ZOO-302CR:(1.4)CELL DIVISION AND CELL CYCLE

Cell Division, Mitosis, and Meiosis

https://bio100.class.uic.edu/lecturesf04am/lect16.htm

Cell Division Functions in Reproduction, Growth, and Repair

Cell division involves the distribution of identical genetic material, DNA, to two daughters cells. What is most remarkable is the fidelity with which the DNA is passed along, without dilution or error, from one generation to the next.

Core Concepts:

- All Organisms Consist of Cells and Arise from Preexisting Cells
  - Mitosis is the process by which new cells are generated.
  - Meiosis is the process by which gametes are generated for reproduction.
- The Cell Cycle Represents All Phases in the Life of a Cell
  - DNA replication (S phase) must precede mitosis, so that all daughter cells receive the same complement of chromosomes as the parent cell.
  - The gap phases separate mitosis from S phase. This is the time when molecular signals mediate the switch in cellular activity.
  - Mitosis involves the separation of copied chromosomes into separate cells
- Unregulated Cell Division Can Lead to Cancer
  - Cell-cycle checkpoints normally ensure that DNA replication and mitosis occur only when conditions are favorable and the process is working correctly.
  - Mutations in genes that encode cell-cycle proteins can lead to unregulated growth, resulting in tumor formation and ultimately invasion of cancerous cells to other organs.

In order to better understand the concept of cell division and genetics, some basic definitions are in order:

- **gene** - basic unit of heredity; codes for a specific trait
- **locus** - the specific location of a gene on a chromosome (locus - plural loci)
- **genome** - the total hereditary endowment of DNA of a cell or organism
- **somatic cell** - all body cells except reproductive cells
- **gamete** - reproductive cells (i.e. sperm & eggs)
- **chromosome** - elongate cellular structure composed of DNA and protein - they are the vehicles which carry DNA in cells
- **diploid (2n)** - cellular condition where each chromosome type is represented by two homologous chromosomes
- **haploid (n)** - cellular condition where each chromosome type is represented by only one chromosome
- **homologous chromosome** - chromosome of the same size and shape which carry the same type of genes
- **chromatid** - one of two duplicated chromosomes connected at the centromere
- **centromere** - region of chromosome where microtubules attach during mitosis and meiosis

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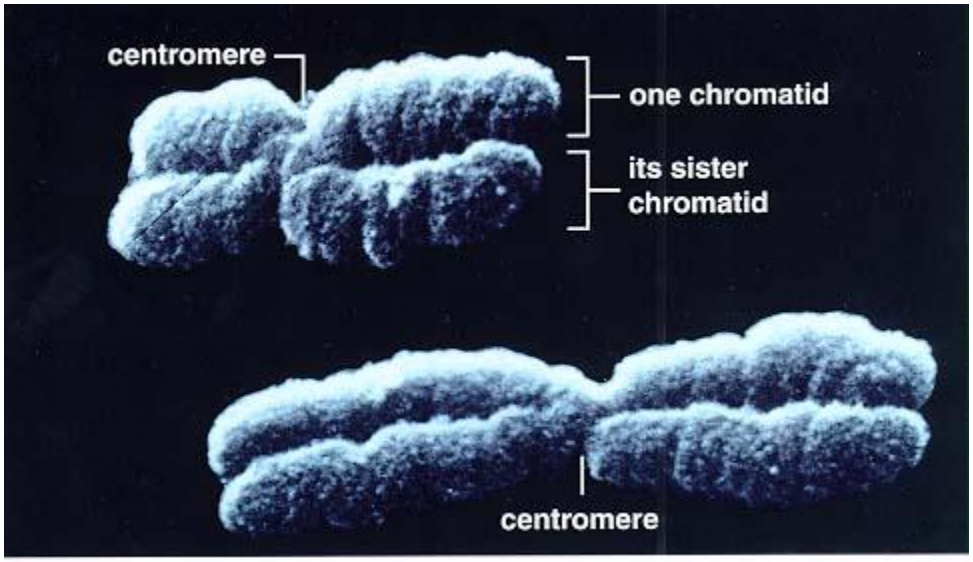
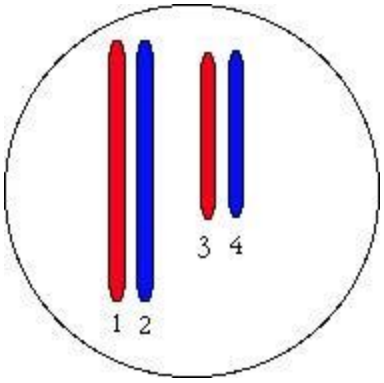
Chromosome structure



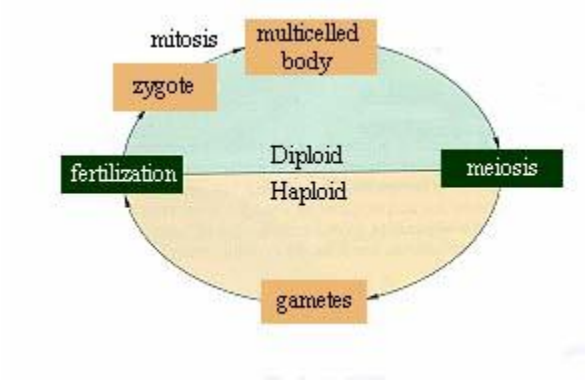
- composed of DNA and protein (histones) all tightly wrapped up in one package
- duplicated chromosomes are connected by a centromere



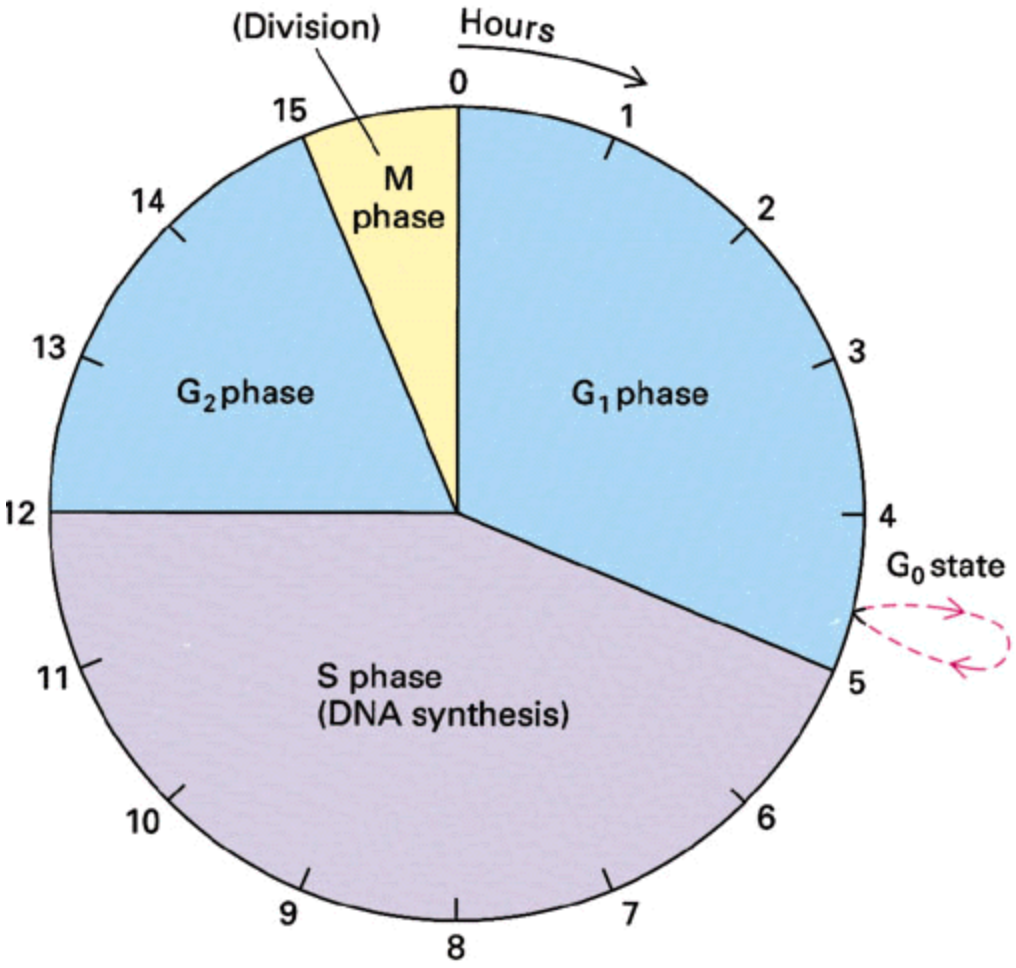
Example - an organism is 2n = 4.

- Chromosomes 1 & 2 are homologous chromosomes
- Chromosomes 3 & 4 are homologous chromosomes
- Chromosomes 1 & 3 came from the mother
- Chromosomes 2 & 4 came from the father

Typical Animal Life Cycle



The Cell Cycle



G1 - first gap

S - DNA synthesis (replication)

G2 - second gap

M - mitosis

- **mitosis** - nuclear/chemical events resulting in two daughter nuclei which have identical genetic material to each other and to the mother cell
- **cytokinesis** - division of the cytoplasm. This usually occurs with mitosis, but in some organisms this is not so

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Mitosis in a Nutshell

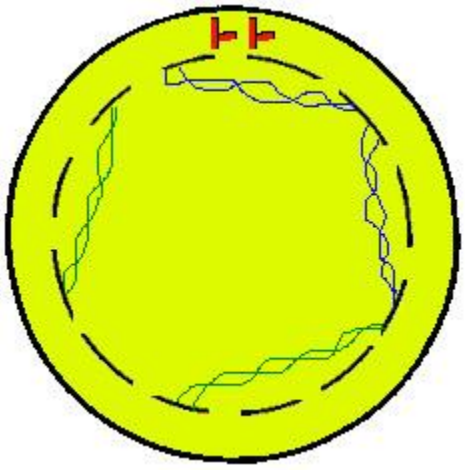
- The stages of the cell cycle can be broken down into six stages:
    - Interphase, Prophase, Metaphase, Anaphase, Telophase

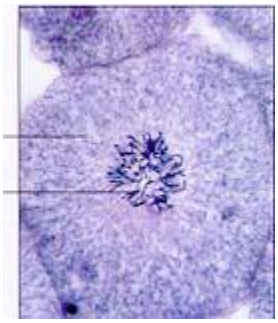
Interphase

- is the "resting" or non-mitotic portion of the cell cycle.
- It is comprised of G1, S, and G2 stages of the cell cycle.

- DNA is replicated during the S phase of Interphase

Prophase - the first stage of mitosis.





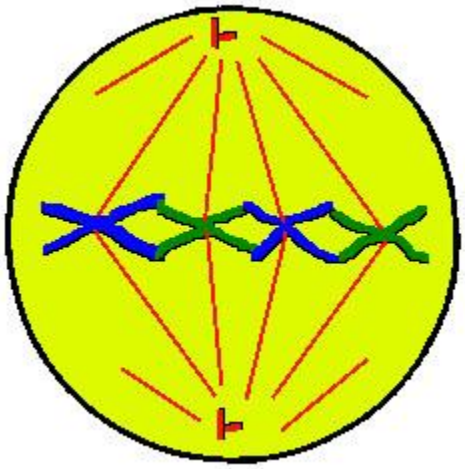
- The chromosomes condense and become visible
- The centrioles form and move toward opposite ends of the cell ("the poles")
- The nuclear membrane dissolves
- The mitotic spindle forms (from the centrioles in animal cells)
- Spindle fibers from each centriole attach to each sister chromatid at the kinetochore

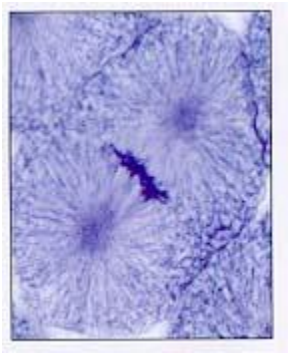
Compare Prophase to the Prophase I and to the Prophase II stages of mitosis.

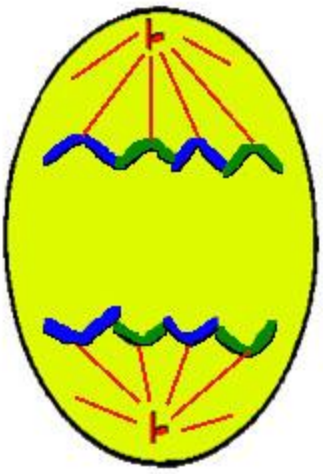
Metaphase

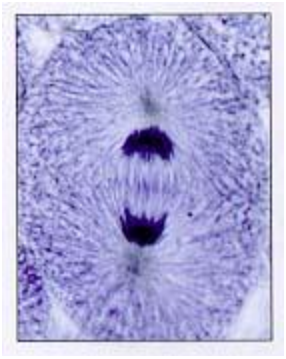
- The Centrioles complete their migration to the poles
- The chromosomes line up in the middle of the cell ("the equator")

Compare Metaphase to the Metaphase I and to the Metaphase II stages of mitosis.









Anaphase

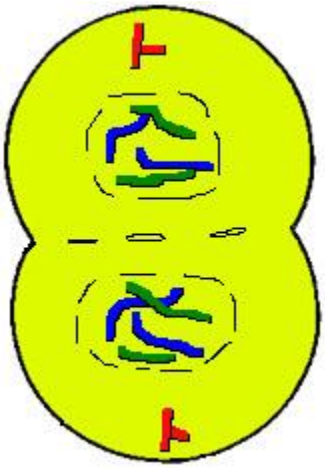
- Spindles attached to kinetochores begin to shorten.
- This exerts a force on the sister chromatids that pulls them apart.
- Spindle fibers continue to shorten, pulling chromatids to opposite poles.
- This ensures that each daughter cell gets identical sets of chromosomes

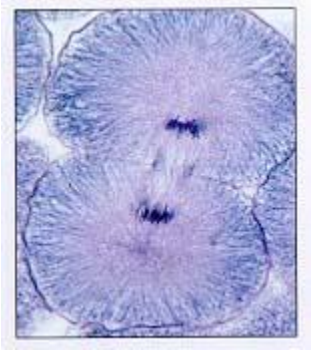
Compare Anaphase to the Anaphase I and to the Anaphase II stages of mitosis.

Telophase

- The chromosomes decondense
- The nuclear envelope forms
- Cytokinesis reaches completion, creating two daughter cells

Compare Telophase to the Telophase I and to the Telophase II stages of mitosis.





Cytokinesis Divides the Cytoplasm

In animal cells, cytokinesis occurs by a process known as **cleavage**

- First, a **cleavage furrow** appears
  - cleavage furrow = shallow groove near the location of the old metaphase plate
- A contractile ring of actin microfilaments in association with myosin, a protein
  - Actin and myosin are also involved in muscle contraction and other movement functions

- The contraction of a the dividing cell's ring of microfilaments is like the pulling of drawstrings
    - The cell is pinched in two

- Cytokinesis in plant cells is different because plant cells have cell walls.
- There is no cleavage furrow
- During telophase, vesicles from the Golgi apparatus move along microtubules to the middle of the cell (where the cell plate was) and coalesce, producing the **cell plate**
    - Cell-wall construction materials are carried in the vesicles and are continually deposited until a complete cell wall forms between the two daughter cells

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Chromosome Separation Is the Key Event of Mitosis

- Mitotic spindle fibers are the railroad tracks for chromosome movement.
    - Spindle fibers are made of microtubules.
    - Microtubules are lengthened and shortened by the addition and loss of tubulin subunits.
    - Mitotic spindle shortening during anaphase is a result of the loss of tubulin subunits.
- A kinetochore motor is the engine that drives chromosome movement.
    - Multiple studies have shown that the kinetochore contains motor proteins that can ⁄walk⁄ along the spindle fiber during anaphase.
    - These proteins presumably remove tubulin subunits, shortening spindle fibers and facilitating the chromosome movement.

Regulation of the Cell Cycle

The cell cycle is controlled by a cyclically operating set of reaction sequences that both trigger and coordinate key events in the cell cycle

- The cell-cycle control system is driven by a built-in clock that can be adjusted by external stimuli (chemical messages)
- **Checkpoint** - a critical control point in the cell cycle where stop and go-ahead signals can regulate the cell cycle
    - Animal cells have built-in stop signals that halt the cell cycles and checkpoints until overridden by go-ahead signals.

    - Three Major checkpoints are found in the G1, G2, and M phases of the cell cycle
- The G1 checkpoint - the Restriction Point
    - The G1 checkpoint ensures that the cell is large enough to divide, and that enough nutrients are available to support the resulting daughter cells.
    - If a cell receives a go-ahead signal at the G1 checkpoint, it will usually continue with the cell cycle
    - If the cell does not receive the go-ahead signal, it will exit the cell cycle and switch to a non-dividing state called G0
    - Actually, most cells in the human body are in the G0 phase
- The G2 checkpoint ensures that DNA replication in S phase has been completed successfully.
- The metaphase checkpoint ensures that all of the chromosomes are attached to the mitotic spindle by a kinetochore.

Cyclins and Cyclin-Dependent Kinases - The Cell-Cycle Clock

Rhythmic fluctuations in the abundance and activity of cell-cycle control molecules pace the events of the cell cycle.

- **Kinase** - a protein which activates or deactivates another protein by phosphorylating them.
- Kinases give the go-ahead signals at the G1 and G2 checkpoints
- The kinases that drive these checkpoints must themselves be activated
    - The activating molecule is a **cyclin,** a protein that derives its name from its cyclically fluctuating concentration in the cell
    - Because of this requirement, these kinases are called **cyclin-dependent kinases**, or **Cdk's**

MPF - Maturation Promoting Factor (M-phase promoting factor)

- Cyclins accumulate during the G1, S, and G2 phases of the cell cycle
- By the G2 checkpoint (the red bar in the figure), enough cyclin is available to form MPF complexes (aggregations of Cdk and cyclin) which initiate mitosis
    - MPF apparently functions by phosphorylating key proteins in the mitotic sequence
- Later in mitosis, MPF switches itself off by initiating a process which leads to the destruction of cyclin
    - Cdk, the non-cyclin part of MPF, persists in the cell as an inactive form until it associates with new cyclin molecules synthesized during interphase of the next round of the cell cycle

PDGF - Platelet-Derived Growth Factors - An Example of an External Signal for Cell Division

PDGF is required for the division of fibroblasts which are essential in wound healing

- When injury occurs, platelets (blood cells important in blood clotting) release PDGF
- Fibroblasts are a connective tissue cells which possess PDGF receptors on their plasma membranes
- The binding of PDGF activates a signal-transduction pathway that leads to a proliferation of fibroblasts and a healing of the wound

Density Dependent Inhibition

- Cells grown in culture will rapidly divide until a single layer of cells is spread over the area of the petri dish, after which they will stop dividing
- If cells are removed, those bordering the open space will begin dividing again and continue to do so until the gap is filled - this is known as **contact inhibition**
- Apparently, when a cell population reaches a certain density, the amount of required growth factors and nutrients available to each cell becomes insufficient to allow continued cell growth

Anchorage Dependence

- For most animal cells to divide, they must be attached to a substratum, such as the extracellular matrix of a tissue or the inside of the culture jar
- Anchorage is signaled to the cell-cycle control system via pathways involving membrane proteins and the cytoskeleton

Cells Which No Longer Respond to Cell-Cycle Controls - Cancer Cells

- Cancer cells do not respond normally to the body's control mechanism.
    - They divide excessively and invade other tissues
    - If left unchecked, they can kill the organism
- Cancer cells do not exhibit contact inhibition
    - If cultured, they continue to grow on top of each other when the total area of the petri dish has been covered
    - They may produce required external growth factor (or override factors) themselves or possess abnormal signal transduction sequences which falsely convey growth signals thereby bypassing normal growth checks
- Cancer cells exhibit irregular growth sequences

- If growth of cancer cells does cease, it does so at random points of the cell cycle
- Cancer cells can go on dividing indefinitely if they are given a continual supply of nutrients
    - Normal mammalian cells growing in culture only divide 20-50 times before they stop dividing

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Meiosis

More definitions:

- **Allele** - alternate forms of the same gene
- **Homozygous** - having two identical alleles for a given gene
- **Heterozygous** - having two different alleles for a given gene
- **Genotype** - genetic makeup of an organism
- **Phenotype** - the expressed traits of an organism

Meiosis in a Nutshell

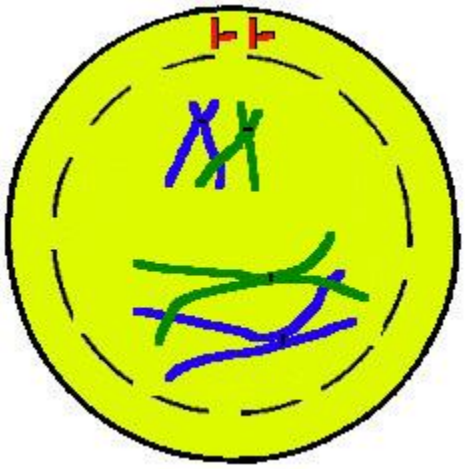
- Meiosis Is a Special Type of Cell Division That Occurs in Sexually Reproducing Organisms
    - Meiosis reduces the chromosome number by half, enabling sexual recombination to occur.
        - Meiosis of diploid cells produces haploid daughter cells, which may function as gametes.
        - Gametes undergo fertilization, restoring the diploid number of chromosomes in the zygote
    - Meiosis and fertilization introduce genetic variation in three ways:
        - Crossing over between homologous chromosomes at prophase I.
        - Independent assortment of homologous pairs at metaphase I:
            - Each homologous pair can orient in either of two ways at the plane of cell division.
            - The total number of possible outcomes = 2n (n = number of haploid chromosomes).
        - Random chance fertilization between any one female gamete with any other male gamete.
- The Role of Sexual Reproduction in Evolution

- Sexual reproduction in a population should decline in frequency relative to asexual reproduction.
  - Asexual reproduction➔No males are needed, all individuals can produce offspring.
  - Sexual reproduction➔Only females can produce offspring, therefore fewer are produced.
- Sexual reproduction may exist because it provides genetic variability that reduces susceptibility of a population to pathogen attack.

The stages of meiosis can be broken down into two main stages, **Meiosis I** and **Meiosis II**

- **Meiosis I** can be broken down into four substages: Prophase I, Metaphase I, Anaphase I and Telophase I
- **Meiosis II** can be broken down into four substages: Prophase II, Metaphase II, Anaphase II and Telophase II

Meiosis I

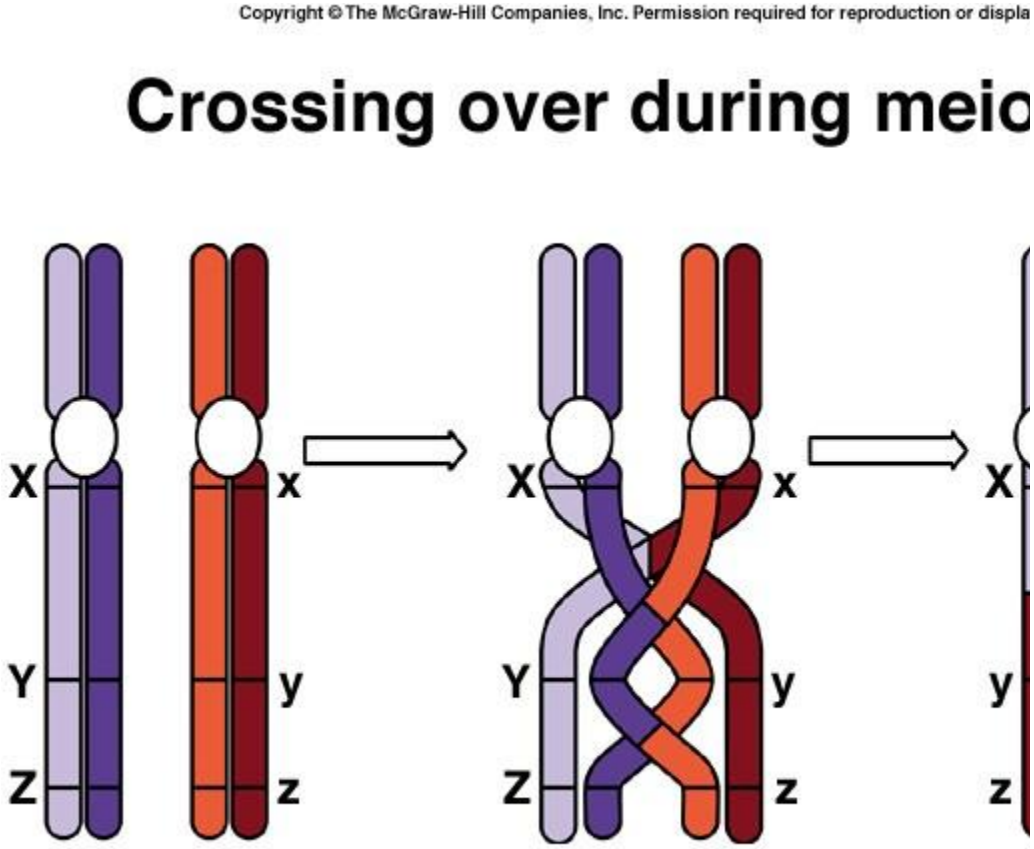


Prophase I - most of the significant processes of Meiosis occur during Prophase I

- The chromosomes condense and become visible
- The centrioles form and move toward the poles
- The nuclear membrane begins to dissolve
- The homologs pair up, forming a tetrad
  - Each tetrad is comprised of four chromotids - the two homologs, each with their sister chromatid
- Homologous chromosomes will swap genetic material in a process known as **crossing over** (abbreviated as XO)
  - Crossing over serves to **increase genetic diversity** by creating four unique chromatids

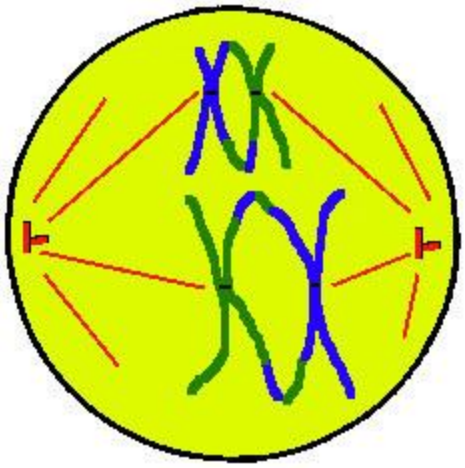
Compare Prophase I to Prophase II and to the Prophase stage of mitosis.

Crossing Over



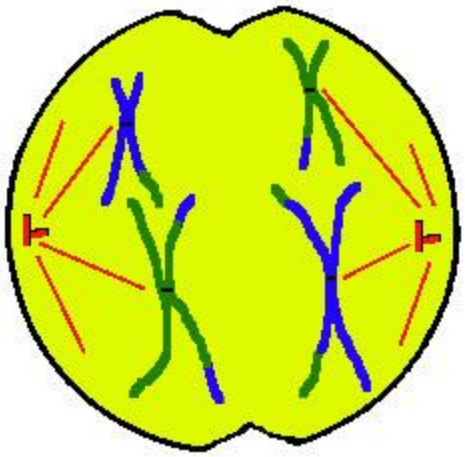
- Genetic material from the **homologous chromosomes** is randomly swapped
- This creates four unique chromatids
- Since each chromatid is unique, the overall genetic diversity of the gametes is greatly increased

Metaphase I



- Microtubules grow from the centrioles and attach to the centromeres
- The tetrads line up along the cell equator

Compare Metaphase I to Metaphase II and to the Metaphase stage of mitosis.

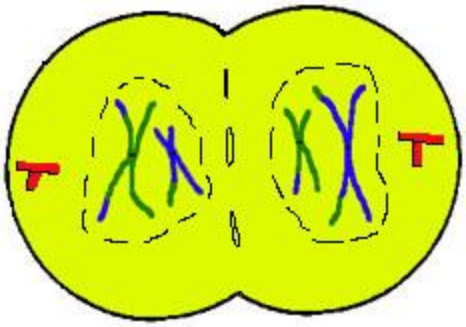


Anaphase I

- The centromeres break and **homologous chromosomes** separate (note that the **sister chromatids** are still attached)
- Cytokinesis begins

Compare Anaphase I to Anaphase II and to the Anaphase stage of mitosis.

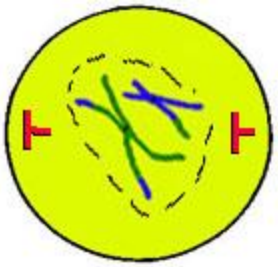
Telophase I

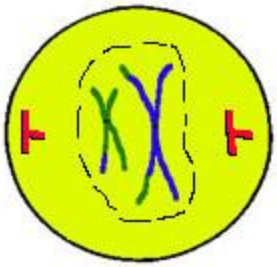


- The chromosomes may decondense (depends on species)
- Cytokinesis reaches completion, creating **two haploid daughter cells**

Compare Telophase I to Telophase II and to the Telophase stage of mitosis.

Meiosis II

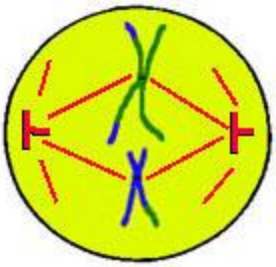


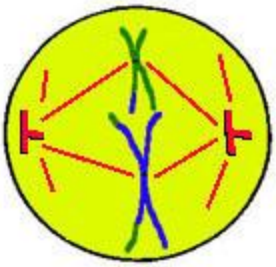


Prophase II

- Centrioles form and move toward the poles
- The nuclear membrane dissolves

Compare Prophase II to Prophase I and to the Prophase stage of mitosis.

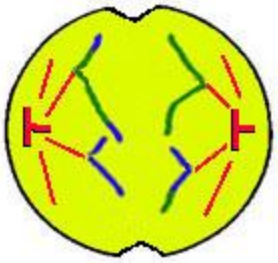


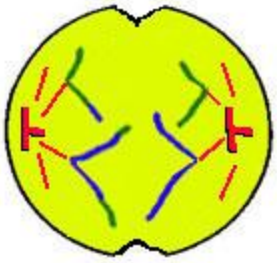


Metaphase II

- Microtubules grow from the centrioles and attach to the centromeres
- The sister chromatids line up along the cell equator

Compare Metaphase II to Metaphase I and to the Metaphase stage of mitosis.





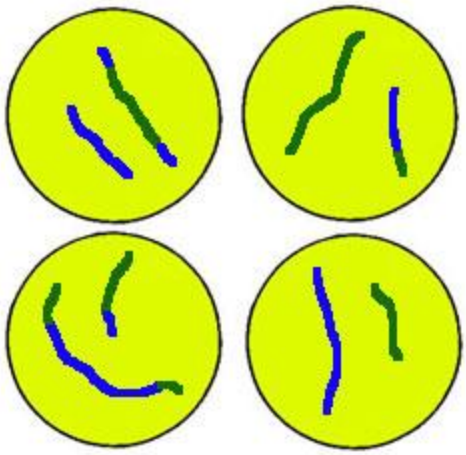
Anaphase II

- The centromeres break and **sister chromatids** separate
- Cytokinesis begins

Compare Anaphase II to Anaphase I and to the Anaphase stage of mitosis.

Telophase II

- The chromosomes may decondense (depends on species)
- Cytokinesis reaches completion, creating **four haploid daughter cells**



Compare Telophase II to Telophase I and to the Telophase stage of mitosis.

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The Mammalian Cell Cycle

An Overview

Jane V. Harper and Gavin Brooks

Summary

In recent years, we have witnessed major advances in our understanding of the mammalian cell cycle and how it is regulated. Normal mammalian cellular proliferation is tightly regulated at each phase of the cell cycle by the activation and deactivation of a series of proteins that constitute the cell cycle machinery. This review article describes the various phases of the mammalian cell cycle and focuses on the cell cycle regulatory molecules that act at each stage to ensure normal cellular progression.

Key Words

14-3-3; anaphase-promoting complex; CDC25; cyclins; cyclin-dependent kinases (Cdks); Cdk inhibitors; cytokinesis; DNA replication; E2F transcription factors; endoreduplication; MAP kinase; pocket proteins; SCF ubiquitin ligases.

1. Introduction

Cell division in mammalian cells is similar to that in other eukaryotes in that it represents an evolutionarily conserved process involving an ordered and tightly controlled series of molecular events. In mammals, the cell cycle consists of five distinct phases: three gap phases—G0, in which cells remain in a quiescent or resting state, and G1 and G2, during which RNA synthesis and protein synthesis occur; S-phase during which DNA is replicated; and M-phase, in which cells undergo mitosis and cytokinesis (**Fig. 1**). G0, G1, S, and G2 are referred to collectively as interphase (i.e., between mitoses). Some cells in the body remain quiescent for their whole lifetime and do not

undergo cell division; however, stimulation of the cell by external factors such as

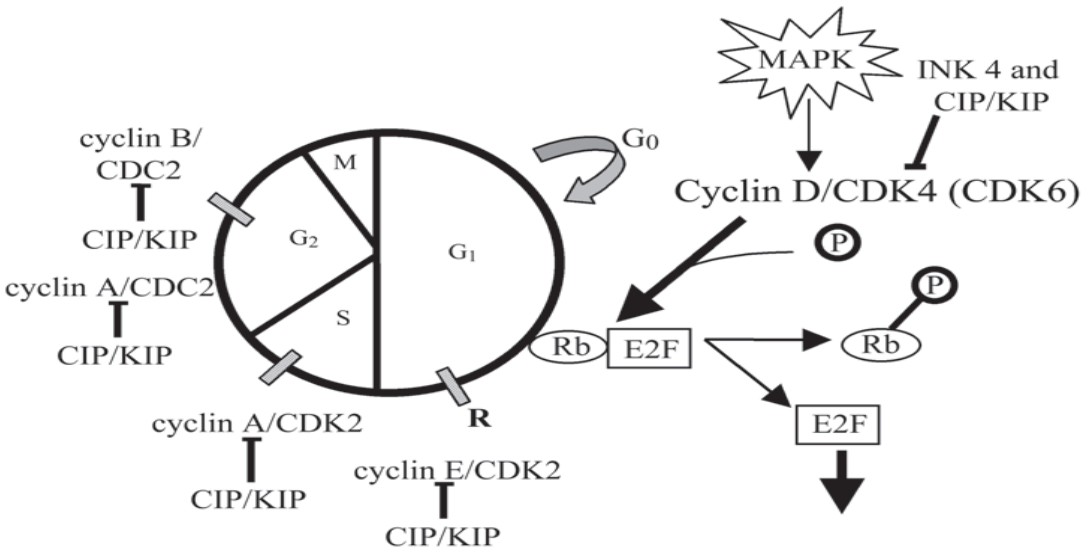

Fig. 1. The five distinct phases of the cell cycle are each controlled by specific cyclin/CDK complexes. The cyclin/CDK complexes in turn are negatively regulated by CIP/KIP and INK4 CDKI family members. E2F transcription factors function at the restriction point (R), leading to the activation of genes essential for DNA synthesis and cell cycle progression. E2F complexed with hypophosphorylated Rb cannot activate transcription. Hyperphosphorylation of Rb causes dissociation from E2F. Cell cycle checkpoints are shown as shaded bars. , inhibition step; _, activation step; Cdk, cyclin-dependent kinase; CIP, Cdk-interacting protein; INK4, inhibitior of Cdk4; KIP, kinase inhibitor protein; MAPK, mitogen-activated protein kinase.
mitogens causes these quiescent cells to reenter the cell cycle and undergo division. Binding of a growth factor molecule to its cell surface receptor can stimulate a number of signaling pathways, an example of which is the Ras-dependent mitogen-activated protein kinase (MAPK) pathway, which plays a major role in entry into G1, as discussed in more detail **Subheading 2.1.** Once cells enter G1, synthesis of the mRNAs and proteins necessary for DNA synthesis occurs, allowing cells to enter S-phase.
The mammalian cell cycle consists of a number of checkpoints that exist to ensure normal cell cycle progression. The primary checkpoint acts late in G1 and is known as the restriction (R) point (**Fig. 1**). Once cells have passed this point, they normally are committed to a round of cell division. Other checkpoints exist in S-phase to activate DNA repair mechanisms if necessary and at the G2/M transition to ensure that cells have fully replicated their DNA and that it is undamaged before they enter mitosis. Finally, there are checkpoint control mechanisms within mitosis to ensure that conditions remain suitable for the cell to complete cell division (cytokinesis).
The length of time for a mammalian cell to progress around the cell cycle and undergo division varies depending on the cell type but on average it takes approx 24 h.
Fig. 1. The five distinct phases of the cell cycle are each controlled by specific cyclin/CDK complexes. The cyclin/CDK complexes in turn are negatively regulated by CIP/KIP and INK4 CDKI family members. E2F transcription factors function at the restriction point (R), leading to the activation of genes essential for DNA synthesis and cell cycle progression. E2F complexed

with hypophosphorylated Rb cannot activate transcription. Hyperphosphorylation of Rb causes dissociation from E2F. Cell cycle checkpoints are shown as shaded bars. , inhibition step; _, activation step; Cdk, cyclin-dependent kinase; CIP, Cdk-interacting protein; INK4, inhibitior of Cdk4; KIP, kinase inhibitor protein; MAPK, mitogen-activated protein kinase.

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Cell cycle time varies in different cell types as a consequence of differences in the time spent between cytokinesis and the restriction point (i.e., G1). The time taken for a cell to pass from S-phase into M is extremely constant between cells and typically is in the region of approx 6 h for S-phase, 4 h for G2 and 1–2 h for mitosis and cytokinesis ***(1)***. Progression through each phase of the cell cycle is under the strict control of various cell cycle molecules, e.g., cyclins, cyclin-dependent kinases (Cdks), and Cdk inhibitors (CDKIs), which themselves are regulated by phosphorylation and dephosphorylation events ***(1)***. The Cdks play a crucial role in regulating cell cycle events once complexed with a cyclin regulatory subunit. Cyclin levels vary dramatically through the cell cycle as a consequence of changes in transcription and ubiquitinmediated degradation (for review *see* **refs. *2*** and ***3***). Cdk activity is negatively regulated by association with various CDKIs. Specific cyclin/Cdk complexes become activated and thereby modulate a distinct phase(s) of the cell cycle (**Fig. 1**). For example, cyclin D/Cdk4(Cdk6) complexes initiate progression through G1 by phosphorylating substrates, such as members of the retinoblastoma (Rb) family of pocket proteins (*see* **Subheading 3.1.**), that eventually lead to the activation of transcription of genes necessary for DNA synthesis and subsequent cell cycle progression; the cyclin E/Cdk2 complex is important in the G1/S transition, where levels peak at the restriction point ***(4**,**5)***; cyclin A/Cdk2 is important during S-phase progression; and cyclin A/CDC2 (also known as Cdk1) and cyclin B/CDC2 are important for progression through G2 and M. The regulation of cyclin synthesis and degradation, in addition to Cdk activity levels, are tightly controlled and is key to ordered progression through the mammalian cell cycle. The following sections will give an overview of the various stages of the mammalian cell cycle and the molecules that regulate progression through each stage of the cycle.

1.1. Cyclins and Cyclin-Dependent Kinases

As for other eukaryotic cells, the mammalian cell cycle is regulated by the sequential formation, activation, and inactivation of a series of cell cycle regulatory molecules that include the cyclins (regulatory subunits) and the Cdks (catalytic kinase subunits) (***2**,**3** ,**6***; *see* also Chap. 16, this volume). Different cyclins bind specifically to different Cdks to form distinct complexes at specific phases of the cell cycle and thereby drive the cell from one phase to another. The cyclins are a family of proteins, which, as their name suggests, are synthesized and destroyed during each cell cycle. To date, eight cyclins have been described that directly affect cell cycle progression: cyclins A1 and A2, B1, -2, and –3, C, D1, -2, and –3, E1 and -2, F, G1 and G2, and H, which all share an approx 150-amino acid region of homology called the *cyclin box* that binds to the N-terminal end of specific Cdks (review, *see* **ref. *2***). Most cyclin mRNAs and proteins show a dramatic fluctuation in their expression during the cell cycle. For example, the expressions of cyclins A and B accumulate transiently at the onset of S-phase and in late G2, respectively, followed rapidly by their degradation via the ubiquitin–proteosome pathway, whereas cyclin D1 levels rise in G1 and remain

elevated until mitosis ***(3,6)***. In contrast, expression of the various Cdk molecules remains relatively constant throughout the cell cycle.

Little information is currently available regarding the recently described cyclins F and G, whereas cyclin H has been shown to form complexes specifically with Cdk7 to produce an enzyme known as Cdk-activating kinase (CAK) that is involved in the activation of CDC2 and Cdk2 kinases by phosphorylating Thr160 and Thr161, respectively ***(7)***. Cyclin H/Cdk7 can also form a tertiary complex with the protein ménage-àtrois-1 (MAT-1), when it modulates gene transcription via RNA polymerase II activity ***(8)***. Another cyclin, cyclin T, has recently been reported in the literature, although it does not appear to be involved with cell cycle progression directly. Cyclin T pairs with Cdk9 and is involved in various cellular processes, including basal transcription, signal transduction, and differentiation (reviewed in **refs. *9*** and ***10***).

The Cdks are a family of serine/threonine protein kinases that bind to, and are activated by, specific cyclins. To date, at least nine Cdks have been described: CDC2 (Cdk1), Cdk2, Cdk3, Cdk4, Cdk5, Cdk6, Cdk7, Cdk8, and CDK9. Cdks 4, 5, and 6 complex mainly with the cyclin D family and function during the G0/G1-phases of the cycle; Cdk2 can also bind with members of the cyclin D family but more commonly associates with cyclins A and E and functions during the G1- phase and during the G1/S transition. As just mentioned, Cdk7 is found in association with cyclin H and is able to phosphorylate either CDC2, Cdk2, or the C-terminal domain of the largest subunit of RNA polymerase II, in addition to the TATA box binding protein or TFIIE ***(7)***. CDC2 is the mitotic Cdk and forms complexes with cyclins A and B that function in the G2- and M-phases of the cell cycle. Cdk8 pairs with cyclin C and is found in a large multiprotein complex with RNA polymerase II, where it is thought to control RNA polymerase II function (reviewed in **ref. *11***). Finally, Cdk9 is a serine/threonine kinase related to CDC2 that pairs with T-type cyclins. The activity of the cyclin T/Cdk9 complex is not cell cycle regulated but is involved in many processes such as differentiation and basal transcription (reviewed in **refs. *9***, ***10***, and ***12***).

As stated above, specific Cdks bind to specific cyclins to form an active complex that integrates signals from extracellular molecules and controls progression through the cell cycle. The Cdk subunit on its own has no detectable kinase activity and requires sequential activation by cyclin binding and subsequent phosphorylation by CAK and dephosphorylation by CDC25 protein phosphatase (*see* **Subheading 6.1.**). This activation process occurs in a two-step manner, as follows:

1. Binding of the cyclin to the Cdk confers partial activity to the kinase. Cyclin binding causes a conformational change in the Cdk molecule, thereby bringing together specific residues involved in orienting ATP phosphate atoms ready for catalysis within the catalytic cleft. These conformational changes also set the stage for subsequent phosphorylation and full activation.
2. Phosphorylation of the cyclin/Cdk complex is performed by CAK which increases Cdk activity approximately 100-fold (13). Phosphorylation occurs on a conserved threonine residue within the T-loop region of the Cdk (Thr160 in CDC2 and Thr161 in other Cdks). Cyclin binding moves the T-loop to expose the phosphorylation site, allowing full activation of the Cdk.

Once activated, the various cyclin/Cdk complexes phosphorylate a number of specific

substrates involved in cell cycle progression. Such substrates include the Rb pocket proteins, lamins, and histones. Evidence exists to suggest that cyclins may be involved in determining the substrate specificity of Cdks (reviewed in ref. 14). For example, cyclin A/Cdk2 and cyclin A/CDC2, but not cyclin B/CDC2, can phosphorylate p107, showing regulation of substrate specificity between kinases complexed with cyclins A and B (15). The E2F-1/DP-1 heterodimer is not a substrate for the active cyclin D-dependent kinases but is efficiently phosphorylated by cyclin B-dependent kinases (16). Interestingly, whereas phosphorylation of E2F-1/DP-1 by cyclin B-dependent kinases does not result in a loss of DNA binding activity, phosphorylation of this same heterodimer by cyclin A-dependent kinases does lead to loss of DNA binding (16). Thus, different Cdk complexes can exert contrasting effects on a common substrate depending on the complexed cyclin. The regulation of Cdks themselves by other molecules can also differ depending upon the bound cyclin. Thus, cyclin A/CDC2 complexes do not require activation by CDC25 phosphatase, whereas cyclin B/CDC2 complexes do ***(17)***.

1.2. Cyclin-Dependent Kinase Inhibitors

The cyclins and Cdks often are referred to as positive regulators of the eukaryotic cell cycle. A family of negative regulators also exists, the CDKIs ***(2,18–20)***. The CDKIs comprise two structurally distinct families, the INK4 (inhibitor of Cdk4) and CIP (Cdk-interacting protein)/KIP (kinase inhibitor protein) families (reviewed in **ref. *21***). The INK4 family includes p14, p15 (INK4B), p16 (INK4A), p18 (INK4C), and p19 (INK4D), which specifically inhibit G1 cyclin/Cdk complexes (cyclin D/CDK4 and cyclin D/CDK6) and are involved in G1-phase control. The CIP/KIP family includes p21 (CIP1/WAF1/SDI1), p27 (KIP1), and p57 (KIP2), which are 38–44% identical in the first 70-amino acid region of their amino termini—a region that is involved in cyclin binding and kinase inhibitory function ***(19,20 ,22)***. The CIP/KIP family displays a broader specificity than the INK4 family, since members interact with, and inhibit the kinase activities of, cyclin E/Cdk2, cyclin D/Cdk4, cyclin D/Cdk6, cyclin A/Cdk2, and cyclin B/CDC2 complexes and also function throughout the cell cycle ***(19)***. Members of the two CDKI families inhibit Cdk activity by distinct mechanisms. Thus, CIP/KIP family members bind to, and inhibit the activity of, the entire cyclin/Cdk complex ***(23)***, whereas INK4 family members block cyclin binding indirectly by causing allosteric changes in the Cdk and hence altering the cyclin binding site; they also act by distorting the ATP binding site that leads to reduced affinity for ATP ***(24)***. In the case of p21, this CDKI has been shown to exist in both active and inactive cyclin/Cdk complexes, and it has been suggested that the stoichiometry of p21 binding to the cyclin/Cdk complex controls activation/inhibition of the complex ***(25)***. In support of this hypothesis, Zhang and colleagues ***(25)*** demonstrated that p21 exists both in catalytically active and inactive cyclin/Cdk complexes and that the addition of subsaturating concentrations of p21 to cyclin A/CDK2 complexes resulted in a progressive increase in Cdk2 activity, suggesting that low concentrations of p21 might function as a cyclin/Cdk assembly factor, whereas the binding of more than one p21 molecule is required to inhibit Cdk2 activity.

The tumour suppressor protein p53 also plays an important role in cell cycle arrest at the G1 and G2 checkpoints subsequent to inducing apoptosis ***(26–28)***. The p53 protein

has a central sequence-specific DNA binding domain and a transcriptional activation domain at its amino-terminus; in response to DNA damage, it can induce the transcription of the CDKI p21, which inhibits the activation of various G1 cyclin/Cdk complexes ***(22**,**27)***.

Antiproliferative signals such as contact inhibition, senescence ***(29)***, extracellular antimitogenic factors ***(30)***, and cell cycle checkpoints, such as p53 ***(31)***, induce expression of p27, p16, p15, and p21, respectively. The role of cell cycle molecules in regulating proliferation is highlighted by the fact that a number of these molecules are found to be mutated or deregulated in numerous tumors. Indeed, it has been suggested that most human tumours result from a mutation or deletion in one or more cell cycle regulators, especially those that control G1/S progression. For example, *p16* is mutated in approximately one-third of all human cancers ***(24**,**32**,**33)***, and *p53* is the most frequently mutated gene identified in human tumors ***(34)***. Also, many types of tumors show low expression levels of *p27*, which is associated with a poor prognosis ***(35)***, and cyclin D1 ***(23**,**36)*** and B1 ***(37)*** are often found at increased levels in breast cancer. Furthermore, there is a direct correlation between inactivation of p53 function and cyclin B1 overexpression in many tumors ***(37)***, although no direct interaction between these two molecules has been shown. However, as a direct consequence of this correlation, it has been proposed that cyclin B1 could be used as a tumor antigen and a cancer vaccine in some instances ***(38)***. Cdks have also been found to be deregulated in some tumors; for example, Cdk4 is mutated in melanoma ***(39**,**40)***, and Cdk2 expression is increased in some breast cancer cells ***(41)***. Indeed, targeting Cdk2 expression with antisense oligonucleotides and RNA interference technologies reduces cellular proliferation in breast tumor cells ***(41)***.

2. The G0/G1 Transition

The mammalian cell cycle is influenced by external signals during the G0- and G1-phases. The mitogen-activated protein kinase (MAPK) cascade is one of the most ubiquitous signal transduction pathways; it regulates several biological processes including progression of the cell cycle. The MAPK cascade consists of three evolutionarily conserved protein kinases, i.e., MAPK kinase kinase (MAPKKK), MAPK kinase (MAPKK), and MAPK, which are activated sequentially in a Ras-dependent manner (reviewed in **ref. *42***).

The MAPK cascade influences cellular proliferation by targeting the cyclin D-dependent kinases ***(43–45)***. Evidence for this comes from the fact that cells that proliferate in the absence of mitogens, for example during embryogenesis, have very little cyclin D-dependent kinase activity ***(46)***.

2.1. Role of MAPK in G1 Cell Cycle Progression

The activation of cyclin D/Cdk4 and cyclin D/Cdk6 complexes is essential for passage through the G1-phase, and they exert their regulation on cell cycle progression by phosphorylating Rb pocket proteins. The Rb pocket protein family serves to repress the activity of the E2F transcription factors that themselves are essential for transcription of genes necessary for entry into S-phase (discussed in more detail in **Subheading 3.**). The Ras/MAPK pathway has been shown to control cyclin D gene expression directly. This is mediated primarily by MAPK, which controls activation of the activation protein-1 (AP-1) and ETS transcription factors that then transactivate the cyclin D promoter that contains specific binding sites for both AP-1 and ETS ***(43**,**47)***. Furthermore,

expression studies using direct inhibitors of cyclin D/Cdk4(Cdk6) complexes (e.g., p21) inhibits Ras-induced proliferation ***(48)***. These data demonstrate that MAPK directly regulates cyclin D expression and, consequently, Cdk4 and Cdk6 activities. The Ras/MAPK pathway also has been shown to regulate Cdks posttranscriptionally by affecting their assembly and catalytic activities. Although the primary role of p21 and p27 is to regulate the activity of Cdks negatively, they are also involved in the assembly of cyclin D/Cdk4(Cdk6) complexes during early G1 (***49***,***50***; *see* **Subheading 1.2.**). The Ras/MAPK pathway has been shown to regulate directly the synthesis of the CIP/KIP family of inhibitors, and it has been demonstrated that growth factor stimulation of quiescent cells causes cell cycle reentry and transient expression of p21 that was dependent on MAPK activity ***(51)***.

Entry into S-phase is partly dependent on proteolytic degradation of p27, and this, in turn, has been shown to be dependent on MAPK activity ***(52***,***53)***. These investigators also observed that expression of Ras resulted in decreased p27 protein levels and an increase in E2F-dependent transcriptional activity ***(53)***.

Taken together, these data provide evidence for a role for the Ras/MAPK pathway in controlling G1/S progression in mammalian cells by a number of mechanisms, including: (1) induction of cyclin D expression and subsequent release of E2F transcription factors following phosphorylation of Rb pocket proteins by cyclin D-dependent kinases; (2) assembly of cyclin A/Cdk2 and cyclin E/Cdk2 complexes by increasing levels of the CDKIs involved in cyclin/Cdk assembly; and (3) decreasing p27 levels.

More recently, a role for MAPK in regulating the G2/M transition has been suggested. Thus, it has been shown that ionizing radiation can activate the MAPK pathway ***(54***,***55)*** and cells expressing a dominant-negative MAPKK are unable to recover from radiation-induced G2/M arrest ***(56)***. Additionally, treatment of cells with a MAPK inhibitor induces G2/M arrest concomitant with a reduction in cyclin B/CDC2 activity ***(57)***. These data suggest that the Ras/MAPK pathway plays a regulatory role at many points during the mammalian cell cycle.

The data discussed above demonstrate regulation of the cell cycle by the MAPK extracellular mitogenic signaling pathway. If the activity of the MAPK pathway were maintained at an abnormally high level, then this could lead to cellular transformation and tumorigenesis. Indeed, cells have developed a safety mechanism in order to counteract this possibility, as shown by the fact that expression of oncogenic Ras or constitutively active MAPKK causes cell cycle arrest with high levels of p21, which is expressed in a p53-dependent manner ***(58***,***59)***.

3. The G1/S Transition

One of the most extensively studied substrates of the cyclin/Cdks is the retinoblastoma (Rb) family of pocket proteins. The phosphorylation status of the Rb pocket proteins plays a major role in controlling E2F transcriptional activity and subsequent S-phase entry by modulating passage through the restriction (R) point in late G1 as discussed in the following sections.

3.1. The Retinoblastoma Pocket Protein Family

The Rb family of pocket proteins comprises a group of tumor suppressor proteins consisting of three members; pRb, p107, and p130. As their name suggests, these proteins contain a pocket region that binds cellular targets. This region also is capable of binding a number of viral oncoproteins such as the adenovirus E1A protein, SV40

large T antigen, and the human papillomavirus 16 E7 protein ***(60)***, demonstrating one mechanism by which tumor viruses can interfere with cell cycle progression in mammalian cells. In addition to phosphorylation events, the functions of different Rb family members are also regulated by changes in expression. During G1, the Rb pocket proteins are found in a hypophosphorylated state in which they bind to members of the E2F transcription factor family (*see* **Subheading 3.2.** below). As cells progress through the cell cycle, these proteins become hyperphosphorylated as a result of phosphorylation by cyclin D/Cdk4(Cdk6) and cyclin E/Cdk2 complexes. Each family member also displays differential expression throughout the cell cycle. Thus, pRb is expressed throughout the cell cycle but is hyperphosphorylated and therefore inactivated in late G1, although by mitosis it becomes dephosphorylated; p130 is highly expressed in G0, whereas levels diminish as cells progress into S-phase, consistent with a role for p130 in maintaining quiescence (reviewed in **refs.** ***61***); and p107 shows a reciprocal expression pattern to p130 such that low levels are found in G0, which then increase as cells progress through G1 into S.

The importance of the Rb family of tumor suppressor proteins in controlling the restriction point is demonstrated by the fact that they are targets of deregulation in most types of human cancer ***(23***,***28***,***62)***; indeed, pRb has been reported to be mutated in approx 30% of all human cancers (reviewed in **ref.** ***63***).

The different actions of Rb pocket proteins with respect to E2F regulation was demonstrated in a study by Hurford et al. ***(64)***. These authors showed that pRb has distinct functions from p107 and p130. They also demonstrated that p107 and p130 functions overlap, since, in cells lacking p107 or p130, there were no changes in E2Fregulated transcription. However, in cells lacking both p107 and p130, or lacking pRb alone, an increase in E2F-regulated transcription was observed ***(64)***.

3.2. The E2F Transcription Factors

Another family of molecules that regulates the G1/S transition is that comprising the E2F transcription factors. To date, seven E2F members have been described (E2Fs 1–7; ***65***), and these molecules exist as heterodimers paired with a DP subunit (**Fig. 2**). Two mammalian DP genes have so far been identified (DP-1 and -2) ***(66)***. E2F and DP proteins contain highly conserved DNA-binding and dimerization domains (**Fig. 2**). The E2F and DP proteins activate transcription in a synergistic manner, and DP proteins appear to act indirectly by enhancing the activity of E2F ***(67)***.

The Rb pocket proteins bind to, and sterically hinder transcriptional activity of, the E2F/DP complex, thereby enabling the E2F transcription factors to act as repressors of

Fig. 2. E2F and DP conserved domain structures. DB represents the DNA binding domain of the E2F and DP family members. E2F7-a and -b contain two domains with high homology to the DNA binding domains of the E2F proteins (DB1 and DB2). The retinoblastoma (Rb) binding domain is located in the transactivation domain of the E2F proteins. Cdk, cyclin-dependent kinase.

gene transcription (reviewed in **ref.** ***65***). Phosphorylation of the pocket protein component of the E2F/pocket protein complex by cyclin D/Cdk4(Cdk6) complexes in the G1-phase of the cycle leads to dissociation of the phosphorylated pocket protein and E2F, followed by E2F-mediated transactivation of promoters of genes necessary for S-phase progression, e.g., dihydrofolate reductase (*DHFR*), *cyclin E*, and *cyclin A* (**Fig.**

1; ***68–70***). With the exception of E2F-7, all members of the seven-member E2F family require heterodimerization with a DP subunit (DP-1 or DP-2) for full activity and share strong homology in their heterodimerization and DNA binding domains, their marked box, and, with the exception of E2F-6, a transactivation domain and a pocket protein binding region that resides within this sequence ***(65,71)***. E2F transcription factors are divided into three main groups: E2Fs 1–3, which play a role in progression from G1 into S-phase of the cell cycle and possess a pRb binding site within their transactivation domain; E2F-4 and -5, which bind to p107 or p130 members of the pocket protein family, and E2F-7—these three play a role in differentiation and proliferation; and E2F-6, which is unique since it lacks both the transactivation and the pocket protein binding domains. E2F-6 (also known as EMA) is thought to regulate cell cycle progression via its role as a transcriptional repressor ***(72–74)***. Although overexpression of E2F-6 did not block cycling NIH3T3 fibroblasts from entry into Sphase of the cycle, there was a significant decrease in S-phase entry when G0-arrested E2F-6 overexpressing cells were stimulated to reenter the cell cycle with serum ***(74)*** More recently, it was suggested that recruitment and interaction of the Polycomb repressor proteins (PcG) are instrumental in mediating the transcriptional repressor function of E2F-6 ***(75)***.

3.2.1. Activation of Transcription by E2F

The precise mechanism by which E2Fs activate transcription is unclear, although studies have shown that the transactivation domain of E2F-1 can interact with cyclic adenosine monophosphate (cAMP) response element binding protein (CBP) ***(76)***. CBP is a transcriptional co-activator and possesses intrinsic histone acetyl transferase (HAT) activity which can modulate chromatin structure and hence gene transcription ***(77)***. Acetylation of histones causes weakening of the interaction between DNA and the nucleosome, thereby making the DNA more accessible for transcription ***(76)***. E2F complexes have also been shown to bend DNA, and this could be important for activation in certain instances ***(78)***.

3.2.2. Subcellular Localization of E2F Transcription Factors

One level of regulation of E2F function occurs through changes in the subcellular localization of individual E2F transcription factors. For example, it has been demonstrated that E2F-4 is expressed in the nucleus and cytoplasm of quiescent cells, but as cells reach S-phase this molecule is found almost exclusively in the cytoplasm ***(79,80)***. This relocation ensures that repressive E2F-4/p107 complexes cannot bind E2F-responsive genes. E2F-4 lacks a nuclear localization sequence (NLS), and therefore it might gain entry to the nucleus by association with DPs or Rb pocket proteins, both of which contain an NLS. Indeed, studies have shown that when it is overexpressed in

cells, E2F-4 is only transported to the nucleus when coexpressed with DP-2, p107, or p130 ***(79,81)***. E2F-5 also lacks an NLS, although nuclear localization has been shown to occur in a DP- and Rb-independent manner such that transport of this E2F to the nucleus is mediated via formation of nuclear pore complexes ***(82)***.

3.2.3. Inactivation of E2F Transcription Factors

Inactivation of E2F is as important as E2F activation for continued progression through the cell cycle. It has been shown that expression of a constitutively active mutant of E2F-1 or DP-1 causes accumulation of cells in S-phase, which leads eventually

to apoptosis ***(83)***. These results imply that inactivation of E2F is required for exit from S-phase.

Inactivation of E2F may be mediated by phosphorylation of E2F and DP subunits, leading to an inhibition of DNA binding activity ***(83–85)***. E2Fs 1–3 have been shown to contain a conserved region that allows enables interaction with cyclin A/Cdk2 or cyclin E/Cdk2; these interactions lead to inhibitory phosphorylation on these transcription factors ***(84)***. There is also evidence for ubiquitin-directed degradation of E2Fs 1–4 ***(86,87)***, which would lead to regulation of DNA binding activity.

3.2.4. Mechanism of pRb-Dependent Repression of E2F Transcriptional Activity

The exact mechanism of pRb-mediated repression has only recently become understood following the discovery that histone deacetylase-1 (HDAC-1) is involved ***(88–90)***. Recruitment of HDAC-1 to the DNA is thought to repress gene activation by altering chromatin structure. Nucleosomal histones have a high proportion of positively charged amino acids that facilitate interaction with negatively charged DNA. Deacetylation is thought to occur on histone tails protruding from the nucleosome ***(91)***, and this increases their positive charge, causing a tighter interaction with DNA, thereby making the DNA less accessible for transcription. Takahashi et al. ***(92)*** observed that high levels of acetylation correlated with activation of E2F-responsive genes in late G1 and at the G1/S border. However, during quiescence, when transcriptional activity is low, histones showed reduced levels of acetylation ***(92)***. They also showed that acetylation of genes occurred in a cell cycle-dependent manner. Thus, during G0 when transcription levels are low, histones display reduced acetylation levels owing to the recruitment of HDAC-1. However, as cells progress through G1 into S-phase, this repression is relieved by HAT (**Fig. 3**). As mentioned earlier in **Subheading 3.2.1.**, it has been shown that E2F is able to interact with both CBP and HAT in vitro and also in transiently transfected cells ***(76)***.

The role of HDAC-1 in repressing gene transcription has been demonstrated further such that HDAC-1 physically interacts with the DHFR promoter to affect cell growth. Thus, an association of HDAC-1 with the DHFR promoter was detected in G0 and early G1, when the gene was silent and also histone H4 showed low acetylation levels. This association then decreased as cells entered S-phase, consistent with an increase in DHFR mRNA levels ***(93)***.

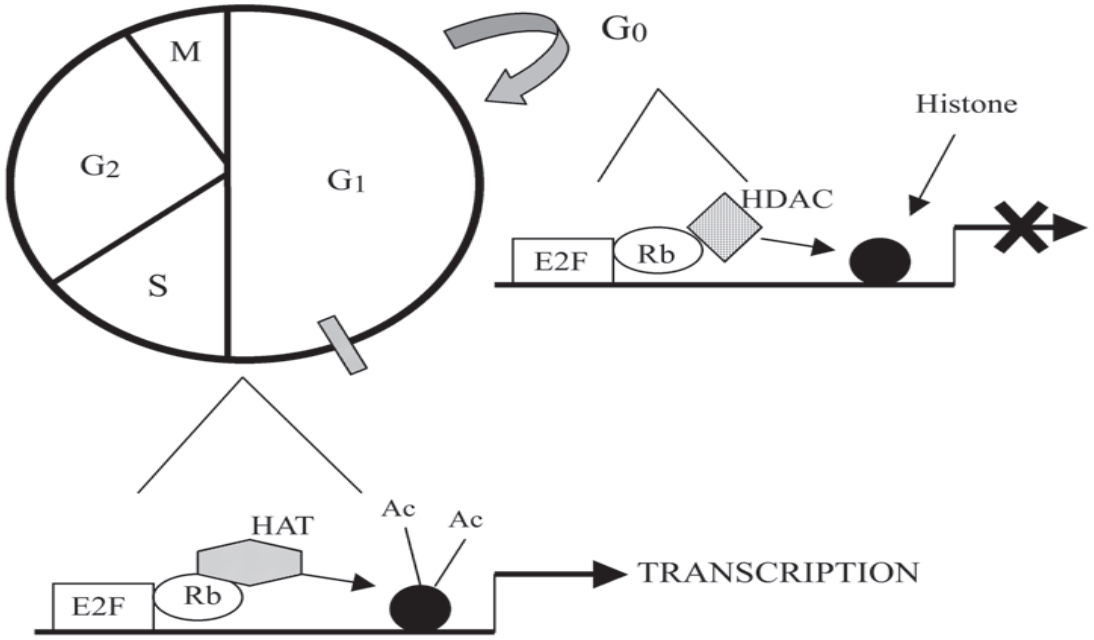

Fig. 3. Histone deacetylase-1 (HDAC-1) is recruited to DNA by retinoblastoma protein (Rb), causing deacetylation and inhibition of transcription during G0. At the G1/S transition, this repression is relieved by the action of histone acetyl transferase (HAT), allowing transcription
of genes necessary for DNA synthesis.

It has been suggested that chromatin-modifying factors may form multienzyme corepressor complexes at promoter regions. Thus, the modifying factor, mSin3, has been shown to form a corepressor complex that acts as a scaffold for the assembly of HDAC-1 repressor complexes ***(94–96)***. The occupancy of E2F-regulated promoters by HDAC-1 and mSin3B in pocket protein-deficient cells was recently assessed ***(97)***. These studies showed that recruitment of HDAC-1, but not mSin3B, was completely dependent on p107 and p130 but not on pRb. These data suggest that specific E2F/Rb complexes are involved in recruitment of chromatin-modifying factors during G0/G1. There is also evidence that the tumor suppressor gene transforming growth factor-□1 (*TGF*-□1), might function by recruitment of HDAC-1 since transgenic mice overexpressing *TGF*-□1 showed enhanced HDAC-1 binding to p130 compared with control animals ***(98)***. Thus, *TGF*-□1 may exert its growth-inhibitory effects by recruitment of HDAC-1.

The model of Rb pocket proteins causing transcriptional repression by association with HDAC-1 is consistent with the model hypothesizing that pRb phosphorylation by cyclin D/Cdk4(Cdk6) complexes relieves E2F transcriptional inhibition. Phosphory-

Fig. 3. Histone deacetylase-1 (HDAC-1) is recruited to DNA by retinoblastoma protein (Rb), causing deacetylation and inhibition of transcription during G0. At the G1/S transition, this repression is relieved by the action of histone acetyl transferase (HAT), allowing transcription
of genes necessary for DNA synthesis.

lation of pRb by Cdk4(Cdk6) initiates intramolecular interactions between the carboxy-terminus of pRB and the pocket region, which displaces HDAC-1 from the pocket, thereby facilitating subsequent phosphorylation of pRb by Cdk2 complexes followed by disassociation from E2F. These results suggest a sequential phosphorylation of pRb by Cdk4(Cdk6) and Cdk2 ***(99)***.

3.3. Role of the Cyclin E/Cdk2 Complex in the G1/S Transition

As cells approach the G1/S border, control of the cell cycle becomes dominated by cyclin E/Cdk2 complexes. It has been demonstrated that overexpression of cyclin E/Cdk2 promotes S-phase entry and that blocking the kinase activity of this complex inhibits progression into S-phase ***(100–102)***. Consistent with its role in S-phase, the cyclin E/Cdk2 complex has been shown to be required for the initiation of DNA replication ***(100–102)***. The importance of phosphorylation of pRb by cyclin E/Cdk2 at the G1/S border has already been discussed (*see* **Subheading 3.1.**).

A recently discovered substrate for cyclin E/Cdk2 has also been shown to be important for S-phase entry. This substrate is a nuclear protein that maps to the ATM locus (NPAT). NPAT was identified from a phage expression library using cyclin E/Cdk2 as a probe and was shown to associate with cyclin E/Cdk2 in vivo using immunoprecipitation studies. The NPAT protein was shown to be present at all stages of the cell cycle in synchronized cells; however, levels peaked at the G1/S boundary and decreased as cells progressed through S. Overexpression of NPAT caused an increase in the number of S-phase cells, suggesting that NPAT expression may be a rate-limiting step for S-phase entry ***(103)***.

Histone gene expression is a major event that occurs as cells pass into S-phase. Histones form part of the nucleosomes that are a fundamental subunit of chromatin, and NPAT has been implicated in the regulation of histone gene expression. Both cyclin E and NPAT have been shown to localize to histone gene clusters at the G1/S border, and phosphorylation of NPAT is required to activate histone gene expression ***(104,105)***. Therefore, evidence exists to show that cyclin E/Cdk2 regulates histone gene expression by phosphorylation of NPAT, a process required for entry into Sphase (*see* **Fig. 4**)

4. S-Phase

S-phase is the point during the cell cycle at which a cell duplicates its chromosomes in readiness for mitosis and cell division ***(1)***. A number of checkpoints exist to ensure that DNA is replicated only once per cycle, that it is fully and correctly replicated, and that replication occurs before cell division. Another important event during S-phase, other than DNA replication, is centrosome duplication. The centrosomes are the primary microtubule organizing center, and failure of cells to coordinate centrosome duplication with DNA replication leads to abnormal segregation of chromosomes, causing genomic instability that can lead to cancer.

4.1. Role of the Cyclin E/Cdk2 Complex in S-Phase Progression

There is much evidence to suggest that DNA synthesis in higher eukaryotes is initiated by activation of Cdk2 ***(23,101,106)***. Cdk2 associates with cyclin E just prior to

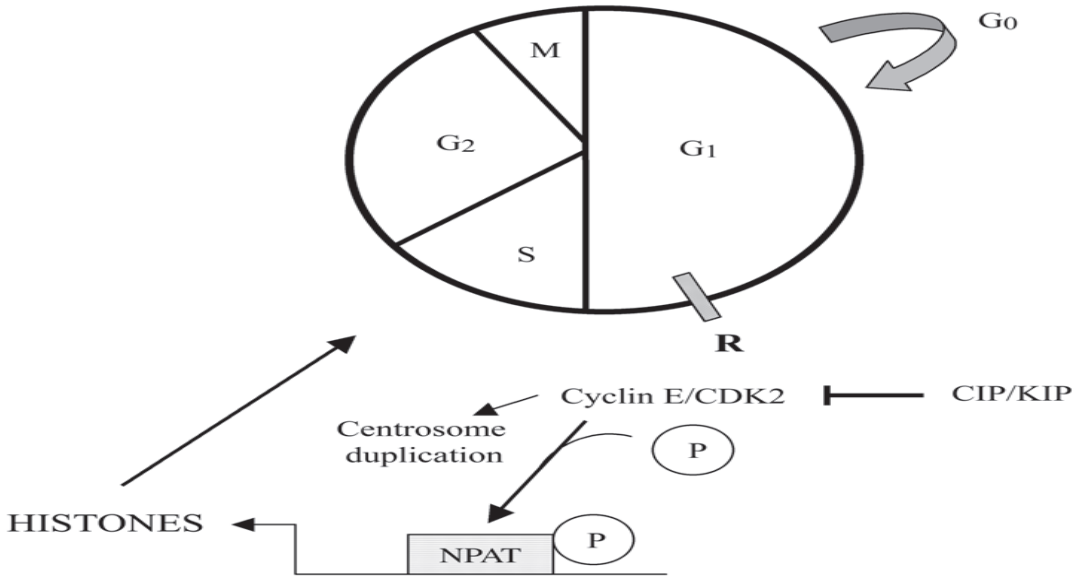

Fig. 4. Activation of NPAT by cyclin E/CDK2 causes histone gene expression necessary for DNA synthesis and S-phase progression. , inhibition step; _, activation step; Cdk, cyclindependent
kinase; CIP, Cdk-interacting protein; KIP, kinase-inhibitor protein.

the onset of S-phase, and the role of this complex in the activation of NPAT and histone gene expression has already been discussed above (*see* **Subheading 3.3.**). A role for cyclin E/Cdk2 in centrosome duplication has also been suggested ***(107)***. Tarapore and colleagues ***(108)*** developed a cell-free centriole duplication system and demonstrated that centrosome duplication was dependent on the presence of cyclin E/Cdk2 complexes. In addition, cyclin E/Cdk2 was shown to phosphorylate nucleophosmin in this model, causing dissociation from centrosomes and subsequent initiation of centrosome duplication ***(108)***.

4.2. Role of the Cyclin A/Cdk2 in S-Phase Progression

The onset of S-phase correlates with formation of cyclin A/Cdk2 complexes. Microinjection of antibodies against Cdk2 complexed with either cyclin A or cyclin E blocks the initiation of DNA synthesis in mammalian cells ***(109,110)***. Cyclin A might be rate-limiting for DNA replication since it can accelerate entry into S-phase when
Fig. 4. Activation of NPAT by cyclin E/CDK2 causes histone gene expression necessary for DNA synthesis and S-phase progression. , inhibition step; _, activation step; Cdk, cyclindependent
kinase; CIP, Cdk-interacting protein; KIP, kinase-inhibitor protein.
—

overexpressed in cells ***(111)***. The fact that depletion of cyclin A by injection of anticyclin A antibody causes inhibition of DNA synthesis suggests that cyclin A plays a role in this process. It has been shown that CDC6 is an intracellular substrate for cyclin A/CDK2 ***(112)***. CDC6 is a protein required for formation of the initiation complex (*see* **Subheading 4.3.2.**), which is necessary for the onset of DNA replication, thereby providing one mechanism by which cyclin A/Cdk2 may regulate DNA replication. CDC6 has been shown to be required for late firing of origins, and this function may

be achieved by phosphorylation following cyclin A/Cdk2 activation, suggesting that this complex may be required for continuation of DNA synthesis in addition to the initiation step ***(113)***. However, it has been demonstrated that microinjection of cyclin A antibodies into cells already progressing through S-phase causes accumulation of cells in G2 ***(109)***, indicating that, in this instance, cyclin A is not required for cells to complete S-phase, and CDC6 may therefore be regulated by other cyclin/Cdk complexes in the cell.

4.3. Cell Cycle Control of DNA Replication in Mammalian Cells

Eukaryotic genomes are extremely large and can range from 107 to greater than 109 bp. Because of this large size, duplication of the eukaryotic genome occurs as a multiparallel process with between 10,000 and 100,000 parallel synthesis sites in human somatic cells (reviewed in **refs.** ***114*** and ***115***). Cells need to ensure that DNA replication occurs at the appropriate time in the cell cycle and also that re-replication does not occur before cells undergo mitosis and cytokinesis. Advances in our understanding of the regulation of these sequential processes have come from numerous studies in yeast systems (reviewed in **ref.** ***114***); *see also* Chapter 1, this volume). These simple model systems have provided much information on the protein complexes involved in the activation and inhibition of DNA synthesis, and a number of homologs have since been identified in higher eukaryotes.

Early experiments carried out in mammalian cells by Rao and Johnson ***(116)*** showed that the initiation of DNA replication is believed to be a two-step process. These investigators showed that fusion of a G1 cell with an S-phase cell triggered DNA replication but that G2 cells were unable to undergo DNA initiation ***(116)***. This led to the notion that an S-phase–promoting factor was required to push cells from G1 into S-phase. The two-step process first involves the assembly of initiation factors at origins of replication and second the triggering of these complexes to activate DNA synthesis by the actions of protein kinases. The following sections will give an overview of those molecules involved in driving DNA initiation and replication.

4.3.1. Origins of Replication

DNA synthesis is known to occur at specific sites on the DNA known as origins of replication. The best characterised origins of replication are those found in *Saccharomyces cerevisiae* and are known as autonomous replication sequences (ARS) ***(117)***.

The ARS contains a highly conserved region of 100–200 bp known as the ARS consensus sequence (ACS), and this is an essential component of the origin of replication to which the origin recognition complex (ORC) binds. The ORC is conserved in all eukaryotes ***(118)***.

Three ORC subunits have been identified in humans, HsORC 1, 2, and 4 ***(119)***, all of which are involved in the initiation of DNA replication by recruitment of specific factors to the DNA. Human ORC has been shown to interact with a HAT, and this may be involved in making the initiation site accessible, thereby facilitating replication ***(120)***.

4.3.2. CDC6 and DNA Replication

A key regulator of DNA replication in mammalian cells is CDC6. Immunodepletion of CDC6 in human cells blocks S-phase entry ***(121,122)*** and has been shown to affect the interaction of ORC with minichromosome maintenance (MCM) proteins but not its interactions with DNA ***(123,124)***. These data suggest that CDC6 may act as an

adaptor protein for interactions of the ORC with other proteins (e.g., MCM proteins). Levels of CDC6 in cycling human cells remain fairly stable during S-phase, G2, and mitosis ***(125,126)***, but lower amounts are present in early G1 when CDC6 is degraded by proteolysis ***(127,128)***. CDC6 does, however, change its subcellular localization during the cell cycle, and it has been shown that nuclear CDC6 is phosphorylated during S-phase and transported to the cytoplasm ***(129)***. Phosphorylation of CDC6 is carried out by cyclin A/Cdk2 and also by Dbf/CDC7. Relocation of CDC6 within the cell might be one way in which cells ensure that re-replication does not occur. However, a substantial amount of CDC6 is found still associated with chromatin during Sphase ***(127)***, suggesting that CDC6 might play roles other than assembly of proteins at the initiation site and may be required for continued synthesis. Because of the relocalization of CDC6 during S-phase, CDC6 must be continually synthesized to account for the fraction associated with chromatin during S-phase ***(130)***.

4.3.3. Minichromosome Maintenance Proteins

The MCM proteins are a complex of six related proteins that form an essential component of the DNA initiation complex. Their requirement for DNA replication has been demonstrated by antibody injection and antisense oligonucleotide experiments ***(131–133)***. The six MCM proteins are not functionally redundant, and deletion of any MCM protein in *S. cerevisiae* or *S. pombe* results in loss of cell viability. In most organisms, the MCM proteins are located in the nucleus throughout the cell cycle ***(134,135)***. In mammalian cells, MCM proteins associate with chromatin in G1, but as cells progress through S-phase they are phosphorylated, and this reduces their affinity for chromatin ***(131,136)***. This may be one way in which cells ensure that replication occurs only once per cycle. In mammalian cells, some MCM proteins copurify with DNA polymerase □̣***(137)***, and evidence exists to suggest that MCMs possess DNA helicase activity ***(138)***. Therefore, it is possible that association of the helicases with a primase forms a mobile primosome that drives discontinuous synthesis.

4.3.4. CDC45

CDC45 is essential for DNA replication in *S. cerevisiae* ***(139–141)*** and this molecule has been shown to interact with MCM family members ***(140,141)***. A human homolog has been identified ***(142)***, and immunoprecipitation experiments indicate that it associates with chromatin periodically throughout the cell cycle. Association of

CDC45 with chromatin may depend on cyclin/CDK complex activity at the G1/S transition ***(143,144)***.

4.3.5. Regulation of DNA Initiation Complexes

Two classes of protein kinases are essential for the initiation of replication, the Cdks and Dbf4/CDC7 kinase.

4.3.5.1. CDKS

A role for Cdk2 in the initiation of replication in higher eukaryotes has been demonstrated in a number of studies; for example, microinjection of antibodies against certain cyclins and Cdks into mammalian cells inhibits S-phase entry ***(110,145)***.

As mentioned in **Subheading 4.2.**, CDC6 is a substrate for cyclin A/Cdk2, and phosphorylation of CDC6 by this complex possibly contributes to the prevention of DNA reinitiation by causing export of CDC6 from the nucleus ***(126,129)***.

MCM proteins also serve as substrates for certain Cdks, and phosphorylation of

MCM proteins causes dissociation from chromatin as cells progress through S-phase. Thus, MCM proteins are substrates for the mitotic complex cyclin B/CDC2 ***(146)***, and this provides a link between mitotic cyclins and the inhibition of reinitiation, ensuring that DNA replication occurs only once before entry into mitosis. It has been shown that MCM2 and -4 are phosphorylated in S-phase and become hyperphosphorylated by G2/M. Both MCM2 and -4 are good in vitro substrates for phosphorylation by cyclin B/CDC2 ***(146,147)***.

4.3.5.2. DBF4/CDC7 KINASE

CDC7 in *S. cerevisiae* and the *S. pombe* homolog, Hsk 1 have been shown to be essential for viability and are directly involved in DNA replication ***(148,149)***. A human homolog of CDC7 has also been identified ***(150–152)***. The human homolog of Dbf4 is regulated transcriptionally ***(153,154)***, with maximal expression during S-phase, which also corresponds to the kinase activity of the Dbf4/CDC7 complex ***(112,153)***. Studies have shown that inactivation of CDC7 in early S-phase prevents firing from replication origins, implicating CDC7 in the initiation of DNA replication ***(155,156)***. Human MCM2 and -3 are both substrates for CDC7 in vitro ***(151,153)***.

4.4. DNA Replication Checkpoints

Various checkpoints serve to inhibit DNA replication in response to partially replicated DNA or DNA damage, to allow the cell sufficient time to repair the damage before undergoing mitosis (*see* **Fig. 1**). Replication checkpoints have been extensively studied in yeast systems, and homologs for the proteins involved have also been identified in higher eukaryotes, including mammals.

4.4.1. The p53-Dependent Pathway

Several phosphatidylinositol (PI)-3-like kinase proteins are believed to be involved in the DNA replication checkpoint, including ATM, ataxia-telangiectasia related protein (ATR), and DNA-dependent protein kinase (DNA-PK) ***(157–160)*** and these ki*130 Harper and Brooks*

nases have been shown to be activated by DNA in vitro ***(161,162)***. The tumor suppressor protein p53 is a downstream target of ATM, and immunoprecipitated ATM can phosphorylate p53 on Ser15, a residue that is phosphorylated in vivo in response to DNA damage ***(163–166)***. DNA damage, occurring, for example, in response to ionizing radiation, leads to stabilization and accumulation of p53, which is involved in activation of a number of cellular responses such as cell cycle checkpoints, genomic stability, gene transactivation, and apoptosis ***(162,167–170)***. p53 is normally associated with the ubiquitin ligase MDM2, such that phosphorylation of p53 on Ser15 leads to its dissociation from MDM2, thereby stabilising the p53 protein ***(163)***. Stabilization of p53 leads to transactivation of the CDKI molecule, p21, which leads to cell cycle arrest ***(171)***.

Other regulators of p53 include ATR and Pin1. Thus, the ATR protein is capable of phosphorylating p53 on Ser15 and may also play a part in activating the p53 checkpoint pathway in response to ultraviolet (UV) and ionizing radiation ***(162,172)***. Pin1 has been shown to regulate the G1/S, G2/M, and DNA replication checkpoints ***(173)*** and is overexpressed in many human cancers ***(174–176)***. A recent report has shown that Pin1 binds phosphorylated p53 and is involved in stabilization of the protein, probably by interfering with the MDM2 interaction, and is also involved with transactivation of p21 in response to DNA damage ***(177)***.

4.4.2. The p53-Independent Pathway

The p53-independent mechanism of cell cycle block in response to unreplicated DNA or DNA damage involves the Rad proteins (reviewed in **refs. *114*** and ***178***). These proteins were first identified in yeast, and mammalian homologs also have been identified. The proteins involved in recognition and processing of the replication perturbation response are Rad1, Rad9, Rad17, and Hus1. The effects of these proteins are mediated by the protein kinases, CDS1 and CHK1, which target proteins involved in cell cycle regulation, for example, the CDC25 dual-specificity protein phosphatases (*see* **Subheading 5.1.**).

DNA-PK is the human homolog of the fission yeast PI-3–like kinase, Rad3, and is activated by proteins that detect sites of DNA strand breakage. Loss of function of these kinases results in inhibition of the checkpoint, suggesting that DNA-PK is important for sensing DNA damage and initiating the checkpoint mechanism ***(179,180)***.

Rad1 has been shown to be similar to proliferating cell nuclear antigen (PCNA) and possesses exonuclease activity ***(181,182)***. PCNA encircles the DNA during replication and retains the polymerase complex on the DNA. PCNA requires several factors in order to load onto DNA, one of which is known as replication factor C (RFC). Rad17 has been shown to share homology with RFC and also has been shown to interact with Rad1 ***(181)***. Rad1, Rad9, and Hus1 have all been shown to interact physically in mammalian cells ***(183,184)***, and it is believed that Rad17 may serve as a recruitment complex for Rad1, Rad9, and Hus1 to sites of DNA damage ***(185)***. Indeed, a recent study has demonstrated that upon replication block, Rad17 is recruited to the sites of DNA damage during late S-phase and that it binds to the Rad1/Rad9/Hus1 complex, enabling its interaction with PCNA ***(186)***.

The two downstream targets of the Rad proteins are the serine/threonine kinases CHK1 and CDS1. These kinases are activated differentially such that CDS1 is involved in mediating responses to unreplicated DNA, and CHK1 is involved in the G2 DNA damage response. CDS1 has been shown to be phosphorylated by ATM ***(187,188)***, and following activation it phosphorylates and inhibits the mitotic activator, CDC25C ***(187–189)***, thereby mediating G2 arrest. CHK1 also phosphorylates CDC25C in vitro ***(190)***. Phosphorylation of CDC25C by CDS1 and CHK1 creates a binding site for the 14-3-3 family of phosphoserine binding proteins (***190***; *see* **Subheading 6.1.2.**). Binding of 14-3-3 has little effect on CDC25C activity, and it is believed that 14-3-3 regulates CDC25C by sequestering it to the cytoplasm, thereby preventing the interactions with cyclin B/CDC2 that are localized to the nucleus at the G2/M transition ***(190,191)***.

The mechanisms by which DNA replication and DNA damage checkpoints exert their effects on cell cycle progression are now becoming clearer. Both p53-dependent and -independent mechanisms exert their effects via complex pathways on key cell cycle regulatory molecules such as p21 and the mitotic regulator, CDC25C (**Fig. 5**). These events occur at specific points in the cell cycle, ensuring that a cell does not proceed through mitosis without a full complement of replicated and intact DNA, thereby ensuring that the genome is passed equally to each of the daughter cells.

5. The G2/M Transition

The G2-phase is another gap phase in the cell cycle in which the cell assesses the

state of chromosome replication and prepares to undergo mitosis and cytokinesis. Cyclin B/CDC2 is the key mitotic regulator of the G2/M transition and was originally identified as the maturation-promoting factor, a factor capable of inducing M-phase in immature *Xenopus* oocytes ***(192–194)***. As is the case with other cyclin/CDK complexes, activation of the cyclin B/CDC2 complex is tightly regulated by phosphorylation and dephosphorylation events and also changes in subcellular localization (reviewed in **refs.** ***195*** and ***196***). The molecules that regulate cyclin B/CDC2 activity receive signals from the checkpoint machinery, as described in **Subheading 4.4.2.** Cyclin A/Cdk complexes also play a role in regulating the G2/M transition.

5.1. Role of the Cyclin B/CDC2 Complex in the G2/M Transition

Cyclin B synthesis begins at the end of S-phase ***(197)***. Two cyclin B isoforms exist in mammalian cells, cyclin B1 and B2. Studies in cyclin B1- and cyclin B2-null mice have confirmed that cyclin B2 is non-essential for normal growth and development ***(198)***. This particular isoform associates with the Golgi and may play a role in Golgi remodelling during mitosis ***(198,199)***. In contrast to cyclin B2, cyclin B1 is thought to be responsible for most of the actions of CDC2 in the cytoplasm and nucleus and it appears to compensate for the loss of cyclin B2 in B2-null mice implying that cyclin B1 is capable of targeting CDC2 kinase to the essential substrates of cyclin B2 ***(198)***. Cyclin B/CDC2 complexes are regulated both positively and negatively by phosphorylation (**Fig. 5**). Phosphorylation of CDC2 on the conserved T-loop region (Thr160) is required for activation, as is the case with all Cdks, and this phosphoryla*132*

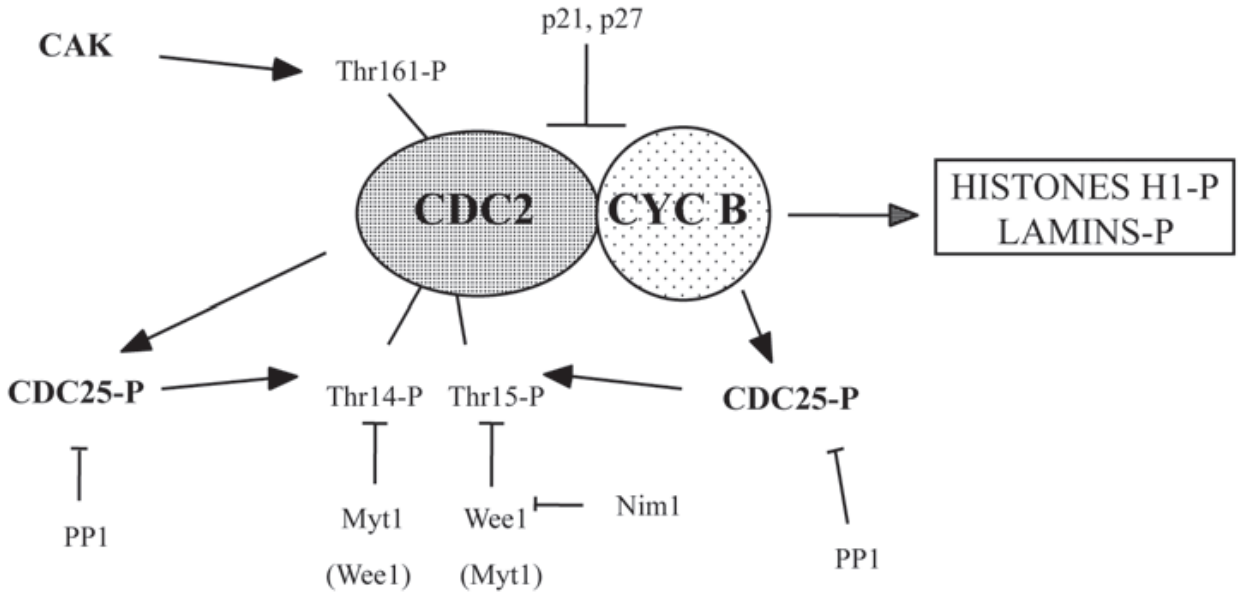

Fig. 5. Regulation of the CDC2/cyclin B complex. The serine/tyrosine kinase, Wee1 catalyzes phosphorylation of Tyr15 on CDC2. Wee1 itself is phosphorylated and inactivated by Nim1 and other unidentified kinases to induce mitosis. Thr14 phosphorylation can be mediated by Wee1 but only once Tyr15 has been phosphorylated. It appears that the Thr/Tyr kinase Myt1 is the critical kinase involved here. Inhibition of CDC2 by wee1 is counteracted by the CDC25

dual-specificity phosphatases. CDC25 is phosphorylated and activated by CDC2/cyclin B (amplification
pathway). Protein phosphatase 1 (PP1) inactivates CDC25 by dephosphorylation of
the same residue that is phosphorylated by CDC2/cyclin B. Full activation of CDC2 requires
Thr161 phosphorylation by Cdk-activating kinase (CAK), which then stabilizes CDC2 association
with cyclin A. , inhibition step; _, activation step.
tion event is mediated by CAK. During G2, cyclin B/CDC2 complexes are held in an
inactive state by phosphorylation of CDC2 Thr14 and Tyr15. Phosphorylation on
Thr14 prevents ATP binding ***(200)***, whereas that on Tyr15 interferes with phosphate
transfer to the substrate owing to its positioning in the ATP binding site on CDC2
(201). These inhibitory phosphorylation events are carried out by the kinases Wee1
and Myt1; Wee1 specifically phosphorylates Tyr15, and Myt1 phosphorylates both
Tyr15 and Thr14, with a stronger affinity for Thr14 ***(202–204)***. Cyclin B/CDC2 becomes
fully activated following dephosphorylation of these sites by the protein phosphatase
CDC25C (**Fig. 5**).

6. Mitosis (M-Phase)

Mitosis (also called karyokinesis) and cytokinesis constitute the shortest phase of
the eukaryotic cell cycle, typically taking around 1–2 h to complete in a mammalian
cell. Mitosis itself comprises five distinct phases as follows:

1. *Prophase*: this stage begins with condensation of the chromosomes in the nucleus
and ends with breakdown of the nuclear envelope. (This latter event occurs over a 1–
2-min interval.)

Fig. 5. Regulation of the CDC2/cyclin B complex. The serine/tyrosine kinase, Wee1 catalyzes
phosphorylation of Tyr15 on CDC2. Wee1 itself is phosphorylated and inactivated by
Nim1 and other unidentified kinases to induce mitosis. Thr14 phosphorylation can be mediated
by Wee1 but only once Tyr15 has been phosphorylated. It appears that the Thr/Tyr kinase Myt1
is the critical kinase involved here. Inhibition of CDC2 by wee1 is counteracted by the CDC25
dual-specificity phosphatases. CDC25 is phosphorylated and activated by CDC2/cyclin B (amplification
pathway). Protein phosphatase 1 (PP1) inactivates CDC25 by dephosphorylation of
the same residue that is phosphorylated by CDC2/cyclin B. Full activation of CDC2 requires
Thr161 phosphorylation by Cdk-activating kinase (CAK), which then stabilizes CDC2 association
with cyclin A. , inhibition step; _, activation step.

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2. *Prometaphase*: at this stage the mitotic spindle forms. Three essential events
must occur in prometaphase if cell division is to proceed normally: (a) the bipolar
spindle axis must be established; (b) the daughter chromatids of each replicated chromosome
must become committed to the opposing spindle poles; and (c) the chromosomes
must become aligned at, or near to, the spindle equator.
3. *Metaphase*: during this stage all chromosomes are bioriented and positioned near
the spindle equator. All chromosomes align themselves along the metaphase plate.
4. *Anaphase*: the sister chromatids that comprise each chromosome separate to form
two independent chromosomes. Anaphase is separated into anaphase A and anaphase B.

5. *Telophase*: this is the final stage of mitosis, in which the chromosomes decondense and a nuclear envelope forms around each set of chromatids. The contractile ring begins to form in readiness for the cell to split into two daughter cells, each with one nucleus.

A number of cell cycle regulatory molecules play pivotal roles in promoting progression through mitosis, including the CDC25 protein phosphatases, the polo-like kinases (PLKs), the 14-3-3 proteins, mitotic cyclin/Cdk complexes, and the anaphasepromoting complex (APC). The role that these individual groups of molecules plays in mitosis is now discussed in more detail.

6.1. The CDC25 Protein Phosphatases

The mammalian CDC25 family of dual-specificity phosphatases consists of three members: A, B, and C ***(205,206)***. CDC25B and C are thought to be the main regulators of mitosis, whereas CDC25A plays a role in regulating the G1/S transition. CDC25B may be involved in the initial dephosphorylation and activation of cyclin B/CDC2, which then initiates the positive feedback loop of CDC25C activation by CDC2 (***207***; *see* **Fig. 5**). CDC25B is also believed to play a role in centrosomal microtubule nucleation during mitosis since overexpression of this molecule causes formation of minispindles in the cytoplasm ***(208)***. CDC25A expression is under transcriptional control of the E2F transcription factors in late G1 ***(209)*** and is involved in activation of cyclin E/Cdk2 and cyclin A/Cdk2 complexes that regulate entry into S-phase (*see* **Subheadings 4.1.** and **4.2.**).

CDC25C is the protein phosphatase that is mainly responsible for dephosphorylation and activation of the cyclin B/CDC2 complex. Treatment of CDC25C with phosphatases in vitro led to reduced CDC25C phosphatase activity, indicating that hyperphosphorylation of CDC25C is required for phosphatase activity during mitosis ***(207,210,211)***. Cyclin B/CDC2 is able to phosphorylate CDC25C (***207,212***; *see* **Fig. 5**) and this initiates a positive feedback loop that induces rapid activation of cyclin B/CDC2 at the G2/M transition. However, the initial trigger of CDC25C activation remains unclear, although CDC25C has been shown to be phosphorylated by cyclin E/Cdk2 and cyclin A/Cdk2 in vitro ***(212)***. PLK-1 is another potential upstream regulatory kinase of CDC25C that might function in vivo ***(213)***.

The role of CDC25C as a key mediator of cyclin B/CDC2 activation has recently been questioned owing to studies performed in CDC25C knockout mice. These mice showed no phenotype with respect to regulation of mitosis and showed no differences

in CDC2 phosphorylation ***(214)***, suggesting that redundancy exists between CDC25 isoforms. Indeed, a role for CDC25A in CDC2 activation has recently been suggested. Destruction of CDC25A by the ubiquitin-mediated pathway serves to ensure that cells do not undergo premature mitosis, and this is achieved by phosphorylation of CDC25A by CHK1. However, Mailand and co-workers ***(215)*** reported that once cells are committed to mitosis, stability of CDC25A undergoes major changes at the G2/M transition owing to phosphorylation on Ser17 and Ser115 that uncouples it from the ubiquitin-mediated degradation pathway. Phosphorylation of CDC25A on these specific residues is mediated by cyclin B/CDC2 and therefore forms part of a positive feedback activation loop whereby CDC25A is stabilized by CDC2, and this is followed by dephosphorylation of CDC2 on Thr14 and Tyr15 ***(215)***.

6.1.1. The Polo-Like Kinases

The polo-like kinases are a family of serine/threonine protein kinases, four of which have been described in mammalian cells: PLK-1, PLK-2 (Snk), PLK-3 (Fnk/Prk), and PLK-4 (Sak a/b) ***(216,217)***. The PLKs share a closely related catalytic domain at their N-termini (50–65% identity at the amino acid level) and a homologous C-terminal domain called the polo box domain (PBD)—of which there are two, PBD1 and PBD2—that is required for directing subcellular localization of the kinase since mutation of this region has been shown to disrupt localization of PLK-1 ***(218,219)***.

PLK protein levels and phosphorylation status are cell cycle-regulated. Thus, PLK1 is undetectable in cells at the G1/S-phase transition; however, levels rise during Sphase, and phosphorylation occurs during G2 ***(220)***. Indeed, PLK1 is important for the G2/M transition, entry into mitosis and exit from mitosis, and is rapidly degraded as the cell exits mitosis and PLK1 activity levels peak at the metaphase–anaphase transition ***(217,220)***. Activation of PLK1 has been found to occur at a similar time to cyclin B/CDC2 activation, and a recent study by Roshak et al. ***(213)*** demonstrated that human CDC25C is a substrate for PLK1 and that phosphorylation caused activation of the phosphatase and subsequent dephosphorylation of cyclin B/CDC2. Other substrates phosphorylated by PLK1 include cyclin B1, Myt1, and the APC.

PLK2 is the least well characterized of the mammalian PLKs, although it is thought to play a role in cell cycle reentry of G0-arrested cells ***(221)***. PLK3 has been implicated in DNA damage control at the G2 checkpoint, where, in mammalian cells, it acts in the p53-mediated stress response pathway ***(222)***. Finally, a cell cycle role for PLK4 has not yet been reported. This particular PLK was isolated from the mouse as two distinct transcripts (a and b) and has since been shown to contain only a single PBD; it also lacks the catalytic motif found in other PLK family members ***(216)***.

6.1.2. The 14-3-3 Proteins

CDC25C phosphatase activity is regulated negatively by phosphorylation on a specific Ser216 residue that creates a binding site for small phosphoserine binding proteins, known as 14-3-3 ***(223)***. A number of 14-3-3 proteins are known to exist, including: 14-3-3 □, □, □, □, □, □***(224)***. CDC25C is localized to the cytoplasm during interphase and is directed to the nucleus just prior to mitosis ***(191,225)***. Binding of 14-3-3 may prevent nuclear localization of CDC25C by masking the NLS, which is in close proximity to the Ser216 residue. In support of 14-3-3 sequestering CDC25C to the cytoplasm during interphase, Ogg et al. ***(226)*** demonstrated that Ser216 is the major phosphorylation site of CDC25C during interphase but not during mitosis. Potential candidate kinases for phosphorylation of Ser216 on CDC25C are CHK1, CDS1, and C-TAK1 ***(227,228)***. CHK1 and CDS1 are both mediators of G2 arrest in response to DNA damage or incomplete replication, as discussed above in **Subheading 4.** Therefore, phosphorylation of CDC25C during interphase creates a binding site for 14-3-3, causing cytoplasmic retention. Dephosphorylation of Ser216 (possibly by CDC25B) at the onset of mitosis results in nuclear localization and subsequent activation of cyclin B/CDC2, as described in **Subheading 6.1.1.** above.

6.2. Subcellular Localization of Cyclin B/CDC2 During G2/M

During interphase, cyclin B/CDC2 complexes are found in the cytoplasm. However, by late prophase, most cyclin B/CDC2 is found in the nucleus following breakdown

of the nuclear envelope ***(229)***. Cyclin B has a cytoplasmic retention sequence (CRS) in the N-terminal region which, when deleted, causes localization of cyclin B to the nucleus ***(230)***. A nuclear export signal (NES) also has been defined within the CRS, and this binds to the export receptor CRM1 ***(231)***; it has been that shown that a specific inhibitor of CRM1 causes accumulation of cyclin B in the nucleus ***(231)***. During mitosis, cyclin B is hyperphosphorylated within the CRS region, and this phosphorylation is thought to disrupt interactions with CRM1 holding cyclin B in the nucleus ***(229,231)***.

The mechanism by which cyclin B enters the nucleus is less well understood. Both cyclin B and CDC2 lack an NLS. Cyclin B1 has, however, been shown to bind importin-b ***(232)***, and this could be one mechanism that mediates cyclin B nuclear localization. The CRS of cyclin B1 is also known to interact with cyclin F, which is found predominantly within the nucleus and contains two NLSs ***(233)***. Interestingly, overexpression of cyclin F causes relocation of cyclin B to the nucleus, suggesting that it may be involved in the import of cyclin B1.

Although cyclin B contains a CRS and an NES, both of which ensure that cyclin B is localized to the cytoplasm, phosphorylation of cyclin B can lead to association with other molecules, resulting in its relocation to the nucleus and thereby allowing access to nuclear substrates.

6.3. Function of Cyclin B/CDC2 During Mitosis

The cyclin B/CDC2 complex is involved in the initiation of a number of mitotic events in both the cytoplasm and the nucleus. During prophase, cyclin B/CDC2 is associated with duplicated centrosomes, and it promotes centrosome separation by phosphorylation of the centrosome-associated motor protein Eg 5 ***(234)***. Cyclin B1/CDC2 and/or cyclin B2/CDC2 complexes are involved in the fragmentation of the Golgi network ***(235)***, and cyclin B/CDC2 also is involved in the breakdown of the nuclear lamina and cell rounding ***(236)***. Thus, the cyclin B/CDC2 complex is involved in completely reorganizing the cell architecture during mitosis.

6.4. Function of Cyclin A/CDC2 During Mitosis

Cyclin A plays important roles at two distinct phases of the cell cycle, G1/S (as discussed in **Subheading 4.2.**) and G2/M. These separate functions coincide with cyclin A binding to two different kinases, CDK2 at the G1/S border and CDC2 during G2. Cyclin A levels are undetectable during G1, and levels begin to rise as cells enter S-phase. By the time a cell enters mitosis, cyclin A levels begin to decline; however, it still is present during prophase, in which it is associated with the centrosomes, although by telophase cyclin A is undetectable ***(109)***. Cyclin A/CDC2 complexes are thought to play a role in activating cyclin B/CDC2 complexes, and recent reports suggest that cyclin A/CDK2 complexes may also act during the G2 checkpoint ***(237,238)***. Exit from mitosis requires degradation of both cyclin A and cyclin B, and this occurs via a ubiquitin-mediated pathway that itself is regulated by the APC pathway (*see* **Subheading 6.5.** below).

6.5. The Anaphase-Promoting Complex

Exit from mitosis requires ubiquitin-mediated degradation of mitotic cyclins via the *cyclin destruction box* ***(239)***, which is regulated by the APC ubiquitin ligase. APC is a

multi-subunit ligase consisting of a number of protein subunits such as APC1, APC2, CDC16, and CDC23 (reviewed in **ref.** ***240***). APC is inactive in the S- and G2-phases of the cell cycle but becomes activated in mitosis as a result of phosphorylation that is believed to be carried out by PLK1 ***(241,242)*** and/or cyclin B/CDC2 ***(241)***. APC requires conversion to an active form by CDC20/Fizzy, and this can only occur following phosphorylation of APC ***(243)***. APC is also required for sister chromatid separation during anaphase by causing destruction of securins, the proteins that hold the sister chromatids together. The securins inhibit the highly conserved enzyme separase during the cell cycle until metaphase, in which it is degraded by the APC ***(244)***. Activation of separase is crucial for the onset of anaphase in all eukaryotic cells.

6.6. Other Cell Cycle Regulatory Molecules Involved in Mitosis

A number of other molecules and protein complexes are involved in regulating normal karyokinesis and cytokinesis in mammalian cells. Such molecules include the securins, separase (*see* **Subheading 6.5.**), and rhabdokinesin-6. Expression of RB6K is regulated during the cell cycle at both the mRNA and protein levels and, similar to cyclin B, reaches maximum levels during M-phase ***(18)***. RB6K localizes in the late stages of mitosis to the spindle midzone and appears on the midbodies during cytokinesis. The functional significance of this localization during cell division has been demonstrated by antibody microinjection studies showing exclusive production of binucleated cells that failed to complete cytokinesis ***(245)***.

7. Cytokinesis

At the end of mitosis the cell must ensure that division is taken to completion by a process called cytokinesis. This occurs following assembly of a cleavage furrow at the site of division that contains actin, myosin, and other proteins that eventually form the contractile ring ***(246)***. Following chromosome segregation, the microtubules bundle in the midregion of the spindle, forming the spindle midzone. As the contractile ring contracts, it creates a membrane barrier between each cell. The spindle midzone remains connected, forming a cytoplasmic bridge until this is finally cut during abcission.

The mid-zone has been shown to contribute to actin ring assembly since placement of an artificial barrier between the spindle midzone and the cell cortex during metaphase caused inhibition of the cleavage furrow, whereas if a barrier was created in early anaphase, cytokinesis proceeded without a problem ***(247)***.

Animal cells divide through the formation of an actomyosin contractile ring at the end of anaphase ***(248)***. As discussed above, the spindle midzone plays a role in contractile ring assembly. Two major classes of proteins are believed to be important in signaling from the spindle midzone to the contractile ring. The first of these are the chromosomal passenger proteins, e.g., the inner centromere proteins that are initially found localized to chromosomes and centromeres and then translocate to the midzone during anaphase ***(249)*** and are involved in chromosome alignment, segregation, and cytokinesis. The second class of proteins are the motor-associated proteins, e.g., Eg5, that are required to maintain the midzone. These proteins localize along the spindles during metaphase and concentrate in the spindle midzone during anaphase ***(250,251)***. Specific Cdks also play a role in cytokinesis, and it has been shown that mammalian cells injected with a nondestructible form of cyclin B undergo anaphase and chromosome segregation but do not form a spindle midzone and fail to undergo cytokinesis

(252), suggesting a role for cyclin B in inhibiting cytokinesis. Cdks may also inhibit myosin, and thereby contractile ring formation, through inhibitory phosphorylation of the myosin regulatory light chain (RLC) ***(253)***. Myosin RLC can be phosphorylated by CDC2, which inhibits its actin-activated ATPase activity in vitro ***(254)***. This inhibitory phosphorylation increases in early mitosis and decreases in anaphase simultaneously with a decrease in CDC2 activation ***(255)***.

8. Endoreduplication

In most eukaryotic cells, S-phase and mitosis are coupled and occur only once during each cell cycle; however, occasionally the sequence of events is interrupted such that the cell undergoes multiple rounds of DNA synthesis in the absence of mitosis. This process is called endoreduplication. Work in yeast has shown that endoreduplication can occur as a result of multiple initiations within S-phase, reoccurring S-phase, or repeated S- and G-phases ***(256)***. In addition, endoreduplication can result in either multiple DNA syntheses within a single nucleus, e.g., megakaryocytes or in multi-nucleated cells, e.g., cardiac myocytes. Little is known about the molecular mechanisms responsible for endoreduplication in multinucleate cells, although a greater understanding of the processes involved might enable cell division to be initiated instead of endoreduplication, which would be useful for replacing damaged cells and would therefore avoid scarring, in terminally differentiated tissues that contain cells such as cardiac myocytes and neurones

9. Destruction of Cell Cycle Regulators During the Cell Cycle: Role Of The SCF Ubiquitin Ligases

The carefully ordered progression of a cell through various stages of the cell cycle is mediated by the timely synthesis and destruction of numerous cell cycle regulatory proteins. Degradation of molecules such as the cyclins occurs via ubiquitin-mediated proteolysis, the specificity of which is controlled by a large number of ubiquitin ligases that themselves are either single subunits or multiprotein complexes that act as recognition factors for substrates to be ubiquitinylated. The SCF complexes represent a large family of ubiquitin ligases that control cell cycle progression. They are so called because they are composed of Skp1, Cul1, and F-box protein as well as Roc1; it is the F-box protein component of the SCF complex that determines substrate recognition. It is well documented that SCF complexes are key in controlling the abundance of cell cycle regulatory proteins, including cyclins, Cdks, and CDKIs ***(257)***. For example, the SCF complex containing the F-box protein Skp2 coordinates the ubiquitinylation of the CDKIs p21 and p27, thereby allowing CDK2 activation at the G1/S border ***(258,259)***.

10. Summary and Conclusions

The mammalian cell cycle is a highly regulated, conserved, and sequential process that is necessary for normal cell growth and development. Our understanding of the mechanisms involved in cell cycle regulation has increased significantly in recent years, as demonstrated by the award of the Nobel Prize for Physiology and Medicine to Leland Hartwell, Paul Nurse, and Tim Hunt in 2001 for their seminal discoveries relating to the cell cycle machinery. Despite this increased understanding, much remains to be learned about the mechanisms involved in controlling growth and proliferation in specific cell types and organs. Extending our knowledge of cell cycle control

in different cell types might help to identify the causes of certain hyperproliferative diseases, including cancer and vascular disease. This then could lead to the development of new therapeutic agents targeting specific cell cycle molecules that become altered in such disorders.