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Cloning by differential screening of a *Xenopus* cDNA coding for a protein highly homologous to cdc2

(cell cycle regulation/kinase cdc2/maturation-promoting factor/deadenylation)

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ABSTRACT **Fertilization of *Xenopus laevis* eggs triggers a period of rapid cell division comprising 12 nearly synchronous mitoses. Protein synthesis is required for these divisions, and new proteins appear after fertilization. Others proteins, however, which are synthesized in the unfertilized egg, are no longer made in the early embryo. To identify such proteins, a differential screen of an egg cDNA library gave nine clones corresponding to mRNAs that are deadenylylated soon after fertilization. The sequence of one of these clones (Eg1) revealed a high homology to $p34^{cdc2}$, the kinase subunit of maturation-promoting factor. Only 12 amino acids in the deduced amino acid sequence were unique to Eg1 when its sequence was compared to all other known examples of cdc2. Despite this strong similarity, however, Eg1 was unable to complement a yeast *cdc2*$^-$ mutant in *Schizosaccharomyces pombe* or a *cdc28* mutant of *Saccharomyces cerevisiae*. Four Eg1 transcripts, two major and two minor, were found in *Xenopus* oocytes and early embryos. These RNAs appeared very early (stage I) in oogenesis and their level remained constant until the midblastula transition, at which time they declined. Eg1 RNA is found in the poly(A)$^+$ fraction of oocytes only between the time of meiotic maturation and fertilization—that is to say, in the unfertilized egg. At fertilization the RNA loses its poly(A) tail and at the same time leaves the polyribosomes.**

For most animals, the developmental period following fertilization is characterized by a period of very rapid cell division called cleavage (1). In the case of *Xenopus*, first cleavage takes place 1.5 hr after fertilization and is followed by 11 almost synchronous cell divisions, which occur every 30 min (2). The onset of transcription is only clearly detected after the 12th cleavage at a stage called the midblastula transition (MBT). The MBT involves the coordinated desynchronization of cell divisions, the appearance of transcription and G_1 and G_2 phases, cell motility, and cell differentiation (2).

In the presence of actinomycin D embryos develop up to the MBT, whereas this development is blocked by puromycin or cycloheximide (3, 4). Similar results have been obtained for oocyte maturation, which is independent of new transcription but requires *de novo* translation (5). Qualitative analyses of the proteins synthesized in oocytes (stage VI), unfertilized eggs, and embryos have shown that during maturation and after fertilization new proteins appear but others are no longer synthesized (6–8). This suggests that the sequential synthesis of specific gene products necessary for maturation (first meiotic division), the metaphase block in the unfertilized egg and cleavages, is regulated at the translational level from the bulk of maternal mRNA. By differential screening of an egg cDNA library we have isolated 11 cDNA clones corresponding to discrete mRNAs that vary in adenylylation and polysome recruitment after fertilization (9). Nine of these clones (denoted Eg1 to Eg9) are deadenylylated after fertilization (9, 10). We have shown that, for at least one of these clones, deadenylylation is associated with release from polysomes (9). These clones correspond therefore to mRNAs whose translation changes soon after fertilization during the very early phase of embryonic development. In the present paper we report the sequence analysis and the characterization at the RNA level of one of these clones (Eg1).‖ It is highly homologous to $p34^{cdc2}$, an essential component of the maturation-promoting factor (MPF) (11, 12). A possible role of Eg1 that is unable to complement cdc2 *Schizosaccharomyces pombe* or cdc28 *Saccharomyces cerevisiae* mutants is discussed.

MATERIALS AND METHODS

Biological Materials. Ovaries were removed from anesthetized *Xenopus laevis* females, and the oocytes were isolated by treatment with dispase and collagenase, successively (13). The oocytes were sorted manually into the different oogenesis stages (14). Ovulated eggs were obtained from females following two injections of human chorionic gonadotrophin. Eggs were fertilized and embryos were cultured at 22°C in F1 modified medium (15).

DNA and RNA Preparation and RNase H Treatment. High molecular weight DNA from erythroblast nuclei was purified as described (16). Total RNA was prepared using the LiCl/urea procedure (17). Poly(A)$^+$ RNA was isolated by oligo(dT)-cellulose chromatography (18). Total RNA prepared from eggs or embryos (4 hr) was treated by RNase H in the presence or in the absence of $(dT)_{12-18}$ (19).

DNA Sequencing. The restriction fragments indicated in Fig. 1 were purified by agarose gel electrophoresis and subcloned into the phagemid Bluescript$^-$. Single-strand DNA was prepared and sequenced by the dideoxy chain-termination method (20).

Northern and Southern Blots. Samples of RNA were separated on agarose gels containing 6% formaldehyde and blotted onto nylon membranes (Hybond, Amersham). Purified inserts were ^{32}P-labeled using random primer (21) to a specific activity of 5×10^8 cpm/μg. Hybridization was carried out in 50% formamide/1% SDS/10× Denhardt's

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Abbreviations: MBT, midblastula transition; MPF, maturation-promoting factor.

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‖The sequence reported in this paper has been deposited in the GenBank data base (accession no. X14227).

solution (0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrrolidone)/10% dextran sulfate/1% sodium pyrophosphate/1 M NaCl/0.05 M Tris·HCl, pH 8, at 42°C overnight. Filters were extensively washed in 0.3 M NaCl/30 mM sodium citrate/0.5% SDS at 65°C.

Ten micrograms of *Xenopus* genomic DNA was digested with *Eco*RI, *Hin*dIII, or *Bam*HI. The samples were fractionated in agarose gels, transferred to nitrocellulose filters, and hybridized with the complete Egl probe. Hybridization and washing conditions were the same as those described for Northern blot analysis.

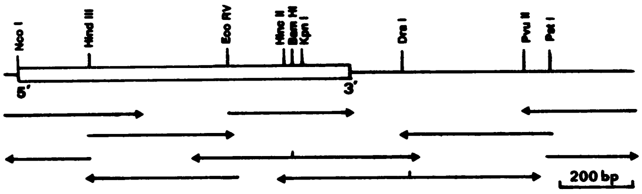
Polysomal and Nonpolysomal RNA Fractionation. One hundred eggs or embryos were homogenized in 5 ml of HKM buffer (20 mM Hepes, pH 7.4/300 mM KCl/10 mM $MgCl_2$) containing 0.5% Nonidet P-40 and 20 μg of polyvinyl sulfate per ml and centrifuged through a 15–30% sucrose gradient for 11 hr at 27,000 rpm in a Beckman SW28 rotor (22). Gradients were monitored at 254 nm by pumping through an I.S.C.O. UV analyzer. The 10S to 80S fraction was collected as the nonpolysomal RNA fraction; the pellet of the gradient contained the polysomal RNA fraction. RNA samples equivalent to five or six eggs or embryos were electrophoresed and blotted for hybridization analysis.

Complementation Assays of *S. cerevisiae cdc28* and *S. pombe cdc2* Mutants. *S. cerevisiae* cells [strain OL128/3C (*MAT***a**, *cdc28-6*, *leu2*, *ural*), a gift of M. Jacquet, Paris XI] were transformed by a modified protoplast method (23, 24) and selected at permissive temperature (26°C) for leucine prototrophy. A 1700-base-pair (bp) *Sma* I–*Dra* I fragment containing the entire open reading frame of the Egl gene was excised from the Bluescript phagemid and cloned into the *Bgl* II site (filled up with Klenow polymerase) of yeast expression vector pEMBLYe30/2 (25). The resulting plasmid, pEMBL4, was used to transform a thermosensitive *cdc28* mutant of *S. cerevisiae* [strain OL128/3C (*MAT***a**, *cdc28-6*, *leu2*, *ural*)] by the protoplast method (23, 24). To test *cdc28* suppression, transformed cells selected at permissive temperature (26°C) were plated on selective medium [0.17% YNB/0.5% ammonium sulfate/28 μg of uracil per ml/2% glucose (26)] and were grown either at permissive or at restrictive temperature (36°C).

Similarly, a haploid h- *S. pombe* strain carrying the temperature-sensitive cdc2 allele *cdc2-33* (27), as well as the *leu1-32* mutation, was transformed to leucine prototrophy at 25°C (the permissive temperature for *cdc2-33*) with plasmid pSM2-Egl and the control plasmids pSM2 and pIRT22. The transformation was carried out as described (28). The transformant colonies were then replica plated to 35°C and examined microscopically after 24 hr. pSM2-Egl was constructed by blunt-end cloning a *Pst* I fragment containing the Egl cDNA into the polylinker *Pvu* II site of the expression vector pSM2 just downstream of the simian virus 40 promoter. This promoter has been shown to work well in the fission yeast (29). pIRT22 contains the wild-type $cdc2^+$ gene.

RESULTS

Egl Sequence Analysis. The sequencing strategy, the nucleotide, and the predicted amino acid sequence of Egl cDNA are shown in Fig. 1. Egl cDNA is 1683 nucleotides long and contains 19 nucleotides of 5′ flanking sequence, an open reading frame of 891 nucleotides, and a 3′ flanking region of 773 nucleotides. The 3′ untranslated region has several features: a 28-nucleotide direct repeat between nucleotides 961 and 1015 and a potential poly(A) signal, AUUAAA (nucleotides 1660–1665). In addition, several sequence motifs, UUUU(A)AU, similar to those shown to be necessary for maturation-specific adenylylation of *Xenopus* mRNAs (30, 31), are also present (Fig. 1). The open reading frame codes for a 297-amino acid polypeptide (M_r 33,948). *In vitro* translation of the mRNA transcribed *in vitro* from Egl cDNA produced a protein that migrates with a M_r of 32,000 on SDS/polyacrylamide gel (data not shown). Evidently, Egl is related to the protein kinase family and contains all of the amino acid motifs that other kinases have (32).

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ACAGCAGTGTCCCCCGGCC ATG GAG AAT TTC CAG AAG GTG GAG AAG ATC GGG GAG GGC   58
                    Met Glu Asn Phe Gln Lys Val Glu Lys Ile Gly Glu Gly   13

ACG TAC GGG GTG GTG TAC AAG GCT CGG AAC AGA GAG ACC GGG GAA ATT GTG GCT  112
Thr Tyr Gly Val Val Tyr Lys Ala Arg Asn Arg Glu Thr Gly Glu Ile Val Ala   31

CTG AAA AAG ATA CGC TTG GAC ACT GAG ACA GAA GGA GTT CCA AGC ACA GCC ATC  166
Leu Lys Lys Ile Arg Leu Asp Thr Glu Thr Glu Gly Val Pro Ser Thr Ala Ile   49

CGG GAA ATT TCC CTC CTC AAA GAA CTG AAC CAT CCC AAC ATT GTG AAG CTT CTT  220
Arg Glu Ile Ser Leu Leu Lys Glu Leu Asn His Pro Asn Ile Val Lys Leu Leu   67

GAT GTC ATT CAT ACG GAA AAC AAG CTC TAC CTT GTC TTC GAG TTC CTT AAC CAA  274
Asp Val Ile His Thr Glu Asn Lys Leu Tyr Leu Val Phe Glu Phe Leu Asn Gln   85

GAT CTG AAG AAA TTC ATG GAC GGT TCA AAC ATT TCT GGA ATT TCA TTG GCC CTA  328
Asp Leu Lys Lys Phe Met Asp Gly Ser Asn Ile Ser Gly Ile Ser Leu Ala Leu  103

GTT AAA AGC TAT CTG TTC CAG TTG CTT CAA GGT TTG GCT TTC TGC CAC TCA CAT  382
Val Lys Ser Tyr Leu Phe Gln Leu Leu Gln Gly Leu Ala Phe Cys His Ser His  121

CGT GTC TTA CAT CGT GAT CTA AAA CCA CAG AAC CTG CTT ATT AAT TCT GAT GGA  436
Arg Val Leu His Arg Asp Leu Lys Pro Gln Asn Leu Leu Ile Asn Ser Asp Gly  139

GCC ATC AAA CTT GCC GAC TTT GGA CTA GCT CGG GCA TTT GGT GTG CCT GTG AGA  490
Ala Ile Lys Leu Ala Asp Phe Gly Leu Ala Arg Ala Phe Gly Val Pro Val Arg  157

ACC TTC ACT CAT GAG GTT GTC ACT CTG TGG TAC AGA GCA CCA GAA ATC CTT CTT  544
Thr Phe Thr His Glu Val Val Thr Leu Trp Tyr Arg Ala Pro Glu Ile Leu Leu  175

GGC TGC AAG TTC TAC TCC ACG GCT GTA GAT ATC TGG AGC CTG GGA TGT ATT TTT  598
Gly Cys Lys Phe Tyr Ser Thr Ala Val Asp Ile Trp Ser Leu Gly Cys Ile Phe  193

GCT GAA ATG ATC ACC AGG AGA GCG CTT TTT CCA GGA GAT TCT GAA ATT GAT CAG  652
Ala Glu Met Ile Thr Arg Arg Ala Leu Phe Pro Gly Asp Ser Glu Ile Asp Gln  211

CTT TTT CGT ATC TTT CGA ACA CTG GGG ACC CCT GAT GAA GTG TCC TGG CCA GGA  706
Leu Phe Arg Ile Phe Arg Thr Leu Gly Thr Pro Asp Glu Val Ser Trp Pro Gly  229

GTC ACA ACT ATG CCA GAT TAC AAG TCA ACC TTT CCC AAG TGG ATC CGA CAG GAC  760
Val Thr Thr Met Pro Asp Tyr Lys Ser Thr Phe Pro Lys Trp Ile Arg Gln Asp  247

TTT AGC AAA GTG GTA CCA CCT TTG GAT GAG GAT GGT AGA GAT CTT TTA GCT CAA  814
Phe Ser Lys Val Val Pro Pro Leu Asp Glu Asp Gly Arg Asp Leu Leu Ala Gln  265

ATG CTT CAG TAT GAC TCC AAC AAA CGG ATT TCT GCT AAA GTA GCG CTG ACC CAT  868
Met Leu Gln Tyr Asp Ser Asn Lys Arg Ile Ser Ala Lys Val Ala Leu Thr His  283

CCT TTC TTC CGA GAT GTG AGC AGA CCT ACT CCA CAT CTG ATT TAGACATACGCGTGGA  926
Pro Phe Phe Arg Asp Val Ser Arg Pro Thr Pro His Leu Ile                  297

ACTGAAAGTACAATGGTTTATAAGATGACCGAAAATGAGCAGCGTTTGTGGAATGACCGAAAATGAGCAGCG  998
                                    <----------------------><-------------
TTTGTGGAATCTTGCAACCCTCTTCTAAGAATGACTTTGTATCCATCAGATCCGTTTCACACCAAACACAGA 1070
---->
AGCAGCAGCATTTTAAAGTAATTTGTTCCTTTTGCAAAATATGTTTTTCATGCTATACGCCAACAAATTTCA 1142

AGTTCTCTTGACATCTAAAGTTTAGGAATCTGCGAAGAATATAAACAGCTAGAATTTTAATCTTATTATTCA 1214

ATTCAGAAAGTCAAATTTTTAGGTCAGTCTCTATAAGCACTTTGGTGCATATTTCATTGGGTGGGTTATTTT 1286

ATATCTTCTAATGCTCTGTGAAGTTTAACAATTGCAATATTCTCTACTGAATTATTCTAGCCAATATCATTC 1358

AACATGTAAGTTTTTTTTAACTGTGAGCTACCTTGTATGCTTGTAATACAGGCACAGCTGGTAGCTCTGTTG 1430

AAATAGGTTATACTGCTTTACAGCATGCTAACTATGAACCATTGGCACTAAAACAACTGGTCTGCAGAAATT 1502

AGATAAAGGTATTAAGAGAAACAACACAACAGACTGCTATTCTGTAAAAAAAAAATATTTTGCACCTTTTAT 1574

TTTTGGATGCAACTGTTTTTAATTTTATGTTTTAACTGTTAATTGTGTGTTTTAGTCTGTACCATTCATGTT 1646

AGTCCTGAAGTTAATTAAAATAATATTATTATTAAAA                                    1683
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FIG. 1. Nucleotide sequence of Egl cDNA and predicted amino acid sequence of the protein. (*Upper*) Restriction map and sequencing strategy. (*Lower*) Nucleotide and amino acid sequences. The 28-nucleotide direct repeat is indicated by dashed arrows. The putative cytoplasmic poly(A) elements are indicated by wavy underlines and the presumptive nuclear poly(A) signal is indicated by a solid underline.

Comparison of the predicted *Xenopus* Egl protein sequence with sequences in the EMBL/Genbank data base showed that Egl was highly homologous to the cdc2 proteins of *S. pombe* (33), chicken (34), mouse (35), and human (36) and to the cdc28 protein of *S. cerevisae* (37): 65.3%, 60.6%, 66.3%, 63.6%, and 64%, respectively (Fig. 2). Therefore, the similarity between *Xenopus* Egl protein and the various cdc2 proteins or cdc28 protein is almost the same as the similarity between *S. pombe* cdc2 and *S. cerevisae* cdc28 (69%) or between *S. cerevisae* cdc28 and human cdc2 (64.5%). Moreover, specific parts of the cdc2 protein sequence, such as the

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XL EG1       MENFQKVEKIGEGTYGVVYKARN RET GEIVALKKIRLDTETEGVPSTAIREISLLKELNHPNI
Cg CDC2      --DYT-I-------------G-H KT- -QV--M-----ES-E-----------------H----
Mo CDC2      --DYI-I-------------G-H -V- -Q---M-----ES-E-----------------R----
Hu CDC2      --DYT-I-------------G-H KT- -QV--M-----ES-E-----------------R----
SP CDC2      ---Y-----------------H KLS -R---M-----ED-S-----------------V-DE-N
SC CDC28  MSGELA-YKRL--V-----------LDL-PGQ-QR--------ES-D----------------KDD--

XL EG1       VKLLDVIHTEN KLYLVFEFLNQDLKKFMDG SNISGISL A LVKSYLFQLLQGLAFCHSHRVL
Gg CDC2      -C-Q--LMQDA R---I----SM----YL-T  IP--QY-DRSR------Y-I----IV-----R---
Mo CDC2      -S-Q--LMQDS R---I----SM----YL-S  IPP-QFMDSST-----H-IM--IV-----R---
Hu CDC2      -S-Q--LMQDS R---I----SM----YL-S  IPP-QYMDSS------Y-I----IV-----R---
SP CDC2   RSNC-R---IL-A-S ---------DM----Y--RISETGAT--DPR--QKFTY--VN-VN----R-II
SC CDC28     -R-Y-IV-S.AH---------DLD--RY-EG  IPKDGP-G-DI--KFMH--CK-I-Y-----I-

XL EG1    HRDLKPQNLLINSDGAIKLADFGLARAFGVTVRTFTHEVVTLWYRAPEILLGCKFYSTAVDIWSLGCIF
Gg CDC2   ----------DDK-V-------------IP--VY----------S---V---SAL---P-----I-T--
Mo CDC2   ----------DDK-T-------------IPI-VY----------S---V---SAR---P-----I-T--
Hu CDC2   ----------DDK-T-------------IPI-VY----------S---V---SAR---P-----I-T--
SP CDC2   ----------DKE-NL---------S---PL-NY---I----------V---SRH---G-----V----
SC CDC28  ----------K--NL--G----------PL-AY---I----------V---G-Q---G--T--I----

XL EG1    AEMITRRALFPGDSEIDQLFRIFRTLGTPDEVSWPGVTTMPDYKSTFPKWIRQDFSKVVPPLDEDGRDL
Gg CDC2   --LA-KKP--H-------------A----NNDV--D-ESLQ---N-----KPGSLGTH-QN-----L--
Mo CDC2   --LA-KKP--H-------------A----NNEV--E-ESLQ---N-----NPGSLASH-KN---NCL-F
Hu CDC2   --LA-KKP--H-------------A----NNEV--E-ESLQ---N-----KPGSLASH-KN---N-L--
SP CDC2   -----R-SP---------EI-K--QV----N-EV-----LLQ-------R-K-M-LH-----NGE--AIE-
SC CDC28  ---CN-KPI-S--------I-K---V----N-AI--DIVYL--F-PS--Q-R-K-L-Q---S--PR-I--

XL EG1    LAQMLQYDSNKRISAKVALTHPFFRDVSRPTPHLI      297
Gg CDC2   -SK--I--PA----G-M--N--Y-D-LDKS-LPANLIKKF 303
Mo CDC2   -SK--V--PA----G-M--K--Y-D-LDNQIKKM       298
Hu CDC2   -SK--I--PA----G-M--N--Y-N-LDNQIKKM       297
SP CDC2   -SA--V--PAH-----R--QQNYL--FH             296
S. CDC28  -DKL-A--PIN----RR-AI--Y-QES               299
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FIG. 2. Amino acid sequence alignment of Eg1 with cdc2 homologs from other species. Abbreviations and source of data: XL EG1, *X. laevis* Eg1 (this paper); Gg CDC2 (34); Mo CDC2, mouse cdc2 (35); Hu CDC2, human cdc2 (36); SP CDC2, *S. pombe* cdc2 (33); SC CDC28, *S. cerevisiae* cdc28 (37), the budding yeast homolog of cdc2.

16-residue "PSTAIR" motif, are present in Eg1, and *in vitro* synthesized Eg1 protein was immunoprecipitated by anti-PSTAIR antibodies and also binds to $p13^{suc1}$ (data not shown).

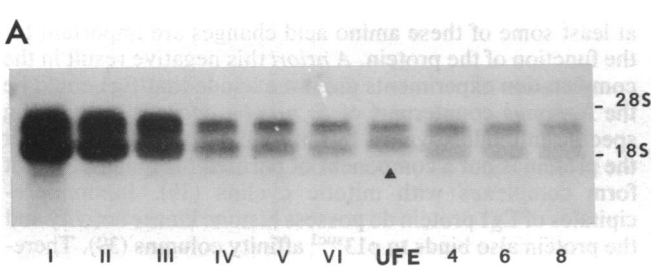
Expression of Eg1 mRNA During Oogenesis and Early Development. Total RNAs extracted from oocytes (stage I to stage VI), eggs, and pre-MBT embryos were subjected to Northern analysis using a ^{32}P-labeled Eg1 cDNA probe (Fig. 3*A*). This probe, made using the entire Eg1 cDNA, essentially hybridized to two major transcripts of about 2 and 3 kilobases (kb) in all samples. In fact, this probe also hybridized to additional transcripts that are less abundant (Fig. 3*B*).

Between stages II and VI of oogenesis total RNA increases about 20- to 30-fold, mainly due to an accumulation of a large amount of rRNA (1). Therefore, the decrease in the autoradiogram signals, observed in Fig. 3*A* for the 2- and 3-kb mRNAs, reflects the dilution of these transcripts in the pool of total RNA. Taking this in account, quantitation of autoradiograms from these Northern analyses confirmed that the number of these transcripts per oocyte or embryo was approximately constant from stage II of oogenesis to the MBT in developing embryos. Total RNAs extracted from several adult tissues (brain, liver, skin, kidney, and testis) were also subjected to Northern analysis using the ^{32}P-labeled probe to the entire Eg1 cDNA. Eg1 transcripts were only detected in testis RNA (data not shown). However, this probe also visualized several transcripts in RNA extracted from exponentially growing *Xenopus* kidney A6 cells (see Fig. 3*B*).

Eg1 Gene Family. We have shown that the cloned Eg1 cDNA hybridizes to several RNAs of different sizes (Fig. 3*B*). This suggests that the mRNA corresponding to the Eg1 cDNA may be transcribed from one of several genes coding for proteins of closely related sequence/function. In this case partial cDNA probes corresponding to the coding sequence should visualize all or most of these transcripts, whereas probes to the 3′ untranslated region, generally less well conserved among related genes, should only hybridize with the homologous mRNA. Therefore subclones of Eg1 cDNA, corresponding to the mainly 5′ coding region (nucleotides 1–213) or to the 3′ untranslated region (nucleotides 1083–1683), were used to produce ^{32}P-labeled probes. Northern analysis of total RNA from oocyte stage VI, eggs, and embryos (6 hr) showed that the 5′ probe visualized a total of five transcripts in oocytes and embryos (2.0, 2.9, 3.0, 3.2, and 3.6 kb) (Fig. 4*A*). With the 3′ probe, however, only the 2-kb mRNA was clearly detected, although faint signals persisted from the other transcripts. These results show, therefore, that the coding regions of the Eg1 transcripts are closely related but their 3′ untranslated regions are different. In addition, this comparative Northern analysis with the 5′ and 3′ probes shows that the cloned Eg1 cDNA corresponds uniquely to the 2-kb mRNA.

To evaluate the number of Eg1-related genes, total *Xenopus* genomic DNA was digested separately with *Eco*RI, *Bam*HI, and *Hin*dIII and subjected to Southern analysis using a ^{32}P-labeled probe to the entire Eg1 cDNA (Fig. 4*B*). Quantitation of the autoradiogram signals, relative to that for Eg1 cDNA, showed that at least four Eg1-related genes may exist in the haploid *Xenopus* genome.

Posttranscriptional Modifications of Eg1 mRNA. Closer examination of the data in Fig. 3*A* shows that the 2-kb transcript appears to be larger in eggs than in either oocytes or embryos. We have previously shown that the Eg1 cDNA was selected by differential screening as an mRNA that is deadenylylated after fertilization (9). To ascertain whether the size differences observed for the 2-kb transcript corresponded to adenylylated or deadenylylated states of the same mRNA, RNAs from eggs and embryos were fractionated by chromatography on oligo(dT)-cellulose. Northern analysis of these poly(A)$^+$ and poly(A)$^-$ fractions from the different samples showed that the slightly larger 2-kb transcript was only present in the poly(A)$^+$ fraction prepared from the eggs (Fig. 5*A*). After fertilization the amount of this larger 2-kb transcript decreased in the poly(A)$^+$ fraction, and there was a concomitant increase of the smaller 2-kb transcript in the poly(A)$^-$ fraction. It should be noted that the 3-kb transcript also passed from the poly(A)$^+$ to the poly(A)$^-$ fractions after fertilization, indicative of a similar postfertilization deadenylylation of this mRNA (Fig. 5*A*). Further evidence that the size difference in the 2-kb transcript was due to a change in the polyadenylylation was obtained by incubating total RNA extracted from eggs and embryos with $(dT)_{12-18}$ in the presence or absence of RNase H. Northern analysis of these samples showed that the size differences of the 2-kb mRNA between the egg and embryo samples were abolished by removal of the poly(A) tract (Fig. 5*B*).



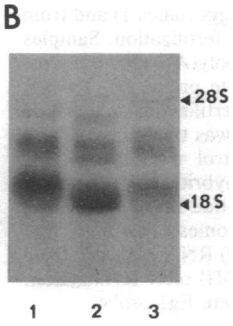


FIG. 3. Northern blot analysis of total RNA extracted from oocytes (stage I to stage VI), unfertilized eggs (UFE), and embryos 4, 6, and 8 hr after fertilization (*A*) or from unfertilized eggs (lane 1), 4-hr embryos (lane 2), and exponentially growing cells corresponding to the A6 cell line (lane 3) (*B*). Each lane was loaded with 10 μg of total RNA, and the Northern analysis was performed using the complete Eg1 probe.

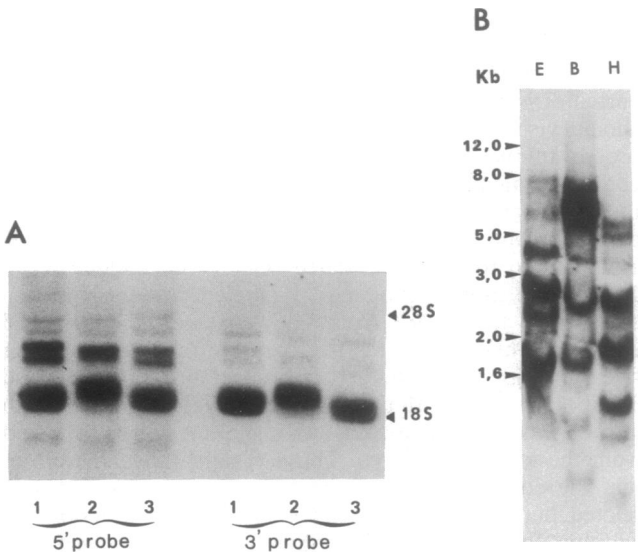


FIG. 4. (*A*) Northern blot analysis of 20 μg of total RNA extracted from stage VI oocytes (lanes 1), unfertilized eggs (lanes 2), and embryos 4 hr after fertilization (lanes 3). Hybridization was performed with the 5′ (mainly coding) region from nucleotide 1 to 213 and a part of the 3′ untranslated region from nucleotide 1083 to 1647. (*B*) Southern blot analysis. Ten micrograms of *Xenopus* genomic DNA was digested with *Hin*dIII (H), *Bam*HI (B), or *Eco*RI (E). The samples were fractionated in agarose gels, transferred to nitrocellulose filters, and hybridized with the complete Egl probe.

In *Xenopus* oocytes and embryos, changes in the polyadenylylation of a mRNA are associated with changes in polysome recruitment (9). Therefore, polysomal and non-

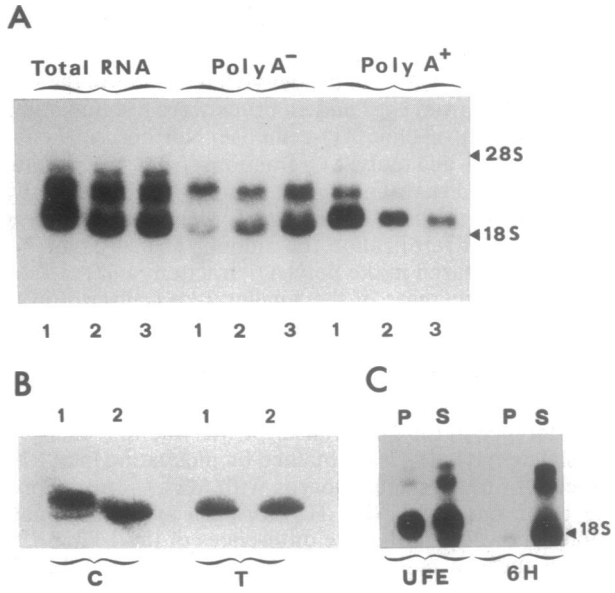


FIG. 5. (*A*) Northern blot analysis of total, poly(A)$^-$, and poly(A)$^+$ RNAs extracted from unfertilized eggs (lanes 1) and from embryos 2 hr (lanes 2) and 6 hr (lanes 3) after fertilization. Samples of total RNA (20 μg), poly(A)$^-$ (20 μg), and poly(A)$^+$ (200 ng) were blotted and hybridized with the Egl complete probe. (*B*) Twenty micrograms of total RNA extracted from unfertilized eggs (lanes 1) and embryos 4 hr after fertilization (lanes 2) was treated by RNase H in the presence (T) or in the absence (control = C) of $(dT)_{12-18}$, transferred to nylon membranes, and then hybridized with the 3′ untranslated region of Egl probe (from nucleotide 1083 to 1647). (*C*) Recruitment of Egl transcripts onto polysomes. Northern blot analysis of polysomal (P) or nonpolysomal (S) RNA prepared from unfertilized eggs (UFE) and embryos 6 hr (6H) after fertilization. Hybridization was performed with the complete Egl probe.

polysomal fractions were prepared from eggs and 6-hr embryos, and the RNA extracted from these fractions was subjected to Northern analysis. The results showed that the 2-kb mRNA visualized by the Egl cDNA probe was present in the polysomal fraction obtained from eggs but was completely absent from this fraction obtained from 6-hr embryos (Fig. 5*C*). When polysomal and non-polysomal fractions were prepared in the presence of EDTA, this transcript was always found in the nonpolysomal fraction (data not shown). Therefore, the postfertilization deadenylylation of this 2-kb mRNA is associated with its release from polysomes.

Biological Role of Egl. The sequence analysis described above suggests that Egl is a protein whose function may be similar to that of cdc2 or cdc28—i.e., a regulator of G_2/M and G_1/S transition during the cell cycle (11, 12). The different analyses at the RNA level that we have performed suggest that this mRNA is translated in the eggs, a time when important changes in the cell cycle status occur. We therefore postulated that Egl could be the *Xenopus* counterpart of cdc2. To test this postulate, we tried to complement *S. cerevisiae cdc28* and *S. pombe cdc2* mutants with Egl. Cells harboring a thermosensitive *cdc28* mutation (strain OL128/3C) were transformed with plasmid pEMBLYe30/2, a replicative plasmid harboring the *LEU2* gene and the phosphoglycerate kinase (PGK) promoter and terminator (25), or with pEMBL4, a pEMBLYe30/2-derived plasmid in which expression of the Egl gene is under control of the PGK promoter. OL128/3C cells were also transformed with a Yep13 plasmid containing the *cdc28* gene. As expected cells containing pEMBLYe30/2 were not able to grow at restrictive temperature (36°C). On the contrary, cells containing the *cdc28* gene on Yep13 (38) were able to grow at 36°C. Finally, cells containing pEMBL4 did not grow at 36°C. Thus the presence of Egl did not suppress the *cdc28* mutation.

Likewise, fission yeast cells carrying the *cdc2-33* mutation could not be rescued by expression of the Egl protein from the plasmid pSM2-Egl. This was shown by transforming a *cdc2-33 leu1-32* haploid strain with pSM2-Egl and with the control vector pSM2 and replica plating the resulting leu$^+$ transformants to the restrictive temperature for *cdc2-33* (see *Materials and Methods*). At the restrictive temperature the cells underwent cell cycle arrest, becoming highly elongated; in contrast, cells transformed with plasmid pIRT22, which contains the wild-type *cdc2*$^+$ gene, were able to grow and divide normally at the high temperature.

DISCUSSION

In the present paper we report the sequence analysis and the characterization at the RNA level of a clone (Egl) selected by differential hybridization. Its protein sequence is highly homologous to known cdc2 protein sequences (33–37). Egl contains the 16-residue PSTAIR region and specifically the tyrosine in position 15, which has been shown to play a key role in mitosis entry through phosphorylation and dephosphorylation processes (11, 12). Only 12 of a total of 297 amino acids were found to be specific to Egl when compared to the other published cdc2 sequences. It should be pointed out that 4 of the amino acids that are missing in Egl are tyrosines. Since Egl cDNA was unable to complement either *cdc2 S. pombe* or *cdc28 S. cerevisiae* mutants, we can postulate that at least some of these amino acid changes are important for the function of the protein. *A priori* this negative result in the completation experiments did not exclude that Egl could be the *Xenopus* counterpart of *S. pombe cdc2*. But, by using specific antibodies against Egl protein, it has been found that the protein is not a component of purified MPF and does not form complexes with mitotic cyclins (39). Immunoprecipitates of Egl protein do possess histone kinase activity and the protein also binds to $p13^{suc1}$ affinity columns (39). There-

fore, although Eg1 does not appear to be functionally equivalent to cdc2, the properties of this protein indicate the need for caution in using p13 beads or PSTAIR antibodies as tools for studying specifically the $p34^{cdc2}$ protein.

In this study we showed that Eg1 RNA is specifically found in the poly(A)$^+$ fraction in the eggs. After fertilization the RNA is deadenylylated and removed from the polysomes. Other studies have shown that during maturation polyadenylylation is required for translation (30, 31, 40–42), and we have previously shown that adenylylation and polysome recruitment as well as deadenylylation and polysome release are also correlated in the embryo (9). Using specific antibodies against Eg1, it has been shown that the protein appears during maturation and is no longer synthesized after fertilization (Tim Hunt, personal communication). This indicates that for Eg1 RNA there is a very close correlation between the adenylylation and the deadenylylation of the RNA and its translation. We have also shown that Eg1 RNA and all of the related transcripts are not specific to oogenesis or early embryogenesis. The same transcripts are also present in exponentially growing cells in culture (A6 cell line) but they are absent in nondividing cells. This strongly suggests that the proteins encoded by these RNAs are involved in proliferation or cell cycle control. But the fact that Eg1 protein is not associated to mitotic cyclins (39) suggests that the protein is not involved in mitosis regulation.

Although as yet we have no indication what the biological role of Eg1 might be, we would like to postulate, based on its regulation, that the protein could play a role in DNA synthesis regulation and perhaps in G_1/S transition. This would be in agreement with the fact that Eg1 protein is absent in nonmaturated oocytes but is present in the eggs where its level is similar to $p34^{cdc2}$ (Tim Hunt, personal communication). It would perhaps be a real problem for meiosis to occur properly if this protein appeared during oogenesis. Moreover, microinjection of anti-sense oligodeoxynucleotides directed against Eg1 RNA in stage VI oocytes does not prevent maturation *in vitro* (data not shown), suggesting that the protein is not involved in meiosis. After fertilization Eg1 protein is no longer synthesized, suggesting that its translation is not necessary for the early development to occur. This is in agreement with the fact that in contrast to mitosis, DNA synthesis is not protein synthesis dependent during *Xenopus* early development. In the fission yeast *S. pombe*, cdc2 acts at two points in the cell cycle: first at "start", where it is required for entry into S phase, and then in late G_2, where it regulates entry into mitosis (12). This is also true for cdc28 in *S. cerevisiae* (43). On the contrary, by microinjection of anti $p34^{cdc2}$ antibodies into serum-stimulated rat fibroblasts it has been shown that only cell division was blocked, but DNA synthesis was unaffected (44). Possibly during evolution in higher eukaryotes two different genes appeared from an ancestral one, which is perhaps still unique in yeast. One of these genes (*cdc2*) would be specifically involved in the control of G_2/M transition and the other (Eg1) would be involved in G_1/S transition. The fact that the human and chicken cdc2 are able to complement the yeast mutants does not exclude this hypothesis because even if in the original cell the gene is involved in only one process it could perfectly well regulate both transitions (G_1/S and G_2/M) in the yeast context. Such a result was obtained with the *ras* human protooncogene product, which was shown to act to regulate cell growth in human and yeast cells but to do so by functioning in distinct and very different pathways in the organisms (45, 46). In higher eukaryotes the use of two different cdc2-like molecules at "start" and mitosis has been hypothesized (11).

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