Cyclin synthesis drives the early embryonic cell cycle

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We have produced extracts of frog eggs that can perform multiple cell cycles *in vitro*. Destruction of the endogenous messenger RNA arrests the extracts in interphase. The addition of exogenous cyclin mRNA is sufficient to produce multiple cell cycles. The newly synthesized cyclin protein accumulates during each interphase and is degraded at the end of each mitosis.

THE early embryonic cell cycle of many organisms is a rapid alternation of interphase and mitotic states. The transition from interphase to mitosis is induced by the appearance of an activity named maturation promoting factor (MPF)[1-3], which is highly conserved[4]. The cytoplasm of meiotic and mitotic cells of a wide range of eukaryotes contains MPF activity, which can be measured by its ability to induce maturation in *Xenopus* oocytes. MPF is now believed to be a protein kinase that initiates a cascade of reactions which lead ultimately to nuclear envelope breakdown, chromosome condensation and the assembly of the mitotic spindle[5]. This view is supported by the recent finding that one of the subunits of MPF is homologous to the gene product of the *cdc2* gene of *Schizosaccharomyces pombe*, whose activity is required for entry into mitosis[6-8]. The gene product of the *cdc2* gene is a protein of relative molecular mass 34,000, referred to as $p34^{cdc2}$, which shares homology with known protein kinases. Immunoprecipitates made from yeast, *Xenopus* and starfish using anti-$p34^{cdc2}$ antisera have protein kinase activity on a number of substrates including histone H1(refs 7-11).

The fluctuation of MPF activity in *Xenopus* oocytes, eggs and embryos is shown schematically in Fig. 1. Fully grown immature oocytes are arrested in prophase of meiosis I with no detectable MPF activity. Secretion of progesterone by the follicle cells that surround the oocyte induces the post-translational activation of an inactive form of MPF and meiosis I (ref. 3). MPF activity then falls before rising again at the onset of meiosis II. The mature oocytes (which pass down the oviduct and emerge as unfertilized eggs) arrest at metaphase of meiosis II by virtue of a calcium-sensitive activity named cytostatic factor (CSF), which stabilizes MPF activity[12]. Fertilization or artificial activation triggers a rise in the intracellular calcium level, the inactivation of CSF, a decline in MPF activity and entry into interphase of the first mitotic cell cycle. At the end of each interphase MPF activity rises transiently, leading to the induction of mitosis; as MPF activity falls the cell enters the next interphase[3].

In a wide variety of organisms protein synthesis is required for the appearance of active MPF in meiosis II and in mitosis[3,13-15]. In the mitotic cell cycles protein synthesis is required during each and every interphase for the induction of the subsequent mitosis. Because early embryos have large stores of the enzymes and structural proteins required for DNA synthesis and mitosis, the requirement for protein synthesis is likely to represent the synthesis of important cell-cycle regulatory molecules. One attractive candidate for a newly synthesized inducer of mitosis is cyclin[15,16]. The cyclins were identified as embryonic proteins that accumulate during interphase and decline precipitously in abundance during mitosis[15]. Cyclin abundance is regulated by controlling the half life of the protein; it is long during interphase and declines during mitosis. A role for cyclin in regulating mitosis was suggested by experiments which show that cyclin mRNA induces maturation in *Xenopus* oocytes[16,17]. Cyclins have now been identified in many organisms, including sea urchins[17], clams[16], starfish[18], *Xenopus*[19], *Drosophila*[20] and *S. pombe*[21-24], and have been divided into two classes, A and B, on the basis of their sizes[15], kinetics of appearance[15] and sequence homology[19].

To study the role of cyclin in the mitotic cell cycle, we turned to extracts of amphibian eggs that would perform the key reactions of the cell cycle[25-31]. We have developed procedures for producing *Xenopus* extracts that undergo multiple cell cycles, extending the work of Lohka and Masui, who produced a single cell cycle *in vitro*[25]. We have used these extracts to show that cyclin is the only newly synthesized protein required to induce mitosis.

Xenopus cyclin *in vivo*

We looked for *Xenopus* cyclins in eggs that had been released from the CSF-mediated metaphase arrest by electrical activation. Shortly after activation the eggs were injected with [^{35}S]methionine, incubated for various times before lysis, and the newly synthesized proteins analysed on SDS-polyacrylamide gels. A closely spaced set of bands, running with apparent relative molecular mass (M_r) of 55,000 (55K), increased in intensity throughout the first cell cycle but then decreased dramatically between 60 and 75 min after activation. This corresponds to the time during the first cell cycle when MPF activity would normally fall at the onset of anaphase. These bands then increased in intensity, only to decline again at the time (85-95 min) when MPF would normally fall (data not shown). The pattern of accumulation and disappearance of the 55K bands suggests that these proteins are cyclins, although they are much less abundant than their counterparts in clams and sea urchins.

In vitro cell cycle

We prepared *Xenopus* egg extracts that undergo multiple cell cycles *in vitro*. Concentrated extracts were prepared from electrically activated eggs that had been washed in a buffer designed to mimic the ionic composition of egg cytoplasm, crushed and fractionated by centrifugation. Demembranated sperm nuclei and an ATP regenerating system were then added to the concen-

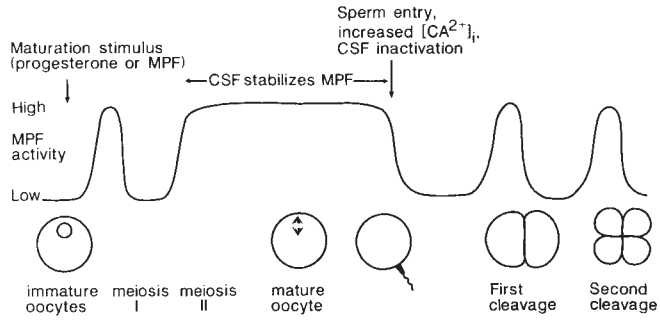
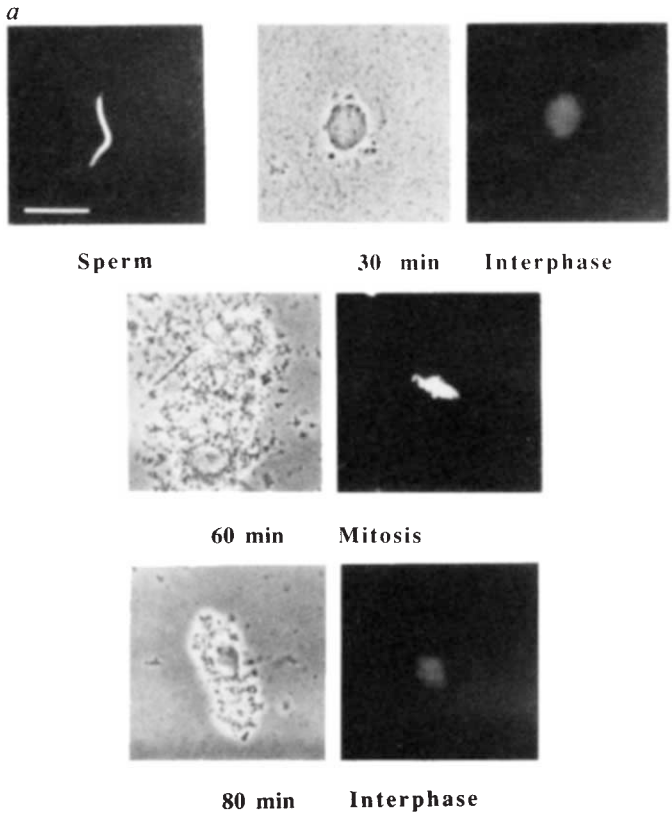


FIG. 1 MPF levels during early *Xenopus* embryonic development. The fluctuation in MPF levels as an immature oocyte passes through meiotic maturation, fertilization and the first two mitotic cell cycles is shown. For further details see the text.

trated cytoplasmic extract. The extracts we have produced have cell-cycle times varying between 35 and 55 min, compared with *in vivo* cycle times of 25–30 min. These extracts, which we refer to as cycling extracts, routinely produce at least three complete cell cycles.

We monitored the progress of the cell cycle in extracts by examining the morphology of the added sperm nuclei in fixed samples stained with a DNA-binding fluorescent dye. We could distinguish five states of the nuclei: interphase, prophase, mitosis and early and late telophase. In the experiment shown in Fig. 2*a*, the sperm nuclei decondensed to form interphase nuclei after 30 min incubation. The nuclear envelope was visible both by phase contrast and immunofluorescence with anti-lamin antibodies (data not shown). At 45 min the nuclei were in prophase,



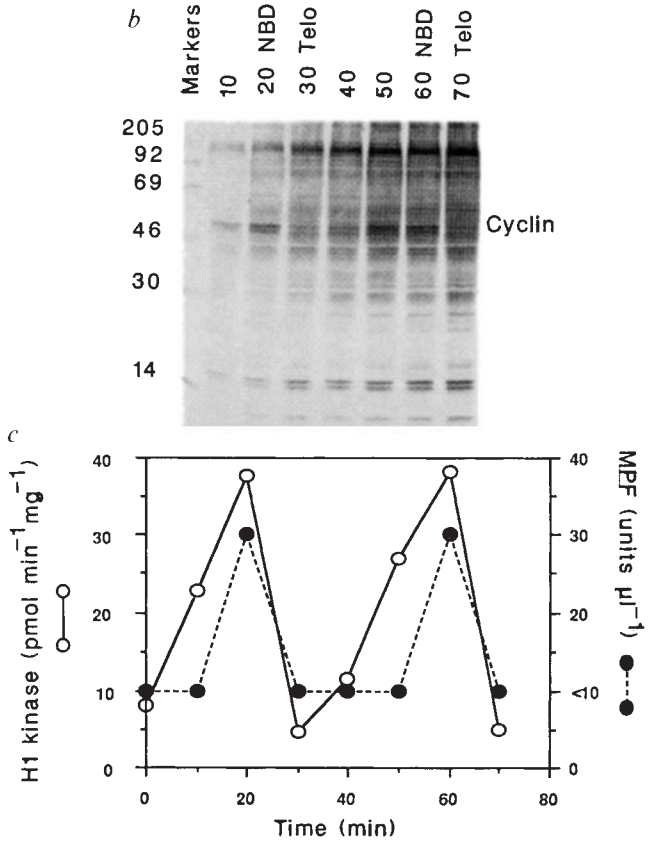


Fig. 2 Behaviour of *in vitro* cell-cycle extracts. *a*, Nuclear morphology at the indicated times of an *in vitro* cell-cycle extract to which sperm nuclei had been added at time zero. Each pair of images consists of a phase contrast image on the left and a fluorescent image of the DNA-binding dye, Hoechst 33342, on the right. Only the fluorescent image of the intact sperm is shown. Scale bar, 20 μm. *b*, Autoradiograph showing the labelled proteins synthesized in an *in vitro* cell-cycle extract to which [^{35}S]methionine had been added at time zero. Samples were taken at the indicated times and either diluted in sample buffer and run on a 12.5% polyacrylamide gel, or fixed for determination of the nuclear morphology. The times of nuclear envelope breakdown (NBD) and telophase (Telo), the positions of the molecular weight markers (in thousands) and the set of cyclin bands are indicated. *c*, The levels of H1 kinase (○—○) and MPF (●·····●) activity during incubation of the extract shown in *b*.

METHODS. Cycling extracts were prepared as follows. Frogs were induced to ovulate and eggs were collected by squeezing into MMR (100 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 0.1 mM NaEGTA, 5 mM NaHEPES, pH 7.8)[46] and dejellied with 2% cysteine, pH 7.9, and then electrically activated in 0.2 ×MMR by two 1-s pulses of 12 V a.c. The activated eggs were washed four times with XB (100 mM KCl, 1 mM $MgCl_2$, 0.1 mM $CaCl_2$, 10 mM KHEPES pH 7.7, 50 mM sucrose) and then twice with XB containing 10 μg ml^{-1} each of leupeptin, chymostatin and pepstatin. Finally, the activated eggs were transferred with a minimal volume of XB to a 5 ml polycarbonate centrifuge tube containing 1 ml of XB plus protease inhibitors containing 100 μg ml^{-1} of cytocholasin B. Ten minutes after activation any residual buffer was removed and the eggs were overlaid with 1 ml Versilube F-50 oil (General Electric, $\rho = 1.03$ g ml^{-1}) and spun at 200*g* for 1 min. The buffer that had been displaced by the oil was then removed and at 15 min after activation the eggs were chilled in an ice bath before being spun for 10 min at 15,000*g* at 2 °C. The cytoplasmic layer was collected by side puncture and the following ingredients were added: MgATP to 1 mM, creatine phosphate to 10 mM, EGTA, pH 7.7, to 0.2 mM and leupeptin, chymostatin, pepstatin and cytochalasin B to 10 μg ml^{-1} each. The extract was then spun again at 15,000*g* for 15 min at 2 °C to remove residual yolk and pigment granules. This extract was stored on ice and used within three hours. All the experiments in this paper were performed with fresh extracts and the final volume of added components never exceeded 20% of the volume of cytoplasmic extract. Sperm nuclei were prepared by a slight modification of the method described by Gurdon[47], stored in 30% glycerol at −70 °C and used at a final concentration of 10^5 per ml in the extract. All incubations were performed at 23 °C. Samples were analysed for morphology by spotting 1 μl extract on a microscope slide and then adding 4 μl fixative (MMR containing 50% glycerol (w/v), 10% formalin and 1 μg ml^{-1} Hoechst 33342) before squashing the drop with a coverslip and examining the nuclei by phase contrast and fluorescent microscopy. MPF was assayed by diluting samples twofold into EB (80 mM potassium β-glycerophosphate, 15 mM $MgCl_2$, 20 mM potassium EGTA, adjusted to pH 7.3 after mixing the components) and assaying them for MPF activity in cycloheximide-treated immature oocytes as described[3]. One MPF unit is the amount of MPF activity which when injected in a volume of 50 nl will induce germinal vesicle breakdown in 50% of injected oocytes. H1 kinase activity was assayed by diluting samples fiftyfold into EB and assaying for H1 kinase activity by adding 10 μl to 6 μl containing 1 mg ml^{-1} of calf thymus histones (a gift of J. Minden[48]), and 1 mM ATP, 0.25 μCi μl^{-1} [γ^{32}P] ATP. The phosphorylated H1 kinase was run on 5–15% gradient polyacrylamide gels and the amount of incorporated phosphate quantified by cutting out and counting the gel bands or by densitometry; H1 kinase activity is expressed as pmol incorporated phosphate min^{-1} mg^{-1} of protein (for counted gel bands) or arbitrary units (for samples assayed by densitometry). All the MPF and H1 kinase assays in this paper were performed on samples that had been diluted into EB and then frozen in liquid nitrogen. To analyse the pattern of protein synthesis [^{35}S]methionine was added to a final concentration of 0.4 mCi ml^{-1} and samples were taken, diluted tenfold into sample buffer and run on 12.5% polyacrylamide gels[49]. Detailed protocols for the preparation of cell-cycle extracts are available on request.

which is characterized by the start of chromosome condensation and the further swelling of the nucleus. Nuclear breakdown occurred by 60 min and clusters of condensed chromosomes were seen. Nuclear breakdown and chromosome condensation occurred in all extracts, but only some extracts formed well defined mitotic spindles. At 70 min the chromosomes had started to decondense into the individual mini-nuclei or karyomeres characteristic of early telophase, and by 80 min interphase nuclei were present once more. At 100 min the nuclei were in prophase and a second round of nuclear breakdown followed at 110 min. The synchrony of the nuclei in these extracts was good: at most time points all the nuclei were in the same morphological stage and no time point contained nuclei in more than two stages. *In vitro*, as *in vivo*, protein synthesis was required in each interphase to allow the occurrence of the next round of nuclear breakdown (data not shown).

The pattern of protein synthesis in cycling extracts was monitored by adding [^{35}S]methionine at the start of the reaction and withdrawing samples at intervals during two cell cycles and analysing them on SDS gels. Figure 2*b* shows the behaviour of the cyclin bands *in vitro*, which is essentially identical to that seen *in vivo*. The intensity of the cyclin bands increased throughout the first interphase and then declined dramatically between nuclear breakdown and the onset of telophase. During the second interphase the intensity of these bands again increased, only to fall again after the second round of nuclear breakdown. These bands are indeed the translation products of the *Xenopus* cyclin B genes that have been cloned on the basis of their homology with sea urchin cyclin: treatment with RNase H and anti-sense oligonucleotides derived from the cloned cyclins destroyed the mRNAs that encode these bands (J. Minshull, T. Hunt, A. W. M. and M. W. K., unpublished data). The two primary cyclin translation products undergo post-translational modification to yield as many as five closely spaced bands on gels[19].

To demonstrate that biochemical events occurring *in vivo* also occur in cell-cycle extracts, we monitored DNA replication and the levels of both MPF and H1 kinase activity, which recent evidence suggests is very closely related to MPF[7,9,11]. Figure 2*c* shows that MPF activity appeared at the time of nuclear breakdown and was undetectable by early telophase. Like MPF, H1 kinase activity peaked at the time of nuclear breakdown and was dramatically diminished by telophase, although appreciable activity was detectable in late interphase and prophase, when MPF was undetectable. This apparent discrepancy may simply reflect the high threshold for the MPF assay. We have also shown that DNA replication only occurs during interphase (data not shown), as previously shown in other *Xenopus* extracts[26,28].

Cyclin induces mitosis

To test whether cyclin synthesis in the absence of other protein synthesis can induce mitosis, we added cyclin mRNA to cell-cycle extracts whose endogenous mRNA had been destroyed. We made such extracts, which we call mRNA-dependent extracts, by using pancreatic RNase to destroy the endogenous mRNA in extracts prepared from activated eggs and then inhibiting the RNase with placental RNase inhibitor, RNAsin[32]. Figure 3*a* compares the pattern of protein synthesis in an mRNA-dependent extract, to which no exogenous mRNA had been added, with that in a mock treated extract to which the RNase inhibitor, but no RNase, had been added. In the mock treated extract cyclin accumulated until 30 min, when breakdown of the nuclear envelope occurred. By 40 min the cyclin bands had declined in intensity and telophase had commenced. In the mRNA-dependent extract the rate of protein synthesis was less than 5% of that of the controls. Over a period of 120 min in the RNase-treated extracts, the nuclei swelled but did not break down. Thus RNase treatment can effectively destroy endogenous mRNA and block entry into mitosis. When a strongly translated mRNA such as the viral RNA of tobacco mosaic virus was added to the mRNA dependent extracts, we obtained 50% of the pretreatment level of total protein synthesis but failed to restore the ability of extracts to enter mitosis (data not shown).

We then asked whether the translation of cyclin mRNA was sufficient to allow mRNA-dependent extracts to enter mitosis. Sea urchin cyclin B mRNA was transcribed *in vitro* from a complementary DNA clone (supplied by J. Pines and T. Hunt[17]). This mRNA was added to an mRNA-dependent extract at three concentrations. For each reaction, [^{35}S]methionine was added at the time of RNA addition. The patterns of protein synthesis and nuclear morphology during the course of this experiment are shown in Fig. 3*b* and *c*, respectively. At a cyclin mRNA concentration of 5 μg ml^{-1}, cyclin protein accumulated, reaching a peak at 40 min, at which point the nuclei had broken down. By 50 min the nuclei were in telophase and the cyclin band had dramatically decreased in intensity. The cyclin band then increased in intensity to a second maximum at 120 min when the nuclei had once more broken down (Fig. 3*b*, *c*). Figure 3*d* shows that in mRNA-dependent cell cycle extracts where the cell cycle is driven by the synthesis of sea urchin cyclin, the activities of MPF and H1 kinase increased in prophase, were maintained at high levels during mitosis, and then declined at the end of mitosis. Thus the synthesis of sea urchin cyclin is sufficient to induce both the morphological and biochemical events characteristic of mitosis.

To test whether the rate of cyclin accumulation affected the length of interphase, we added different concentrations of cyclin mRNA to the mRNA-dependent extracts. At 2.5 μg ml^{-1} of cyclin mRNA, the accumulation of cyclin was slightly slower and nuclear envelope breakdown did not occur until 50 min; at 60 min telophase had begun and the cyclin abundance had declined markedly (Fig. 3*b*). At this concentration of mRNA a second round of nuclear envelope breakdown did not occur during the experiment. Finally, at the lowest concentration of mRNA, cyclin accumulated at a slower rate and the nuclei never entered mitosis, nor did the intensity of the labelled cyclin band ever decline (Fig. 3*b*). In experiments that were carried out for longer times, the translation of cyclin mRNA added to mRNA-dependent extracts has induced as many as three rounds of nuclear envelope breakdown. These experiments suggest that cyclin synthesis is sufficient both to induce mitosis and to allow progression from mitosis to the next interphase, and that its rate of accumulation affects the length of interphase.

We have also tested the ability of the *Xenopus* cyclins to induce mitosis. Transcripts of either *Xenopus* B1 or B2 cyclin clones (provided by J. Minshull and T. Hunt) drove the *in vitro* cell cycle (data not shown). Both cyclin proteins increased in abundance during interphase, reached peak levels at nuclear envelope breakdown and had declined by the onset of telophase.

Discussion

We have developed an *in vitro* cell-cycle extract that faithfully reproduces the key features of the cell cycle in early *Xenopus* embryos and performs multiple cell cycles. The endogenous mRNA in such an extract could be destroyed, arresting the cell cycle in interphase and producing an mRNA-dependent extract. In an mRNA-dependent extract, the translation of sea urchin cyclin B or either of two *Xenopus* cyclin B mRNAs was sufficient to drive multiple cell cycles. These results imply that cyclin synthesis can satisfy the protein synthesis requirement both for the activation of MPF and the entry into mitosis, as well as for the degradation of cyclin, inactivation of MPF and progress to the next interphase. The amount of cyclin protein present at mitosis in mRNA-dependent extracts programmed with cyclin mRNA is not substantially different from that translated from the endogenous cyclin mRNA in mock treated extract. This demonstrates that cyclin made in the absence of synthesis of other protein can induce mitosis at about the same level as when the protein is made in untreated extracts.

One limitation to the conclusion that cyclin synthesis is

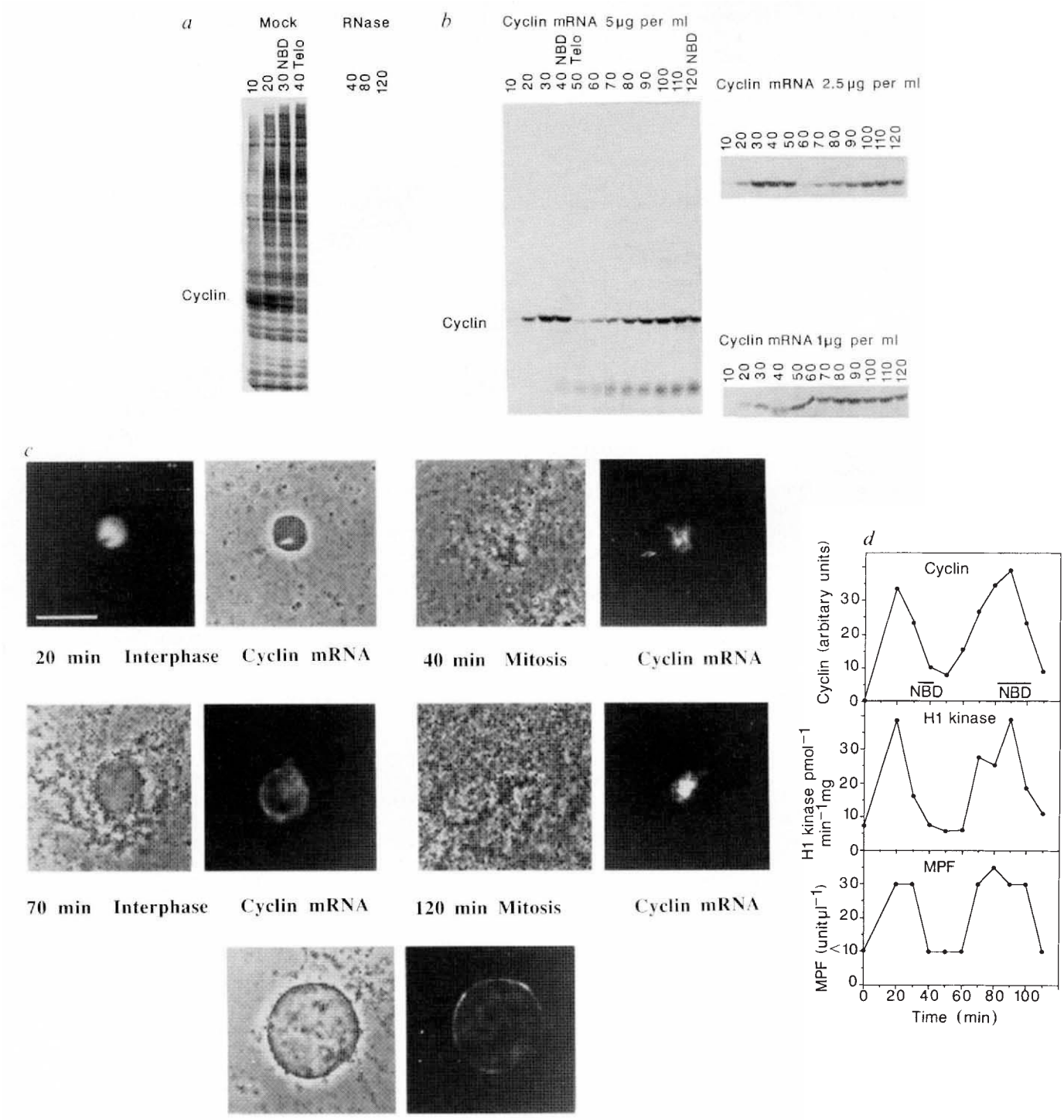


FIG. 3 Cyclin synthesis is sufficient to drive the embryonic cell cycle. *a*, The pattern of protein synthesis in mock treated and RNase-treated extracts. Cell cycle extracts prepared from activated eggs were treated with RNasin (gift of David Drechsel) alone (Mock) or with RNase followed by RNasin (RNase). [^{35}S]methionine was added to the extracts, which were then incubated and samples were removed at the indicated times and run on 10% polyacrylamide gels. The times of nuclear envelope breakdown (NBD) and telophase (Telo) and the position of cyclin are indicated. *b*, The pattern of protein synthesis in mRNA-dependent extracts with different doses of added sea urchin cyclin mRNA. [^{35}S]Methionine and cyclin mRNA were added 10 min after the addition of RNasin and samples were taken for analysis on 10% polyacrylamide gels and for nuclear morphology at the indicated times. The times of nuclear envelope breakdown and telophase are indicated. *c*, Nuclear morphology in the mRNA-dependent extracts with and without added sea urchin cyclin mRNA whose pattern of protein synthesis is shown in *a* and *b*. Each pair of images consists of a phase-contrast image and a fluorescent image of the DNA-binding dye Hoechst 33342, as described in Fig. 2. Scale bar, 20 μm. *d*, The fluctuations in cyclin abundance and MPF and H1 kinase activity in an mRNA-dependent extract to which 5 μg ml^{-1} of sea urchin cyclin mRNA had been added. The times of nuclear breakdown are indicated (NBD).

METHODS. Cycling extracts were prepared and incubated with a final concentration of 0.25 μg ml^{-1} of boiled pancreatic RNase at 10 °C for 20 min before adding 0.05 volume of a solution of placental RNase inhibitor (RNasin, OD_{280} = 0.21) and incubating for a further 10 min at 10 °C and then adding calf liver transfer RNA to 50 μg ml^{-1}, [^{35}S]methionine to 0.4 mCi ml^{-1} and sea urchin cyclin B mRNA in 100 mM KCl and 1 mM $MgCl_2$. The mRNA was transcribed by T7 polymerase from the sea urchin cyclin B cDNA clone as described[17] and then treated with RNase-free DNase to destroy the template DNA. Cyclin abundance was measured by densitometry.

sufficient to drive the cell cycle is that some protein synthesis occurs between activation and the RNase treatment that produces mRNA-dependent extracts. The ability of cyclin synthesis to induce multiple rounds of nuclear envelope breakdown demonstrates that cyclin synthesis is sufficient to induce mitosis in a cell cycle where no other protein synthesis has occurred. Although these experiments show that cyclin synthesis is sufficient to drive later cell cycles, it does not prove that other proteins synthesized in the first cell cycle are not required for the first cell cycle. In the accompanying paper[33] we demonstrate that cyclin protein made in a cell-free translation system can induce entry into mitosis when it is added to an extract where cycloheximide had been used to inhibit protein synthesis before the start of the first mitotic cell cycle. This experiment also shows that the translation of residual endogenous mRNA in the extracts, or of mRNA newly transcribed from the added sperm nuclei, does not supply proteins that are necessary for the induction of mitosis.

The length of the cell cycle increased as the amount of cyclin mRNA was reduced, suggesting that cyclin accumulation has to occur to some critical level to induce entry into mitosis. Thus at least a fraction of the length of interphase in embryonic cell cycles represents the time required to accumulate cyclin to this critical level. This time can be estimated by measuring the time in interphase at which the addition of inhibitors of protein synthesis no longer blocks the occurrence of the next mitosis[34,35]. This time varies in different embryonic cell cycles, *in vivo*, but is always less than half the length of interphase, suggesting that there are slow steps between the accumulation of cyclin and the activation of MPF and occurrence of mitosis.

The lack of other newly synthesized proteins in the mRNA-dependent extracts strongly suggests that when the cyclin band disappears at the end of mitosis, it does so as a result of proteolysis, rather than as a result of some form of post-translational modification which alters its gel mobility so that it is obscured by other bands. Finally, the stability of the newly synthesized cyclin at the lowest dose of cyclin mRNA shows that the rapid degradation of cyclin is not induced unless it is present in doses sufficient to induce the extract to enter mitosis.

Recent mRNA ablation experiments have shown that cyclin synthesis is necessary for entry into mitosis. In these experiments it was necessary to cleave the mRNAs for both *Xenopus* cyclin B1 and B2 to prevent entry into mitosis[19], confirming the functional redundancy we have seen for these two proteins. The demonstration that cyclin synthesis is both necessary and sufficient for entry into mitosis strongly suggests that cyclin is the only newly synthesized protein required for the induction of mitosis in *Xenopus* embryos.

The ability of cyclin synthesis to induce mitosis suggests an attractive model for the early embryonic cell cycle (Fig. 4*a*). In this model, cyclin activates the protein kinase activity of $p34^{cdc2}$ that constitutes MPF activity. An opposing activity inactivates this $p34^{cdc2}$ kinase activity. The level of MPF activity is thus controlled by the ratio of the activities of cyclin and the inactivator. Early in interphase cyclin levels are low and the activity of the inactivator is dominant, keeping MPF in its inactive form. Cyclin accumulates during interphase until its activity exceeds that of the inactivator. At this point, MPF activation begins and ultimately triggers a cascade of reactions which lead to the morphological and biochemical events of mitosis. We postulate that MPF induces the degradation of cyclin. Once cyclin has been destroyed, the inactivator is once more dominant, MPF is inactivated and the cell cycle progresses into the next interphase. In this model both interphase and mitosis are unstable states. The instability of interphase is caused by the accumulation of cyclin, whereas that of mitosis is due to the ability of MPF to induce degradation of cyclin. This simple model of the early *Xenopus* cell cycle predicts that cyclin degradation is required to exit from mitosis. The accompanying paper[33] verifies this prediction and examines the mechanism by which cyclin activates MPF.

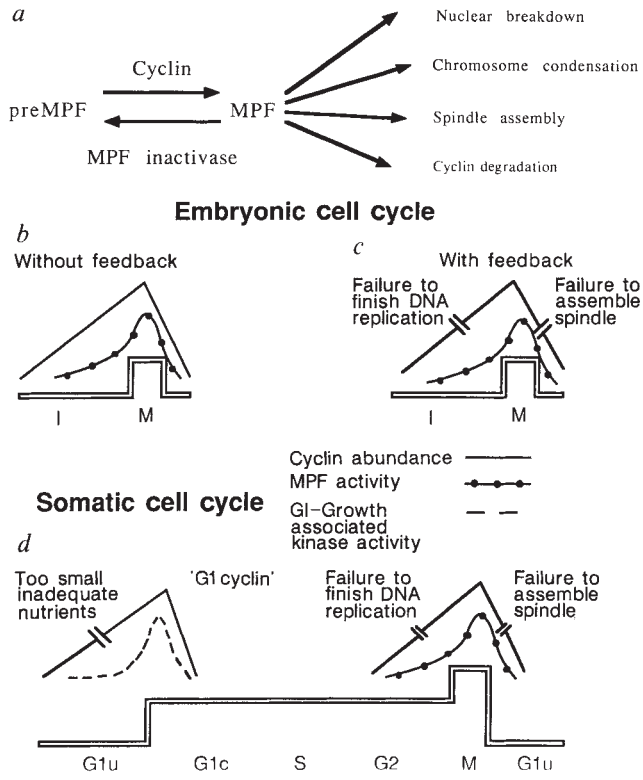


FIG. 4 A model for the cell cycle. *a*, An overview of the proposed reactions controlling passage through the cell cycle. *b*, A diagram of alternation of interphase (I) and mitosis (M), and the levels of cyclin (——) and MPF activity (●—●) in an early embryonic cell cycle without feedback controls. *c*, A diagram of an embryonic cell cycle with feedback controls (II). *d*, A schematic view of a somatic cell cycle showing the role of a Gl cyclin in activating a Gl-specific $p34^{cdc2}$ kinase activity (— —) to drive the cells from a state where they are not committed to the mitotic cell cycle (Glu), to a state where they are committed to enter DNA synthesis and ultimately progress to mitosis (Glc). See text for further details.

In this model the accumulation of cyclin initiates a pathway that leads to the post-translational activation of $p34^{cdc2}$ to a form that has MPF activity. During interphase in early *Xenopus* embryos, none of the events in this pathway, except the concentration of cyclin, seems to be regulated, making cyclin the trigger of mitosis. In the more tightly controlled cell cycle of somatic cells, other steps in the pathway that leads to MPF activation, such as cyclin or $p34^{cdc2}$ phosphorylation, may be subject to regulation. One example of this may be the post-cellularization divisions of *Drosophila* embryos. In these cell cycles, cyclin synthesis is required, but it is the accumulation of the gene product of the *string* gene that appears to trigger mitosis[36]. The *string* gene is the homologue of the *S. pombe cdc25* gene, which acts as a dose-dependent activator of the mitosis-inducing function of *cdc2* (ref. 37).

The cyclin-based oscillator in frog extracts lacks feedback controls and will continue to cycle even when processes such as DNA synthesis or spindle assembly are inhibited by aphidicolin and nocodazole (data not shown; see Fig. 4*b*). The lack of feedback controls in the extract is in good agreement with the previous demonstrations that the oscillations in MPF activity were unaffected by inhibiting DNA synthesis or microtubule polymerization[3,38,39]. In all somatic cells and in the embryos of many organisms the cell cycle manifests feedback controls that prevent the initiation of one step in the cell cycle until the previous step has been successfully completed[40]. The cyclin-based oscillator may be converted to such a cell cycle by imposing controls on the accumulation and degradation of

cyclin (Fig. 4*c*). One example is the action of CSF, which appears to stabilize MPF activity by blocking the degradation of cyclin[35]. In this case, inactivation of CSF and progress into the mitotic cell cycle is made dependent upon an increase in cytoplasmic calcium concentration induced by fertilization. It is easy to imagine that CSF or a related molecule could act in somatic cells to prevent the degradation of cyclin until the spindle has been successfully assembled. In this case the signal that inactivates CSF would be generated by a pathway that monitors some parameter of spindle assembly, such as stable attachment of the kinetochores to microtubules. In sea urchin and clam embryos, microtubule depolymerization greatly increases the length of mitosis and stabilizes cyclin[15]. The completion of DNA synthesis could be used to regulate either the accumulation of cyclin, or some post-translational step in the activation of MPF. The latter possibility is suggested by the ability of aphidicolin to block sea urchin embryos in interphase, even though cyclin accumulates to very high levels (T. Hunt, personal communication).

In early embryos, regulation of the cell cycle appears to be limited to the control of the entry into and the exit from mitosis. Somatic cells possess an additional control point located in G1, where cells can either become committed to passage through the cell cycle or remain in a resting state. This commitment point has been named 'Start' in studies on yeasts and the restriction point in mammalian tissue culture cells[40,41]. Three lines of evidence fuel the speculation that passage through Start requires the accumulation of a molecule related to cyclin that induces a particular spectrum of $p34^{cdc2}$ kinase activity (Fig. 4*d*). First, experiments on tissue culture cells suggest that the accumulation of an unstable protein to some critical level is required to pass the restriction point[42]. Second, the *cdc2* gene of *S. pombe* is required at Start as well as for the induction of mitosis[43]. The Start function of *cdc2* is independent of the genes that regulate its mitotic function, *cdc25*, *weel* and *cdc13*, the *S. pombe* B-type cyclin, suggesting both that the substrate specificity and kinase activity of $p34^{cdc2}$ at Start and the induction of mitosis are different, and that a second group of genes that has not yet been identified exists to modulate the activity of $p34^{cdc2}$ at Start[8]. Third, homologues of both cyclin and $p34^{cdc2}$ have been identified among mutants which affect the ability of mating pheromones to arrest the cell cycle of *S. cerevisiae* at Start[44,45] (W. Courchesne and J. Thorner, personal communication). These lines of evidence strongly suggest that the interaction between members of the cyclin and $p34^{cdc2}$ families will play a key part in the regulation of Start as well as in the induction of mitosis. □

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The role of cyclin synthesis and degradation in the control of maturation promoting factor activity

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We show that cyclin plays a pivotal role in the control of mitosis. A proteolysis-resistant mutant of cyclin prevents the inactivation of maturation promoting factor and the exit from mitosis both *in vivo* and *in vitro*. We have used a fractionated extract to study the activation of MPF by added cyclin protein.

THE activation of a protein complex called maturation promoting factor (MPF) induces cells to enter mitosis and meiosis. In the accompanying paper[1] we show that cyclin is the only newly synthesized protein required to induce MPF activity and the entry into mitosis in the cell cycle of early *Xenopus* embryos. This finding implies that in early embryonic cell cycles, none of the other components of MPF needs to be synthesized in each cell cycle, and that all steps in MPF activation and inactivation other than cyclin accumulation are post-translational events. Here we investigate the role of cyclin synthesis and degradation