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Cell Biology

Coordinated synthesis and degradation of cdc2 in the mammalian cell cycle

(cell synchronization/phosphotyrosine/p34^{cdc2}/cdc2 transcription/primary human fibroblasts)

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ABSTRACT **The product of the *cdc2* gene (cdc2 or p34^{cdc2}), the catalytic subunit of M phase-promoting factor (MPF), is held at a constant steady-state level throughout the cell cycle. In this report, we show that the constant concentration is maintained by a coordinated regulation of protein synthesis and degradation. At the end of each mitosis, *cdc2* transcription is shut off, and the mRNA is rapidly degraded. A 12-fold activation of *cdc2* gene transcription occurs every round of the cell cycle at the G_1/S transition, in a growth factor-dependent manner. The increase in mRNA correlates with the accumulation of newly synthesized cdc2 during S and G_2 phases. At the onset of mitosis, the translation of cdc2 mRNA is shut off. During G_1 phase, the cdc2 protein has a relatively long half-life of 18 hr, so cdc2 made in the previous cell cycle is maintained. Once synthesis is activated at G_1/S, a concurrent mechanism of degradation is activated, and the protein half-life is reduced to 7.5 hr. By the end of interphase, new cdc2 accounts for 75–85% of the total cdc2 pool. In addition, we show that >75% of the new cdc2 complexes with cyclin, suggesting that a majority of the new cdc2 functions as MPF.**

A key component in the regulation of the eukaryotic cell cycle is the protein kinase encoded by the *cdc2* gene (also called cdc2 or p34^{cdc2}). This gene was first identified in *Schizosaccharomyces pombe* to encode an essential function for cell-cycle progression (8). The *cdc2* gene is evolutionarily conserved, such that human cdc2 can rescue temperature-sensitive loss-of-function yeast cdc2 mutants (1). As a kinase, cdc2 has been shown to phosphorylate multiple substrates and has been implicated in the activation of several cellular processes including chromosome condensation, nuclear envelope breakdown, mitotic spindle formation, and the activation of DNA replication (reviewed in ref. 2).

Activation of the cdc2 kinase activity requires cyclins, and the cdc2–cyclin complexes are regulated by the phosphorylation state of both components (reviewed in ref. 3). During late interphase, cdc2 is complexed with mitotic cyclins and is kept inactive as a kinase by phosphorylation on threonine and tyrosine residues (reviewed in ref. 4). At the G_2/M transition, this complex is dephosphorylated to become the active maturation-promoting factor (MPF) (5, 6). The mouse cell FT210, which has a temperature-sensitive cdc2 protein, arrests at G_2/M, establishing that MPF activity is required for the successful induction of mitosis in higher eukaryotes (7). In yeast, the requirement of cdc2 at the G_1/S transition has been clearly shown through genetic analyses (8), while in higher eukaryotes, its role at G_1/S is not completely understood.

In all cycling cells, the total level of cdc2 protein remains constant throughout the cycle, while the kinase activity of cdc2 oscillates in a cell cycle-dependent manner. For this reason, little attention has been focused on the regulation of cdc2 protein expression. In *Schizosaccharomyces pombe*, the level of cdc2 mRNA does not appear to be regulated in the cell cycle (9). However, in mammalian cells, several groups have shown that cdc2 mRNA and protein levels decrease when cells are made quiescent and increase during S phase after serum stimulation (5, 10). Even in cycling cells, the level of cdc2 mRNA is not constant, and an increase in cdc2 mRNA is correlated with progression from late S to G_2 in HeLa cells (11). In this report we show that despite the constant level of cdc2 protein, cdc2 translation in NIH 3T3 cells is activated at the G_1/S transition and is inactivated at the G_2/M boundary. The accumulation of new cdc2 is accompanied by a concurrent mechanism of degradation, resulting in the old pool of cdc2 protein being largely replaced each round of the cell cycle. In addition, we show that a significant majority of this new cdc2 is assembled in the MPF complex.

MATERIALS AND METHODS

Cell Culture and Synchronization. NIH 3T3 murine fibroblasts, human HeLa cells, or primary human foreskin fibroblasts (hFF) were cultured at 37°C in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (vol/vol) calf serum (HyClone). Metaphase-arrested cells and the S/G_2 adherent cells were obtained as described (5). After removal of nocodazole, fluorescence-activated cell sorting (FACS) analyses (FACScan flow cytometer; Becton Dickinson) indicated that at hour 0, 95% of the cells are metaphase cells; at hour 4 after release, 80% are in G_1; and at hour 14 after release, 75% are in S phase. Protein synthesis was arrested in cultured cells by the addition of 25 μg of cycloheximide (Sigma) per ml, while DNA synthesis was arrested by using 10 μg of aphidicolin (Sigma) per ml (12). Defined serum-free medium contained 3:1 (vol/vol) DMEM/Ham's F-12 medium, 0.075% bovine serum albumin, and 5 μg of transferrin and 5 ng selenium per ml.

Metabolic Labeling. In the pulse-labeling experiments, 1×10^6 cells were incubated with methionine-free minimal essential medium (Flow Laboratories) supplemented with 10% dialyzed calf serum, 2 mM L-glutamine, and 10 mM Hepes (pH 7.2) for 20 min at 37°C, followed by fresh methionine-free medium containing 200 μCi (1 μCi = 37 kBq) of Tran^{35}S-label (a mixture of [^{35}S]methionine and [^{35}S]cysteine; specific activity, >1000 Ci/mmol; ICN) for 15 min at 37°C. Where indicated, cells were chased with DMEM supplemented with 10% calf serum and 300 mg of nonradioactive L-methionine per liter. In experiments where continuation of the cell cycle was required, labeling was with 20 μCi of Tran^{35}S-label in medium supplemented with 50 μM of L-methionine (Sigma). To follow DNA synthesis, [*methyl*-^{3}H]thymidine (ICN) incorporation was measured as described (5).

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Abbreviations: MPF, M phase-promoting factor; hFF, human foreskin fibroblasts; Tyr(*P*), phosphotyrosine.

RNA Extraction and Northern Blots. Total RNA was extracted from cell cultures as described by Chomczynski and Sacchi (13), and was analyzed by standard Northern blotting techniques (14). Northern blots were probed with either the mouse cdc2 cDNA supplied by Jeffry Corden (Johns Hopkins School of Medicine) or a fragment of the murine β-actin cDNA.

Immunoprecipitation and Immunoblotting. Immunoprecipitations with anti-human cdc2 C-terminal antibodies were performed as described (5). The cdc2 C-terminal peptide (seven-amino acid peptide: Asp-Asn-Gln-Ile-Lys-Lys-Met) was used as a competing antigen. ^{35}S-containing gels were soaked in 20% (wt/vol) 2,5-diphenyloxazole (Sigma) in glacial acetic acid for 60 min prior to drying and exposing to film. Immunoblottings with anti-cdc2 or anti-Tyr(P) antibodies were performed as described (5). Immunoblottings with anti-cyclin B antibodies were performed as described (19). Bands were visualized by using ^{125}I-labeled protein A (ICN) and were quantitated by using an LKB laser densitometer.

RESULTS

Synthesis of cdc2 Protein Is Cell Cycle Dependent. The rate of cdc2 synthesis was determined in NIH 3T3 cells that were synchronized in G_1, S/G_2, or M phase of the cell cycle. Each cell population was pulse-labeled for 15 min with [^{35}S]methionine, and cdc2 in whole-cell lysates was immunoprecipitated. Synthesis of cdc2 was detected in exponentially growing cells (Fig. 1*A*, lanes EXP), and a similar rate of synthesis was found in cells at S/G_2 (Fig. 1*A*, lanes S/G_2). The cdc2 synthesis rate was greatly reduced in both G_1 and mitosis (Fig. 1*A*, lanes G_1 and M). The steady-state levels of cdc2 protein in whole-cell lysates were determined by immunoblotting with anti-cdc2 peptide antibody (Fig. 1*B*). Relatively constant levels of total cdc2 protein were detected at all stages of the cycle. In accordance with previous results (15), modified forms of the protein, which migrated more slowly on SDS/PAGE, were detectable by Western blotting in S/G_2 cells but not in G_1 or mitotic cells. These results signify that phosphorylation of cdc2 is coordinated with new synthesis.

After establishing a variable rate of cdc2 synthesis, we expanded the study to determine the pattern of cdc2 synthesis as cells progress through interphase. NIH 3T3 cells were blocked in metaphase (hour 0), released, and allowed to continue through the cell cycle. Within 90 min after release, 95% of the cells had completed mitosis. DNA synthesis was detected 6–8 hr after release from metaphase as determined by [^{3}H]thymidine incorporation (Fig. 2*E*). The total level of cdc2 protein as determined by immunoblotting was relatively constant as cells progressed from metaphase, through G_1, and into S phase (Fig. 2*A*), whereas the rate of cdc2 synthesis increased as cells entered and continued through S phase (Fig. 2*B*). A 10-fold increase in the rate of cdc2 synthesis relative to total protein synthesis was observed as cells transit from G_1 into S phase (Fig. 2*C*). The increase in synthesis was again correlated with the appearance of Tyr(*P*) on cdc2, which also increased as cells progressed through S phase (Fig. 2*D*). Indeed, when protein synthesis was shut off with cycloheximide, accumulation of Tyr(*P*) on cdc2 was also arrested (data not shown). This data shows a correlation between cdc2 synthesis, tyrosine phosphorylation, and DNA synthesis. To determine if cdc2 synthesis is dependent on DNA replication, aphidicolin, an inhibitor of DNA synthesis, was added at hour 4 in the time-course. DNA synthesis was completely blocked by aphidicolin (Fig. 2*E*), and yet the pattern of cdc2 synthesis was not affected (Fig. 2*C*). In addition, in cells that were synchronously arrested at "G_1/S" by treatment with hydroxyurea (see ref. 12), cdc2 synthesis and tyrosine phosphorylation were fully activated (not shown). These data suggest that the activation of both cdc2 synthesis and tyrosine phosphorylation occur at or before the G_1/S boundary and are not dependent on concurrent DNA synthesis.

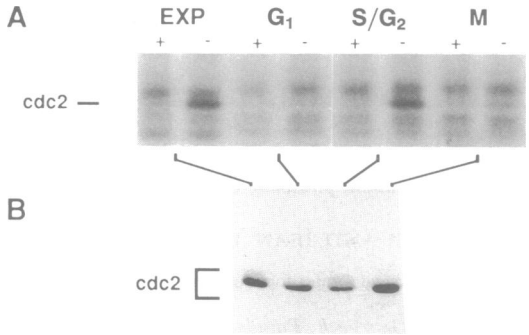

FIG. 1. Synthesis of cdc2 is cell cycle-regulated. Lanes: EXP, exponentially growing population of NIH 3T3 cells; M, cells arrested in metaphase with nocodazole; S/G_2, adherent cells remaining after nocodazole treatment; G_1, cells released from metaphase arrest for 4 hr. (*A*) cdc2 translation rate measured by [^{35}S]methionine pulse-labeling followed by anti-cdc2 immunoprecipitation. Lanes indicated + or − represent the presence or absence of competing antigenic peptide in the immunoprecipitation reaction. (*B*) Immunoblot of 20 μg of total-cell lysate probed with an antibody against mammalian cdc2.

cdc2 Synthesis Is Correlated with cdc2 mRNA Level in the Cell Cycle. Previous reports have shown that the level of mammalian cdc2 mRNA is not constant during the cell cycle (11) and could explain the observed cell cycle-regulated synthesis. Therefore, the cdc2 mRNA level in the NIH 3T3 cell-cycle time course was measured by Northern blotting (Fig. 3). Northern blots were probed with either a murine cdc2 cDNA (Fig. 3*A*) or, as an internal control, a murine β-actin cDNA (Fig. 3*B*). In NIH 3T3 cells, a single band of 1.4 kilobases (kb) was detected by the cdc2 cDNA probe. A plot of the cdc2 mRNA signal normalized to the β-actin mRNA signal is shown in Fig. 3*C*. A high level of stable cdc2 mRNA was detected in metaphase-blocked cells (0 hr) followed by a rapid degradation as cells entered G_1 (2–4 hr). cdc2 mRNA began to accumulate as cells entered S phase (8 hr) and reached a maximum at G_2/M (18 hr). Nuclear run-on analysis showed a 12-fold activation of *cdc2* transcription as cells progressed from G_1 to S phase (not shown). The increase in the level of cdc2 mRNA is therefore largely due to regulation at the level of *cdc2* gene transcription. There was a direct correlation between cdc2 mRNA levels and accumulation of new cdc2 during interphase (compare Fig. 3 *C* and *D*); however, cdc2 synthesis was inhibited in metaphase-arrested cells despite a high concentration of mRNA.

Since the expression of cdc2 is activated at or before the G_1/S transition, it raised the possibility that this was in direct response to growth factors (16). Metaphase-arrested NIH 3T3 cells were released into G_1 (3 hr) at which time half of the cultures were placed into defined serum-free medium, while the other half remained in complete medium as control cells. Since these cells entered and exited mitosis in the presence of serum, they were expected to remain competent for proliferation. Indeed, the level of c-myc mRNA was found to be identical between the control cells and cells released into serum-free medium (Fig. 4). However, cells in the serum-free medium did not enter S phase despite the high level of c-myc mRNA (Fig. 4, [^{3}H]thymidine incorporation bars). It appears that cells released from metaphase arrest by nocodazole have a strict requirement for the continued presence of serum to progress into S phase. The level of cdc2 mRNA was low in the absence of serum (Fig. 4). These results show that

activation of *cdc2* transcription is tightly coupled to the entry into S phase in every round of the cell cycle.

Degradation of cdc2 Protein Is Also Cell Cycle Dependent. Since accumulation of new cdc2 increases 10-fold during S phase, it is curious that the total level of cdc2 protein remains relatively unchanged (see Fig. 2). Either the amount of newly synthesized cdc2 is so small that its contribution to the total level is undetectable by immunoblotting, or the new synthesis is significant and there is a concurrent mechanism of degradation. We experimentally determined the pool size of new cdc2 and found that the new cdc2 made up at least 40% of the total pool at the end of each S phase. This result suggests an increased degradation of cdc2 when synthesis is activated. The half-life of newly synthesized cdc2 was determined in a pulse–chase experiment (Fig. 5*B*). A logarithmic-scale graph of the decay of labeled cdc2 protein gives a half-life of 7.5 hr (Fig. 5*C*). The cdc2 protein half-life has to be much longer than 7.5 hr in G_1 because during those 8 hr, there is very little cdc2 synthesis and the steady-state level of cdc2 is unchanged (see Fig. 2). We have found that [^{35}S]methionine pulse–chase could not be used to determine the half-life of cdc2 during G_1 for two reasons. Pulse-labeling with a high concentration of radioactivity (200 μCi/ml) prevented entry into mitosis, presumably because of radiation damage. As a result, a G_1 population could not be obtained. We have determined that cells will continue to cycle under lower levels of radiation (see *Materials and Methods*), but the efficiency of labeling is not high enough to sustain a chase experiment. Instead, the decay of G_1 cdc2 was determined by the steady-state level of cdc2 protein on immunoblots in G_1 cells treated with cycloheximide (Fig. 5*A*). Under these conditions, there was no significant degradation of total Coomassie-stained proteins (not shown). A logarithmic-scale graph of the decay of G_1 cdc2 protein gives a half-life of 18 hr (Fig. 5*C*). These experiments show that, in addition to the cell-cycle regulated expression of cdc2, there is also a cell cycle-dependent mechanism of cdc2 degradation.

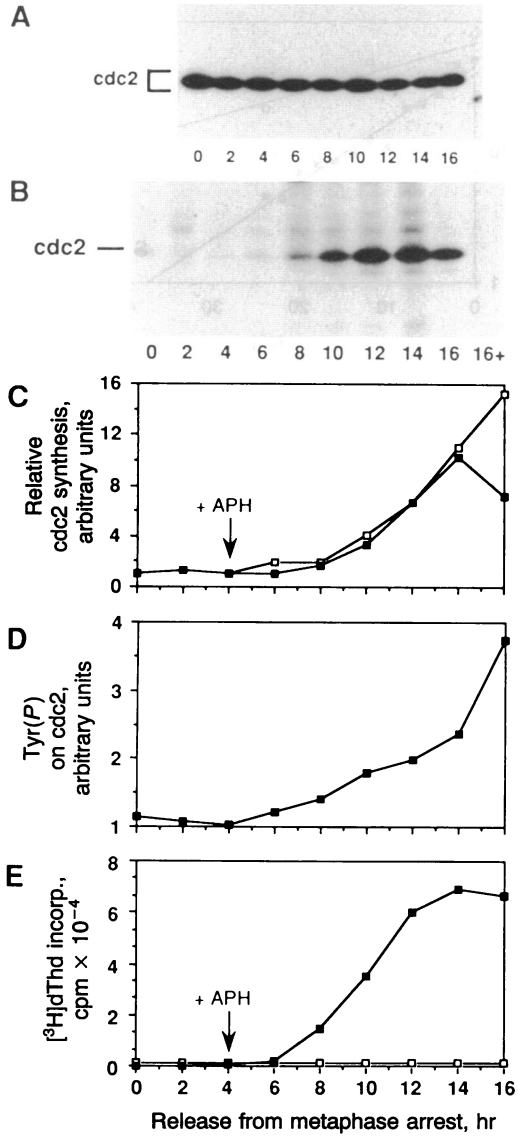

FIG. 2. Rate of cdc2 synthesis during the cell cycle. Cells were blocked at metaphase with nocodazole and released. Hour 0 represents cells arrested in metaphase and subsequent numbers represent hours after release from metaphase. (*A*) Immunoblot of 20 μg of total-cell lysate probed with anti-cdc2 antiserum. (*B*) cdc2 translation rate measured by [^{35}S]methionine pulse-labeling followed by anti-cdc2 immunoprecipitation. Lane 16+ shows an immunoprecipitation reaction in which competing antigenic peptide was included. (*C*) Quantitation of cdc2 synthesis relative to total trichloroacetic acid-precipitable ^{35}S cpm (cdc2 ^{35}S/total ^{35}S). Untreated cells (■) are compared with cells treated with 10 μg of aphidicolin per ml (□) added at hour 4 (+ APH). (*D*) Quantitation of the amount of Tyr(*P*) on cdc2 as determined by anti-Tyr(*P*) immunoblotting (values are averages of four experiments). (*E*) Incorporation (incorp.) of [^{3}H]thymidine in control cells (■) and in cells treated with 10 μg of aphidicolin per ml (□) added at hour 4 (+ APH).

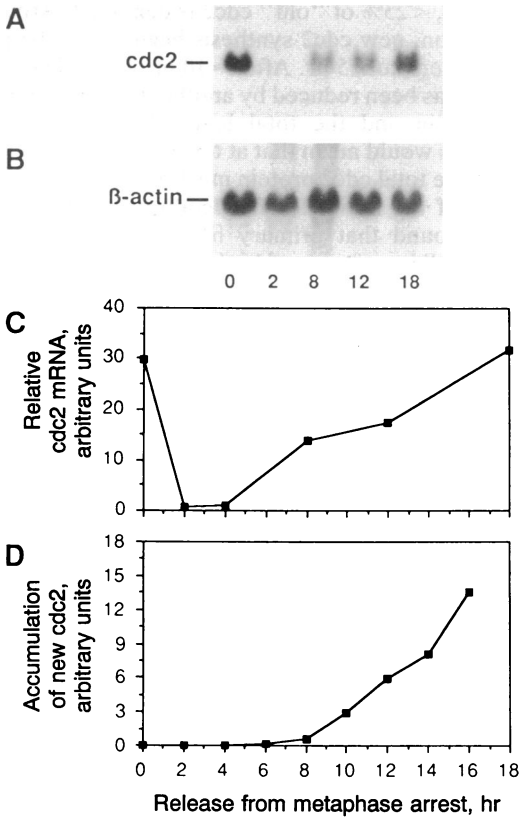

FIG. 3. Levels of cdc2 mRNA during the cell cycle. NIH 3T3 cells were arrested in metaphase (hour 0) and released into the cell cycle (hours 2 through 18) as in Fig. 2. (*A* and *B*) At each time point, 15 μg of total RNA was subjected to Northern analysis and probed with either a murine cdc2 cDNA probe (*A*) or a murine β-actin cDNA probe (*B*). (*C*) Quantitation of the cdc2 mRNA signal normalized to the β-actin mRNA signal (values are averages of three experiments). (*D*) Time course of new cdc2 accumulation as NIH 3T3 cells progress from metaphase through S phase. Cells released from the block of metaphase by nocodazole were labeled continuously with [^{35}S]methionine. At each time point, cell lysates were immunoprecipitated with anti-cdc2 antibodies, and the amount of ^{35}S-labeled cdc2 is plotted.

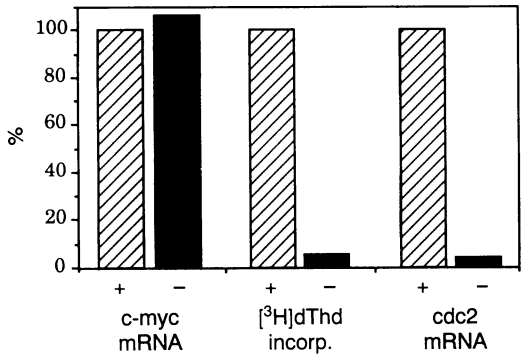

FIG. 4. Activation of *cdc2* transcription requires serum. Metaphase-arrested cells were released either into complete medium with 10% serum (+) or into defined serum-free medium (−). After 18 hr, levels of mRNA for cdc2 or c-myc were determined by Northern analysis and were normalized to the signal for β_2-microglobulin, which remained constant with or without serum. [^{3}H]Thymidine incorporation (incorp.) was measured at the time of mRNA harvest. Values obtained in defined serum-free medium (−) are shown as a percent of the signal in the control medium (+).

The half-life values can be used to calculate the theoretical pool size of new cdc2 at the end of each interphase. In NIH 3T3 cells, the time between metaphase and the G_1/S transition is ≈8 hr. During this time, the cdc2 half-life is 18 hr; consequently, <25% of "old" cdc2 is degraded. Around the G_1/S transition, new cdc2 synthesis begins, and the protein half-life shortens to 7.5 hr. After ≈10 hr of S and G_2 phases, "old" cdc2 has been reduced by another 60%, while the total cellular protein and the total level of cdc2 have nearly doubled. This would mean that at the end of each interphase, 75–85% of the total cdc2 protein must be newly synthesized.

Majority of the New cdc2 Is Modified in Primary Fibroblasts. We found that primary hFF have less total cdc2 protein per cell by a factor of 15 than do their immortalized/transformed counterparts—e.g., NIH 3T3 and HeLa cells. Fig. 6*A* shows immunoblots of total cell lysates from exponentially growing HeLa cells or hFF. Fig. 6*A Top* was probed with anti-cdc2 antibody, which showed a 15-fold difference in the steady-state level of cdc2 protein between the two cell types. However, the level of Tyr(*P*) on cdc2 from the two cell types was the same (Fig. 6*A Middle*). Interestingly, the level of cyclin B, a component of MPF, was also the same in the two cell types (Fig. 6*A Bottom*). The immunoblot data shown for HeLa cells is identical to that for NIH 3T3 cells (not shown). This suggests that cells such as NIH 3T3 and HeLa have an excess pool of free cdc2 protein, the significance of which is unclear. When pulse–chase experiments were carried out in NIH 3T3 or HeLa cells (as in Fig. 5), it was difficult to resolve the various modified forms of cdc2 because of the large pool of unmodified cdc2 protein. Therefore, in our determination of the fate of newly synthesized cdc2, we used hFF. When these primary human cells were subjected to pulse–chase with [^{35}S]methionine, we observed a protein half-life of 8 hr, similar to that in NIH 3T3 cells (see Fig. 5*C*). In addition, a majority of the newly made cdc2 (>75%) appeared in the slower migrating, modified forms of cdc2 (Fig. 6*B Upper*). This modification has been shown to be caused by phosphorylation on tyrosine and threonine residues (17) and is preceded by complex formation with cyclin B (18). Modified cdc2 is the major form that is in complex with the mitotic cyclins and is used as MPF (19). Fig. 6*B Lower* is a quantitation of the intensities of the individual bands throughout the chase as a percent of the total cdc2 signal. Within 2 hr, >75% of the new cdc2 had been modified, suggesting that a majority of the newly synthesized cdc2 complexes with cyclin B becomes phosphorylated and ultimately goes on to function as MPF.

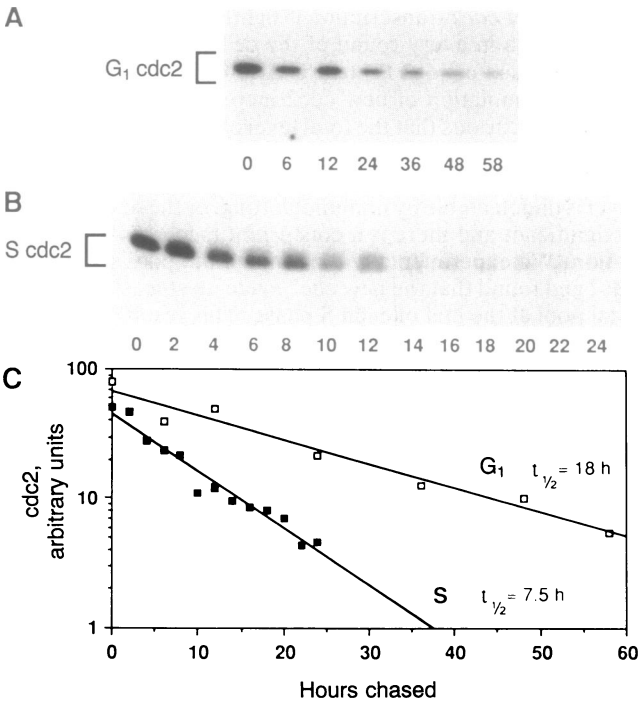

FIG. 5. Degradation of cdc2 is cell cycle-regulated. (*A*) Anti-cdc2 immunoblot of 20 μg of total-cell lysate from NIH 3T3 cells released from metaphase arrest in the presence of cycloheximide. Lanes are labeled by the time in hours after addition of cycloheximide and show degradation of cdc2 protein during G_1. (*B*) Immunoprecipitation of cdc2 from exponentially growing NIH 3T3 cells pulse-labeled with [^{35}S]methionine followed by an unlabeled methionine chase. Lanes are labeled by hours chased and show degradation of cdc2 protein made during S phase. (*C*) cdc2 protein half-life data is quantitated and graphed on a semilogarithmic plot. Curves yield a G_1 half-life of 18 hr (□) and an S-phase half-life of 7.5 hr (■).

DISCUSSION

We have shown that the expression of mammalian cdc2 oscillates in a cell cycle-specific manner. Cells progressing through G_1 have low levels of cdc2 mRNA (Fig. 3) and protein translation (Fig. 1). The activation of cdc2 expression appears to occur at or before the G_1/S boundary, and the pattern of cdc2 expression is directly correlated with, but not dependent on, DNA replication (Fig. 2). When cells reach late S and G_2, the mRNA and new cdc2 accumulation is maximal (Fig. 3; see also ref. 11), similar to other cell cycle-regulated genes such as those encoding the mitotic cyclins, *cdc25*, and *nimA* (19–21). We have routinely observed a 30–50% overall reduction in total protein translation during mitosis, but this is not sufficient to explain the >90% reduction in cdc2 translation, especially given the high mRNA levels at metaphase. It is possible that active MPF or some downstream activity somehow negatively regulates cdc2 translation. Finally, after metaphase there is a rapid and complete degradation of cdc2 mRNA (Fig. 3) identical to the degradation of the mitotic cyclins and their mRNAs (19, 22). Furthermore, similar time-course studies conducted in HeLa cells yielded identical results (data not shown), indicating that this mechanism of regulation is not unique to NIH 3T3 cells.

We have presented evidence suggesting that there are two pools of cdc2. After mitosis and throughout G_1, all of the cdc2 protein is "old" protein, originating in the previous cell cycle. During G_1, the cell maintains the total level of cdc2 protein by imposing a long cdc2 half-life. At the G_1/S transition, cdc2 gene expression is activated. The coordination of a high rate of synthesis and a faster rate of degradation allows the cell to maintain the total steady state of cdc2

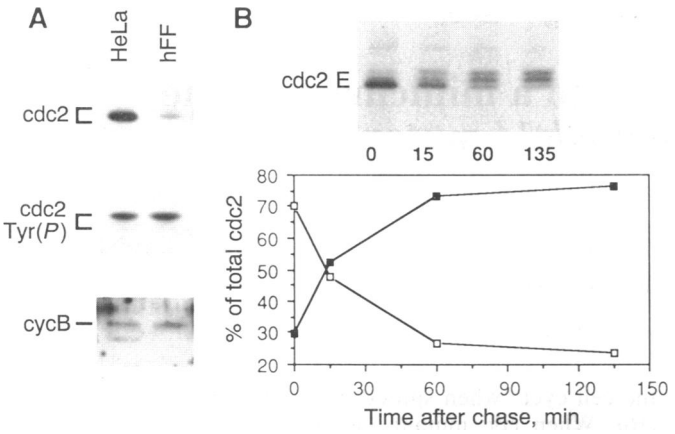

FIG. 6. Newly synthesized cdc2 is modified. (*A*) Immunoblots comparing the levels of cdc2 protein, Tyr(*P*) on cdc2, or cyclin B (cycB) protein. Twenty micrograms of total-cell lysate from either exponentially growing HeLa cells or primary hFF was analyzed. (*B Upper*) Anti-cdc2 immunoprecipitations from exponentially growing primary hFF pulse-labeled with [^{35}S]methionine followed by unlabeled methionine chase. Three discernible bands are indicated, and the time chased is in minutes. (*B Lower*) Quantitation of individual bands plotted as a percent of the total cdc2 signal. □, Lower, unmodified form of cdc2; ■, combination of both of the slower migrating, modified forms of cdc2.

protein. By the end of G_2, a majority of the total cdc2 protein is newly synthesized. In addition, we have shown that a large percentage of the "new" cdc2 is modified into a pre-MPF form. This is supported by the correlation of cdc2 synthesis and tyrosine phosphorylation (Fig. 2) and is consistent with the fact that the maximum accumulation of new cdc2 is at G_2/M. It is yet unclear if only new cdc2 is used as MPF. Nevertheless, the data poses the interesting question of why a cell sets up two different pools of cdc2 in the cell cycle.

The expression of cdc2 is activated each round of the cell cycle. We have shown that this activation occurs at or before the G_1/S transition, not in late S and G_2 as previously suggested by McGowan *et al.* (11). Furukawa *et al.* (23) have proposed that expression of c-myc is a prerequisite for activation of cdc2 expression. Our data shows that c-myc expression is clearly not sufficient for cdc2 activation. While cdc2 expression is dependent on growth factors, it is curious that cells with constitutive growth factor signals (i.e., HeLa cells) still impose a similar cell cycle regulation on cdc2 expression. This leads us to speculate that the activation of cdc2 expression is instead a result of progression through G_1 and may ultimately be dependent on S-phase entry.

The total intracellular level of cdc2 protein also appears to be tightly regulated. We have shown that when the expression of cdc2 is activated, a mechanism of concurrent degradation is also activated. This results in significantly different cdc2 protein half-lives, depending on the cell's position in the cell cycle. Differential half-lives may be the mechanism by which proteins such as proliferating cell nuclear antigen (PCNA) maintain their constant total protein level while dramatically varying their synthesis rate (24). The substantial difference in the total level of cdc2 between primary hFF and HeLa (or NIH 3T3) cells indicates that the intracellular concentration of p34 protein can be set at different levels, since we have found no difference in the amount of cdc2 mRNA between these two cell types (data not shown). It is unclear if this increase in cdc2 level contributes to the transformed/immortalized state.

Newly synthesized cdc2 may play a specific role in the cell cycle of eukaryotic cells. Broek *et al.* (25) have proposed that newly synthesized cdc2 directs the cell to the start of S phase. In fission yeast, certain mutants allow the destruction of cdc2 during late S or G_2, and when synthesis is restored, these cells will reactivate DNA replication and become polyploid (25). Furukawa *et al.* (23) have shown that addition of antisense oligonucleotides against the AUG of the cdc2 mRNA are sufficient to block human T cells at the G_1/S boundary after their activation from quiescence. We attempted to inhibit cdc2 translation by using identical procedures in either synchronously or asynchronously cycling populations of NIH 3T3, HeLa, or hFF. We observed no decrease in the translation rate of cdc2 under a variety of conditions (data not shown). We did observe a general, nonspecific decrease in [^{3}H]thymidine incorporation with either antisense or nonsense oligonucleotides. Although the antisense method may work in primary T cells, it does not appear to be a useful method for determining new cdc2 function in other cell lines. Murray and Kirschner have shown that in the embryonic system of *Xenopus*, newly synthesized cyclin B is all that is required for successful mitotic division in egg extracts (26). Therefore, cyclin B can complex with the maternal stockpile of old cdc2 in frog eggs. In mammalian somatic cells, however, we were unable to determine if the old cdc2 from the previous cell cycle can complex with cyclin B. Nevertheless, it is clear that a majority of the new cdc2 becomes modified, suggesting complex formation with cyclin B and ultimately a role as MPF.

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