Characterization and Expression of Mammalian Cyclin B3, a Prepachytene Meiotic Cyclin*  

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We report the identification and expression pattern of a full-length human cDNA and a partial mouse cDNA encoding cyclin B3. Cyclin B3 (CCNB3) is conserved from Caenorhabditis elegans to Homo sapiens and has an undefined meiotic function in female, but not male Drosophila melanogaster. We show that H. sapiens cyclin B3 interacts with cdk2, is localized to the nucleus, and is degraded during anaphase entry after the degradation of cyclin B1. Degradation is dependent on sequences conserved in a destruction box motif. Overexpression of cyclin B3 is expressed in HeLa cells, it is nuclear, interacts with cdk2 and cdc2, and is associated with a modest histone H1 kinase activity (15). In Drosophila, cyclin B3 is expressed in both mitotic and meiotic cells. In mitotic cells, it is required when either cyclin A or cyclin B1 is absent. Its abundance is down-regulated during the metaphase to anaphase transition, following degradation of cyclin B1 (16). Overexpression of a nondegradable cyclin B3 is associated with chromatin decondensation defects at the end of mitosis (17). Cyclin B3 is essential for fertility; unlike cyclin B1 deficiency, infertility of cyclin B3-deficient flies is restricted to the female germ line and does not appear to involve defects in ovary structure or in meiosis I entry (16).

We report the cloning of a full-length human cyclin B3 cDNA and a partial mouse cyclin B3 cDNA. Like the previously described chicken and Drosophila homologs, when mammalian cyclin B3 expression is enforced in mitotic cells, it is nuclear, is degraded upon anaphase entry following cyclin B1 degradation, and it is a poor activator of cdk2 kinase. We could not detect endogenous cyclin B3 in cell lines from a wide variety of cell types. In contrast, we readily observed cyclin B3 mRNA and protein in prepachytenocytes, specifically during the leptotene and zygote spermatocytes. The expression pattern of mammalian cyclin B3 suggests that it may be important for events occurring in early meiotic prophase I.

Cyclins are positive regulatory subunits of the cyclin-dependent kinases (cdks).1 Cyclins increase kinase activity by inducing structural changes in the cdk (1). Cyclins also provide substrate binding motifs (2, 3), signals that localize cdk4 (4, 5), and motifs that allow the incorporation of the cdk into higher order molecular complexes (6, 7). Although first identified as regulators of mitotic cell cycle transitions, cdk-cyclin complexes have other roles: cyclin C-cdk8 and cyclin H-cdk7 regulate transcription as part of the polymerase II holoenzyme (8–10), pho80-pho85 regulates the localization of the pho4 transcription factor (11), p35-cdk5 regulates neuronal function (12, 13), and cyclin A1, with an unidentified cdk, regulates the pachytenocyte to diplotene transition during male gametogenesis (14).

Originally cloned from a chicken cDNA library, cyclin B3 is expressed at very low levels in virally transformed hematopoietic cell lines and early embryonic stages of chicken. When Gallus gallus cyclin B3 is expressed in HeLa cells, it is nuclear, interacts with cdk2 and cdc2, and is associated with a modest histone H1 kinase activity (15). In Drosophila, cyclin B3 is expressed in both mitotic and meiotic cells. In mitotic cells, it is only required when either cyclin A or cyclin B1 is absent. Its abundance is down-regulated during the metaphase to anaphase transition, following degradation of cyclin B1 (16). Overexpression of a nondegradable cyclin B3 is associated with chromatin decondensation defects at the end of mitosis (17). Cyclin B3 is essential for fertility; unlike cyclin B1 deficiency, infertility of cyclin B3-deficient flies is restricted to the female germ line and does not appear to involve defects in ovary structure or in meiosis I entry (16).

MATERIALS AND METHODS

Identification of Cyclin B3 cDNAs—The sequence data for the human cyclin B3 cDNA, mouse cyclin B3 cDNA, and L23 BAC have been submitted to the GenBank database base under the accession numbers AJ416458, AJ416459, and AJ416884—6, respectively.

Antibodies—We generated and purified our own human cyclin B3 antibody based on procedures we described previously (18). Briefly, a fragment of cyclin B3 spanning amino acids 997–1395 was cloned into pGEX-5X (Amersham Biosciences). Recombinant protein was purified on glutathione-agarose beads (Sigma) and used as antigen. A bacterially expressed cyclin B3 (amino acids 997–1395) tagged with His6 at the C terminus was coupled to CNBr-activated Sepharose (Sigma) and used to affinity purify the antibody.

We also used antibodies to cdk2 (M2, Santa Cruz), cdk2 (A12, Santa Cruz).
Cyclin B3

FIG. 1. Evolutionary conservation of cyclin B3. A, cyclin B3 proteins from human, chicken, Drosophila, and C. elegans. Solid boxes indicate the conserved amino acids. The conserved regions of the putative destruction box (db, gray) and cyclin box (cb, black) are 48–62 and 274–290 amino acids in length, respectively. Conservation in each region is shown as percent identity (% ID) after pairwise alignment. Numbers to the right indicate the length of the protein. B, cyclin B3 is most closely related to cyclins of the A and B type. Cyclin box sequences from C. elegans (Ce), chicken (Gg), mouse (Mm), human (Hs), and Drosophila (Dm) were used for the ClustalX program.

Cruz), and mouse IgG (Zymed Laboratories Inc.) in the course of this work.

Immunoprecipitation and Kinase Assay—In vitro translation and reaction translations were performed as prescribed by the manufacturer (Promega). For producing transcripts, cyclin B3 and cyclin B1 the full-length cDNAs were cloned into pCITE1 from Clontech. To generate the destruction box mutant (pEGFP-C3–62A), mutations R60A, F63A, and N68A were introduced into the pEGFP-C3-cyclin B3 construct by site-directed mutagenesis (QuikChange, Stratagene).

HeLa cells cultured in Dulbecco's modified Eagle's medium were synchronized in G2 phase by a thymidine/aphidicolin block as described previously (19). The plasmids pEGFP-C3-cyclin B3, pEYFP-C3-cyclin B3, pEGFP-C3–3XA, and ECFP-N1-cyclin B1 (a gift from Anja Hagting) were diluted in 10 mM Tris and 1 mM EDTA (pH 8) to 50 ng/μl. Human cyclin B3 can associate with cdk2.

FIG. 2. Human cyclin B3 can associate with cdk2. A, mobility of in vitro translated cyclins. Rabbit reticulocyte lysate was programmed with mRNA encoding the cyclins indicated above each lane, and [35S]methionine-labeled products were resolved on 10% SDS-polyacrylamide gels and detected by autoradiography. HMG1 is the cyclin box-containing fragment (amino acids 918–1395) of full-length cyclin B3 (see “Results”). The migration of molecular weight markers is indicated on the left. B, cyclin B3 binds cdk2 but fails to activate its histone H1 kinase activity. After translation of the mRNA (indicated above each lane), reactions were stopped with Nonidet P-40 radioimmune precipitation buffer (see “Materials and Methods”) and to immunoprecipitate cdk2 or cdk2 as indicated below the panels. A nonspecific antibody (RoM) was used as a negative control. Immunoprecipitates were assayed for histone H1 kinase activity (top panel), for cyclin interaction (middle panel), and for the presence of the cdks (immunoblot with the PSTAIRE antibody, which detects both cdc2 and cdk2). The migration of molecular weight markers is indicated on the left. C, antibody against cyclin B3 coprecipitated cdk2 and did not cross-react with cyclin B1. Lysates were programmed with either cyclin B3 or cyclin B1 (indicated above each lane), and translation was stopped by the addition of Nonidet P-40 radioimmune precipitation assay buffer. They were subsequently incubated with either affinity-purified cyclin B3 antibodies (cycB3) or a nonspecific antibody (RoM) as indicated below the panels, and the resulting precipitated proteins were resolved by SDS-PAGE, and the presence of the cyclin or cdk2 was determined by autoradiography (top panel) and immunoblot (bottom panel), respectively. The migration of cdk2 is indicated with an arrow. The asterisk represents the cross-reactive heavy chain of the antibodies used for immunoprecipitation. 10% of the programmed lysate was applied directly to the gel (last two lanes on the right).

In Vivo Protein Destruction Assay—To produce fluorescently labeled cyclin B3, the cDNA was cloned into pEGFP-C3 or pEYFP-C3 (Clontech). To generate the destruction box mutant (pEYFP-C3–3XA), mutations R60A, H63A, and N68A were introduced into the pEYFP-C3-cyclin B3 construct by site-directed mutagenesis (QuickChange, Stratagene).
lin-eosin. Both the sense and antisense probe contained sequences from nucleotide 1 to 429 of the 972 nucleotides in the partial mouse cDNA. This represents the coding portion of the transcript.

RNA in situ hybridization on human tissues was performed as described previously (22). Tissue sections from testis, adrenal glands, kidney, liver, pancreas, spleen, thyroid, brain, and ovary were prepared both as individual slides containing tissue pairs and as part of a multitissue array. Slides were hybridized at 42°C overnight to the sense probe and antisense probes (nucleotides 4082–4424).

Northern Analysis—Multiple tissue Northern blots with human or mouse adult tissues were obtained commercially (Clontech).

RESULTS

Identification of Human Cyclin B3—In the course of screening a cDNA library for p27-interacting proteins, we identified a clone we labeled HMG1.1. It contained an open reading frame (ORF) homologous in a 270–290-amino acid region containing a cyclin box motif to cyclin B3, previously identified in chicken, flies, and worms.

To obtain a full-length cDNA, we screened a testis cDNA library and searched data bases for end sequence-tagged clones with additional 5'-coding sequences. Compiling these results, we determined that full-length human cyclin B3 cDNA encoded an ORF of 1,395 amino acids, corresponding to a protein of 125 kDa (Fig. 1A). HMG1.1 was encoded in the region spanned from nucleotide 3119 to 4540 of this cDNA. While we were preparing our manuscript, a cDNA from pooled Wilms tumors (GenBank accession no. AI73558) encoding a full-length ORF was identified, and Lozano and colleagues (25) identified a full-length transcript encoding an identical ORF. A cyclin B3 cDNA was also isolated as a cdk5-binding protein in a two-hybrid screen.

There were two highly conserved regions among cyclin B3 proteins from different organisms (Fig. 1A). Similarities were restricted to the termini and included a putative destruction box motif of the RXFXXXX type at the N terminus (human amino acids 60–68) and a cyclin box motif (human amino acids 1160–1245) at the C terminus (Fig. 1A). The Caenorhabditis
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sequence lacked similarity at the N terminus. The significance, if any, of the nonconserved region in the middle of the protein is not clear. As noted during the cloning of the chicken homolog (15), human cyclin B3 was most similar to cyclins of the A and B types (Fig. 1).

Human Cyclin B3 Can Form a Complex with cdk2 but Not with cdc2—We examined the ability of human cyclin B3 to form complexes with and activate cdk2 and cdc2. Because the full-length protein was predicted to be considerably larger than chicken and Drosophila cyclin B3, we also tested HMG1.1, which encoded a 55-kDa C-terminal fragment of cyclin B3 including the cyclin box. We individually expressed human cyclin B3, HMG1.1, or human cyclin B1 in rabbit reticulocyte lysates and assayed the cdk immunoprecipitates from these lysates for associated cyclin and histone H1 kinase activity (Fig. 2). Full-length cyclin B3 translated at its predicted molecular mass of 120 kDa, HMG1.1 translated at 55 kDa, and cyclin B1 at 60 kDa, respectively (Fig. 2A).

cdk2-associated kinase activity was detected in lysate programmed with cyclin B1 mRNA but not in lysate programmed with cyclin B3 or HMG1.1 (Fig. 2B). Similar amounts of cdk2 were precipitated from the lysates, and nearly equimolar amounts of cyclin were coprecipitated. cdc2-associated kinase activity was apparent in cyclin B1-programmed lysate but not in cyclin B3-programmed lysates. These results indicate that cyclin B3 can interact with cdk2 but not with cdc2.

Expression of cyclin B3 blocks cells in M phase. A, representative data from flow cytometric analysis. In this figure we show the data collected for cotransfection of 293T cells with 10 μg of cyclin B3, cyclin B3XA mutant, or empty vector and a GFP expression vector. The plots are indicated at the top of each column. The top plots are the comparison of the GFP staining (y axis) and the DNA content (x axis) and the gates we used to call GFP status. The middle plots are histograms of the DNA content in the GFP (−) population. The bottom plots are histograms of the DNA content in the GFP (+) population. The percent of cells in each gate is indicated in the dot plot. B, immunoblot. Proteins were extracted from parallel transfected cultures, and the amount of cyclin B3 was determined by immunoblotting with human cyclin B3 antisera. 5 x 10⁵ cells were obtained from cultures transfected with the DNAs indicated above each set of lanes (increasing amounts are represented by triangles). Extracts were prepared by solubilizing cells directly in Laemmli sample buffer. M indicates a mock transfected culture, and U is an asynchronously growing culture of 293T cells. C, representative data from 8 h postmicroinjection of cyclin B3 expression vectors. Cells were injected with 50 ng/μl plasmid, and pictures were taken at 8 h. A representative phase contrast (left) and fluorescence (right) field from GFP-cyclin B3- and GFP-cyclin B3XA-injected cells are shown. GFP+ cells appear white. Mitotic cells are round. In some cases cells that are not expressing cyclin B3 have entered mitosis and are marked with an asterisk.
Cyclin B3

in lysate programmed with full-length cyclin B3 or HMG1.1. cdc2 was precipitated in similar amounts from the different lysates, and the lack of activity correlated with the failure to coprecipitate cyclin B3 or HMG1.1 (Fig. 2B). No significant H1 kinase activity was immunoprecipitated with a mock antibody from any lysate or with cdk antibodies from unprogrammed lysate. Similar cdk interactions and an absence of histone H1 kinase activity were seen when either the GST-HMG1.1 fusion protein or a protein produced in baculovirus-infected cells was incubated with extracts from exponentially growing HeLa cells, MANCA cells, or testes prepared from adult mice, and complexes isolated on glutathione-agarose (negative data not shown).

Reciprocally, immunoprecipitation of cyclin B3 with an affinity-purified antibody coprecipitated cdk2 from cyclin B3- but not cyclin B1-programmed lysates (Fig. 2C). These precipitates did not have histone H1 kinase activity (data not shown). This antibody did not cross-react with cyclin B1 (Fig. 2C). A mock antibody did not precipitate cdk2, cyclin B3, or cyclin B1. The affinity-purified cyclin B3 antibody did not react with recombinant cyclins A, D1, D2, D3, and E produced in baculovirus-infected Sf9 cells (data not shown). We conclude that human cyclin B3 can bind efficiently to cdk2 but not cdc2; however, it was unable to induce H1 kinase activity.

**Human Cyclin B3 Accumulates in the Nucleus**—We next examined the localization of human cyclin B3 tagged with GFP in mitotic cells. Nuclear accumulation of fluorescence was observed for an N-terminal GFP fusion protein (GFP-WT) in HeLa cells (Fig. 3A). Equivalent results were obtained with 293T and MDA468 cells (data not shown). On the other hand, C-terminal GFP-tagged protein accumulated in the cytosol (Fig. 3A). YFP and CFP have distinct fluorescence emission spectra, allowing the simultaneous detection of two proteins (26). Coinjection of vectors expressing cyclin B1-CFP and YFP-B3 showed that in interphase cells marked by cytoplasmic accumulation of cyclin B1, cyclin B3 accumulated in the nucleus (Fig. 3B). Endogenous cyclin B3 protein accumulated in the nucleus of human spermatocytes in testis (see Fig. 7A), suggesting that nuclear localization was likely to be more physiologic.

**Cyclin B3 Can Be Degraded during Mitosis, after Cyclin B1 Degradation, and in a Destruction Box-dependent Manner**—We next examined whether human cyclin B3 was degraded during mitosis. We injected G2-phase HeLa cells with an N-terminally tagged GFP- or YFP- cyclin B3 expression construct and monitored the fluorescence in single cells during real time mitosis. In mitosis, cells typically completed metaphase alignment (designated t = 0) 33–36 min after nuclear envelope breakdown and initiated telophase movements 15–18 min afterward (data not shown). In several experiments, fluorescence associated with injection of wild-type cyclin B3 in single cells began to decline in late metaphase and declined rapidly shortly after entering into anaphase (half-maximally at ~9 min) (Fig. 4A), suggesting that human cyclin B3 is destroyed over the metaphase to anaphase transition.

Next using cells injected with a pair of YFP-B3 and B1-CFP constructs, we defined the timing of cyclin B3 degradation relative to cyclin B1 degradation. Most B1-CFP/YFP-B3-expressing cells underwent a seemingly normal mitosis and began to destroy B1-CFP at the start of metaphase (more than half-maximal at t = 0) (Fig. 4B). In these cells, YFP-B3 degradation, both the onset and the half-maximal timing, occurred roughly 12 min after B1-CFP degradation (Fig. 4B).

Triple alanine substitution in the destruction box motif (R60A/F63A/N68A, the 3XA mutant) prevented the decrease in cyclin B3-associated fluorescence after completion of anaphase B (Fig. 4A, right, cells 1 and 2). Fluorescence of wild-type cyclin B3 was reduced by 78 ± 5% (mean ± S.D.) from the metaphase amount at this time (Fig. 4A, left). This indicated that mitotic destruction of cyclin B3 was dependent on the destruction box sequence.

**Cyclin B3 Can Accelerate Progression through G2 and Interferes with Mitotic Exit**—To determine whether the expression of cyclin B3 would affect cell cycle progression, we cotransfected 293T cells with increasing amounts of vectors encoding cyclin B3 and GFP and examined the distribution of cells in each phase of the cycle by flow cytometry 36 h later using a FACS analysis platform. Cyclin B3 expression did not significantly affect the cell cycle distribution at any amount (Fig. 5A and Table I). On the other hand, cells transfected with 5 μg of cyclin B3XA expression vector accumulated in G2/M phase of the cell cycle, and those transfected with 10 μg of cyclin B3XA expression vector accumulated in both G2/M and G1 (Table I). As expected, cyclin B3 did not accumulate to the amounts of cyclin B3XA (Fig. 5B). This suggests that accumulated cyclin B3 might accelerate progression through S, inhibit progression through G2, or inhibit mitotic exit, and at very high levels might even retard the G1 to S transition.

Because cyclin B3 exerts effects on the G2/M population at lower doses, we chose to address the effects of cyclin B3 expression on G2 progression and mitotic exit. To accomplish this we injected plasmids expressing either GFP-cyclin B3 or the nondegradable mutant into G2-synchronized cells. Four fields were imaged 2 h later and scored for mitotic GFP-positive cells. Mitotic cells are easily identifiable by their rounded morphologic appearance (e.g. see Fig. 5C). 0 of 48 cyclin B3-expressing cells were mitotic; however, 22 of 30 cells expressing the nondegradable mutant were. This suggests that accumulation of cyclin B3 might trigger premature mitotic entry. We then looked 8 h later, a time at which some noninjected cells would progress through G2, divide, and enter G1 (Fig. 5C). In two separate experiments, very few cells expressing cyclin B3 were in M phase (7 of 99 and 1 of 71), and those were in anaphase. All other cells were in G1 or G2. In contrast, in two experiments with cells expressing the nondegradable mutant, 70 of 85 and 39 of 55 were arrested in mitosis. The vast majority of these were in anaphase, with only 11 of 70 or 10 of 39 in metaphase or past the point of cleavage furrow ingestion. These observations indicate that accumulated cyclin B3 can trigger premature mitotic entry and inhibit progression from M to G1 by arresting or delaying cells in anaphase. These observations might reflect bona fide functions of cyclin B3 or be the result of cyclin B3 interfering with the normal regulation of mitotic events. Consequently, it was important to identify the cell types in which cyclin B3 was usually expressed.
Expression of Human Cyclin B3 mRNA Is Restricted to Testis in Mature Tissues—We next examined the expression of human cyclin B3 sequences in normal human tissues. Using a probe from the 3′/H11032-conserved region (nucleotides 4082–4424) and two probes from the nonconserved sequences (nucleotides 404–1129 and 3133–3416) (Fig. 6A), we detected an abundant signal only in testis by Northern analysis of mRNA prepared from adult tissues (Fig. 6B). While this manuscript was in preparation, similar Northern results were reported for different N- and C-terminal probes (25). These signals were only apparent as a single 5 kb band, suggesting that the B3 probe sequences might be contiguous. To demonstrate this more directly, we performed reverse transcription (RT)-PCR assays (P1, P2, P3, P4) using overlapping primer sets (Fig. 6A) and were able to amplify products of the appropriate size and sequence from testis mRNA (Fig. 6C). As a control for the same reverse transcriptase reactions, PCR for glyceraldehyde-3-phosphate dehydrogenase amplified similar yields from testis and thymus mRNA, at different amounts of template (Fig. 6C and data not shown). This result is in marked contrast to that of Lozano and colleagues (25) who reported mRNA in other tissues after two rounds of nested RT-PCR. However, our single round PCR protocol was sufficient to detect a single copy of cDNA per cell, and we were unable to detect cyclin B3 in thymus. It is possible that the mRNA was cell type-specific; however, we were unable to detect any in situ hybridization signals in multiple organs listed under “Materials and Methods” (negative data not shown).

We screened a number of cell lines for cyclin B3 mRNA and protein. We could not detect cyclin B3 mRNA or protein in exponentially growing MDA468 (breast epithelial), HL-60 (pro-myelocytic leukemia), K-562 (chronic myelogenous leukemia), MANCA (B cell), SW480 (colon), A549 (lung), G361 (melanoma), G401 (embryonic kidney), or NT2/d1 (fibroblast-like embryonal carcinoma) cells (negative data not shown). We observed a weak signal after RT-PCR in HeLa cells, consistent
with our cDNA library screen, but we could not detect the mRNA by Northern or the protein by immunoblot, immunoprecipitation, or immunofluorescence (data not shown). Thus, the normal expression of cyclin B3 mRNA was highly restricted to the testis, suggesting that it may have a tissue-specific role in mammals.

**Human Cyclin B3 Is Expressed in a Distinct Subset of Germ Cells**—The restricted expression of mRNA in the tissue Northern blot and our inability to detect it in many different cell lines suggested that cyclin B3 may have a cell type-specific function. The normal human testis contains four predominant cell types: Sertoli cells, Leydig cells, germ cells, and supporting fibroblast-like cells; consequently, we looked for the cell type(s) that express cyclin B3. We detected cyclin B3 protein in the nucleus within a seminiferous tubule, and the organization of the chromatin in positive cells were consistent with being in early pachytene spermatocytes (30). This allows

A similar expression pattern was seen with cyclin B3 mRNA, which was detected at the periphery of intratubular cells in testis sections (Fig. 8, A and B). Sense probes were negative (Fig. 8, C and D). No mRNA was detected in any other tissues on the array (negative data not shown; organs are listed under “Materials and Methods”). This suggests that cyclin B3 was expressed in the germ cells of the adult human testis.

**Mouse Cyclin B3 mRNA Is Expressed in Leptotene and Zygotene Stages of Spermatogenesis and in the Developing Fetal Ovary**—Because the staging of spermatogenesis is more defined in the mouse, we wanted to examine the expression of cyclin B3 in mouse testis. However, our cDNA probes and antibodies to human cyclin B3 do not recognize mouse RNA and protein, respectively. Therefore we first identified cyclin B3-related mouse genomic sequences in a single BAC clone by nucleic acid hybridization, and we used these to derive sequences for primers to amplify an RT-PCR product from mouse testis. Ultimately, we isolated a cDNA by 3’–rapid amplification of cDNA ends-PCR, which we named TR. The TR cDNA encoded a single ORF of 238 amino acids which was highly similar to the C terminus (amino acids 1153–1295) of human cyclin B3 (70% identity). This mouse cDNA and genomic sequences mapped to a region of the X chromosome XA2-A3 (data not shown) which was syntenic with the region Xp11.22–11.23 (28), where human cyclin B3 was mapped (25).

During the first wave of mouse spermatogenesis, tubules progress through the different stages of meiosis in a fairly synchronous fashion. mRNA expression was not detected at day 7.5 (data not shown). Rare tubules were positive at D8.5 (Fig. 9) and increased in frequency from day 9.5 (Fig. 9) through day 12.5 (data not shown). Many of these signals localized to cells at the center of the positive tubules. At the same time, no expression was detected in histologically undeveloped tubules that contained only spermatogonia and Sertoli cells. The postnatal developmental age (29), the center location within a seminiferous tubule, and the organization of the chromatin in positive cells were consistent with being in early meiosis I, most likely in the leptotene stage.

By D14.5, tubules containing predominantly leptotene or zygotene spermatocytes expressed cyclin B3 mRNA, but precocious tubules that had progressed to the pachytene stage did not (Fig. 9). Thus, the expression patterns of cyclin B3 mRNA during the first spermatogenic wave indicated that cyclin B3 was expressed during a narrow window of meiosis, beginning at the onset of the first meiotic prophase and ending by the pachytene stage.

In adults, tubules can be classified into 12 stages based on the morphologic appearance of the cells (30–33). This allows

**Fig. 7. Human cyclin B3 protein is expressed in developing male germ cells.** Tissue sections were prepared, mounted on a tissue array, and stained with cyclin B3 antibody as described under “Materials and Methods.” Reactive protein was detected by peroxidase reaction (45) and slides counterstained with hematoxylin (blue). This makes the positive cells appear brown/purple on a blue background. A, testis (×100); B, testis (×200) with an insert at ×1,000 showing two Sertoli cells (Sr) that are unstained near two staining intratubular germ cells; C, liver (×100); D, pancreas (×100). These are representative fields from at least three different experiments. Negative staining in the brain, lung, thymus, and spleen on the array is not shown.

**Fig. 8. Human cyclin B3 mRNA is expressed in intratubular cells of the testis.** Sections from testis and other tissues on the array were hybridized to either antisense RNA (A and B) or sense RNA (C and D) encoding cyclin B3 as indicated under “Materials and Methods.” Only the results with testis are shown. Hybridized probe was detected by fluorescence (red) and cell nuclei by DAPI (blue). Magnification: A and C, ×200; B and D, ×400. Results are representative of two independent experiments.
expression of mRNA to be determined at each stage of spermatogenesis. mRNA expression was detected in tubules at the transition between stages VIII and IX (Fig. 10) and was more pronounced in tubules at stages X, XI, and XII, but was not observed from stages I through VII. No significant signal was detected with the sense probe (not shown). At higher magnification, signals could be localized further to specific cell types in each tubule. No signal was detected in preleptotene spermatocytes, even at higher exposures, and no expression was detected in the pachytene, diplotene, or secondary spermatocytes from stages IX through XII (data not shown). Thus, once spermatocytes reached the pachytene stage of the first meiotic prophase, the expression of cyclin B3 mRNA was down-regulated.

In neonatal females meiosis is largely completed following birth; however, it is ongoing during fetal development. We were able to detect cyclin B3 mRNA expression in the fetal ovary but not the adult (Fig. 11). This suggests that cyclin B3 has functions in both the male and female germ cells during meiosis.

**DISCUSSION**

Transitions in the mitotic cell cycle are regulated by the accumulation and destruction of cyclin proteins. Periodic accumulation of cyclins may also regulate specific transitions in the meiotic cell cycle. In mammals, progression from premeiotic DNA synthesis into meiotic prophase I may be controlled by the regulated expression of cyclin A2 (34, 35). The appearance of cyclin A1, at least in males, is important for pachytene (14, 36), and its disappearance may be a prerequisite for the transition into the first meiotic division. Likewise, cyclin B1 and cyclin B2 may participate in the control of the transition from the first
to the second meiotic divisions (37–39). Here, we report that cyclin B3 accumulates during leptotene and zygotene and is reduced in abundance before pachytene (Fig. 12). This places cyclin B3 into a unique interval of meiotic prophase I.

After entry into prophase I at leptotene, germ cells embark upon the assembly of the synaptonemal complex, a proteinaceous complex upon which sisters align. Axial element formation begins on chromosomes; these elements incorporate central and transverse elements to form the synaptonemal complex as cells progress through zygotene (40). Prior to leptotene, cells condense their chromosomes (41, 42), perhaps to facilitate synaptonemal complex formation or recombination. Meiotic recombination occurs sometme in pachytene (43). Although many proteins of the synaptonemal complex and recombination machinery have been identified, what controls their assembly and function between the end of premeiotic DNA synthesis and the entry into pachytene is not clear. Because cyclins play roles regulating earlier (D-cyclins (46) and cyclin A2) and later (cyclin A1 and B1 cyclins) transitions in gametogenesis, it is reasonable to suspect that they will play roles in the events of early prophase I.

Finally, we must ask what is the testis-specific cdk partner of cyclin B3, and where is it localized? We have shown that cyclin B3 can interact with cdk2, albeit as a poor activator of histone H1 kinase activity. This could be interpreted to suggest that cyclin B3 might have a different catalytic partner or might be affecting substrate choice (or both). We established that cdk2 can be a bona fide partner for cyclin B3 because we could score the cyclin B3-cdk2 interaction in a number of systems (bacterial or insect cell produced proteins, proteins produced by in vitro translation in rabbit reticulocyte or wheat germ lysates, and by immunoprecipitation from HeLa cells expressing cyclin B3), but all fail to activate cdk2-associated H1 kinase activity. Nevertheless, chromosomal cdk2 is present in leptotene and zygotene cells (44). To explore the possibility that this is a cyclin B3-associated complex perhaps with catalytic activity toward specific substrates, we need to develop antibodies reactive to the mouse protein.

Enforced cyclin B3 expression is able to induce accumulation of cells in anaphase and at even higher doses in G1 phase. The molecular mechanism by which enforced cyclin B3 induced these blocks is not fully defined. However, the nonproductive association of cyclin B3 with cdk2 makes it reasonable to suggest that the G1 arrest might be associated with the ability of cyclin B3 to titrate cdk2, preventing the accumulation of sufficient amounts of cyclin E-cdk2 or cyclin A-cdk2 complexes. The first block, seen at the lower amounts of cyclin B3, is the anaphase block. Similar results were observed in Drosophila that expressed a nondegradable mutant of cyclin B3 under control of a heat shock promoter. How cyclin B3 affects anaphase exit is not clear, and we have been unable to detect an associated histone H1 kinase activity in these cells. This suggests two possibilities: it might be a cdk-independent mechanism or a mechanism in which the cyclin is interfering with the regulated proteolysis of another component.

In summary, these data place cyclin B3 in an overlooked cell cycle window of meiosis, the transition from premeiotic S phase cells to recombination-permissive pachytene cells. Our data concernig the restricted expression of cyclin B3 to leptotene and zygotene cells is consistent with two possible functional roles. First, cyclin B3 might play a role in the formation of the synaptonemal complex. Alternately or additionally, cyclin B3 expression might prevent precocious pachytene entry. An understanding of cyclin B3 function must await further studies on the effect of dysregulating cyclin B3 expression and on identification of cyclin B3-interacting proteins in the leptotene and zygotene cells.

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