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Dominoes and Clocks: The Union of Two Views of the Cell Cycle

ANDREW W. MURRAY AND MARC W. KIRSCHNER

We review the recent advances in understanding transitions within the cell cycle. These have come from both genetic and biochemical approaches. We discuss the phylogenetic conservation of the mechanisms that induce mitosis and their implications for other transitions in the cell cycle.

THE CELL CYCLE IS THE SET OF EVENTS THAT IS RESPONSIBLE for the duplication of the cell. The recent advances in our understanding of the cell cycle have come from two approaches. Geneticists attempted to understand the cell cycle by analyzing mutations that arrested the cell cycle of somatic cells at specific points, whereas embryologists and physiologists examined natural points of cell cycle arrest and the agents that induced the embryonic cell cycle to proceed.

The genetic approach to the somatic cell cycle evolved from prokaryotic genetics in the 1950s and 1960s. With genetics, researchers successfully explained complicated processes, such as phage morphogenesis, as a linear sequence of events. The most extreme models of these processes suggested that they would resemble metabolic pathways: the initiation of each step in the pathway would be dependent on the completion of the preceding step, because the product of the earlier step was the substrate for the latter one; specific genes were assumed to execute each step. When this approach was applied to yeast, first in the budding yeast by Hartwell and his colleagues (*1*) and later in the fission yeast by Nurse and his colleagues (*2*), the result was a description of the cell cycle as a set of dependent reactions. The basis of this dependency is discussed in the accompanying review by Hartwell and Weinert (*3*). The physiological and embryological approach was championed by researchers who favored marine and amphibian eggs. They argued that eggs and oocytes were the simplest systems for studying the basic processes of the cell cycle, because they were specialized for rapid cell division. The result of their investigations was a description of the cell cycle as a biochemical machine that oscillated between two states, mitosis and interphase, and whose oscillations were independent of the completion of many of the cell cycle events. Initially the two views of the cell cycle, one as a set of dependent reactions (the domino theory) and the other as a biochemical oscillator (the clock theory), seemed incompatible.

The cell fusion experiments of Rao and Johnson (*4*) supported both points of view. The fusion of cells in mitosis with cells in any other state induced some form of mitotic response in the interphase nucleus and supported the embryological model of distinct mitotic and interphase cytoplasmic states, with the mitotic state dominant over all interphase states (*4*). Fusion experiments, however, also supported the idea of a dependent cell cycle, since in any fusion between two interphase cells at different stages of the cell cycle, the advanced nucleus waits for the completion of events in the retarded nucleus before progressing in the cell cycle (*4*).

In this review we discuss recent evidence from both traditions that has led to a unified view of the eukaryotic cell cycle. This synthesis suggests that a single biochemical mechanism underlies the cell cycle in all eukaryotic organisms. We have concentrated on the reactions that regulate progress through the cell cycle and do not discuss the mechanism of individual cell cycle events such as DNA synthesis or nuclear envelope breakdown and reformation.

A. W. Murray is in the Departments of Physiology and Biochemistry and Biophysics and M. W. Kirschner is in the Department of Biochemistry and Biophysics, School of Medicine, University of California at San Francisco, San Francisco, CA 94143–0448.

Identification of a Key Regulator of the Cell Cycle

The frog oocyte and egg have been widely used to study the biochemical reactions underlying the regulation of the cell cycle. The major cell cycle events from the oocyte through the early cell divisions are shown in Fig. 1, and the points at which natural cell cycle arrest occurs are indicated. The fully grown frog oocyte is arrested at G2, preceding the first meiotic division. Secretion of progesterone by the follicle cells induces the oocyte to proceed rapidly through meiosis I and prophase of meiosis II, before arresting at metaphase of meiosis II. The mature oocyte is released from the ovary, passes down the oviduct, and emerges as an unfertilized egg. Fertilization relieves the metaphase arrest and initiates a series of rapid (30 min in *Xenopus*), nearly synchronous, cell cycles that proceed without any detectable G_1 or G_2 phases until there is an abrupt change to a more complex and asynchronous cell cycle after 12 divisions (*5*). Although the initial studies of meiotic maturation focused on extracellular signals like progesterone, a major innovation was the use of cytoplasmic transfer by microinjection to identify intracellular regulators of meiosis. Masui and Markert (*6*) and Reynhout and Smith (*7*) both showed that the unfertilized egg contained a cytoplasmic activity (not progesterone), that could induce immature oocytes to undergo meiotic maturation. They named this activity maturation promoting factor or MPF. The oocytes that were injected with the unfertilized egg cytoplasm rapidly passed through meiosis and arrested at the normal metaphase arrest of the unfertilized egg (*6, 7*). When the oocyte entered the first meiotic division, it generated more MPF activity than was injected into it (*8, 9*). After meiosis I MPF activity disappeared, but reappeared and was maintained at high levels as the oocyte entered, and was stably arrested at, second meiotic metaphase in the unfertilized egg (*9*), suggesting that MPF is associated with the metaphase state.

The physiological induction of meiotic maturation by progesterone involves a complicated pathway, including a transient drop in cyclic adenosine monophosphate (cAMP) and a requirement for protein synthesis. Many agents, such as cAMP-dependent kinase inhibitors, sulfhydryl reagents, and ras protein, can induce protein synthesis–dependent oocyte maturation (*10, 11*). MPF is defined by its ability to bypass the protein synthesis requirement for inducing meiosis I and therefore seems to be the immediate inducer of the meiotic state. MPF activity is functionally conserved in eggs and oocytes of distantly related species, such as frogs and starfish.

MPF is found in both mitotic and meiotic cell cycles. MPF activity is present in extracts of yeast (*12, 13*) and mammalian cells (*14, 15*) and frog blastomeres (*6, 9,16*) that are in mitosis, but is not present in extracts prepared from interphase cells. MPF has recently been purified from frog and starfish eggs and is a protein kinase that phosphorylates histone H1 (*17, 18*). MPF seems to be identical to the growth-associated H1 kinase that was known for many years to appear during mitosis in a number of organisms (*19*). The catalytic subunit of MPF is probably $p34^{cdc2}$, the homolog of the product of the key fission yeast–cell cycle control gene, *cdc2*.

Although MPF stimulates oocytes to enter meiosis and is associated with the mitotic state, its exact role in mitosis was unclear. The function of MPF was illuminated by examining the role of protein synthesis in the early embryonic cell cycle. [In this review we will use the term embryonic cell cycle to refer to the early synchronous cleavage divisions and somatic cell cycle to cell cycles occurring after the completion of embryogenesis. The changes in the cell cycle that occur between the end of the synchronous cleavages and the end of embryogenesis are discussed in the article by O'Farrell *et al.* in this issue (*20*).] In the embryonic cell cycles of both frogs (*21*) and sea urchins (*22, 23*), protein synthesis was required for each mitotic division; in the absence of protein synthesis, cells arrested after DNA synthesis and before mitosis. Protein synthesis was required only during the first part of interphase, suggesting that there were posttranslational steps before the entry into M phase (*22, 24*).

Injection of partially purified MPF into protein synthesis–arrested embryos induced all of the events of mitosis (*21*), suggesting (i) that the protein synthesis requirement was for the synthesis of MPF itself or for the synthesis of some activator of MPF and (ii) that MPF was the key regulator of mitosis. MPF activity fluctuates during the early cleavage stages; it increases as cells enter mitosis and falls as they leave mitosis and enter interphase (Fig. 2). The rise in MPF was dependent on protein synthesis (*9*). It was not clear whether the newly synthesized component was MPF itself or an activator of MPF.

Was the oscillation of MPF itself responding to the nuclear or centrosome cycle or was it being driven by some autonomous biochemical cycle? Time-lapse movies of *Xenopus* and sea urchin eggs that were arrested from division by blocking microtubule assembly or by removing the nucleus and centriole continued to undergo periodic contractions of their cortex with the same period as the normal division cycle (*25, 26*). Measurements were made of MPF activity in enucleated (*16*) or colchicine-treated (*27*) frog eggs and were found to fluctuate with the same periodicity as the cortical contractions. Thus MPF oscillations can occur independently of the nucleus, the centriole, and microtubules. The experiments on MPF strongly suggest that its activation and inactivation drives the mitotic cycle in all eukaryotic organisms.

Cyclin and the Protein Synthesis Requirement for Entry into M Phase

What are the proteins that must be synthesized in order for the embryonic cell cycle to enter mitosis? Hunt and his colleagues examined protein synthesis in the first few cell cycles of sea urchin

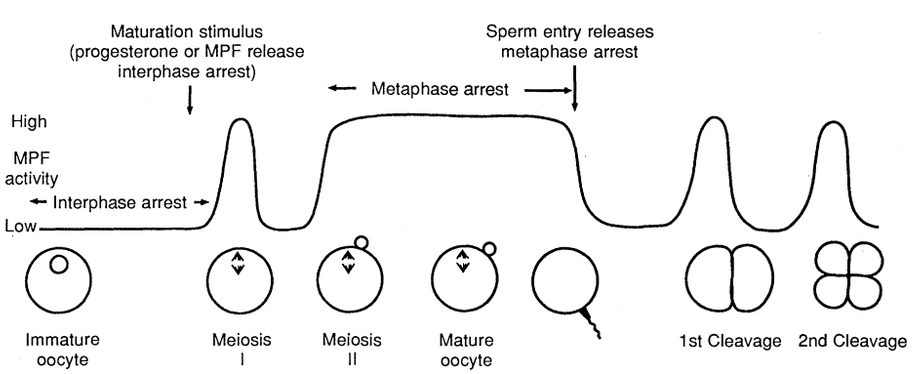

Fig. 1. The early frog life cycle. The appearance of the frog egg and oocyte and the level of MPF activity are shown during early early development. Points of cell cycle arrest and the stimuli that release these arrests are indicated.

eggs (*28*). Continuous labeling with [^{35}S]methionine showed that the abundance of most newly synthesized proteins increased continuously after fertilization, with one exception. A prominent protein of 56 kD, accumulated until the first mitotic division and then disappeared at the end of mitosis; this protein accumulated in the next interphase and again disappeared at mitosis. Pulse-labeling experiments showed that the fluctuation of the concentration of this protein was due to variations in its half-life rather than its rate of synthesis; the half-life was long in interphase and short in mitosis. Because of its cyclic appearance, this protein was named cyclin. Unfortunately, a totally unrelated protein, now thought to be an auxiliary protein of DNA polymerase δ (*29*), was called both proliferating cell nuclear antigen (PCNA) and cyclin (*30*). It is generally agreed that cyclin should be reserved for a specific class of proteins, discovered first in sea urchins and now known to be ubiquitous, that appear and disappear in the mitotic cycle. On the basis of sequence comparisons, cyclins have been divided into two classes (A and B) and most organisms contain both types (*31–39*).

Although the kinetics of cyclin accumulation (Fig. 2A) suggested that it might be MPF or an activator of MPF, there was no direct evidence for this hypothesis. The first link with MPF came when Swenson, Farrell, and Ruderman (*31*) injected the messenger RNA (mRNA) for clam cyclin A into *Xenopus* oocytes and induced maturation. A similar result was obtained by Pines and Hunt (*32*) with sea urchin cyclin B mRNA. Both groups had reservations in interpreting these experiments as showing that cyclin is MPF or an immediate activator of MPF, rather than one of many agents that perturb the progesterone pathway. These reservations stem from the failure to show that cyclin protein could induce maturation in the absence of protein synthesis (the definition of MPF activity). These reservations now seem justified because in clams, meiosis I can be induced in the absence of protein synthesis (*40*). Clam oocytes already contain cyclin B (*40*). Clam oocytes already contain cyclin B (probably in an inactive form), whereas in frogs the synthesis of a noncyclin protein (c-Mos) is required for maturation induced by progesterone (*41*). Despite these reservations, these experiments raised the possibility that the synthesis of cyclin was required for the activation of MPF in both mitosis and meiosis.

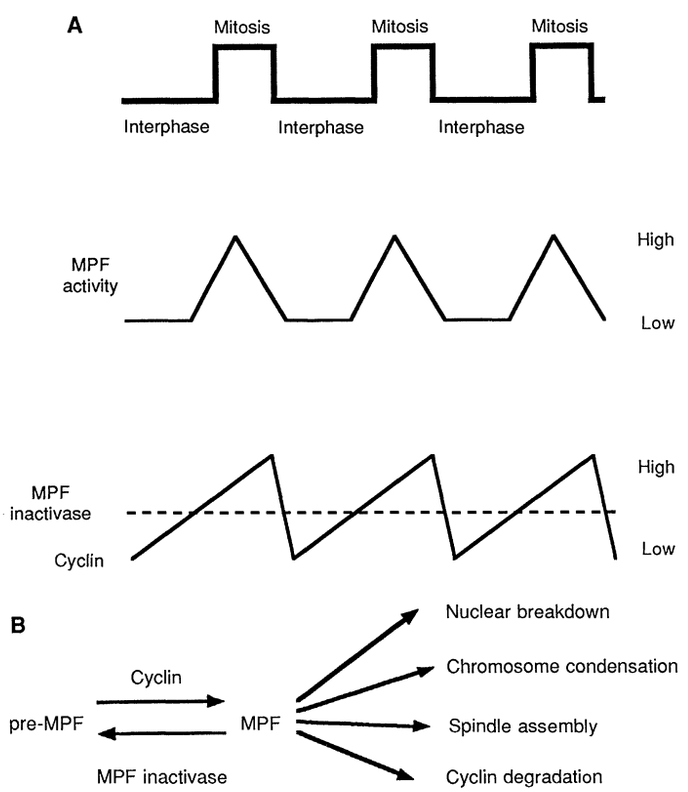

Fig. 2. (**A**) Fluctuation of cyclin and MPF levels in the embryonic cell cycle. From top to bottom the three traces show the alternation of mitosis and interphase, the level of MPF activity, and the abundance of cyclin superimposed on the activity of a hypothetical MPF inactivator. The cell cycle proceeds from left to right. (**B**) A model for the embryonic cell cycle. Pre-MPF denotes inactive forms of MPF, and MPF indicates the active form.

In order to demonstrate that cyclin synthesis had a direct role in the activation of MPF, it was necessary to turn to cell-free systems. Lohka and Masui (*42*) pioneered this approach for the cell cycle by making extracts that performed a single cell cycle. Hutchison *et al.* (*43, 44*) and our laboratory (*45, 46*) modified these preparations and made extracts of activated frog eggs that would go through multiple cell cycles as assayed by nuclear and chromosomal morphology (*44, 45*), DNA replication (*43, 44, 47*), oscillations in MPF and H1 kinase activity, and periodic accumulation and degradation of the frog cyclin homologs (*45*). These cell cycle extracts actively synthesized proteins and, when blocked in protein synthesis, arrested in interphase (*44, 45*). By degrading the endogenous mRNA with ribonuclease, it was possible to make protein synthesis in these extracts dependent on added exogenous mRNAs. The destruction of the endogeneous mRNA arrests the cell cycle in interphase. However, when sea urchin or frog cyclin mRNA was added to the nuclease-treated extracts, the cell cycle resumed (*45*). Under these circumstances the only protein made was cyclin, which accumulated and was destroyed at each mitosis, demonstrating that cyclin synthesis was sufficient to drive the embryonic cell cycle. The normal events of the cell cycle were entrained to the accumulation of cyclin, with the length of the cell cycle increasing as the rate of cyclin synthesis declined (*45*). Minshull *et al.* (*34*) performed a complementary experiment to demonstrate that specific destruction of endogenous cyclin B RNA arrested the embryonic frog cell cycle in interphase. Thus cyclin synthesis is both necessary and sufficient for entry into mitosis.

Cyclin is stable in cells that are arrested in meiosis (*46*) and mitosis (*28*), implying that cyclin degradation is required for cells to exit meiosis or mitosis. This idea was tested directly by producing a mutant cyclin, with 90 amino acids deleted from the NH_2-terminus, that was fully capable of activating MPF and driving cells into mitosis. However, this protein was not degraded, and as a consequence produced a stable metaphase arrest (*46*). Injection of the mRNA for the truncated cyclin into cleaving eggs also arrested cells at metaphase (*46*), showing that cyclin degradation was required to exit mitosis in vivo as well as in vitro.

A simple cell cycle model (Fig. 2) can be deduced from these experiments. In its original form this model postulated both an MPF inactivase (whose activity remained constant through the cell cycle) and an inactive from of MPF that could be activated by cyclin (*48*). During interphase, translation of cyclin mRNA causes cyclin protein to accumulate to a threshold at which the rate of activation of MPF by cyclin exceeds the rate of MPF inactivation by the inactivase, leading to the activation of MPF. Active MPF, which is a kinase, directly phosphorylates some of the proteins involved in the structural changes (such as nuclear envelope breakdown and chromosome condensation) during mitosis and also phosphorylates regulatory molecules that in turn lead to posttranslational modifications of other mitotic substrates. One of the consequences of the activation of MPF is the activation of the process of cyclin degradation. The disappearance of cyclin causes the destabilization of MPF by the constitutively present inactivase. Phosphatases reverse the phosphorylation of the protein substrates and result in the reestablishment of the interphase structures. The loss of MPF activity also turns off cyclin degradation so that cyclin can accumulate in the next cycle and

initiate another round of MPF activation. This model is consistent with all of the known components of the cell cycle, with the exception of the postulation of the MPF inactivase. If cyclin is a component of MPF required to maintain its activity, the inactivase may not be necessary. There is not yet any evidence for any enzymatic activity of cyclin, and its sole function may be to modulate the protein kinase activity of $p34^{cdc2}$, the catalytic subunit of MPF.

Genetic Analysis of the Somatic Cell Cycle

Genetic investigations of the cell cycle have concentrated on two yeasts: the budding yeast, *Saccharomyces cerevisiae* (*1, 49*), and the fission yeast, *Schizosaccharomyces pombe* (*2, 50*). In both organisms, investigators isolated a large collection of cell division cycle (*cdc*) mutants that arrest the cell cycle at a specific point and then analyzed the interactions of these mutants in detail. This analysis has revealed several of the organizing principles of the cell cycle and identified genes whose activity is required for the crucial transitions within the cell cycle. The study of cell cycle mutants has increased our understanding of how individual cell cycle steps (such as DNA synthesis and mitosis) are coordinated so that the events occur in the right order. The general principle of this coordination is that the initiation of each cell cycle step is dependent on the completion of the preceding step (*1*). Thus the initiation of mitosis is dependent on the completion of DNA synthesis, and the completion of mitosis is dependent on proper assembly of the mitotic spindle (*3*).

The analysis of cell cycle mutants has also revealed how cells maintain a constant average size over many cell divisions. This size regulation requires that the continuous events of the cell cycle, collectively referred to as cell growth, are coordinated with the cycle of stepwise events that includes DNA synthesis, centrosome duplication, and mitosis. If there is no coordination between growth and the stepwise events, the average cell size can be maintained only if the doubling time for cell mass is exactly equal to the length of the cycle of stepwise events. Genetic and physiological analysis of the budding yeast has shown that, in general, the cycle of stepwise events can be completed more rapidly than the cell can double its mass (*1*). These two processes are coordinated at a point in the cell cycle early in interphase that has been named Start (*51*).

Passage through Start is required to initiate the cycle of stepwise events that will lead to budding, DNA synthesis, spindle pole–body duplication, and ultimately mitosis (*49*). Coordination between growth and the stepwise events is ensured by requiring that cells reach a minimum size before the passage through Start can occur (*52*). In addition to its role in size control, Start is also the point in the yeast cell cycle at which cells respond to at least two types of external signal. One of these is the concentration of nutrients. Cells that are starved for any one of several nutrients are unable to pass Start (*53*). This starvation response ensures that cells do not attempt to pass through the cell cycle in the absence of proper nutrients, even if they are large enough to pass Start. Passage through Start can also be blocked by the presence of mating pheromones that arrest haploid yeast cells (allowing them to conjugate) at a point in the cell cycle just before Start (*49*). A point analogous to Start occurs during G_1 of mammalian tissue culture cells; it has been referred to as the Restriction point (*54, 55*) [discussed in the accompanying review by Pardee (*56*)]. In this review we will use the term Start to refer to the G_1 control point in both yeast and mammalian cells.

Two types of mutations that arrest the budding yeast cell cycle before Start have been isolated. One class arrests cells as unbudded, nongrowing cells and mimics nutrient starvation (*57*). The genes identified by these mutants are involved in generating the signals (such as elevated levels of cAMP) that report nutrient availability. The second class of mutants arrest cells as growing, unbudded cells. Mutants in some of these genes [*CDC36* and *CDC39* (*58*)] mimic the presence of mating pheromones, but mutants in the *CDC28* gene are defective in an activity that appears to be important in inducing passage through Start (*59*). The postulated role of *CDC28* in passage through Start is akin to that of MPF in inducing mitosis.

In the budding yeast the *CDC28* gene is required to induce passage through Start (*60*), and in the fission yeast the *cdc2* gene induces both passage through Start and mitosis (*61*). The experiments of Beach *et al.* (*62*) demonstrated that the *cdc2* gene of fission yeast and *CDC28* gene of budding yeast were functionally equivalent; the *CDC28* gene complemented mutations in *cdc2*. The protein product of these genes are homologous (*63, 64*). These findings have been extended by Lee *et al.* (*65*), who found a human homolog of *cdc2* that could complement mutations in *cdc2* in fission yeast. The product of the *cdc2* gene and its homologs in other organisms is referred to as $p34^{cdc2}$ and has homology to known protein kinases (*63, 64, 65*). In yeasts, Start and the induction of mitosis are controlled by regulating the activity of these gene products rather than their amount (*66, 67*). The protein kinase activity that can be precipitated by antisera to $p34^{cdc2}$ varies during the cell cycle (*66, 67*). In fission yeast the *cdc13* gene is also required for the induction of mitosis (*36*). A physical interaction between the *cdc2* and *cdc13* has been demonstrated (*68*).

In the fission yeast, the genetic analysis of the induction of mitosis has identified genes that regulate a major cell cycle transition, but are not essential for its execution. In particular, two genes, *wee1* and *cdc25*, act antagonistically to control the entry into mitosis (*69, 70*). Increasing the ratio of *wee1* to *cdc25* activity increases the cell size required for entry into mitosis, whereas decreasing this ratio decreases the critical cell size (*70*). The cell cycle can proceed in the absence of both genes. However, overexpression of *cdc25* in a strain mutant for *wee1* causes premature and often lethal entry into mitosis (*70*). In fission yeast much of the cell cycle regulation in response to cell size and nutrient availability occurs at the entry into mitosis rather than Start (*71*). It seems likely that signals that influence the entry into mitosis act in some way to alter the ratio of *wee1* and *cdc25* activities. Both *wee1* (*69*) and *nim1* (*72*), a gene that negatively regulates *wee1* activity, show homology to known protein kinases, suggesting that much of the regulation of mitosis involves changes in the pattern of protein phosphorylation.

The observation that the *CDC28* gene in budding yeast appears to function only at Start [but for a different view see (*73*)], whereas the homologous *cdc2* gene in fission yeast functions both at Start and mitosis (*61*), has led to the notion that the cell cycles of these two organisms may be fundamentally different. Specifically, it has been suggested that mitosis is initiated at Start in the budding yeast. Recent evidence suggests that this may not be the case and that homologs of *wee1* and *cdc25* have a role in the control of mitosis in budding as well as fission yeasts. Budding yeast contains a homolog of the fission yeast *cdc25* gene, and the simultaneous deletion of this gene and overexpression of the fission yeast *wee1* gene produce a G_2-like arrest in budding yeast, suggesting that the initiation of mitosis and the passage through Start are distinct events in budding yeast as well as fission yeast (*74*). The *Drosophila* gene named string, which is required for the later embryonic cell cycles, and whose accumulation appears to determine when those divisions occur, is homologous to *cdc25* (*75*).

A model for the pathway that leads to the induction of mitosis has been constructed from genetic investigations (Fig. 3). The entry into mitosis requires the coordinated activity of the *cdc2* and *cdc13* gene products and is regulated by the balance between the activities of *cdc25*, *nim1*, and *wee1* and by implication by changes in protein

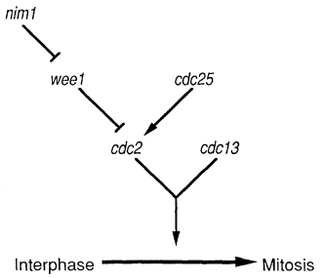

Fig. 3. A model for the induction of mitosis in the fission yeast. Gene products are shown as stimulating (→) or inhibiting (⊣) the activity of other gene products. The *cdc2* and *cdc13* gene products are shown acting in concert to induce mitosis. Based on (*72*).

phosphorylation. However, it is not clear what causes the ratio of these activities to change during the cell cycle. In addition, this model can explain the entry into mitosis, but it does not explain how mitosis is terminated. It is likely that protein phosphatases are important in the exit from mitosis. In both fission yeast (*76*) and *Aspergillus nidulans* (*77*), mutations in genes for type 1 protein phosphatases prevent the exit from mitosis. In fission yeast, overexpression of the type 1 phosphatase mutations can inhibit the entry into mitosis under a particular set of conditions (*78*). Whether the primary target of these phosphatases is MPF itself or the substrates that MPF phosphorylates is not yet known.

A Unified View of the Cell Cycle

A unified view of the eukaryotic cell cycle has emerged from the demonstration that key components of the embryonic and somatic cell cycles are the products of homologous genes. Thus a variety of experiments with immunological and affinity reagents have demonstrated that one of the subunits of MPF from frogs (*79, 80*), starfish (*18, 81*), and probably humans (*82*) (where MPF activity has not been assayed directly) is $p34^{cdc2}$. In addition, the product of the fission yeast *cdc13* gene has been shown to be homologous to cyclin (*36–39*). There is physical (*68, 83*) and genetic evidence (*36*) for an association between cyclin and $p34^{cdc2}$, and it has been proposed that MPF is a complex between these two proteins (*68, 83*).

There is now some agreement about the events that lead to the induction of mitosis. In cycling cells the level of $p34^{cdc2}$ is constant during the cell cycle, but cyclin must accumulate during interphase. Cyclin and $p34^{cdc2}$ must then associate to form a complex that as yet lacks MPF activity (*82*). This complex then undergoes poorly characterized posttranslational modifications [probably involving the dephosphorylation of $p34^{cdc2}$ and the phosphorylation of cyclin (*82*)] that lead to its conversion into active MPF. The activity of *cdc25* accelerates these changes, whereas that of *wee1* retards them. Once activated, MPF induces the degradation of cyclin, leading to the inactivation of MPF and the return to interphase (*45, 46*). Although the destruction of cyclin is necessary to inactivate MPF, it is currently unclear whether it alone is sufficient for the inactivation of MPF. One additional protein that may be required for the inactivation of MPF is p13, the product of the fission yeast *suc1* gene (*84, 85*).

We already know of a number of gene products that regulate the activation and inactivation of MPF; many more are likely to be discovered. Even if all these components are phylogenetically conserved, they will probably be present in different amounts in the cell cycles of different organisms, and different cell types within the same organism. Thus the components that are rate-limiting for the activation of MPF and whose accumulation, activation, or both appear to drive the entry into mitosis will differ in different cell cycles. For instance, there is evidence that the rate of cyclin accumulation is rate-limiting for the induction of mitosis in the early embryonic cell cycles of the frog embryo (*45*), but not in cell cycles 14 to 16 of the early fly embryo (*35*) or in the fission yeast cell cycle (*68*). In both the latter cases, it is likely that the activity of *cdc25* is rate-limiting for the entry into mitosis: in fission yeast, increasing the dosage of the *cdc25* gene increases the rate of entry into mitosis (*70*), and in cycle 14 of fly embryos, the time at which the transcription of *cdc25* is first detected predicts the time of entry into mitosis (*75*).

The existence of common components suggests that the somatic and embryonic cell cycles must be fundamentally similar. The primary distinction between these cycles is the much greater use of feedback controls in the somatic cell cycle. These controls generate dependency relations within the cell cycle and allow external factors to influence cell cycle progress. We view the cell cycle as being regulated by the activity of MPF and consider that there are only two functionally different levels of MPF activity, high and low, and that the change between them is very rapid. These two levels of MPF define two cell cycle states; cells with high levels of MPF are in mitosis, and those with low levels of MPF are in interphase. Since anaphase is initiated by the fall of MPF, in our definition it lies within interphase. Although this view may seem offensive to structural students of mitosis, we believe that it is the correct perspective in terms of cell cycle regulation. Both high and low levels of MPF activity eventually lead to stable cell cycle states: a configuration of the cell that does not change dramatically over time (Fig. 4).

In the case of mitosis, the stable state is metaphase. We distinguish this stable state from the cell cycle transition that leads to it: the reorganization of the interphase nucleus into a metaphase spindle. The process of reorganization induced by the transition includes the events of the stages described cytologically as prophase and prometaphase. We believe that the multiple changes that constitute this transition are initiated by a single event, the rapid rise in MPF activity, and that the complex sequence of events that produce the metaphase state is not a reflection either of processes that are activated by different levels of MPF activity (during the rise in MPF activity), or of intricate regulatory interactions between the constituent processes. We prefer to view this transition as the sum of several independent pathways, such as chromosome condensation, nuclear envelope breakdown, and spindle assembly, each of which is a series of biochemical reactions that is initiated by active MPF. Within each of these pathways, dependencies may arise because the product of one step in the pathway is required as the substrate for the next.

The inactivation of MPF leads to the stable interphase state: a cell containing an intact nucleus that has completed DNA replication. The fall in MPF activity induces a series of events (anaphase, the reorganization of the nucleus, and the initiation of DNA replication) that lead from metaphase to the stable interphase state. Anaphase and reformation of the nucleus appear to be parallel, independent processes that are both initiated by the decline in MPF activity. In contrast, there is evidence that the initiation of DNA replication is dependent on the reformation of the nuclear envelope (*86*).

It seems likely that all cell cycles are organized so that transitions between cell cycle states are initiated by changes in the activity of protein complexes containing $p34^{cdc2}$. The complexity of these transitions reflects a number of parallel pathways, each composed of a series of biochemical steps that are initiated by the changes in $p34^{cdc2}$ activity. Within this overall framework the somatic cell cycle (Fig. 5) differs from that of embryonic cells in two major respects. The first is the presence of a system of feedback controls that ensure that the processes that occur during the transition to one cell cycle state are completed before the transition to the next cell cycle state is initiated [discussed in the accompanying review by Hartwell (*3*)].

The second specialization of the somatic cell cycle is the subdivi-

sion of interphase into two states by Start, the regulated cell cycle transition that allows the coordination of the stepwise events of the cell cycle with cell growth and external signals. After entering interphase and before passing Start, the cell completes the processes of anaphase, nuclear envelope assembly, and cytokinesis, but is prevented from replicating its DNA or its microtubule organizing center (MTOC). This defines a new stable cell cycle state, a cell with an intact nucleus that has not replicated its DNA or its microtubule organizing center (MTOC). This defines a new stable cell cycle state, a cell with an intact nucleus that has not replicated its DNA and has a single MTOS. After Start has occurred, the cell is committed to the initiation of DNA replication and proceeds to a stable state with an intact nucleus and replicated DNA and MTOC. In this view of the somatic cell cycle there are three stable states: metaphase, pre-Start interphase (in traditional terms the part of G_1 before Start), and postreplication interphase (traditionally called G_2). In budding yeast, Start is the main point at which the cell cycle is controlled in response to cell size and external conditions (*49*), whereas in the fission yeast much of this control is exerted at the initiation of mitosis (*50*). Mammalian cell cycle regulation has been proposed to occur mainly at Start (*87*), but examples of control over G_2 have also been reported (*88*).

The requirement for *cdc2* activity for both passage through Start and for the induction of mitosis (*61*) suggests that the mechanisms that bring about these two cell cycle transitions are fundamentally similar. This conclusion is reinforced by the execution of cell size and nutrient controls at Start in budding yeast and entry into mitosis in fission yeast. Specifically, we postulate that $p34^{cdc2}$ interacts with a cyclin-like molecule at Start to induce a protein kinase activity that leads to the commitment to DNA replication.

Evidence that cyclin is involved at Start comes from the study of the $DAF1^c$ mutant [a similar mutant in this gene had been previously isolated as *whi1-1* (*89*)], which prevents the pre-Start cell cycle arrest that mating pheromones induce in haploid budding yeast cells (*90*). In the absence of mating pheromones, $DAF1^c$ cells go through Start at a smaller size than wild-type cells. In contrast, deletion of the *DAF1* gene leads to cells passing Start only at a size substantially larger than wild-type cells (*90*). Thus this gene appears to have a role in setting the critical size for passage through Start, suggesting that mating pheromones may arrest the cell cycle by effectively setting the cell size required to pass Start to be infinitely large. When the dominant $DAF1^c$ and *whi1-1* mutants were cloned and sequenced, they proved to be COOH-terminal deletions of a cyclin-like molecule (*89, 90*). Since deletions of a sea urchin cyclin (albeit at the NH_2-terminal) rendered it resistant to proteolysis (*46*), it is tempting to speculate that the $DAF1^c$ mutant produces a gene product that is more stable than its wild-type counterpart. In wild-type cells, Start would require the accumulation of a cyclin-like molecule to some critical concentration. Mating pheromones would arrest the cell cycle by decreasing the half-life of this protein, preventing it from ever reaching the critical level required to pass Start. However, in $DAF1^c$, mating pheromones would be unable to destabilize the truncated cyclin and would therefore fail to arrest the cell cycle. Two other budding yeast cyclin genes have been isolated as suppressors of *cdc28* mutants (*91*). Deletion of both these genes and $DAF1^c$ leads to cell cycle arrest in, G_1 whereas deletions of one or two of the cyclin-like genes are viable (*92*), suggesting that there is functional redundancy among the cyclins that are involved in passage through Start.

The idea that all the major control points of the cell cycle are regulated by the interaction between $p34^{cdc2}$ and cyclin-like molecules suggests a biochemical basis for the feedback controls that regulate the cell cycle. One such control prevents cells from exiting

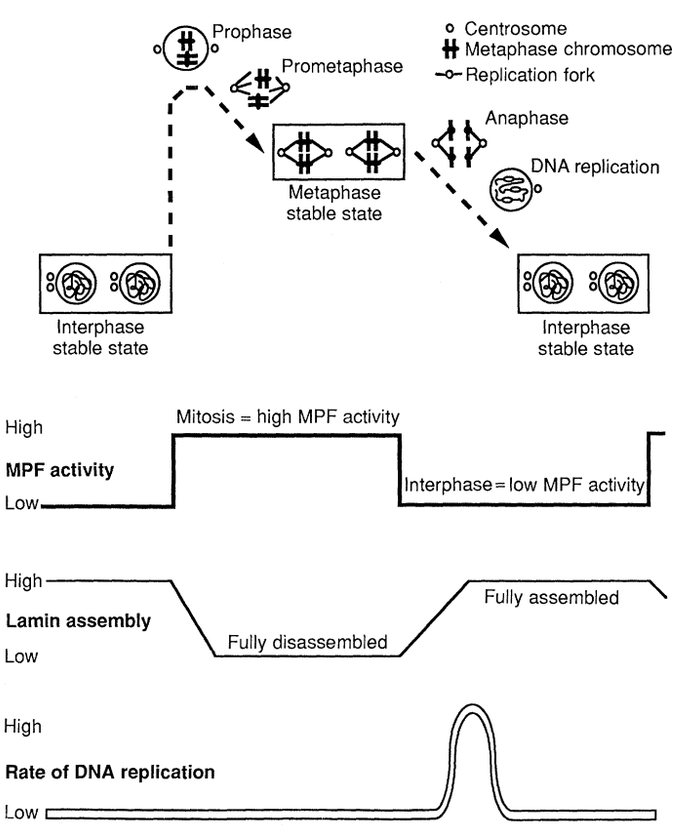

Fig. 4. A representation of the embryonic cell cycle. One and a half cell cycles are shown proceeding from left to right. The stable states are boxed and the transitional intermediates are shown besides the arrows that connect the stable states. The vertical distance between interphase and prophase indicates that the activation of MPF makes the intact interphase nucleus energetically and kinetically unstable. The three lower traces from top to bottom show the activity of MPF, the extent of nuclear lamin assembly, and the rate of DNA replication. Note that lamin assembly and disassembly and DNA replication only occur during the transitions between stable states.

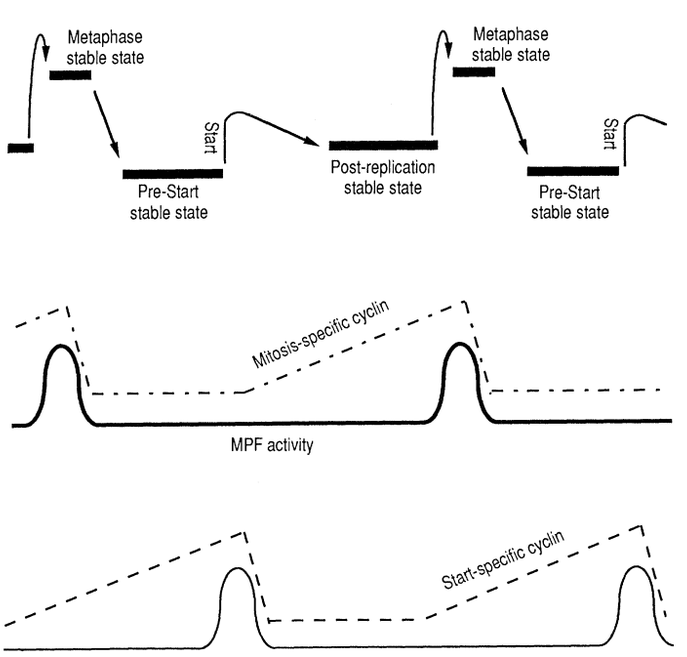

Fig. 5. A representation of the somatic cell cycle. The somatic cell cycle is shown as consisting of three stale states: metaphase, pre-Start interphase, and post-replication interphase. The transitions between these states are indicated by arrows.

mitosis until the spindle has been correctly assembled. We postulate that a signal generated by some aspect of an incorrectly assembled spindle (such as kinetochores that are not stably attached to spindle microtubules) prevents the degradation of cyclin and therefore the exit from mitosis. A precedent for feedback control over the exit from mitosis exists in unfertilized frog eggs, which are arrested in metaphase of meiosis II by virtue of an activity named cytostatic factor (CSF) (*93*). The presence of CSF renders cyclin stable in the presence of high levels of MPF (*46*). The CSF-mediated cell cycle arrest is released by fertilization, which triggers an increase in the cytoplasmic calcium concentration that leads to the inactivation of CSF (*94*). CSF activity is not found during the early mitotic cell cycles of the frog embryo (*93*). We speculate that a CSF-like activity exists in somatic cells and prevents them from leaving mitosis until the activity is inactivated as a result of a signal that is generated by the proper assembly of the mitotic spindle.

Somatic cells and some early embryos possess feedback controls that prevent cells that have not finished DNA replication from entering mitosis. These controls might act on the accumulation or posttranslational activation of cyclin. The latter possibility is suggested by the observation that when sea urchin eggs that are treated with aphidicolin fail to enter mitosis, they accumulate cyclin but fail to phosphorylate it (*95*). In control cells, the appearance of this phosphorylation correlates with the appearance of MPF activity (*96*).

Unresolved Issues

Although this model successfully explains some features of the cell cycle, it still leaves a great many unanswered questions. The simple embryonic cell cycle without feedback controls is closest to a complete description. There is evidence that cyclin accumulation leads to the activation of MPF, which in turn leads to the destruction of cyclin and the inactivation of MPF (*34, 45, 46, 83*). Although this basic scheme explains the entry into and exit from mitosis, it is not an explanation of how multiple cycles of MPF activity are produced. In general, collections of reactions in which the product of one reaction influences the rate of another eventually reach a steady state. In order to produce a system that continually cycles, it is necessary to introduce time delays (for instance, a lag between MPF activation an the occurrence of cyclin degradation) and autocatalysis (for instance, the ability of MPF to catalyse MPF activation).

In order to identify the features that produce multiple cell cycles, it will be necessary to understand the detailed mechanism of MPF activation, cyclin degradation, and MPF inactivation. This will include:

1) Identifying currently unknown components involved in these processes.

2) Determining the role of cyclin and $p34^{cdc2}$ phosphorylations. Phosphorylation of cyclin has been reported to correlate with the activation of MPF (*82, 97*), whereas an increase in $p34^{cdc2}$ phosphorylation has been reported to occur on release of quiescent cells from pre-Start arrest (*67, 98*). Decreases in the overall phosphorylation of $p34^{cdc2}$ (*99, 100*) and in the amount of phosphotyrosine in $p34^{cdc2}$ (*101*) have been reported to correlate with, and may be required for, the activation of MPF. However, there have also been reports that the overall phosphorylation (*82*) and tyrosine phosphorylation (*102*) of $p34^{cdc2}$ increase on entry into mitosis.

3) Determining the mechanisms of cyclin degradation and MPF inactivation. In vivo the degradation of cyclin leads to the rapid inactivation of MPF (*46*). If the association of cyclin with $p34^{cdc2}$ is required for both the maintenance and establishment of MPF activity (even in the absence of other proteins) then no other steps are required for the inactivation of MPF. However, if cyclin is not required under all circumstances for the maintenance of MPF activity, then the in vivo inactivation of MPF may require a specific MPF inactivase. There is evidence for forms of MPF in vitro that lack cyclin (*18*), and activities that prevent the activation of MPF (*103*) and may inactivate MPF.

4) Understanding how the key cell cycle regulators like MPF influence specific biochemical and structural changes. Histone H1 is the only identified substrate of MPF that may be involved in the induction of mitosis (*17, 18*), and it seems unlikely that all the changes of mitosis are induced by phosphorylating H1.

A number of additional unanswered questions exist for the somatic cell cycle. One of these concerns the effect of Start on the ability to replicate chromosomal DNA. In all cells the chromosomal DNA is replicated only once in each cell cycle, as a result of some modification of the DNA template during replication (*47, 104*). In embryonic cells, where the DNA replication machinery is active throughout the cell cycle, passage through mitosis reverses this modification to allow a new round of DNA replication (*105*). Does the exit from mitosis reverse the template modification in somatic cells, or is passage through Start also required?

If $p34^{cdc2}$ and cyclin-like molecules are controlling both Start and the entry into mitosis, how are these two cell cycle transitions differentiated from each other? The fission yeast *cdc13* gene, which is a member of the cyclin B family, is required for entry into mitosis, but not for passage through Start (*36, 39*). Perhaps there are both mitosis-specific (cyclin B) and Start-specific cyclins (cyclin A)? Other possible differences between Start and mitosis include (i) substrates for $p34^{cdc2}$-containing kinases that are present at one point in the cell cycle, but not at the other point and (ii) the use of different $p34^{cdc2}$-like molecules at Start and mitosis. There are multiple members of this protein family (*106, 107*), although at least in fission yeast the genetic evidence demonstrates that the same protein is used both at Start and mitosis (*61*).

How is the cell cycle constructed so that Start and mitosis always alternate with each other? There must be some regulatory interaction between these two $p34^{cdc2}$-driven cell cycle transitions. For instance, a regulatory network might make it impossible to accumulate a mitosis-specific cyclin until the cell had passed Start, and impossible to accumulate a Start-specific cyclin until the cell had exited from mitosis.

Even if the questions posed above are answered, and the feedback controls described by Hartwell (*3*) are understood, we will not have achieved a complete description of the cell cycle, much less a "grand unified theory." First, there are cell cycle components that current models do not include. In *Aspergillus*, Osmani and Morris have identified and characterized *nimA* as a gene whose activity is required for entry into mitosis (*108, 109*) and *bimE* as a gene that prevents premature entry into mitosis (*110*). Neither of these genes is homologous to any of the regulators of the cellcycle identified in other organisms (*110*).

Second, there is strong evidence that there are other cycles, independent of both $p34^{cdc2}$ and cyclin, that exist within the cell cycle. Mitchison and his collaborators showed that even when the fission yeast cell cycle was arrested by temperature-sensitive *cdc2* mutants, the activity of the enzyme nucleotide diphosphate kinase (NDPK) and the rate of CO_2 production underwent periodic fluctuations (*111, 112*). In frog embryos (*113*), periodic centrosome duplication continues to occur even after the cell cycle has been arrested in interphase by protein-synthesis inhibitors. The basis of these cycles is obscure, as are the mechanisms that normally entrain them to the cyclin-$p34^{cdc2}$ cycle.

Despite these deficiencies, our knowledge of the biochemistry of cell cycle transitions is at last on a firm foundation. To a large extent

this knowledge has been gained without illuminating the mechanisms by which these transitions induce specific cell cycle events such as DNA and centrosome replication, nuclear envelope breakdown, or spindle assembly. Hopefully this omission will be remedied by the next cycle of cell cycle research.

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