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## The role of cyclin synthesis, modification and destruction in the control of cell division

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### Summary

This paper reviews our current knowledge of the cyclins based on observations of the oocytes and eggs of sea urchins, clams and frogs. Cyclins are proteins found in all eukaryotes whose special property is rapid destruction at specific stages in the cell cycle. The cyclins fall into three families. A-type cyclins have been found in clams, flies and frogs. B-type cyclins have been found in clams, flies, frogs, sea urchins and fission yeast. A more distantly related family of three genes is found in *Saccharomyces cerevisiae*. B-type cyclins appear to be required for cells to enter mitosis, and their destruction is thought to be necessary for exit from mitosis. We describe evidence in support of these ideas, and describe various conditions under which cyclin destruction is delayed or deranged. We conclude with a discussion of the relationship between the cyclins and maturation- (or M phase-) promoting factor and some ideas on how the cyclins may work.

### Introduction

Cell division has attracted biologists' attention from the earliest days; the first edition of Wilson's textbook *The Cell in Development and Inheritance* devoted two whole chapters to the subject (Wilson, 1896). Cell division has been studied from a wide range of different perspectives, depending on the particular interests of the investigators and what techniques were available to them. Our own entry into this field arose naturally but by chance from studies of protein synthesis in the oocytes and eggs of clams and sea urchins. Fertilization of the eggs of both species leads to a rapid increase in the rate of synthesis of a new set of proteins, and protein synthesis is required for cleavage. At first it was the mechanism of this translational control that attracted our attention (Rosenthal *et al.* 1980), but we soon came to appreciate that the proteins specified by this class of mRNA often served interesting roles in early development. Indeed, one of them proved to encode the small subunit of ribonucleotide reductase, the enzyme that provides DNA precursors (Standart *et al.* 1985); among the others were cyclins.

The cyclins are proteins whose concentration follows a sawtooth oscillation in step with cleavage. They are encoded by abundant maternal mRNAs. The level of cyclin

Key words: cyclins, cell cycle, protein synthesis, proteolysis, mitosis.

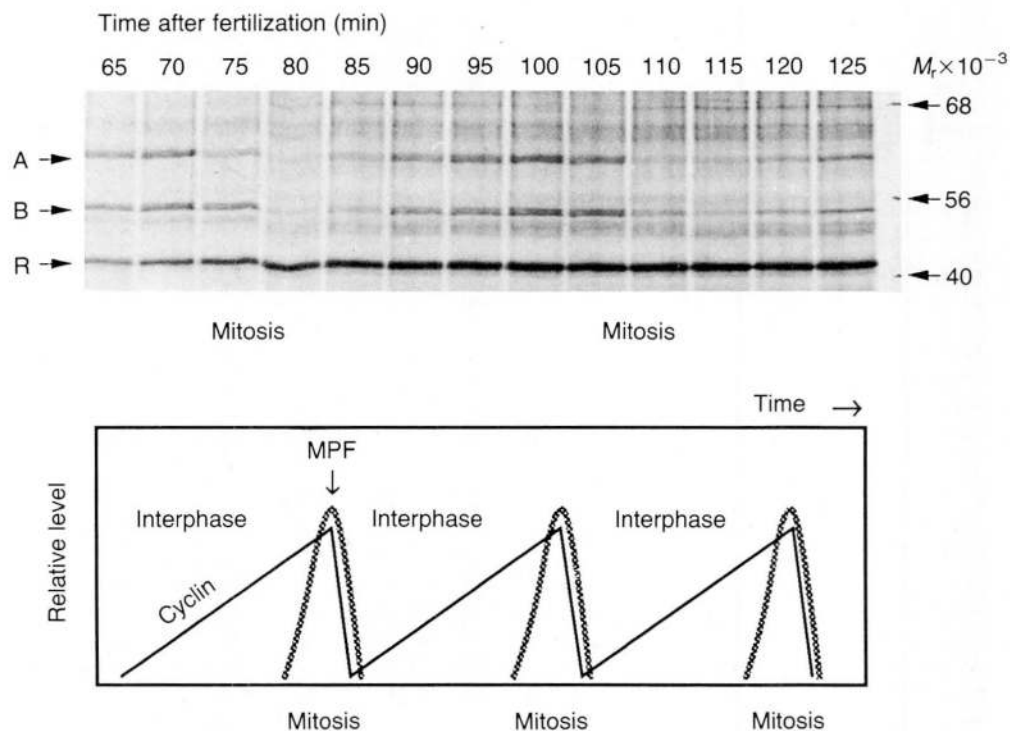


Fig. 1. The relationship between cyclin and MPF. The top panel shows the labelling patterns of cyclin A (A), cyclin B (B), and ribonucleotide reductase small subunit (R) with [<sup>35</sup>S]methionine during the first two mitotic cycles in fertilized clam oocytes. The lower panel shows a simplified diagram of the relationship between cyclin synthesis and destruction and the phases of the cell cycle. MPF activity appears abruptly at the G<sub>2</sub>→M transition, and falls abruptly when cyclin is destroyed just before the metaphase→anaphase transition. It is not known how the continuous increase in cyclin is translated into the switch-like entry into M phase.

mRNAs does not change during the first few hours of development, nor does the rate of cyclin synthesis show significant cell cycle-related fluctuations. Cyclin levels fall because of their rapid destruction just before the onset of anaphase, as shown in Fig. 1; new synthesis steadily replenishes the cyclin pool once the short destruction period has ended. Cyclins were first recognized in fertilized sea urchin eggs and clam oocytes, thanks to their strong labelling with [<sup>35</sup>S]methionine and their sudden and obvious disappearances (Evans *et al.* 1983; Swenson *et al.* 1986; Westendorf *et al.* 1989). They were subsequently found in starfish and *Urechis caupo* eggs (Standart *et al.* 1987; E. Rosenthal, personal communication). The behaviour of these proteins immediately suggested that they played a role in the regulation of the cell cycle, and could explain very easily why protein synthesis was necessary for cleavage (Hultin, 1961; Wilt *et al.* 1967; Wagenaar, 1983; Dubé, 1988). Direct evidence that synthesis and destruction of cyclin are necessary for entry into and exit from mitosis has only recently been obtained (Minshull *et al.* 1989; Murray and Kirschner, 1989; Murray



Fig. 2. Sequence comparisons between cyclins in the central 200 residues. This figure displays all the published cyclin protein sequences (omitting the *S. cerevisiae* cyclin-like proteins WHI1<sup>+</sup>, CLN1 and CLN2), aligned in the central conserved region. The listing starts just before a conserved acidic patch in the B-type cyclins, at residue 121 (in frog B1) and ends just beyond the second tryptophan residue at residue 327. Completely conserved amino acids are listed as the consensus. Printed in bold are the sequences EV-EEYKL, which appear only in A-type cyclins and FLRR-SK which is characteristic of B-type cyclins. The program MULTALIGN was used to make the alignments (Barton and Sternberg, 1987). The sequences are found in the following publications: clam, Swenson *et al.* 1986 and Westendorf *et al.* 1989; sea urchin, Pines and Hunt, 1987; cdc13, Boohar and Beach, 1988 and Hagan *et al.* 1988; frog, Minshull *et al.* 1989; *Drosophila*, Lehner and O'Farrell, 1989; human, Pines and Hunter, 1989).

*et al.* 1989). Remarkably, in cleaving frog eggs at least, the protein synthesis requirement for entry into mitosis appears to be entirely explained by the requirement for cyclin (Murray and Kirschner, 1989).

Cyclins from higher eukaryotes contain 400–450 amino acids and can be classified according to their sequence into A- and B-types. Fig. 2 presents a compilation of all the currently published sequences in the central region of about 200 residues where they show maximum similarity. Both A- and B-type cyclins are present in clams, flies and frogs, the organisms where thorough searches have been conducted. In extracts of frog eggs, it is much easier to detect the B-type cyclins than the A-types by [<sup>35</sup>S]methionine labelling. A-type cyclins have yet to be identified with certainty in sea urchin and starfish eggs. Only in cleaving clam embryos are both A- and B-type cyclins synthesized at similar high rates, as Fig. 1 shows.

During the last year, cyclins have been identified in a wide variety of eukaryotic

species and cell types. The *cdc13<sup>+</sup>* gene in *Schizosaccharomyces pombe* is a cyclin (Booher and Beach, 1988; Solomon *et al.* 1988; Goebel and Byers, 1988; Hagan *et al.* 1988); cDNA clones have been obtained from clams (Swenson *et al.* 1986; Westendorf *et al.* 1989), sea urchin (Pines and Hunt, 1987), *Drosophila* (Lehner and O'Farrell, 1989; Whitfield *et al.* 1989), *Xenopus* (Minshull *et al.* 1989), humans (Pines and Hunter, 1989), and starfish (Labbé *et al.* 1989b). The sequences of the cyclin-like proteins from *Saccharomyces cerevisiae* are much more distantly related to the others, and they seem to be required at *START* rather than mitosis (Nash *et al.* 1988; Hadwiger *et al.* 1989; Cross, 1989, this volume). These genes (*WHI1<sup>+</sup>* (= *DAF1*), *CLN1* and *CLN2*) were isolated by very different genetic screens from the other cyclins and it remains to be seen if such proteins are present in higher eukaryotes, and whether they have the property of cell cycle stage-specific destruction in budding yeast. Conversely, it is not yet known whether or not *S. cerevisiae* has A- and B-type cyclins.

In oocytes and eggs, the cyclins are encoded by relatively abundant 'masked' maternal mRNAs that are not translated to a significant extent before fertilization. After fertilization they load rapidly onto polysomes (Rosenthal *et al.* 1980; Evans *et al.* 1983). Studies of the kinetics of polypeptide synthesis and destruction in the first two mitotic cells cycles in cleaving clam and sea urchin eggs show that cyclins are stable during interphase, and are destroyed in the space of five minutes or less just before the chromosomes part at the metaphase–anaphase transition of the cell cycle (Swenson *et al.* 1986; Westendorf *et al.* 1989). The accumulation and destruction of the *cdc13<sup>+</sup>* gene product (cyclin B) follows these kinetics in *S. pombe* (Booher *et al.* 1989) as do cyclins B1 and B2 in *Xenopus* (Murray and Kirschner, 1989). By contrast, Lehner and O'Farrell (1989) found less clear-cut behaviour on the part of cyclin A in *Drosophila*. Their data are more consistent with the idea that only a certain fraction of the cyclin is destroyed in each cell cycle. *Drosophila* eggs contain a maternally-provided supply of cyclin A, which does not disappear at each division. Rather, its concentration falls progressively during the early pre-cellular nuclear divisions and the rate of loss accelerates towards the end of cleavage. This behaviour could be explained if a fixed amount of cyclin per nucleus was destroyed at each division cycle, so that the rate of destruction increased as the square of the number of division cycles. Perhaps only a certain active fraction of the cyclin enters the nucleus and only this (initially small) fraction is destroyed in a cell cycle-dependent manner. It should be noted that most studies of cyclins up to now have concentrated on their behaviour in eggs and early embryos. Studies in somatic cells are just beginning (Pines and Hunter, 1989).

The cyclins found in higher eukaryotes do not appear to have an intrinsically short half-life. Thus, if fertilized sea urchin or clam eggs are prevented from entering mitosis, either by addition of emetine before the stage at which cyclin has reached its critical threshold, or by inhibition of DNA synthesis, cyclin is as stable as any other protein specified by maternal mRNA (Hunt and Ruderman, unpublished observations, and see Fig. 5). Murray and Kirschner (1989) have similarly shown that cyclin is stable in extracts of unfertilized frog eggs ('CSF-arrested extracts'), which do not undergo the metaphase→anaphase transition. Cyclin is also stable in

activated extracts that fail to enter mitosis because of insufficient supplies of cyclin. The rule appears to be that cyclin is normally only unstable during a short window around the time of anaphase onset in mitosis or meiosis.

### **Clam and sea urchin cyclin mRNA make frog oocytes enter M phase**

The first experiment to offer more direct support that cyclin had a role in promoting the  $G_2 \rightarrow M$  transition was performed by Swenson *et al.* (1986), who showed that injection of synthetic clam cyclin A mRNA into stage VI *Xenopus* oocytes caused their maturation into eggs. This experiment has been repeated with sea urchin maternal mRNA and with synthetic sea urchin cyclin B mRNA (Pines and Hunt, 1987). The newly synthesized sea urchin cyclin protein was a prominent labelled gel band, which disappeared from the frog oocytes at about the time of white spot formation, presumably at the end of first meiotic metaphase. Thus, frog oocytes can both respond to and destroy a heterologous cyclin. As little as 0.3 ng of 'synthetic' cyclin mRNA, the lowest dose tested, still gave 100% maturation, and from the minimum effective dose of total mRNA we estimated that 0.01 ng of natural mRNA should be sufficient. This makes cyclin mRNA one of the most potent artificial inducers of oocyte maturation.

To determine whether cyclin mRNA was the sole active maturation-promoting principle in sea urchin maternal mRNA, we specifically ablated cyclin mRNA with antisense oligodeoxyribonucleotides and RNase H before injection into the frog oocytes (Minshull and Hunt, 1986). This treatment abolished the mRNA's maturation-promoting factor (MPF)-like activity, whereas in a control in which the mRNA for ribonucleotide reductase was cut, the ability of the mRNA to promote maturation was unaffected. These results confirmed the MPF-like activity of cyclin mRNA. It remains a question, however, what this result means in terms of the natural mechanism of oocyte maturation. This may be yet another example of the ever-growing list of interesting agents that are active in the oocyte maturation assay, which include insulin, cAMP-dependent protein kinase inhibitors, *ras* and *mos* oncogenes (Maller and Krebs, 1980; Maller, 1985; Birchmeier *et al.* 1985; Sagata *et al.* 1988).

The recent finding that translation of *c-mos* mRNA is required for progesterone-induced maturation of *Xenopus* oocytes, and that *c-mos* mRNA can induce maturation (Sagata *et al.* 1988) came as a surprise to us, since we had interpreted the protein synthesis requirement for *Xenopus* oocyte maturation in terms of cyclin synthesis. However, we had performed antisense oligonucleotide experiments in which the *Xenopus* B-type cyclins were ablated by microinjection of antisense oligonucleotides and found that maturation (as judged by white spot formation and germinal vesicle breakdown) could occur in the almost complete absence of translatable cyclin B mRNA (Minshull and Colman, unpublished results). This result appears to conflict with the studies in which injected cyclin B mRNA induced maturation. At present, we can only offer a speculative reconciliation of these data. It is known that clam oocytes contain a maternal store of cyclin B *protein* as well as a

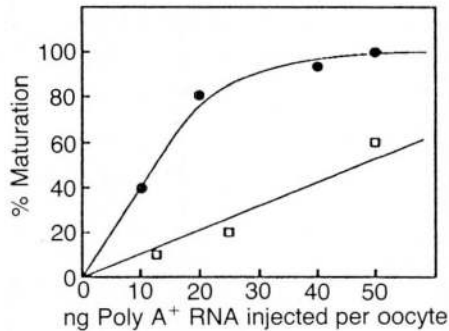


Fig. 3. Maturation response of *Xenopus* oocytes to RNA prepared from *Xenopus* oocytes and eggs. Poly(A)<sup>+</sup> RNA was prepared from *Xenopus* oocytes and eggs as described in Materials and methods and tested for its maturation-promoting activity by injecting 50 nl of mRNA solution. The injected oocytes were examined between 6 h and 8 h after injection and finally scored at 16 h. No uninjected controls matured, and progesterone-treated oocytes showed 100% maturation. (●—●), egg mRNA; (□—□), oocyte mRNA.

store of A- and B-type cyclin mRNA, which appears to be sequestered in discrete particles (Westendorf *et al.* 1989). If frog oocytes contained a similar store of cyclin protein, the role of *c-mos* could be to release or activate it. Microinjected cyclin mRNA would bypass the *mos* requirement by placing cyclin protein directly in the oocyte cytoplasmic compartment. It should be noted that attempts to produce maturation of oocytes by injection of cyclin protein made in a reticulocyte lysate have not yet proven successful, so although we assume it should, we do not actually know if pure cyclin protein possesses MPF activity. The resolution of these puzzles should shed considerable light on a much-studied system.

### ***Xenopus* oocytes contain (at least) four different cyclin mRNAs**

When we repeated the mRNA injections that had been successful in the case of sea urchin maternal mRNA, using *Xenopus* egg or oocyte poly A<sup>+</sup> RNA, we found that *Xenopus* egg poly A<sup>+</sup> RNA had MPF-like activity. As shown in Fig. 3, mRNA from frog eggs was about five times as active at promoting the maturation of *Xenopus* oocytes as poly A<sup>+</sup> RNA from oocytes. Moreover, oocyte poly A<sup>+</sup> RNA showed low but detectable MPF-like activity while egg poly A<sup>+</sup> RNA did not (data not shown). These results suggested that a mRNA encoding an MPF-like activity was present in non-adenylated form in oocytes, and that this mRNA lengthened its poly A tail during meiotic maturation. Such a class of mRNA is commonly found in eggs and oocytes (Rosenthal *et al.* 1983; Dworkin *et al.* 1985).

We searched for cyclin mRNAs in a *Xenopus* egg cDNA library, using a consensus oligonucleotide based on a conserved region between clam and urchin cyclins, and a sea urchin cDNA clone as probes. The first positive clone to be sequenced showed strong homology to sea urchin cyclin and clam cyclin B. The same library was subsequently rescreened with the oligonucleotide alone, and about 20 independent

new clones were sequenced. These clones all corresponded to a second cyclin B mRNA. Both cyclins were found as full-length coding region clones in  $\lambda$ gt10 oocyte cDNA libraries (Minshull *et al.* 1989). They corresponded to mRNAs of roughly equal abundance, with about  $10^7$  molecules of each message per oocyte. Their sequences, together with comparison with cyclins from other organisms, were published by Minshull *et al.* (1989). It is interesting that the first 100 amino acids contain only 28 identical residues, whereas the last 300 show almost 64% identity.

We were surprised that the second cyclin we found was a cyclin B, and not a cyclin A. Two considerations led us to expect frogs to possess A-type cyclins. First, *Spisula* and *Drosophila* contained both types of cyclin, and second, C. Ford and colleagues observed a [ $^{35}$ S]methionine-labelled band that was larger than the B-type cyclins, but which showed typical cyclin disappearance in cycling extracts (Ford, personal communication). Having seen these data, we rescreened the  $\lambda$  libraries and picked clones that gave a weak signal with a B-type probe and a relatively stronger signal with a consensus oligonucleotide. The sequences of representative members of this family were clearly A-type cyclins, of which there appear to be two closely related but different forms. The A-type cyclins contain a sequence EV-EEYKL shortly after the conserved MR-IL-DWL *motif* shown by all cyclins. A-type cyclins lack the cyclin B *motif* FLRR-SK (bold print in Fig. 2), which should be a target for cyclic AMP-dependent protein kinase. It is probably also significant that the A-type cyclins lack an acidic B-type cyclin *motif* that Westendorf *et al.* (1989) have suggested may bind a metal ion. This acidic region marks the start of the highly conserved 200-residue 'cyclin box' shown in Fig. 2. The possible metal-binding region is shown below:-

EDIDKDDGDNPQLCSEYAKD	sea urchin ( <i>Arbacia punctulata</i> )
EDIDKDDGDNPQLCSEYAKE	sea urchin ( <i>Strongylocentrotus purpuratus</i> )
KDVDADDDGNPMLCSEYVKD	frog B1
EDIDADDGGNPQLCSDYVMD	frog B2
QNI DANDKENPQLVSEYVND	clam B
DDLDAEDWADPLMVSEYVVD	cdc13 from <i>S. pombe</i>

Synthesis of the A-type cyclins is more difficult to detect in cell-free systems from frog eggs than that of cyclins B1 and B2, whereas as Fig. 1 shows, cyclin A is as abundant as cyclin B in early clam embryos. Immunoprecipitates using anti-cyclin A antibodies reveal that frog cyclin A is in fact synthesized at a similar level to that of individual B-type cyclins. We have not yet isolated an A-type cyclin from sea urchin eggs (though there is preliminary evidence for its existence) and at present, the function of the A-type cyclins is not known.

### Cell-free systems from *Xenopus* eggs need cyclin to enter M phase

Several groups have used cell-free systems from *Xenopus* eggs based on the original description of Lohka and Masui (1983). Most of these systems show aspects of entry into mitosis, including breakdown of the nuclear envelope and chromosome condensation (Lohka and Masui, 1983; Blow and Laskey, 1985; Lohka and Maller,



1985; Newport and Spann, 1987; Hutchison *et al.* 1987; Murray and Kirschner, 1989). Protein synthesis is required for the first entry into mitosis and for the second and subsequent rounds of DNA synthesis. To test whether cyclin synthesis accounted for the protein synthesis requirement, we inhibited synthesis of cyclins B1 and B2 with antisense oligonucleotides. We found that entry into the mitosis-like state and the second round of DNA synthesis was prevented provided both cyclins were completely cut (Minshull *et al.* 1989). In the best experiments, no general inhibition of protein synthesis occurred. We found that complete loss of either B-type cyclin alone did not inhibit entry into mitosis. Cyclins B1 and B2 thus appear to be functionally interchangeable, at least in this cell-free system.

It will be important to discover when translation of the various cyclin mRNAs in *Xenopus* occurs, and to find out what pools – if any – of cyclin protein exist in the stage VI oocyte.

### **Cyclin is post-translationally modified, probably phosphorylated, at the onset of M phase**

As we first observed in sea urchin embryos (particularly *Lytechinus pictus*, *Strongylocentrotus purpuratus* and *Sphaerechinus granularis*), cyclin is converted into a form that migrates more slowly on one-dimensional polyacrylamide gels at around the time the fertilized eggs begin to enter mitotic prophase (Fig. 4). Similar mobility shifts are seen when sea urchin cyclin was translated in *Xenopus* oocytes (Pines and Hunt, 1987), and in the endogenous *Xenopus* B-type cyclins (Minshull *et al.* 1989). In the sea urchin eggs, the new form represents a conversion of previously synthesized polypeptide, since as the lower panel in Fig. 4 shows, it still occurs after inhibition of protein synthesis with emetine. We believe that the conversion is due to phosphorylation, because the converted form can be returned to its primary form by treatment with acid or alkaline phosphatase (Golsteyn, Stewart and Hunt, unpublished observations). We were able to obtain conversion of cyclin to its phosphorylated form in a cell-free system. First, cyclin mRNA was translated in a reticulocyte lysate. In the next step, sea urchin extract, ATP and phosphatase inhibitors were added to the labelled substrate mix (Standart *et al.* 1987). Using this assay, the converting enzyme has been partially purified. Its apparent molecular weight is between 20K and 40K ( $K=10^3 M_r$ ) as measured by gel filtration, and the same activity is found in interphase extracts (from unfertilized eggs) and mitotic extracts (from fertilized eggs). Thus, there is no evidence for cell cycle-related oscillations in the activity of this presumed protein kinase, which suggests that control of phosphorylation may be achieved by alterations in the activity of phosphatase(s) (Golsteyn and Hunt, unpublished observations).

When DNA synthesis is prevented by aphidicolin in fertilized sea urchin eggs, they do not enter mitosis (Killian *et al.* 1985; Sluder and Lewis, 1987), and as shown in Fig. 5, cyclin is extremely stable. Moreover, the mobility shift is also inhibited in those species that display it (data not shown). It is not yet clear, however, whether cyclin phosphorylation is necessary to trigger mitosis, or if its phosphorylation is a

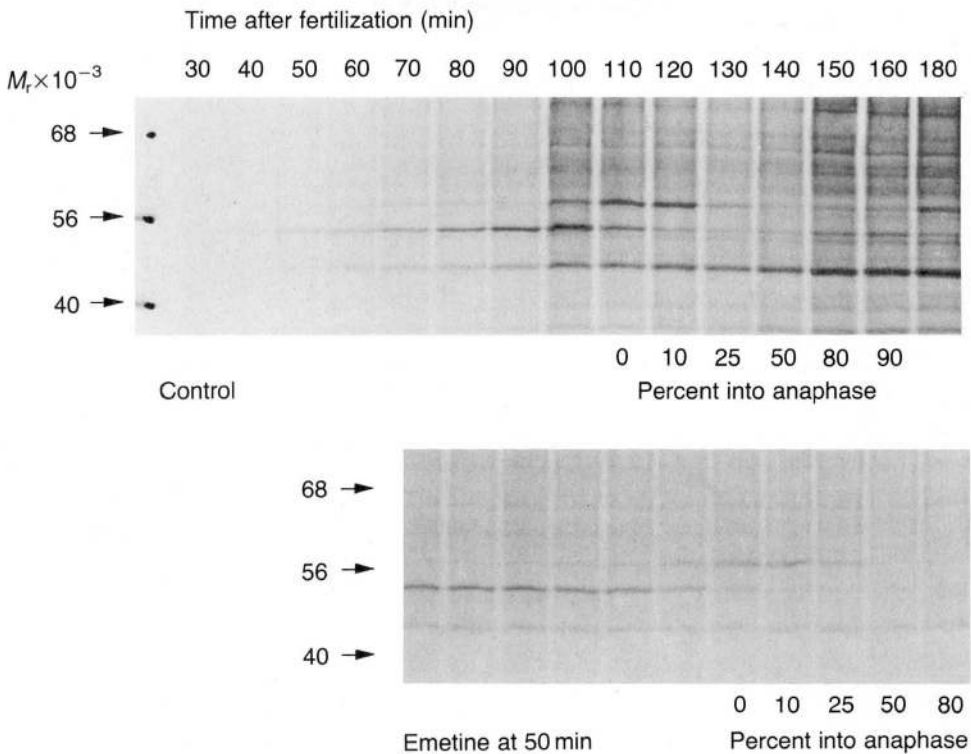


Fig. 4. Post-translational modification of cyclin in sea urchin eggs. Fertilized eggs from the sea urchin *Lytechinus pictus* were incubated with [ $^{35}\text{S}$ ]methionine and samples taken for analysis on SDS-polyacrylamide gels and autoradiography. Protein synthesis was blocked by the addition of emetine at 50 min in a portion of the culture. Samples were also taken for fixation and staining with Hoechst 33342 to visualize the chromosomes, and scored for completion of the metaphase→anaphase transition. It was not possible to determine the time of entry into M phase. Note that emetine delayed the cell cycle.

consequence of entering M phase (Meijer *et al.* 1989). These observations suggest that cyclin may be activated by post-translational modification, and that it may not be active as a mitotic inducer in its ground state. The identification of the activity that performs the conversion and its regulation are clearly important matters.

#### **In clam embryos, the rapid proteolysis of cyclin requires a correctly formed mitotic spindle**

The first evidence that destruction of cyclin might be necessary for passage through the cell cycle came from studies of colchicine-treated and of parthenogenetically activated clam eggs. Colchicine-treated cells lack spindles altogether, and the parthenogenetically activated embryos have monopolar instead of bipolar mitotic spindles, because they lack centrosomes (Kuriyama *et al.* 1986). In both cases, the

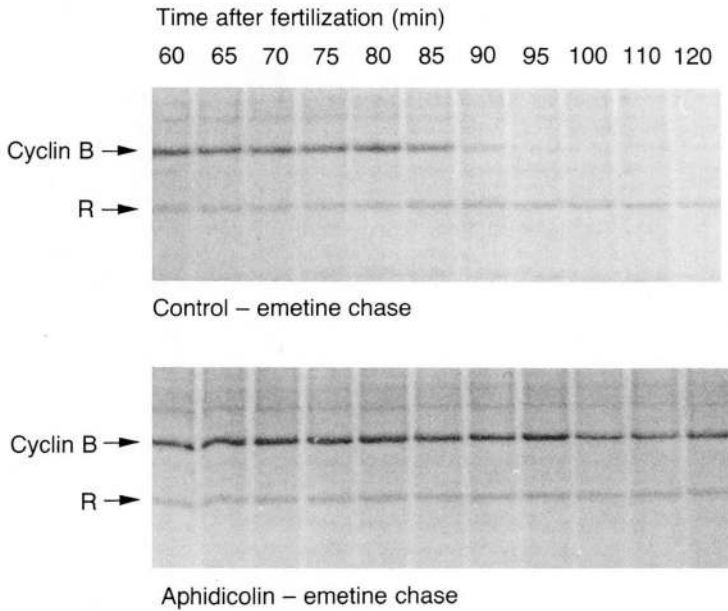


Fig. 5. Aphidicolin blocks cyclin destruction in sea urchin eggs. Fertilized eggs of the sea urchin *Arbacia punctulata* were incubated with [ $^{35}$ S]methionine. Aphidicolin was added to one portion after 5 min and protein synthesis in both samples was blocked by addition of 100  $\mu$ M-emetine at 60 min. Samples from each culture were analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography. The control cells entered mitosis and divided, whereas the aphidicolin-treated cells remained in interphase throughout. Cyclin B from *Arbacia* does not display the mitosis-associated shift in mobility seen in *Lytechinus*. R, ribonucleotide reductase small subunit.

embryos enter first mitosis at the normal time, but remain in M phase for much longer than the normal 10 min. Fig. 6 shows that the level of cyclin B remains high in the presence of colchicine instead of showing its usual rapid fall. Cyclin destruction is activated, but it only proceeds at its normal high rate after a considerable delay, with the result that cyclin levels are held at a more or less constant high level. If protein synthesis is now blocked with emetine, cyclin levels eventually drop to zero, as shown in Fig. 6B. The cells enter interphase (i.e., the chromosomes decondense and nuclei reform) when cyclin finally disappears. Controls in which protein synthesis was not inhibited stayed in mitosis throughout the period shown. A similar experiment performed on parthenogenetically-activated eggs is shown in Fig. 7. In this case, the control (top panel) shows almost complete destruction of cyclin A at the normal time, and its level stays low thereafter. The destruction of cyclin B is initiated later than normal, after which its level remains more or less constant and high, because destruction of cyclin B occurs after a (roughly) 50 min lag. Thus, addition of emetine at 80 min (middle panel) and 100 min (bottom panel) leads to the complete loss of cyclin B about 50 min later, at which point the cells leave mitosis and re-enter interphase. It is not clear why cyclin A destruction proceeds more rapidly

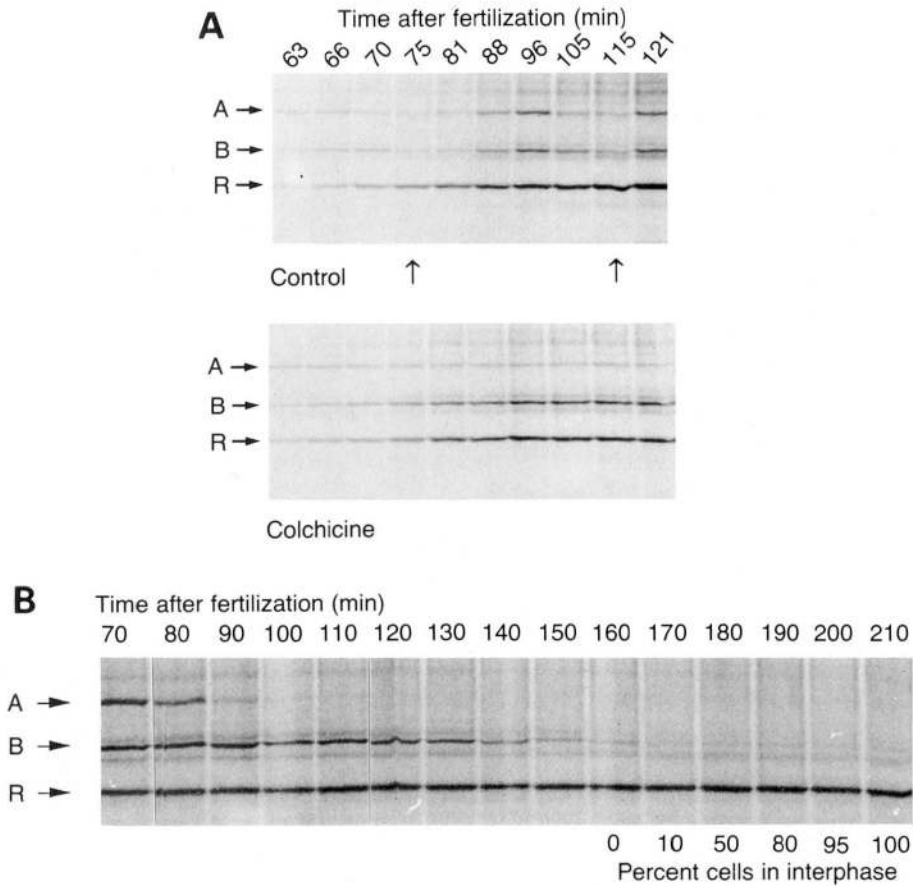


Fig. 6. Colchicine disturbs cyclin A and B oscillations in fertilized clam embryos. In panel A, a batch of clam oocytes was fertilized and allowed to pass through meiosis. [ $^{35}\text{S}$ ]methionine was added at 50 min, after which the culture was divided in two and 20  $\mu\text{M}$ -colchicine added to one portion. Samples were taken into trichloroacetic acid and analyzed by SDS-polyacrylamide gels and autoradiography exactly as described by Evans *et al.* (1983). The arrows beneath the control embryo autoradiograph indicate the times of first and second mitosis. Note that cyclin A destruction occurs earlier than that of cyclin B in the controls. Cyclin A (A) also displays a shorter lifespan than cyclin B (B) in the colchicine-treated embryos. In panel B, a culture of colchicine-treated embryos labelled with [ $^{35}\text{S}$ ]methionine was treated with 100  $\mu\text{M}$ -emetine at 70 min. Parallel samples were taken for running on gels or fixation and staining with orcein to visualize condensed chromosomes. Numbers below the autoradiograph indicate the fraction of cells that had lost staining chromosomes and had reformed nuclei (interphase). R, ribonucleotide reductase small subunit.

more rapidly than cyclin B destruction, or what gives cyclin B its curious 50 min life expectancy. There must be clues to the destruction mechanism in these observations.

Parthenogenetically-activated oocytes eventually exit from mitosis (albeit asynchronously) even when protein synthesis proceeds normally. Such cells pass rapidly through interphase, and repeat the greatly prolonged M phase. Though these cells

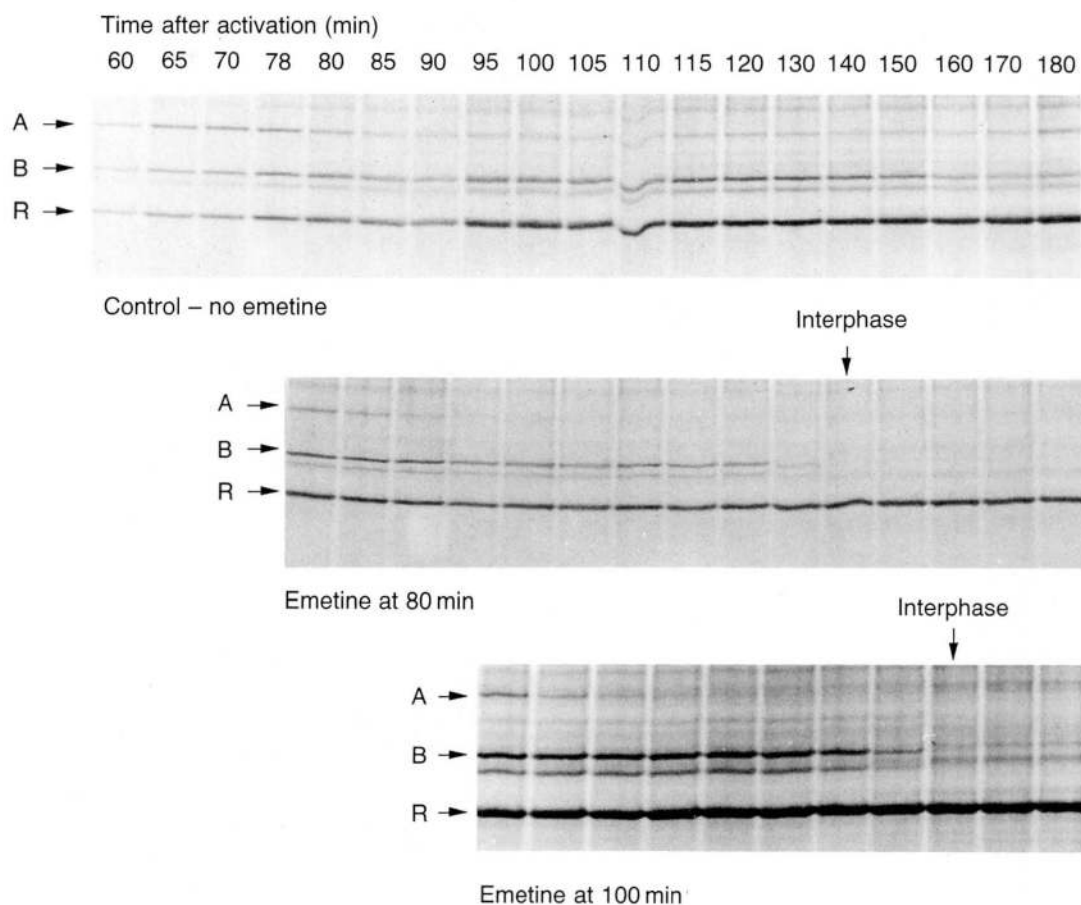


Fig. 7. Inhibition of protein synthesis advances exit from mitosis in parthenogenetically-activated clam embryos. Clam oocytes were activated by exposure to sea water containing 50 mM-KCl for 10 min. They were returned to regular sea water, and [ $^{35}$ S]methionine was added to the culture at 50 min (after second polar body emission). Samples were removed at 80 and 100 min and made 100  $\mu$ M in emetine, which immediately blocks further protein synthesis (data not shown). All cells had entered first mitosis by 80 min. The controls remained in mitosis, judged by their orcein-staining mitotic chromosomes and lack of nuclei, until 170 min; a small fraction of cells with nuclei were seen at 180 min. In the '80 min emetine' culture, all cells had nuclei by 140 min, and in the '100 min emetine' culture, 100% of cells were in interphase by 160 min. Note that the decay of cyclin A (A) occurs rapidly, while cyclin B (B) decays as though it had a life expectancy of about 50 min. R, ribonucleotide reductase small subunit.

never divide they eventually accumulate large amounts of DNA in a single giant nucleus.

There is thus a striking correlation between loss of cyclin and entry into the next cell cycle, and if these results are to be taken at face value, it seems that very little cyclin is required to keep cells in M phase, for cyclin levels fall extremely low before the chromosomes decondense.

### **Protease inhibitors stabilize cyclin and block starfish oocytes in M phase**

The protease inhibitors leupeptin, soybean trypsin inhibitor or antipain block 1-methyladenine-treated starfish oocytes in first meiotic metaphase, and MPF levels rise normally but stay high (Picard *et al.* 1985). We found that antipain prevented cyclin destruction (Picard, Pines and Dorée, unpublished), and we presume that this accounts for the meiotic arrest. We also found that PMSF blocked maturing clam oocytes in first meiotic metaphase, although the same concentrations of PMSF did not block the second meiotic metaphase→anaphase transition or the subsequent mitotic divisions. This suggests that the cyclin proteolysis that occurs at first meiosis relies on a somewhat different mechanism than subsequent divisions, which may be connected with the absence of true interphase between the meiotic divisions. The interpretation of these results is not very secure, however, since the concentration of protease inhibitors required to cause cell cycle arrest is high, and they may act through unknown side effects. It would obviously be helpful to be able to inhibit the destruction of cyclin with precise specificity, but this has not yet proven possible.

The identification and characterization of the destruction mechanism is clearly a matter of extreme interest. The protease is highly specific in the sense that cyclins appear to be the only targets among proteins specified by maternal mRNA. Moreover, the protease shows precise temporal regulation, for cyclin is only unstable during mitosis, and then only briefly during the moments preceding the metaphase–anaphase transition. The significance of the requirement for a correctly formed bipolar mitotic spindle for correctly timed cyclin destruction is not clear, and in the cleavage cell cycles of *Xenopus*, the destruction of cyclin is not inhibited by colchicine (Murray and Kirschner, 1989). One interpretation of this difference between different organisms is that cyclin destruction *per se* does not require an intact, bipolar spindle. Rather, it may reflect a regulatory mechanism capable of detecting misplaced chromosomes. Such a mechanism would be absent in early frog embryos, and would presumably be acquired after the midblastula transition. We note that different cyclins are destroyed at slightly different times; does this mean that each cyclin has its own protease, or that each cyclin has its own way of becoming susceptible to a common destruction machinery? At present we cannot tell if the protease is constitutive and the cyclins are targeted for destruction at a particular time, or if the protease itself is regulated.

### **The relationship between cyclin and MPF**

Entry into meiotic and mitotic prophase is accompanied by the appearance of a cytoplasmic activity known as MPF (Masui and Markert, 1971; Smith and Ecker, 1971; reviewed by Maller, 1985; Ford, 1985). MPF activity appears periodically even if the egg cannot cleave, provided protein synthesis continues (Newport and Kirschner, 1984; Gerhart *et al.* 1984). MPF activity falls precipitously at the end of metaphase (Gerhart *et al.* 1984). It is now known that one of the components of MPF is the product of the *cdc2*<sup>+</sup> gene or its homologue(s) in higher organisms (Dunphy *et al.* 1988; Gautier *et al.* 1988; Arion *et al.* 1988; Labbé *et al.* 1988a). The level of

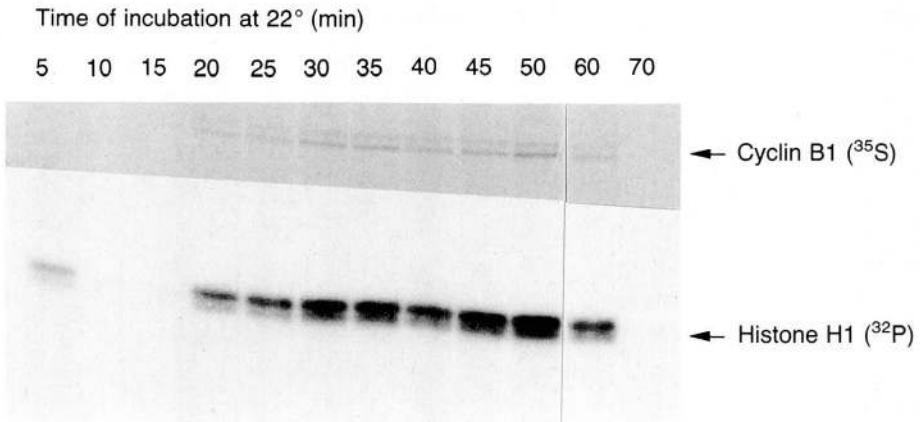


Fig. 8. Synthesis and destruction of cyclin B1 during a mitotic cycle in an extract of *Xenopus* eggs. An extract of A23187-activated *Xenopus* eggs was made as described by Murray and Kirschner (1989) and incubated at 22° with [<sup>35</sup>S]methionine. At the indicated times, samples were removed into buffer with anti-cyclin B1 antibody and the immune complexes harvested with protein A-Sepharose. The resin was washed and incubated with  $\gamma$ -<sup>32</sup>P ATP, histone H1 and a peptide inhibitor of cAMP-dependent protein kinase (Cicirelli *et al.* 1988), mixed with sample buffer and analyzed by SDS-polyacrylamide gel and autoradiography. The <sup>35</sup>S was detected by a sheet of Amersham Hyperfilm  $\beta$ -max, and the <sup>32</sup>P by a second overlaying sheet of Fuji Rx backed with an intensifying screen. Cyclin was first detectable (as a doublet) at 20 min and was destroyed between 60 and 70 min. The histone kinase activity seen at 5 min is a reproducible finding.

this protein kinase subunit does not vary during the cell cycle. Protein synthesis is required to activate the histone H1 kinase activity of MPF (Gerhart *et al.* 1984; Meijer and Pondaven, 1988); newly synthesized cyclin is a prime candidate for the role of activator of p34<sup>cdc2</sup>. The destruction of cyclin at the end of metaphase presumably leads to loss of mitotic kinase activity. From the very beginning, it seemed that there must be a close connection between cyclin and MPF and evidence in favour of this idea is now rapidly accumulating. Thus, Draetta *et al.* (1989) have shown that antibodies against p34<sup>cdc2</sup> coprecipitate cyclin from clam eggs. Moreover, p13<sup>suc1</sup> affinity columns bind cyclin (Draetta *et al.* 1989; Meijer *et al.* 1989). We have raised polyclonal antibodies against *Xenopus* cyclin B1 and B2 produced by overexpression in *Escherichia coli* and find that these antibodies specifically immunoprecipitate radiolabelled cyclins from cycling extracts of activated *Xenopus* eggs. Fig. 8 shows that these immunoprecipitates display histone H1 kinase activity. Moreover, the autophosphorylated 45K component of highly purified MPF (Lohka *et al.* 1988) is recognised by anti-cyclin antisera (Gautier and Minshull, unpublished observations). At earlier stages of purification of MPF, these antibodies recognise a 56K phosphorylated component, which appears to correspond to cyclin B2. In sea urchins, Meijer *et al.* (1989) found that cyclin copurified with histone H1 kinase activity through several different purification steps, and there was good correlation between the extent of phosphorylation of cyclin and kinase activity. However, the

exact composition and stoichiometry of these  $p34^{cdc2}$ /cyclin complexes remains to be determined.

### How does cyclin work?

There is now very strong evidence that cyclin is necessary for the appearance of MPF activity and that MPF disappears due to destruction of cyclin (Minshull *et al.* 1989; Murray and Kirschner, 1989; Murray *et al.* 1989). The metaphase  $\rightarrow$  anaphase transition thus appears to be triggered by proteolysis. At present, however, we know little more than this. In particular, we do not know how cyclin acts (we discuss two kinds of model below). How cyclin is destroyed and what turns the destruction mechanism on and off are extremely important issues about which we know almost nothing.

Two general kinds of model could explain how cyclin activates MPF, and how the destruction of cyclin leads to the end of mitosis. The first and presently more widely favoured model, summarized in Fig. 9, is based on the recent finding from several laboratories that cyclin is associated with  $p34^{cdc2}$  in the high molecular weight histone H1 kinase/MPF complex (Draetta *et al.* 1989; Meijer *et al.* 1989; Labbé *et al.* 1989b). It is assumed that the activity of the  $p34^{cdc2}$  kinase requires cyclin

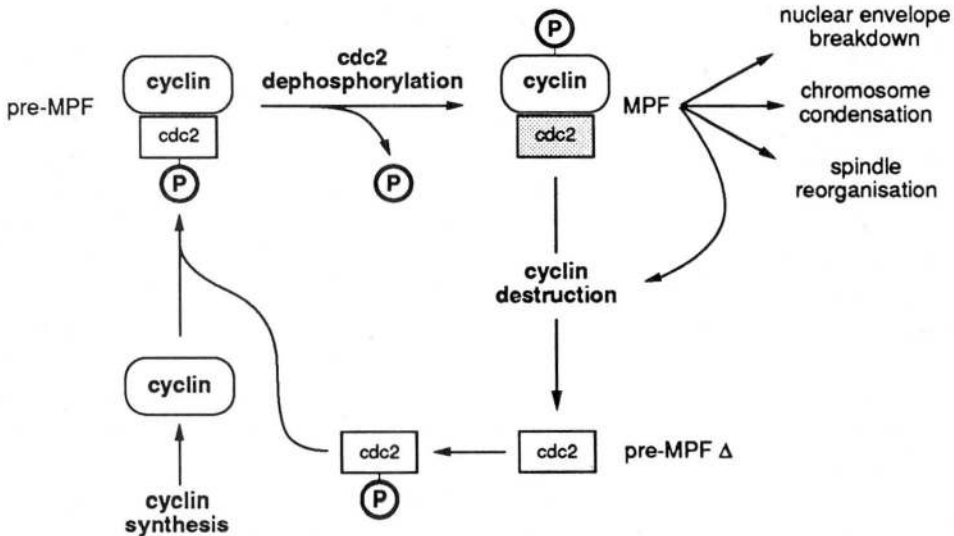


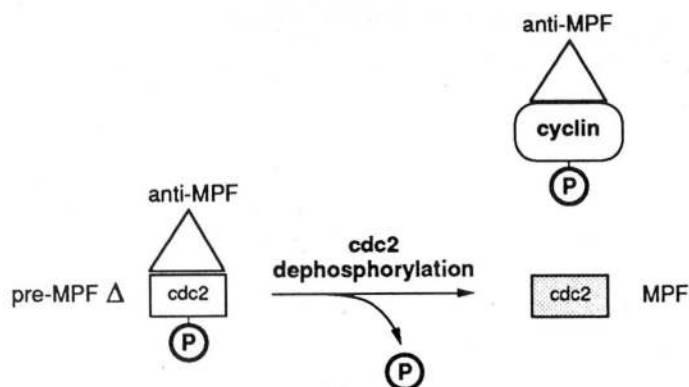
Fig. 9. A model to describe the role of cyclin in the activation of MPF. The diagram shows a simplified cycle in which  $p34^{cdc2}$  is seen as associating with cyclin to form MPF, which possesses histone H1 kinase activity. In accord with current data, we show  $p34^{cdc2}$  as being dephosphorylated and cyclin as being phosphorylated in the most active form of the kinase (Meijer *et al.* 1989; Dunphy and Newport, 1989; Morla *et al.* 1989; Labbé *et al.* 1989a). When cyclin has been proteolyzed,  $p34^{cdc2}$  is called pre-MPF  $\Delta$  to indicate the deletion of cyclin (Murray *et al.* 1989). Pre-MPF contains cyclin and requires post-translational modification to activate it. It is probable that MPF contains  $p13^{suc1}$ , but the cycle of its association with  $p34^{cdc2}$  is not yet known. We believe this model is more likely to be correct than the versions shown in Fig. 10.



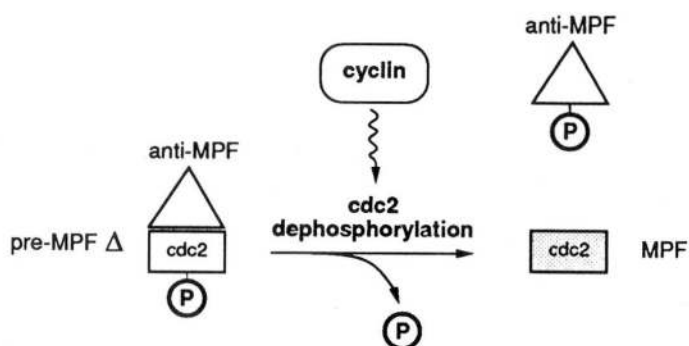
for its activity. According to this view, destruction of cyclin would directly inactivate the mitotic protein kinase. It is a simple matter to add extra features to this model in the form of covalent modifications to cyclin and p34<sup>cdc2</sup> that could regulate the activity of the complex in response to cellular signals. There is persuasive evidence that dephosphorylation of p34<sup>cdc2</sup> is required to activate the kinase (Dunphy and Newport, 1989; Gautier *et al.* 1989; Morla *et al.* 1989). This 'stoichiometric' model does not easily explain why some investigators find low molecular weight forms of histone H1 kinase (Labbé *et al.* 1988*b*), and it is a little uncomfortable that p34<sup>cdc2</sup> appears to be in such large molar excess over cyclin. Labbé *et al.* (1989*a*) estimated that p34<sup>cdc2</sup> is present at a concentration of about 4  $\mu\text{M}$  in starfish oocytes, whereas we estimate that even at the height of its accumulation, cyclin is present at no more than a tenth this concentration, and probably less. Similar concerns are raised by recent experiments by Meijer *et al.* (1989), who used affinity chromatography to purify the H1 kinase from sea urchin eggs, and found that it contained a large excess of p34<sup>cdc2</sup> over cyclin. Perhaps this is a feature of eggs and very early embryos, however, because somatic human cells appear to contain a more or less balanced complement of the two subunits in late G<sub>2</sub> cells (Draetta *et al.* 1988; Pines and Hunter, 1989).

The alternative model assigns a catalytic or triggering role to cyclin. According to this view, cyclin would activate p34<sup>cdc2</sup> in some as yet undetermined way, possibly by direct modification of p34<sup>cdc2</sup> but more likely by preventing the action of an inhibitor of p34<sup>cdc2</sup> activity. *Xenopus* and human cells are known to contain an entity known as anti-MPF, and Cyert and Kirschner have described another inhibitor of MPF activation they call INH (Cyert and Kirschner, 1988). Assuming that anti-MPF does not correspond to the cyclin protease (which may or may not be the case), destruction of cyclin would allow anti-MPF to regain activity and shut down mitotic protein kinase activity. There are certain indications that the catalytic model may be a better description of the way that cyclin works. Thus, Dorée and his colleagues have purified a form of MPF that does not contain intact cyclin (Labbé *et al.* 1988*b*, 1989*a*). Moreover, Murray, Solomon and Kirschner (personal communication) found that removal (by immunoprecipitation) of cyclin from a preparation of partially purified MPF did not lead to loss of activity. They also demonstrated that varying the concentration of cyclin affected the rate of activation rather than the final level of MPF activity (Murray *et al.* 1989). Finally, during the St Andrews meeting in 1989, Hutchison showed an experiment in which a *Xenopus* egg cell-free system treated with aphidicolin exhibited the usual destruction of cyclin at the end of a cell-free first cell cycle, yet histone H1 activity remained high (Hutchison *et al.* 1989, this volume). All these data suggest that it is possible to have high MPF activity without cyclin, and would be easily explained if cyclin acted by keeping an inhibitor of MPF at bay. Thus, if the inhibitor were removed or inactivated by alternative means, whether biochemical or pharmacological, cyclin would not be required to keep histone H1 kinase/MPF active. The two types of model are illustrated schematically in Fig. 10.

'Catalytic' models do not readily explain why p34<sup>cdc2</sup> associates so intimately with cyclin, a finding confirmed in several laboratories (Draetta and Beach, 1988; Draetta



Cyclin acts stoichiometrically as anti-anti-MPF



Cyclin acts catalytically by activating *cdc2* phosphatase and inactivating anti-MPF by phosphorylation

Fig. 10. Two alternative 'catalytic' models to explain how cyclin works. The upper cartoon shows cyclin as binding to and thereby removing a hypothetical anti-MPF subunit, thus activating  $p34^{cdc2}$ . According to this diagram, anti-MPF acts stoichiometrically but both it and cyclin might act catalytically, for example to alter the phosphorylation state of  $p34^{cdc2}$ . It is easy to generate more elaborate versions of this model with positive feedback. In the lower model, cyclin modifies the hypothetical inhibitor, which allows  $p34^{cdc2}$  dephosphorylation.

*et al.* 1989; Meijer *et al.* 1989; Labbé *et al.* 1989b), but are not incompatible with this apparent 'fact'. The stoichiometry of the interaction as well as the presence or absence of other subunits like  $p13^{suc1}$  (Brizuela *et al.* 1987) remain to be determined.

## Conclusion

The behaviour of the cyclins appears to shed a little light on a difficult theoretical biological problem, that of creating all-or-none switches from continuous processes. Entry into prophase and exit from mitotic metaphase seem to be regulated and irreversible processes, and what we now need to discover are the factors besides the

steadily rising level of cyclin that are necessary to trigger entry into mitosis. It should be clear that though cyclin is necessary, it is not sufficient for the  $G_2 \rightarrow M$  transition, whose timing depends on post-translational events. The enzymes that catalyze these events will no doubt be regulated in interesting ways. Likewise, the abrupt destruction of cyclins that is arguably their most intriguing property demands explanations in molecular, enzymological and cellular physiological terms. There is much to learn.

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