Development 121, 2047-2056 (1995)
Printed in Great Britain © The Company of Biologists Limited 1995

Two CDC25 homologues are differentially expressed during mouse development

Dineli Wickramasinghe[1], Sandy Becker[2], Mary K. Ernst[1], James L. Resnick[1], John M. Centanni[1], Lino Tessarollo[1], Laura B. Grabel[2] and Peter J. Donovan[1,*]

[1]ABL-Basic Research Program, NCI-Frederick Cancer Research and Development Center, P.O. Box B, West 7th Street at Fort Detrick, Frederick, MD 21702, USA
[2]Biology Department, Hall-Atwater Laboratories, Wesleyan University, Lawn Avenue, Middletown, CT 06459, USA

*Author for correspondence

SUMMARY

The *cdc25* gene product is a tyrosine phosphatase that acts as an initiator of M-phase in eukaryotic cell cycles by activating $p34^{cdc2}$. Here we describe the cloning and characterization of the developmental expression pattern of two mouse *cdc25* homologs. Sequence comparison of the mouse genes with human *CDC25* genes reveal that they are most likely the mouse homologs of human *CDC25A* and *CDC25B* respectively. Mouse *cdc25a*, which has not been described previously, shares 84% sequence identity with human *CDC25A* and has a highly conserved phosphatase domain characteristic of all *cdc25* genes. A glutathione-S-transferase-cdc25a fusion protein can hydrolyze para-nitrophenylphosphate confirming that cdc25a is a phosphatase. In adult mice, *cdc25a* transcripts are expressed at high levels in the testis and at lower levels in the ovary, particularly in germ cells; a pattern similar to that of *twn*, a *Drosophila* homolog of *cdc25*. Lower levels of transcript are also observed in kidney, liver, heart and muscle, a transcription pattern that partially overlaps, but is distinct from that of *cdc25b*. Similarly, in the postimplantation embryo *cdc25a* transcripts are expressed in a pattern that differs from that of *cdc25b*. *cdc25a* expression is observed in most developing embryonic organs while *cdc25b* expression is more restricted. An extended analysis of *cdc25a* and *cdc25b* expression in preimplantation embryos has also been carried out. These studies reveal that *cdc25b* transcripts are expressed in the one-cell embryo, decline at the two-cell stage and are re-expressed at the four-cell stage, following the switch from maternal to zygotic transcription which mirrors the expression of *string*, another *Drosophila* homolog of *cdc25*. In comparison, *cdc25a* is not expressed in the preimplantation embryo until the late blastocyst stage of development, correlating with the establishment of a more typical G_1 phase in the embryonic cell cycles. Both *cdc25a* and *cdc25b* transcripts are expressed at high levels in the inner cell mass and the trophectoderm, which proliferate rapidly prior to implantation. These data suggest the *cdc25* genes may have distinct roles in regulating the pattern of cell division during mouse embryogenesis and gametogenesis.

Key words: cdc25, cell cycle, mouse, preimplantation embryo, chromosomal localization, mouse

INTRODUCTION

Genetic and biochemical analysis of species as divergent as yeast and humans has facilitated the identification of many cell division cycle (cdc) genes that universally control the eukaryotic cell cycle. cdc25 plays an important role in the eukaryotic cell cycle by acting as a mitotic inducer. Initially, cdc25 was identified in *Schizosaccharomyces pombe* (*S. pombe*; Russell and Nurse, 1986) and was shown to be the tyrosine phosphatase which dephosphorylates the tyrosine-15 residue on the $p34^{cdc2}$ kinase and activates it (Gould and Nurse, 1989; Dunphy and Kumagai, 1991; Gautier et al., 1991; Millar et al., 1991a; Strausfeld et al., 1991; Lee et al., 1992). $p34^{cdc2}$, initially identified in *S. pombe*, is a highly conserved serine-threonine kinase found in all eukaryotes examined. In frogs, $p34^{cdc2}$ in concert with cyclin B forms an active kinase, termed maturation promoting factor (MPF) (Gautier et al., 1988) which was first identified in oocytes as an activity that caused resumption of meiosis (Masui and Markert, 1971; Smith and Ecker, 1971). The oscillation of MPF activity was also observed in mitotic cell cycles in *Xenopus* embryos (Gerhart et al., 1984). Wee1 and Mik1 have been characterized as the tyrosine kinases responsible for inactivation of MPF by phosphorylating $p34^{cdc2}$ on tyrosine 15 (Featherstone and Russell, 1991; Parker et al., 1991; Lundgren et al., 1991; Lee et al., 1992). Therefore, the tyrosine phosphatase cdc25 acts as a positive regulator of MPF at entry into M-phase.

Multiple *cdc25* genes have been identified in flies, humans, rats, and mice. All of the genes isolated are capable of rescuing a temperature-sensitive *cdc25* mutant of *S. pombe*, while sequence identity defines a highly conserved region common to all tyrosine phosphatases (Moreno and Nurse, 1991; Millar

and Russell, 1992). In *Drosophila*, two *cdc25* homologs, *twine* and *string* (*twn* and *stg*), have been identified (Edgar and O'Farrell, 1989; Alphey et al., 1992). *Twn* is expressed in developing male and female germ cells and both male and female mutants of *twn* are sterile, demonstrating that *twn* plays a role in gametogenesis (Alphey et al., 1992; Courtot et al., 1992; White-Cooper et al., 1993). A different role has been suggested for *stg*. Embryos mutant for *stg* arrest at the G_2 phase of the fourteenth cell cycle and do not divide or progress further in development (Edgar and O'Farrell, 1989). Maternally derived *stg* mRNA supports the first 13 cell cycles of the syncitial embryo and a switch to zygotic transcription occurs at cycle 14. These observations suggest that *stg* is required for mitosis during *Drosophila* embryogenesis (Edgar and O'Farrell, 1989, 1990). Thus, distinct functions and patterns of expression of the different *cdc25* genes are observed during *Drosophila* development.

In mouse, little is known about the functions and expression patterns of *cdc25* genes. Only two mouse genes, homologous to human *CDC25B* and *CDC25C*, have been identified (Kakizuka et al., 1992; Nargi and Woodford-Thomas, 1994). Expression studies, limited to late postimplantation stages and some adult tissues, hint at differential expression of these genes. Here, we identify a new mouse *cdc25* gene, homologous to human *CDC25A*, which we designate *cdc25a*. A highly conserved phosphatase domain is shared by cdc25a and the other mammalian cdc25 gene products described previously. Functional characterization of cdc25a demonstrates phosphatase activity in a standard para-nitrophenylphosphate (PNPP) assay. Mapping studies indicate that *cdc25a* is genetically distinct and unlinked to *cdc25b* (manuscript in preparation). To extend the expression analysis in pre- and postimplantation stages of development, in situ hybridization was carried out in addition to northern and RNase protection analysis. The side-by-side comparison of the distribution of transcripts of the two *cdc25* genes, *cdc25a* and *cdc25b*, illustrates unique expression patterns and suggest that these genes play distinct roles during mouse development.

MATERIALS AND METHODS

Cloning and sequencing *cdc25a*

A PCR-based strategy was employed to isolate mouse *cdc25* genes. Degenerate oligonucleiotides, designed to identify human *CDC25C*, were used for PCR amplification (Sadhu et al., 1990). NIH 3T3 fibroblast RNA was isolated and reverse transcribed as described by Sambrook et al. (1989) for use as a template. The PCR products were cloned into *Cla*I and *Xho*I sites in Bluescript as described previously (Sadhu et al., 1990). Two distinct, but related, clones pME10 and pME11 (200 bp and 225 bp inserts, respectively) were identified and sequenced. These two clones were used to probe oligo dT-primed lambda zap cDNA libraries of 8.5 dpc mouse embryos (a gift from Dr J. Gerhart) or NIH 3T3 cells (Stratagene). The longest clones identified with each probe were sequenced completely. Two cDNA fragments of 1.1 kb and 1.9 kb were obtained by screening an 8.5 dpc embryo library using the pME10 probe, and were found to be homologous to human *CDC25A*. Open reading frames were identified in the 1.1 kb and the 1.9 kb fragments coding for 192 amino acids and 436 amino acids respectively. To obtain the 5′ end of the *CDC25A* homolog a 5′ RACE was carried out (Frohman and Martin, 1989) using RACE-ready kidney cDNA (Clonetech Lab) according to the manufacturer's published protocol. The gene-specific primer used for the first PCR reaction was 5′-TACTCTCCTGTAGATGTGACG-3′. A degenerate primer based on the sequence of the first six amino acids from human *CDC25A* (5′-ATGG$^{A}_{G}$CTIGGICCIAGICC-3′) and gene specific primer (5′-TCTAGAGAATCAGAGTGGCTC-3′) were used for the second PCR reaction. A 3′ overlap region spanning bases 222 to 336 was identified with the 1.9 kb cDNA clone. The 5′ sequence obtained by PCR is shown in Fig. 1 and represents bases 1 to 336. In addition, a 1.0 kb cDNA fragment was obtained from a NIH 3T3 fibroblast library with pME11, which was identical to *cdc25b* (*cdc25M2*; the mouse homolog of human *CDC25B*) (Kakizuka et al., 1992).

Expression of GST-cdc25a fusion protein and identification of phosphatase activity

The 1.1 kb *Eco*RI fragment of *cdc25a*, isolated from the oligo dT-primed 8.5 dpc lambda Zap cDNA library (see above), was used for expression studies. The *Eco*RI fragment containing the C-terminal region of *cdc25a*, including the catalytic region and phosphatase box, was subcloned into pGEX1 (Pharmacia). Transformants in the correct orientation were identified and grown in L Broth overnight. Cells were grown to mid-logarithmic phase, IPTG-treated and harvested as described (Lee et al., 1992). The glutathione-S-transferase-cdc25a (GST-cdc25a) fusion protein, purified according to the protocol of Lee et al. (1992), was used for PNPP assays, where hydrolysis of the phosphate group of the substrate PNPP is used to demonstrate phosphatase activity (Guan et al., 1990). The PNPP assays were carried out using 1, 2, 3, or 5 μg of the fusion protein for 60 minutes or 0.6 μg of fusion protein for time intervals ranging from 15 minutes to 60 minutes. Absorbance was measured at 410 nm on a spectrophotometer. GST protein alone was used as a control in these assays.

Analysis of *cdc25a* mRNA expression

A multiple tissue northern blot (Clonetech Labs, Lot no. 36344) was used to analyze *cdc25a* mRNA levels in different mouse tissues. 2 μg of poly(A)$^+$ RNA were electrophoresed, transferred to a nylon membrane, prehybridized and hybridized according to the published protocol (Clonetech Labs, Multiple Tissue Northern Blot Protocol). Prehybridization and hybridization were carried out at 50°C in 5× SSPE, 10× Denhardt's solution, 100 μg/ml salmon sperm DNA and 2% SDS. A 1.1 kb *Eco*RI fragment of the *cdc25a* cDNA was labeled with [α-^{32}P] dCTP using the multi-prime method (Amersham) and used as probe. Blots were rinsed in 2× SSC, 0.5% SDS and washed in 0.1× SSC, 0.1% SDS at 50°C according to the manufacturer's protocol and exposed to film with intensifying screens.

RNase protection analysis of *cdc25a* expression during postimplantation development

Total RNA was made from postimplantation mouse embryos (7.5 to 15.5 dpc), from isolated organs of 13.5 dpc and 16.5 dpc embryos and from adult testis and ovary using RNAzol B (Cinna/Biotex) by the method of Chomczynski and Sacchi (1987). An antisense *cdc25a* probe was generated from a linearized *Pvu*II fragment of digested pME10 containing the 200 bp insert of *cdc25a* using T7RNA polymerase and [α-^{32}P]CTP (800 Ci/mM). The RNA probe was subsequently treated with DNAse, gel purified and approximately 10,000 cpm hybridized overnight with each sample of embryo RNA. RNase digestion and analysis was carried out according to the protocol of Zinn et al. (1983). tRNA (Sigma) was run in parallel as a control, while a β-actin probe was used on the same samples as a loading control.

In situ hybridization analysis of *cdc25a* and *cdc25b* in postimplantation embryos and adult organs

In situ hybridization was carried out on postimplantation embryos (10.5 days post-coitus (dpc) to 17.5 dpc) according to the protocol of Tessarollo et al. (1993). RNA probes were synthesized using S^{35}-labeled UTP and T7 polymerase. Probes synthesized for *cdc25a* spanned bases 222 to 2106 while the *cdc25b* probe spanned 1454 to

2560 bases (Kakizuka et al., 1992). Control sense probes were synthesized using T3 polymerase and were used in comparison.

In situ hybridization analysis of *cdc25a* and *cdc25b* in preimplantation mouse embryos

Female B6C3F1 mice (Jackson Labs) were stimulated to ovulate with pregnant mare's serum gonadotropin (Sigma). 24 hours later the mice were injected with human chorionic gonadotropin (Sigma) and housed with male B6C3F1 mice overnight. The presence of a vaginal plug indicated a successful mating. Fertilized eggs at the one-cell stage were obtained from these mice by dissection and flushing of the oviduct. Embryos were fixed immediately or cultured further for 1-4 days to obtain 2-cell, 4-cell, 16-cell, morula and blastocyst stage embryos. These embryos of different ages were introduced into oviducts isolated from recipient females, fixed overnight in 4% paraformaldehyde at 4°C and dehydrated through an ethanol series.

```
       ATGGAACTGGGCCCGAGCCCCCCACCGCGCCGCCTGTTCTTCGCTTGCAGCCCCACGCCT   60
  1    M  E  L  G  P  S  P  P  P  R  R  L  F  F  A  C  S  P  T  P
       GCGTCGCAGCCCACGGGGAAGATGCTGTTTGGCGCATCAGCTGCTGGCGGACTGTCCCCT  120
 21    A  S  Q  P  T  G  K  M  L  F  G  A  S  A  A  G  G  L  S  P
       GTCACCAACCTGACGGTCACCATGGACCAGCTGGAAGGACTGGGCAGTGACTGTGAGAAA  180
 41    V  T  N  L  T  V  T  M  D  Q  L  E  G  L  G  S  D  C  E  K
       ATGGAAGTGAGAAATAACAGCAGTCTACAGAGAATGGGCTCCTCAGAATCGACCGATTCA  240
 61    M  E  V  R  N  N  S  S  L  Q  R  M  G  S  S  E  S  T  D  S
       GGTTTCTGTCTAGATTCTCCTGGGCCCTTGGACAGTAAAGAAAACCTTGAAATTTCCCTG  300
 81    G  F  C  L  D  S  P  G  P  L  D  S  K  E  N  L  E  I  S  L
       ACGAGAATAAATTCCCTACCTCAGAAGCTCCTGGGATGTAGCCCAGCCCTGAAGAGGAGC  360
101    T  R  I  N  S  L  P  Q  K  L  L  G  C  S  P  A  L  K  R  S
       CACTCTGATTCTCTAGACCAAGACACCTTTCACCTCATCGACCAGGATAAAAATAAAGAA  420
121    H  S  D  S  L  D  Q  D  T  F  H  L  I  D  Q  D  K  N  K  E
       AATGAAGCATTTGAACCCAAAAAGCAATACGACCTGCATCTTTCGTCACATCTACAGGAG  480
141    N  E  A  F  E  P  K  K  Q  Y  D  L  H  L  S  S  H  L  Q  E
       AGTAAAGATCTTTACACACAGAGGCAGAATTCAGCCCAGCTCGGAATGCTATCTTCAAAT  540
161    S  K  D  L  Y  T  Q  R  Q  N  S  A  Q  L  G  M  L  S  S  N
       GAAAGTGAATCAGGAAATTTCAGTCCTCTTTTTACTCCCCAGTCACCTGTAAAAGCCACT  600
181    E  S  E  S  G  N  F  S  P  L  F  T  P  Q  S  P  V  K  A  T
       TTGTCCGATGAGGATGATGGCTTCATAGACCTTCTGGATGGCGAGAATTTAAAGAATGAT  660
201    L  S  D  E  D  D  G  F  I  D  L  L  D  G  E  N  L  K  N  D
       GAGGAGACCCCTACTAGCATGGTAAGCCTCTGGACAGCTCCCCTTGTCATGAGGAGACCT  720
221    E  E  T  P  T  S  M  V  S  L  W  T  A  P  L  V  M  R  R  P
       GCAAACCTTGCCGATCGGTTGCGGGGGGCTGTTTGACTCCCCTTCCCCGTGCGGCTCCAGC  780
241    A  N  L  A  D  R  L  R  G  L  F  D  S  P  S  P  C  G  S  S
       ACTCGGGCGGTGTTGAAGAGAGCAGACCGGTCTCACGAGGAGCCTCCTCGGGGTACAAAG  840
261    T  R  A  V  L  K  R  A  D  R  S  H  E  E  P  P  R  G  T  K
       AGGAGGAAGAGTGTGCCCAGCCCTGTGAAGGCGAAGGCGGATGTTCCGGAGCCCGCCCAG  900
281    R  R  K  S  V  P  S  P  V  K  A  K  A  D  V  P  E  P  A  Q
       CTTCCATCCCAGTCTCTATCCCTGATGTCTTCCCCCAAAGGAACCATTGAGAACATTTTG  960
301    L  P  S  Q  S  L  S  L  M  S  S  P  K  G  T  I  E  N  I  L
       GACAGTGACCCAAGAGACCTTATAGGAGATTTCTCCAAGGGTTATCTCTTTAATACCGTC 1020
321    D  S  D  P  R  D  L  I  G  D  F  S  K  G  Y  L  F  N  T  V
       TCTGGGAAGCATCAGGATTTGAAATATATTTCTCCAGAAATTATGGCATCTGTTTTGAAT 1080
341    S  G  K  H  Q  D  L  K  Y  I  S  P  E  I  M  A  S  V  L  N
       GGCAAGTTTGCCAGTCTCATTAAAGAGTTTGTTATCATTGACTGCCGATACCCATATGAG 1140
361    G  K  F  A  S  L  I  K  E  F  V  I  I  D  C  R  Y  P  Y  E
       TATGAAGGAGGTCACATCAAGGGTGCCGTGAATCTGCACATGGAAGAAGAGGTTGAAGAC 1200
381    Y  E  G  G  H  I  K  G  A  V  N  L  H  M  E  E  E  V  E  D
       TTCTTACTTAAGAACCCTATTGTGCCTACTGATGGCAAGCGTGTCATTGTCGTGTTCCAC 1260
401    F  L  L  K  N  P  I  V  P  T  D  G  K  R  V  I  V  V  F  H
       TGTGAGTTTTCCTCTGAAAGAGGCCCTCGAATGTGCCGATATGTGAGAGAGAGAGATAGG 1320
421    C  E  F  S  S  E  R  G  P  R  M  C  R  Y  V  R  E  R  D  R
       CTCGGCAATGAGTACCCCAAGCTCCACTACCCTGAGCTGTATGTCCTGAAAGGGGGATAC 1380
441    L  G  N  E  Y  P  K  L  H  Y  P  E  L  Y  V  L  K  G  G  Y
       AAGGAGTTCTTTTTGAAGTGCCAGTCTCACTGTGAACCCCCCAGCTACCGGCCAATGCAC 1440
461    K  E  F  F  L  K  C  Q  S  H  C  E  P  S  Y  R  P  M  H
       CATGAAGACTTTAAAGAAGACCTAAAGAAGTTCCGCACCAAGAGCCGGACCTGGGCAGGA 1500
481    H  E  D  F  K  E  D  L  K  K  F  R  T  K  S  R  T  W  A  G
       GAAAAGAGGAAAAGAGAGATGTACAGTCGCCTGAAGAAGCTCTGAAGCCAAATGGTAGCA 1560
501    E  K  R  K  R  E  M  Y  S  R  L  K  K  L  *
       GCCTGAGCTTCTCTGCCCCGTCCCTTTTCCCTTTGTCGCAGAGCAGTAAGCAAAGGGGCC 1620
       AGCTGTACGGTAACCTGGAGAAAGACCTGGGCCTTCCATTCCTTGGACCTGTCTCCTACA 1680
       CTCCCAGGTTGGAGCCTAGGCACCCTGCCGTTACACTCTTCTGTTCTGTAAGAGTCCTTC 1740
       CCTGTCAGGACTGTCTGCCAAGCTGACAAGCACAGCACAGACTGGAGTCTGCAACACCGT 1800
       GTTAGCTGCTCTCACTGAGCATCCCATGAAGAAGGCCTTATTGGTAGACCCTGGCCTGTC 1860
       ACAGGAGAGAAAAGCCGCCAAGTGCTGCTGGCCAAATACCAAAGATAGGCTGCAAGGGAG 1920
       GAGAGGGCCTCGTGGATGACTCTTAACTTTAATTTATTCAGCTTCATCAATTATTTTATT 1980
       ATTGTTTTAATTCAAGACTTTTACTTTTCTGCTTCATTAAGTCAAAATACTGCCATTCTA 2040
       GGTAGAGTTTTATCATCCTAGGAGCTACCTCTACTTTTAATTTAAAAAAAAAAAAAGAA 2100
       GGAATT
```

Fig. 1. Sequence of mouse *cdc25a* and the predicted amino acids. cDNA was isolated from 8.5 dpc mouse embryo library by hybridization to a PCR fragment derived using degenerate primers to *string*. Two overlapping clones represent the full length cDNA derived *cdc25a*. A 1.9 kb cDNA sequence represents the 3′ end of the gene and is shown starting at nucleotide 220 with a predicted open reading frame from amino acid 74. The 5′ cDNA sequence was obtained by 5′ RACE-PCR (see Materials and Methods) and overlapped with the 1.9 kb fragment at predicted amino acid residues 74 to 112. The Met at position 1 corresponds to the known start site of CDC25A.

```
CDC25C   DITITQMLEE ...DSNQGHL IGDFSKVCAL PTVSGKHQDL KYVNPETVAA
cdc25c   DMNAIQMEEE ...ECGSELL IGDFSKVCVL PTVPGKHPDL KYISPDTVAA
CDC25B   .....HDEIE NLLDSDHREL IGDYSKAFLL QTVDGKHQDL KYISPETMVA
cdc25b   .....H.EIE NILDSDHRGL IGDYSKAFLL QTVDGKHQDL KYISPETMVA
CDC25A   LASSPKGTIE NILDNDPRDL IGDFSKGYLF HTVAGKHQDL KYISPEIMAS
cdc25a   LMSSPKGTIE NILDSDPRDL IGDFSKGYLF NTVSGKHQDL KYISPEIMAS

                                        B
CDC25C   LLSGKFQGLI EKFYVIDCRY PYEYLGGHIQ GALNLYSQEE LFNFFLKKPI
cdc25c   LLSGKFQSVI ERFYIIDCRY PYEYLGGHIL GALNLHSQKE LHEFFLRKPV
CDC25B   LLTGKFSNIV DKFVIVDCRY PYEYEGGHIK TAVNLPLERD AESFLLKSPI
cdc25b   LLTGKFSNIV EKFVIVDCRY PYEYEGGHIK NAVNLPLERD AETFLLQRPI
CDC25A   VLNGKFANLI KEFVIIDCRY PYEYEGGHIK GAVNLHMEEE VEDFLLKKPI
cdc25a   VLNGKFASLI KEFVIIDCRY PYEYEGGHIK GAVNLHMEEE VEDFLLKNPI

                              Phosphatase Box
CDC25C   VPLDTQKRII IVFHCEFSSE RGPRMCRCLR EEDRSLNQYP ALYYPELYIL
cdc25c   VPLDIQKRVI IVFLCEFSSE RGPRMCRSLR EKDRALNQYP ALYYPELYIP
CDC25B   APCSLDKRVI LIFHCEFSSE RGPRMCRFIR ERDRAVNDYP SLYYPEMYIL
cdc25b   MPCSLDKRII LIFHCEFSSE RGPRMCRFIR ERDRAANDYP SLYYPEMYIL
CDC25A   VPTD.GKRVI VVFHCEFSSE RGPRMCRYVR ERDRLGNEYP KLHYPELYVL
cdc25a   VPTD.GKRVI VVFHCEFSSE RGPRMCRYVR ERDRLGNEYP KLHYPELYVL
```

Fig. 2. Comparison of cdc25 proteins from human and mouse. Conserved domains across all cdc25 proteins have been boxed. Box A represents an invariant sequence GKHQDLKY found in all mammalian cdc25 genes. Box B represents a short sequence of homology (identity and conserved substitutions) XIXDCRYPYEYXGGHIXXAXNL. The phosphatase box depicts the motif identified as required for phosphatase activity. Invariant amino acids in all proteins are shaded. (cdc25b sequence shown is from the clone described in Materials and Methods and corresponds to that of Kakizuka et al., 1992; *cdc25c* sequence shown is taken from Nargi and Woodford-Thomas, 1994.)

Oviducts containing embryos were embedded in paraffin and 6 μm sections were cut and collected on siliconized slides. Sections were dewaxed in xylene, rehydrated through an ethanol series and refixed in 4% paraformaldehyde at 4°C (20 minutes). Sections were then treated with proteinase K (20 μg/ml for 7.5 minutes), fixed again in 4% paraformaldehyde (5 minutes), incubated in 0.25% acetic anhydride in 0.1 M triethanolamine (10 minutes) and again dehydrated. Slides were hybridized overnight under coverslips in a moist chamber at 50-55°C with 1-5 ng/μl of digoxigenin-labeled RNA probe in 75 μl hybridization solution. Probes were generated according to the protocol in the Genius Kit (Boehringer Mannheim Biochemicals). The probes for *cdc25a* and *cdc25b* were derived from pME10 and pME11, respectively using the T7 promoter and each was approximately 220 bases in length. Control (sense) probes were also synthesized similarly for pME10 and pME11 using the T3 promoter.

Following hybridization, slides were washed at high stringency (20-30 minutes at 65°C in 50% formamide, 2× SSC), RNase treated for 30 minutes at 37°C, and again washed at high stringency as before. After equilibrating in Buffer 1 from the Genius Kit, the slides were incubated in a 1:1000 dilution of alkaline phosphatase-conjugated anti-digoxi-

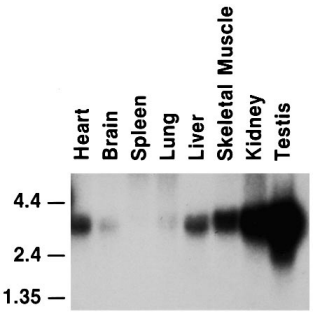

Fig. 3. Northern analysis of *cdc25a* mRNA expression in various adult mouse tissues. A 1.1 kb *Eco*RI cDNA fragment of *cdc25a* was used to probe a multiple tissue northern blot. 2 μg poly(A)$^+$ selected RNA obtained from various adult tissues were used for the northern analysis (see Materials and Methods).

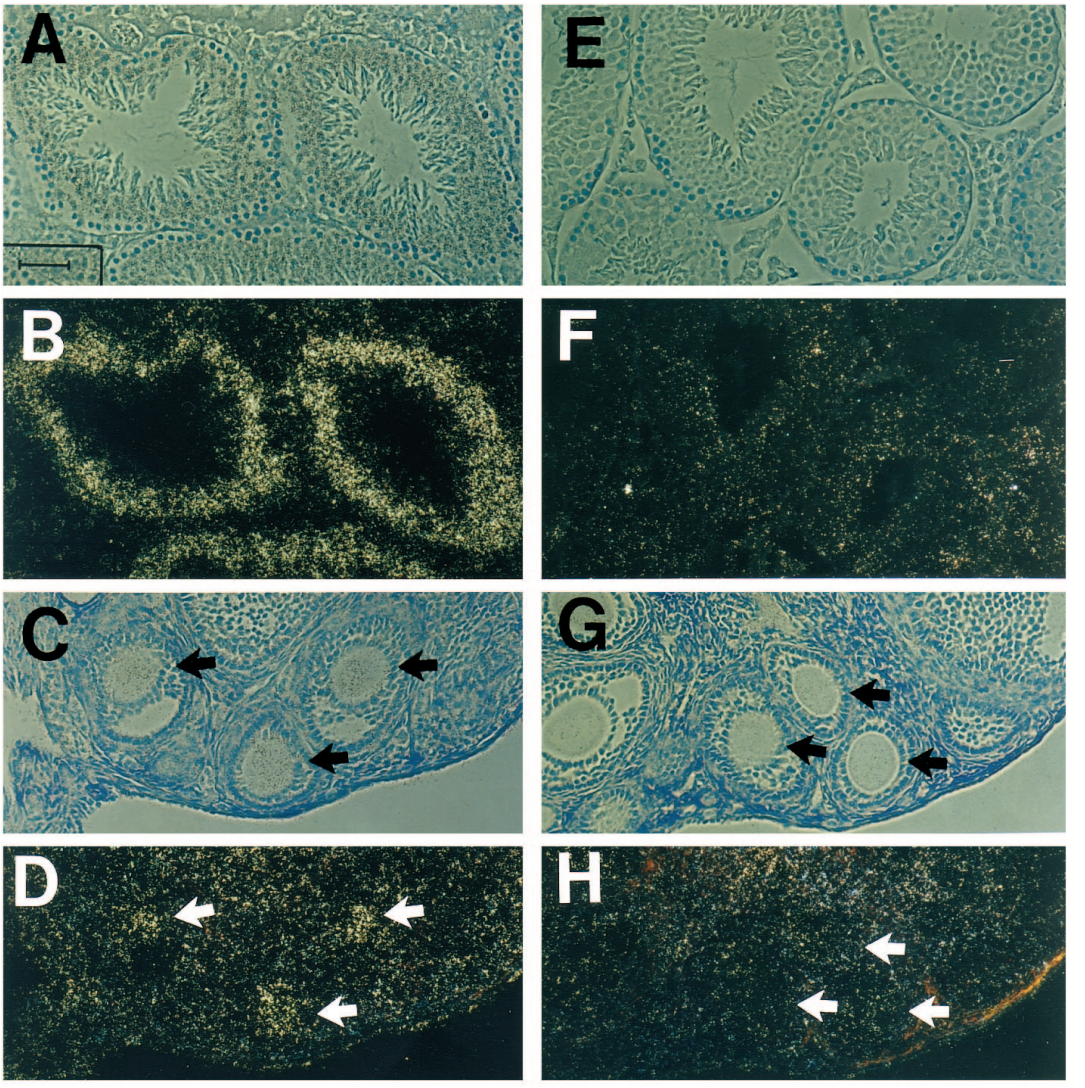

Fig. 4. In situ hybridization analysis of *cdc25a* and *cdc25b* in adult testis and ovary. Transverse sections of adult testis hybridized with probes for *cdc25a* (A,B) and *cdc25b* (E,F) and adult ovary hybridized with *cdc25a* (C,D) and *cdc25b* (G,H). A,C,E,G are bright-field images while B,D,F,H are the corresponding dark-field images photographed at ×200 magnification. Arrows in C,D,G,H point to oocytes in ovarian follicles. Scale bar in A indicates 20 μm.

genin antibody (Boehringer Mannheim Biochemicals) and washed extensively in several changes of Buffer 1, before overnight incubation in substrate (Buffer 3 and the staining solution from the Genius Kit). The reaction was stopped by incubation for 10 minutes with TE, followed by 10 minutes in water, and finally gelvatol and coverslips were placed on the slides for photography and storage. Slides were analyzed using a confocal laser scanning microscope (CLSM; Zeiss, Germany). A ×40 oil immersion, Nomarski objective (N.A. 1.3, acrostigmatic lens) was used to identify embryos using a scan rate of 8 seconds. The Nomarski images are represented by the blue channel and the reflected peroxidase-digoxigenin labeled probe is represented by the red channel. All manipulations on the CLSM were according to the protocol described by Tsarfaty et al. (1992, 1994).

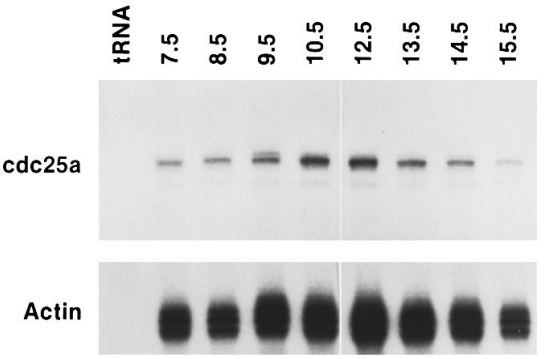

Fig. 5. RNA samples from 7.5 dpc to 15.5 dpc were analyzed by RNase protection using a 200 bp α-^{32}P-labeled *cdc25a* fragment as described in Materials and Methods. The control for loading (β-actin) is shown for each sample in the lower panel. tRNA was used as a negative control.

RESULTS

Isolation of mouse *cdc25* cDNAs

A comparison of known cdc25 protein sequences from different species reveals a conserved carboxy-terminal region

spanning approximately 100 amino acids. To isolate the mouse *cdc25* cDNAs, PCR products were obtained using degenerate primers designed from the conserved carboxy terminal of the *Drosophila stg* gene (Sadhu et al., 1990) using NIH *3T3* cDNA as template. Two distinct PCR-derived clones (pME10 and pME11) were used as probes to screen an 8.5 dpc oligo dT-primed mouse embryo or a NIH *3T3* cDNA library. In the first screen, using pME10 as probe, two cDNAs of 1.1 and 1.9 kb were obtained and were found upon sequence analysis to represent an identical gene (Fig. 1). The 1.9 kb fragment is shown spanning bases 220 to 2106, and the 1.1 kb fragment spans bases 961 to 2106 and is therefore entirely contained within the 1.9 kb fragment (Fig. 1). Sequence comparison reveals 84% identity with human *CDC25A* while 63% and 65% identity is shared with human *CDC25B* and *CDC25C* respectively. Amino acid sequence comparison demonstrates these clones share 77% identity with the human CDC25A protein (Fig. 2). This clone represents the third mouse *cdc25* gene identified and can be designated *cdc25M3* but to conform with the human gene nomenclature we propose designating it *cdc25a*. The *cdc25a* start site obtained by 5′ RACE-PCR corresponds to the Met at position 1 of the human *CDC25A*.

A second screen of a NIH *3T3* fibroblast cDNA library using pME11 as a probe yielded a 1.0 kb cDNA identical to *cdc25M2* (Kakizuka et al., 1992) which is the mouse homolog of human *CDC25B* and which we will refer to as *cdc25b*. Both mouse clones, *cdc25a* and *cdc25b*, reveal a shared amino acid motif (XXDCRYPYEYXG-GHIXXAXNL and KRX-IXXFXCEFSSERG-PRMCR) with all other mammalian *cdc25* genes (Fig. 2). The motif FHCXXXXXR, which flanks the active site of protein tyrosine phosphatases, is included in this conserved region of mouse *cdc25a* suggesting potential tyrosine phosphatase activity. Amino acid sequence comparison demonstrates that *cdc25a* and *cdc25b* share a low overall identity of 29% and 34% respectively with the *Drosophila stg* gene and 34% and 32% identity respectively with the *Drosophila* gene *twn*.

Expression of GST-cdc25a fusion protein and identification of phosphatase activity

In order to determine if *cdc25a* acts as a phosphatase, a GST-cdc25a fusion protein was assayed for its ability to hydrolyse PNPP (Guan et al., 1990, 1991). The GST-cdc25a fusion, constructed using the C-terminal 198 amino acids of cdc25a, was used to demonstrate a conversion of PNPP to p-nitrophenol.

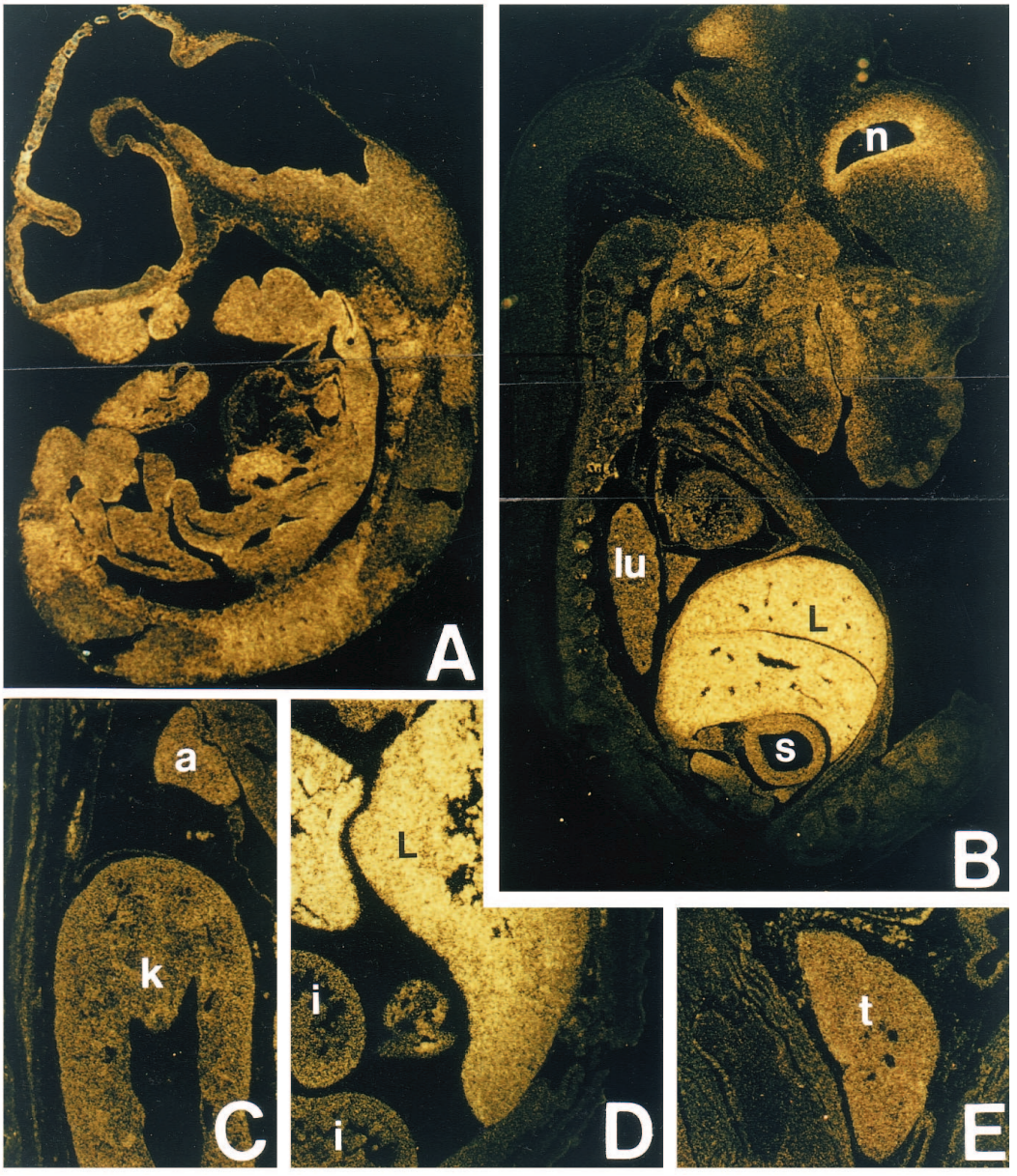

Fig. 6. Developmental expression of *cdc25a* in postimplantation embryos. Dark-field images of 10.5 dpc (A), 13.5 dpc (B) embryos (×25 magnification) and various tissue from 17.5 dpc embryos (C,D,E) (×50 magnification) are shown. Embryonic tissue is identified with the following letter designations: n, neuroepithelium; lu, lung; s, stomach; L, liver; a, adrenal gland; k, kidney; i, intestine; t, thymus.

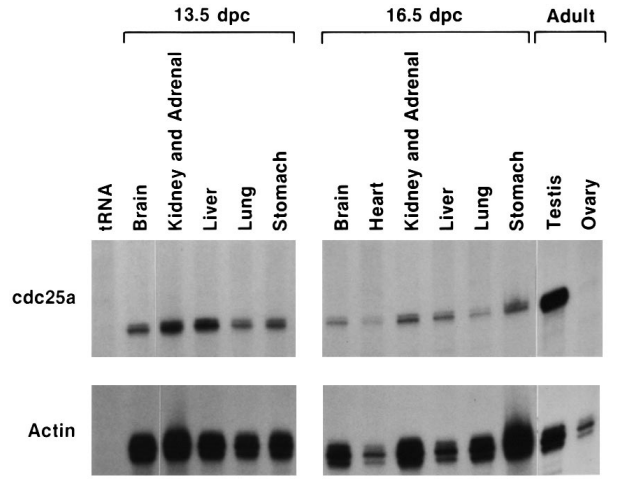

Fig. 7. RNase protection analysis of *cdc25a* in tissue of 13.5 dpc and 16.5 dpc embryos, and adult gonads. Expression of *cdc25a* RNA is shown in brain, kidney and adrenal gland, liver, lung and stomach of 13.5 dpc and 16.5 dpc embryos. RNase protections of *cdc25a* RNA of adult testis and ovary are also shown. tRNA is included as a negative control while β-actin is included as a loading control for each sample (lower panel).

GST-cdc25a fusion protein or GST protein alone were used in the assays. The kinetic and concentration-dependent hydrolysis of PNPP indicates that cdc25a-GST is a phosphatase (data not shown). GST protein alone did not hydrolyse PNPP. Thus, the prediction that cdc25a is a phosphatase can be confirmed and, furthermore, suggest that it is functionally similar to other members of the cdc25 family.

cdc25a mRNA expression in adult tissue and during postimplantation development

In order to analyze *cdc25a* expression in adult animals and postimplantation embryos, we carried out northern analysis, in situ hybridization and RNase protection assays. Northern analysis reveals that a 3.4 kb *cdc25a* transcript is expressed in some adult tissues (Fig. 3). High levels of *cdc25a* transcript were detected in adult testis while lower levels were detected in kidney, liver, heart, and skeletal muscle and none were detected in spleen and lung (Fig. 3). Interestingly, in situ hybridization analysis of *cdc25a* and *cdc25b* show varying patterns of RNA expression in the adult testes and ovary. *cdc25a* is highly expressed in the adult testes particularly in primary and secondary spermatocytes, but the probe does not appear to hybridize to spermatogonia or spermatids (Fig. 4A,B). In contrast, *cdc25b* is poorly expressed in primary and secondary spermatocytes and in somatic tissue (Fig. 4E,F). In the ovary, *cdc25a* is primarily expressed in the oocytes of early preantral and antral follicles (Fig. 4C,D; arrows), while a lower level of expression is observed in granulosa cells and in other somatic tissue. In contrast, lower levels of *cdc25b* transcripts are observed in oocytes (Fig. 4G,H; arrows) while some hybridization is also observed in the somatic cells of the ovary. To characterize further the developmental expression of *cdc25a*, RNase protections were carried out on RNA isolated from different stages of postimplantation development from 7.5 dpc to 15.5 dpc. *cdc25a* expression was observed throughout and appears to be essentially unchanged across this developmental spectrum in comparison to the β-actin controls (Fig. 5). These data show that *cdc25a* RNA expression does not fluctuate significantly between 7.5 dpc and 15.5 dpc of postimplantation mouse development while *cdc25a* transcript levels vary in adult tissue.

A detailed comparative analysis of *cdc25a* and *cdc25b* expression was carried out on 10.5 to 17.5 dpc embryos by in situ hybridization. Previous studies have described the expression pattern of *cdc25b* in postimplantation embryos (Kakizuka et al., 1992). *cdc25a* is expressed in most developing tissue and is observed ubiquitously in the 10.5 dpc embryo (Fig. 6A). For example, the developing heart, primitive gut, liver primordia, the branchial arch, and the developing brain, in particular the proliferative-neuroepithelium, express *cdc25a*. Similarly, in the 13.5 dpc (Fig. 6B) and the 17.5 dpc embryo *cdc25a* is expressed in most tissues (Fig. 6C,D,E). The developing liver of the 13.5 dpc embryo expresses high levels of *cdc25a* while expression levels decrease by 17.5 dpc. A similar pattern of *cdc25a* expression is observed in the developing kidney and adrenal gland of the 13.5 dpc and 17.5 dpc embryo. *cdc25a* expression in the brain is particularly apparent in the developing neuroepithelium of all embryos examined. In contrast, *cdc25b* reveals a more restricted pattern of expression. *cdc25b* transcripts are detected primarily in developing liver and neuroepithelium in 13.5 dpc embryos while the signal decreases in the 17.5 dpc embryo (data not shown) in agreement with previous results (Kakizuka et al., 1992). Since widespread embryonic expression of *cdc25a* was observed consistently in several in situ hybridization experiments, RNase protections were carried out to independently confirm these results. RNase protection analysis was carried out on RNA obtained from organs isolated from 13.5 dpc and 16.5 dpc embryos. *cdc25a* expression is detected in embryonic brain, heart, kidney and adrenal gland, liver, lung and stomach (Fig. 7).

cdc25a and *cdc25b* expression is regulated during preimplantation development

To examine the expression pattern of *cdc25a* and *cdc25b* during preimplantation development, in situ hybridization experiments were carried out on one-cell, two-cell, four-cell (Fig. 8), eight-cell (not shown), sixteen-cell, morula and blastocyst stage embryos (Fig. 9). Distinct patterns of expression were observed for *cdc25a* and *cdc25b*. *cdc25a* transcripts were not detectable in the early embryo stages (one-, two- and four-cell, Fig. 8A,C,E respectively; eight-cell (data not shown), 16-cell and morula, Fig. 9A,C) when compared to the sense controls for each experiment (data not shown). The expression of *cdc25a* is first detected as a very strong signal at the blastocyst stage (Fig. 9E), both in trophectoderm and inner-cell mass (ICM). In contrast, *cdc25b* is observed early in preimplantation embryogenesis. *cdc25b* expression is detected at the one-cell stage (Fig. 8B) followed by a decrease in signal intensity at the two-cell stage (Fig. 8D). However, the *cdc25b* RNA is detectable at a higher level in the four-cell stage (Fig. 8F) and is expressed through the eight-cell (data not shown), 16-cell and morula stages (Fig. 9B,D, respectively). Maximal expression of *cdc25b* is observed at the blastocyst stage (Fig. 9F) in the trophectoderm and ICM. Numerous embryos from two or more experiments were analyzed at each stage of development and consistently demonstrate the expression patterns

described. Confocal images were generated under uniform conditions for all stages shown. Since all embryos processed were in interphase and none were in mitosis, *cdc25a* and *cdc25b* expression is described for embryos at interphase. In addition, embryos were also examined with regard to compartmentalization of the *cdc25* signal within the cell. However, the signal is prevalent in both the nuclear and cytoplasmic compartments. Absence of signal in some regions appears to be due to side preparation and was not a consistent observation.

carried out on preimplantation embryos (this study), postimplantation embryos (this study; Kakizuka et al., 1992) and adult tissue (this study; Kakizuka et al., 1992; Nargi and Woodford-Thomas, 1994). Analysis of adult tissue shows *cdc25a* is expressed at a high level in testis and at lower levels in ovary, which correlates with meiotic and mitotic activity in these organs. A more detailed in situ hybridization analysis of *cdc25a* and *cdc25b* expression in testis and ovary reveal varied patterns of expression in male and female germ cells. It is interesting to note that *cdc25a* is expressed in oocytes from most

DISCUSSION

This study identifies a new mouse homolog of cdc25. Cloning and sequence comparison of cdc25a reveals conservation of a phosphatase domain which is common to all cdc25 family members. As predicted from sequence analysis, cdc25a demonstrates phosphatase activity. Furthermore, we describe a distinct pattern of expression of two mouse *cdc25* genes which correlate with distinct functions for each gene.

Sequence comparison reveals that cdc25a is the mouse homolog of human CDC25A sharing 83% similarity between the two proteins and also demonstrates that cdc25a shares highly conserved regions with other cdc25 homologs (Fig. 2). The 'phosphatase motif' FHCXXXXXR characteristic of all tyrosine phosphatases, including the *cdc25* genes, is completely conserved in cdc25a, suggesting that it is a bonafide cdc25 family member. The phosphatase activity of cdc25a was confirmed by demonstrating that a GST-cdc25a fusion protein could dephosphorylate the substrate PNPP. A similar response is observed in PNPP assays carried out with other cdc25 homologs, suggesting that cdc25a could act to dephosphorylate $p34^{cdc2}$ kinase.

Expression studies of *cdc25* genes in mouse were

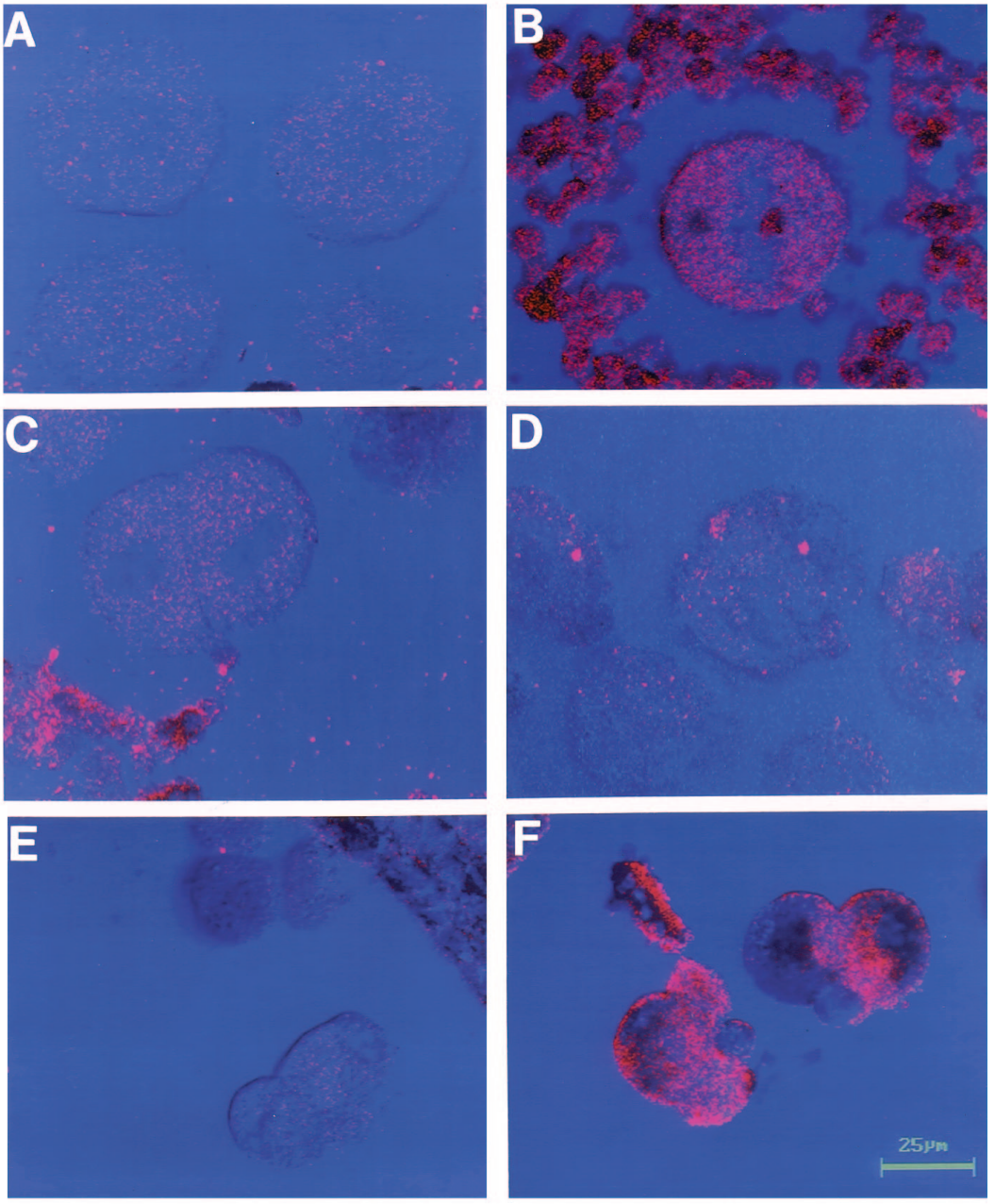

Fig. 8. In situ hybridization of *cdc25a* and *cdc25b* in preimplantation embryos. One- to 16-cell, morula and blastocyst embryos were introduced into oviducts and processed to carry out in situ hybridization analysis (see Materials and Methods). Confocal microscope images were generated and the Nomarski images are represented in blue while the digoxigenin-HRP-labeled *cdc25a* probe is represented in red. One-cell (A,B), two-cell (C,D) and four-cell (E,F) embryos are shown. *cdc25a* was used as the probe in A,C and E while *cdc25b* was used in B,D and F. Scale bar (in F), 25 μm.

stages of follicular development and primary and secondary spermatocytes which represent stages of meiotic germ cells. In contrast, *cdc25b* is not highly expressed in the germline of the ovary and testis. These results suggest that *cdc25a* may play a role in meiosis, which is analogous to the role of *twn* in *Drosophila* gametogenesis. However, it is important to note that, unlike *twn*, *cdc25a* is also expressed in other somatic tissue such as brain, heart, liver, kidney, and skeletal muscle. These observations collectively suggest that *cdc25a* may have a dual function in mitosis and meiosis. The observation of *cdc25a* transcript expression in non-dividing tissue such as heart and skeletal muscle may reflect posttranscriptional and posttranslational regulation of *cdc25a* activity. Indeed, recent evidence suggests that *cdc25* itself may be regulated by phosphorylation (Kumagai and Dunphy, 1992; Ogg et al., 1994). A similar observation of *cdc25b* expression in non-dividing adult tissue has been reported (Kakizuka et al., 1992). *cdc25b* is expressed predominantly in spleen, lung, heart, brain, intestine, and muscle (Kakizuka et al., 1992) while *cdc25c* is expressed in spleen and thymus (Nargi and Woodford-Thomas, 1994). These results demonstrate that *cdc25* genes display varied patterns of expression in adult tissue.

RNase protections and northern analysis of postimplantation embryos reveal that *cdc25a* is expressed from 7.5 dpc to 15.5 dpc of gestation and does not change significantly at these stages. In contrast, *cdc25b* is expressed at 10.5 dpc, peaks at 13.5 dpc, and decreases at 17.5 dpc (Kakizuka et al., 1992). In situ hybridization and RNase protection analysis of postimplantation tissue reveals that *cdc25a* is ubiquitously expressed in most developing tissue, while *cdc25b* has a more restricted pattern of expression. *cdc25b* expression is mostly confined to proliferative liver and neural tissue as observed in this study and by Kakizuka and co-workers (1992). In contrast, *cdc25a* is expressed not only in proliferative liver and neuroepithelium, but also in developing heart, kidney, adrenal gland, stomach, intestine, and lung. *cdc25a* transcripts are observed in the 13.5 dpc embryonic liver and kidney but have decreased by 17.5 dpc, which correlates with a loss of mitotic activity and increased differentiation of these tissues. Collectively, these observations show that *cdc25a* and *cdc25b* are expressed differentially during postimplantation development.

In situ hybridization analysis of preimplantation embryos demonstrate that *cdc25a* is expressed only at the blastocyst stage. *cdc25b i*s expressed at the one-cell stage, decreases to background levels at the two-cell stage and is observed once again from the four-cell to the blastocyst stage. Interestingly,

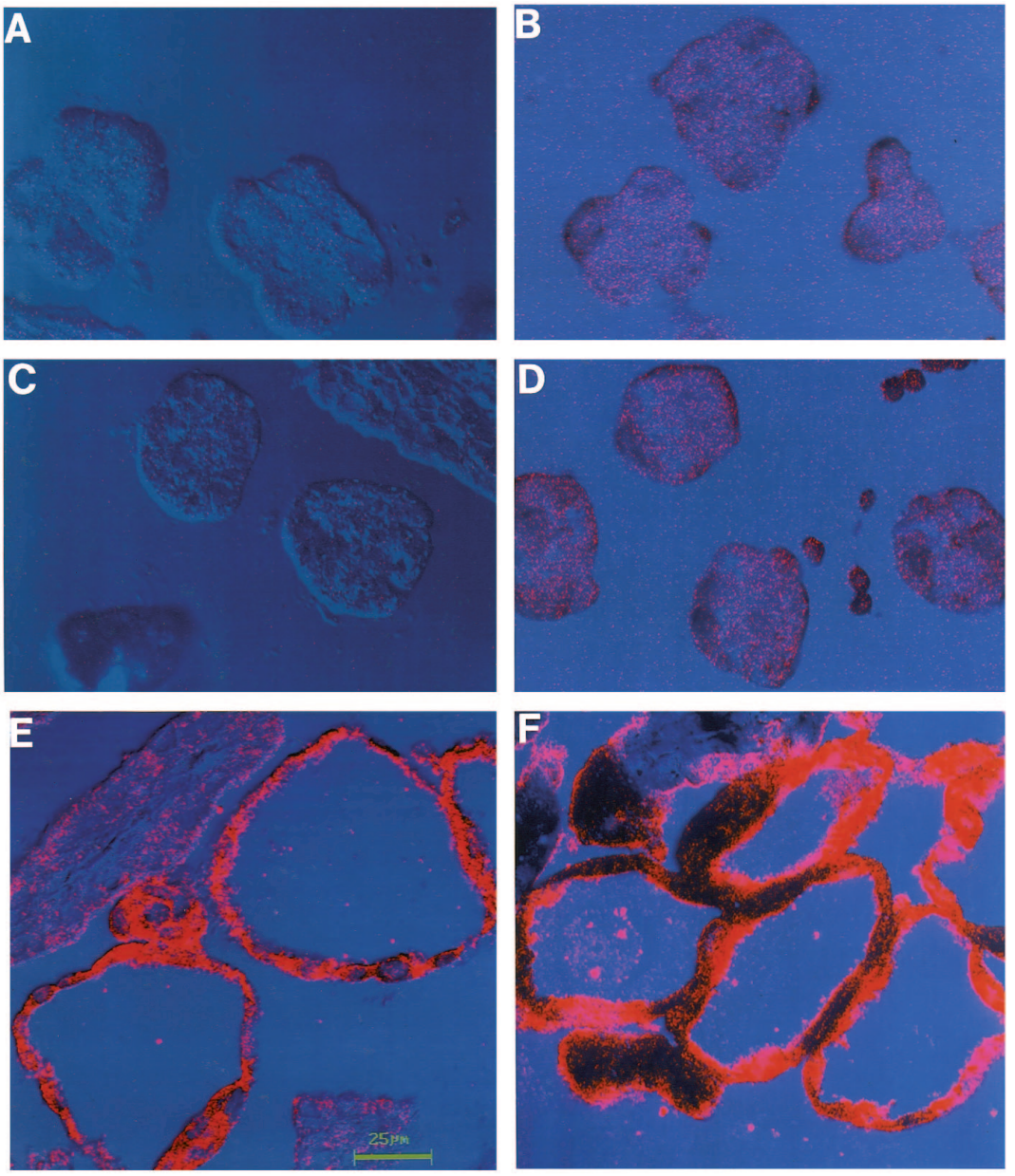

Fig. 9. In situ hybridization analysis of 16-cell (A,B), morulae (C,D) and blastocyst stage (E,F) embryos are shown. Images were generated as described in Fig. 8. *cdc25a* was used as a probe in A,C and E and *cdc25b* was used in B,D and F. Scale bar (in F), 25 μm.

we note that *cdc25a* transcripts are expressed in ovarian oocytes, but are not observed in the one-cell embryo. *cdc25b* transcripts, on the other hand, are expressed in oocytes at low levels and are present in one-cell embryos. Maximal expression of *cdc25b* is observed at the blastocyst stage. *cdc25c* expression has not been examined in embryos. Taken together, these results demonstrate a different pattern of expression for two *cdc25* mouse homologs, suggesting a distinct role for each of these genes. It is interesting to note that the onset of *cdc25a* and *cdc25b* expression is coincident with two important developmental transitions in the early embryo. Expression of *cdc25a* is first detected in the ICM and the trophectoderm of the blastocyst stage which gives rise to the embryo proper and extra-embryonic membranes respectively at implantation. Proliferative activity occurs at this stage in preparation for implantation (for reviews see Rossant, 1986; Cruz and Pedersen, 1991). Expression of *cdc25b* decreases at the two-cell stage and is re-expressed at the four-cell stage, which correlates well with the transition from maternal to zygotic transcription control in early mouse development (for review see Pratt, 1989; Telford et al., 1990; Schultz, 1993). An analogous transition is observed in the *Drosophila* embryo and is controlled by the *stg* gene (Edgar and O'Farrell, 1989).

Cell cycle-specific expression and functions of the different *CDC25* genes have been examined in mammalian cells. CDC25C protein is expressed throughout the cell cycle in HeLa cells (Millar et al., 1991b) while CDC25A protein is observed predominantly at G_1 (Jinno et al., 1994). Microinjection of CDC25C antibodies arrest cells at G_2 while CDC25A arrest cells at G_1 (Millar et al., 1992; Hoffman et al., 1994; Jinno et al., 1994). These results suggest that CDC25C and CDC25A perform different functions in the cell cycle. The G_1/S cell cycle transition in mammals is thought to be controlled by the cyclin dependent kinases (cdks) and is negatively regulated by Thr14/Tyr15 phosphorylation of $p33^{cdk2}$ (Gu et al., 1992; Sebastian et al., 1993). Thus, dephosphorylation of $p33^{cdk2}$ is necessary for its activation. Recently, CDC25A has been shown to dephosphorylate $p33^{cdk2}$ in complex with cyclin E, in vitro (Hoffman et al., 1994). In addition, CDC25A phosphatase activity is detectable in cells at G_1 and S phase of the cell cycles but not in G_2 and M-phases (Hoffman et al., 1994). These results suggest that a distinct G_1/S role is played by cdc25A. It is tempting to speculate that different mouse *cdc25* genes may also have distinct cell cycle-regulatory functions. cdc25a is first expressed at the blastocyst stage which correlates with establishment of a more 'typical' G_1 phase in the mouse embryo (Chisholm, 1988) corroborating the observation of a G_1 role for CDC25A as reported recently (Jinno et al., 1994; Hoffman et al., 1994). In contrast to the emerging G_1/S phase role for CDC25A, it is interesting to note that cdc25a is also expressed in meiotic germ cells and may function during meiosis in addition to mitosis. This observation suggests that cdc25a may play an analogous role to twn in *Drosophila* gametogenesis. However, since *cdc25a* is not exclusively expressed in meiotic cells, unlike *twn* in *Drosophila*, overlapping and compensatory roles for mammalian *CDC25* genes may have evolved. The mouse embryo offers an excellent model for examining this proposition further.

In conclusion, we have identified two distinct *cdc25* mouse genes. Furthermore, these genes demonstrate distinct developmentally regulated patterns of expression which coincide with major developmental transitions during early embryogenesis and gametogenesis. The data suggest that key cell cycle regulators such as cdc25 could play an important role in regulating mitosis in the developing mouse embryo and meiosis in the germline.

We thank Paul Russell, Tony Hunter and Byron Sebastian for sharing sequence information at the initial stages of the project. We also thank Helen Piwnica-Worms and Scott Ogg for providing vectors and for advice on purifying fusion proteins. Sue Hong and Lynn Bixler are acknowledged for technical help with sequencing and embryo collection respectively, while Jim Resau is thanked for generating confocal images. Leslie Lock is thanked for helpful comments and critical reading of the manuscript. We also thank Cathy Hahn and Madeline Wilson for excellent secretarial assistance. This research was sponsored in part by the National Cancer Institute, DHHS, under contract N01-CO-74101, with ABL.

REFERENCES

Alphey, L., Jiminez, J., White-Cooper, H., Dawson, L., Nurse, P. and Glover, D. (1992). *Twine*, a *cdc25* homolog that functions in the male and female germline of Drosophila. *Cell* **69**, 977-988.

Chisholm, J. C. (1988). Analysis of the fifth cell cycle of the mouse development. *J. Reprod. Fert.* **84**, 29-35.

Chomczynski, P. and Sacchi, N. (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Analyt. Biochem.* **162**, 156-159.

Courtot, C., Fankhause, C., Simanis, V. and Lehner, C. F. (1992). The *Drosophila cdc25* homolog *twine* is required for meiosis. *Development* **116**, 405-416.

Cruz, Y. P. and Pedersen, R. A. (1991). Origin of embryonic and extraembryonic cell lineages in mammalian embryos. In: *Animal Applications of Research in Mammalian Development* (ed. N. First), pp. 147-203. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

Demetric, D. J. and Beach, D. H. (1993). Chromosome mapping of human cdc25A and cdc25B phosphatases. *Genomics* **18**, 144-147.

Dunphy, W. G. and Kumagai, A. (1991). The cdc25 protein contains an intrinsic phosphatase activity. *Cell* **67**, 189-196.

Edgar, B. A. and O'Farrell, P. H. (1989). Genetic control of cell division patterns in the Drosophila embryo. *Cell* **57**, 177-187.

Edgar, B. A. and O'Farrell, P. H. (1990). The first post-blastoderm cell cycles of Drosophila embryogenesis are regulated in G2 by string. *Cell* **62**, 469-480.

Featherstone, C. and Russell, P. (1991). Fission yeast p107 Wee1 mitotic inhibitor is a tyrosine/serine kinase. *Nature* **349**, 808-811.

Frohman, M. A. and Martin, G. R. (1989) Rapid amplification of cDNA ends using nested primers. *Technique* **1**, 165-170.

Galaktionov, K. and Beach, D. (1991). Specific activation of cdc25 tyrosine phosphatases by B type cyclins: evidence for multiple roles for mitotic cyclins. *Cell* **67**, 1181-1194.

Gautier, J., Norbury, C., Lohka, M., Nurse, P. and Maller, J. L. (1988). Purified maturation-promoting factor contains the product of a Xenopus homolog of the fission yeast cell cycle control gene cdc2+. *Cell* **54**, 433-439.

Gautier, J., Solomon, M. J., Booher, R. N., Bazan, J. F. and Kirschner, M. W. (1991). cdc25 is a specific tyrosine phosphatase that directly activates $p34^{cdc2}$. *Cell* **67**, 197-211.

Gerhart, J., Wu, M. and Kirschner, M. (1984). Cell cycle dynamics of an M-phase-specific cytoplasmic factor in Xenopus *laevis* oocytes and eggs. *J. Cell Biol.* **98**, 1247-1255.

Gould, K. L. and Nurse, P. (1989). Tyrosine phosphorylation of the fission yeast cdc2+ protein kinase regulates entry into mitosis. *Nature* **342**, 39-45.

Gu, Y., Rosenblatt, J. and Morgan, D. O. (1992). Cell cycle regulation of CDK2 activity by phosphorylation of Thr 160 and Tyr 15. *EMBO J.* **11**, 3995-4005.

Guan, K., Broyles, S. S. and Dixon, J. E. (1991). A Tyr/Ser protein phosphatase encoded by vaccinia virus. *Nature* **350**, 359-362.

Guan, K., Haun, R. S., Watson, S. J., Geahlen, R. L. and Dixon, J. E. (1990). Cloning and expression of a protein tyrosine phosphatase. *Proc. Natl. Acad. Sci.* **87**, 1501-1505.

Hoffman, I., Draetta, G. and Karsenti, E. (1994). Activation of the

phosphatase activity of human cdc25A by a cdk2-cyclin E dependent phosphorylation at the G_1/S transition. *EMBO J.* **13**, 4302-4310.

Jinno, S., Suto, K., Nagata, A., Igarashi, M., Kanaoka, Y., Nojima, H. and Okayama, H. (1994). cdc25A is a novel phosphatase functioning early in the cell cycle. *EMBO J.* **13**, 1549-1556.

Kakizuka, A., Sebastian, B., Borgemeyer, U., Hermans-Borgmeyer, I., Bolado, J., Hunter, T., Mooekstra, M. F. and Evans, R. M. (1992). A mouse cdc25 homolog is differentially and developmentally expressed. *Genes Dev.* **6**, 578-590.

Kumagai, A. and Dunphy, W. G. (1992). Regulation of the cdc25 protein during the cell cycle in Xenopus extracts. *Cell* **70**, 139-151.

Lee, M. S., Enoch, T., Piwnica-Worms, H. (1994). *Mik*1 encodes a tyrosine kinase that phosphorylates $p34^{cdc2}$ on tyrosine 15. *J. Biol. Chem.* **269**, 30530-30537.

Lee, M. S., Ogg, S., Xu, M., Parker, L. L., Donaghue, D. J., Maller, J. M. and Piwnica-Worms, H. (1992). $cdc25^{+}$ encodes a protein phosphatase that dephosphorylates $p34^{cdc2}$. *Mol. Biol. Cell.* **3**, 73-84.

Lundgren, K., Walworth, N., Booher, R., Dembski, M., Kirschner, M. and Beach, D. (1991). Mik1 and Wee1 cooperate in the inhibitory tyrosine phosphorylation of cdc2. *Cell* **64**, 1111-1122.

Masui, Y. and Markert, C. L. (1971). Cytoplasmic control of nuclear behavior during meiotic maturation of frog oocytes. *J. Exp. Zool.* **177**, 129-145.

Millar, J. B. A., McGowan, C. H., Lenaers, G., Jones, R. and Russell, P. (1991a) $p80^{cdc25}$ mitotic inducer is the tyrosine phosphatase that activates $p34^{cdc2}$ kinase in fission yeast. *EMBO J.* **10**, 4301-4309.

Millar, J. B. A. and Russell, P. (1992). The cdc25 M-phase inducer: an unconventional protein phosphatase. *Cell* **68**, 407-410.

Millar, J. B. A., Blevitt, J., Gerace, L., Sadhu, K., Featherstone, C. and Russell, P. (1991b). p55cdc25 is a nuclear protein required for the initiation of itosis in human cells. *Proc. Natl. Acad. Sci. USA* **88**, 10500-10504.

Moreno, S. and Nurse, P. (1991) Clues to action of cdc25 protein. *Nature* **351**, 194.

Nargi, J. L. and Woodford-Thomas, T. A. (1994). Cloning and characterization of a cdc25 phosphatase from mouse lymphocytes. *Immunogenetics* **39**, 99-108.

Ogg, S., Gabrielli, B. and Piwnica-Worms, H. (1994). Purification of a serine kinase that associates with and phosphorylates human cdc25c on serine 216. *J. Biol. Chem.* **269**, 30461-30469.

Parker, L. L. Atherton-Fessler, S., Lee, M. S., Ogg, S., Falk, J. L., Swenson, K. I. and Piwnica-Worms, H. (1991). Cyclin promotes the phosphorylation of $p34^{cdc2}$ in a wee1 dependent manner. *EMBO J.* **10**, 1255-1263.

Pratt, H. P. M. (1989). Marking time and making space: chronology and topography in the early mouse embryo. *Int. Rev. Cytol.* **117**, 99-130.

Rossant, J. (1986). Development of extraembryonic cell lineages in the mouse embryo. In: *Experimental Approaches to Mammalian Embryonic Development* (eds. J. Rossant and R. A. Pedersen), pp. 97-120. Cambridge: Cambridge University Press.

Russell, P. and Nurse, P. (1986). cdc25 function as an inducer of the mitotic control of fission yeast. *Cell* **45**, 145-153.

Sadhu, K., Reed, S. I., Richardson, H. and Russell, P. (1990). Human homolog of yeast cdc25 mitotic inducer is predominantly expressed in G2. *Proc. Natl. Acad. Sci. USA* **87**, 5139-5143.

Sambrook, J., Fritsch, E. and Maniatis, T. (1989). *Molecular cloning: A Laboratory Manual*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press .

Schultz, R. M. (1993). Regulation of zygotic gene activation in the mouse. *BioEssays* **15**, 531-538.

Sebastian, B., Kakizuka, A. and Hunter, T. (1993). Cdc25M2 activation of cyclin dependent kinases by dephosphorylation of threonine-14 and tyrosine-15. *Proc. Nat. Acad. Sci. USA* **90**, 3521-3524.

Smith, L. D. and Ecker, R. E. (1971). The interaction of steroids with *Rana pipiens* oocytes in the induction of maturation. *Dev. Biol.* **25**, 232-247.

Strausfield, U., Labbe, J.C., Fesquet, D., Caradore, J. C., Picard, A., Sadhu, K., Russell, P. and Doree, M. (1991). Dephosphorylation and activation of a $p34^{cdc2}$/cyclin B complex *in vitro* by human CDC25 protein. *Nature* **351**, 242-245.

Telford, N. A., Watson, A. J. and Schultz, G. A. (1990). Transition from maternal to embryonic control in early mammalian development: a comparison of several species. *Mol. Reprod. Dev.* **26**, 90-100.

Tessarollo, L., Tsoulfas, P., Martin-Zanca, D., Gilbert, D., Jenkins, N. A., Copeland, N. G. and Parada, L. F. (1993) trk-C, a receptor for neurotropin 3, is widely expressed in the developing nervous system and in non-neuronal tissues. *Development* **118**, 463-475.

Tsarfaty, I., Resau, J., Rulong, S., Keydar, I., Faletto, D. and Vande Woude, G. (1992). The met proto-oncogene receptor and lumen formation. *Science* **257**, 1258-1261.

Tsarfaty, I., Rong, S., Resau, J., Shen, R., da Silva, P. and Vande Woude, G. (1994). Met signaling in mesenchymal to epithelial conversion. *Science* **263**, 98-101.

White-Cooper, H., Alphey L. and Glover, D. M. (1993). The *cdc25* homologue *twine* is required for only some aspects of the entry into meiosis in *Drosophila*. *J. Cell. Sci.* **106**, 1035-1044.

Zinn, K., DiaMaio, D. and Maniatis, T. (1983). Identification of two distinct regulatory regions adjacent to the β-interferon gene. *Cell* **34**, 865-879.

(Accepted 12 April 1995)