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Caenorhabditis elegans cyclin A- and B-type genes: a cyclin A multigene family, an ancestral cyclin B3 and differential germline expression

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SUMMARY

We have cloned cDNAs for *Caenorhabditis elegans cyclins A1, B* and *B3*. While *cyclins A1* and *B* are most closely related to either A- or B-type cyclins of other species, *cyclin B3* is less related to these cyclins. However, this cyclin is most similar to the recently identified chicken *cyclin B3*. Our identification of a *Caenorhabditis* homolog demonstrates that *cyclin B3* has been conserved in evolution. *cyclin A1* is a member of an A-type multigene family; however the *cyclin A1* cDNA only recognizes a single band on northern blots. A single-sized RNA is also observed for the *cyclin B3* cDNA. In contrast, three different transcripts are observed for the *cyclin B* cDNA. Based on our analyses using RNAs from germline-defective mutants and from populations enriched for males, one *cyclin B* transcript is specific to the paternal germline. The two other *cyclin B* transcripts, as well as the *cyclin A1* and *cyclin B3* transcripts, are most abundant in the maternal germline and are only present at low levels in other tissues. Moreover, the 3′ untranslated regions of each *Caenorhabditis* cyclin cDNA possess several copies of potential translational control elements shown in *Xenopus* and *Drosophila* maternal cyclin mRNAs to function during oogenesis and early embryogenesis.

Key words: cyclin, *Caenorhabditis*, *Ascaris*, 3′ UTR, translational control element

INTRODUCTION

The cyclin family of genes has been cloned from numerous organisms (Evans et al., 1983; Swenson et al., 1986; Standart et al., 1987; Pines and Hunt, 1987; Lehner and O'Farrell, 1989; Pines and Hunter, 1989; Westendorf et al., 1989; Whitfield et al., 1989; Minshull et al., 1989; Ghiara et al., 1991; Gallant and Nigg, 1994). Cyclin A and B proteins accumulate during interphase, before being rapidly degraded upon exit from M-phase. Cyclin protein accumulation and destruction are critical events for the control of mitosis and meiosis (Minshull et al., 1989; Murray and Kirschner, 1989; Lehner and O'Farrell, 1989; Labbe' et al., 1989; Westendorf et al., 1989). Mitotic destruction of A- and B-type cyclins requires an amino-terminal motif known as the destruction box and degradation has been shown to occur by the ubiquitin pathway (Glotzer et al., 1991; Hunt, 1991). The C-terminal cyclin box is required for association with a *cdc2*-like kinase subunit. Activation of these kinases, either $p34^{cdc2}$ or $p34^{cdk2}$, is dependent on this association. The cyclin A/cdk2 complex is maximal at S phase, while cyclin A/cdc2 and B/cdc2 are maximal at the G_2/M border (Minshull et al., 1990; Whitfield et al., 1990; Clarke et al., 1992). The general role of cyclins in kinase activation has been well established; however, the specific roles of different cyclins in mitosis and meiosis remain elusive.

Both cyclin A and B RNAs have been shown to be abundantly supplied maternal messages in the *Drosophila* embryo, as well as in other organisms. While cyclin A RNA is uniformly distributed in the *Drosophila* oocyte and embryo, cyclin B RNA is enriched in the posterior of the oocyte and is later concentrated in the germline precursors of the embryo, the posterior pole cells (Whitfield et al., 1989; Dalby and Glover, 1993). The enrichment of cyclin B RNA occurs in the region of the polar granules, large cytoplasmic granules that form at the posterior of the fly oocyte and are subsequently incorporated into the embryonic germline precursor pole cells. Polar granules have been shown to be necessary for germline development. vasa, a *Drosophila* polar granule component, and a putative RNA binding helicase, co-localizes with *Drosophila* cyclin B RNA. Embryos from mutant *vasa* mothers that lack polar granules fail to localize cyclin B RNA (Raff et al., 1990). These findings could imply that cyclin B RNA is involved in germline development/determination.

Maternal cyclin RNAs from *Xenopus* and *Drosophila* have been shown to be regulated by several different elements in their 3′ UTRs (untranslated regions). In frog oocytes, maternally supplied cyclin messages are stored and translationally repressed. This regulation has been attributed to adenylation control elements, ACEs (also called CPE, cytoplasmic polyadenylation elements), located within the 3′ UTRs (e.g. see Sheets et al., 1994). In *Drosophila*, two regions within the cyclin B 3′ UTR have been found to exert distinct functions.

One region, narrowed down to a 181 nt segment, directs the posterior localization of the message in the oocyte and embryo; and the second, a small conserved element, represses the translation of this RNA in the embryonic pole cells (Dalby and Glover, 1992, 1993). This translational control element, TCE, has the same 11 nt bipartite sequence as the NRE, nanos response element, found in the 3′ UTRs of the *Drosophila hunchback* and *bicoid* RNAs, regulated by the nanos protein in the early embryo. However, the spacing between the two halves of the element differs for the TCE and NRE (Wharton and Struhl, l991; Dalby and Glover, 1993). The variation in spacing could direct the recognition of these same sequences by different embryonic proteins.

In cloning and characterizing the A- and B-type genes from the free-living nematode *Caenorhabditis elegans*, several unexpected features have emerged. We present evidence for a large, multigene family of A-type cyclins in *Caenorhabditis*. We also report a *Caenorhabditis cyclin B3* gene that is both A- and B-like. The only other *cyclin B3* gene has been recently reported in the chicken (Gallant and Nigg, 1994). That the *B3* gene exists in both nematode and chicken demonstrates it is likely of ancestral origin. In addition, we report that each cyclin shows a different pattern of expression when analyzed in the *Caenorhabditis* germline, with the indication that one of three *cyclin B* transcripts is uniquely involved in spermatogenesis.

MATERIALS AND METHODS

PCR reactions

Degenerate oligonucleotide primers were designed for PCR (polymerase chain reaction) amplification of A- and B-type cyclins. The cyclin B primers were designed using the nematode codon bias and the amino acids sequences ILIDWL and IASKYEE (located in the cyclin box, corresponding to amino acids 196-261 in clam; Westendorf et al., 1989). For ease in cloning, restriction sites (*Bam*HI and *Eco*RI for the B-type and *Bam*HI and *Sal*I sites for the A-type) were added. The primers for the B-type cyclins (with degenerate positions indicated in parentheses) were: 5′-TAA TGG ATC CAT (TC)CT (TC)AT (TC)GA (TC)TG GCT-3′ and 5′-CGC CGA ATT C(CT) TC(CT) TC(AG) TA(CT) TT(AG) GA(AG) GC(AG) AT-3′. For the A-type cyclin the primers were: 5′-GCA CTG CAG ATG CG(ACGT) GC(ACGT) AT(ACGT) (TC)T(ACGT) (GA)T(ACGT) GA(TC) TGG-3′ and 5′-GCA GTC GAC GG(GATC) GG(AG) (AT)A(GATC) AT(CT) TC(CT) TC(AG) TA(TC) TT-3′. Genomic DNA was used as template for the cyclin B amplifications. Mixed stage, oligo(dT)-primed cDNA (provided by M. Granato and R. Schnabel, Tübingen, FRG) was the template for the cyclin A amplifications. PCR reaction conditions were carried out as described by Kocher et al. (1989), with a standard 50 μl reaction mixture containing either 50 ng of *Caenorhabditis* genomic DNA or 50 ng of *Caenorhabditis* cDNA and 25 pmole of each primer. Thirty cycles of amplification were performed in a temperature cycler with denaturation (94°C) for 1 minute, primer annealing (54°C) for 1.5 minutes and primer extension for 0.5 minute, followed by a 10 minute incubation at 72°C. The reaction products were isolated from an 8% acrylamide gel and cloned into various plasmid vectors. The vectors in which the cDNAs were cloned are: pSK− for *cyclin B* and *cyclin B3*; pKS+ for *cyclin A1* (Stratagene, La Jolla, CA).

Sequence analysis

The *Caenorhabditis cyclin A1, B* and *B3* cDNA clones, as well as the original PCR products, were isolated and sequenced on both strands using dideoxy chain termination with Sequenase Version 2.0 (US Biochemical, Cleveland, OH). Subclones for sequencing *cyclins B* and *B3* were created using Erase-A-Base exonuclease III deletions (Promega, Madison, WI). The *cyclin A1* cDNA was sequenced using primers spaced at approximately 300 bp intervals. Selected regions of the *cyclin B3* genomic clone were also sequenced. The *cyclin A1* genomic clone has been sequenced as part of the *Caenorhabditis* sequencing project (Wilson et al., 1994).

cDNA, genomic library and yeast artificial chromosome (YAC) screens

The *Caenorhabditis cyclin A1, B* and *B3* PCR products were labelled by random priming in the presence of [α-^{32}P]dATP (New England Nuclear, Boston, MA) (Feinberg and Vogelstein, 1983), and probes were used to screen a mixed stage *Caenorhabditis* cDNA library provided by Dr R. Barstead, Washington University, St Louis, MO. Hybridization conditions were: 65°C, 5× SSPE (20× is 3 M NaCl, 0.2 M NaH_2PO_4, 0.02 M EDTA, pH 7.4), 4× Denhardt's solution (50× is 1% Ficoll, 1% polyvinylpyrrolidone, 1% BSA), 0.2% SDS and denatured salmon sperm DNA at 200 μg/ml. The highest stringency wash conditions were 0.5× SSPE with 0.2% SDS at 65°C. The *Caenorhabditis cyclin A1, B* and *B3* cDNA clones were used to screen N2 (wild type) *Caenorhabditis* genomic libraries either constructed in our laboratory (Roussell and Bennett, 1993) or provided by Dr C. Link, University of Colorado, Denver, CO. Hybridization and wash conditions were the same as above. The *Caenorhabditis cyclin A1, B* and *B3* cDNA clones were labelled and hybridized to yeast artificial chromosome (YAC) grid filters containing overlapping segments of *Caenorhabditis* genomic DNA that were provided by Dr R. Waterston, Washington University, St Louis, MO. YAC locations for all three cDNAs were confirmed by cosmid fingerprinting of genomic clones for *cyclin B*, *cyclin B3*, and two genomic *cyclin As* by Dr A. Coulson at the MRC Laboratory, Cambridge, England. Conditions for genomic Southern analyses were the same as for the library screenings.

Growth and maintenance of worms

Wild type *Caenorhabditis elegans* var. Bristol (N2 strain) (Brenner, 1974), and temperature-sensitive (ts) mutant strains were cultivated in liquid culture (Roussell and Bennett, 1993). Synchronous third to fourth stage larval worms (L3/L4) were produced by subjecting gravid adults to a 40% sodium hypochlorite (commercial bleach), 0.5 M NaOH solution for about 5 minutes, which dissolves adult tissue and leaves the eggs intact. The eggs were hatched in low salt M9 solution (Brenner, 1974) and then fed, resulting in the establishment of a synchronous liquid culture that was observed until >90% of the worms were either L3s or L4s, at which time RNA was extracted. The germline proliferation mutant strain *glp-4* (*bn2* allele) (Beanan and Strome, 1992) was provided by Dr S. Strome, Indiana University, Bloomington, IN. The feminizing mutant *fem-1* (*hc17* allele) (Argon and Ward, 1980; Spence et al., 1990) was provided by the *Caenorhabditis* Genetics Center while it was located at the University of Missouri, Columbia, MO. The masculinizing mutant *fem-3 (gf)*(*q24* allele) (Barton et al., 1987) was provided by Dr J. Kimble, University of Wisconsin, Madison, WI. The *him-5* strain (*e1490* allele) (Hodgkin et al., 1979) was provided by Dr S. Ward while at the Carnegie Institution of Washington, Baltimore, MD. *him-5* males were collected by straining through 35 μm mesh as described by Klass and Hirsh (1981). For the temperature-sensitive strains, worms were grown for several generations in liquid culture and harvested at the permissive temperature (15°C); a matching liquid culture was grown for an additional generation at the restrictive temperature, 25°C (Roussell and Bennett, 1993).

RNA isolation

Poly(A)$^+$ RNA was isolated from *Caenorhabditis* wild-type (N2) mixed stage and third to fourth (L3/L4) larval stage worms, from three temperature-sensitive (ts) mutant strains, and from males strained

from populations of *him-5* worms. RNAs were isolated as described by Bennett and Ward (1986).

Northern analysis

Probes were prepared by [α-^{32}P]dATP random prime labelling and hybridized to blots of *Caenorhabditis* poly(A)$^+$ RNA. Hybridization conditions were 42°C, 5× SSPE, 4× Denhardt's, 0.1% SDS, 50% formamide and 200 μg/ml denatured salmon sperm DNA. Wash conditions were as stringent as 0.5× SSPE, 0.2% SDS and 65°C. For the detection of the cyclin genes from the related parasitic nematode *Ascaris lumbricoides* var. *suum*, northern blots were hybridized at 37°C and washed to 57°C; Southern blots were hybridized and washed at 57°C. Quantifications were performed using an Image Quant Phosphor Imager (Molecular Dynamics).

RESULTS

Cloning and sequence analysis of *Caenorhabditis cyclin* genes

In order to investigate the potential role of cyclins in the germline, A- and B-type cyclins have been identified from the free-living nematode *Caenorhabditis elegans.* Polymerase chain reactions (PCRs) with cyclin-specific primers were performed and the PCR products were cloned. Sequence analyses revealed three different cyclin-like sequences. Each PCR product was used to screen a *C. elegans* mixed-stage cDNA library. Potentially full-length cDNAs for each were sequenced.

The *cyclin A1* cDNA is 2111 bp long and has an open reading frame of 485 amino acids (Fig. 1A). The predicted amino acid sequence is most similar to A-type cyclins, with a 42% identity to the clam cyclin A (Westendorf et al., 1989) in the cyclin box region (see Fig. 1A, bracketed region). As evidence of a trans-spliced message, the last 11 nts of the 22 nt spliced leader sequence, SL1, are detected at the 5′-most end of the *cyclin A1* cDNA (Fig. 1A). The spliced leader sequence, a 22 nt sequence that is added to the 5′ end of mRNAs from a small nonpolyadenylated transcript, is found on the 5′ ends of most nematode mRNAs (Krause and Hirsh, 1987). The identification of SL1 sequences on nematode cDNAs often signifies the cDNA is full-length. Moreover, the size of the *cyclin A1* cDNA is approximately the same as the mRNA (see Fig. 3A).

The *cyclin B* cDNA is 1501 bp long, begins with a partial SL1 sequence, and encodes an open reading frame of 361 amino acids (Fig. 1B). The *Caenorhabditis* cyclin B is most closely related to B-type cyclins from other species, with 45% identity in the cyclin box when compared to clam cyclin B (66 identical amino acids out of 147 amino acids) (Westendorf et al., 1989). A genomic clone corresponding to the single-copy *cyclin B* gene has been isolated. The *cyclin B* genomic clone physically maps to Linkage Group IV (chromosome 4) just to the right of the *mec-3* and the *him-8* genes. The map position was first determined by hybridization to a YAC (yeast artificial chromosome) grid containing overlapping segments of *C. elegans* genomic DNA and then by cosmid fingerprinting (carried out by A. Coulson at the MRC Laboratory, Cambridge, England).

The *cyclin B3* cDNA sequence is 1462 bp long and encodes an open reading frame of 385 amino acids (Fig. 1C). The *Caenorhabditis cyclin B3* cDNA is quite divergent when the predicted amino acids within the cyclin box are compared with other previously reported A- or B-type cyclins (29% identical to clam cyclin A and 33% identical to clam cyclin B; Westendorf et al., 1989). However, the *C. elegans cyclin B3* cDNA is much more closely related to the recently reported chicken *cyclin B3* gene (Gallant and Nigg, 1994). *C. elegans* and chicken cyclin B3 share 50% identity in the cyclin box. Allowing conserved amino acid changes, the similarity to the chicken cyclin B3 predicted protein rises to 66% (97 matching amino acids out of 147). The *C. elegans cyclin B3* genomic clone physically maps to Linkage Group V between the *unc-76* and *rsn-4* genes.

The evolutionary relationship as suggested by comparing the cyclin box regions of *Caenorhabditis*, clam and vertebrate cyclins is illustrated in Fig. 1D.

The original sequence proposed for the amino-terminal cyclin B destruction box had a consensus of R-ALG(NDE)I-N, followed by a lysine-rich region. Deletion of this sequence eliminated the destruction of the sea urchin cyclin protein by the ubiquitin pathway (Glotzer et al., 1991; Hunt, 1991). Putative destruction boxes are also present in the N-terminal regions of the *C. elegans* cyclins A1, B and B3 (boxed regions in Fig. 1A,B and C). The sequences in these *Caenorhabditis* cDNAs have two or three of the conserved amino acids: arginine, leucine and asparagine, **R**xx**L**xxxx**N**, like those in other species, but they lack the other conserved amino acids. The putative *Caenorhabditis* cyclin A1 and cyclin B destruction boxes are ten amino acids in length and are alike in containing the novel conserved amino acids LKPS, placed R--L-**LKPS**N (Fig. 1A and B). As we have not tested these potential destruction boxes, our designations must be considered preliminary; however, as nematodes are evolutionarily distant from sea urchins and *Xenopus*, these results could indicate that only the **R**xx**L** motif is necessary for destruction box function. It is known that both the arginine and leucine are critical for cyclin degradation (Glotzer et al., 1991; Stewart et al., 1994).

Cyclin A belongs to a multigene family

Several results indicate that there are multiple *cyclin A* genes in *Caenorhabditis*. When the *cyclin A1* cDNA was hybridized to a YAC grid containing overlapping regions of *Caenorhabditis* genomic DNA, multiple YACs, from various genomic locations, were positive. In addition, when a single *Eco*RI restriction fragment from the center of the *Caenorhabditis cyclin A1* cDNA (from nts 322 to 1564) was hybridized to a Southern blot of *Caenorhabditis* genomic DNA digested with several restriction enzymes, multiple fragments ranging in size from ~1.3 kb to >10 kb were detected (Fig. 2). In screening a *Caenorhabditis* genomic library, two different *cyclin A* genomic clones, *A1* and *A2*, were isolated, while several different genomic clones also containing a ~1.3 kb hybridizing fragment were not further pursued. The *Eco*RI fragments corresponding to these two genomic clones are indicated with arrows (Fig. 2, lane 3). Physical mapping has indicated these two genomic clones are not linked. The *cyclin A1* genomic clone maps to Linkage Group III (chromosome 3) on the same cosmid as the *glp-1* gene, while the *cyclin A2* genomic clone maps to Linkage Group II (chromosome 2) to the left of the *lin-4* gene. The *cyclin A1* genomic clone has already been sequenced (Wilson et al., 1994), revealing that the hybridizing genomic *cyclin A1* fragment contains three small, 45 nt, 50 nt, and 82 nt introns and no additional *Eco*RI sites. Therefore,

A

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5′ CGAAGTTTGAGATTCCGACACAGCGCACCCCTAGGGACCAATTATCAAGCGCTTTTGAGAGCAGCAGATCGCGACGAG
M R S A L S L K P S N G N A A K S Q A V N N K N V I K N A P L G G K L T R Q I G T
S N L L Q Q A L P S K K I D E S P I I K I D A K D S F K V F E D Q E P E K E N S S
E N V D A T E K D S N V I P A E D N N M I H E L E R K M E E K S R A E K L K F K F
M Q T R D N S D I T S R F S E P P S E F S V L C D D D D C D K V S V A S S T F T T
S V R A T F S S F H F D E N Q R K K E F G K E E A V K[K I Q K K A A K E A R D D S
M F S S E E F F P D I I K Y M L H R Q T K N R A S H E C F D I Q S Q V N E E M R T
I L I D W F S D V V K E Y N F Q K E T F H L A V S L V D R A L S M F N I D K M R F
Q L V G T T S M M I A V K Y E E I F P P E I E D F A L I T D N T Y R V P D I L L M
E R F L L G K F D F V V A M P T S S W F G T C F A K R M N F T K K M R N T V H Y L
L E L S L I D V H F L R Y R P S D I A A A A C C F A N L Q A D V E S W P Q K M V D
D T G I S T E D F V D V L R D L H R M Y L N A S T A D F K S I F Y N Y S E T A Q M
E V A L L P A P T D K L R S M F P S I F V T A P K S S N D S S S P Q]*
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B

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                                                    5′ GAAGTTTGAGCGAAAGGCCTCAAT
M L R A T N N R R T S N N V E K D S L Q M A K H G N G P L K P V N A Q G L Q T K R
E A R E I L A L K P S N P A P V E T A[Q K S Q R I N L Q D A E T K C L A M A D D I
Y K Y L V H H E K K Y L L E E C F M E G G E P T P K M R R I L V D W L V Q V H V R
F H L T P E T L H L T V F I L D R M L Q K K V T S K A D L Q L L G I S A M F V A S
K F E E V Y L P D I H D Y E F I T E N T Y S K K Q I L A M E Q T I L N S L N F D L
S C P S S L V F L R C L S R I L S E N D A S P I D N Q A F C Y T Y N I S K C L G E
L A L L D S V M A S T P R S H I A S A S M I I A L E V H P V D G I E A E N A V S V
I C K Q L G A S K K V I E D A V A L L A E V S Y K N F K Q G K L V A I K N K Y Q S
S K L A Q V S N L M T D D V L E K I N R M G Q N A K V D A S E M E]*
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C

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                                                    5′  CGAGCATTCAAA
M M L R S Q A K N V D L T S Q A D S R H Q Q K R K Q A E Q L D A L K N P S E P A A
K K Q H S K G L T E L R A H I S G F K I D S A K R D P L G K S R T S R R D V E N L
P P Q K S R Y V D[P C P H Y D Y D L E E A G N P D S I S D Y A Q G I F D Y Y R H R
E V H F R V R K Y L H K H P E V D V K T R A I L I D W M V E I Q E T F E L N H E T
L Y N A V K L T D M Y L C K T K N V D K N T I Q K L A C V A I F I A A K Y D E R S
P P L V D D L I Y L S G D R F S R D E L L A M E R E L F A T V G Y D L G S P L S Y
R Y L R R F G R V C R V D M K T L T M G R F I L E T S L M V Y E Y A M V S Q S R L
A A A A F V L A M R M L D K N N E Y E W N P V L E K Y S G F T G E E V M P L V E H
M N H I L H F S K D K W A Q L T S V R Q K Y S H E V F F H V A S I P M L P D T L K
V V D S H T Y A P V P M L S Y P]*
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D

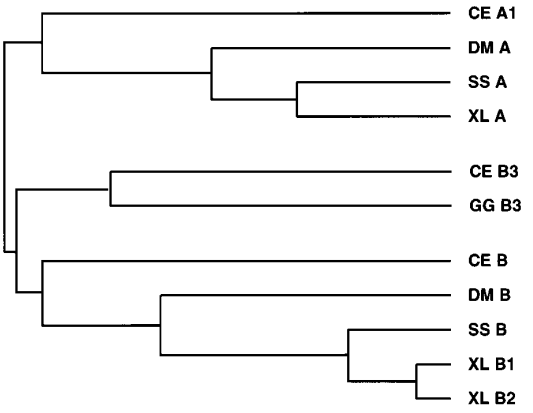

Fig. 1. Sequence analyses of *Caenorhabditis* cyclins A1, B and B3. Predicted amino acid sequences of the cyclin A1 (A), B (B) and B3 (C) cDNAs. The putative cyclin box is bracketed in each sequence. The putative destruction box is boxed in each sequence while the partial spliced leader (SL1) is underlined at the 5′ end of both cyclin A1 and cyclin B. The sequence used to construct degenerate primers for PCR amplification and the sequence that was amplified by PCR are shown in each sequence by arrows. The *cyclin B3* genomic clone was partially sequenced (the 5′ end of the gene), with the discovery that 3 nt were missing from the cDNA. Therefore the genomic sequence was merged with the *cyclin B3* cDNA sequence at aa 8-9. (D) Dendrogram resulting from a comparison of cyclin boxes of *Caenorhabditis* cyclins A1, B, and B3 with cyclin boxes from A- and B-types from other species. The dendrogram was created using the program CLUSTAL of the PCGENE DNA analysis software. Cyclin box sequences used for the alignment were: *C. elegans* cyclin A1, aa 192-485 (this paper); *D. melanogaster* cyclin A, aa 183-491 (Lehner and O'Farrell, 1989); *S. solidissima* cyclin A, aa 144-422 (Swenson et al., 1986); *X. laevis* cyclin A, aa 144-418 (Minshull et al., 1990); *C. elegans* cyclin B3, aa 92-384 (this paper); *G. gallus* cyclin B3, aa 126-403 (Gallant and Nigg, 1994); *C. elegans* cyclin B, aa 61-361 (this paper); *D. melanogaster* cyclin B, aa 237-530 (Lehner and O'Farrell, 1990); *S. solidissima cyclin B*, aa 149-428 (Westendorf et al., 1989); *X. laevis* cyclin B1, aa 116-397 (Minshull et al., 1989); *X. laevis* cyclin B2, aa 111-392 (Minshull et al., 1989). The same dendrogram was also obtained when other vertebrate cyclin boxes were used instead of the *Xenopus* cyclin boxes (not shown). The length of the horizontal branches is inversely correlated with the similarity of the proteins.

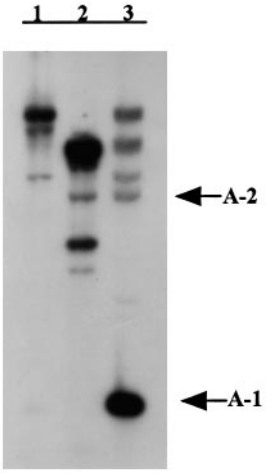

Fig. 2. Genomic Southern analysis of *Caenorhabditis cyclin A* genes. Lane 1, *Bam*HI; lane 2, *Hin*dIII; lane 3, *Eco*RI enzyme digested genomic DNA was hybridized with a ^{32}P-labelled *cyclin A1* cDNA *Eco*RI fragment that spans nt 322 to nt 1564. Each lane contained 5 μg of *Caenorhabditis* genomic DNA. The 4.7 kb and 1.3 kb fragments, indicated, correspond to the isolated *cyclin A1* and *cyclin A2* genomic clones (results not shown). To assure limit digestions, 5 units of enzyme/microgram genomic DNA were used, with overnight incubation. Therefore the pattern of hybridization, which has been consistent throughout multiple Southern analyses, is not likely to be due to partial digestion.

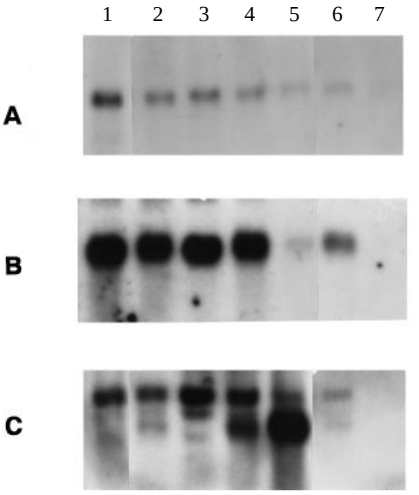

Fig. 3. Levels of *Caenorhabditis cyclins A1, cyclin B* and *cyclin B3* RNA in wild-type and several germline mutants. Each lane contained 3 μg of poly(A)$^+$ RNA from different temperature-sensitive germline mutants grown at the permissive (15°C) or restrictive (25°C) temperature. Expression of *Caenorhabditis cyclins A1* (A), *cyclin B3* (B), and *cyclin B* (C). Lane 1, N2 (wild type) RNA; lane 2, *fem-1* RNA 15°C; lane 3, *fem-1* RNA 25°C; lane 4, *fem-3 (gf)* RNA 15°C; lane 5, *fem-3 (gf)* RNA 25°C; lane 6, *glp-4* RNA 15°C; lane 7, *glp-4* RNA 25°C. The *Caenorhabditis* vitellogenin gene, an intestinal message (Spieth and Blumenthal, 1985), was used as a loading control to ensure equivalent loading of the RNA samples (results not shown). The exposure for B is approximately one third that for A and one half that of C.

based on Southern analysis, YAC hybridization, and cosmid fingerprinting, the *cyclin A* genes appear to be part of a multigene family, with several other *cyclin A* genomic clones yet to be identified.

The exact number of A-type *C. elegans* genes remains unknown. Hybridization to the 1.3 kb fragment is consistently five-six times stronger than to the four larger *Eco*RI fragments, implying there could be as many as nine or ten *cyclin A* genes in *Caenorhabditis*. Completion of the *Caenorhabditis* genome sequencing project will identify all the *cyclin A* genes. The *Caenorhabditis cyclin A*- and *B*-type cDNAs do not cross-hybridize with one another (results not shown); therefore it is unlikely that any of the multiple restriction fragments detected by the *cyclin A1* cDNA correspond to the B-type genes.

Expression of the *Caenorhabditis* cyclins in mixed stage worms

To examine expression of the cyclin genes, a northern blot of *Caenorhabditis* mixed stage poly(A)$^+$ RNA was hybridized with labelled *Caenorhabditis cyclin A1, B* or *B3* cDNAs (Fig. 3A-C, lanes 1). The *cyclin A1* cDNA, although hybridizing to multiple restriction fragments by Southern analysis, surprisingly only detects a single 2.2 kb message (Fig. 3A, lane 1). The *cyclin B3* cDNA hybridizes to a 1.6 kb message (Fig. 3B, lane 1). The *cyclin B* cDNA hybridizes to three distinct messages of 1.7 kb, 1.5 kb and 1.3 kb, with the two smaller *cyclin B* messages only observed upon long exposures (Fig. 3C, lane 1). The three *cyclin B* transcripts could be produced by multiple promoters, differing lengths of poly(A) tails, the use of multiple polyadenylation sites for the addition of the poly(A) tail, by alternative splicing or by a combination of these. We have tested several of these possibilities. Experiments designed to detect differences in the length of the poly(A) tail by hybridization of oligo(dT) to poly(A)$^+$ RNA followed by RNase H digestion have shown that the three *cyclin B* transcripts are still detectable after RNase H treatment, although each *cyclin B* transcript is slightly smaller in size due to the removal of the poly(A) tail (not shown). This result implies that the three *cyclin B* transcripts are not due to three different lengths of the poly(A) tail. Two potential polyadenylation signal motifs are present in the *cyclin B* 3′ UTR (Fig. 4A). Use of the first consensus polyadenylation signal would be predicted to produce a 1.3 kb transcript while the predicted use of the second polyadenylation signal sequence would produce the larger 1.7 kb transcript. To test the hypothesis that at least two of the *cyclin B* messages might be due to the use of these different polyadenylation sites, a 172 nt probe specific for the 3′-most end of the *cyclin B* cDNA was hybridized to wild-type, N2, poly(A)$^+$ RNA (Fig. 4A). As predicted if these sites are used, the results of hybridization with the 3′-most probe only show the longest 1.7 kb transcript (Fig. 4B, lane 3), while hybridization of the same N2 poly(A)$^+$ RNA with the full-length *cyclin B* cDNA or a similar sized internal fragment detects all three B1 transcripts (Fig. 4B, lanes 1 and 2). Similarly, a probe containing the first polyadenylation signal (at 15-20 nts after the termination codon) detects all three B1 transcripts (not shown). Therefore, these results rule out differing tail length due simply to polyadenylation, and imply that the 1.3 kb and the 1.7 kb transcripts are produced using two different polyadenylation sites.

Germline expression of *Caenorhabditis* cyclins

Germline development in the wild-type *C. elegans* hermaphrodite begins with mitotic germline proliferation in the third larval stage. Spermatogenesis begins at the late third to early fourth larval stage, and mature sperm are stored. In the late fourth larval stage, the hermaphrodite switches to the production of oocytes that are made throughout adulthood. In wild-type animals sperm numbers often determine brood size; the worm ceases oocyte production when the supply of sperm is exhausted. To establish the expression patterns of the cyclin genes in the adult germline, we made use of several temperature-sensitive mutant strains of *Caenorhabditis* that are defective at particular points of germline development. We expected that most of the cyclin transcripts would be found in

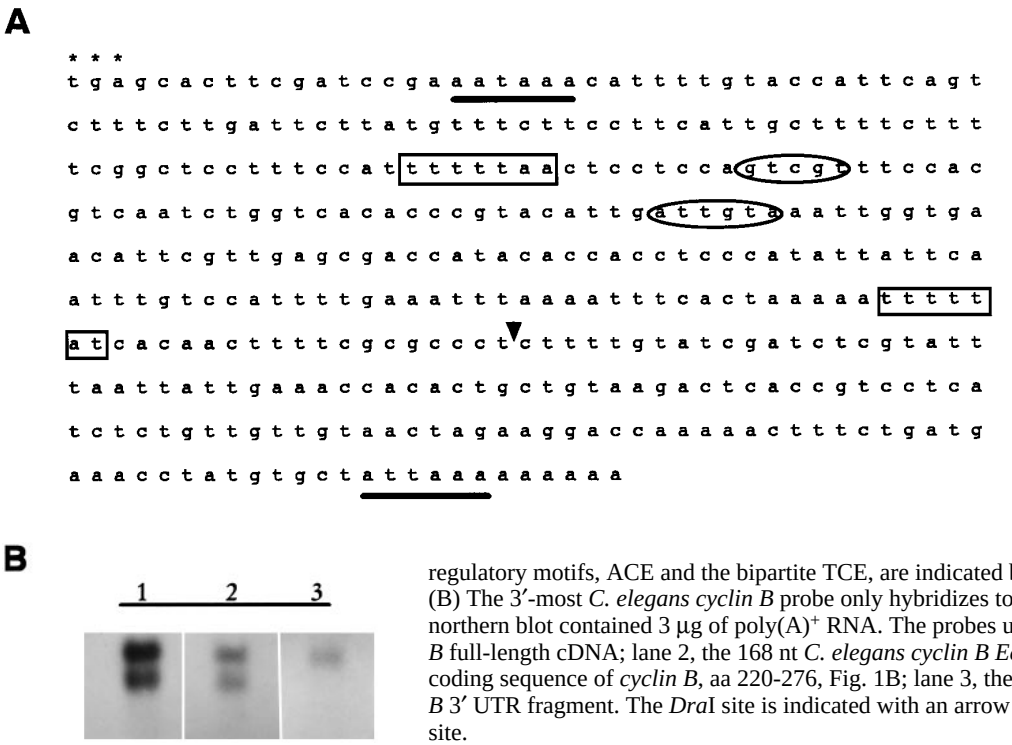

Fig. 4. Differential use of polyadenylation sites in *C. elegans cyclin* B. (A) The 394 nt 3′ UTR sequence of the cyclin B cDNA is shown. The two potential polyadenylation sites are underlined in bold. The potential regulatory motifs, ACE and the bipartite TCE, are indicated by boxes and ovals, respectively. (B) The 3′-most *C. elegans cyclin B* probe only hybridizes to the 1.7 kb message. Each lane on this northern blot contained 3 μg of poly(A)$^+$ RNA. The probes used were: lane 1, the *C. elegans cyclin B* full-length cDNA; lane 2, the 168 nt *C. elegans cyclin B Eco*RI-*Hin*cII fragment containing coding sequence of *cyclin B*, aa 220-276, Fig. 1B; lane 3, the 172 nt *Eco*RI-*Dra*I *C. elegans cyclin B* 3′ UTR fragment. The *Dra*I site is indicated with an arrow in 4A; the *Eco*RI site is the 3′ cloning site.

the germline; only the germline is undergoing cell divisions in the adult *C. elegans* worm. We were also most interested in determining whether the three cyclin B messages are all of similar germline origin.

The three *Caenorhabditis* temperature-sensitive (ts), germline-defective mutants used in these studies were: *fem-1, fem-3 (gf)*, and *glp-4*. While each strain shows germline defects at 25°C, no somatic defects are observed at 25°C for any of these three strains and each is phenotypically wild type at 15°C. *fem-1* (the *hc17* allele) is a loss-of-function feminizing mutant that produces wild-type levels of oocytes but no sperm at 25°C (Spence et al., 1990). *fem-3 (gf)* (*q24*, a weak allele) is a gain-of-function masculinizing mutant that produces many-fold more sperm than wild type and no oocytes at 25°C (Barton et al., 1987). *glp-4* (the *bn-2* allele) is a germline proliferation mutant that at 25°C is germline defective and only slowly produces a few germ cell nuclei; no oocytes or sperm are produced (Beanan and Strome, 1992).

Caenorhabditis cyclin A1 cDNA was hybridized to a northern blot of poly(A)$^+$ RNA from wild type and from each of these germline-defective mutants grown at 15°C or 25°C. Levels of cyclin A1 RNA in the *fem-1* mutants (producing oocytes but no sperm) show no reduction at the non-permissive temperature, 25°C (Fig. 3A, compare lanes 2 and 3). *fem-3 (gf)* and *glp-4* mutants grown at the restrictive temperature, 25°C, at which no oocytes are produced show greatly reduced levels of the *cyclin A1* 2.2 kb message as compared to animals grown at the permissive temperature, 15°C (Fig. 3A, lanes 4 and 5, lanes 6 and 7). Based on these northern analyses, the *Caenorhabditis cyclin A1* appears to be primarily transcribed in the maternal germline. That is, when normal numbers of oocytes are produced, the *cyclin A1* levels are wild type. The absence of oocytes results in a dramatic reduction in the *cyclin A1* message. While *cyclin A1* expression is primarily maternal, there is also a component that is present even when oocytes are missing, as seen in the *fem-3* and *glp-4* RNAs isolated at the restrictive temperature (Fig. 3A, lanes 5 and 7). This low level of *cyclin A1* RNA could reflect message that is produced during mitotic germ cell proliferation (*fem-3*, and to a smaller extent, *glp-4*), or message present in the adult somatic tissues.

As an additional means of measuring somatic transcription, we have made use of the complementary nematode species, *Ascaris lumbricoides* var. *suum. Ascaris* is a large parasitic nematode; its size allows tissue dissection. When *Ascaris* intestinal poly(A)$^+$ RNA is hybridized with the *Caenorhabditis cyclin A1* cDNA, a transcript is detected (not shown). However, in contrast to *Caenorhabditis*, the ascarid worm grows throughout its adult life, with cell proliferation continuing in the adult intestine and therefore the somatic *cyclin A* transcript in *Ascaris* may not relate to whether somatic *cyclin A* transcripts exist in *Caenorhabditis* adults.

As with *cyclin A1*, there is little or no reduction in the *cyclin B3* RNA levels in the *fem-1* mutants at 25° C (no sperm) (Fig. 3B, compare lanes 2 and 3). In contrast, when *cyclin B3* RNA levels in the *fem-3* and *glp-4* are compared at 15°C and 25°C, the *cyclin B3* 1.6 kb message is seen to be greatly reduced at the nonpermissive temperature (25°C) in both mutants, with only long exposures showing any detectable hybridization (Fig. 3B, lanes 4 and 5, 6 and 7). Quantification of the *cyclin B3* message using these RNAs shows that 80% or more of the *cyclin B3* message is maternally supplied. *Caenorhabditis cyclin B3* also cross-hybridizes to a single message in *Ascaris* intestinal poly(A)$^+$ RNA (not shown). These results imply that the *Caenorhabditis cyclin B3* message is primarily maternal (correlated with the presence of oocytes) in the adult hermaphrodite with a low level expression likely attributed to the proliferating germ cell nuclei and/ or somatic tissues. The

hybridization to *Ascaris* RNA also implies that the ancestral *cyclin B3* gene is present in other nematode species.

The *Caenorhabditis cyclin B* gene was also analyzed using wild type and these germline-defective mutant RNAs (Fig. 3C). The *fem-1* and *fem-3* RNAs show essentially wild-type levels of *cyclin B* RNA at 15°C (Fig. 3C, lanes 2 and 4), as expected for the permissive temperature. In *fem-1* RNA at the restrictive temperature, the 1.7 kb and the 1.5 kb transcripts are detected (Fig. 3C, lane 3) at consistent, but slightly increased levels (as are those for *cyclin A1* and *cyclin B3*, Fig. 3A and 3B, lane 3). We predict that these small increases may be due to the retention of oocytes observed in the *fem-1* mutant. None of the three *cyclin B* RNAs is detectable in *glp-4* mutants at 25°C (Fig. 3C, lane 7), except upon much longer exposures. Comparison of the levels of the 1.7 kb *cyclin B* transcript from wild type, *fem-1, fem-3(gf),* and *glp-4* RNAs allows us to conclude that the largest *cyclin B* transcript is primarily maternal. When oocyte numbers are reduced (*glp-4* or *fem-3*) (Fig. 3C, lanes 5 and 7) the 1.7 kb message is significantly reduced. However, low levels of the 1.7 kb message are still detected without the presence of oocytes; therefore it is likely that this message is also expressed in the proliferating mitotic germ nuclei or in the soma. Positive hybridization of *Caenorhabditis cyclin B* to a single message in adult *Ascaris* intestinal RNA makes it likely that there is somatic *cyclin B* RNA in this related nematode (not shown). The 1.5 kb *cyclin B* transcript is only present when oocytes are being produced and is much less abundant than the 1.7 kb message in the RNAs tested. Surprisingly, in the *fem-3(gf)* strain (Fig. 3C, lane 5), producing many-fold more sperm and no oocytes at the restrictive temperature, the 1.3 kb *cyclin B* message is much enhanced, with a low level of the 1.7 kb transcript also detected. The levels of the 1.3 kb transcript vary dramatically and consistently with the presence and numbers of sperm, making it a candidate for a message specific to spermatogenesis. Because the 1.3 kb transcript is likely to be fully contained within the 1.7 kb transcript, it is not possible to generate a hybridization probe specific for the shorter 1.3 kb message.

As an independent means to test whether the *cyclin B* 1.3 kb transcript is exclusive to the paternal germline, we isolated poly(A)$^+$ RNA from *C. elegans* male worms. We were able to obtain enough males to isolate poly(A)$^+$ RNA by using the *him-5* strain (the *e1490* allele) that produces ~20% males, many-fold more males than the ~0.5% males in a wild-type population (Hodgkin et al., 1979). (*C. elegans* males are produced as a result of infrequent X-chromosome nondisjunction in the hermaphrodite worm; with the rate of nondisjunction increased in the *him-5* strain.) Males are significantly smaller than adult hermaphrodites; therefore males were selected by size filtration of a synchronous *him-5* population. With male poly(A)$^+$ RNA, levels of the 1.3 kb message are significantly increased (Fig. 5A, lane 1). Levels of the 1.3 kb message in the L3/L4 animals, at the stage when spermatogenesis is beginning and before oogenesis has begun, are somewhat above those in the mixed-stage (mostly adult) animals (Fig. 5A, lanes 2 and 3). In contrast, the 1.7 kb message is undetectable in either the male or the L3/L4 populations, while it is the major *cyclin B* transcript in the mixed-stage N2 RNA (Fig. 5A, lane 3). A probe corresponding to the small RNA coding for the sperm-specific, major sperm protein, MSP (Burke and Ward, 1983), was used as a control (Fig. 5B).

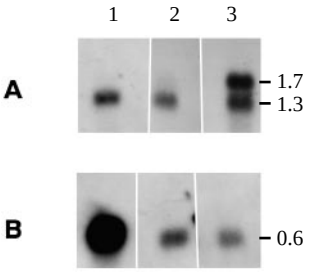

Fig. 5. The 1.3 kb *cyclin B* transcript appears specific to the paternal germline. In this northern blot, each lane contained 3 μg of poly(A)$^+$ RNA. Lane 1, *him-5* RNA; lane 2, staged L3/L4 RNA; lane 3, N2 mixed-stage (mostly adult) RNA. The probes used were: (A) the full-length *C. elegans cyclin B* cDNA; and (B) the full-length, 600 bp, *C. elegans* MSP cDNA (Burke and Ward, 1983). A pharyngeal myosin gene (Miller et al., 1986) was hybridized as a control to ensure equivalent loading of the RNA samples (not shown); for male RNA the vitellogenin probe used in Fig. 3 is not an appropriate control, as male worms do not produce vitellogenin (Schedin et al., 1991).

MSP shows the same pattern of hybridization as does the 1.3 kb *cyclin B* transcript. Therefore, while the 1.7 kb and the rare 1.5 kb *cyclin B* transcripts are primarily maternal, the 1.3 kb *cyclin B* transcript appears specific to spermatogenesis. The evidence in hand doesn't allow us to distinguish whether the smallest *cyclin B* message is important for mitotic proliferation or meiotic divisions during spermatogenesis. The levels of this message are increased with the *fem-3* mutant that produces 10- to 20-fold more germ cell nuclei and sperm than normal (Fig. 3C, lane 5). Even at the permissive temperature for *fem-3* worms, the 1.3 kb message is greatly increased (Fig. 3C, lane 4), implying some leakiness of this phenotype at 15°C.

In comparing the steady-state levels of the *cyclin A1*, *cyclin B* and *cyclin B3* transcripts by northern analyses, the *cyclin B3* transcript appears to be much more abundant than *cyclin A1* or *cyclin B* (Fig. 3). While we consider it likely that the differences in abundance observed in these studies are due to differential transcription, we cannot rule out the possibility that the results observed might not be attributed to differential stability of the *cyclin A1, B* or *B3* messages in the germline, which is not easily testable in *C. elegans*. It is also apparent that while expression of all three cyclin genes is reduced when oocytes are absent, the reduction is most dramatic for *cyclin B3* (Fig. 3, lanes 4 and 5). Because this study represents the first comparisons of the A- and B-type cyclins that include the report of a *cyclin B3* gene, it remains to be determined if this profile is unique to the *C. elegans* germline.

DISCUSSION

Our interest in cloning *Caenorhabditis* cyclins relates to their potential roles in the the germline of this free-living nematode. In the course of our analyses of the A- and B-type cyclins, several distinct features of the genomic organization of these cyclins in *C. elegans* became apparent.

C. elegans cyclin A1 cDNA is in a multigene family

We have isolated *cyclin A* genomic clones corresponding to two *cyclin A* genes. On the basis of Southern and YAC grid analyses, it is likely that there are several other *cyclin A* genes. The mouse genome contains two *cyclin A* genes as well as multiple *cyclin B* genes. There are six B-like, *SCB, cyclin* genes in *S. cerevisiae* (Chapman and Wolgemuth, 1992; Ghiara et al., 1991; Epstein and Cross, 1992; Schwob and Nasmyth, 1993).

Therefore, a multigene family of *cyclin* genes is not unexpected. However, a family of *cyclin A* genes with as many as ten members is unprecedented.

One can question why *C. elegans* has uniquely elected to expand the *cyclin A* genes into a multigene family. Perhaps multiple *cyclin A*s are needed to facilitate the rapid cell divisions that occur as the worm develops from egg to larvae in about 12 hours. This rapid embryonic division contrasts sharply with the early *Ascaris* embryo that divides about once a day (Azzaria and McGhee, 1992). And, in contrast to *Caenorhabditis*, *Ascaris* has only a single *cyclin A* gene (J. Richards and K. Bennett, unpublished results). Both *Ascaris* and *Caenorhabditis* differ from *Drosophila* in that they begin zygotic transcription as early as the 4- to 8-cell stage (Cleavinger et al., 1989; Edgar et al., 1994). In *C. elegans* multiple genes might be necessary to produce enough transcript if each gene has a rather weak promoter. Alternatively, many of the *cyclin A* genes may have evolved tissue-specific functions.

While there are multiple *cyclin A* genes in *Caenorhabditis*, a single 2.2 kb transcript is detected using the *cyclin A1* cDNA probe. This single transcript may reflect exclusive expression from the *cyclin A1* gene, with the other *cyclin A* genes representing pseudogenes. However, only a few cases of pseudogenes have been reported in *Caenorhabditis* (for example, see Ward et al., 1988). Alternatively, multiple *cyclin A* cDNAs could produce the same-sized message. Evidence supports the latter possibility. We have sequenced several other putative cyclin A PCR products from a cDNA library and have found several slightly different *Caenorhabditis* cDNAs represented (J. Richards, unpublished results). There is precedence in *C. elegans* for multiple genes producing a single transcript. In *Caenorhabditis* the major sperm protein, MSP, genes constitute a very large family of more than 50 members of expressed genes and pseudogenes from which a single-sized, abundant transcript is produced (Fig. 5B) (Burke and Ward, 1983).

C. elegans cyclin B3: an ancestral gene

In contrast to *cyclin A*, Southern hybridization of *cyclin B* or *B3* cDNA probes to *Caenorhabditis* genomic DNA suggests that both are single copy genes (not shown). A comparison of B-type cyclins from higher metazoan species suggests that *cyclin B3* has been conserved throughout evolution (Fig. 1D). Thus we predict that *cyclin B3*, which so far has only been described in chicken and *Caenorhabditis*, should be found in other organisms as well. The cross-hybridization to *Ascaris* and the recent identification of a *Drosophila cyclin B3* gene supports this suggestion (this paper and J. A. Knoblich and C. F. Lehner, unpublished results). Fig. 1D also suggests that *cyclin B3* has diverged from B-type cyclins early in evolution. In contrast, the pair of closely related cyclins, B1 and B2, that have so far only been described in vertebrate species, seem to have diverged later in evolution (Fig. 1D). Therefore, it is unlikely that a *cyclin B1* or *B2* gene will be found in *C. elegans*.

cyclin B produces three differentially expressed transcripts

The *cyclin B* gene produces three uniquely sized transcripts that appear to be differentially expressed in the maternal and paternal germlines. The presence of two potential polyadenylation signal sequences in the *cyclin B* 3′ UTR as well as hybridization results using specific *cyclin B* 3′ UTR probes suggests that the 1.7 kb and 1.3 kb transcripts arise from polyadenylation at different sites (Fig. 4B, lanes 1-3). We predict that the 1.3 kb *cyclin B* transcript is produced during spermatogenesis through use of the consensus poly(A) signal (at nts 15-20 after the termination codon, Fig. 4A). Polyadenylation at this first site would produce a short 3′ UTR lacking both the potential ACE and TCE sequences, while use of the more distal polyadenyation signal would result in the longest 1.7 kb message. How the rarest 1.5 kb *Caenorhabditis cyclin B* transcript is made is unknown. There is precedence for multiple *cyclin B* transcripts in *Drosophila* and in the mouse. The *Drosophila cyclin B* cDNA codes for two transcripts expressed in the female germline. The shorter 2.3 kb *Drosophila cyclin B* transcript is produced by alternative splicing and is missing 393 nts from the central region of the longer 2.7 kb *cyclin B* transcript (Dalby and Glover, 1992). In the mouse the *cyclin B1* cDNA hybridizes to four differentially expressed transcripts, with two of these transcripts also differing in the choice of polyadenylation sites, resulting in changes in the 3′ UTR length (Chapman and Wolgemuth, 1992). As in *C. elegans*, the sperm-specific mouse *cyclin B1* transcript is the product of a more-5′ polyadenylation signal, Chapman and Wolgemuth (1992). From evidence that the 1.7 kb maternal *cyclin B* message of *Caenorhabditis* contains a 3′ UTR likely to be absent in the 1.3 kb sperm-specific transcript, we speculate that the *C. elegans* 3′ UTR contains critical translational control signals. This untranslated region may also contain a region specifying localization, of similar function to that found in the *Drosophila cyclin B* 3′ UTR. These regions and signals may have been eliminated in the paternal message because they are either unnecessary or deleterious. This model can be tested in vivo by producing transgenic worms with reporter constructs with or without these sequences.

Potential 3′ regulatory motifs

Two putative regulatory motifs, ACE and TCE, have been shown to be important for the regulation of clam, mouse, *Xenopus* or *Drosophila* maternal mRNAs (Standart et al., 1990; Huarte et al., 1992; Sheets et al., 1994; Dalby and Glover, 1993). ACE (also called a cytoplasmic polyadenylation element, CPE) is a U-rich sequence, with a loose consensus of A/UUUUUAU/A. The ACE is required both in *Xenopus* and in mouse for the deadenylation (storage) and, in consort with the poly(A) signal, for the readenylation (translational activation) of maternal messages containing this motif (McGrew et al., 1989; Fox et al., 1989; Huarte et al., 1992; Bachvarova, 1992; Simon et al., 1992; Sheets et al., 1994). The second regulatory element present in the *Drosophila cyclin B* 3′ UTR is the bipartite TCE (translational control element) that functions to repress the translation of this mRNA in the pole cells of the early *Drosophila* embryo (Dalby and Glover, 1993). The consensus sequence of the TCE is GUUGU-X_{23}-AUUGUA.

All three *Caenorhabditis* cyclin cDNAs contain putative ACE and TCE elements (Fig. 4A and not shown). The *cyclin A1* cDNA contains a 578 nt 3′ UTR with three potential ACE motifs and three potential TCE motifs, each a match of 9/11 nucleotides with the *Drosophila* TCE. The *cyclin B* cDNA contains a 394 nt 3′ UTR with two ACE motifs and one potential TCE sequence, a 10/11 nucleotide match to the *Drosophila* TCE (Fig. 4A). The *cyclin B3* cDNA contains a

307 nt 3′ UTR with one ACE and two potential TCE sequences of a 9/11 nucleotide match to the TCE. In each case the spacing between the bipartite motif is more like that of the *Drosophila cyclin B* TCE than like the NRE elements in the *hunchback* and *bicoid* genes. We have also reported NRE/TCE-like elements in the *C. elegans glh-1* gene (Roussell and Bennett, 1993). Microinjections into the *C. elegans* gonad of a beta-galactosidase reporter RNA containing putative 3′ regulatory sequences have recently been used as functional assays for two 3′ UTR elements (Goodwin et al., 1993; Evans et al., 1994). Therefore, the prospective 3′ UTR elements in the cyclin genes can be tested.

What role might these two elements play in the *C. elegans* cyclin genes? As they do in other organisms, we predict that the ACEs repress translation of the maternal *cyclin* messages during oogenesis. Consistent with this prediction, it appears that the paternal *C. elegans cyclin B* transcript lacks ACEs (Fig. 4B). We also predict that NRE/TCEs will function to repress translation in localized areas in the early *C. elegans* embryo, as they do in *Drosophila*. The *C. elegans* ACEs and TCEs could effect the same message at different points in development if the specific binding proteins that complex with these motifs are spatially or temporally limited.

Caenorhabditis as a genetic model for cyclins

We predict that *Caenorhabditis* may prove a useful model for genetic studies of the cyclins. To date no mutation in a *cyclin B* gene has been identified in any higher eucaryote. The physical map position of the *C. elegans cyclin B* gene is in the immediate vicinity of the *mec-3* and the *him-8* genes, both of which have been genetically and physically mapped. This location closely corresponds to the genetic map positions of nine lethal mutations that have been isolated in a mutagenesis screen on LG1V (chromosome 4) (Clark and Baillie, 1992). These nine candidates can each be tested for rescue with the *cyclin B* gene, using the techniques of DNA transformation, now routine with *C. elegans*.

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