Sequences within the Conserved Cyclin Box of Human Cyclin A Are Sufficient for Binding to and Activation of cdc2 Kinase

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Cyclins are pivotal in the coordinate regulation of the cell cycle. By physical association, they are able to activate at least one of the cyclin-dependent kinases, cdc2. How this association between the catalytic moiety and cyclins leads to subsequent activation of the kinase remains unclear. In this report, we describe experiments to investigate this event at a physical level. Our approach was to map the regions required on the cyclin A molecule for interaction with cdc2. We have mapped the contact regions to two small noncontiguous stretches of amino acids, residues 189 to 241 and 275 to 320, both located within the conserved cyclin box domain of the protein. We have further shown that this region not only represents a contact site for cdc2 but apparently represents an intact functional domain with respect to cdc2 activation. This region alone is sufficient to stimulate maturation when injected into immature *Xenopus laevis* oocytes. This observation implies that events leading to the activation of cdc2 kinase can be mediated through small regions of the cyclin molecule that are located in the cyclin box. These regions contain some of the most highly conserved residues found between all the cyclin members so far identified. This suggests that the cyclin family members may have conserved a similar mechanism to bind and activate cyclin-dependent kinases.

Cyclins were first identified in cleaving invertebrate embryos as proteins whose abundance oscillated dramatically with progression through the cell cycle (12, 52, 54). A significant role for cyclins in the regulation of cell division was implicated by the observation that microinjection of clam cyclin A into immature frog oocytes could stimulate them to enter M phase (54). The ability of cyclins to activate arrested oocytes stems from their ability to stimulate an inactive pool of an M-phase-inducing factor known as maturation-promoting factor (MPF) (33, 49). MPF is a protein kinase whose catalytic subunit is the cdc2 protein kinase (1, 10, 15). In cycling cells, MPF is activated by association with a B-type cyclin, leading to phosphorylation of appropriate substrates to promote entry into mitosis (7, 14, 28). Similarly, in the fission yeast *Schizosaccharomyces pombe*, the activity of the cdc2 kinase at the G₂/M transition is also regulated by association with a cyclin B homolog, cdc13 (3, 7, 35). In the budding yeast *Saccharomyces cerevisiae*, a family of cyclin B-like proteins is believed to be involved in the G₂/M control of the cdc2 homolog CDC28 (16, 53). In addition to regulating the G₂/M transition, genetic studies of yeasts have defined a role for cyclins at the G₁/S boundary (43). In *S. cerevisiae*, the cdc2 homolog CDC28 is regulated in G₁ by association with cyclin-like molecules known as CLN1, CLN2, and CLN3 (22, 36). Activation of CDC28 by CLNs is essential to allow cells to pass through START (45, 56).

In mammalian cells, control of this G₁/S transition or "restriction point" (40) is poorly understood; however, a role for cyclins now appears likely (9, 26, 30, 55). The timing of activation of the cyclin A-associated kinase in interphase has suggested that the cyclin A-cdc2 complex may function to regulate entry into or exit from S phase (17, 41). Microinjection of cyclin A antibodies or antisense oligonucleotides further implicates a function during S phase (18, 39). Recent

studies have revealed several novel cyclins known as cyclins C, D, and E (25, 32, 34, 57). These new cyclins share a region of homology with all the other cyclins isolated to date. This conserved domain is known as the cyclin box (38) and corresponds to a region of approximately 100 amino acids towards the middle of the linear sequence of the cyclin protein. The timing of expression of cyclins D and E makes them good candidates for G₁ cyclins, since their RNA levels peak during G₁/S. Further studies with cyclin E show that its associated kinase activity (9, 26, 55) and a physical association with the transcription factor E2F both peak during G₁ (30).

Members of the cyclin family are thus able to regulate the cell cycle at multiple stages, by controlling the activity of the catalytic subunit(s) to which they are bound. The steady-state level of cdc2 protein does not fluctuate through the cell cycle (6), but its kinase activity is cell cycle regulated, apparently as a result of its dependence on cyclins for its stimulation. It has been established that cyclin binding is an essential prerequisite for cdc2 kinase activity (50); thus, cyclin synthesis is a rate-limiting step for the activation of the kinase. There is a close correlation between the abundance of cyclins A and E and associated histone H1 kinase activity (9, 41). In the case of cyclin B, there is an apparent lag period between accumulation of cyclin B and subsequent activation of the kinase, suggesting that other events are required for the full activation of enzymatic activity (5, 6, 50). The activity of the cyclin B-cdc2 kinase peaks at mitosis and then sharply declines concomitant with cyclin degradation (6, 7, 35, 44, 50). Physical association between cyclins and cdc2 therefore appears not to be sufficient for activation but serves to trigger a series of key posttranslational phosphorylation events of the cdc2 subunit, leading to maximal activity and the precise timing of mitosis (6, 8, 13, 20, 21, 27, 37, 42).

The exact mechanism by which the physical interaction of a cyclin with its catalytic subunit confers on the kinase the ability to become subsequently activated is unclear. To

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investigate the initial steps in this activation process, we sought to determine which regions of the cyclin molecule are required for binding to *cdc2* and whether these regions alone are sufficient to promote activation of the kinase. We used human cyclin A and human p34^{cdc2} for this study.

MATERIALS AND METHODS

Coimmunoprecipitation of cyclin A polypeptides with *cdc2*.

Coimmunoprecipitations were performed as previously described (11) with modifications. Briefly, cyclin A cRNA was synthesized by in vitro transcription of human cDNA with T7 RNA polymerase (Promega). The cyclin A polypeptides were synthesized by in vitro translation of the cRNA in rabbit reticulocyte lysate (Promega) in the presence of [³⁵S]methionine (NEN). Forty microliters of in vitro-translated protein was mixed with cold detergent lysate prepared from approximately 10⁶ human ML1 (myeloid leukemia) cells for 2 h at 4°C. The mixture was divided into two halves. One half was incubated with *cdc2*-specific antiserum G6 (6), a rabbit polyclonal antiserum raised to a carboxy-terminal peptide of *cdc2*; the other half was incubated with normal rabbit serum as a negative control. Complexes were also coprecipitated with a *cdc2* affinity reagent, p13 beads, which was prepared as described previously (3). The protein complexes were resolved on 10 or 15% polyacrylamide gels (29) and detected by fluorography (2). One microliter of each of the in vitro-translated products was also resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) to confirm efficient translation.

Oligonucleotide primer design and PCR. A polymerase chain reaction (PCR) strategy similar to that described previously (23) was used to generate the cyclin A mutants. Briefly, two primers (30-mers) were used to generate each of the amino- and carboxy-terminal mutants. As an example, for the amino-terminal deletion NTdl173, the first primer was identical to sequences downstream of the codon for methionine 173, 5'-GCCAGTGAGTGCCATGGAAGTACCAGACTA3', with 11 additional nucleotides added to the 5' end of the primer, including a restriction site for *NcoI*. The positioning of the primer was such that the ATG within the *NcoI* site corresponded in position to the ATG at residue 173. This ensured that after being cut with *NcoI* and ligated into the vector, the sequence would be in frame. The second primer was 5'-TTACTTTCCTAGGGAACAAAAGATTCTAC3'; the first 15 nucleotides from the 3' end of this primer are complementary to the cyclin A sequence coding for amino acids 425 to 432 (432 is the stop codon) followed by a *BamHI* recognition sequence and 7 additional nucleotides at the 5' end. For the carboxy-terminal mutants, the 5' primer was complementary to sequences encoding the first six amino acids of cyclin A, with an *NcoI* recognition site flanked by nine nucleotides added to the 5' end.

The PCR mixture included 0.8 mM neutralized deoxynucleoside triphosphate (Pharmacia), 1 μM concentrations of each of two oligonucleotide primers, 100 ng of double-stranded cyclin A cDNA, 16.6 mM (NH₄)₂SO₄, 67 mM Tris-HCl (pH 8.8), 6.7 mM MgCl₂, 10 mM 2-mercaptoethanol, and 1 U of *Thermus aquaticus* DNA polymerase (New England Biolabs) in a final volume of 50 μl. After the mixtures were covered with 50 μl of mineral oil, the reactions were carried out in a DNA thermal cycler (Perkin Elmer) at three temperatures (94°C for 1 min, 55°C for 1 min, and 72°C for 1.5 min) for 30 cycles.

The PCR-amplified DNA fragments were examined by agarose gel electrophoresis. The appropriately sized frag-

ments were cut from the gel and purified with a GeneClean kit (Bio 101). After digestion with *NcoI* and *BamHI*, the fragments were cloned into the *NcoI*-*BamHI* site of pBlue-script.globin (23).

To generate the internal mutants, two separate PCR fragments were generated. For example, to generate mutant Δ241-275, we generated a fragment encoding residues 1 to 241 with an *NcoI* site at the 5' end and an *EcoRI* site at the 3' end. The second fragment encoded residues 275 to 432, with an *EcoRI* site at the 5' end and a *BamHI* site at the 3' end. After being cut by the appropriate restriction enzymes, the fragments were ligated together and then cloned into pBlue-script.globin as described above.

Bal 31 deletions. Thirty micrograms of human cyclin A cDNA was linearized at the 3' end of the coding region with *XbaI*. The linear DNA was resuspended in buffer containing 20 mM Tris-HCl (pH 8.0), 12 mM CaCl₂, 12 mM MgCl₂, 200 mM NaCl, and 1 mM EDTA. One and one-half units of *Bal 31* exonuclease (New England Biolabs) was added, and the mixture was incubated at 30°C. To obtain multiple deletions, a time course of treatment with the exonuclease was performed. Samples were taken every 20 s, and the reaction was halted by addition of 20 mM EGTA [ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid]. It was demonstrated previously that under these conditions we could remove approximately 60 nucleotides from each end per min. Sets of samples from three time points were pooled and ethanol precipitated. Each DNA sample was treated with T4 DNA polymerase and Klenow fragment to generate blunt ends by standard protocols (47). After treatment with calf intestinal phosphatase, *XbaI* linkers were ligated to the DNA ends. The recircularized plasmids were transformed into competent *Escherichia coli*. Six colonies were picked from each pool. DNA was prepared and then linearized with *XbaI*, which cuts within the vector at the 3' end of the cDNA. Linearized plasmids were electrophoresed on a 1% agarose gel, and a total of 12 clones were picked on the basis of their sizes to cover a range of deletions up to approximately 300 nucleotides from the 3' end.

Microinjection of cyclin A cRNAs into *Xenopus laevis* oocytes. Oocytes were prepared for microinjection as described previously (54) with modifications. After removal from a female frog, the immature oocytes were treated with 1 mg of collagenase-dispase (Boehringer Mannheim) per ml for 2 h at room temperature. The oocytes were then washed extensively with Barth's saline and stored at 19°C until use. Approximately 50 oocytes were injected for each cRNA to be tested. Capped cRNAs were prepared as described above. Brome mosaic virus RNA was obtained from Promega. RNA concentrations used for injection ranged between 0.2 and 1 ng per oocyte. Progesterone was used at a final concentration of 5 μg/ml.

RESULTS

In vitro binding assay for cyclin A. We have investigated the binding of cyclin A to *cdc2*, using an in vitro binding assay as shown in Fig. 1A and as described in Materials and Methods. Cyclin A protein was synthesized by translation of cRNA in rabbit reticulocyte lysate in the presence of [³⁵S]methionine. The labeled translates were mixed with cold detergent lysates of ML1 cells, a human myeloid leukemia cell line, to allow complexes to form between the radioactively labeled cyclin protein and cellular proteins derived from the ML1 lysate. The complexes were then coprecipitated with antibodies raised against a carboxy-

FIG. 1. In vitro binding assay for cyclin A and cdc2. (A) Scheme for the in vitro binding assay. In vitro-translated human cyclin A was mixed with a Nonidet P-40 lysate of ML1 cells. Protein complexes were precipitated with anti-cdc2 reagents p13 and G6. (B) The complexes were then resolved on a 10% polyacrylamide gel. Protein complexes were precipitated with the following: lane 1, p13 beads, the protein product (p13) of the *suc1* gene of *S. pombe* cross-linked to Sepharose beads; lane 2, Sepharose beads alone; lane 3, G6, a polyclonal rabbit antiserum raised against a carboxy-terminal peptide of cdc2; and lane 4, normal rabbit serum (NRb). In lane 5, 1 μ l of in vitro-translated cyclin A was loaded.

terminal peptide of cdc2 (G6) (6) or with a cdc2 affinity reagent, p13 Sepharose beads (3). Bound proteins were subjected to SDS-PAGE and then to fluorography. Figure 1B shows that cyclin A could bind to cdc2 when either anti-cdc2 antibodies or p13 Sepharose beads were used as a means of isolation of these complexes.

In addition to full-length cyclin A protein being produced by the in vitro translation reactions, a series of polypeptides that arise largely from translational initiation at internal AUG codons were synthesized. As can be seen in Fig. 1B, a proportion of these shorter polypeptides was coprecipitated with anti-cdc2 antibodies, suggesting that the carboxy-terminal 33 kDa of the cyclin A protein contains all the sequences required for binding to cdc2.

Amino-terminal residues of cyclin A are dispensable for binding to cdc2. To deduce more precisely the amino acids of the cyclin A molecule required for the interaction with cdc2, we generated an extensive series of mutations, using a PCR strategy similar to that described previously (23), as outlined in Materials and Methods. These mutants were cloned into an in vitro transcription-translation vector containing a globin promoter to enhance transcription efficiency. cRNAs were made from these mutants by transcription with T7 RNA polymerase and then translated in rabbit reticulocyte lysate in the presence of [35 S]methionine. Mutant translation products were then tested for their ability to coprecipitate with cdc2 by using the anti-cdc2 antibody G6 as described above. The mutants to be described were also tested for binding to p13 Sepharose beads, which gave identical results (data not shown). Three deletion mutants, NTdl148, NTdl158, and NTdl173, all bound with the same affinity to cdc2 as the wild-type protein (Fig. 2), indicating that as many as 172 amino acids could be deleted from the amino terminus of cyclin A without impairing its association with cdc2. The

deletion mutant construct NTdl148 gave rise to two polypeptides, a full-length protein and a shorter species that comigrated with NTdl173. We believe that the shorter protein is likely to be a product of degradation rather than of internal initiation at methionine 173, since it failed to bind to cdc2 and its abundance varied greatly in independent translations.

FIG. 2. Amino-terminal mutants binding to cdc2. (A) In vitro binding of mutants to cdc2. Forty microliters of each in vitro-translated protein was mixed with cell lysate for 2 h. This mixture was divided in half, and complexes were isolated with the anti-cdc2 antiserum G6 or, as a negative control, normal rabbit serum (NRb) as indicated above the lanes. Complexes were resolved on a 12% polyacrylamide gel. (B) In vitro-translated products of the amino-terminal mutants (1 μ l loaded). Molecular weight markers (in thousands) are indicated.

FIG. 3. Carboxy-terminal mutants binding to cdc2. (A) In vitro binding of mutants to cdc2. Forty microliters of each in vitro-translated protein was mixed with cell lysate, except for the wild type, of which 20 μ l was used. After 2 h of mixing, the lysates were divided in half, and complexes were isolated with the anti-cdc2 antiserum G6 or, as a negative control, normal rabbit serum (NRb) as indicated above the lanes. Complexes were resolved on an SDS-10% polyacrylamide gel. (B) In vitro-translated products of the carboxy-terminal mutants (1 μ l loaded). Molecular weight markers (in thousands) are indicated on the left of the figure. N1, full-length cyclin A.

There was a dramatic drop in binding activity with any further deletion of amino-terminal residues, with NTdl189 still able to bind to cdc2 but with at least a two- to threefold reduction in affinity (Fig. 2A, lane 7). All detectable binding was lost with the removal of a further 11 amino acids (NTdl200 [Fig. 2A]). Interestingly, this boundary for high-affinity binding to the cdc2 protein corresponded closely to the beginning of the conserved cyclin box region (38). These data are summarized in Fig. 4 and indicate that the first 189 residues of cyclin A (of a total of 432 amino acids) were not required for complex formation with cdc2.

Carboxy-terminal mutations destabilize cyclin A. To examine the requirement for carboxy-terminal residues in binding of cyclin A to cdc2, we treated a linearized cyclin A cDNA construct with *Bal* 31 exonuclease as described in Materials and Methods. A time course of treatment with the exonuclease was performed, and 20 clones were isolated on the basis of their sizes to obtain serial deletions of the carboxy terminus ranging in size from 4 nucleotides (CTdl428) to 300 nucleotides from the 3' end. These clones were subsequently sequenced and tested for binding to cdc2 by the in vitro mixing assay described above. The carboxy-terminal mutants translated poorly, so for these experiments the amount of wild-type protein used as the control was halved to keep protein levels approximately comparable within the mixing experiment (Fig. 3). We were surprised to find that only very minor deletions could be tolerated in this region. The removal of 10 residues from the carboxy terminus (CTdl422) still allowed the remaining cyclin A polypeptide to bind to cdc2, but the removal of a further 21 amino acids completely abolished the association (CTdl401). We believe that these deletions of cyclin A disrupted binding to cdc2, not by removing amino acid residues involved in direct contact with

FIG. 4. Binding of cyclin A and cdc2: amino- and carboxy-terminal mutations are summarized. The shaded region represents the conserved cyclin box. The number of amino acid (aa) residues is indicated. Binding efficiencies are represented as wild type (+++), moderately reduced (++), weak (+), or background (-).

cdc2 but by disrupting the conformation of the protein in some way. Studies described below clearly show that binding occurred in the absence of the carboxy terminus of cyclin A. Two other carboxy-terminal mutants, generated by PCR, behaved differently. A deletion mutant, CTdl369, lacking the last 63 residues could bind weakly to cdc2, but with CTdl320 no association was detectable (data not shown). These results are summarized in Fig. 4.

Binding sites for cdc2 map to the cyclin box. To map further the contact site(s) on cyclin A for cdc2, we generated a number of internal deletions of the cyclin A protein. Secondary structures were predicted by the Chou and Fasman algorithm (4) and were used as a guideline for where deletions might be tolerated. For example, the smallest deletion, Δ 241-275, was chosen since this region was predicted to be alpha-helical in structure. This particular cyclin A mutant was indeed still able to bind to cdc2, suggesting that the deletion did not perturb the structure of the protein, at least within the contact region for cdc2. Larger deletions 3' to this region (for example, Δ 241-313 and Δ 241-375) yielded proteins that were unable to bind to cdc2, although SDS-PAGE of the products showed that they were synthesized (data not shown). These results are summarized in Fig. 5A.

The final set of mutants to be generated combined the deletions that have been described above. The internal deletion Δ 241-275 was incorporated into cyclin A proteins that had had either amino-terminal or carboxy-terminal residues or both removed. Curiously, the introduction of this 34-amino-acid internal deletion into the cyclin box of cyclin A (Δ 241-275), in combination with an amino-terminal truncation of 172 amino acids (NTdl173), allowed subsequent removal of the carboxy-terminal residues without affecting binding activity. This was in contrast to similar carboxy-terminal deletions made in the context of the wild-type cyclin A backbone (Fig. 3). This finding allowed us to map the contact sites on cyclin A more precisely. Mutants NTdl173/ Δ 241-275/CTdl369 and NTdl173/ Δ 241-275/CTdl320 both bound with affinities that were approximately 70% of wild-type levels, as shown in Fig. 5B, suggesting that the majority of the carboxy terminus is not required for binding.

We went on to remove residues from each boundary in five-amino-acid intervals in an attempt to narrow down contact sites more finely. The only smaller mutant to bind with reasonable affinity was NTdl189/ Δ 241-275/CTdl320, whose affinity was comparable to that observed with the

FIG. 5. Binding of cyclin A and cdc2: internal and minimal mutations. (A) Summary of binding data of internal and minimal mutants. The shaded region represents the conserved cyclin box. The number of amino acid (aa) residues is indicated. Binding efficiencies are represented as moderately reduced from wild-type efficiency (++), weak (+), or background (-). (B) In vitro binding of a minimal mutant to cdc2. Forty microliters of each in vitro-translated protein was mixed with cell lysate for 2 h. The mixture was divided in half, and complexes were isolated in lanes 1 and 3 by using the anti-cdc2 antiserum G6. As a negative control, normal rabbit serum (NRB) was used in lanes 2 and 4. Complexes were resolved on a 15% polyacrylamide gel. One microliter of each in vitro-translated (IVT) protein was loaded in the right panel. N1, full-length cyclin A. Molecular weight markers (in thousands) are indicated.

amino-terminal mutant NTdl189, i.e., about 30 to 40% of the wild-type affinity. Removal of any additional residues reduced binding below the detection levels of this assay (data not shown). These results are summarized in Fig. 5A and indicate that two short, noncontiguous amino acid stretches of cyclin A, residues 189 to 241 and 275 to 320, are sufficient to bind to cdc2 in vitro. Both these regions map to the well-conserved cyclin box found in all cyclins sequenced to date.

The cyclin box is sufficient to activate cdc2. To determine whether these physical domains represented functional regions of cyclin A, we examined whether these mutants were able to induce maturation of metaphase-arrested unfertilized *X. laevis* oocytes. It has previously been documented that injection of clam cyclin A into immature oocytes was sufficient to stimulate maturation and to cause germinal vesicle breakdown (54).

Capped human cyclin A cRNA was microinjected into 50 collagenase-treated immature oocytes. In parallel experiments, clam cyclin A RNA was injected as a positive control and BMV RNA was injected as a negative control. Maturation of the oocytes was scored by the appearance of a white spot on the pigmented animal pole of the oocyte, an indication that germinal vesicle breakdown had occurred. It was found that cyclin A from humans worked as well as clam cyclin A in this assay, with maturation occurring in the majority of eggs within an equal time frame per microgram of cRNA (data not shown). We thus proceeded to test the activities of the human cyclin A deletion series by using this assay. For the purpose of these experiments, we chose mutants that were able to bind to cdc2 at close-to-wild-type levels. Each construct was tested with at least four different batches of oocytes; each batch was also scored for maturation induced by progesterone. The percentage of oocytes

stimulated by progesterone was used as a guideline for the viability of the individual batches of oocytes, and our results from injection of the mutant RNAs were normalized against this percentage. As shown in Fig. 6, the ability to stimulate oocyte maturation correlated well with the ability to bind to cdc2. A surprising observation was that one of the smallest mutants, NTdl173/Δ241-275/CTdl320, which encodes residues 173 to 241 and 275 to 320, was also capable of stimulating maturation. This result demonstrates that these

CYCLIN A MUTANT	BINDING TO CDC2	% OOCYTE MATURATION						
		1	2	3	4	5	6	7
Wild type	+++	132	82	96	82	75	60	90
NTdl148	+++	nd	70.5	113	57.6	63	nd	0
NTdl173	+++	nd	nd	82	62	68	nd	74.3
NTdl200	-	16	0	20	0	12.5	0	nd
NTdl294	-	0	0	4	0	5.7	nd	nd
CTdl369	+	95.3	54.9	nd	63	nd	nd	65
CTdl320	-	10.9	nd	0	0	nd	0	4.3
NTdl173/Δ241-275/CTdl320	++	nd	nd	nd	69	80	90	89.3

FIG. 6. Microinjection of capped cRNA of cyclin A deletion mutants into *X. laevis* oocytes. Approximately 50 eggs per sample received injections. Results from separate batches of oocytes are expressed as percent maturation of injected eggs divided by percent maturation in 5 μg of progesterone per ml to normalize for variability in viability. nd, not determined. Binding efficiencies are described in the legend to Fig. 4.

all the cloned human cyclins and both cdc2 and cdk2 (data not shown). This result apparently does not reflect the *in vivo* situation, where other governing factors must help determine binding specificity.

Conservation of the contact regions between cyclins and their catalytic partners would imply that at least the initial steps in the activation of the kinase may also be conserved. In addition, other important regulatory events must occur to fully activate the kinase, which may lead to the distinctions that exist between the different kinase complexes, such as the difference in timing of the cyclin A-cdc2 and the cyclin B-cdc2 kinase activities. These differences presumably allow each kinase complex to play a subtly different role in the regulation of the cell cycle. Events that are likely to affect activation of the kinase include the timing of expression and subcellular localization of the cyclins, which might allow differential activation of similar catalytic subunits. Cyclins A and B appear to associate preferentially with different phosphorylated forms of cdc2 (7, 41, 48). Since phosphorylation of cdc2 occurs coincidentally with cyclin association, this observation would imply that association with different cyclins may promote different posttranslational modification of the kinase subunit. Differences have also been detected in the phosphorylated forms of cdk2 found in association with cyclins A and E (9, 31).

The mechanism and timing of degradation of the different cyclin proteins are also likely to be important in the differential regulation of the catalytic subunits by altering the steady-state levels of cyclin protein present in the cell. For such processes, other regions of the cyclin molecule may be required. In mitotic cyclins, the amino-terminal sequences have been shown to contain sequences that target these cyclins to be degraded by the ubiquitin pathway (19). The carboxy terminus may be required for structural stability of the cyclin molecule, since in the yeast CLNs and human cyclins C and D, this domain contains PEST sequences (22, 32), which have been suggested to target proteins for rapid (and constitutive) degradation (46).

These studies indicate that small regions conserved among cyclins are sufficient for binding and activation of the catalytic subunit to which they are bound. Further experiments are necessary to understand the processes by which these kinase complexes become distinct.

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