A Long Twentieth Century of the Cell Cycle and Beyond

Review

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Only those exacting editors at *Cell* could seriously ask you to review the past century of cell cycle research and to predict the course of research for the next century, and to do it all in 10 pages! It is unrealistic to try to be comprehensive in such a review, and so I will focus on what I consider to be the important principles underlying the cell cycle, with less emphasis on detailed descriptions of molecular mechanisms which would degenerate into lists of genes and proteins. Referencing will be minimal, and will be restricted to reviews, a few key primary publications, and to books written in English for summaries of the earlier literature.

Let us begin at the end of the century by summarizing what is now known about the cell cycle. We know that the cell cycle is the universal process by which cells reproduce, and that it underlies the growth and development of all living organisms. The most important events of the cell cycle are those concerned with the copying and partitioning of the hereditary material, that is replicating the chromosomal DNA during S phase and separating the replicated chromosomes during mitosis. Controls operate that regulate onset of these events and compensate for errors in their execution. The molecular basis of these controls is highly conserved from simple unicellular eukaryotes such as yeast to complex metazoans such as ourselves. The precision with which cell cycle events are executed ensures the survival of living organisms, while loss of this precision increases genomic instability, an important factor in the formation of cancer. The mitotic cell cycle is modified to a meiotic cycle during gamete formation, leading to a reduction in chromosome number that is essential for sexual reproduction, and to an increase in genetic variation that is a driving force for evolution. Thus, the cell cycle plays a central role in the operation and development of all life, and in ensuring the continuity of life across generations. These discoveries were made over a period that extends into the previous century, and so the scope of this review will be similarly extended, hence "A Long Twentieth Century of the Cell Cycle."

Discovery, Growth, and Heredity

Study of the cell cycle began with the discovery of cell division. The concept of a cell was well established by the mid-nineteenth century, but understanding of how cells were reproduced remained confused, partly because Schleiden and Schwann, the major proponents of the cell theory, thought that cells arose from within preexisting cells by a process somewhat similar to precipitation or crystallization (Schwann, 1857). Greater clarity came with Nägeli and Remak who correctly described the division of plant and animal cells, and with Virchow who promoted the idea that all cells were produced by the fission of preexisting cells (for a pithy, historical account of this period, see Harris, 1999). At around the same time, Kolliker realized that early embryonic cleavage represented a series of cell divisions producing cells that eventually became differentiated into various tissues and organs (for a great near contemporary account of cell work at this time, see Wilson, 1925). This idea was extended further during the 1870s and 1880s by Pringsheim, Strasburger, and Hertwig (Sharp, 1921), who recognized that eggs and sperm were single cells which became joined together at fertilization, so even the most complex multicellular organisms passed through a single celled stage. Thus, cell division was established as the basis of growth and development of both animals and plants.

Improvements in microscopes and microscopic techniques led to a detailed description of the changes occurring to the chromosomes during mitosis, the most conspicuous event of cell division. A critical feature observed by Flemming and Strasburger during the 1880s was the appearance of elongate chromosomal threads formed from the nucleus, which then split lengthways before shortening and thickening later in mitosis (Wilson, 1925; Flemming, 1965). Van Beneden later showed that the longitudinal halves of each split chromosome separated apart into the two daughter nuclei, and that the chromosomes of a fertilized nematode egg were derived in equal numbers from the egg and sperm. With this discovery, Weissman came to the important conclusion that the chromosomes were the basis of heredity, and that germ cells formed a continuous line of heredity between the generations (Sharp, 1921; Wilson, 1925). The link between the cell cycle and genetics was further strengthened by the rediscovery of Mendel's Laws of Inheritance by DeVries, Correns, and Tschermak at the turn of the century. Mendel's postulate that a zygote contains two sets of "qualities" whilst the maternal and paternal gametes have a single set, paralleled the generation of haploid and diploid sets of chromosomes during meiosis, fertilization, and mitosis. Mendel's abstract laws could thus be explained by the concrete behavior of the chromosomes during the cell cycle.

This pioneering work was confirmed during the first two decades of the twentieth century, placing the cell cycle firmly at the centre of the growth, development and heredity of all living organisms (Wilson, 1925). At this time there were also speculations relevant to the control of the cell cycle. Hertwig proposed the concept of the karyoplasmic ratio (Wilson, 1925), arguing that there is a constancy between nuclear and cytoplasmic volume, and that cell cleavage takes place when this ratio becomes unbalanced. Implicit in this concept is the idea that progression through the cell cycle is coordinated with cellular growth, an idea which was only developed further later in the century. The period between 1920 and 1950 was somewhat of a Dark Ages for the cell cycle. The most interesting work dealt with the various chromosomal changes that can occur during mitosis

and meiosis, and their significance for genetic transmission (Darlington, 1958). However, for new insights into the events and controls of the cell cycle, we have to move on three decades to work started in the 1950s.

Events of the Cell Cycle

The events concerned with the replication and partition of the chromosomes are common to all cell cycles, because with few exceptions, a newly divided cell needs to receive a full genome complement to survive. Chromosomes are present in low copy number, and so special mechanisms are required to ensure their precise replication and partition. The double-helical base-paired structure of DNA provided a deeply satisfying solution to the problem of how replication can be so precise (Watson and Crick, 1953). Also at this time, microspectrophotometric (Swift, 1950) and autoradiographic studies (Howard and Pelc, 1953) in eukaryotic cells showed that DNA replication occurs during a restricted part of interphase called S phase. This work led to the eukaryotic cell cycle being divided into S phase and M phase or mitosis, with the gap before S phase being called G1 and after S phase G2 (Mitchison, 1971). In prokaryotes, chromosome replication and partition need not be temporally separated, and can overlap during the cell cycle of rapidly growing bacteria. It seems likely that the separation of these processes into S phase and M phase and the controls which regulate their onset both evolved as DNA content increased during the emergence of eukaryotic cells. The discovery of S phase also identified two key problems still important today: how does the machinery of DNA replication work, and what determines the onset of S phase during the cell cycle?

The first of these problems has been worked on for the past 40 years and has resulted in the gradual unravelling of the molecular mechanisms and enzymology of the process of DNA replication, starting with the pioneering discovery of a DNA polymerase (Kornberg et al., 1956). Important advances were the development of the T4 (Morris et al., 1975), *E. coli* and SV40 (Li and Kelly, 1984) in vitro DNA replication systems, the discovery that an RNA primer was needed to initiate DNA synthesis (Brutlag et al., 1971), and the identification of enzymes such as the topoisomerases, helicases, ligases, primases, and polymerases required to unwind the DNA strands and to synthesize new copies (Kornberg and Baker, 1992). The enzymes operate together in replication complexes, which generate bidirectionally organized replicating forks. Initiation of DNA replication was found to occur at specific origin regions defined by distinct DNA sequence motifs in prokaryotic and viral systems, and in the budding yeast (Stinchcomb et al., 1979). However, such unique DNA sequence specificity is not found in most eukaryotes studied, an example being *Xenopus* eggs (Mechali and Kearsey, 1984). Higher eukaryotic origins appear to have a more extended structure probably reflecting a degenerate organization and possibly a role for higher order nuclear structure. The end of the century has seen increased attention directed toward the molecular mechanisms by which replication complexes are built at origins. Chromatin associated origin recognition complexes (ORCs), identified because they bind yeast origins, are thought to act as "landing pads"

for the replication complexes (Bell and Stillman, 1992). A key step for this is carried out by the initiator protein Cdc6p/Cdc18p, which loads Mcm proteins onto chromatin to "license" DNA for replication (Blow and Laskey, 1988; Diffley, 1996). Licensing ensures that no DNA is replicated twice during an S phase, and that there is only one S phase each cell cycle. Once replication complexes are built and activated, a series of replicating forks are set up along the chromosomes generating bubbles that eventually fuse together to complete DNA replication.

The next major event of the cell cycle is mitosis (see Figures 1 and 2). Early work up to the 1960s focused on descriptive and structural studies, moving from light microscopy to electron microscopy and biochemical analysis. The mitotic spindle was first described by Boveri at the turn of the century as a system of astral rays extending between the centrosomes (Wilson, 1925). Improvements in fixation and electron microscopy demonstrated that the spindle was made up of microtubules (Harris, 1961), and isolation of the mitotic apparatus and the identification of a colchicine binding protein (Mazia, 1961) eventually led to the discovery that microtubules were composed of tubulin polymers (Kiefer et al., 1966). Fluorescence imaging and in vitro assays revealed that microtubules oscillate between growing and shrinking states, a process called dynamic instability (Mitchison and Kirschner, 1984). Microtubular organizing centres (MTOCs) were found to be located at centrosomes and at kinetechores, and shown to seed new microtubular growth, and to capture and stabilize preexisting microtubules preventing their shrinkage (Nicklas, 1997). Tubulin subunit turnover and microtubular motors can move chromosomes (Inoue and Salmon, 1995), and motors may also contribute to the building of mitotic spindles by organizing and bundling microtubules (Vernos and Karsenti, 1996).

These properties provide a satisfying although still incomplete explanation for the events of mitosis. Initially the centrosomes duplicate, separate, and then generate a microtubular spindle between them. This establishes a bipolarized cellular state that is an essential early step of mitosis. The chromosomes, composed of two sister chromatids, become condensed, and each chromatid has a kinetochore located at its centromere, which is able to capture spindle microtubules. A stable configuration of chromosomes on the spindle is only achieved when one sister chromatid kinetochore becomes attached to microtubules emanating from one pole of the spindle, and the other sister chromatid kinetochore becomes similarly attached to the other pole (Nicklas, 1997). This is the crucial step of mitosis because when this is achieved, the replicated DNA molecules of each chromosome become separately oriented toward opposite poles of the cell. At this point cohesion between sister chromatids is lost (Nasmyth, 1999), and the chromatids move apart to form two nuclei that become separated by cytokinesis.

Controls, Concepts, and Methods

Controls of the cell cycle regulate the onset of events such as S phase and mitosis, and ensure that these events occur in the correct sequence, are coordinated with cellular growth, and are corrected for errors in their
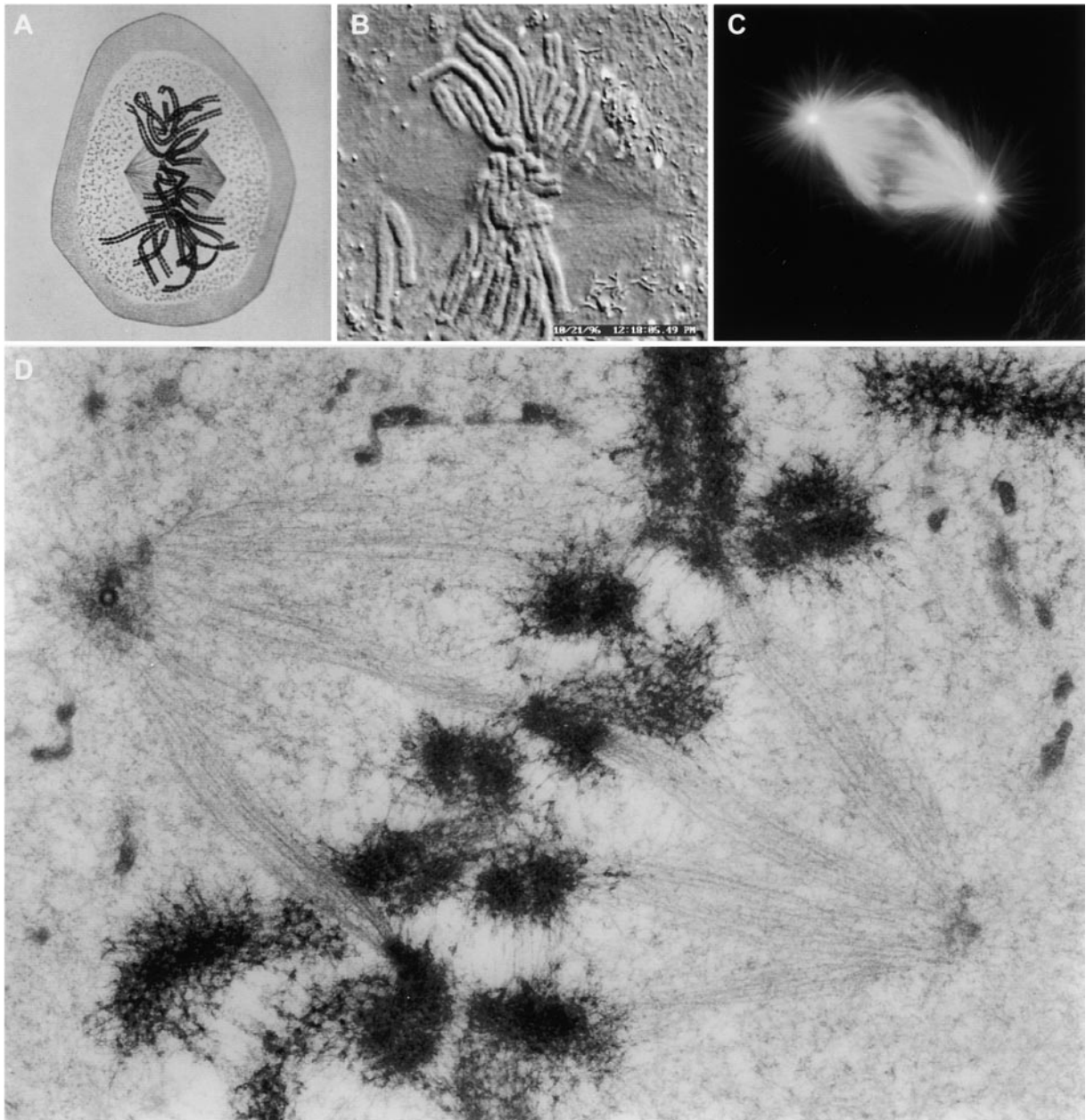
Figure 1. A Long Twentieth Century of Imaging Mitosis

The cell cycle culminates at the metaphase stage of mitosis, and passage through metaphase is guarded by a checkpoint that delays anaphase until all the chromosomes are properly attached to the spindle. The composite figure illustrates the metaphase spindle as depicted through the years. The spindle was first described and drawn by Flemming in 1992 (A) from the visual inspection of stained sections cut from fixed and embedded salamander epithelia (Flemming, 1882). Although it can be seen in living cells by polarization light microscopy, it is not normally visible by those imaging modes that allow for chromosome behavior to be detailed in vivo (e.g., DIC light microscopy, as in [B]). As microtubules are the major structural component of the spindle, it is readily apparent after labeling fixed cells for the immunofluorescent localization of microtubules (C) or in sections viewed in the electron microscope, a technique developed in the mid–twentieth century (D). (A)–(C) are of newt cells, and (D) is from a PtK1 cell. (Figure kindly provided by Conly L. Rieder, Division of Molecular Medicine, Wadsworth Center, N. Y. State Department of Health, Albany, New York.)

execution. Early speculations about controls included roles for such diverse processes and components as energy reservoirs, heat labile division structures, and limit cycle oscillators (for a fine review, see Mitchison, 1971). In the last 25 years that there has been more consistent progress in understanding these controls, largely as a consequence of better conceptualization of the problems combined with more effective methodologies. An important conceptual advance was the idea that the cell cycle should be considered as a temporally organized sequence of events analogous to simple developmental systems such as phage morphogenesis
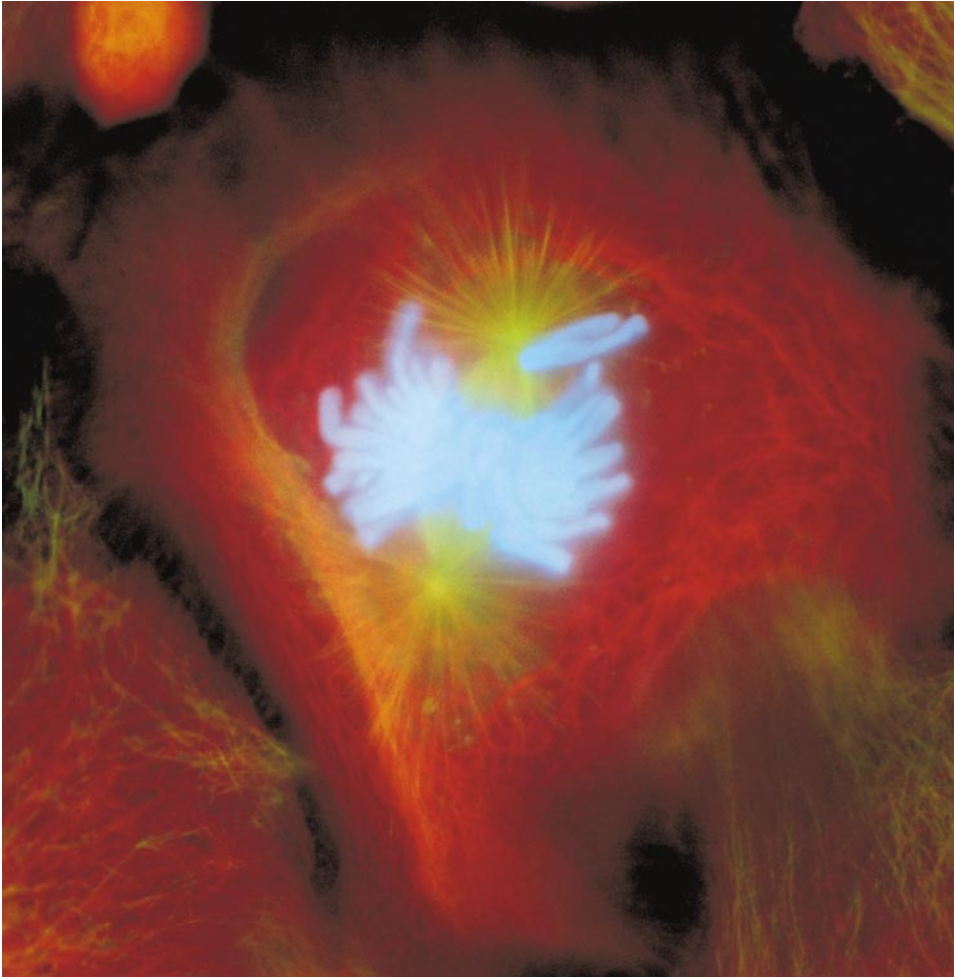
Figure 2. Imaging Mitosis at the Turn of the Millennium

Currently available light microscope technology allows detailed and spectacular imaging of spindle organization in metaphase cells. This shows a salamander (newt) lung cell, fixed in metaphase, and photographed by epifluorescence after labeling the chromosomes (blue) and DAPI, and staining the microtubules (green) and keratin filaments (red) by indirect immunofluorescence methods. This cell contains a single monooriented chromosome that will delay anaphase onset until it becomes properly bioriented and positioned near the spindle equator. In epithelia, the spindle is often surrounded by a cage of intermediate filaments that formerly surrounded the nucleus. (Figure kindly provided by Conly L. Rieder, Division of Molecular Medicine, Wadsworth Center, N. Y. State Department of Health, Albany, New York.)

(Hartwell, 1974). This thinking focused attention on the way in which different cell cycle events were linked together in an orderly sequence. Later events were often found to be dependent upon the successful completion of earlier events, and it was reasoned that these dependencies could be of two types, either directly coupled or based on a linking signaling control. Direct coupling is hard-wired, like sequential substrate–product relationships in a metabolic pathway, and is most relevant for sequential events in processes involving direct molecular interactions like building DNA replication complexes. Failure of an early step in this type of process fails to generate the correct "product" required as the "substrate" for the next step, making the later step dependent on the earlier one. By contrast, dependencies operating through a signaling control can link more distant events separated either in space within the cell or in time between different phases of the cell cycle. For example inhibiting S phase by reducing the supply of deoxyribonucleotides blocks the temporally distant and unrelated event of mitosis. This block requires a set of proteins acting in a signal transduction pathway, which communicate the fact that S phase is incomplete to the effectors of mitosis. This idea was developed further to generate the concept of the checkpoint control (Hartwell and Weinert, 1989). At different points in the cell cycle, the cell "checks" if an earlier event, such as S phase, has been properly executed before proceeding to a later event, such as mitosis. The checkpoint concept also covers other situations such as blocking mitosis after DNA damage until the damage is repaired, a mechanism which helps ensure faithful genomic transmission.

A second important conceptual advance was the idea that certain cell cycle events acted as major rate-limiting steps for cell cycle progression. Extending earlier ideas about trigger points and division proteins, an analogy was made between cell cycle control and the control of flux through a metabolic pathway (for a relevant review

of flux control, see Kacser and Porteous, 1987) leading to the proposal that certain steps in the cell cycle might be rate limiting for cell cycle progression (Nurse, 1975). Growth of the cell was thought to be an important factor in this control by restraining specific rate-limiting steps. Certain cells such as amphibian oocytes and eggs are very much enlarged, and in these cases cell mass is no longer limiting and cell cycle progression is regulated by a timer or oscillator (Murray and Hunt, 1993). The steps that are rate limiting might also vary in different circumstances shifting control from one step to another in the regulatory network (Kacser and Porteous, 1987).

These conceptual advances were complemented by development of powerful new experimental approaches. One was the application of genetics coupled with molecular biology, which was particularly effective for cell cycle studies with the yeasts (Hartwell et al., 1973; Nasmyth and Reed, 1980). Cell cycle mutants were isolated, the genes defined by these mutants identified, physiologically characterized, cloned by complementation, and the cloned gene used as the starting point for subsequent biochemical analysis. A second experimental approach was the use of complex cell extracts derived from amphibian or marine invertebrate oocytes or eggs to generate in vitro systems able to carry out steps of the cell cycle in vitro (Lohka and Masui, 1983). The ability to deplete and purify certain components from these complex cell-free extracts also allowed a biochemical analysis of these steps. This allowed the purification of maturation-promoting factor (MPF), a factor that promotes the onset of M phase (Lohka et al., 1988). Both approaches complemented each other, and the fact that the cell cycle and its control turned out to be highly conserved meant that studies could be made and compared in a variety of biological systems, each with its own advantages.

Cell Cycle Engines

Factors that could advance cell cycle progression were good candidates for components which act as rate-limiting steps in the cell cycle, and were identified genetically in fission yeast by mutants which accelerated cell division, and in amphibian eggs by the purification of MPF. A network of genes was characterized in yeast that regulated the onset of mitosis; core to this network was the Cdc2p protein kinase activated by the Cdc25p protein phosphatase and inhibited by the Wee1p protein kinase (Nurse, 1990) MPF was identified in *Rana* oocytes induced to enter M phase as part of the egg maturation process by injection with cytoplasm derived from eggs in M phase (Masui and Markert, 1971). MPF purified from *Xenopus* (Lohka et al., 1988) contained two proteins, one of 34 kDa that cross reacted with antibodies raised against the yeast Cdc2p protein kinase, and another protein that was shown to be a cyclin. Cyclins were originally discovered by workers searching for proteins that fluctuated in level through the cell cycle of cleaving marine invertebrates (Evans et al., 1983). It was reasoned that proteins behaving in this manner were important for controlling cell division. This work led to the identification of cyclin-dependent kinases (CDKs) made of a catalytic protein kinase subunit and a cyclin subunit. They were shown to act as universal cell cycle regulators

from yeast to mammals by the cloning of the human *CDC2* gene by complementation of a cdc2 mutant in fission yeast (Lee and Nurse, 1987). Interestingly, a cell cycle periodic CDK-like activity had been proposed as a mitosis regulator in the slime mold *Physarum* a decade previously, but experimental limitations of the slime mold unfortunately prevented this initial work from being developed further (Bradbury et al., 1974).

It has been proposed that CDKs act as a "cell cycle engine" (Murray and Hunt, 1993), driving cells through the cell cycle. Different CDKs control the onset of S phase and M phase (van den Heuvel and Harlow, 1993), and increasing the activity of these CDKs can advance both events. CDKs are regulated by the availability of the cyclin subunit, by changes in phosphorylation of a catalytic site tyrosine residue controlled by Cdc25p and Wee1p, and by association with CKI inhibitors (Nurse, 1990; Morgan, 1995). In metazoan cells CDKs act in early G1 to activate E2F-dependent transcription of genes required for S phase, in late G1 to initiate S phase, and finally in G2 to initiate mitosis. In the yeasts the range of CDKs is more limited and in certain circumstances the same CDK appears to initiate S phase at a low activity and mitosis at a high activity (Fisher and Nurse, 1996). The fact that a gradual increase in a single CDK activity can drive cells through the whole sequence of cell cycle events might indicate that a similar regulatory situation operated in the primeval eukaryotic cell. The higher levels of CDK activity present during G2 have also been shown to block initiation of a further S phase (Hayles et al., 1994), probably by regulating activity of the Cdc6p/Cdc18p initiator. This control helps to ensure that there is only one S phase in each cycle. Only when CDK activity falls as a consequence of cyclin proteolysis is the block over S phase initiation relieved and cells exit mitosis. The fall in CDK activity probably promotes replication complexes to form on origins of replication allowing cells to prepare for the S phase of the next cell cycle.

Two other regulatory processes that are very important for cell cycle progression are proteolysis and transcription. Controlled proteolysis plays a direct role in CDK regulation by controlling cyclin levels, and also contributes to other cell cycle steps such as the changes in sister-chromatid cohesion that occur when chromatids separate at anaphase/telophase (Murray and Hunt, 1993; Nasmyth, 1999). Proteolysis could contribute to the irreversibility of cell cycle transitions, or could be necessary to change the activities of complexes or protein machines important for these transitions. Cell cycle periodic transcription (Muller, 1995) of genes required for the onset of S phase are regulated during G1 by the E2F family of transcription factors, and if these genes are activated earlier in G1 then S phase can be advanced.

Checkpoint Mechanisms

The checkpoint concept has been a valuable aid to understanding the cell cycle (Hartwell and Weinert, 1989). The most studied checkpoints are the DNA damage and replication controls that block mitosis when DNA is damaged or DNA replication is incomplete. These checkpoints have surveillance systems that detect either specific DNA structures indicative of ongoing repair

or replication, or the presence of protein complexes engaged in repair or replication. A signal transduction pathway is then activated that either blocks onset of mitosis by maintaining Cdc2p tyrosine phosphorylation and preventing CDK activation, or blocks at a later stage of mitosis through other mechanisms. Another checkpoint blocks S phase after DNA damage and in mammalian cells this control requires the p53 tumor suppressor gene (Murray and Hunt, 1993).

The spindle checkpoint arrests mitotic progression if the spindle is not assembled, or if all the chromosomes are not properly oriented and attached to the spindle. It was revealed by showing that displacements of a chromosome from the spindle blocked further mitotic progression and by isolating yeast mutants that continue to divide even in the absence of a fully functional spindle (Hoyt et al., 1991; Li and Murray, 1991; Nicklas, 1997). The spindle checkpoint is thought to operate by monitoring whether kinetochores and microtubules are properly associated. If they are not, then the cohesion of sister chromatids is maintained and microtubules fail to shorten, and as a consequence the sister chromatids do not move apart to opposite poles of the cell (Chen et al., 1996). This control ensures that the precise replication of DNA at the molecular level leads to the precise segregation of the replicated DNA at the cellular level.

Checkpoints controls are essential for maintaining genomic stability. Failures of these checkpoints allow cells to divide when DNA is damaged or incompletely replicated, or when chromosomes are incorrectly partitioned, resulting in increased genetic damage. This is likely to be crucial for the generation of cancer, as suggested by the observation that p53 is required to block S phase after DNA damage and that checkpoint-activated cell cycle arrest can lead to programmed cell death (Murray and Hunt, 1993).

Turn of the Millennium
What will we learn next about the cell cycle? For the next 10–20 years, work will focus on the more obvious problems evident today. Given the importance of CDKs in cell cycle control, it is surprising how little is known about the interface between CDK activities and the implementation of cell cycle events. We need to know the molecular mechanisms by which CDKs directly or indirectly initiate DNA replication, prevent another S phase during G2, and bring about mitotic events such as chromosome condensation, nuclear envelope breakdown, and microtubular reorganization. This will require identification of CDK substrates in vivo, working out how substrate specificity is altered in different CDKs, and determining how quantitative changes in CDK activity can bring about either S phase or mitosis. Further molecular structural studies will lead to better understanding of how CDK activity is regulated, clarifying the effects of phosphorylation, and of interactions with different substrates and CDK inhibitors. It is also becoming evident that changes in the subcellular localization of CDKs and their regulators are important in controlling cell cycle progression.

The next obvious problem is the molecular basis of cell cycle checkpoint mechanisms. Checkpoint surveillance systems are of particular interest because they are extraordinarily sensitive, being able to detect tiny amounts of damage or minor errors, and to respond to sophisticated inputs of information such as assessments of the precise position and attachment status of a chromosome within a spindle. These studies are opening up a whole new area of signaling, whereby aspects of the intracellular environment are monitored and this information is communicated to other parts of the cell. Checkpoints have also provided innovative ways to think about new therapies for cancer. If failures in checkpoint controls turn out to be a frequent feature of cancerous cells and potential therapeutic targets exposed by checkpoint defects can be identified, then common strategies might be developed against a wide variety of cancers. This would present a more promising approach than unspecific attempts to block cell cycle progression, which are less likely to distinguish between cancerous and normal cells. Investigations of checkpoint controls in animal models and human cancers will be required to test the validity of this approach.

The molecular mechanisms underlying cell cycle steps such as the decondensation of DNA prior to replication, the assembly of the DNA replication apparatus, the condensation of chromosomes at mitosis, the cohesion of sister chromatids, the changes in microtubular dynamics at mitosis, and the process of cytokinesis, should soon be elucidated. There might also be other cell cycle steps that have yet to be discovered. Special partitioning mechanisms might exist for low copy number components or structures such as mitochondria to ensure that a sufficient number is inherited by both newly divided cells. Partitioning of such structures could involve association with the mitotic apparatus, or they could be temporarily converted to high copy components by fragmentation prior to cell division. Light microscopic observations of tagged proteins in living cells and monitoring patterns of gene activity revealed by genome-wide expression studies, should contribute to the identification of hitherto unrecognized cell cycle steps or events.

Our improved understanding of how the mitotic cell cycle operates needs now to be applied to the meiotic cell cycle. Meiosis differs from mitosis in two important ways. First, S phase is followed by two M phases, and second, chromosome number becomes reduced at meiosis I. The suppression of S phase between meiosis I and II may be due to sufficient CDK activity remaining after meiosis I to prevent the initiation of a further S phase, whilst the reduction in chromosome number may be due to the persistence of sister chromatid cohesion during mieosis I so that chromatids segregate to a single pole. If the developmental switch between meiotic and mitotic cycles could be manipulated, then it might be possible to carry out genetic life cycles in vitro by forcing diploid cells to undergo a reductional division to produce haploid "gametes" that could be fused to generate a diploid zygote. Such cells might also have high levels of recombination increasing rates of gene replacement.

A further topical subject for study is the interface between the cell cycle and development. Studies of this problem have already been started in *Drosophila* and have revealed that CDK regulation changes as embryos develop. In embryos and during tissue repair and maintenance, cells are stimulated to grow and to enter the cell cycle by signals generated by whole organ or organism

controls. The major cell cycle regulators need to respond to growth controls and developmental signals to ensure that cell division occurs with the correct spatial pattern in the organism and generates the right organ size. Insights will be obtained by knowing how the appropriate signal transduction pathways influence the activity of cell cycle regulators.

Into the Next Century

Parts of this final section may read as if I have been taking drugs, but rather wilder speculations are required when predicting advances that may occur far in the future. One of the major objectives in the next century should be a complete description and understanding of the eukaryotic cell cycle. This is achievable because although the cell cycle is a complex process, it is less complicated than most other developmental phenomena, and moreover its universality means that it can be studied in a wide range of biological systems making it less likely that any specific problem will become technically intractable. The aim should be to develop a full description of the molecular machines that make up the modules responsible for the different steps of cell cycle progression, to determine how these modules are linked together, and to demonstrate how their operation brings about the reproduction of the cell.

An important starting point for this program will be the whole genome sequences of the simple but distantly related budding and fission yeasts. These organisms do little else other than grow and divide, and their systematic functional analysis and comparison should allow the gene set underlying the eukaryotic cell cycle to be fully identified. The universal features of this gene set can be determined by comparison with other genomes, and these comparisons will also provide insight into the evolution of the cell cycle. The next objective will be the description of the molecular machines required to execute cell cycle steps . At the forefront here will be in vitro assays using complex systems like *Xenopus* egg cell-free extracts and reconstituted assays with purified components. The operation of these machines in vivo can be confirmed using genetically tractable organisms and by real time monitoring of the appropriate molecules in cycling cells.

These machines then need to be linked and integrated together to define the modules and overall regulatory networks required to bring about the reproduction of the cell. This task will require system analyses that emphasize the logical relationships between elements of the networks, information flow between the modules, how the networks operate dynamically and spatially within the cell, and how more global cellular characteristics such as increase in cell mass or elapse of time interface with the cell cycle. Conventional analyses of regulatory pathways seek for "first causes" upstream in the pathway and retreat into an infinite regress of regulators of regulators. In contrast the real objective should be to work out how the overall regulatory network responds to the changes in these global cellular characteristics.

Another objective will be to understand how a cell duplicates itself in space during the cell cycle. This involves problems ranging from the establishment of bipolarity at mitosis to the production of two identically organized spatial objects at cell division. It requires working out how signaling and organizing mechanisms operate beyond local molecular interactions and extend to the longer distances found at the scale of a cell. There could be roles for molecular gradients or for other fields that extend throughout a cell, and for self-organizing structures made up molecular polymers such as microtubules and their associated motors.

These experimental analyses will need to be complemented by new data handling methodologies and fresh conceptual approaches, both to deal with the high level of information generated, and to gain insight into how the regulatory networks and molecular machines actually operate. Moreover, it might also require a more profound break with the traditional thinking that has been so effective for cell and molecular biology to date. A Kantian "common sense" view of the world based on our everyday experiences has dominated past thinking. We imagine molecular mechanisms and cellular functions in terms of mind pictures of objects interacting with each other like tiny billiard balls organized together in linear causal pathways. This common sense view has difficulty dealing both with regulatory networks in which linear causality might not be the dominating feature, or with phenomena that exhibit complex changes in space and time. Dealing with these system properties, which ultimately must underlie our understanding of all cellular behavior, will require more abstract conceptualization than biologists have been used to in the past. A useful metaphor could be the changes in thinking associated with physics at the beginning of this century. Newtonian physics operates in the three-dimensional space and time of everyday experience and is easily contemplated by a human mind, which has evolved to function in such an environment. With relativity and quantum mechanics, physics moved from this accessible common sense world into a far more abstract one, much more difficult for the human mind to imagine and conceive. Perhaps a proper understanding of the complex regulatory networks making up cellular systems like the cell cycle will require a similar shift from common sense thinking. We might need to move into a strange more abstract world, more readily analyzable in terms of mathematics than our present imaginings of cells operating as a microcosm of our everyday world.

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