Cyclin synthesis and degradation and the embryonic cell cycle

ANDREW W. MURRAY

Department of Biochemistry and Biophysics, University of California at San Francisco,
San Francisco, CA 94143-0448, USA

Summary

I discuss recent advances in the study of somatic and embryonic cell cycles. In the frog embryonic cell cycle, cyclin is the only newly synthesized protein required to activate maturation-promoting factor and induce mitosis. Diminishing the rate of cyclin synthesis increases the length of interphase. Cyclin degradation is required for the progression from mitosis to interphase. Comparison of the frog embryonic cell cycle to other cell cycles suggests that all cell cycles will rely on the same closely conserved set of components. However, the component that is rate-limiting for any step in the cell cycle will vary in different cell cycles.

Introduction

For many years the investigation of embryonic and somatic cell cycles had proceeded independently. The identification of p34\textsuperscript{cdk2}, the protein product of a major somatic cell cycle control gene, as a subunit of maturation-promoting factor (MPF), the protein complex that induces mitosis in embryonic cell cycles (Dunphy et al. 1988; Gautier et al. 1988; Labbé et al. 1988), has linked the two previously separate areas of cell cycle research. In this article I will briefly discuss the genesis of the conventional views of the embryonic and somatic cell cycles, describe how the synthesis and degradation of a single protein, cyclin, can drive the embryonic cell cycle, and finally discuss ways of rationalizing the differences between the embryonic and somatic cell cycles.

The somatic cell cycle

The key advances in our understanding of the somatic cell cycle have come from genetic studies on two yeasts: the budding yeast, \textit{Saccharomyces cerevisiae} and the fission yeast, \textit{Schizosaccharomyces pombe}. This approach was pioneered by Hartwell (reviewed by Hartwell, 1978), who argued that the underlying logic of the cell cycle could be revealed by isolating mutants that arrested the cell cycle at specific stages, studying their phenotypes and examining the interaction between different cell cycle mutants. He and his colleagues found that the cell cycle could be largely described as a series of steps where the initiation of each step required the completion of the preceding step in the cell cycle. Thus cells which have not completed DNA synthesis fail to enter mitosis, and cells which have failed to correctly assemble a mitotic spindle do not make the transition between metaphase and anaphase (Hartwell,

Key words: cell cycle, MPF, proteolysis, cdc2.
Fig. 1. The somatic cell cycle in budding yeast. The cell cycle is divided into four stages: G\textsubscript{1}, S, G\textsubscript{2} and M. The main points in the cell cycle where progress is dependent on earlier events in the cell cycle are shown as STOP signs labelled with the conditions that must be met in order for the cell cycle to continue. START is shown as a single point in G\textsubscript{1}. Passage through START requires adequate nutrients and an attainment of a critical size. At the edge of the circle the appearance of budding yeast cells at different points in the cell cycle is shown (the nucleus is shown solid).

1978). (I believe that it makes good sense to regard this transition as marking the end of mitosis and the beginning of the subsequent interphase.) This type of cell cycle has been described as a dependent cell cycle and this view is supported by experiments in which tissue culture cells in different stages of the cell cycle were fused to each other (Rao and Johnson, 1970). The dependent cycle can be viewed either as a traditional biochemical pathway where the product of each step acts as a substrate for the next, or as a series of essentially independent events that are linked to each other by feedback controls. The latter view is illustrated in Fig. 1, where the feedback controls have been illustrated as points where the cell cycle stops until certain conditions have been met: the boundary between G\textsubscript{2} and mitosis, which cannot be crossed until DNA synthesis is complete; the boundary between metaphase and anaphase, whose passage requires the correct assembly of the mitotic spindle, and a special point called Start where cells must have attained a certain minimum size and have sufficient nutrients available in order to commit themselves to passage through the next cell cycle. Between the end of mitosis and Start, cells can enter a specialized resting phase called G\textsubscript{0}; they cannot then re-enter the mitotic cell cycle without passing through Start. The strongest evidence for feedback controls is the existence of mutants and drugs that destroy the normal dependency relations in the cell cycle: for instance the rad9 mutant of budding yeast allows cells that have failed to repair DNA damage to pass through mitosis (and in doing so kill themselves; Weinert and Hartwell, 1988), while tissue culture cells that are arrested in S phase can be induced to enter mitosis by treatment with caffeine (Schlegel and Pardee, 1986).
Nurse and his colleagues have performed a genetic analysis of the cell cycle of the fission yeast (reviewed by Lee and Nurse, 1988). Their main interest has been the induction of mitosis. The key player in this transition is the product of the cdc2 gene known as p34cdc2, whose ability to induce mitosis is increased by the activity of the cdc25 gene and diminished by the activity of the wee1 gene (Fantes, 1979). Altering the relative dosage of the wee1 and cdc25 genes affects the minimum cell size required to enter mitosis, and extreme alterations lead either to arrest in G2 as very large cells (no cdc25 function), or entry into mitosis at a cell size that is so small that it kills the cells (excess of cdc25 function in the absence of wee1 function; Russell and Nurse, 1986). Since the cell cycle length of fission yeast is mostly regulated by controlling the length of G2, cell size and nutrient availability must interact in some way with the gene products that control the activity of cdc2.

Intriguingly, the activity of cdc2 is also required for passage through Start (Nurse and Bissett, 1981), although the cdc25 and wee1 genes do not appear to influence the rate of this transition. The fission yeast cdc2 is functionally interchangeable and homologous with the budding yeast CDC28 gene (Beach et al. 1982). In budding yeast, the activity of CDC28 is clearly required for passage through Start but has not been shown to be required for the induction of mitosis, although it can clearly act in the induction of mitosis when it is expressed in the fission yeast (Beach et al. 1982).

The embryonic cell cycle

Studies on the cell cycle of early embryos began with the cytologists of the late 19th and early 20th century (reviewed by Wilson, 1928) and received their modern stimulus from the discovery of a protein complex which could induce meiosis when injected into immature oocytes (Masui and Markert, 1971; Reynhout and Smith, 1974) and was therefore named maturation-promoting factor (MPF). MPF is conventionally assayed by its ability to induce maturation in frog oocytes and has been detected by this assay in extracts from cells in either mitosis or meiosis from a wide variety of eukaryotes, demonstrating both its ubiquity and its functional conservation during evolution (Kishimoto and Kanatani, 1977).

MPF has recently been purified from frog eggs by Lohka and Maller and shown to be a protein kinase with strong activity towards histone H1 (Lohka et al. 1988). A number of criteria suggest that MPF is identical to the growth-associated H1 kinase previously identified in extracts of mitotic tissue culture cells (J. Maller, personal communication). Immunological tests demonstrate that one of the subunits of MPF purified from frog or starfish eggs is the product of the homologue of the cdc2 gene (Dunphy et al. 1988; Gautier et al. 1988; Labbé et al. 1988) and a similar conclusion has been reached for the mitotic H1 kinase of HeLa cells studied by Draetta and Beach (1988).

The work of Kirschner, Gerhart, Newport and their colleagues has shown that the embryonic frog cell cycle is driven by fluctuations in the activity of MPF (reviewed by Kirschner et al. 1985). High levels of MPF activity induce entry into mitosis (Newport and Kirschner, 1984), while MPF activity decreases as the cells leave.
mitosis and enter interphase (Gerhart et al. 1984). Unlike the somatic cell cycle there are no detectable feedback controls in this embryonic cell cycle: inhibiting DNA synthesis, microtubule assembly, or even removing the egg nucleus all fail to prevent the regular oscillation of interphase and mitotic states (Hara et al. 1980). This cell cycle has therefore been called an autonomous oscillator, to indicate that there is some biochemical machinery that oscillates in a way that drives nuclear events but is not dependent upon them (Fig. 2).

Protein synthesis is required during interphase of each embryonic cell cycle for the occurrence of the subsequent mitosis (Wagenaar, 1983), suggesting that at least one of the components required for the induction of mitosis must be synthesized de novo in each cell cycle. Since the protein synthesis requirement can also be met by the injection of MPF (Newport and Kirschner, 1984) it appears that the newly synthesized proteins must act somewhere on the pathway of events that leads to the activation of MPF. Hunt and his colleagues discovered cyclin, a prominent candidate for a newly synthesized inducer of MPF activity, when they examined the pattern of protein synthesis in early sea urchin embryos (Evans et al. 1983). The abundance of cyclin increases during each interphase only to decline precipitously at the end of each mitosis. The level of cyclin is regulated entirely by controlling the half-life of the newly synthesized protein: it is long during interphase and becomes extremely short at the end of mitosis (Evans et al. 1983).

**Cyclin induces mitosis**

The kinetics of cyclin appearance and disappearance suggest an attractive model for
the embryonic cell cycle (Murray, 1987; Murray and Kirschner, 1989). Fig. 3 shows this model, where we suggest that there are two competing activities: cyclin, which stimulates the conversion of an inactive form of MPF into an active one, and a so far hypothetical MPF inactivase which converts the active form of MPF into the inactive one (Fig. 3). While cyclin abundance fluctuates during the cell cycle, the activity of the MPF inactivase remains constant. The last postulate is that MPF induces its own degradation as well as inducing nuclear envelope breakdown, chromosome condensation and spindle assembly. These components create a version of the autonomous oscillator. At the beginning of each interphase the level of cyclin is low, so that the activity of the MPF inactivase is dominant and MPF is kept in its inactive form. As interphase proceeds the level of cyclin increases until the rate of MPF activation exceeds that of MPF inactivation and MPF activity increases, leading to the induction of mitosis. However, the appearance of MPF activity leads ultimately to the destruction of cyclin, the subsequent inactivation of MPF and exit from mitosis.

A number of lines of evidence support this scheme. Clam cyclin A (Swenson et al., 1986) or sea urchin cyclin B (Pines and Hunt, 1987) mRNAs can induce meiotic maturation when injected into immature frog oocytes. As far as the exit from mitosis is concerned, in both clams (T. Hunt and J. Ruderman, personal communication) and flies (Lehner and O'Farrell, 1989) cyclin is destroyed immediately before the onset of anaphase, and in starfish, protease inhibitors can block the exit from meiosis.
I (Picard et al. 1985). Suggestive as these lines of evidence are, none of them directly demonstrates that cyclin accumulation induces mitosis, or that cyclin degradation is required for the exit from meiosis.

To address these issues, we turned to in vitro cell cycle extracts of frog eggs that were prepared by an adaptation of the method pioneered by Lohka and Masui (1983). These cell cycle extracts are capable of several cell cycles that proceed at rates similar to those of the in vivo cell cycle (Hutchison et al. 1987; Murray and Kirschner, 1989) and closely mimic the properties of the in vivo cell cycle: in interphase the nuclei are intact, DNA synthesis occurs (Blow and Watson, 1987; Hutchison et al. 1987) and the activity of MPF and H1 kinase are low (Murray and Kirschner, 1989); as mitosis is initiated the activity of MPF and H1 kinase increases, the chromosomes condense, nuclear envelope breakdown occurs and at the end of mitosis cyclin is degraded (Hutchison et al. 1988; Murray and Kirschner, 1989).

The cell cycle extracts can be treated with pancreatic RNase followed by RNase inhibitor, so that their mRNA is destroyed but they retain the ability to synthesize proteins in response to exogenous mRNA added after the RNase treatment. These mRNA-dependent extracts are arrested in interphase but can be induced to start cycling by adding pure sea urchin or frog cyclin B mRNA. In each cycle, cyclin accumulates during interphase and is then destroyed at the end of mitosis, demonstrating that cyclin synthesis, in the absence of other protein synthesis, is sufficient to induce the entry into mitosis (Murray and Kirschner, 1989). By specifically destroying cyclin mRNA, Minshull et al. (1989) have shown that cyclin B synthesis is necessary for the induction of mitosis in frog cell cycle extracts. The finding that cyclin synthesis is both necessary and sufficient for the induction of mitosis in frog egg extracts makes it likely that in vivo, cyclin is the only newly synthesized protein required for the induction of mitosis.

Is cyclin accumulation the rate-limiting step, or trigger, of mitosis in frog embryos? In the mRNA-dependent extracts, the length of the cell cycle increases as the amount of added cyclin mRNA decreases, suggesting that cyclin accumulation can be rate-limiting for the induction of mitosis (Murray and Kirschner, 1989). On the other hand, experiments in vivo show that inhibiting protein synthesis during the second half of interphase does not prevent or delay the next mitosis (Karsenti et al. 1987; Wagenaar, 1983). The combination of these observations suggest that interphase of the frog embryonic cell cycle may be divided into two stages: an initial stage during which cyclin must be accumulated to some critical level in order to ensure the subsequent entry into mitosis, and a later stage during which post-translational events lead to the activation of MPF. Since the rate of cyclin synthesis will affect the duration of the initial stage but will not grossly affect the rate of the post-translational modification step, increasing the rate of cyclin synthesis will not be able to diminish the length of interphase indefinitely. I will address the identity of the triggers of mitosis in other cell cycles below.

The role of cyclin destruction in the exit from mitosis was investigated by constructing a truncated cyclin that was capable of inducing mitosis but not of being degraded in mitotic cytoplasm. Introduction of the mRNA for this protein either
into frog cell cycle extracts or intact frog eggs arrested the cell cycle in metaphase but did not prevent the destruction of the endogenous full length cyclin (Murray et al. 1989). This experiment demonstrates that the truncated cyclin is a dominant inhibitor of the exit from mitosis and therefore that cyclin degradation is required for exit from mitosis.

Structure of cell cycles
How can we relate the autonomous oscillations of the embryonic cell cycle to the dependent pathways of the somatic one? The cyclin-based cell cycle described above appears to match the properties required of the autonomous oscillator. In this scheme mitosis and interphase alternate because both cell cycle states are unstable: interphase because the accumulation of cyclin leads to the induction of mitosis, and mitosis because the activity of MPF leads ultimately to the destruction of cyclin. We speak of the trigger of mitosis as the step in the activation of MPF that is rate-limiting for the induction of mitosis. Even if the set of reactions required for the activation of MPF is evolutionarily conserved, the step that is rate-limiting may be different in different organisms and in different cell cycles in the same organism. For instance, although the rate of cyclin accumulation appears to be rate-limiting for at least one part of interphase in the early embryonic frog cell cycle, it is clearly not rate-limiting for the post-cellularization cell divisions of the Drosophila embryo (Edgar and O'Farrell, 1989; Lehner and O'Farrell, 1989). In this case the rate-limiting step appears to be the synthesis of the homologue of the cdc25 gene that is probably required for the slow post-translational steps after cyclin accumulation has been completed. If this step is very slow relative to the rate of cyclin accumulation, then the rate of cyclin synthesis would have to be very drastically reduced before it became rate-limiting. Because there is no requirement for cdc25 synthesis in the early frog cell cycle, this function must either not be required, or more likely supplied by maternally inherited stores of the cdc25 protein.

In the frog embryonic cell cycle, the cyclin-based oscillator runs without any feedback controls to entrain it to events of the nuclear cycle. However, in some embryonic cell cycles feedback controls do exist, and it is easy to imagine that these act through cyclin (Fig. 4). Thus in sea urchin embryos inhibition of DNA synthesis blocks the phosphorylation of cyclin as well as entry into mitosis (N. Standart and T. Hunt, personal communication). It is tempting to speculate that cyclin phosphorylation is one of the post-translational steps required for the activation of MPF and that the protein kinase that performs this modification can only be activated by some signal that is generated when DNA replication is completed. In contrast, this kinase would be constitutively active in frog embryos.

In both clams and sea urchins the depolymerization of microtubules increases the length of mitosis (Sluder et al. 1986) and stabilizes cyclin during mitosis (Evans et al. 1983), suggesting the existence of a feedback system that can detect incorrectly assembled spindles and as a result inhibit cyclin degradation. Although frog embryos lack this control, unfertilized frog eggs provide a clue as to what one of its
components might be. When frog oocytes are induced to mature they pass rapidly through meiosis I and then arrest in metaphase of meiosis II. This arrest is mediated by an activity that has been named cytostatic factor (CSF), and is preserved as the mature oocyte passes down the oviduct and is laid as an unfertilized egg (Masui, 1974; Masui and Markert, 1971). The activity of CSF is calcium-sensitive (Masui, 1974) so that the rise in intracellular calcium concentration that is induced upon fertilization leads to the inactivation of CSF, allowing MPF levels to decline and the cell cycle to progress into interphase. One of the effects of CSF activity is to stabilize cyclin in metaphase cytoplasm (Murray et al. 1989), suggesting that CSF may arrest the cell cycle by preventing the degradation of cyclin. In frogs, CSF activity does not appear during the early embryonic cell cycles, which may account for the lack of feedback controls seen after fertilization. In embryos that do have feedback controls it is possible that CSF, or some related activity, mediates the mitotic feedback control by requiring some aspect of mitotic spindle assembly, rather than fertilization, to generate the signal required for CSF inactivation.

Proper coordination of the cell cycle requires that the chromosomal DNA is replicated once and only once before the initiation of mitosis. The studies of Rao and Johnson (1970) demonstrate that over-replication is prevented by some mechanism that modifies the chromosomes during the course of replication so that they cannot be re-replicated within the same cell cycle even if the DNA replication machinery
remains active within the same cell. In early embryonic cell cycles this re-replication block is removed during passage through mitosis (Newport and Kirschner, 1984) so that DNA replication begins as soon as chromosome decondensation and nuclear reformation begins. Blow and Laskey (1988) suggest that the re-replication block is removed as a consequence of the physical act of nuclear envelope breakdown, although the alternative possibility that nuclear envelope breakdown and the removal of the re-replication block are independent events that both require the activation of MPF has not been rigorously excluded.

In somatic cells DNA replication requires passage through Start, suggesting that the block to re-replication might be removed as a consequence of passage through Start, rather than through mitosis. If this possibility is true, then in some sense the mitotic and DNA replication cycles are under separate controls and therefore require coordination. This coordination may be facilitated by the fact that both Start and mitosis require the activity of the cdc2 gene product (Nurse and Bisset, 1981), especially if the two different activities of this protein are induced by different accessory proteins at the two cell cycle transitions. At mitosis the activation of cdc2 requires the activity of both cdc25 and the fission yeast cyclin B homologue, cdc13 (Booher and Beach, 1988; Hagan et al. 1988; Solomon et al. 1988). The finding that cdc13 in fission yeast and cyclin A and B in clams are associated with p34cdc2 (Draetta et al. 1989) suggests that the association of cyclin with p34cdc2 may both activate the protein kinase activity of p34cdc2 and determine its substrate specificity. Since neither cdc13 nor cdc25 activity are required for passage through Start, it is tempting to suggest that either cyclin A or some undiscovered member of the cyclin family might act at Start to activate p34cdc2 to a pattern of substrate specificity to produce the biochemical changes that correspond to passage through Start (Fig. 4). In the budding yeast one such cyclin has been identified as the product of the gene variously referred to as whi1 and DAF1. Mutants in this gene which truncate the protein (possibly making it resistant to degradation) allow cells to pass Start at much smaller sizes than normal (Cross, 1988; Nash et al. 1988). If there are different cyclins responsible for the Start and mitosis-inducing activities of p34cdc2 in the somatic cell cycle, then the orderly progress of the cell cycle requires that the accumulation and/or activation of these different cyclins alternate during the cell cycle. This could be achieved by making the accumulation of the mitosis-specific cyclin dependent on the occurrence of Start and the accumulation of the Start-specific cyclin dependent on the occurrence of mitosis. In the embryonic cell cycle, Start and mitosis may occur as a single event, either by making the kinetics of the Start and mitosis-specific cyclins identical, or by making both Start (the removal of the block to re-replication of DNA?) and mitosis dependent on the same cyclin.

How many clocks run the cell cycle? If there are separate but intertwined clocks for mitosis and DNA replication may there not also be other clocks for other cell cycle events? This question has been clearly answered in the affirmative by Mitchison and his colleagues who have discovered two clocks which continue to run when the other cell cycle clocks have been stopped by temperature-sensitive mutations in cdc2 (Creanor and Mitchison, 1986; Novak and Mitchison, 1986). Under these conditions
these two clocks, whose molecular mechanisms are currently mysterious, run with slightly different periods. It seems likely that we still have a great deal to learn about timekeeping and coordination in the cell cycle.

I am extremely grateful to Marc Kirschner for his support, hospitality and for many stimulating discussions, and to my colleagues in the cell cycle field for many valuable discussions that have shaped my thinking. I am a Lucille P. Markey Scholar and am supported by a grant from the Lucille P. Markey Charitable Trust.

References


