The EMBO Journal vol.9 no.10 pp.3233 - 3240, 1990

p40^{MO15}, a *cdc2*-related protein kinase involved in negative regulation of meiotic maturation of *Xenopus* oocytes

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Communicated by A.Colman

The clone MO15 which codes for a 40 kd protein (p40^{MO15}) with 40% amino acid identity to the human *cdc2* protein kinase has been isolated from a *Xenopus* cDNA library using a synthetic oligonucleotide probe. MO15 mRNA is accumulated during oogenesis, becomes de-adenylated during meiotic maturation, and is degraded after the mid-blastula-transition stage of embryogenesis. Translation of p40^{MO15} is restricted to non-mature oocytes. Specific inhibition of p40^{MO15} synthesis in stage VI oocytes by antisense oligonucleotide depletion of MO15 mRNA increases the rate of progesterone induced H1 kinase activation and oocyte maturation. This effect can be reversed by subsequent injection of synthetic MO15 mRNA. These results suggest that p40^{MO15} is involved in negatively regulating meiosis.

Key words: cdc2-related/kinase/maturation/oocytes/*Xenopus*

Introduction

Immature stage VI *Xenopus* oocytes are arrested in prophase of meiosis I. The resumption of meiosis, or oocyte maturation, is triggered by the release of progesterone by the follicle cells surrounding the oocyte, in response to circulating gonadotrophic hormone (reviewed by Smith, 1989). Completion of meiosis I and arrest in metaphase of meiosis II are required before fertilization can occur. The early/intermediate events of maturation which follow the binding of progesterone at the oocyte membrane are still poorly understood. The results from a number of studies are equivocal, indicating that many of these events involve the activity (or inactivity) of protein kinases such as cAMP-dependent protein kinase (PKA) and protein kinase C (PKC), and phosphatases such as phosphatase-1 (reviewed by Maller, 1985 and Smith, 1989). Nevertheless, changes in the pattern of phosphorylated proteins (Maller and Smith, 1985) and the appearance of M-phase specific phosphorylated proteins (Davis *et al.*, 1983) provide further evidence for the involvement of phosphoproteins. These events lead ultimately to the activation of maturation promoting factor (MPF), a protein complex which positively regulates the G_2/M transition of both meiotic and mitotic cell-cycles in eukaryotes (reviewed by Maller, 1985). Once activated, MPF brings about the late events of oocyte maturation and germinal vesicle breakdown (GVBD) such as chromatin condensation, disassembly of the nuclear lamina and spindle formation. MPF, which itself has protein kinase activity, has now been purified extensively (Lohka *et al.*, 1988). Subsequent studies have shown that it contains a heterodimer of p34^{cdc2} and cyclin B (Dunphy *et al.*, 1988; Gautier *et al.*, 1988, 1990). p34^{cdc2} is a serine/threonine kinase encoded by the *Xenopus* homologue of the fission yeast *Schizosaccharomyces pombe* cell-cycle control gene *cdc2* (Hindley and Phear, 1984). Genetic analysis of *cdc2* mutants has indicated that the function of this gene is essential for cells to pass through both the G_1/S and G_2/M transitions of the cell-cycle (Nurse and Bisset, 1981). Consequently, activation of p34^{cdc2}, as a component of MPF, has been thought of as the key regulatory event which is required for oocytes to resume meiosis (Murray, 1989; Hunt, 1989).

The mechanisms which cause and maintain prophase arrest in oocytes are still not known, although it has been proposed that one or more phosphorylated 'maturation proteins' (MpPs) are involved (Maller and Krebs, 1977). Several candidate proteins which are rapidly dephosphorylated after progesterone treatment have been described, but no evidence to confirm their involvement has been obtained (Boyer *et al.*, 1986). Such hypothetical proteins could function by blocking the translation or activation of proteins which are required to resume meiosis, and indeed the early/intermediate events of maturation, preceding the activation of MPF, do require protein synthesis (Wasserman and Masui, 1975). From studies in yeast, frog and human cells it is known that active p34^{cdc2} kinase is complexed with cyclin protein (Booher and Beach, 1987; Draetta and Beach, 1988; Pines and Hunter, 1989; Gautier *et al.*, 1990). Accumulation of cyclin B has been shown to drive the activation of p34^{cdc2} in the early embryo mitotic cell-cycle (Murray and Kirschner, 1989; Minshull *et al.*, 1989), but this is unlikely to be the case during the resumption of meiosis since a significant store of cyclin is already present and associated with p34^{cdc2} in the oocyte (Gautier *et al.*, 1990; Roy *et al.*, 1990). However, the proto-oncogene product p39$^{c\text{-}mos}$ is required for meiotic maturation (Sagata *et al.*, 1989) and is not synthesized significantly in the oocyte until maturation is induced (Watanabe *et al.*, 1989). Cyclin B is a potential substrate for p39$^{c\text{-}mos}$ kinase (Roy *et al.*, 1990) and conceivably accumulation of p39$^{c\text{-}mos}$ activity during maturation may directly activate the p34^{cdc2}–cyclin complex.

Activation of the p34^{cdc2} M-phase kinase also requires its dephosphorylation on tyrosine residues (Gautier *et al.*, 1989; Dunphy and Newport, 1989; Moria *et al.*, 1989; Gould and Nurse, 1989). Thus, an alternative model for regulation of MPF activity would require in turn regulation of the p34^{cdc2} tyrosine phosphatase involved. Precocious activation of MPF can be induced in oocytes by okadaic acid inhibition of phosphatases which may well be involved in controlling this tyrosine phosphatase (Cyert and Kirschner, 1988; Gorris *et al.*, 1989; Felix *et al.*, 1990). In addition to p34^{cdc2} and

$p56^{cdc13}$ (a yeast cyclin gene product), the execution of mitosis, in fission yeast, also involves the *cdc25* and *suc1* gene products (Moreno *et al.*, 1989). Fission yeast *suc1* gene product blocks tyrosine dephosphorylation of $p34^{cdc2}$ in a cell-free system (Dunphy and Newport, 1989), but it is not yet clear if and how these genes may function in *Xenopus* oocyte maturation.

Clearly protein kinases play an important role in regulating and bringing about meiotic maturation. With the intention of isolating new components of this regulatory pathway, we have been isolating protein kinase genes which are expressed in *Xenopus* oocytes. Using the strategy of homology probing with synthetic oligonucleotides, the sequences of which were deduced from the conserved amino acid domains of serine/threonine kinases (Hanks *et al.*, 1988), we have obtained cDNA clones corresponding to S6 kinase II and PKA (unpublished data). We describe in this paper the isolation and characterization of one clone, MO15, which codes for a 40 kd protein ($p40^{MO15}$) with 40% amino acid identity to human $p34^{cdc2}$. MO15 is not a *Xenopus cdc2* homologue, but, probably represents a new member of a growing family of *cdc2*-related proteins. Expression of MO15 is restricted to the oocyte and specific inhibition of $p40^{MO15}$ synthesis by antisense oligonucleotide injection suggests that this protein may be involved in negatively regulating meiosis.

Results

Cloning and characterization of MO15 cDNA

Several redundant oligonucleotides with sequences deduced from the conserved catalytic domains of serine/threonine kinases (Hanks *et al.*, 1988) were synthesized and used to screen cDNA libraries. One of these oligo probes, CDC2.4, was used to isolate clone 4.5 from a *Sau*3A digested cDNA library in phage M13. Sequencing of clone 4.5 indicated that it probably encodes a protein kinase, therefore it was in turn used to isolate the larger clone MO15 from a full-length cDNA library constructed from mature oocyte poly(A)$^+$ RNA in λ-ZAP. MO15 contained a 1.36 kb insert, which when sequenced revealed an open reading frame with two potential start sites coding for predicted proteins of 40.9 kd and 39.7 kd respectively (see Figure 1). The second AUG is in a more favourable context to act as initiation codon (Kozak, 1984), and analysis of the endogenous protein synthesized by oocytes indicated that the second AUG is in fact used preferentially *in vivo* (see below and Figure 4a). All of the highly conserved amino acid residues found in protein kinase nucleotide binding and catalytic sites are represented in the predicted amino acid sequence (Figure 1). A computer search indicated that MO15 protein has homology to most protein kinases present in the NBRF protein database, strongly suggesting that it is itself a protein kinase. The most homologous protein detected was the human $p34^{cdc2}$ protein kinase, with ~40% of the matched residues being identical (see Figure 2). In addition to this overall homology, regions known to be highly conserved and characteristic of the *cdc2* gene family, such as the PSTAIRE box (Lee and Nurse, 1987), are partially represented in MO15 (8/15 amino acids, see Figure 2). This places MO15 in the group of cdc2-related proteins which includes PSK-J3 found in a human HeLa cell cDNA library (Hanks, 1987) and KSS1, KIN28 and PHO85 found in *Saccharomyces cerevisiae* (Courchesne *et al.*, 1989; Simon *et al.*, 1986; Toh-e *et al.*, 1988). The sequences of these cdc2-related proteins are also compared in Figure 2, and clearly the level of homology is not compelling enough to suggest that MO15 is a homologue to any one of them.

MO15 mRNA levels during oogenesis and early development

A Northern blot of RNA extracted from stage VI oocytes indicated that a single MO15 transcript, ~1.4 kb in size, is expressed (see Figure 3a, lane 1). The 1.36 kb cDNA clone is therefore nearly full-length. Equivalent amounts of MO15 mRNA were also found in defolliculated stage VI oocytes (data not shown), indicating that the transcripts are indeed contained in the oocyte. By comparison with the signal obtained from known amounts of synthetic mRNA we estimate that the abundance of MO15 mRNA is low, at ~1.5 pg/oocyte. In order to begin to characterize the

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1    GAATTCGGGTCACATTCGCGCTCCCGTCGCGCCGGTAGTAGCGTGAAGTAGGTGTGGCCA
61   AAGTGTTAAAGGTAGATGTTGGCAATAGACTGTGGGAAAGGGGCTGCAGGGAGAATGGAA
                    [M  L  A  I  D  C  G  K  G  A  A  G  R ]M  E

121  GGTATAGCGGCGAGAGGGGTGGACGTGCGGTCCCGTGCCAAGCAGTATGAGAAGCTGGAT
       G  I  A  A  R  G  V  D  V  R  S  R  A  K  Q  Y  E  K  L  D

181  TTCCTAGGCGAAGGGCAGTTTGCCACTGTTTATAAAGCCCGAGACAAGAATACTGATAGA
       F  L  G  E  G  Q  F  A  T  V  Y  K  A  R  D  K  N  T  D  R
             *     *           *

241  ATTGTGGCCATTAAAAAGATTAAACTGGGGCATAGAGCAGAAGCTAATGATGGGATAAAC
       I  V  A  I  K  K  I  K  L  G  H  R  A  E  A  N  D  G  I  N
             *     *                                    *

301  AGAACAGCGCTGAGAGAGATAAAGCTACTACAGGAGTTAAGCCATCCTAATATCATTGGG
       R  T  A  L  R  E  I  K  L  L  Q  E  L  S  H  P  N  I  I  G
                      *

361  CTACTGGATGCTTTTGGACACAAATCCAACATAAGCCTGGTGTTTGATTTTATGGAGACG
       L  L  D  A  F  G  H  K  S  N  I  S  L  V  F  D  F  M  E  T

421  GATCTTGAGGTCATAATAAAAGATACCAGCTTGGTATTAACTCCAGCTCATATTAAATCA
       D  L  E  V  I  I  K  D  T  S  L  V  L  T  P  A  H  I  K  S
                                                  [-----15-1-

481  TACATGCTGATGACTCTTCAAGGCTTGGAATACTTACACCATCTCTGGATCCTCCACAGA
       Y  M  L  M  T  L  Q  G  L  E  Y  L  H  H  L  W  I  L  H  R
       -----]

541  GATTTAAAGCCGAATAATTTGCTGCTGGATGAAAATGGGGTCCTTAAACTTGCTGATTTT
       D  L  K  P  N  N  L  L  L  D  E  N  G  V  L  K  L  A  D  F
             *           *                                    *  *

601  GGTCTTGCAAAGTCATTTGGGAGTCCAAACAGAATATATACTCATCAAGTGGTAACAAGG
       G  L  A  K  S  F  G  S  P  N  R  I  Y  T  H  Q  V  V  T  R
             *

661  TGGTACCGATCTCCTGAGTTGCTGTTTGGTGCTAGAATGTATGGTGTGGGAGTTGACATG
       W  Y  R  S  P  E  L  L  F  G  A  R  M  Y  G  V  G  V  D  M
                      *                                       *

721  TGGGCAGTTGGCTGCATCTTAGCTGAGCTGCTACTTCGGGTGCCTTTTTTACCTGGGGAT
       W  A  V  G  C  I  L  A  E  L  L  L  R  V  P  F  L  P  G  D
                *

781  TCTGATCTAGACCAGCTAACGAGAATATTTGAAACTTTGGGAACACCAACTGAAGAGCAG
       S  D  L  D  Q  L  T  R  I  F  E  T  L  G  T  P  T  E  E  Q
       [-----15-2------]

841  TGGCCGGGCATGTCCAGCCTTCCAGACTATGTTGCCTTTAAAAGTTTTCCAGGGACACCA
       W  P  G  M  S  S  L  P  D  Y  V  A  F  K  S  F  P  G  T  P

901  CTGCACCTCATTTTTATTGCTGCTGGCGATGATCTTCTTGAACTCCTGCAAGGTTTATTT
       L  H  L  I  F  I  A  A  G  D  D  L  L  E  L  L  Q  G  L  F

961  ACCTTTAATCCGTGTGCAAGATGCACAGCATCCCAGGCACTTCGAAAAAGGTATTTTAGC
       T  F  N  P  C  A  R  C  T  A  S  Q  A  L  R  K  R  Y  F  S
                         *

1021 AACAGACCGGCACCAACCCCAGGAAACTTGTTGCCAAGACCCAATTGTTCTATAGAAGCA
       N  R  P  A  P  T  P  G  N  L  L  P  R  P  N  C  S  I  E  A

1081 TTAAAGGAGCAGCAAAATCTCAACCTGGGAATAAAGAGAAAGCGGACAGAAGGCATGGAT
       L  K  E  Q  Q  N  L  N  L  G  I  K  R  K  R  T  E  G  M  D

1141 CAAAAAGACATTGCTAAGAAGTTGAGTTTTTGACTCCAGACAATGTAGACCATACGCCAA
       Q  K  D  I  A  K  K  L  S  F

1201 GCCTGAGAGAAAACTGCTGCTATAAACAGAGACGTCAGAGACCGGTCCATGAAATGAACT
1261 AAAACTTGTAATATATGTAAATACTGAGTTGCGTGATCCGTAACAGAACTAGTCATTAAA
1321 ATAATACTTTCTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
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Fig. 1. The nucleotide and deduced amino acid sequence of MO15. Both of the potential start codons are underlined and in italics; the 13 predicted N-terminal amino acids translated from the first AUG are distinguished by italics and bracketed. Highly conserved amino acids present in all serine/threonine kinases are in bold type and indicated by an asterisk. The amino acids showing identity with the cdc2 PSTAIRE motif (Lee and Nurse, 1987) are underlined. The regions of MO15 mRNA complementary to the antisense oligonucleotides 15-1 and 15-2 are bracketed [---15-1---] and [---15-2---].

expression of MO15, total RNA was extracted from oocytes and early embryos at various stages of development. Figure 3a, lanes 2–11 shows that MO15 transcripts accumulate during oogenesis and, following fertilization, persist through early cleavage until they are mostly degraded following MBT. MO15 mRNA is just detectable in gastrula RNA on longer exposure of the blot. In contrast, cytoskeletal actin transcripts which were hybridized on the same blot (using trace amounts of probe) to control for recovery and non-specific degradation, are shown to be still present in total RNA extracted from gastrulae (see Figure 3a, lane 11). The loss of most MO15 transcripts following MBT is therefore a specific effect. No MO15 mRNA could be detected in later stages of embryo (up to swimming tadpole) nor in adult liver, spleen, testes or skeletal muscle (data not shown). An apparent shift in mobility of the transcripts present in mature oocytes, eggs and embyros, compared with stage VI oocytes, suggested that the mRNA is de-adenylated during oocyte maturation, as are many other maternal transcripts (Dworkin and Dworkin-Rastl, 1985; Hyman and Wormington, 1988). This was confirmed by selecting for poly(A)$^+$ RNA using oligo(dT) cellulose. Figure 3b shows the MO15 mRNA present in unbound and bound oligo(dT) fractions prepared from the total RNA samples shown in Figure 3a. MO15 transcripts are found in both fractions from oocytes up to stage VI, but following maturation are exclusively in the unbound poly(A)$^-$ fraction. Cytoskeletal actin transcripts, which were hybridized on the same blot, are also de-adenylated during maturation (at a slower rate than MO15 transcripts), but adenylated transcripts reappear in gastrulae (see Figure 3b).

Altering the expression of genes by means of specific antisense oligonucleotide or synthetic mRNA injection can potentially provide information about their function. Antisense oligonucleotide-mediated degradation of endogenous oocyte transcripts has been demonstrated for several genes (Dash *et al.*, 1987; Shuttleworth and Colman, 1988), although complete removal of some transcripts has proved difficult to achieve. The single oocyte nucleus does not have the capacity to replace pg quantities of mRNA within the time-course of such experiments (Horrell *et al.*, 1987), therefore the effects of oligo injection persist long after the oligo has been degraded. We have synthesized two antisense

```
          1                                                  50
  MO15    MEGIAARGVD VRSRAKQYEK LDFLGEGQFA TVYKARDKNT DRIVAIKKIK
CDC2hs    ME........ ......D-T- IEKI---TYG V---G-H-T- GQV--M---R
PSK-J3
  KSS1        M--TIT FDIPS.--KL V-LI---AYG --CS-IH-PS GIK------.
 KIN28             MKVNME-T- EKKV---TY- V--LGCQHS- G-KI---E--
 PHO85             MSSSS-FKQ -EK--N-TY- ----GLN-T- GVY--L-EV-

          51                                                 100
  MO15    LGHRAEANDG INRTALREIK LLQEL.SHPN IIGLLD.... .AFGHKSNIS
CDC2hs    -E...SEEE- VPS--I---S --K--.R--- -VS-Q-.... .VLMQD-RLY
PSK-J3                                                     KVT
  KSS1    ...QPFSKKL FVTRTI---- --RYFHE-E- --SI--KVRP VSIDKLNAVY
 KIN28    TS...-FK-- LDMS-I--V- Y---MQ.--- V-E-I-.... .I-MAYD-LN
 PHO85    -....DSEE- TPS--I---S -MK--.K-E- -VR-Y-.... .VIHTENKLT

          101                                                150
  MO15    LVFDFMETDL EVII..KD.. TSL.VLTPAH IKSYMLMTLQ .GLEYLHHLW
CDC2hs    -I-E-LSM-- KKYL...-SI PPGQYMDSSL V---LYQI-- .-IVFC-SRR
PSK-J3    ---EHVDQ-- RTYL.D-APP PG-....--E TIKDLMRQFL R--DF--ANC
  KSS1    --EEL----- QKV-..NNQN SGFST-SDD- VQYFTYQI-R .A-KSI-SAQ
 KIN28    --LE-LP--- --V-..--KS I...LF---D --AW-----R GVYHC.-RNF
 PHO85    ---E--DN-- KKYMDSRTVA NTPRG-ELNL VKYFQWQL-- G--AFC-ENK

          151                                                200
  MO15    ILHRDLKPNN LLLDENGVLK LADFGLAKSF GSPNR..... ..IYTHQVVT
CDC2hs    V-------Q- --I-DK-TI- -------RA- -I-I-..... ..V---E---
PSK-J3    --------E- --VTSG-TV- -------RIY .SYQW..... ..AL-PV---
  KSS1    VI---I--S- ---NS-CD-- VC-----RCL A-SSDSRETL VGFM-EY-A-
 KIN28    ---------- --FSPD-QI- V------RAI PA-H..... .EIL-SN---
 PHO85    --------Q- --INKR-Q-- -G-----RA- -IP..... VNTFSSE---

          201                                                250
  MO15    RWYRSPELLF GARMYGVGVD MWAVGCILAE LLLRVPFLPG DSDLDQLTRI
CDC2hs    L------VLL -SAR-STP-- I-SI-T-F-- -ATKK-LFH- --EI---F--
PSK-J3    L---A--V-L Q
  KSS1    ----A--IML TFQE-TTAM- I-SC------ MVSGK-LF-- RDYHH--WL-
 KIN28    ----A----- --KH-TSAI- I-S--V-F-- -M--I-Y--- QN-V--MEVT
 PHO85    L---A-DV-M -S-T-STSI- I-SC------ MITGK-LF-- TN-EE--KL-

          251                                                300
  MO15    FETLGTPTEE QWPGMSS..L PDYV.AFKSF PGTPLHLIFI A...AG..DD
CDC2hs    -RA----NN- V--EVE-..- Q--KNT-PKW KPGS-..... -SHVKNLDEN
PSK-J3
  KSS1    L-V----SF- DFNQIK-KRA KE-IANLPMR -PL-WETVW. ..SKTDLNP-
 KIN28    -RA-----DR D--EV--FMT YNKLQIYPPP SRDE-RKR-- -...-S..EY
 PHO85    -DIM---N-S L--SVTK..- -K-NPNIQQR -PRD-RQVLQ PHTHEPLDGN

          301                                                350
  MO15    LLELLQGLFT FNPCARCTAS QALRKRYFSN RPAPTPGNLL PRPNCSIEAL
CDC2hs    GLD--SKMLI YD-AK-ISGK M--NHP--ND LDNQIKKM
PSK-J3
  KSS1    MID--DKMLQ ---DK-IS-A E---HP-LAM YHD-SDEPEY -PL-LDD-FW
 KIN28    A-DFMC-ML- M--QK-W--V -C-ESD--KE L-P-SDPSSI KIR-
 PHO85    -MDF-H--LQ L--DM-LS-K ---HHPW-AE YYHHAS

          351                                384
  MO15    KEQQ.....N LNLGIKRKRT EGMDQKDIAK KLSF
CDC2hs
PSK-J3
  KSS1    -LDNKIMRPE FFFEVPIEML KD-LYDELM- TME
 KIN28
 PHO85
```

Fig. 2. Comparison of the deduced amino acid sequence of clone MO15 with that of CDC2Hs (human cdc2; Lee and Nurse, 1987), PSK-J3 (human cdc2-related clone from HeLa cells; Hanks, 1987), *KSS1*, *KIN28* and *PHO85* (*S.cerevisiae* cdc2-related genes; Courchesne *et al.*, 1989; Simon *et al.*, 1986; Toh-e *et al.*, 1988). Alignments were created using University of Wisconsin Genetics Computer Group DNA analysis software.

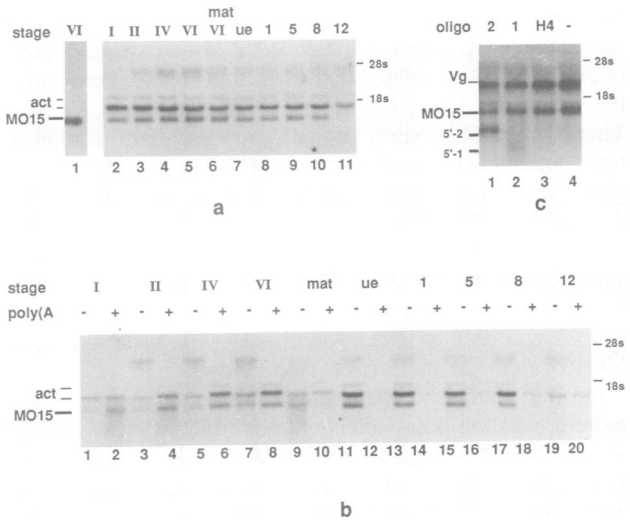

Fig. 3. Northern blot analysis of MO15 mRNA. (**a**) Total RNA, extracted from lanes 1–5, stage VI, I, II, IV and VI oocytes (VI, I–VI); lane 6, mature stage VI oocytes (mat VI); lane 7, unfertilized eggs (ue); lanes 8–11, stage 1, 5, 8 and 12 embryos (1, 5, 8, 12), was electrophoresed on denaturing formaldehyde agarose gels and blotted onto nitrocellulose membrane. Lanes 2–11 of the blot were hybridized with both MO15 and cytoskeletal actin ^{32}P-labelled RNA probes. Lane 1 was hybridized with MO15 probe alone. The positions of the 1.4 kb MO15 transcripts (MO15), cytoskeletal actin transcripts (act) and rRNA (18S and 28S) are indicated. (**b**) The total RNA used in (a) was fractionated using oligo(dT) cellulose and the unbound (−) and bound fractions (+) were fractionated, blotted and probed as in (a). (**c**) Total RNA was extracted from oocytes, 15 h after injection of lane 1, antisense oligo 15-2; lane 2, antisense oligo 15-1; lane 3, antisense oligo H4; lane 4, uninjected oocytes then electrophoresed and blotted as in (a). The blot was probed with both MO15 and Vgl ^{32}P-labelled RNA probes. The positions of full-length MO15 transcripts (MO15), the MO15 5′-cleavage fragments from oligo 15-1 injected oocytes (5′-1) and oligo 15-2 injected oocytes (5′-2), Vg1 transcripts (Vg) and rRNA (18S and 28S) are shown.

MO15 oligos (oligo 15-1 and 15-2, see Figure 1) and tested their effect on MO15 transcripts. Figure 3c shows total RNA extracted from oocytes 15 h after injection with either oligo 15-1, oligo 15-2, or a control oligo (antisense histone H4) and uninjected oocytes. Both antisense MO15 oligos caused specific degradation of MO15 transcripts, as assessed by reduced amounts of full-length mRNA and also the appearance of 5′-cleavage fragments of the predicted size. The 3′-fragment generated by RNase H cleavage of mRNA *in vivo* has previously been shown to be unstable (Shuttleworth and Colman, 1988). Densitometric scans of the autoradiograph indicated MO15 mRNA depletion of only 12% in oligo 15-1-injected oocytes, and 54% in oligo 15-2-injected oocytes, in this experiment. The control H4 oligo has no effect on MO15 transcripts, and Vg1 mRNA which was probed on the same blot to control for non-specific degradation, was unaffected by injection of any of the oligos. In other experiments, such as that shown in Figure 5c, 65% of MO15 mRNA was depleted by oligo 15-1 injection and 78% by oligo 15-2 injection, indicating that the efficacy of oligo injection can vary between experiments, however mRNA cleavage is always seen to occur.

Expression of MO15 protein in vitro and in vivo

The complete 1.35 kb MO15 insert was sub-cloned into the transcription vector SP64T (Krieg and Melton, 1984) and used to prepare capped synthetic mRNA. The translation of this mRNA was checked in both rabbit reticulocyte lysate and *Xenopus* egg cell-free systems, and by microinjection into stage VI *Xenopus* oocytes. In all three-cases a doublet of proteins was translated with sizes of ~40 kd, confirming the predicted size(s) for MO15 protein (data not shown). This doublet of proteins probably represents utilization of both potential start codons of MO15. The presence of the globin mRNA 5′-untranslated leader in the synthetic mRNA derived from SP64T may be responsible for this, since the endogenous MO15 protein found in oocytes migrates as a single band corresponding to the smaller band of this doublet (see below and Figure 4a, lanes 1 and 2). This synthetic mRNA was used to investigate the possible involvement of MO15 in oocyte maturation (see below).

In order to analyse the expression of endogenous MO15 protein, a rabbit polyclonal antibody (15SSRV) was raised to a 14.8 kd fusion protein expressed in *Escherichia coli*. The protein contains the N-terminal 11 amino acids from bacteriophage T7 gene 10 followed by the C-terminal 122 amino acids of MO15. This region of MO15 protein shows minimum homology to $p34^{cdc2}$ and other protein kinases. Both proteins of the doublet produced by translating synthetic MO15 mRNA in oocytes are immunoprecipitated efficiently by immune antiserum (20−30% of input labelled protein) but not preimmune serum from the same rabbit (data not shown). In addition, *Xenopus* $p34^{cdc2}$ translated from synthetic *cdc2* mRNA in oocytes is not immunoprecipitated by 15SSRV (data not shown).

Immunoprecipitation of ^{35}S-labelled proteins extracted from immature stage VI oocytes produced a 40 kd protein ($p40^{MO15}$) which is not precipitated by preimmune serum (see Figure 4a, lanes 2 and 5). Preincubation of the immune serum with MO15 bacterial fusion protein blocked $p40^{MO15}$ precipitation (see Figure 4b, lanes 1 and 2), while microinjection of oocytes with each of the two different antisense MO15 oligonucleotides (15-1 and 15-2), before labelling, reduced the amount of $p40^{MO15}$ made by the oocytes (see

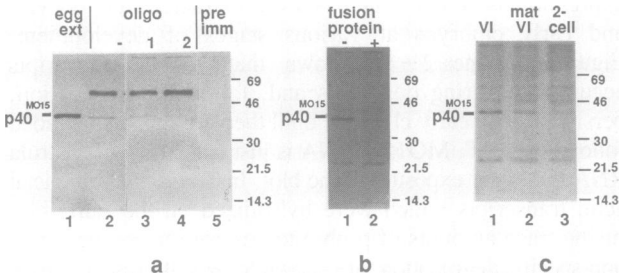

Fig. 4. (a) Immunoprecipitation of [^{35}S]methionine-labelled $p40^{MO15}$ protein from lane 1, a *Xenopus* egg cell-free extract containing synthetic MO15 mRNA; lane 2, uninjected stage VI oocytes; lane 3, antisense oligo 15-1 injected oocytes; lane 4, antisense oligo 15-2 injected oocytes. Oocytes were labelled for 6 h, 15 h after injection of oligos, homogenized then immunoprecipitated using antiserum 15SSRV. Lane 5, [^{35}S]methionine-labelled protein from uninjected stage VI oocytes, immunoprecipitated using preimmune serum. The positions of molecular weight markers are shown.
(**b**) Immunoprecipitation of [^{35}S]methionine-labelled $p40^{MO15}$ protein from stage VI oocytes using lane 1, antiserum 15SSRV (−); lane 2, antiserum 15SSRV which was preincubated for 30 min at 4°C with MO15 fusion protein (+). (**c**) Immunoprecipitation of [^{35}S]-methionine-labelled $p40^{MO15}$ protein from lane 1, stage VI oocytes (VI); lane 2, mature stage VI oocytes (mat); lane 3, two-cell embryos (2-cell). Oocytes and embryos were injected with [^{35}S]methionine, homogenized and equal TCA-precipitable counts were immunoprecipitated with antiserum 15SSRV. The positions of protein molecular weight markers are shown.

Figure 4a, lanes 3 and 4), proving that this protein is derived from MO15 mRNA. Densitometry indicated that oligos 15-1 and 15-2 reduced $p40^{MO15}$ synthesis by 50% and 80% respectively, in this experiment. Total protein synthesis was not affected, quantitatively or qualitatively in oligo-injected oocytes (data not shown), and incorporation of ^{35}S into a contaminating 58 kd protein present in the immunoprecipitates is likewise not affected (see Figure 4a), sugesting that injection of these oligos does not result in non-specific effects on the synthesis of proteins other than $p40^{MO15}$. $p40^{MO15}$ comigrated with the smaller protein of the doublet translated in an mRNA dependent *Xenopus* egg cell-free extract programmed with synthetic MO15 mRNA (see Figure 4a, lanes 1 and 2), suggesting that the second AUG is used preferentially during translation of endogenous MO15 mRNA. The contaminating 58 kd protein, which is not precipitated by preimmune serum (Figure 4a, lanes 2−5) is significantly reduced when more stringent washing procedures are used (see Figure 4b and c). Moreover, preincubation of immune serum with MO15 bacterial fusion protein had no effect on its precipitation (data not shown), indicating that this contaminant is not closely related to MO15.

The adenylation status of an mRNA has generally been considered to reflect its translational status; de-adenylation being correlated with down-regulation and release of the mRNA from polysomes (Hyman and Wormington, 1988). To confirm that this was the case with MO15 mRNA in maturing oocytes and embryos, [^{35}S]methionine was injected into non-mature and mature stage VI oocytes and fertilized eggs. The proteins labelled in a 2 h period following injection were analysed by immunoprecipitating equal TCA-precipitable counts using the antibody 15SSRV. During the short labelling period, immature oocytes synthesized detectable amounts of $p40^{MO15}$, mature oocytes synthesized less, and in fertilized eggs (by then two-cell embryos) the 40 kd protein was only just detectable (see

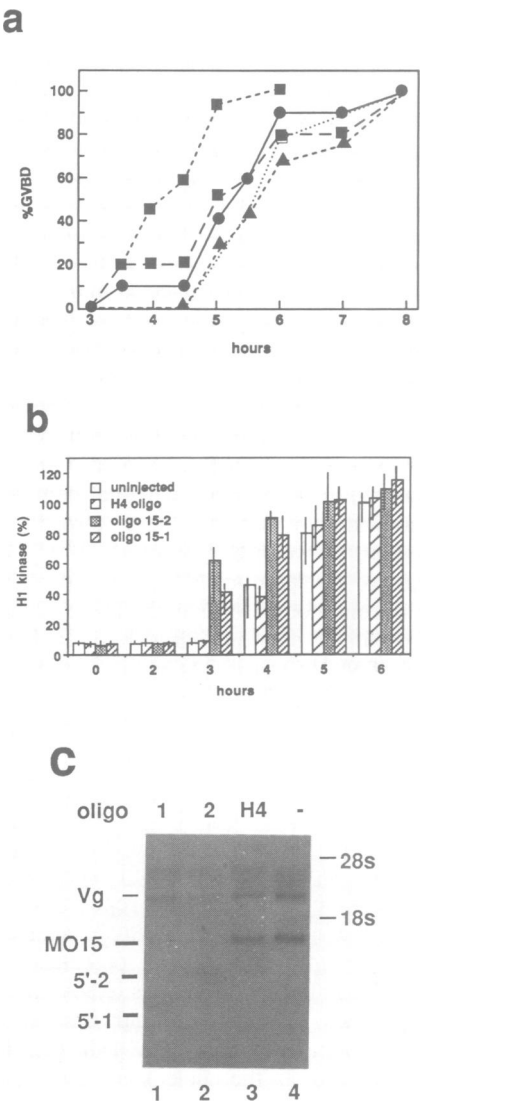

Fig. 5. (**a**) The kinetics of progesterone induced GVBD in oligo 15-1-injected oocytes, ■- - -■; oligo *cdc2*-injected oocytes, □· · · ·□; MO15 mRNA-injected oocytes, ▲- - -▲; uninjected oocytes, ●—●. Oocytes from a primed frog were injected and incubated for 15 h, then treated with progesterone. Some of the oligo 15-1-injected oocytes were injected with MO15 mRNA 2 h before addition of progesterone (oligo 15-1 followed by MO15 mRNA-injected oocytes, ■— —■). The number of oocytes with an animal pole white spot was scored at various times up to 8 h after addition of progesterone, by which time 100% of uninjected oocytes had undergone GVBD. (**b**) The rate of H1 kinase activation in oligo 15-1-injected, oligo 15-2-injected, oligo H4-injected and uninjected oocytes. Oocytes from a primed frog were injected, incubated for 15 h, then treated with progesterone. Four oocytes were removed and individually homogenized at various times after the addition of progesterone, up to 6 h. ($GVBD_{50}$ for uninjected oocytes occurred at 5 h). The H1 kinase activity present in the homogenates was determined by an *in vitro* assay using [γ-^{32}P]ATP and histone H1. The relative amounts of H1 kinase, expressed as a percentage of that found in the uninjected control oocytes at 6 h, was plotted as a mean value (with dispersion) for each sample of four oocytes. (**c**) Total RNA was extracted from oligo-injected and uninjected oocytes in the same experiment as that shown in (b). Lane 1, oligo 15-1-injected oocytes; lane 2, oligo 15-2-injected oocytes; lane 3, control oligo H4-injected oocytes; lane 4, uninjected oocytes. The RNA was analysed by electrophoresis and blotting as described in Figure 3. The blot was probed with both MO15 and Vgl ^{32}P-labelled RNA probes. The positions of full-length MO15 transcripts (MO15), the MO15 5′-cleavage fragments from oligo 15-1 injected oocytes (5′-1) and oligo 15-2 injected oocytes (5′-2), Vg1 transcripts (Vg) and rRNA (18S and 28S) are shown.

Figure 4c). This confirms that a reduction in $p40^{MO15}$ translation accompanies the de-adenylation of MO15 transcripts during maturation, and demonstrates that expression of $p40^{MO15}$ is largely restricted to immature oocytes. In fact, similar amounts of $p40^{MO15}$ are synthesized by stage IV and stage VI oocytes (data not shown), suggesting that expression occurs throughout vitellogenesis.

The effects of altered $p40^{MO15}$ expression on oocyte maturation

To investigate whether or not reduced synthesis of $p40^{MO15}$ has any effect on progesterone induced oocyte maturation, the kinetics of GVBD (assessed by the appearance of the animal pole white spot) were compared in oligo 15-1 injected, control (antisense cdc2) oligo-injected and uninjected oocytes. 15 h after injection the oocytes were treated with progesterone. Figure 5a shows the proportion of oocytes undergoing GVBD, expressed as a percentage of the total number of responding oocytes for each treatment, at various times after addition of progesterone. Oligo 15-1 injection advanced the time at which 50% of oocytes had matured ($GVBD_{50}$) by ~1.25 h when compared with uninjected oocytes. The control *cdc2* antisense oligo injection did not affect the rate of maturation. In other experiments, an antisense histone H4 oligo and an oligo complementary to the T7 RNA polymerase promoter likewise had no effect on the rate of maturation (data not shown).

Injected oligos are reported to be rapidly degraded in oocytes (Woolf *et al.*, 1990), and this was confirmed by injecting ^{32}P-labelled MO15 mRNA into oocytes 15 h after injection of oligo 15-1, extracting RNA after a further 8 h and comparing the amount of labelled RNA with that extracted from non-oligo-injected oocytes. No differences in stability of the RNA were detected (data not shown). When synthetic, capped MO15 mRNA was injected into oligo 15-1 injected oocytes, 2 h before the addition of progesterone, the effect on rate of GVBD was reversed and the oocytes matured with the same kinetics as uninjected oocytes (see Figure 5a). This provides further proof of the specificity of the effect of oligo 15-1 injection. Surprisingly, injection of synthetic MO15 mRNA into control oocytes did not delay the rate of GVBD (see Figure 5a). Injection of synthetic mRNA *per se* does not affect the kinetics of GVBD, since in other experiments oocytes injected with synthetic prolactin mRNA likewise matured with the same kinetics as uninjected oocytes (data not shown).

The appearance of a white spot at the animal pole is arguably a subjective assessment of maturation, and not always indicative of true maturation. An alternative method of determining the onset of GVBD is to assay for the appearance of M-phase specific H1 kinase activity. Although this activity may not be attributable entirely to one enzyme (e.g. $p34^{cdc2}$), it does serve as an indicator for the onset of M-phase. Figure 5b shows the relative levels of H1 kinase activity present in oligo 15-1, oligo 15-2, control (antisense histone H4) oligo and uninjected oocytes at various times after addition of progesterone. Both antisense MO15 oligos, but not the control H4 oligo, accelerated the rate at which H1 kinase activity appeared following addition of progesterone, independently confirming the results for oligo 15-1 on rate of GVBD (Figure 5a), and demonstrating that oligo 15-2 has a similar effect on maturation. This provides further evidence for the specificity of the effect of antisense oligo injection. Figure 5c shows a Northern blot analysis

of total RNA, extracted from oligonucleotide-injected and uninjected oocytes in the same experiment as that shown in Figure 5b, indicating that on this occasion both oligo 15-1 and oligo 15-2 caused effective degradation of MO15 mRNA. Densitometric scans indicate 65% removal by oligo 15-1 and 78% removal by oligo 15-2. The relative effects of oligos 15-1 and 15-2 on H1 kinase activation have been found to vary, probably as a result of the experimental variation indicated by mRNA analysis (cf. Figures 3c and 5c). Another potential complication may also affect interpretation; the 5′-cleaved fragment of MO15 mRNA in oligo 15-2-injected oocytes (see Figures 3c and 5c) potentially codes for a 28 kd truncated MO15 polypeptide. This polypeptide would not carry epitopes recognized by the C-terminal antibody 15SSRV, therefore its existence has not been confirmed. Such a truncated MO15 polypeptide may influence H1 kinase activation in oligo 15-2-injected oocytes if it retained some activity.

Discussion

A small but growing number of cdc2-related kinases have been reported, these include *KIN28*, *PHO85*, *KSS1* and *FUS3* all of which have been identified in *S.cerevisiae* by genetic studies (Courchesne *et al.*, 1989; Toh-e *et al.*, 1988; Simon *et al.*, 1986; Elion *et al.*, 1990). The isolation of PSK-J3 from human HeLa cells provides an indication that such kinases are also expressed in vertebrate cells (Hanks, 1987). The sequence of $p40^{MO15}$ shows a similar degree of homology to $p34^{cdc2}$ as do these other cdc2-related proteins, and clearly it is not a *Xenopus* cdc2 homologue. Not surprisingly, expression of $p40^{MO15}$ failed to rescue function in *S.pombe* mutant CDC2.33 cells (Shuttleworth, MacNeil and Nurse; unpublished data). Some of the amino acid identity between $p40^{MO15}$ and $p34^{cdc2}$ is due to the conserved nucleotide binding and catalytic domains found in all serine/threonine kinases, but the presence of a partially represented PSTAIRE motif and other cdc2 specific residues suggest that MO15 is more closely related to cdc2 than to other known kinases. We have recently identified two more distinct cdc2-related genes that are expressed in *Xenopus* oocytes, using polymerase chain reaction (PCR) amplification from maternal RNA, and these are currently being characterized. Sequence homology unfortunately does not allow prediction of function; the evolutionary distance between yeast and vertebrates would permit considerable functional divergence, and as yet no function has been described for PSK-J3. However, the yeast genes *FUS3* and *KSS1* have both been implicated in the events which regulate cell-cycle progression in response to mating pheromone in *S.cerevisiae*; *FUS3* mediating G_1 arrest and *KSS1* mediating release from G_1 arrest (Elion *et al.*, 1990; Courchesne *et al.*, 1989). This indicates that at least some cdc2-related genes, although only distantly related to cdc2, have cell-cycle-related functions. The results presented here for MO15, showing an involvement with G_2/M arrest prior to meiosis, suggest that it too may have a cell-cycle regulatory function analogous to *FUS3* which causes G_1 arrest prior to conjugation.

The effects of reduced $p40^{MO15}$ synthesis on maturation are specific, as far as can be determined: (i) injection of antisense MO15 oligos had no detectable effect on transcripts other than MO15 nor on the translation of proteins other than $p40^{MO15}$, (ii) two independent antisense MO15 oligos (and no other oligos tested) accelerated the rate of H1 kinase activation and maturation, and (iii) the effect could be reversed by the injection of synthetic MO15 mRNA. The failure of synthetic MO15 mRNA injection alone to delay GVBD is unexpected, but may be explained by the presence or absence of other rate-limiting components in the oocyte which may be required for $p40^{MO15}$ activity. In any case, the results of such overexpression experiments should be interpreted with some caution. For example, expression of $p34^{cdc2}$, from synthetic mRNA injected into oocytes, results in the accumulation of large quantities of labelled protein, only a small proportion of which is phosphorylated and binds to $p13^{suc}$ beads (J.Shuttleworth, unpublished observation). The ability of the oocyte to utilize such 'exogenous' translation products appropriately cannot be predicted. Similarly, injection of synthetic cyclin B mRNA in *Xenopus* oocytes induces maturation (Ruderman, personal communication), yet it is now known that the oocyte already contains a significant store of cyclin B (Roy *et al.*, 1990); clearly the accumulation of synthetic mRNA-derived protein does not mimic the normal course of events. The data on $p40^{MO15}$ has been interpreted on a qualitative basis; although values for removal of MO15 transcripts and reductions in $p40^{MO15}$ synthesis are noted, until the steady-state levels of $p40^{MO15}$ and the nature of its active form are known no quantitative correlations can be drawn. This information is currently being sought. However, the low abundance of MO15 mRNA (1−5 pg/oocyte), might suggest (but does not prove) that $p40^{MO15}$ is present at low levels relative to $p34^{cdc2}$ for example. This comparison may be of interest since the way in which cdc2 is thought of as a key regulator is now being reappraised in the light of: (i) the high abundance of $p34^{cdc2}$ protein, (ii) the growing list of potential 'structural' substrates such as histone H1; lamins, RNA polymerase II, nucleolar proteins transcription and translation factors (reviewed by Moreno and Nurse, 1990) and (iii) the fact that $p34^{cdc2}$ is found in both the nucleus and cytoplasm, and is associated with structural elements of the mitotic cell (Bailley *et al.*, 1989). The role of $p34^{cdc2}$ may well be that of a 'workhorse' directly involved in reorganizing cellular activities and architecture for mitosis (Peter *et al.*, 1990) rather than a control element responsible for initiating a regulatory cascade of events.

The precise function of $p40^{MO15}$ cannot be determined from these experiments, and speculation is limited by the lack of any certain knowledge of the nature of the regulatory events involved in arrest and resumption of meiosis. Conceivably, arrest could be maintained either by the presence of a negative regulator (e.g. the hypothetical MpP) or the requirement to accumulate a positive regulator (MPF), or both. In these models $p40^{MO15}$ could function directly or indirectly in maintaining negative regulation or inhibiting the accumulation of the positive regulator. The lag between progesterone treatment and onset of GVBD can vary considerably between oocytes removed from different frogs, in both the same and different laboratories (LaMarca *et al.*, 1985; Smith, 1989); GVBD can take <5 h or >10 h, and in extreme cases some oocytes mature spontaneously in the absence of progesterone while others fail to respond at all. This probably reflects differing environmental conditions or the physiological status of the female. Injection of the frog with gonadotrophin before removal of oocytes improves synchrony of the response and reduces the time taken to undergo GVBD (Reynhout *et al.*, 1975; LaMarca *et al.*,

1985). In contrast to oocytes responding to progesterone, oocytes induced to mature by injection of active MPF rapidly and consistently undergo GVBD in <3 h. The variability must therefore be associated with events preceding MPF activation, either with release of the block to meiosis or the rate at which early/intermediate events can be performed (Smith, 1989). These observations suggest that the mechanisms of meiotic arrest may be complex, involving several components acting sequentially, in which case some oocytes may be arrested at different points. Alternatively the levels and/or activity of rate limiting components may vary between unstimulated frogs.

Since $p40^{MO15}$ expression is restricted to non-mature oocytes, this would suggest that its function is indeed associated with processes occurring before or during maturation. This is consistent with $p40^{MO15}$ either maintaining arrest of meiosis or negatively regulating the events leading to MPF activation, if indeed these are distinct processes. $p40^{MO15}$ does not appear to be involved in the events which follow MPF activation; whereas antisense oligo 15-1 injection has a positive effect on progesterone induced H1 kinase activation (see above), no effect on the rate of maturation induced by MPF injection was observed (unpublished observation). In addition, the shortened lag phase in oligo 15-1 and oligo 15-2 injected oocytes does not reflect bypass of early events requiring protein synthesis, since these oocytes do not mature in the presence of cycloheximide (unpublished observation). Antisense oligo 15-1 or 15-2 injected oocytes do not mature in the absence of progesterone, but until a more effective removal of MO15 transcripts (and newly synthesized $p40^{MO15}$) can be achieved this observation is not very informative. Reduced $p40^{MO15}$ synthesis in oligo-injected oocytes must however lead to reduced levels of functional $p40^{MO15}$ protein, either by allowing depletion of steady-state levels or limiting the amount of newly synthesized protein available. This could specifically alter the balance of phosphorylation/dephosphorylation events occurring in the oocyte and accelerate the rate of progesterone induced GVBD in a way similar to that induced non-specifically by, for example, injection of $pp60^{v\text{-}src}$ (Spivak *et al.*, 1984). A more detailed understanding of how $p40^{MO15}$ functions will hopefully emerge with our continuing studies on its synthesis, steady-state levels, phosphorylation and activity.

Materials and methods

Oocytes and eggs

Stage VI oocytes were obtained from frogs and maintained in modified Barth's medium as described by Colman (1984a). In some experiments (where indicated) the frog was primed 2 days before isolation of oocytes by injection of 100 U of FSH (Folligon, Intervet Laboratories Ltd). Oocytes were matured by incubation in 10 μg/ml progesterone. Unfertilized eggs were obtained as described by Gurdon (1977).

Oocyte injection

20 nl aliquots of solutions containing either 2 mg/ml oligonucleotide or 1 mg/ml synthetic mRNA, each in water, were injected into the cytoplasm of oocytes as described by Colman (1984b). Oligonucleotide and RNA-injected oocytes were usually incubated at 20°C for 15 h before use, unless otherwise stated.

Synthetic oligonucleotides

Synthetic oligonucleotides for use as probes or antisense reagents were synthesized by the solid-phase phosphoramidite method, using an Applied Biosystems model 380A DNA synthesizer. Fully deprotected oligonucleotides were purified using Millipore Sep-pak C18 columns and dissolved in water. The sequences of the oligos were as follows:

redundant probe CDC2.4; TT(C/T)TG(A/C/T)GG(C/T)TT(C/T)AA(A/G)TC
antisense oligo 15-1; TAAATCTCTGTGGAGGA.
antisense oligo 15-2; GCTGGACATGCCCGGCC.

The relative positions of the antisense oligos in MO15 mRNA are indicated in Figure 1. The antisense H4 oligo is described in Shuttleworth and Colman (1988). The antisense *cdc2* oligo was designed to a sequence present in *Xenopus cdc2* cDNA clone provided by John Newport (unpublished data).

Construction and screening of cDNA libraries

The library constructed from *Sau*3A digested *Xenopus* egg cDNA in M13mp9 was provided by Tim Hunt and Jeremy Minshull. The library was screened using oligo probe CDC2.4 which was end-labelled using [γ-^{32}P]ATP and polynucleotide kinase. Hybridization was performed in 6 × SSPE, 5 × Denhardt's solution, 100 μg/ml yeast tRNA, 5 ng/ml of labelled oligo at 37° for 18 h. Filters were washed in 6 × SSPE, 0.1% SDS at 37°C. The full-length *Xenopus* cDNA library was constructed from oocyte RNA in λ-ZAP. Total RNA prepared from mature oocytes, as described by Kressman *et al.* (1978) was fractionated on oligo(dT) cellulose and the poly(A)$^+$ RNA used to synthesize cDNA using standard procedures. *Eco*RI linkers were added and the cDNA was cloned into *Eco*RI digested phage λ-Zap (Promega). The library was screened with prime-cut probe prepared from M13 clone 4.5 single-stranded DNA using [α-^{32}P]-dGTP and Klenow fragment. Hybridization was performed in 5 × SSPE, 50% formamide, 5 × Denhardt's solution, 100 μg/ml yeast tRNA and 0.1% SDS at 42°C for 18 h. Filters were washed in 0.2 × SSPE, 0.1% SDS at 60°C.

DNA sequencing

Suitable restriction fragments generated with *Bam*HI, *Eco*RI, *Kpn*I, *Ssp*I, *Taq*I and *Ava*I from clone MO15 were subcloned into M13mp18 or mp19 and sequenced using standard dideoxynucleotide chain-termination techniques with bacteriophage T7 DNA polymerase and [α-^{35}S]dATP.S.

RNA synthesis

For preparing synthetic, capped MO15 and cdc2 mRNA, the *Eco*RI inserts from each clone were subcloned into the SP6 transcription vector SP64T (Krieg and Melton, 1984). Capped transcripts were synthesized as described by Krieg and Melton (1987). The *Xenopus cdc2* cDNA was provided by John Newport.

RNA analysis

Total RNA was extracted from oocytes and embryos and analysed by Northern blotting of denaturing formaldehyde agarose gels as described by Shuttleworth and Colman (1988). MO15, Vg1 and cytoskeletal actin [α-^{32}P]UTP-labelled synthetic RNA probes were prepared as described by Krieg and Melton (1987). The Vg1 cDNA clone is described by Dale *et al.* (1989) and the cytoskeletal actin cDNA was provided by Tim Mohun.

Protein analysis

[^{35}S]Methionine labelling of oocytes, homogenization, immunoprecipitation and SDS−PAGE were performed as described by Dale *et al.* (1989), except for the immunoprecipitates shown in Figure 4a, which were performed using only three washes in buffer containing 100 mM NaCl.

Histone H1 kinase assay

Individual oocytes were homogenized in 10 μl of 100 mM NaCl, 20 mM Tris−HCl pH 7.6, 1% Triton X-100, 15 mM EGTA, 80 μM Na β-glycerophosphate, 10 mM NaF, 1 mM phenylmethylsulphonylfluoride, 10 μg/ml leupeptin, 10 μM pepstatin. H1 kinase activity was assayed using 5 μl of homogenate in the *in vitro* assay described by Meijer *et al.* (1989).

Preparation of antibody

A 570 bp *Ssp*I−*Eco*RV fragment from clone MO15, containing coding region for the 122 C-terminal amino acids of $p40^{MO15}$, was subcloned into the end-filled *Bgl*I site of plasmid pET3a (Rosenberg *et al.*, 1987). The recombinant plasmid was used to transform *E.coli* BL21(DE3)pLysS then induced to express high levels of fusion protein (Studier and Moffatt, 1986). The 14 kd fusion protein, consisting of 11 amino acids from the bacteriophage T7 gene 10 protein followed by the C-terminal polypeptide coded by MO15, formed insoluble inclusion bodies which were partially purified by centrifugation of a freeze−thaw lysate from the bacterial culture in the presence of Triton X-100 and EDTA. The protein was solubilized in 2% SDS, electrophoresed on an SDS−polyacrylamide gel, and the band cut out then emulsified in Freund's complete adjuvant. Rabbits were immunized

with ~100 μg of protein then boosted at 2 week intervals with 50 μg of protein prepared in Freund's incomplete adjuvant. The antiserum collected from rabbits was stored in aliquots at −20° and characterized as described in Results.

Acknowledgements

We thank Tim Hunt and Jeremy Minshull for providing their M13 library and encouragement; Paul Nurse and Stewart MacNeill for *S.pombe* transfections; John Newport for making available his *Xenopus cdc2* clone; Jim Maller for providing purified MPF; Glenn Matthews for translating MO15 in his mRNA dependent *Xenopus* egg extract. This work was funded by the Wellcome Trust.

References

Bailly,E., Doree,M., Nurse,P. and Bornens,M. (1989) *EMBO J.*, **8**, 3985−3995.
Booher,R. and Beach,D. (1987) *EMBO J.*, **6**, 3441−3447.
Boyer,J., Asselin,J., Belle,R. and Ozon,R. (1986) *Dev. Biol.*, **113**, 420−428.
Colman,A. (1984a) In Hames,B.D. and Higgins,S.J. (eds), *Transcription and Translation: A Practical Approach*. IRL Press, Oxford, pp. 271−300.
Colman,A. (1984b) In Hames,B.D. and Higgins,S.J. (eds), *Transcription and Translation: A Practical Approach*. IRL Press, Oxford, pp. 49−69.
Courchesne,W.E, Kunisawa,R. and Thorner,J. (1989) *Cell*, **58**, 1107−1119.
Cyert,M.S. and Kirschner,M.W. (1988) *Cell*, **53**, 185−195.
Dale,L., Matthews,G., Tabe,L. and Colman,A. (1989) *EMBO J.*, **8**, 1057−1065.
Dash,P., Lotan,I., Knapp,M., Kandel,E. and Goelet,P. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 7896−7900.
Davis,F.M., Tsao,T.Y., Fowler,S.K. and Rao,P.N. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 2926−2930.
Draetta,G. and Beach,D. (1988) *Cell*, **54**, 17−26.
Dunphy,W.G. and Newport,J.W. (1989) *Cell*, **58**, 181−191.
Dunphy,W.G., Brizuela,L., Beach,D. and Newport,J. (1988) *Cell*, **54**, 423−431.
Dworkin,M.B. and Dworkin-Rastl,E. (1985) *Dev. Biol.*, **112**, 451−457.
Elion,E.A., Grisafi,P.L. and Fink,G.R. (1990) *Cell*, **60**, 649−664.
Felix,A.-M., Cohen,P. and Karsenti,E. (1990) *EMBO J.*, **9**, 675−683.
Gautier,J., Matsukawa,T., Nurse,P. and Maller,J. (1989) *Nature*, **339**, 626−629.
Gautier,J., Norbury,C., Lohka,M., Nurse,P. and Maller,J. (1988) *Cell*, **54**, 433−439.
Gautier,J., Minshull,J., Lohka,M., Glotzer,M., Hunt,T. and Maller,J. (1990) *Cell*, **60**, 487−494.
Gorris,J., Hermann,J., Hendrix,P., Ozon,R. and Merlevede,W. (1989) *FEBS Lett.*, **245**, 91−94.
Gould,K.G. and Nurse,P. (1989) *Nature*, **342**, 39−45.
Gurdon,J.B. (1977) *Proc. R. Soc. B.*, **198**, 211−247.
Hanks,S.K. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 388−392.
Hanks,S.K., Quinn,A.M. and Hunter,T. (1988) *Science*, **245**, 42−52.
Hindley,J. and Phear,G.A. (1984) *Gene*, **31**, 129−134.
Horrell,A., Shuttleworth,J. and Colman,A. (1987) *Genes Dev.*, **1**, 433−444.
Hyman,L.E. and Wormington,M.W. (1988) *Genes Dev.*, **2**, 598−605.
Hunt,T. (1989) *Curr. Opinion Cell Biol.*, **1**, 268−274.
Kozak,M. (1984) *Nucleic Acids Res.*, **12**, 857−872.
Kressman,A., Clarkson,S.G., Pirrotta,V. and Birnstiel,M.L. (1978) *Proc. Natl. Acad. Sci. USA*, **75**, 1176−1180.
Krieg,P.A. and Melton,D.A. (1984) *Nucleic Acids Res.*, **12**, 7057−7070.
Krieg,P.A. and Melton,D.A. (1987) *Methods Enzymol.*, **155**, 397−415.
LaMarca,M.J., Westphal,L.M. and Rein,D.A. (1985) *Dev. Biol.*, **109**, 32−40.
Lee,M. and Nurse,P. (1987) *Nature*, **327**, 31−35.
Lohka,M.J., Hayes,M.K. and Maller,J.L. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 3009−3013.
Maller,J. (1985) *Cell. Diff.*, **16**, 211−221.
Maller,J.L. and Krebs,E.G. (1977) *J. Biol. Chem.*, **252**, 1712−1718.
Maller,J.L. and Smith,D.S. (1985) *Dev. Biol.*, **109**, 150−156.
Meijer,L., Arion,D., Golsteyn,R., Pines,J., Brizuela,L., Hunt,T. and Beach,D. (1989) *EMBO J.*, **8**, 2275−2282.
Minshull,J., Blow,J.J. and Hunt,T. (1989) *Cell*, **56**, 947−956.
Moreno,S. and Nurse,P. (1990) *Cell*, **61**, 549−551.
Moreno,S., Hayles,J. and Nurse,P. (1989) *Cell*, **58**, 361−372.
Moria,A.O., Draetta,G., Beach,D. and Wang,J.Y.J. (1989) *Cell*, **58**, 193−203.
Murray,A.W. (1989) *Nature*, **342**, 14−15.
Murray,A.W. and Kirschner,M.W. (1989) *Nature*, **339**, 275−286.
Nurse,P. and Bisset,Y. (1981) *Nature*, **292**, 558−560.
Peter,M., Nakagawa,J., Dorée,M., Labbé,J.C. and Nigg,E.A. (1990) *Cell*, **61**, 591−602.
Pines,J. and Hunter,T. (1989) *Cell*, **58**, 833−846.
Reynhout,J.K., Taddei,C., Smith,L.D. and LaMarca,M.J. (1975) *Dev. Biol.*, **44**, 375−379.
Rosenberg,A.H., Lade,B.N., Chul,D.-S., Lin,S.-W., Dunn,J.J. and Studier,F.W. (1987) *Gene*, **56**, 124−135.
Roy,L.M., Singh,B., Gautier,J., Arlinghaus,R.B., Nordeen,S.K. and Maller,J.L. (1990) *Cell*, **61**, 625−631.
Sagata,N., Daar,I., Oskarsson,M., Showalter,S.D. and Vande Woude,G.F. (1989) *Science*, **245**, 643−645.
Shuttleworth,J. and Colman,A. (1988) *EMBO J.*, **7**, 427−434.
Simon,M., Seraphin,B. and Faye,G. (1986) *EMBO J.*, **5**, 2697−2701.
Smith,L.D. (1989) *Development*, **107**, 685−699.
Spivak,J., Erickson,R. and Maller,J. (1984) *J. Mol. Cell. Biol.*, **4**, 1631−1634.
Studier,F.W. and Moffatt,B.A. (1986) *J. Mol. Biol.*, **189**, 113−130.
Toh-e,A., Tanaka,K,. Uesono,Y. and Wickner,R.B. (1988) *Mol. Gen. Genet.*, **214**, 162−164.
Wasserman,W.J. and Masui,Y. (1975) *Exp. Cell. Res.*, **91**, 381−388.
Watanabe,N., Vande Woude,G.F., Ikawa,Y. and Sagata,N. (1989) *Nature*, **342**, 505−511.
Woolf,T.M., Jennings,C.G.B., Rebagliati,M. and Melton,D.A. (1990) *Nucleic Acids Res.*, **18**, 1763−1769.

Received on April 26, 1990; revised on June 22, 1990

Note added in proof

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number X53962 X. LAEVISMO15cDNA.