS, Dormann HL, Ueberheide BM, Garcia BA, Shabanowitz J, Hunt DF, Funabiki H, Allis CD. Regulation of HP1- chromatin binding by histone H3 methylation and phosphorylation. Nature 2005;438:1116-22.
52. Joo HY, Zhai L, Yang C, Shuyi Nie, Erdjument-Bromage H, Tempst P, Chang C, Wang H. Regulation of cell cycle progression and gene expression by H2A deubiquitination. Nature 2007;449:1068-72.