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Isolation of the murine cyclin B2 cDNA and characterization of the lineage and temporal specificity of expression of the B1 and B2 cyclins during oogenesis, spermatogenesis and early embryogenesis

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SUMMARY

A cDNA encoding the murine cyclin B2 (*cycB2*) was isolated from an adult mouse testis cDNA library as part of studies designed to identify cyclins involved in murine germ cell development. This *cycB2* cDNA was then used to examine the pattern of *cycB2* expression during male and female germ cell development and in early embryogenesis, and to compare this expression with the previously characterized expression of *cycB1*. A single 1.7 kb *cycB2* transcript was detected by northern blot hybridization analysis of total RNA isolated from midgestation embryos and various adult tissues. Northern blot and in situ hybridization analyses revealed that *cycB2* expression in the testis was most abundant in the germ cells, specifically in pachytene spermatocytes. This is in contrast to the highest levels of expression of cycB1 being present in early spermatids. In situ analysis of the ovary revealed *cycB2* transcripts in both germ cells and somatic cells, specifically in the oocytes and granulosa cells of growing and mature follicles. The pattern of *cycB1* and *cycB2* expression in ovulated and fertilized eggs was also examined. While the steady state level of *cycB1* and *cycB2* signal remained constant in oocytes and ovulated eggs, signal of both appeared to decrease following fertilization. In addition, both *cycB1* and *cycB2* transcripts were detected in the cells of the inner cell mass and the trophectoderm of the blastocyst. These results demonstrate that there are lineage- and developmental-specific differences in the pattern of the B cyclins in mammalian germ cells, in contrast to the co-expression of B cyclins in the early conceptus.

Key words: cell cycle, spermatogenesis, oogenesis, cyclin B

INTRODUCTION

Germ cell development involves mitotic proliferation, meiotic recombination followed by reduction divisions and subsequent differentiation of highly specialized cells, the egg and the sperm. Although these events occur in both male and female germ cell development in mammals, the temporal pattern of their progression is quite different. In mouse, primordial germ cells (PGCs) enter the genital ridge by day 13.5 post-coitum (p. c.). Male germ cells arrest in mitosis and remain arrested until a few days after birth, at which time gonocytes resume mitosis. By day 8 of postnatal (p. n.) development, the first wave of spermatogenetic cells has entered meiosis I (Nebel et al., 1961). By day 22 p. n., these germ cells have completed both meiotic division I and II and have begun the complex morphological changes of spermiogenesis, which transform haploid spermatids into highly specialized spermatozoa (Nebel et al., 1961).

Female mammalian germ cells, on the other hand, enter the embryonic ovary, complete their mitotic divisions and enter into meiosis, arresting at prophase of meiosis I (MI). At birth, oocytes are found in primordial follicles, surrounded by a single layer of granulosa cells. Puberty is marked by the recruitment of a pool of oocytes into a growth period in response to cyclic variations in gonadotropins. During this growth period, the oocyte remains arrested in prophase of MI, but the oocyte itself increases in size. Concomitantly, the number of granulosa cells surrounding the oocyte increases. After completion of this growth and proliferative phase to form the mature follicle, the oocyte is stimulated to resume meiosis. MI is completed and the ovum is ovulated while arrested in MII, awaiting fertilization to complete the second meiotic division (rev. Peters, 1969; Buccione et al., 1990).

The genes involved in controlling the specialized mitotic and meiotic cycles of germ cell differentiation are only now being identified. Maturation promoting factor (MPF) was originally described as an activity found in unfertilized frog eggs capable of inducing germinal vesicle breakdown (GVBD) and resumption of meiosis when injected into immature oocytes (Masui and Markert, 1971). Cytoplasm

from eggs of various organisms, including mouse, has been shown to induce meiotic maturation of *Xenopus laevis* (Sorenson et al., 1985) or starfish oocytes (Hashimoto and Kishimoto, 1988), demonstrating that the meiosis-inducing function of MPF is evolutionarily conserved. Cytoplasm from mitotically active embryonic cells (Wasserman and Smith, 1978) and mammalian tissue culture cells at the G_2 phase of the cell cycle (Sunkara et al., 1979) is also capable of inducing meiotic maturation of *Xenopus* oocytes, suggesting that a common factor is capable of driving cells into M phase of mitotic and meiotic cell cycles.

MPF, now referred to as either the maturation or M phase promoting factor, is composed of two subunits, p34^{cdc2} and cyclin B (Dunphy et al., 1988; Gautier et al., 1988; Labbe et al., 1989). *cdc2*, which was initially identified in the fission yeast *Schizosaccharomyces pombe*, encodes a 34×10^3 M_r serine/threonine protein kinase (Simanis and Nurse, 1986). In fission yeast, p34^{cdc2} kinase activity is required at two points in the cell cycle, the G_1-S and the G_2-M phase transitions (rev. Nurse, 1990). Cyclins, which were originally identified in marine invertebrates, have since been identified in a variety of species ranging from yeast to man (rev. Pines, 1991). The association of cyclin with p34^{cdc2} is required for regulating the phosphorylation state of p34^{cdc2} which in turn controls its kinase activity (rev. Clarke and Karsenti, 1991; Fleig and Gould, 1991). Cyclin may also be involved in conferring substrate specificity on the *cdc2* kinase (rev. Hunt, 1991c).

Both *cdc2* and *cyclin B* are members of gene families. *cdc2*-related or cyclin dependent kinases (*cdk*) have been identified in a number of different species, including human (Elledge and Spottswood, 1991; Tsai et al., 1991; Meyerson et al., 1992), *Xenopus* (Blow and Nurse, 1990; Paris et al., 1991; Gabrielli et al., 1992), *Drosophila* (Lehner and O'Farrell, 1990) and goldfish (Hirai et al., 1992). There is evidence that these *cdk* genes function earlier than *cdc2* in the cell cycle, during G_1-S phase (Fang and Newport, 1991; Elledge et al., 1992; Rosenblatt et al., 1992).

Cyclins have been divided into three major groups, G_1, A and B, based on amino acid homology and timing of their appearance during the cell cycle (rev. Minshull et al. 1989b; Hunt, 1991b; Pines, 1991). Not only are there several groups of cyclins, but there are multiple members in at least two of the groups, including the B-type cyclins (rev. Pines, 1991). Multiple B-type cyclins have been identified in organisms as evolutionarily divergent as *Saccharomyces cerevisiae*, *Xenopus*, mouse and human. Genetic mapping using the human cyclin B1 cDNA as a probe revealed the presence of at least nine B1-related sequences in the mouse genome (Hanley-Hyde et al., 1992; Lock et al., 1992). The roles of the different B-type cyclins are not understood. In general, the B-type cyclins appear during the G_2/M phase transition of the cell cycle, although several recently identified yeast B-type cyclins have been shown to function earlier, specifically in S phase (Epstein and Cross, 1992; Fitch et al., 1992).

We have used mouse gametogenesis as a model system in which to study genes important in the in vivo regulation of the mammalian mitotic and meiotic cell cycles, in particular, the cyclins. Mouse spermatogenesis in particular provides an excellent system to study genes involved in cell cycle control. In the adult testis, one can examine cells in various stages of differentiation within a single lineage, from the self-renewing stem cells, the spermatogonia, to the meiotically dividing spermatocytes, as well as the post-meiotic germ cells, the spermatids. We have previously observed the developmentally regulated pattern of expression of murine *cycB1* in both the male and female germ cells (Chapman and Wolgemuth, 1992). We now describe the isolation of a second murine B-type cyclin, *cyclin B2* (*cycB2*). The pattern of *cycB2* expression during male and female germ cell development was assessed by northern blot and in situ hybridization analyses. *cycB2* is also expressed in a regulated pattern in the testis and ovary, but in a pattern quite distinct from that of *cycB1*. The fate of the *cycB1* and *cycB2* transcripts that accumulate in the fully grown oocytes, during meiotic maturation, at fertilization and during early embryogenesis was addressed by in situ hybridization analysis.

MATERIALS AND METHODS

Source of tissues

Swiss Webster mice, obtained from Charles River (Wilmington, DE) or C57Bl/6J mice, obtained from Jackson Laboratory (Bar Harbor, ME), were used as the source of all adult tissues and cell populations. Adult testes and ovaries were obtained from mice older than 35 days. Ovaries obtained from mice 22 days p. n. were also used for in situ hybridization analysis as indicated. Embryos were collected at the days noted [day 0.5 post-coitum (p. c.) being the day the vaginal plug is detected] and dissected free of extra-embryonic membranes and placentae. The mouse mutant strain *atrichosis (at)* (*ATEB/Le a/a dat/deb*) was obtained from the Jackson Laboratory (Bar Harbor, ME). Dissected tissue specimens were frozen in liquid nitrogen prior to RNA isolation. Dissected tissues for in situ analysis were fixed in 4% paraformaldehyde in 1× PBS (1× PBS: 130 mM NaCl, 7 mM Na_2HPO_4, 3 mM NaH_2PO_4) overnight at 4°C.

Collection of ovulated and fertilized eggs

C57Bl/6J females were superovulated by intraperitoneal injection with 5 I. U. pregnant mare serum gonadotropin (PMSG, Sigma) and 48 hours later with 5 I. U. human chorionic gonadotropin (HCG, Sigma). These females were then either (1) killed 24 hours later to obtain ovulated eggs, (2) mated to C57Bl/6J males and killed 24 hours later to obtain fertilized eggs or, (3) mated to B6 males and killed at day 3.5 p. c. to obtain blastocyst stage embryos. Ovulated eggs were dissected from the ampullae into M16 media (Hogan et al., 1986), cumulus cells were removed by treatment with 300 μg/ml hyaluronidase (Sigma) in M2 media (Hogan et al., 1986) and then moved to fresh M2 media to remove the hyaluronidase. Fertilized eggs were dissected from the ampullae into M2 media and checked visually for the presence of two pronuclei. At day 3.5 p. c., blastocysts were flushed from the uterus into 1× PBS. Ovulated eggs, fertilized eggs and blastocysts were collected with a micropipet and reinjected into host ampullae. The ampullae were then dissected and fixed in 4% paraformaldehyde overnight at 4°C before paraffin embedding for in situ analysis.

Probes

All DNA probes were labeled with [^{32}P]dCTP (DuPont, Wilmington, DE) using Amersham's Multiprime DNA labeling systems (Amersham, Arlington Heights, IL) to a specific activity greater

than 10^9 cts/minute/μg. The human cyclin B1 (HsCycB1) and cyclin B2 (pHsCycB2-2) cDNAs were generous gifts of B. Futcher (Cold Spring Harbor Laboratory). A 1465 bp *Bam*HI-*Sac*I insert of HsCycB1 and a 1300 bp *Sac*II-*Bgl*II insert of pHsCycB2-2 were used as probes for screening a testis cDNA library. A 330 bp *Pst* I fragment of p3.3 (see Fig. 1A) was used as a probe for screening an embryo cDNA library. Sense and antisense RNA probes were transcribed from linearized plasmids using T3 or T7 RNA polymerase (Promega Biotech, Madison, WI), respectively, in the presence of [^{32}P]UTP (DuPont) or [^{35}S]UTP (DuPont) according to protocols suggested by the manufacturer. A [^{32}P]UTP-labeled antisense RNA probe generated from linearized p3.3 was used for northern blot hybridization analyses. Antisense and sense [^{35}S]UTP-labeled RNA probes were generated from linearized p3.3 and p18.2, hydrolyzed to 0.1 kb according to Cox et al. (1984) and were used for in situ hybridization analyses. p18.2 is the plasmid containing the entire coding region of murine cyclin B1 (Chapman and Wolgemuth, 1992).

Screening of testis and embryo cDNA libraries

The preparation of the adult mouse testis cDNA library has previously been described (Chapman and Wolgemuth, 1992). The day 10.5 mouse embryo library was a generous gift of J. Ruiz (Harvard University). Both cDNA libraries were prepared using Stratagene's ZAP-cDNA Synthesis Kit (Stratagene, La Jolla, CA). For each library, 1×10^6 recombinant plaques were screened in duplicate according to Maniatis et al. (1982). Filters were prewashed in 50 mM Tris pH 8.0, 1 M NaCl, 1 mM EDTA, 0.1% SDS at 50°C for 1 hour and prehybridized in 5× SET (1× SET: 100 mM NaCl, 30 mM Tris-HCl pH 8.0, 2mM EDTA), 1× Denhardt's (0.02% Ficoll 400, 0.02% BSA (Fraction V), 0.02% polyvinylpyrolodone), 20 mM $NaPO_4$ pH 7.0, 1% SDS, 0.1 mg/ml denatured and sheared salmon sperm DNA, 0.1 mg/ml *Escherichia coli* DNA at 50°C for 4 hours. Hybridization conditions were identical to those of prehybridization with the addition of ^{32}P-labeled probes to a final concentration of 1×10^6 cts/minute/ml of hybridization solution. Hybridizations were performed at 50°C for 16-20 hours. Filters were washed at a final stringency of 2× SSC (1× SSC: 150 mM NaCl, 15 mM sodium citrate) at room temperature for the testis library and 0.2× SSC, 0.1% SDS at 50°C for the embryo library, air dried and exposed to Kodak XAR film with intensifying screens at −70°C for 16-20 hours. Positive clones were purified by two rounds of plaque purification. Inserts from the tertiary screen were in vivo excised according to Stratagene's protocol. The resulting phagemid contained the cDNA of interest inserted at the *Eco*RI and *Xho*I site of pBluescript SK(−).

DNA sequencing and analysis

Clones were sequenced using the double-stranded sequencing procedure for the Perkin Elmer Cetus *AmpliTaq* Sequencing Kit (Norwalk, CT) as described by the manufacturer. DNA sequence analysis was also performed on an Applied Biosystems Model 373A DNA sequencer (Applied Biosystems, Foster City, CA). Sequences were analyzed using the IBI Pustell Sequence Analysis Software (Pustell, 1988) and the Sequence Analysis Programs for the VAX (Devereux et al., 1984).

RNA isolation and northern blot hybridization analysis

RNA was isolated using the method described by Cathala et al. (1983) or by Auffray and Rougeon (1980). RNA samples were electrophoresed on denaturing 0.8% agarose/2.2 M formaldehyde gels, blotted onto Nytran (Schleicher & Schuell, Keene, NH) membranes and crosslinked by ultraviolet irradiation and baking at 80°C in vacuo for 2 hours. Even loading of the gels and integrity of the RNA were checked by ethidium bromide staining. Hybridizations with a ^{32}P-labeled riboprobe were performed according to Krumlauf et al. (1987). The filters were prehybridized at 65°C for 4 hours in 60% formamide, 5× SSC, 5× Denhardt's, 20 mM $NaPO_4$ pH 7.0, 1% SDS, 0.1 mg/ml sheared and denatured salmon sperm DNA. Hybridization conditions were identical to prehybridization with the addition of dextran sulfate to 7%, tRNA (baker's yeast) to 0.1 mg/ml, poly(A) to 10 μg/ml and [^{32}P]UTP-labeled antisense riboprobe to a final concentration of 10^7 cts/minute/ml of hybridization mixture. Hybridizations were performed at 65°C for 16-20 hours. The filters were washed at a final stringency of 0.2× SSC, 1% SDS at 80°C for 1-2 hours and exposed to Kodak XAR films with intensifying screens at −70°C for the indicated time periods. Sizes of the transcripts were determined by comparison to the ribosomal RNA bands.

In situ hybridization analysis

Paraffin-embedded tissues were cut into 8 μm sections and analyzed by in situ hybridization using the procedure described by Jaffe et al. (1990). Slides were exposed for the indicated time periods. Slides were viewed on a Leitz photomicroscope under brightfield and epiluminescence optics. Photomicrographs were taken using Kodak film.

RESULTS

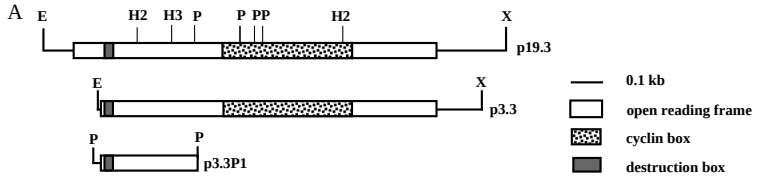
Cloning and sequence analysis of mouse cyclin B2

To identify the cyclins involved in the mitotic and meiotic stages of mouse germ cell development, an adult mouse total testis cDNA library was screened under reduced stringency using human cyclin B1 and B2 cDNAs as probes. Several cDNAs that hybridized with the human B1 cyclin were isolated and characterized (Chapman and Wolgemuth, 1992). Sequence analysis of a 1.2 kb clone, designated p3.3, which hybridized to both the human cyclin B1 and B2 probes, revealed homology to the human cyclin B2 protein (Xiong et al., personal communication; S. Reed, personal communication). Comparison of the sequence to the previously identified B2 cyclins and the absence of an in-frame initiating methionine in the sequence indicated that this cDNA was not full length. In addition, northern hybridization analysis using an antisense riboprobe generated from this 1.2 kb clone revealed a 1.7 kb transcript in total RNA isolated from both adult mouse testes and mid-gestation embryos (data not shown; see below).

To identify a cDNA encoding the entire murine cyclin B2 protein, a day 10.5 post-coitum (p. c.) mouse embryo cDNA library and the adult testis cDNA library were screened using a probe generated from the 5′ region of p3.3 (p3.3P1, Fig. 1A). The longest cDNA, p19.3, was isolated from the embryo library and was approximately 1.5 kb. Sequence analysis of p19.3 revealed an in-frame initiation codon in good agreement with the consensus sequence for the initiation of translation in vertebrates (Kozak, 1987). Restriction maps of p19.3 and p3.3 are shown in Fig. 1A. The nucleotide sequence of p19.3 and predicted amino acid sequence are shown in Fig. 1B. The open reading frame is 1194 nucleotides and is capable of encoding a protein of 398 amino acids with a predicted molecular weight of 44.5×10^3 M_r.

The putative protein contains both the characteristic cyclin box and the destruction box. The cyclin box is the region of shared homology among all cyclins (Minshull et al., 1989b). The destruction box is characteristic of B-type cyclins and is believed to trigger the ubiquitin-mediated pathway of degradation (Glotzer et al., 1991; rev. Hunt,

Fig. 1. (A) Restriction map of p19.3. Location of restriction enzyme sites are shown in relation to the open reading frame (open box) of the mouse *cycB2* cDNA, p19.3. The filled boxes represent the location of the 'destruction box' and the 'cyclin box' as indicated. The location of the probes used in these studies, p3.3 and p3.3P1, are also shown in relation to p19.3. Restriction enzymes are abbreviated as follows: *Eco*RI (E), *Hin*dII (H2), *Hin*dIII (H3), *Pst*I (P)and *Xho*I (X). (B) Nucleotide sequence of p19.3. The nucleotide and deduced amino acid sequence of the mouse *cycB2* cDNA are shown. A termination codon at the end of the coding region is indicated by an asterisk (*). The boxed region indicates the amino acid region that is most highly conserved among cyclins, the 'cyclin box'. Two polyadenylation signals in the 3′ untranslated region are underlined. The sequence begins with an *Eco*RI linker and includes the 3′ untranslated region up to the position of the poly(A) tail. The EMBL accession number for murine cyclin B2 is X66032. (C) Sequence comparison of murine cycB2 and cycB1 proteins. Alignment of the amino acid sequences of murine cycB2 (top line) and murine cycB1 (bottom line) are shown. (D) Sequence comparison of murine and human cycB2 proteins. Alignment of the amino acid sequences of murine cycB2 (top line) and murine cycB1 (bottom line) are shown.



B

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    1  GAATTCGGCACGAGTGGACGCGGAGCGGCGGGGCCCTGACCCTCCCAACGGTGTCGCAGACCGGAGTGGCTGTGCTCGTCCCGACTTGCCAGGGCGCCCT

       M  A  L  L  R  R  P  T  V  S  S  D  L  K  N  I  D  T  E  V  I  P  K  A  K  S  H  V  T  I  R  R  A
  101  CATGGCGCTGCTCCGACGCCCGACGGTGTCCAGTGATTTGAAGAATATTGACACAGAAGTTATTCCTAAAGCCAAGAGCCATGTGACTATCCGGCGGGCA

       V  L  E  E  I  G  N  K  V  R  N  R  T  T  Q  V  A  K  K  P  Q  N  T  K  V  P  A  L  S  T  K  V  T  N
  201  GTTTTAGAAGAAATTGGAAATAAAGTTAGAAACAGAACCACTCAGGTGGCGAAGAAACCTCAGAACACCAAAGTACCAGCTCTGTCCACCAAAGTGACAA

       V  N  K  Q  P  K  P  T  A  S  V  K  P  V  Q  M  E  A  L  A  P  K  D  R  P  P  A  P  E  D  V  S  M
  301  ATGTCAACAAGCAGCCGAAACCCACAGCCTCTGTGAAACCAGTGCAGATGGAGGCGCTAGCTCCCAAGGATCGTCCTCCCGCCCCTGAGGATGTCTCCAT

       K  E  E  S  L  C  Q  A  F  S  D  A  L  L  C  K  I  E  D  I  D  N  E  D  R  E  N  P  Q  L  C  S  D
  401  GAAGGAAGAGAGCCTCTGCCAAGCTTTCTCTGATGCTCTGCTCTGCAAGATCGAGGACATAGATAACGAGGACAGGGAGAACCCTCAGCTCTGCAGTGAC

       Y  V  K  D  I  Y  Q  Y  L  R  Q  L  E  V  L  Q  S  I  N  P  H  F  L  D  G  R  D  I  N  G  R  M  R  A
  501  TACGTGAAGGACATCTACCAGTACCTCAGGCAGCTTGAGGTTTTACAGTCCATTAATCCACACTTCTTAGATGGAAGAGATATAAATGGACGTATGCGTG

       I  L  V  D  W  L  V  Q  V  H  S  K  F  R  L  L  Q  E  T  L  Y  M  C  I  G  I  M  D  R  F  L  Q  A
  601  CCATCCTGGTGGACTGGCTGGTCCAAGTCCATTCCAAGTTTCGGCTTCTGCAGGAAACTCTGTACATGTGCATTGGCATCATGGACCGGTTCCTGCAGGC

       Q  L  V  C  R  K  K  L  Q  V  V  G  I  T  A  L  L  L  A  S  K  Y  E  E  M  F  S  P  N  I  E  D  F
  701  TCAGCTGGTCTGCCGGAAGAAACTGCAGGTGGTCGGGATCACAGCTCTGCTCCTGGCTTCCAAATATGAGGAGATGTTTTCTCCAAATATCGAAGACTTT

       V  Y  I  T  D  N  A  Y  T  S  S  Q  I  R  E  M  E  T  L  I  L  K  E  L  K  F  E  L  G  R  P  L  P  L
  801  GTTTATATCACAGATAATGCTTACACCAGTTCCCAAATCCGAGAAATGGAGACTCTGATTTTGAAAGAGTTGAAATTTGAGTTGGGCCGACCTTTGCCAC

       H  F  L  R  R  A  S  K  A  G  E  V  D  V  E  Q  H  T  L  A  K  Y  L  M  E  L  T  L  V  D  Y  D  M
  901  TTCACTTCTTAAGGCGAGCCTCAAAAGCCGGAGAGGTGGATGTTGAACAGCACACTTTAGCCAAGTACCTGATGGAGCTGACGCTCGTCGACTATGACAT

       V  H  Y  H  P  S  Q  V  A  A  A  A  S  C  L  S  Q  K  V  L  G  Q  G  K  W  N  L  K  Q  Q  Y  Y  T
 1001  GGTGCACTACCATCCTTCTCAGGTGGCAGCCGCTGCCTCTTGCCTGTCTCAGAAGGTGCTGGGCCAAGGAAAATGGAATTTGAAGCAGCAGTATTACACA

       G  Y  M  E  S  E  V  L  E  V  M  Q  H  M  A  K  N  V  V  K  V  N  D  N  R  T  K  F  I  A  V  K  N  K
 1101  GGCTACATGGAGAGTGAAGTCCTGGAAGTCATGCAGCACATGGCCAAGAACGTTGTGAAAGTCAATGACAACCGTACCAAGTTCATCGCTGTCAAGAACA

       Y  A  S  S  R  L  L  K  I  K  H  D  P  Q  L  N  S  K  I  I  K  D  L  V  S  P  L  L  G  S  P  *
 1201  AGTATGCCAGCAGCAGACTCCTGAAGATCAAGCACGATCCTCAGCTGAACTCCAAAATCATCAAAGACCTGGTCTCCCCTCTGCTGGGCAGCCCCTAGGC

 1301  ACTGGACTCCGATCCTGCGCTTCTCAGATCCTGTATGTATTTTATTCTAGTTTACATCACAAACCTCTTCTCAGACTCATTTTCTAATTGTGTATTGATG

 1401  AAAAATAAAGCTATTGATTTTCTTATACTATCCTGTGTATGTTTGTATACTCTACACTGAAGAAATGTAGAATGGCCTACAATAACTCTGCTCTGTATTG

 1501  AAGGAAAATAAAGCTATTGATCTACTTA  1528
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C

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    1 ..MALLRRPTVSSDLK.....NIDTEVIPKAKSHVTIR.RAVLEEIGN
      : : |...:..: |       .: ..|...| |...:| |..|::|||
    1 MALRVTRNTKINAENKAKVSMAGAMRVPVTVTAASKPGLRPRTALGDIGN

   41 KV......RNRTTQVAKKPQNTK..VPALSTKVTNVN.......KQPKP
      ||      | . .. ||. ...|  |.||...|..|          :|.|
   51 KVSEELQARVPLKREAKTLGTGKGTVKALPKPVEKVPVCEPEVELAEPEP

   75 TASVKPVQMEALAP.....KDRPPAPEDVS...MKEESLCQAFSDALLCK
      .:.:..|. | |.|      .:..|.| ::|    || |||||.:|.
  101 EPELEHVREEKLSPEPILVDNPSPSPMETSGCAPAEEYLCQAFSDVILA.

  117 IEDIDNEDRENPQLCSDYVKDIYQYLRQLEVLQSINPHFLDGRDINGRMR
      :.|:|.:| .:|.|||:|||||.|||||  ||:.|.:|:||::.|.||
  150 VSDVDADDGADPNLCSEYVKDIYAYLRQLEEEQSVRPKYLQGREVTGNMR

  167 AILVDWLVQVHSKFRLLQETLYMCIGIMDRFLQAQLVCRKKLQVVGITAL
      |||:|||:||: |||||||:||.:::|:|||:|.  |.:|.|:||:|||:
  200 AILIDWLIQVQMKFRLLQETMYMTVSIIDRFMQNSCVPKKMLQLVGVTAM

  217 LLASKYEEMFSPNIEDFVYITDNAYTSSQIREMETLILKELKFELGRPLP
      ::|||||:.|:|:||.::|:|.||. |||:||  ||: |.|.||||||
  250 FIASKYEEMYPPEIGDFAFVTNNTYTKHQIRQMEMKILRVLNFSLGRPLP

  267 LHFLRRASKAGEVDVEQHTLAKYLMELTLVDYDMVHYHPSQVAAAASCLS
      |||||||.||||||||||||||.::|:|||: |||:||:| ||.
  300 LHFLRRASKVGEVDVEQHTLAKYLMELSMLDCDMVHFAPSQIAAGAFCLA

  317 QKVLGQGKWNLKQQYYTGYMESEVLEVMQHMAKNVVKVNDNRTKFIAVKN
      |:|:.|.|. . |.| :| |..:|.||||:|||||.|| . || :.|||
  350 LKILDNGEWTPTLQHYLSYSEDSLLPVMQHLAKNVVMVNCGLTKHMTVKN

  367 KYASSRLLKIKHDPQLNSKIIKDLVSPLLGSP* 399
      |||.|:  ||.  :|||:.::.:| .::  .
  400 KYAASKHAKISTLAQLNCTLVQNLSKAVTKA*  431
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D

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    1 MALLRRPTVSSDL.........KNIDTEVIPKAKSHVTIRRAVLEEIGN
        ||||||||||          .||||:| .|.|||||||.|||||||
    1 .MALRRPTVSSDLAAQVAKKAQNENIDTGVNSKVKSHVTIRRTVLEEIGN

   41 KVRNRTTQVAKKPQNTKVPALSTKVTNVNKQPKPTASVKPVQMEALAPKD
      :| .|          |||| .||.|||||| |||||..|||| ||||:
   50 RVTTR.........TKVPVQPTKTTNVNKQLKPTASVPKVQMEKLAPKG

   91 RPPAPEDVSMKEESLCQAFSDALLCKIEDIDNEDRENPQLCSDYVKDIYQ
      ..|.|||||||.|||||||||||||||||:|||||||||||||||||||
   90 PSPTPEDVSMKEENLCQAFSDALLCKIEDIDNEDWENPQLCSDYVKDIYQ

  141 YLRQLEVLQSINPHFLDGRDINGRMRAILVDWLVQVHSKFRLLQETLYMC
      ||:|||||||||||||||||||||||||||:|||||||||||||||||||
  140 YLKQLEVLQSINPHFLDGRDINGRMRAILVGWLVQVHSKFRLLQETLYMC

  191 IGIMDRFLQAQLVCRKKLQVVGITALLLASKYEEMFSPNIEDFVYITDNA
      :|||||||||.| |:|||||:|||||||  ||||||||||||||||||||
  190 VGIMDRFLQVQPVSRKKLQLVGITALLALSKYEEMFSPNIEDFVYITDNA

  241 YTSSQIREMETLILKELKFELGRPLPLHFLRRASKAGEVDVEQHTLAKYL
      ||||||||||||||||||||||||||||||||||||||||||||||||||
  240 YTSSQIREMETLILKELKFELGRPLPLHFLRRASKAGEVDVEQHTLAKYL

  291 MELTLVDYDMVHYHPSQVAAAASCLSQKVLGQGKWNLKQQYYTGYMESEV
      ||||:|||||.||.|||||||||||||||||||||||||||||| |.||
  290 MELTLIDYDMVHYPPSKVAAAASCLSQKVLGQGKWNLKQQYYTGYTENEV

  341 LEVMQHMAKNVVKVNDNRTKFIAVKNKYASSRLLKIKHDPQLNSKIIKDL
      ||||||||| |||||:| ||||||:|||||:||:  |||||||| :|||
  340 LEVMQHMAKMVVKVNENLTKFIAIKNKYASSKLLRSAHDPQLNSKRVKDL

  391 VSPLLGSP* 399
      .|||:|..
  390 ASPLIGRS* 397
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1991a). Where colinear, p19.3 and p3.3 are identical. Interestingly, p19.3 contains two consensus polyadenylation signals in its 3′ untranslated region. Both polyadenylation signals appear to be used in mRNAs from testis and embryos, since representative cDNAs of both classes were isolated from both libraries. The use of the alternative polyadenylation signals generates mRNAs that differ by approximately 100 nt in their 3′ untranslated regions.

Analysis of the predicted protein product of p19.3 revealed that it is more closely related to the B2 cyclins than to the B1 cyclins. Similarities to the B2 cyclins of *Xenopus* (Minshull et al., 1989a), chicken (Gallant and Nigg, 1992) and human, were 76%, 79% and 90%, respectively, at the amino acid level. This is in contrast to the similarities of 67.5%, 68% and 68% when compared to the cyclin B1 of *Xenopus* (Minshull et al., 1989a), mouse (Chapman and Wolgemuth, 1992) and human (Pines and Hunter, 1989), respectively. Comparisons of the murine cycB2 and cycB1 proteins and of the murine and human cycB2 proteins are shown in Fig. 1C and D, respectively. The gene encoding p19.3 has thus been designated murine *cyclin B2*, *cycB2*.

Expression of murine *cycB2* in adult and embryonic samples

Northern blot hybridization analyses, using an antisense riboprobe generated from p3.3, were performed to determine the tissue distribution of *cycB2* transcripts. A 1.7 kb transcript was detected in total RNA isolated from adult spleen and intestine, but was not observed in total RNA isolated from adult brain, heart, lung, liver or kidney (data not shown). A 1.7 kb *cycB2* transcript was also detected in the two germ cell-containing tissues, the ovary and testis, as well as in day 12.5 p. c. embryos (Fig. 2). A 1.7 kb transcript was also detected by northern analysis of total RNA isolated from mid-gestation embryos, from day 10.5 to day 17.5 p. c. and from day 11.5 p. c. placenta (data not shown).

To determine the cellular specificity of *cycB2* expression in the adult testis, whether germinal or somatic, total RNA was isolated from the testes of mice homozygous and heterozygous for the *atrichosis* (*at*) locus. Mice homozygous for the *at* locus are devoid of germ cells, but have the normal complement of somatic cell types, including Leydig, Sertoli and peritubular myoid cells (Hummel, 1964). The heterozygous littermates have the normal somatic and germ cell complements and are fertile. By northern blot hybridization, a 1.7 kb transcript was detected in total RNA from *at/+* testes, but not in RNA isolated from the testes of *at/at* mice (Fig. 2). The absence of a *cycB2* transcript in the homozygous mutant testis indicated that the *cycB2* mRNA was most likely localized to the germ cell compartment of the testis, as has been demonstrated for a number of genes expressed in the testis, i.e. *Hox 1.4* (Wolgemuth et al., 1987) and *Zfp-37* (Burke and Wolgemuth, 1992). Alternatively, it is possible that *cycB2* is also expressed in the somatic cells but requires the presence of germ cells for its proper expression, as was observed for the level of POMC expression in the somatic cell compartment, which was influenced by the presence or absence of germ cells (Gizang-Ginsberg and Wolgemuth, 1987).

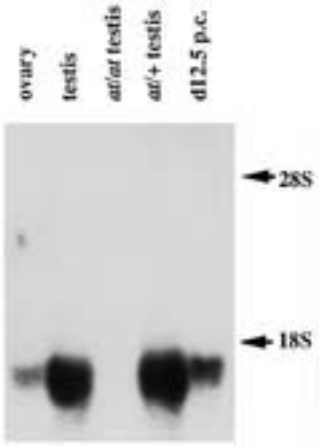

Fig. 2. Distribution of *cycB2* transcripts in mouse tissues. Each sample contained 20 μg of total RNA from day 12.5 p. c. embryos, ovary and from the testes of adult SW, *at/+* and *at/at* mice as indicated. Hybridization was with a ^{32}P-labeled p3.3 antisense riboprobe. Exposure time: 24 hours.

In situ hybridization localizes *cycB2* transcripts to spermatocytes and early spermatids

To localize *cycB2* expression further to particular testicular cells, in situ hybridization using ^{35}S-labeled sense and antisense RNA probes was performed. The results of in situ hybridization analysis of *at/+* and *at/at* testes are shown in Fig. 3. In agreement with the results from northern blot hybridization experiments, no signal above background was observed over the somatic cells in the germ-cell-deficient (*at/at)* testes (Fig. 3A,B). Furthermore, no signal was detected over the somatic cells in the heterozygous, fully fertile *at/+* sections (Fig. 3C-H). This indicated that the *cycB2* transcript detected by northern analysis in the testes samples was indeed due to germ cell and not somatic cell expression. No signal above background was detected in either *at/+* or *at/at* testis sections using the sense probe (data not shown).

Expression of *cycB2* was readily detected within tubules from the testes of the fertile heterozygous mice (Fig. 3C-H). However, the most intense labeling was observed over only a subset of these tubules. Analyses of histological cross-sections of seminiferous tubules have revealed that germ cells at distinct stages of spermatogenesis are always found in specific patterns of association with one another (Oakberg, 1956; rev. Russell et al., 1990). These defined groups of cellular associations arise because a new round of spermatogenesis is initiated every 12 days, but the entire process lasts 35 days. The various cellular associations can be divided into twelve distinct stages, which together make up a complete cycle of the seminiferous epithelium (Oakberg, 1956; rev. Russell et al., 1990). Determination of the stage of a specific tubule allows for more precise identification of the spermatogenic cells present in the tubule.

The stages of individual tubule indicated by Roman numerals in Fig. 3C-H were classified according to Oakberg (1956) and Russel et al. (1990). A stage I tubule, which contains primarily early round and elongating spermatids, is shown in Fig. 3C and D. The darkly staining cells in the basal compartment of the tubule are spermatocytes that have just entered the pachytene stage of meiosis I. No signal above background was detected in these early pachytene spermatocytes. *cycB2* transcripts were, however, detected in the early round spermatids, located in the middle layer of the tubule. By stage VII, a low level of signal was

detected in the spermatocytes that had reached the mid-pachytene stage (Fig. 3E,F). No *cycB2* signal was observed in the preleptotene spermatocytes that lie in the basal compartment of the tubule. The highest level of *cycB2* signal was detected in stage X-XI tubules, which contain predominantly pachytene and diplotene spermatocytes in the

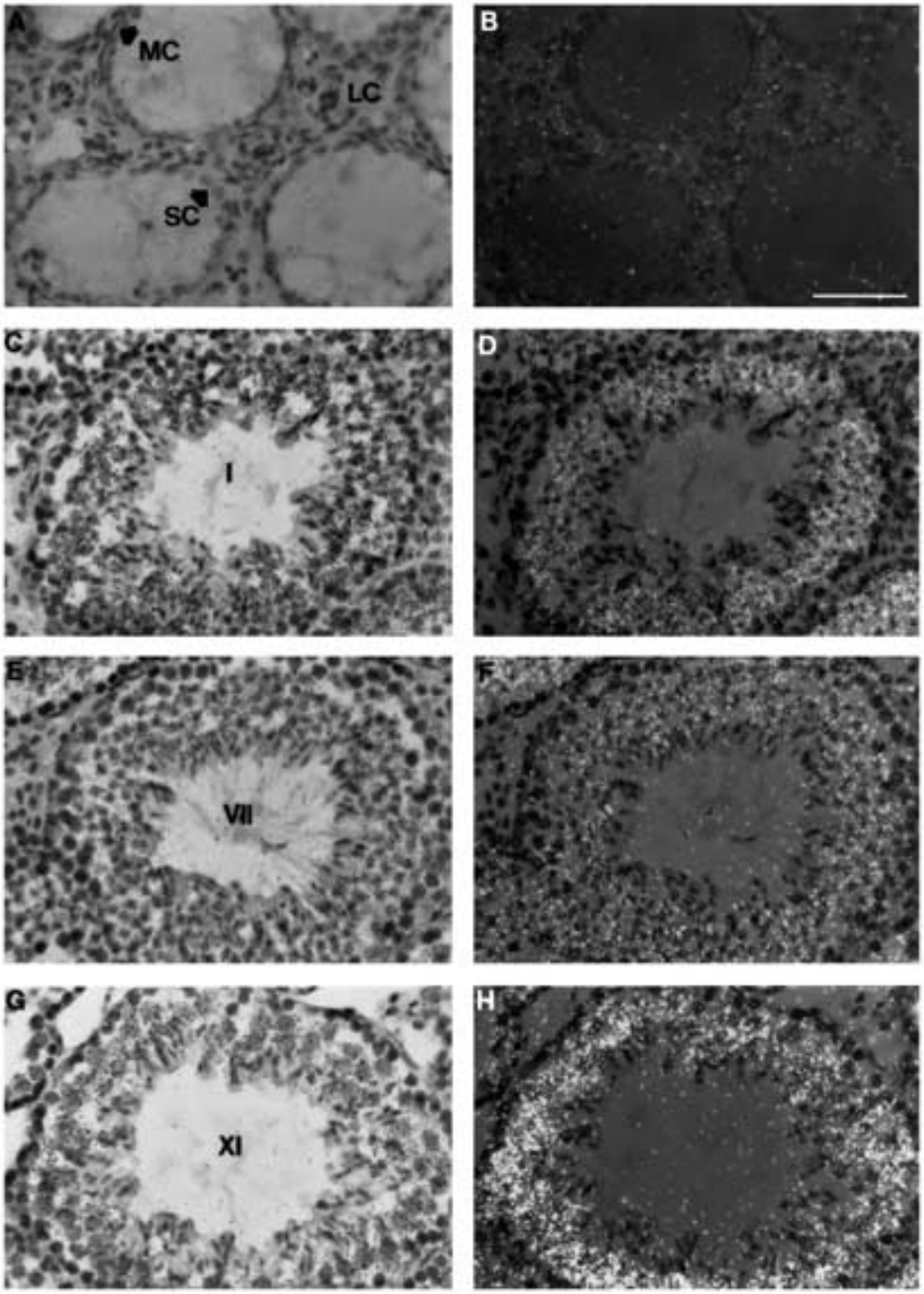

Fig. 3. Cellular localization of *cycB2* transcripts in the testis by in situ hybridization. Testis sections were photographed using bright-field (A, C, E and G) and epiluminescence (B, D, F and H) optics. Hybridization was with a ^{35}S-labeled antisense p3.3 riboprobe. (A,B) Testis sections from *at/at* mice; (C-H) Testis sections from *at/+* mice. The stages of the seminiferous epithelium are designated by Roman numerals in C-H. Cell types are designated in A as follows: Leydig cells (LC), Sertoli cells (SC) and peritubular myoid cells (MC). Exposure time: 10 days. Size bar, 100 μm.

center layer of the tubule. A stage XI tubule is shown in Fig. 3G,H. Spermatocytes in leptotene and zygotene of meiosis I are also present in stage X-XI tubules; however, no signal above background was detected. In situ hybridization analysis of testes from day 7 p. n. mice, where the spermatogonia are clearly seen, did not reveal any *cycB2* signal above background in these mitotically dividing germ cells (data not shown). Secondary spermatocytes are found in only a very small portion of the tubules present in the adult testis due to the brevity of this particular stage. Nonetheless *cycB2* transcripts were also detected in these cell types (data not shown). No signal above background was detected over the elongating spermatids found in the adluminal region of the tubules (Fig. 3C-H). This pattern of cycB2 expression is in striking contrast with that of *cycB1* expression, wherein *cycB1* transcripts were detected at low levels in late pachytene spermatocytes and at highest levels in the round spermatids (Chapman and Wolgemuth, 1992). These distinct patterns of expression in male germ cells are summarized in the diagram in Fig. 4.

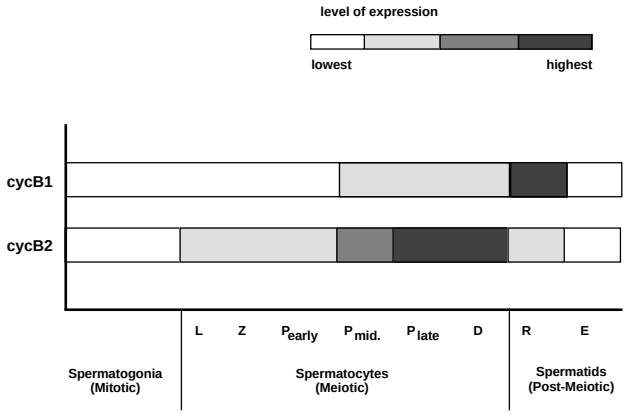

Fig. 4. Summary of *cycB1* and *cycB2* expression during spermatogenesis. The relative levels of *cycB1* and *cycB2* expression are indicated by the stippled box. The spermatogenic cell types are indicated as follows: leptotene (L), zygotene (Z), pachytene (P), diplotene (D), round spermatids (R) and elongated spermatids (E). The pachytene stage is divided into early, middle (mid.) and late stages.

Localization of *cycB2* signal to both germinal and somatic cells in the ovary

Northern blot hybridization analysis revealed that *cycB2* transcripts were also present in the ovary. To determine if *cycB2* was expressed in the germ cells, as in the testis, or in the somatic cells, sections of ovaries were examined by in situ hybridization (Fig. 5). Developing ovarian follicles were staged by the size of the oocyte and by the number of layers of granulosa cells surrounding the oocyte according to Pedersen and Peters (1968). Oocytes that have not entered the growth phase, called resting oocytes, are found in follicles staged 1 through 3a, while growing oocytes are found in follicles staged 3b through 6. Fully grown oocytes are found in follicles of stages 7 and 8, in which meiosis resumes. No *cycB2* signal was detected in the resting oocytes (Fig. 5A,B). *cycB2* signal was first detected at low levels in growing oocytes of follicles staged 3b. *cycB2* transcripts were detected in oocytes as they continued to grow, through to the antral stages (Fig. 5A-D). No obvious difference was noted in the level of *cycB2* transcripts in oocytes in later stage follicles. The pattern of accumulation of *cycB2* transcripts in the growing oocytes was thus quite similar to that observed for *cycB1* transcripts (Chapman and Wolgemuth, 1992).

cycB2 expression was also readily detected in somatic cells of developing follicles, the granulosa cells. This is in contrast to *cycB1*, which is expressed most abundantly in the germ cells of the ovary and at very low levels in the granulosa cells of larger follicles (Chapman and Wolgemuth, 1992). *cycB2* transcripts were detected over the granulosa cells, in particular granulosa cells of follicles in stage 5 and larger (Fig. 5A-D). Within the granulosa cells of stage 7 and 8 follicles (antral follicles), *cycB2* transcripts were not evenly distributed. Rather, *cycB2* transcripts were most abundant over those granulosa cells closest to the oocyte, the cumulus granulosa cells (Fig. 5C,D). Within the adult ovary, there are also follicles that stop growing and begin to degenerate. These are known as atretic follicles (Peters, 1969). When compared to a healthy follicle, the level of *cycB2* signal over the granulosa cells in atretic follicles appeared to be diminished while a low level of *cycB2* signal was still detectable in the oocytes (data not shown).

cycB1 and *cycB2* transcripts are present following ovulation and in the early embryo

The above results on *cycB2* and our previous observations on *cycB1* expression (Chapman and Wolgemuth, 1992) demonstrate that these murine B-type cyclins are expressed in a developmentally regulated manner in the adult ovary, but in quite distinct patterns. *cycB1* is expressed most abundantly in the germ cells, particularly in growing oocytes, while *cycB2* is expressed at apparently comparable levels in both the growing oocyte and in a subset of the somatic cells of the developing follicle. To determine whether the relative levels of *cycB1* and *cycB2* mRNAs change as oocytes undergo meiotic maturation and fertilization, in situ hybridization was used to examine *cycB1* and *cycB2* expression in histological sections of ovarian oocytes, ovulated eggs and fertilized eggs (Fig. 6). As previously observed, *cycB1* and *cycB2* transcripts were detected in oocytes (Fig. 6A,D). While both *cycB1* and *cycB2* transcripts were detected in ovulated (Fig. 6B,E) and fertilized eggs (Fig. 6C,F), the relative levels of both appeared to decrease in the fertilized eggs.

To determine whether both *cycB1* and *cycB2* are expressed during early embryonic development, blastocyst-staged embryos were examined by in situ hybridization analysis. *cycB1* and *cycB2* transcripts were detected in cells constituting both the inner cell mass and the trophectoderm of the blastocyst (Fig. 7). Thus while *cycB1* and *cycB2* are expressed differentially in specific somatic and germinal lineages during male and female germ cell development, they are co-expressed in both the embryonic and extraembryonic cell lineages at apparently comparable levels during the early stages of embryogenesis.

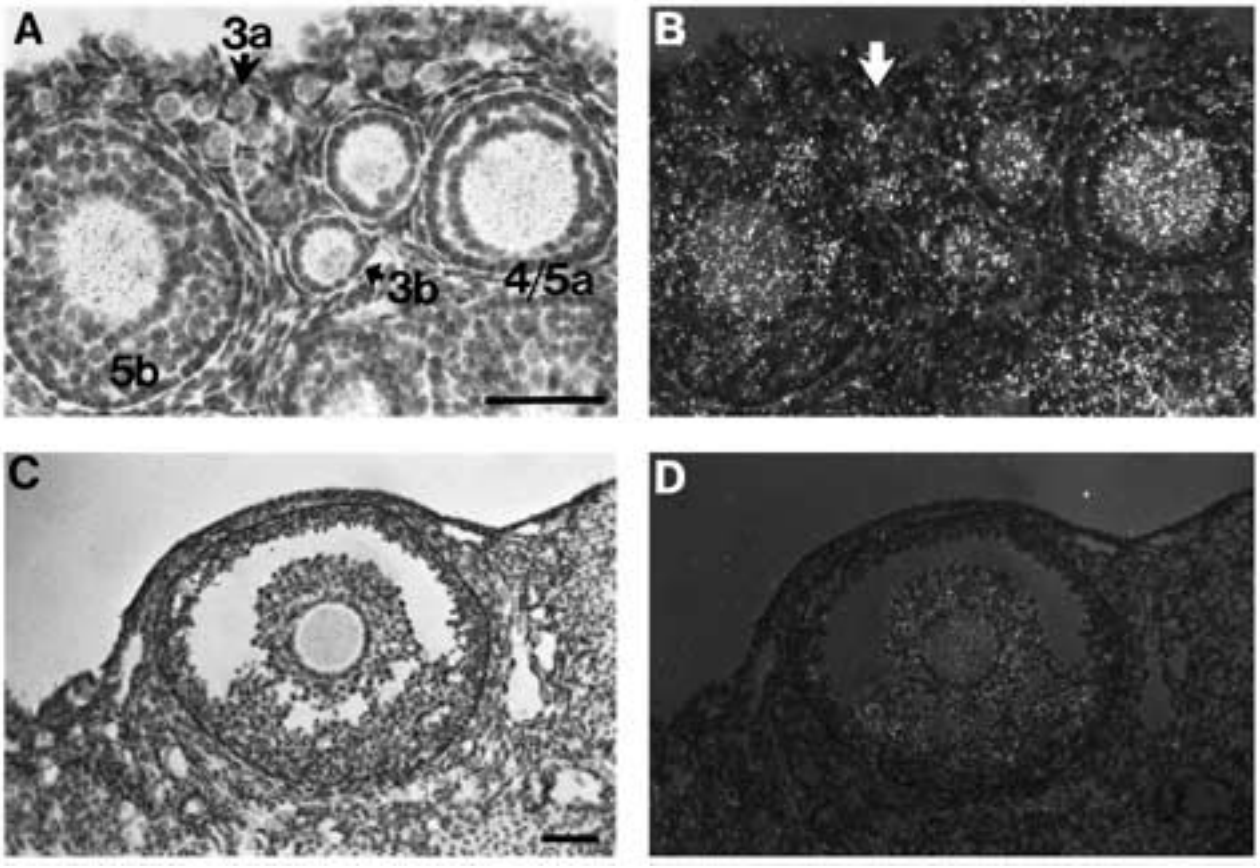

Fig. 5. Cellular localization of *cycB2* signal in the mouse ovary by in situ hybridization. Photomicrographs were taken using bright-field (A,C) and epiluminescence (B,D) optics. (A,B) Sections of a day 22 p. n. mouse ovary; (C,D) sections of an ovary from a mouse older than 35 days. The stages of the follicles are indicated in A. Resting oocytes, found in follicles stage 3a, are indicated by a white arrow in B. Hybridization was with a ^{35}S-labeled antisense p3.3 riboprobe. Exposure time: 14 days. Size bars, 100 μm.

DISCUSSION

Isolation of murine *cycB2*

Given the distinct temporal and chronological progression of mitosis and meiosis in female and male germ cells in mammals, it is likely that there are unique cell cycle control points in each lineage. Although the adult testis contains germ cells undergoing both mitotic and meiotic cell divisions, little or no attention has been focused on the role of the cell cycle genes in these processes. The high degree of evolutionary conservation of the genes that control the cell cycle has facilitated the isolation of their homologs in various species. We were interested in examining cyclin B expression during the mitotic and meiotic cell cycles of mouse germ cell development. In this paper, we present the isolation and sequence of a cDNA encoding a new murine B-type cyclin, *cycB2*. The putative *cycB2* protein contains the characteristic 'cyclin box' and 'destruction box' and shows higher homology to the previously identified cyclin B2 proteins than the cyclin B1 proteins. *cycB2* encodes a 1.7 kb transcript that was detected in both the adult ovary and testis by northern blot analysis. Since *cycB1* had previously been shown to be expressed in the testis and ovary, specifically in germ cells, the pattern *cycB2* expression during germ cell development was determined, with particular focus on whether its expression was similar to or distinct from that of *cycB1*.

Cyclin B expression in the testis

Northern blot and in situ hybridization analysis revealed that, in the testis, *cycB2* was present at highest levels in the meiotically dividing spermatocytes. This is in contrast to the distribution of *cycB1* transcripts, which were most abundant in the post-meiotic germ cells in the testis, the spermatids. Lower levels of *cycB2* mRNAs were also detected in the early round spermatids. It is curious that neither *cycB2* nor *cycB1* signal was detected in the mitotically dividing spermatogonia. This could be because the level of *cycB1* and *cycB2* transcripts were simply below the level of sensitivity of the in situ hybridization procedure. Alternatively, since at least nine cyclin B loci have been localized in the mouse genome (Lock et al., 1992; Hanley-Hyde et al., 1992), a different cyclin B could be responsible for activating p34^{cdc2} in these cells.

Cyclins function as the regulatory subunit of the p34^{cdc2}/cyclin complex. Synthesis of cyclin B is capable of driving cells into M phase of mitotic and meiotic cell cycles (Minshull et al., 1989a; Murray and Kirschner, 1989; Murray et al., 1989; Westendorf et al., 1989). Based on this knowledge of cyclin function in cell cycle progression, the

pattern of *cycB2* expression in the testis more closely follows a predicted pattern of expression than does that of *cycB1*. High levels of *cycB2* transcripts in the meiotically dividing germ cells lends further support for a *cycB2* role in the meiotic cell divisions of germ cell development. Our finding of high levels of *cycB1* transcripts in the post-meiotic male germ cells was surprising (Chapman and Wolgemuth, 1992). However, since we are only now beginning

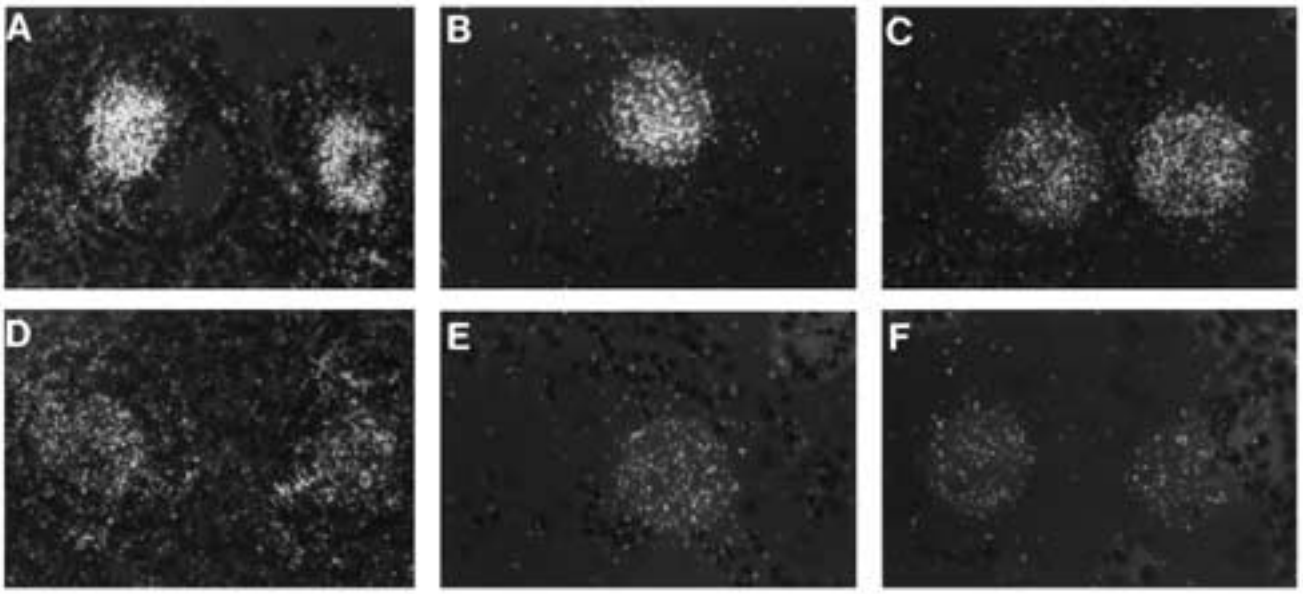

Fig. 6. *cycB1* and *cycB2* transcripts in oocytes, ovulated eggs and fertilized eggs. Photomicrographs were taken using epiluminescence optics. Hybridization was with ^{35}S-labeled antisense p18.2 riboprobe representing *cycB1* (A-C) and ^{35}S-labeled antisense p3.3 riboprobe representing *cycB2* (D-F). Exposure time: 21 days. Size bar, 100 μm.

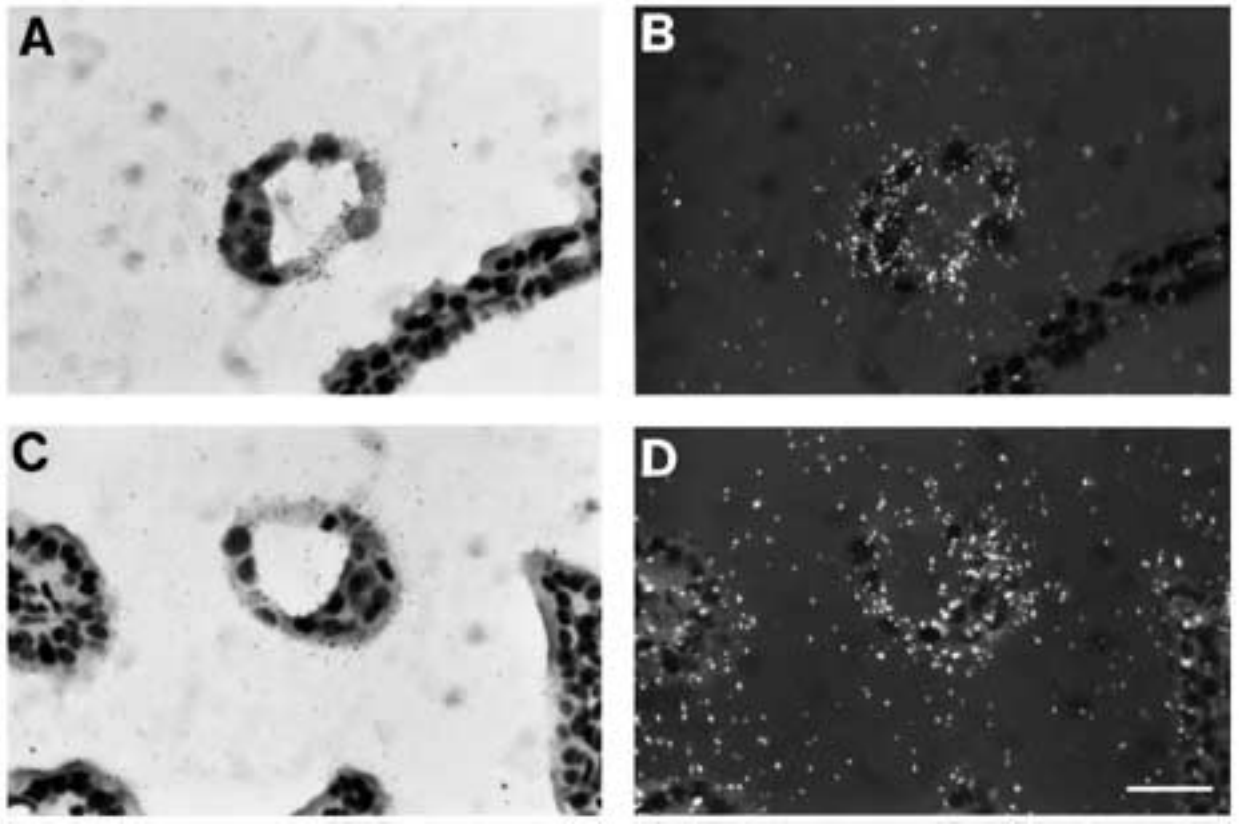

Fig. 7. *cycB1* and *cycB2* signal in blastocysts. Photomicrographs were taken using bright-field (A,C) and epiluminescence (B,D) optics. (A,B) Hybridized with a ^{35}S-labeled antisense p18.2 (*cycB1*); (C,D) hybridized with a ^{35}S-labeled antisense p3.3 (*cycB2*). Exposure time: 21 days. Size bar, 100 μm.

to understand the way in which cyclins regulate *cdc2* kinase activity, these results may implicate a function for *cycB1* which does not involve cell cycle progression. Instead, *cycB1* could be involved in directing kinase activity to specific substrates which themselves are involved in germ cell differentiation.

Cyclin expression during oogenesis and early embryogenesis

Our results on *cycB1* and *cycB2* expression during murine oogenesis also revealed surprisingly different patterns. In situ analysis revealed that, whereas *cycB1* transcripts were most abundant in the growing oocytes, *cycB2* transcripts were present in the granulosa cells of growing and mature follicles, as well as in growing oocytes. The steady state levels of *cycB1* and *cycB2* did not change dramatically following ovulation; however, the levels of both appeared to drop following fertilization. In stage 7 and 8 follicles, *cycB2* transcripts were most abundant over the granulosa cells closest to the oocyte. In these antral follicles, the granulosa cells can be divided into two groups. The mural granulosa cells are attached to the basement membrane that encloses the follicle and the cumulus granulosa cells are attached to the oocyte (rev. Buccione et al., 1990). In the rat ovary, the cumulus granulosa cells have been shown to divide more frequently than the mural granulosa cells in these antral follicles (Hirshfield, 1986). Our results are therefore consistent with a role for *cycB2* in mitotic proliferation.

It is not yet known whether cycB1 and cycB2 are functionally interchangeable in vivo. *cycB1* and *cycB2* cDNAs have been identified in only a few species and studies on the regulation of the cell cycle in higher eukaryotes has been largely confined to vertebrate cell lines in vitro. In one of the few in vivo studies, expression of both *cycB1* and *cycB2* was shown to peak around the G_2-M phase transition in regenerating rat liver (Lu et al., 1992). Mouse *cycB1* and *cycB2* have been shown to be co-expressed in both embryonic and extraembryonic cell lineages in early embryogenesis (our above observations) as well as in a variety of adult tissues, including spleen, intestine, ovary and testis (Chapman and Wolgemuth, unpublished observations). By determining the lineage and stage-specific sites of expression of *cycB1* and *cycB2* in the ovary and testis, we have discovered a surprising uncoupled expression of the two genes in specific cells. We observed that *cycB1* and *cycB2* are co-expressed but are differentially modulated in a number of cell types (pachytene spermatocytes, early spermatids and cumulus granulosa cells; see Fig. 4).

In the only other vertebrate system in which *cycB1* and *cycB2* expression during oogenesis has been studied in detail, Kobayashi et al. (1991) found that in *Xenopus*, the steady state level of *cycB1* and *cycB2* transcripts remained constant from the earliest stages of oogenesis until after fertilization. This is in contrast to our observations that during mouse oogenesis, the steady state level of *cycB1* and *cycB2* transcripts increased as the oocytes grew and dropped following fertilization. These distinct patterns of cyclin message accumulation may be due to differences in the reliance of later development on stored mRNAs and proteins in the two species. In *Xenopus*, zygotic transcription does not begin until after the twelfth mitotic division, at the time of the midblastula transition (Newport and Kirschner, 1982a,b). Mouse zygotic transcription, however, begins by the two-cell stage (Braude et al., 1979; Flach et al., 1982). Therefore the accumulation of maternal RNAs and proteins in the egg is critical for the early stages of amphibian development, whereas it may not play such a vital role in mouse development.

While the levels of *cycB1* and *cycB2* transcripts did not change during *Xenopus* oogenesis, the levels of protein did (Kobayashi et al., 1991). cycB2 was the major cyclin protein during oogenesis. The level of cycB1 protein reached that of cycB2 in the matured egg. An interesting possibility suggested by these observations was that the MPF kinase prior to MI was different than that present in the MII-arrested eggs. We have shown that both *cycB1* and *cycB2* transcripts are present in mouse oocytes, ovulated eggs and fertilized eggs. Whether the levels of *cycB1* and *cycB2* transcripts reflect the corresponding amounts of protein remains to be determined. It will be interesting to see whether different MPF species exist during mouse oogenesis as exist in *Xenopus*. It is not clear whether different cyclins can direct $p34^{cdc2}$ to specific substrates (rev. Hunt, 1991c). However, it is interesting to speculate that the differential pattern of *cycB1* and *cycB2* expression observed in the germ and somatic cell lineages of the testis and ovary may reflect differences in the preferred $p34^{cdc2}$ substrate. Furthermore, some of these substrates may not be involved with cell cycle progression per se, but rather with other cellular events such as chromatin remodeling and nuclear condensation.

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