A novel cyclin gene from *Drosophila* complements *CLN* function in yeast

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In the yeast *Saccharomyces cerevisiae* three G1-S cyclins, or CLNs, have been identified that trigger the G1-S transition of the cell cycle. The regulation of the G1-S transition is particularly intriguing in *Drosophila* both because G1 is added to the cell cycle developmentally and G1-S regulators may drive the polytene cell cycle. To identify potential G1-S regulators from *Drosophila*, a cDNA expression library was constructed in which Kc cell cDNAs were placed in a high-copy *S. cerevisiae* vector under the control of the constitutive ADH1 promoter. Following transformation into an *S. cerevisiae* strain lacking all three CLN gene products, we identified one *Drosophila* cDNA that complemented the yeast G1 cyclins and restored growth to near wild-type levels. The *CLNDm* gene is present as a single copy in the *Drosophila* genome and encodes a 1.2-kb mRNA. DNA sequence analysis reveals that although this gene has cyclin homology, it is a new member of the cyclin gene family. *CLNDm* mRNA expression correlates with periods of maximal cell division throughout *Drosophila* development. The transcript is most abundant in early embryos, and it is present in low levels in larvae, pupae, and adults. *Drosophila* embryos hybridized in situ to this cyclin gene show uniform expression of the message throughout the embryo, with diminishing expression as embryogenesis proceeds.

[Key Words: Cell cycle; *Drosophila*; cyclin; *CLN*; *S. cerevisiae*]

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During *Drosophila* development the cell cycle undergoes several dramatic changes [Fig. 1]. The early cleavage divisions of embryogenesis are controlled by maternal components and rapidly oscillate between S phase and mitosis without detectable intervening gap (G) phases [Foe and Alberts 1983, Campos-Ortega and Hartenstein 1985]. Following cellularization and blastoderm formation, at ~3 hr after fertilization, a zygotically regulated G2 phase is added to the cell cycle [Edgar and O’Farrell 1989]. This is followed by three postblastoderm mitotic divisions that occur in spatially and temporally regulated domains [Foe 1989]. In at least the first two of these cycles 14 and 15), S phase follows mitosis almost immediately, such that if a G1 phase is present it must be <5 min in length [Edgar and O’Farrell 1990]. After cycle 16, either a G1 phase is added to the cell cycle or an existing G1 is lengthened greatly, as evidenced both by a gap prior to S phase and by the detection of cells with a 2C DNA content [Edgar and O’Farrell 1990, Smith and Orr-Weaver 1991]. During the latter half of embryogenesis, only the neural cells continue in a mitotic cycle with G1, S, G2, and M phases.

*Drosophila* genes involved in regulating the entry into mitosis have been identified, and they illustrate the extreme evolutionary conservation of cell cycle control. In a number of organisms the G3-M transition has been shown to be governed by maturation-promoting factor [MPF], a complex of the cdc2 and cyclin proteins with serine-threonine kinase activity [for review, see Lewin 1990]. *Drosophila* homologs to cyclin A and B have been cloned and, in the case of cyclin A, demonstrated genetically to be essential for mitosis (Lehner and O’Farrell 1989; Lehner and O’Farrell 1990b; Whitfield et al. 1990). The *Drosophila* string gene, a homolog of the *Schizosaccharomyces pombe* cdc25 gene (a positive regulator of the *cdc2* protein kinase), is the critical regulator responsible for triggering entry into mitosis during the three postblastoderm divisions of embryogenesis [Edgar and O’Farrell 1989, 1990]. A second gene capable of rescuing *S. pombe* cdc25 mutants has been isolated, and it has been subsequently shown to be a *Drosophila* cdc25 homolog [Jimenez et al. 1990, L. Alphey and D. Glover, pers. comm.]. Two cdc2 homologs have been identified in *Drosophila*, only one of which complements the *S. pombe* mutant [Jimenez et al. 1990, Lehner and O’Farrell 1990a]. One or both of these could be targets of the two *Drosophila* cdc25 genes.

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Regulators of the G₁–S transition and the entry into S phase have not been identified in Drosophila. The cdc2 gene has been shown in the yeasts S. pombe and Saccharomyces cerevisiae to regulate both the G₁–S and G₂–M transitions [Nurse and Bissett 1981; Piggott et al. 1982; Reed and Wittenberg 1990]. Therefore, it is likely that at least one of the two Drosophila cdc2 homologs will function in controlling entry into S phase, but this remains to be established. The regulation of the G₁–S transition is a particularly interesting problem in Drosophila, as a G₁ phase is added to the cell cycle, or greatly lengthened, during embryogenesis in response to developmental signals. The identification of Drosophila G₁–S regulators will facilitate understanding how this alteration of G₁ occurs. In addition, because the endo cell cycle (S–G) is independent of G₂–M string control, it is of interest to determine whether this novel cell cycle is also independent of G₁–S regulators [Smith and Orr-Weaver 1991].

In S. cerevisiae the CDC28 gene, the homolog of cdc2, controls the G₁–S transition by interacting with three redundant G₁-specific cyclins, CLN1, CLN2, and CLN3 [Richardson et al. 1989]. The CLN cyclins act to regulate the G₁–S transition because strains mutated for all three CLN genes arrest in G₁. Furthermore, dominant, gain-of-function alleles of CLN result in precocious entry into S phase [Cross 1988; Nash et al. 1988]. The levels of the CLN1 and CLN2 transcripts and proteins vary with the cell cycle and are maximal in G₁. Although the CLN3 transcript does not change with the cell cycle, presumably the activity of the CLN3 protein is modulated [Wittenberg et al. 1990]. Thus, the levels or activity of the CLN proteins appear to trigger the G₁–S transition, most likely by the association of these proteins with the CDC28 protein to form an active kinase. The CLN2 protein has been shown to associate with the CDC28 protein in an active kinase complex [Wittenberg et al. 1990].

The recent isolation of four G₂–M cyclins from S. cerevisiae [Ghiara et al. 1991; Surana et al. 1991] implies that although CDC28 is responsible for both the G₁–S and G₂–M transitions, its specificity is dictated by associated cyclin proteins.

The isolation of G₁–S regulators is important for the investigation of cell cycle regulation in Drosophila. We chose to isolate Drosophila homologs to the CLN genes by functional complementation because this has the stringent criterion that the complementing genes are capable of conducting the same biological function. Nevertheless, the possibility remains that the properties exhibited by a Drosophila gene in the yeast cell may not accurately reflect its true function in the fly. Transformation of an S. cerevisiae strain mutated for all three CLN genes with a cDNA expression library yielded a novel Drosophila cyclin gene that complements the CLN function. This cyclin gene is expressed maximally during developmental periods when cell division is occurring.

**Results**

**Isolation of a functional CLN homolog from Drosophila**

Many cell cycle regulators are evolutionarily conserved and can complement the same function in heterologous species. For example, the human cdc2 gene complements cdc2⁻ S. pombe mutants [Lee and Nurse 1987]. We used such an approach to search for possible G₁ cyclins in Drosophila by screening for Drosophila cDNAs capable of complementing cln⁻ deficiencies in S. cerevisiae. During the Drosophila life cycle, cells that utilize a cycle with both G₁ and G₂ phases make up only a small percentage of the total cell population. We therefore constructed the cDNA library using mRNA obtained from actively dividing Drosophila Kc cells. Kc cells are an embryonically derived cell line but utilize a cell cycle that contains both a G₁ and a G₂ phase [Echalier 1976], thereby increasing the chances of isolating G₁ regulatory factors.

We screened for rescue of growth of an S. cerevisiae strain mutated for the CLN genes by transforming the strain with the Drosophila Kc cell cDNA library that had been constructed in a yeast expression vector [Fig. 2]. Loss of all three S. cerevisiae CLN genes is lethal; therefore, the viability of the host strain [884-21B] lacking all three CLN genes [cln1, cln2, cln3] was sustained by the presence of the CLN3 gene on a UR43-marked CEN plasmid. After transformation of this strain with the cDNA library, the UR43-marked CLN3 plasmid was removed by the plasmid shuffle technique using 5-fluoro-orotic acid (5-FOA) selection [Boeke et al. 1987], and the colonies that retained the ability to grow without the CLN3 gene were selected. Cells lacking the CLN3 plasmid should grow only if they contain a Drosophila gene capable of complementing the G₁–S defect of the cln triple mutant. To compensate for the possibility that the Drosophila gene would complement the yeast
**DNA from the single positive isolate, temporarily designated CLNDm until assigned to a cyclin class, was electroporated into Escherichia coli to recover the cDNA-containing plasmid. The transformants contained only one plasmid, with an insert size of ~1.1 kb. The purified CLNDm plasmid was restested for ability to complement CLN function by transforming strain 884-21B[pW16]. This strain, isogenic with 884-21B, contains a plasmid with the yeast CLN3 gene under the control of the GAL1 promoter, so the strain is able to grow on galactose but not on medium containing glucose. However, the CLNDm transformants of this strain grew quite well on medium containing glucose. Thus, under two different selection criteria, the CLNDm gene complemented the cln− deficiency.**

To verify that the cDNA insert was derived from Drosophila, we probed a Southern blot of genomic Drosophila DNA with the CLNDm insert. Five different restriction digests of Drosophila DNA hybridized to CLNDm in patterns indicative of a single-copy gene, as all but one digest yielded a single band by Southern analysis. Moreover, the CLNDm DNA hybridized to polyclonal bands 88D,E on larval salivary gland squashes (data not shown). These results show that the DNA is derived from Drosophila and that the gene is present in only a single copy in the Drosophila genome.

**Rescue of the cln− phenotype in yeast**

The growth and morphology of the yeast strain rescued by the CLNDm gene was examined to determine how well the Drosophila gene complemented the mutant yeast phenotype. The rescued strain showed seemingly wild-type growth as evidenced by the colony size on YPD plates (Fig. 3) or synthetic complete medium (data not shown). When examined under the microscope, however, the cell shapes were often aberrant, with many large cells having long projections and squared or elongated cell morphology. In addition, chains of incompletely separated cells were observed. This morphology may be the result of the overproduction of the Drosophila CLN homolog, as the same aberrant morphology was seen in the isogenic strain containing the CLN3 gene overproduced by the galactose promoter. Because the growth rate of the CLNDm transformants was near wild type and the altered morphology may be a nonspecific stress response, we did not pursue this issue further.

Strain 884-21B containing CLNDm was tested for its ability to respond to α-factor in a halo assay. The CLNDm-containing strain displayed the same, or slightly greater, sensitivity to α-factor as a pFC204 (CLN3) transformant of the same strain (data not shown). Therefore CLNDm not only complements the cln growth defect but is also responsive to the mating pheromone signal for cell cycle arrest.

**DNA sequence of CLNDm**

The CLNDm gene was sequenced to determine whether it was homologous to previously identified genes, espe-
Figure 3. Comparison of the growth rate of a transformant with CLNDm with a transformant of CLN3. The growth rates of strain 884-21B, triply mutated for the cln genes, are compared when transformed with CLNDm in the high-copy expression vector pBL15 [left] versus the starting strain containing CLN3 on the CEN vector pFC204 [right]. Growth was for 3 days at 30°C on YPD plates.

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The gene sequence was not found in the GenBank data base. The DNA sequence of 1084 bp (Fig. 4) encodes a putative open reading frame of 267 amino acids with a consensus translation start sequence 5' to the first AUG [Caveney 1987], and a putative polyadenylation signal 3' to the coding sequence [Fitzgerald and Shenk 1981]. A poly(A) tail of 16 bp is present in the cDNA clone. The open reading frame encodes a polypeptide of ~31 kD, with an estimated pl of 5.95.

Comparison of CLNDm with previously identified cyclins, using either the PIMA or IALIGN protein homology alignment programs [Dayhoff et al. 1983; Deveraux et al. 1984; Smith and Smith 1990], indicated that CLNDm, though clearly unique, has homology to cyclins. Both programs identified the same region of the CLNDm gene as homologous to the other cyclins, the "cyclin box." This box consists of ~100 amino acids located between amino acids 39 and 138 in the CLNDm protein. The regions aligned between CLNDm, human cyclin D [Xiong et al. 1991], mouse cyl3 [one of several mouse cyclin D's recently identified; Matsushima et al. 1991], and CLN1 by the IALIGN program are shown in Figure 5. The region of CLNDm that appears most conserved is the LLAPCLLASK stretch. However, CLNDm is quite diverged from other members of the cyclin family. The cyclin box of CLNDm is ~20% identical to other cyclins and 45% similar if conservative amino acid changes are considered.

Using the IALIGN program, we quantitatively compared the CLNDm gene with seven cyclin genes: human cyclin D and mouse cyl3, the CLN1, CLN2, and CLN3 genes from S. cerevisiae [Nash et al. 1988; Hadwiger et al. 1989]; and the Drosophila G2-M cyclins A and B [Leh-
The cell cycle changes that occur during embryogenesis are indicated. In pairwise comparisons of Figure 5 Amino acid sequence comparison of CLNDm with two cyclin D's and CLNI. The alignments obtained from the IALIGN program are indicated. In pairwise comparisons of CLNDm with human cyclin D [A], mouse cycl1 [B], or CLNI [C], the same region of CLNDm was identified by the computer. The numbers indicate the amino acid position within each protein sequence. Identical residues are designated by vertical bars, conserved amino acids are shown by asterisks (*). The following groups of amino acids were classified as conserved: \( \text{N, D, E, Q; K, R; S, T, A, G, P; F, W, Y; I, V, L, M} \) (Dayhoff et al. 1983). Obtained by comparing the real sequences versus the sequences. Thus, an alignment score of 5.08 means that the probability of obtaining the similarities by chance is \( 1.5 \times 10^{-7} \), and a score of 3.63 has a probability \( 1.3 \times 10^{-4} \). Of those compared, the only other cyclin that showed homology to CLNDm was CLNI [Fig. 5]; however, its alignment score is not significant. CLNDm is very different from the other cyclins tested.

CLNDm is predicted to encode the lowest molecular mass protein in the cyclin family (31 kD), although the predicted cyclin D protein is small as well (34 kD; Xiong et al. 1991). CLNDm also parallels cyclin D and CLNI in having its cyclin box located in the amino-terminal region of the protein. In contrast with the CLNI protein, the predicted CLNDm protein does not appear to contain a PEST region, a region associated with proteins with a short intracellular half-lives (Rogers et al. 1986).

While this manuscript was in preparation we learned that Pierre Leopold and Patrick O'Farrell had isolated a Drosophila cyclin-like gene by using a similar complementation strategy. Their gene appears to be the same as ours, as evidenced by the same cytological location. They have designated their gene was expressed transcriptionally to determine when in development the transcript parallels CLNI in having its cyclin box located in the amino-terminal region of the protein. In contrast with the CLNI protein, the predicted CLNDm protein does not appear to contain a PEST region, a region associated with proteins with short intracellular half-lives (Rogers et al. 1986).

We wanted to examine when in development the Drosophila CLN gene was expressed transcriptionally to determine whether its expression was enhanced at times when cell division was occurring or during developmental periods when most cells were in the endo cell cycle. The cell cycle changes that occur during embryogenesis have been described (Fig. 1). During the larval and pupal stages, the majority of cells are in the endo cell cycle, and mitotic divisions occur only in imaginal or neural tis-
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Figure 6. Northern analysis of Drosophila CLNDm mRNA in Kc cells and during development. Northern blots of poly(A)^+ RNA were hybridized to the Drosophila CLNDm cDNA. As a control for the amount of mRNA loaded, the same blots were probed with actin-SC DNA (Fryberg et al. 1983) (bottom). The developmental stages shown are a composite of two different Northern blots in which lanes labeled ovary through third-instar are from one blot, and lanes labeled pupae and adult are from another. (A) Molecular weight standards (bases); Kc cell mRNA, mRNA from 8- to 24-hr staged embryos. (B) Poly(A)^+ RNA from ovary, 0- to 2-, 2- to 5-, 5- to 8-, and 8- to 24-hr staged embryos, second-instar larvae, third-instar larvae, pupae, adult female, and adult male.

Localization of the CLNDm transcript in embryos

The developmental Northern blot suggested that the transcript was expressed in dividing cells and not in polytene tissues. To examine whether the transcript was localized to cells undergoing cell division we performed digoxygenin whole-mount in situ hybridization to wild-type embryos at different developmental stages using the CLNDm DNA as a probe [Fig. 7]. The level of CLNDm transcript is high in preblastoderm embryos and diminishes progressively during embryogenesis [Fig. 7A,B]. Although the staining is faint by germ-band extension, the staining is more intense than in control embryos without probe or those probed with vector sequences [Fig. 7B,C; data not shown]. We cannot be certain of the exact developmental point at which the transcript can no longer be detected above background.

No tissue-specific localization of the CLNDm transcript was detected from preblastoderm to cuticle deposition, the latest developmental time point that we could analyze using this technique [Fig. 7A,B; data not shown]. The CLNDm message levels were not detectably higher either in mitotically dividing cells or in polytene cells. This is in contrast to the string gene, the Drosophila homolog of S. pombe cdc25, which labels specific postblastoderm mitotic domains [Fig. 7D] (Edgar and O’Farrell 1989). Thus, it appears that CLNDm is not preferentially transcribed in mitotic cells, but because the message is present in low levels it is possible that we failed to detect some specific labeling. The fact that the gene does not appear to be expressed at higher levels in dividing cells does not preclude a direct role by CLNDm in the Drosophila cell cycle. Neither Drosophila cyclins A or B nor the cdc2 gene are regulated transcriptionally during the cell cycle (Jimenez et al. 1990; Lehner and O’Farrell 1990a,b; Whitfield et al. 1990).

Discussion

We have identified a novel cyclin gene from Drosophila that complements the CLN function in S. cerevisiae. Yeast strains triply mutated for the CLN genes grow at near wild-type levels in the presence of CLNDm. Per-

Figure 7. Distribution of CLNDm transcript during embryogenesis. Whole-mount embryos were hybridized in situ with the CLNDm or string cDNA, which had been labeled by incorporation of digoxygenin dUTP by random priming. Hybridization was detected by an alkaline phosphatase histochemical reaction. (A) Preblastoderm embryo hybridized to CLNDm; (B) Germ-band-extended embryo hybridized to CLNDm; (C) preblastoderm embryo hybridized without probe and stained; (D) germ-band-extended embryo hybridized with string. All embryos are oriented with anterior to the left and dorsal up. Bar, 50 μm.
haps unexpectedly, cells bearing CLNDm respond to α-factor, implying that the pheromone signal transduction pathway is able to regulate CLNDm. Whereas CLNDm contains a cyclin box and is clearly a member of the cyclin family, the sequence of CLNDm is quite divergent from the other cyclins and represents a new branch on the cyclin family tree. Of the published cyclin genes, CLNDm is closest to cyclin D. However, this gene has been independently isolated and assigned to a new class of cyclins, cyclin C (Leopold and O’Farrell 1991). CLNDm is the same gene as Droso-phiya C. When expressed in high copy in yeast, CLNDm can regulate the G1–S transition. Complementation is a stringent criterion of gene function, which strongly implies that CLNDm may regulate the cell cycle in Drosophila. Although it is possible that CLNDm may control the G1–S transition in Drosophila, this conclusion must be regarded as tentative for two reasons. The precedent exists for genes from one species to complement yeast mutations, yet normally have a different function, as is the case with mammalian ras genes. Whereas RAS proteins control adenylate cyclase in S. cerevisiae, they do not do so in mammalian cells (Hall 1990). Furthermore, both S. pombe and human cyclin B, which normally regulate G2–M, have been reported to rescue yeast chi mutants (cited in Xiong et al. 1991). Second, although members of the cyclin family share the cyclin box, individual members are highly diverged and show only 20% homology within this region. Because the conservation is so limited it is possible that the cyclin box represents a structural motif, and it may be diagnostic solely of a kinase interaction domain rather than a cell cycle function. When initially identified, the homeo box was thought to be diagnostic of a gene involved in developmental determination. As more examples of homeo box-containing proteins have been identified, it has become clear that this domain represents a DNA-binding motif that is not restricted to developmental genes.

Despite these cautionary notes, we think it most likely that CLNDm is involved directly in regulating the cell cycle in Drosophila. The expression of CLNDm during development closely parallels the extent of cell division. Developmental Northern analysis showed that the CLNDm mRNA is present maximally during the early stages of embryogenesis when the cleavage and postblastoderm divisions occur. The low levels of CLNDm transcript during larval, pupal, and adult male stages correlate with the relatively infrequent cell division occurring at these times. In addition to its expression pattern correlating with a role of CLNDm in the cell cycle, of the published cyclin genes CLNDm is most homologous to the cyclin D class. Mouse cyclin D has been shown to be regulated by colony-stimulating factor, and the timing of its expression suggests that this cyclin may be involved in S-phase commitment (Matsushima et al. 1991).

At present, we cannot distinguish between a role of CLNDm in G1–S versus G2–M regulation. In situ hybridizations to embryos showed that the transcript was distributed uniformly throughout the embryo, showing neither a preponderance in G1 or G2 cells nor an increased abundance in cells in a dividing cell cycle. Although it would have been informative, it is not surprising that the transcripts for this cyclin gene do not fluctuate with the cell cycle. Cyclin A and B message levels are constant during the cell cycle in Drosophila (Lehner and O’Farrell 1989, 1990b), and so far only the string gene has been demonstrated to be transcriptionally regulated in the Drosophila cell cycle (Edgar and O’Farrell 1989). The fact that the CLNDm mRNA is present at low levels during larval stages when many tissues are in the endo cell cycle implies that CLNDm is not involved in the endo cell cycle. Nevertheless, CLNDm may play a role if it is subject to post-transcriptional regulation. Alternatively, another as yet unidentified cyclin may regulate the endo cell cycle. Several mouse cyclin D’s with different tissue specificities have been identified (Matsushima et al. 1991), raising the possibility that a family of cyclins with developmental specificity may exist in Drosophila.

Analysis of the CLNDm protein levels during the mitotic and endo cell cycles will provide further insights into the function of this gene. Such an analysis will be facilitated greatly by the spatial and temporal map of the mitotic divisions and polytene S phases that has been defined for Drosophila embryogenesis (Foe 1989, Smith and Orr-Weaver 1991). The accumulation and disappearance of the cyclin A and B proteins during the Drosophila cell cycle have been suggestive of their roles in mitosis (Lehner and O’Farrell 1990b, Whitfield et al. 1990). Characterization of the CLNDm protein may also clarify whether this gene functions in the endo cell cycle or is restricted to regulating the early embryonic and, possibly, germ-line divisions. Ultimately, the isolation of mutations within the CLNDm gene, taking advantage of the ability to apply a genetic approach in Drosophila, will permit us to elucidate fully the contribution of this novel cyclin gene to the cell cycle.

Materials and methods

Strains

S. cerevisiae strain 884-21B[pW16], MATa arg4·Bcl ura3·Δns leu2-3,113 trp1-1° ade1·TRP1 cln1·CLN1 cln2·LEU2 cln3·pW16, URA3 CEN4 ARS1 CLN3 Amp’ colE1 was obtained from Fred Cross (Rockefeller University) (Richardson et al. 1989). The arg4, ura3, and leu2 mutations are nonreverting. The pW16 plasmid in this strain was replaced with pFC204, URA3 CEN4 ARS1 CLN3 Amp’ colE1 [obtained from Fred Cross] to generate a strain that transformed efficiently. Strain 884-21B[pW16] will not grow on glucose because the complementing CLN3 gene is under the control of the galactose promoter. Transformants of pFC204 were selected by their ability to grow on glucose. After serially streaking onto YDP several times, colonies that had lost the pW16 plasmid were identified by Southern analysis. E. coli HB101 [Sambrook et al. 1989] and XL1 blue (Stratagene) were described previously.

Expression vector construction

The S. cerevisiae expression vector pBL15 contains the cDNA-cloning site downstream from the constitutive ADH1 promoter and upstream of the ADH1 termination sequence. Plasmid pBL15 was derived from plasmid pDB15 [Becker et al. 1991] by replacing the URA3 marker with the ARG4 marker by digesting
with NdeI and StuI to remove the majority of the URA3 sequence, and blunt-end-ligating a 3.3-kb PstI fragment containing the entire ARG4-coding region into the vector ends.

cDNA library construction

Drosophila Kc cell mRNA was kindly provided by Paul Kaufman (Whitehead Institute). Full-length cDNA was prepared by using the cDNA synthesis kit from BRL but substituting SuperScript RNase H⁻ reverse transcriptase (BRL) for the reverse transcriptase supplied with the kit. Reverse transcriptase reactions were performed at 42°C to improve processivity. The double-stranded cDNA was blunt end ligated to self-incompatible BstXI monomer linkers [Invitrogen], which prevents more than one linker from being added per cDNA molecule end. The cDNA was then ligated into pBluescript vector DNA that had been linearized with BstXI. This cDNA library was transformed into E. coli HB101, and 1 x 10⁹ independent transformants were obtained. The CsCl₂-purified Drosophila cDNA obtained from this transformation was used to transform S. cerevisiae for the CLN complementation screen.

CLN complementation screen

S. cerevisiae 884-21Bl[pFC204] was transformed with the Drosophila Kc cell cDNA library by using the lithium acetate method [Schiestl and Gietz 1989], and Arg² transformants were selected at 30°C on plates lacking arginine. Transformants were then replica plated onto synthetic complete medium containing 5-FOA, only cells that are Ura⁻ will survive on 5-FOA (Boeke et al. 1984) and were hybridized with either nick-translated or random-primed probes in aqueous solution at 65°C. Northerns blots were hybridized to probes labeled by random priming in 50% formaldehyde at 42°C as described previously (Orr-Weaver and Spradling 1986). For quantification of mRNA levels, X-ray film was preflashed and the intensity of exposure was quantitated on a Zeineh Soft Laser Scanning Densitometer [Biomed Instruments].

In situ hybridization

In situ hybridizations to polytene chromosomes and embryos were done with a wild-type Canton S stock. Polytene chromosome squashes and hybridizations were performed as described previously [Engels et al. 1986], with the modification of hybridization overnight at 30°C to increase detection. In situ hybridizations to embryos were performed essentially as described [Tautz and Pfeifle 1989], with the modification that the initial paraformaldehyde fixation was performed by using 2 ml of 4% paraformaldehyde/0.2 ml DMSO/8 ml heptane. The digoxigenin-labeled probe was made as described in the Genius Kit (Boehringer Mannheim), with the modifications that the hexanucleotide mix was used at double the recommended concentration and the ethanol-precipitated probe was resuspended in 100 μl of hybridization buffer. For digoxigenin labeling of CLNDm the cDNA insert was isolated and digested with Alul prior to labeling. The 1.4-kb EcoRI restriction fragment of the string cDNA was isolated, digested with Alul, and labeled [Edgar and O'Farrell 1989]. As a control for background hybridization, Bluescript vector was labeled and used as a probe. Ten microliters of probe were used per hybridization. Salivary gland in situ hybridizations were examined under phase on a Zeiss Axiohot microscope with a Plan-Neofluar 40x objective. Embryos were examined by using Nomarski optics and a Plan-Neofluar 25x objective.

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Note added in proof

The human cyclin C gene referred to as unpublished has been published by D. Lew, V. Dulić, and S. Reed. 1991. Cell 66: 1197–1206.

Sequence data described in this paper have been submitted to EMBL/GenBank data libraries under accession number X62948.

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