Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 2060–2064, March 1996
Developmental Biology

A developmental timer regulates degradation of cyclin E1 at the midblastula transition during *Xenopus* embryogenesis

JOHN A. HOWE AND JOHN W. NEWPORT*

Department of Biology, University of California, San Diego, La Jolla, CA 92093-0347

Communicated by John Gerhart, University of California, Berkeley, CA, November 28, 1995 (received for review August 14, 1995)

ABSTRACT **We have analyzed cyclin E1, a protein that is essential for the G_1/S transition, during early development in *Xenopus* embryos. Cyclin E1 was found to be abundant in eggs, and after fertilization, until the midblastula transition (MBT) when levels of cyclin E1 protein, and associated kinase activity, were found to decline precipitously. Our results suggest that the reduced level of the cyclin E1 protein detected after the MBT does not occur indirectly as a result of degradation of the maternally encoded cyclin E1 mRNA. Instead, the stability of cyclin E1 protein appears to play a major role in reduction of cyclin E1 levels at this time. Cyclin E1 protein was found to be stable during the cleavage divisions but degraded with a much shorter half-life after the MBT. Activation of cyclin E1 protein turnover occurs independent of cell cycle progression, does not require ongoing protein synthesis, and is not triggered as a result of the ratio of nuclei to cytoplasm in embryonic cells that initiates the MBT. We therefore propose that a developmental timing mechanism measures an ≈5-hr time period, from the time of fertilization, and then allows activation of a protein degradative pathway that regulates cyclin E1. Characterization of the timer suggests that it might be held inactive in eggs by a mitogen-activated protein kinase signal transduction pathway.**

Cyclin proteins are regulatory subunits of a family of cell cycle controlling kinases, or cyclin-dependent kinases (cdks), that are required for a number of cell cycle events (1, 2). In higher eukaryotes cdk2 is thought to play an essential role in control of S phase (3, 4) and is regulated by A- and E-type cyclins. A number of observations suggest that cyclin E/cdk2 complexes are required for initiation of S phase. Cyclin E is periodically expressed during the cell cycle and maximally activates cdk2 at the G_1/S transition (5, 6). Overexpression of cyclin E in tissue culture cells diminishes growth factor requirements and promotes premature entry into S phase (7–9), while microinjection of cyclin E antibodies during G_1 inhibits entry into S phase (10). Furthermore, during early *Drosophila* development down regulation of cyclin E is required for exit from the embryonic cell cycle, and ectopic expression of cyclin E in cells that normally arrest in G_1 results in progression through an extra cell cycle (11). Cyclin A is also required for S-phase progression (12–14) and is unique among cyclins in that it forms functional complexes with both the mitotic regulator cdc2, as well as cdk2 (15, 16). Cyclin A is detected in active complexes with cdk2 shortly after cyclin E/cdk2 complexes are first detected (5, 6), and antibody ablation and antisense RNA experiments have indicated that cyclin A is essential for S phase (12, 13). A currently popular model suggests that cyclin E/cdk2 complexes initiate S phase, after which cyclin E is degraded, and further S-phase progression is regulated by cyclin A/cdk2 complexes (2).

The atypical embryonic cell cycles in organisms such as *Drosophila* and *Xenopus* provide useful models to study regulation of cell cycle control molecules. In *Xenopus*, fertilization is followed by a stage of rapid cellularization during which the egg divides synchronously 12 times in 6 hr. These early cell cycles are biphasic, consisting only of S and M phases that last ≈15 min each. After the 12th cleavage division, embryos go through the midblastula transition (MBT). At this time zygotic transcription is initiated and the cell cycle elongates (17, 18). The first two cell cycles immediately following the MBT are longer (50 and 100 min, respectively) most likely due to an expansion in the length of S phase (19). The third cell cycle after the MBT is the first that is substantially expanded, with a length that ranges from 3 to 6 hr. This expansion occurs at the onset of gastrulation when further development and cell division first become dependent on zygotic transcription (20).

We have been studying emergence of the somatic cell cycle after the MBT to learn more about regulation of cyclin proteins and mRNAs. In a previous study, we found that the maternal pools of cyclins A1 and A2 mRNAs were stable prior to the onset of gastrulation (EGT; early gastrula transition) and then were abruptly degraded (21). At this time, cyclin A2 mRNAs, but not cyclin A1 messages, were replaced by zygotic transcription. In addition, we found that cyclin A1 protein was stable during interphase prior to the EGT but turned over rapidly during interphase after the EGT. These results suggested that activation of degradative programs that control cyclin A levels in the somatic cell cycle could be first activated at the onset of gastrulation. Further characterization suggested that the components of these degradative systems were present in the egg, or synthesized shortly after fertilization, but held inactive until the EGT by a developmental timing mechanism.

To determine whether other cyclins were regulated by degradative pathways activated during early development, we undertook a study of one of the *Xenopus* E-type cyclins, cyclin E1. During cleavage cell cycles in *Xenopus*, cdk2 is associated primarily with E-type cyclins and only low concentrations of cyclin A/cdk2 complexes have been detected (ref. 22; J.A.H., unpublished data). Cyclin E1 has previously been shown to be synthesized during oogenesis and detected at high concentrations during the cleavage stages, but it disappears between developmental stages 8 and 10.5 (22). Here we show that cyclin E1 protein is stable during the cleavage divisions but turns over with a decreased half-life during interphase after the MBT. Although the time in development when cyclin E1 becomes unstable is distinct from the EGT the proteolytic system that controls E1 turnover appears to be regulated by a similar timing mechanism. We show that although the degradation of cyclins A and E occurs at different times during development, the timers that regulate the onset of these two degradative processes share a number of features.

MATERIALS AND METHODS

Embryos. *Xenopus* eggs were fertilized *in vitro* as described (17). Fertilized eggs were dejellied 30 min after fertilization

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: MBT, midblastula transition; EGT, early gastrula transition; MAPK, mitogen-activated protein kinase; MAPKK, mitogen-activated protein kinase kinase; CHX, cycloheximide; HU, hydroxyurea; cdk, cyclin-dependent kinase.

*To whom reprint requests should be addressed.

using 2% L-cysteine (pH 7.8), washed five times in 0.25× MMR solution (100 mM NaCl/20 mM KCl/20 mM $CaCl_2$/10 mM $MgCl_2$/5 mM Hepes, pH 7.4), and maintained at ambient temperature (23°C–24.5°C). When appropriate, hydroxyurea (HU) or cycloheximide (CHX) was added to the culture medium to final concentration of 30 mM or 100 μg/ml, respectively. In some cases, to enhance the rate of CHX uptake, embryos were incubated in Danilchik's solution without calcium (23) beginning 2 hr after fertilization. Fertilized eggs where microinjected in 1× MMR containing 5% Ficoll to help maintain the integrity of the plasma membrane. All injections were carried out using a Picospritzer II (General Valve Corp., Fairfield, NJ). Coenocytial eggs were produced by centrifugation of fertilized eggs in MMR containing 5% Ficoll, over a cushion of 50% Ficoll, for 5 min at 1000 rpm in a tabletop IEC (Needham Heights, MA) clinical centrifuge.

Extracts, p13-Sepharose Purification, Immunoprecipitation, and H1 Kinase Assays. Fertilized eggs were collected at relevant time points and flash frozen in liquid nitrogen. Developmental staging was according to Niewkoop and Faber (24). Cytosolic extracts were made by solubilizing frozen embryos in extraction buffer [EB; 20 mM Hepes, pH 7.3/80 mM β-glycerophosphate/20 mM EGTA/15 mM $MgCl_2$/50 mM NaF/1 mM $NaVO_4$/0.5 mM phenylmethylsulfonyl fluoride (PMSF)/0.5% NonidetP40 (NP-40)] and clarified by centrifugation in a tabletop centrifuge for 15 min at 4°C. For immunoprecipitations and p13-Sepharose purification, cytosolic extracts from 10–20 embryos were diluted with EB and 20 μl of p13-Sepharose beads (25) or the appropriate antibodies and 30 μl of a 50% slurry of protein A-Sepharose beads (Pharmacia) was added. After a 1-hr incubation with constant rotation, beads were washed four times with bead wash buffer (50 mM Tris·HCl, pH 7.5/5 mM NaF/250 mM NaCl/5 mM EGTA/5 mM EDTA/0.5 mM PMSF/0.1% NP40). Proteins purified by immunoprecipitation or by p13-Sepharose precipitation were diluted in 1:4 sample buffer, boiled, and separated by PAGE. For measuring cyclin E1-associated histone H1 kinase activity, cyclin E1 immunoprecipitates from four embryos were washed two additional times with H1 kinase buffer (40 mM Hepes, pH 7.3/20 mM $MgCl_2$/1 mM dithiothreitol) and then diluted with 60 μl of H1 kinase buffer containing 0.5 mM ATP/0.2 mg of histone H1 per ml, and 10 μCi of [γ-^{32}P]ATP (1 Ci = 37 GBq). After incubation at room temperature for 15 min, the reactions were stopped by addition of 4× sample buffer, boiled, and separated by PAGE. Phosphorylated forms of histone H1 were detected on dried gels by autoradiography and quantitated by Cherenkov counting.

Western Blotting and Antibodies. Proteins were transferred to Immobilon-P membranes (Millipore) for 2 hr at 55 V. Membranes were blocked with PBST (127 mM NaCl/8 mM Na_2PO_4/2.7 mM KCl/1.1 mM KH_2PO_4/0.1% Tween) containing 5% dried milk powder, rinsed with PBST, and then incubated with diluted primary antibodies for 1 hr. The filters were then extensively washed with PBST. Primary antibodies were detected with horseradish peroxidase-coupled secondary antibodies followed by enhanced chemiluminescence using an ECL kit (Amersham).

The cyclin E1 antibody used in these studies was raised in rabbits against a cyclin E1 peptide (SVRSRKRKADVA; amino acid residues 23–34) using the services of Research Genetics (Huntsville, AL). Whole serum was affinity purified against the peptide antigen coupled to Affi-Gel 10 agarose beads (Bio-Rad) according to the protocol described (26). The anti-peptide cdk2 and cyclin A2 antibodies have been described (4, 21) and the cyclin A1 antibody, a kind gift of J. Maller (University of Colorado, Denver, CO) was raised in sheep against a full-length cyclin A1 fusion protein. The full-length cyclin E1 DNA sequence was obtained by PCR from the published sequence (22).

RESULTS

To analyze cyclin E1 protein during embryogenesis, eggs were collected at 1-hr intervals following fertilization and assayed for cyclin E1 protein concentration and cyclin E1-associated H1 kinase activity. Cyclin E1 protein was detected in unfertilized eggs and in eggs collected during the first 5 hr after fertilization at approximately equimolar concentrations (Fig. 1*A*). However, after the 5-hr time point the amount of cyclin E1 protein detected decreased rapidly and was almost undetectable at 7 hr. The H1 kinase activity associated with cyclin E1 complexes followed an almost identical profile during this time frame (Fig. 1*A*, E1-H1K). The level of cyclin E1-associated kinase activity was high and constant during the first 6 hr after fertilization and then decreased rapidly between 6 and 7 hr. The decrease in H1 kinase activity associated with E1 complexes was not due to a reduction in the levels of the catalytic kinase subunit of cyclin E1, cdk2, as this protein was detected at equal concentrations at each time point assayed. Together these results demonstrate that cyclin E1 protein levels begin to decline rapidly 6 hr after fertilization at a time point that closely corresponds to the MBT. This time point is distinct from the EGT when the embryonic A-type cyclin, cyclin A1, begins to degrade (Fig. 1*B*; ref. 21).

The precipitous decrease in cyclin E1 levels that we have observed 6 hr after fertilization correlates temporally with the MBT. This coincidence is consistent with the MBT triggering some molecular mechanism that is responsible for regulating the concentration of cyclin E1 protein. However, cell cycle progression after the MBT could also be required for reduction in cyclin E1 levels. To distinguish between these possibilities we asked whether cyclin E1 levels declined in eggs in which cell cycle progression was blocked at the MBT. For this experiment, fertilized eggs were arrested at the MBT with the ribonucleotide reductase inhibitor HU. In the presence of this drug, fertilized eggs divide normally up to the MBT (20); however, because of depletion of the maternal pool of deoxyribonucleotides at this point, the cells arrest in the first

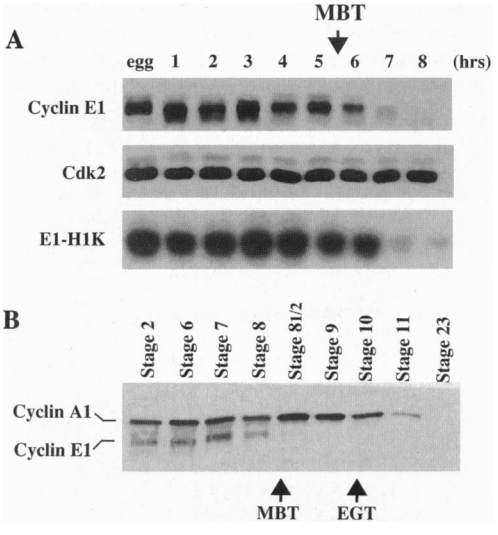

FIG. 1. (*A*) Cyclin E1 protein levels and associated H1 kinase activity during early development. Cyclin–cdk complexes from 10 eggs, collected at the indicated times after fertilization, were concentrated on p13-Sepharose beads, separated by SDS/PAGE, and immunoblotted with anti-peptide antibodies against either cyclin E1 (*Top*) or cdk2 (*Middle*). For measurement of E1-associated H1 kinase activity, cyclin E1 immunoprecipitates were assayed for H1 kinase activity as described. Histone H1 proteins were separated by SDS/PAGE and phosphorylated forms were detected by autoradiography (*Bottom*). (*B*) Cyclin E1 and cyclin A1 protein levels in embryos. p13-Sepharose precipitates from staged embryos were immunoblotted with antibodies made against cyclin A1 protein and a cyclin E1 peptide.

interphase of the 12th cell cycle and further cell division does not occur. Cyclin E1 protein levels were monitored in such arrested embryos and were found to decrease over the exact time course as in control embryos (Fig. 2*A*). The reduction in protein levels was specific for cyclin E1 as the level of cyclin A2 was actually found to increase after the MBT in HU-arrested embryos (Fig. 2*B*). These results suggest that cell cycle progression after the MBT is not required to induce reduction in cyclin E1 protein levels.

The data presented above show that cyclin E1 levels begin to decline at the MBT and that this process is not dependent on cell division past the MBT. Therefore, it is possible that the reduction in cyclin E1 levels, like the MBT itself, is activated when the embryo achieves a critical nuclear/cytoplasmic ratio (17, 18). To test this possibility, cell division was inhibited before the MBT with the protein synthesis inhibitor CHX. In the presence of CHX, resynthesis of the A- and B-type cyclins is inhibited in embryos after mitosis, and embryonic cells become arrested in interphase. For the experiments described here, CHX was added 2 or 4 hr after fertilization—i.e., 3.5 and 1.5 hr before the MBT. After arrest, cyclin E1 was monitored at subsequent time points. After CHX addition 4 hr after fertilization (the eighth cell cycle), one extra division occurred and the eggs arrested in the ninth cell cycle (500 cells). The cyclin E in these arrested eggs remained stable until the temporal equivalent of the MBT and then declined precipitously between 5 and 6 hr (Fig. 3*A*). Similarly, when CHX was added to fertilized eggs at 2 hr, the eggs arrested after the fourth or fifth divisions (16–32 cells). Again, in these embryos, as in controls, a precipitous decline in cycle E1 levels was observed between 5 and 6 hr after fertilization (Fig. 3*B*). In some experiments in which CHX was added 2 hr after fertilization, we detected only a modest reduction in cyclin E1 levels between 5 and 6 hr after fertilization. We suspect that this variation may be correlated with egg quality. Despite this caveat, these experiments show that the abrupt reduction in cyclin E1 levels between 5 and 7 hr after fertilization occurs even when the embryonic cells do not reach the nuclear/cytoplasmic ratio that is required to initiate the MBT. Therefore, our results show that, although these two events occur at the same time during normal development, the timing mechanisms controlling each process appear to be distinct. In addition, these experiments suggest that the reduction in cyclin

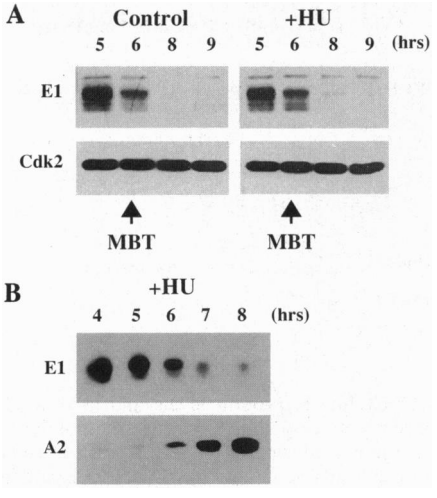

FIG. 2. Cyclin E1 and cyclin A2 protein levels in eggs treated with HU. Fertilized eggs were treated with HU 2 hr after fertilization and collected at the indicated time points. Cyclin–cdk2 complexes were partially purified by p13-Sepharose precipitation and then separated by SDS/PAGE. (*A*) Immunoblots using antibodies against either cyclin E1 or cdk2. (*B*) Immunoblots using cyclin E1 or cyclin A2 antibodies.

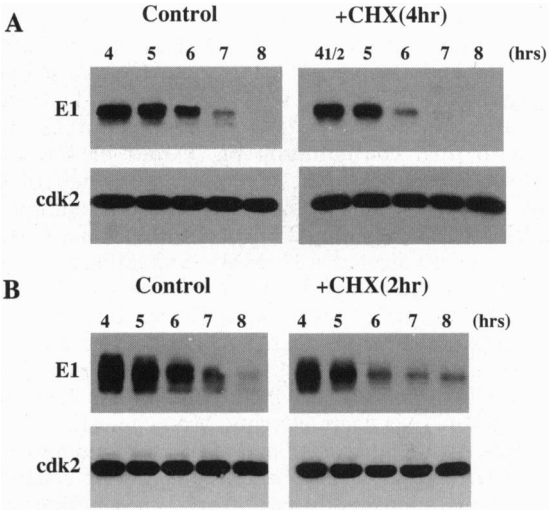

FIG. 3. Cyclin E1 protein levels in eggs treated with CHX either 4 hr after fertilization (*A*) or 2 hr after fertilization (*B*). Cyclin E1 and cdk2 were detected as described in the legend to Fig. 1.

E1 protein levels at a time period equivalent to the MBT occurs primarily as a result of a change in cyclin E stability at this time. In cleavage stage eggs, as is the case in egg extracts (22), cyclin E1 appears to be a stable protein, but after the MBT it turns over rapidly with a half-life of ≈30 min.

The findings described above suggest that a change in the stability of cyclin E1 protein at the MBT is required for its decline at this time in development. Mechanistically, this implies that cyclin E1 protein turns over with a decreased half-life at this time because of activation of a new protein degradative system that is developmentally regulated. Because inhibition of protein synthesis at time points as early as 2 hr after fertilization did not affect the timed turnover of cyclin E1, we suspected that the molecular components of this degradative pathway are either present in unfertilized eggs or are synthesized shortly after fertilization. If this were the case, the degradative system would have to be maintained in an inactive form until 5 hr after fertilization and then activated. Recently, we have proposed that such timing mechanisms are responsible for activating mRNA and protein degradative systems at time points equivalent to the MBT and the onset of gastrulation (S. Guadagno, K. Milarski, and J.W.N., unpublished data; ref. 21). Characterization of the degradation of cdc2 mRNA at the MBT has shown that this message can be stabilized when egg cytoplasm or purified recombinant Mos protein kinase is reintroduced into fertilized eggs 1 hr after fertilization. It was therefore of interest to determine whether Mos kinase might play a role in inhibiting the timed degradation of cyclin E1.

Mos kinase is the gene product of the c-*mos* protooncogene and is a component of cytostatic factor (27), an activity known to mediate the second meiotic metaphase arrest in unfertilized eggs by stabilizing mitosis-promoting factor (28). Mos protein kinase is synthesized only during oocyte maturation (29) and disappears shortly after fertilization (30). To determine whether Mos kinase might also inhibit the timer that controls turnover of cyclin E1, Mos kinase protein was reintroduced into eggs 60 min after fertilization. Injection of Mos kinase caused the eggs to arrest in the second mitosis after fertilization as expected (31). We found that cyclin E1 could be detected in control and Mos-injected embryos in equimolar concentrations 4 hr after fertilization, but 7 hr after fertilization, when cyclin E1 was almost undetectable in control embryos, readily detectable levels of cyclin E1 could be detected in Mos-injected embryos (data not shown). This experiment suggested that Mos kinase could inhibit the developmentally regulated degradation of cyclin E1.

A number of studies have shown that Mos kinase indirectly activates mitogen-activated protein kinase (MAPK) by directly up-regulating mitogen-activated protein kinase kinase (MAPKK; refs. 32 and 33). Furthermore, in eggs, activation of MAPK serves to inhibit the ubiquitin-mediated proteolysis of cyclin B. Therefore, it was of interest to determine whether activation of the MAPK pathway could inhibit the developmentally timed degradation of cyclin E1. To examine this possibility, a constitutively activate form of MAPKK protein (34) was injected into eggs 1 hr after fertilization. This protein was found to arrest embryos in mitosis at the two-cell stage, as is the case when constitutively active MAPK is injected into fertilized eggs (35). At 4 hr, the amount of cyclin E1 present in injected and control eggs was the same. However, 7.5 hr after fertilization, when cyclin E1 had declined to very low levels in control eggs, it was readily detected in the eggs that had been injected with MAPKK (Fig. 4). This experiment shows that activation of the MAPK pathway inhibits the timing mechanism that normally controls turnover of cyclin E1 protein 6 hr after fertilization.

Our data thus far are consistent with a model in which a timing mechanism activated at fertilization measures an ≈5-hr period and then activates new proteolytic programs. In addition, Mos kinase and activated MAPKK can inhibit this degradative program, possibly by inhibiting or resetting the timer when reintroduced into eggs 1 hr after fertilization. To determine whether the timer could be inhibited at later times after fertilization, we injected activated MAPKK ≈4 hr after fertilization. For these experiments, it was necessary to inhibit cellularization of the embryo to allow diffusion of the injected MAPKK protein throughout the cytoplasm. To inhibit cellularization, embryos were lightly centrifuged 1 hr after fertilization. This procedure generates eggs in which cleavage is inhibited but DNA replication and MPF oscillations continue normally and the MBT occurs at the normal time (17, 36). Cyclin E1 protein levels were found to decline in such coenocytial eggs at time points that were comparable to control embryos (Fig. 4). However, when MAPKK was injected into coenocytial eggs ≈4 hr after fertilization, cyclin E1 levels were found to remain high at time points after which cyclin E1 had turned over in control eggs (Fig. 4). This result suggests that the timing mechanism that developmentally regulates the turnover of cyclin E can be blocked by MAPKK at time points as late as 4 hr after fertilization.

Although cyclin E1 protein appears to turn over at the MBT in a fashion that is independent of mRNA levels, we were nevertheless interested in the fate of the maternal cyclin E1 message. To address this point, Northern blot analysis of total mRNA isolated from fertilized eggs was used to monitor cyclin E1 message during early embryogenesis. These experiments showed that cyclin E1 mRNA was detected throughout the first 10 hr of development in nearly identical quantities at each time point assayed (Fig. 5). Although this result would suggest that cyclin E1 mRNA was stable before and after the MBT, degradation of the maternal E1 message could be masked by new zygotic transcription of cyclin E1 mRNAs at the MBT. To examine this possibility, it was necessary to limit the contribution of new transcription after the MBT. To do this, embryos were incubated with CHX at stage 3/4 (2 hr after fertilization). Although this treatment does not formally inhibit transcription, levels of new transcripts are greatly reduced because embryos are arrested with only 16–32 nuclei compared to >4000 that are present in embryos after the MBT when zygotic transcription is initiated. Embryos treated with CHX were found to contain levels of cyclin E1 mRNAs comparable with the levels in control embryos until stage 10 (8 hr after fertilization in this experiment). At stage 10.5, however, E1 mRNAs were not detected in CHX-treated embryos but could be readily detected in control embryos (Fig. 5). This finding suggests that the embryonic pools of cyclin E1 message, like the maternal cyclin A1 and A2 messages, are degraded at the EGT (21)—i.e., 2–3 hr after the period when cyclin E1 protein levels become unstable.

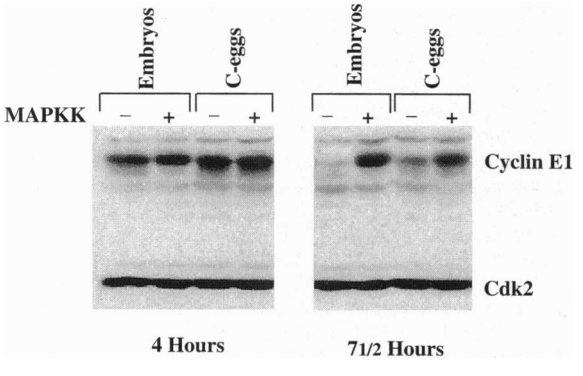

FIG. 4. Cyclin E1 protein levels 4 or 7.5 hr after fertilization in embryos or in coenocytial eggs (indicated as C-eggs) after injection of activated MAPKK protein. Constitutively activated MAPKK protein (2–5 ng) was injected 1 hr after fertilization for embryos and ≈3.75 hr after fertilization for coenocytial eggs. Cytosolic extracts were separated by SDS/PAGE and immunoblotted with anti-peptide antibodies against cyclin E1 and cdk2.

DISCUSSION

In this report, we have shown that cyclin E1 protein levels precipitously decline during *Xenopus* development over a time frame that is coincident with the MBT. The reduction in cyclin E1 protein at this time appears to result primarily from a change in the stability of cyclin E1 protein. Cyclin E1 protein is stable during both interphase and mitosis of the embryonic cell cycle (ref. 22 and these studies), but it turns over rapidly immediately after the MBT.

Our data suggest that the components that constitute the proteolytic system required for cyclin E1 turnover are present in the egg or are synthesized shortly after fertilization but held in an inactive form until the MBT. Activation of this proteolytic system does not require cell cycle expansion, *de novo* zygotic transcription, ongoing protein synthesis, or the nuclear/cytoplasmic ratio thought to trigger the MBT. Instead, we propose that cyclin E1 turnover is controlled by a developmental timing mechanism that measures an ≈5-hr time period after fertilization and then allows the cyclin E1 degradative system to be activated. Degradation of cyclin E1 protein at the MBT is temporally distinct from the EGT when cyclin A1

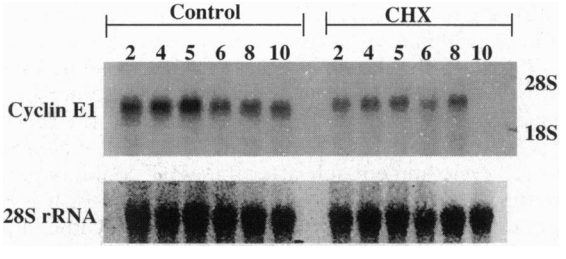

FIG. 5. Northern blot analysis of cyclin E1 mRNA during early development. Fifteen-microgram aliquots of total RNA, isolated from control or CHX-treated embryos at the indicated times (hr) after fertilization, were separated on formaldehyde/agarose gels, transferred to nitrocellulose, and probed with random-primed ^{32}P-labeled cyclin E1 DNA fragments. The filters were then washed to high stringency and exposed to x-ray film (*Upper*). To check the integrity of the RNA samples and to ensure that approximately equal amounts of RNA were analyzed, the formaldehyde/agarose gel was stained with ethidium bromide and the gel section containing the 28S rRNAs was photographed (*Lower*). CHX was added 2 hr after fertilization to a final concentration of 100 μg/ml.

protein becomes unstable, but we have observed that the maternal cyclin E1 message, like the cyclin A1 and A2 messages, does become unstable at the EGT. On the other hand, the turnover of cyclin E1 protein at the MBT is coincident with degradation of the maternal mRNAs that encode cdc2 and cdk2 at the MBT (S. Guadagno, K. Milarski, and J.W.N., unpublished data). The degradative systems that control turnover of these mRNAs also appear to be regulated by a cell autonomous timing mechanism. Together these results suggest that distinct degradative timing mechanisms play important roles in clearing maternal programs during early development.

In an attempt to characterize the timing mechanism responsible for activating cyclin E1 turnover, we found that reintroduction of Mos kinase, or a constitutively active version of MAPKK, which is a downstream component of the Mos signal transduction pathway, inhibited the timed degradation of cyclin E1. These findings suggest that Mos kinase may inactivate the timing mechanism in eggs and that degradation of Mos after fertilization allows activation of the timer. It is worth noting that the timing mechanism that regulates the maternal pools of cdk2 and cdc2 messages can also be inhibited by injection of Mos kinase, and it is tempting to speculate that the timers could be mechanistically related.

Although we have not identified embryonic constituents that form the molecular basis of the timer, our working model predicts that the timer either generates a positive regulator of the cyclin E1 turnover pathway or inactivates a factor that inhibits the degradative pathway. Therefore, the time lag between fertilization and the onset of cyclin E1 degradation represents the time required either to generate sufficient concentrations of an activator or to inactivate an inhibitor below a threshold concentration. Injection of activated MAPKK protein into coenocytial eggs 4 hr after fertilization inhibited cyclin E1 degradation after the MBT and is consistent with a model in which an activator, or repressor, of the cyclin E1 proteolytic pathway is progressively modified during the cleavage stages. For example, if the timer were responsible for progressive degradation of an inhibitor of the cyclin E1 degradation machinery, then injection of activated MAPKK might inhibit further turnover of this inhibitor, thereby blocking the timed activation of the cyclin E1 degradative system 5–6 hr after fertilization.

In terms of cell cycle regulation, our results demonstrate that cyclin E1 is stable throughout both S and M phases in the early embryonic cell cycles. This situation is unique to these embryonic cycles as cyclin E1 is expressed periodically during the somatic cell cycle and maximal amounts of cyclin E1 mRNA, protein, and H1-associated kinase activity occur at the G_1/S transition (5, 6, 37). Cyclin E protein turns over with a short half-life of <30 min in rat tissue culture cells (K-A. Won and S. Reed, personal communication), and it is possible that the proteolytic system that normally regulates the stability of cyclin E1 during the somatic cell cycle is activated for the first time in eggs 5–6 hr after fertilization. Thus, the modest elongation of the cell cycle immediately after the MBT could, in part, be due to the time required to synthesize cyclin E1 as well as other components required for DNA synthesis that become limiting at the MBT. If this is true, then it suggests that the events associated with the onset of the MBT may be regulated by at least two different types of timers, one that measures the nuclear/cytoplasmic ratio and another that controls the onset of proteolytic events. It will be interesting to determine whether these two timers are redundant or cooperative in nature.

We would like to thank Jim Maller for supplying the cyclin A1 antibody and Sam Mansour and Natalie Ahn for activated MAPKK protein. Thanks to Jeff Stack and Joseph Schulz for many helpful suggestions. This work was supported by National Institutes of Health Grant GM 33523 to J.W.N. and a fellowship from the Medical Research Council (Canada) to J.A.H.

1. King, R. W., Jackson, P. W. & Kirschner, M. W. (1994) *Cell* **79,** 563–571.
2. Sherr, C. (1994) *Cell* **79,** 551–555.
3. Blow, J. J. & Nurse, P. (1990) *Cell* **62,** 855–862.
4. Fang, F. & Newport, J. W. (1991) *Cell* **66,** 731–742.
5. Dulic, V., Lees, E. & Reed, S. I. (1992) *Science* **257,** 1958–1961.
6. Koff, A., Giordano, A., Desai, D., Yamashita, K., Harper, J. W., Elledge, S., Nishimoto, T., Morgan, D. O., Franza, B. R. & Roberts, J. M. (1992) *Science* **257,** 1689–1694.
7. Ohtsubo, M. & Roberts, J. M. (1993) *Science* **259,** 1908–1911.
8. Resnitzky, D. M., Gossen, M., Bujard, H. & Reed, S. (1994) *Mol. Cell. Biol.* **14,** 1669–1679.
9. Wimmel, A., Lucibello, F., Sewing, A., Adolph, S. & Muller, R. (1994) *Oncogene* **9,** 995–997.
10. Ohtsubo, M., Theodora, A. M., Schumacher, J., Roberts, J. M. & Pagano, M. (1995) *Mol. Cell. Biol.* **15,** 2612–2624.
11. Knoblich, J., Sauer, K. Jones. L., Richardson, H., Saint, R. & Lehener, C. (1994) *Cell* **77,** 107–120.
12. Girard, F., Strausfeld, U., Fernandez, A. & Lamb, N. J. C. (1991) *Cell* **67,** 1169–1179.
13. Pagano, M., Pepperkok, R., Ansorge, W. & Draetta, G. (1992) *EMBO J.* **11,** 961–971.
14. Guadagno, T., Ohtsubo, M., Roberts, J. M. & Assoian, R. (1993) *Science* **262,** 1572–1575.
15. Tsai, L-H, Harlow, E. & Meyerson, M. (1991) *Nature (London)* **353,** 174–177.
16. Desai, D., Gu, Y., & Morgan, D. O. (1992) *Mol. Cell. Biol.* **3,** 571–582.
17. Newport, J. & Kirschner, M. W. (1982) *Cell* **30,** 675–686.
18. Newport, J. & Kirschner, M. (1982) *Cell* **30,** 687–696.
19. Dasso, M. & Newport, J. W. (1990) *Cell* **61,** 811–823.
20. Newport, J. W. & Dasso, M. (1989) *J. Cell Sci. Suppl.* **12,** 149–160.
21. Howe, J. A., Howell, M., Hunt, T. & Newport, J. W. (1995) *Genes Dev.* **9,** 1164–1176.
22. Rempel, R. E., Sleight, S. & Maller, J. (1995) *J. Biol. Chem.* **270,** 6843–6855.
23. Peng, H. B. (1991) in *Methods in Cell Biology*, eds. Kay, B. K. & Peng, H. B. (Academic, San Diego), pp. 657–662.
24. Niewkoop, P. D. & Faber, J. (1967) *Normal Table of Xenopus laevis* (North–Holland, Amsterdam).
25. Dunphy, W. G. & Newport, J. W. (1989) *Cell* **58,** 181–191.
26. Harlow, E. & Lane, D. (1988) *Antibodies: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 511–551.
27. Sagata, N. Watanabe, N. Vande Woude, G. F. & Ikawa, Y. (1989) *Nature (London)* **342,** 515–518.
28. Newport J. W. & Kirschner, M. W. (1984) *Cell* **37,** 731–742.
29. Sagata, N., Oskarsson, M., Copeland, T., Brumbaugh, J. & Vande Woude, G. F. (1988) *Nature (London)* **335,** 519–526.
30. Watanabe, N., Vande Woude, G. F., Ikawa, Y. & Sagata, N. (1989) *Nature (London)* **342,** 505–511.
31. Yew, N., Mellini, M. L. & Vande Woude, G. F. (1991) *Nature (London)* **355,** 649–652.
32. Nebreda, A. R. & Hunt, T. (1993) *EMBO. J.* **12,** 1979–1986.
33. Shibuya, E. K. & Ruderman, J. V. (1993) *Mol. Cell. Biol.* **4,** 781–790.
34. Mansour, S. J., Matten, W. T., Herman, A. S., Candia, J. M., Rong, S., Fukasawa, K. F., Vande Woude, G. F. & Ahn, N. G. (1994) *Science* **265,** 966–970.
35. Haccard, O., Sarcevic, B., Lewellyn, A., Hartley, R., Roy, L., Izumi, T., Erikson, E. & Maller, J. (1993) *Science* **262,** 1262–1265.
36. Kimelman, D., Kirschner, M. & Scherson, T. (1987) *Cell* **48,** 399–407.
37. Lew, D. J., Dulic, V. & Reed, S. I. (1991) *Cell* **66,** 1197–1206.