Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 3011–3015, April 1996
Developmental Biology

Isolation of *Drosophila* cyclin D, a protein expressed in the morphogenetic furrow before entry into S phase

(cyclin-dependent kinase/two-hybrid/interaction trap)

RUSSELL L. FINLEY, JR.*†‡, BARBARA J. THOMAS§¶, S. LAWRENCE ZIPURSKY§‖, AND ROGER BRENT*†**

*Department of Molecular Biology, Massachusetts General Hospital, 50 Blossom Street, Boston, MA 02114; †Department of Genetics, Harvard Medical School, Boston, MA 02115; and §Department of Biological Chemistry, ‖The Howard Hughes Medical Institute and The Molecular Biology Institute, University of California, Los Angeles, CA 90024

Communicated by Elizabeth F. Neufeld, University of California, Los Angeles, CA, October 6, 1995 (received for review July 21, 1995)

ABSTRACT **During *Drosophila* development, nuclear and cell divisions are coordinated in response to developmental signals. In yeast and mammalian cells, signals that control cell division regulate the activity of cyclin-dependent kinases (Cdks) through proteins such as cyclins that interact with the Cdks. Here we describe two *Drosophila* cyclins identified from a set of Cdk-interacting proteins. One, cyclin J, is of a distinctive sequence type; its exclusive maternal expression pattern suggests that it may regulate oogenesis or the early nuclear divisions of embryogenesis. The other belongs to the D class of cyclins, previously identified in mammalian cells. We show that *Drosophila* cyclin D is expressed in early embryos and in imaginal disc cells in a pattern that anticipates cell divisions. Expression in the developing eye disc at the anterior edge of the morphogenetic furrow suggests that cyclin D acts early, prior to cyclin E, in inducing G_1-arrested cells to enter S phase. Our results also suggest that, although cyclin D may be necessary, its expression alone is not sufficient to initiate the events leading to S phase.**

In yeast or mammalian cells in culture, progression through the cell cycle requires the activity of cyclin-dependent kinases (Cdks) (1, 2). Extracellular signals that regulate cell proliferation result in changes in Cdk activity during the G_1 phase of the cycle (3, 4). During *Drosophila melanogaster* development, cell cycles are regulated in different phases, from the early nuclear divisions of embryogenesis that lack gap phases, to cell divisions with regulated G_2 and, eventually, G_1 phases (5, 6). Although the specific developmental signals that control these divisions are not fully understood, it is likely that the response to them is mediated, as in vertebrates, by Cdk-interacting proteins [Cdk interactors (Cdis)] that affect Cdk activity. Some *Drosophila* Cdis have been identified, including cyclins A and B, which were shown to be important for postblastoderm divisions (7–9) and for early cleavage divisions in the case of cyclin A (10); the "String and Twine" phosphatases, which may activate Cdks by dephosphorylating them at the G_2–M transition (11–13); and cyclin C, which was isolated by its ability to complement yeast cyclin mutants (14, 15). Recently, a *Drosophila* E-type cyclin was isolated and shown to be necessary to drive certain embryonic cells into S phase in the neurogenic region of the epidermis and in endoreduplicating tissue (16, 17). However, *Drosophila* counterparts to a number of mammalian Cdis that regulate cell division in response to extracellular signals, such as cyclin D (4, 18), have not been identified.**

MATERIALS AND METHODS

Yeast Strains and Plasmids. Yeast strain EGY48 (*MATα ura3 his3 trp1* 3LexAop-*LEU2*::*leu2*) has been described (19, 20). *HIS3* 2-μm bait plasmids for expressing LexA-DmCdc2, LexA-DmCdc2c (20), LexA-Hairy (21), or LexA-BcdΔ160 (22) have been described.

Libraries. We made three *Drosophila* cDNA libraries for expression of cDNA-encoded proteins fused to a transcription activation domain in yeast. Details of the library constructions are available on request (http://xanadu.mgh.harvard.edu). Briefly, cDNA was made from poly(A)$^+$ RNA derived from *Drosophila* embryos (0 to 12 hr; provided by S. Abmayr and T. Maniatis, Harvard University), ovaries (provided by G. Jimenez and D. Ish-Horowicz, Imperial Cancer Research Fund), or mixed discs (provided by J. Fisher Vize and R. Lehman, Whitehead Institute) as described (20) and inserted into the yeast expression vector pJG4-5 (20). The embryonic library (RFLY1) has 4×10^6 independent members, and 90% of the plasmids have cDNA inserts of 0.5–2.9 kb (average size, 1 kb). The ovary library (RFLY3) has 3.5×10^6 independent members, and 83% of the plasmids have inserts of 0.3–1.5 kb (average size, 800 bp). The disc library (RFLY5) has 4.0×10^7 independent members, and 92% of the plasmids have inserts of 0.3–2.1 kb (average size, 900 bp).

Interactor Hunts. Two hunts for *D. melanogaster* (Dm) DmCdc2 and DmCdc2c interactors were performed as described (20, 23). In the first, we transformed EGY48 that expressed LexA-DmCdc2 with RFLY1 library DNA and collected 6.6×10^5 transformed colonies. From these, 6.6×10^6 viable cells were plated onto galactose medium lacking leucine, and 100 Leu$^+$ colonies were picked. Of these, 8 were galactose-dependent Leu$^+$, indicating that the Leu$^+$ phenotype depended on the expression of the cDNA. The library plasmids from these 8 were rescued, and the cDNA inserts were sequenced; they represented four unique cDNAs—Cdi2, Cdi3, Cdi7, and Cdi3ΔN (a shorter version of Cdi3). In the second selection, we transformed EGY48 that expressed LexA-Dmcdc2c with RFLY1 DNA and collected 5×10^6 transformants. From these, 7.5×10^7 viable cells were plated onto galactose medium lacking leucine, and 145 Leu$^+$ colonies were picked, 18 of which were galactose-dependent Leu$^+$. Of the 18, 9 encoded Cdi3 and 9 encoded four additional Cdis: Cdi4, Cdi5, Cdi11, and Cdi12. All strains also contained the *lacZ* reporter, pJK103, so that some interactions resulted in blue color on 5-bromo-4 chloro-3 indolyl β-D-galactoside (X-Gal) plates (22, 23) (not shown).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: Cdk, cyclin-dependent kinase; Cdi, Cdk interactor.

‡Present address: Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit, MI 48201.
¶Present address: National Cancer Institute, National Institutes of Health, Building 37, Room 4C3, 37 Convent Drive, Bethesda, MD 20892.
**To whom reprint requests should be addressed.

††The sequences reported in this paper have been deposited in the GenBank data base (accession nos. U41808 for *D. melanogaster* cyclin D, U41809 for *D. melanogaster* cyclin J, and U40077 for *D. melanogaster* cks).

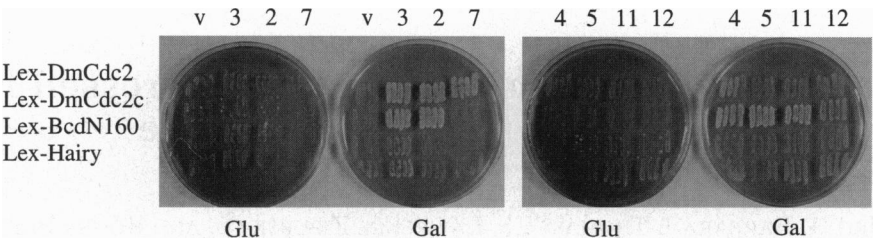

FIG. 1. *Drosophila* Cdis. Seven cDNAs that encode Cdis were isolated in hunts for interactors with DmCdc2 and DmCdc2c (see *Materials and Methods*). Four derivatives of EGY48 that expressed LexA-DmCdc2, LexA-DmCdc2c, LexA-Hairy, or LexA-BcdN60 were transformed with the library vector pJG4-5 (v) or derivatives of pJG4-5 that expressed from the *GAL1* promoter activation domain fusions to Cdi2 (lanes 2), Cdi3 (3), Cdi4 (4), Cdi5 (5), Cdi7 (7), Cdi11 (11), or Cdi12 (12). Four individual colonies from each transformation were patched to minimal selection plates (not shown) and then replica plated to minimal selection plates lacking leucine but containing either glucose (Glu) or galactose plus raffinose (Gal). Galactose-dependent growth indicates an interaction between the Cdi and the LexA fusion (20).

Sequence Analysis. The amino acid sequences of 75 cyclin proteins from Genbank including, from various species, 21 B-type, 14 A-type, 7 E-type, 11 D-type, 3 C-type, 2 F-type, 2 G-type, 1 H-type, and several yeast cyclins, were aligned by using the Wisconsin Package (Genetics Computer Group) PILEUP program and visual inspection. A cyclin consensus sequence was derived consisting of the 20 residues that are either identical or have conservative replacements (methionine or leucine; tryptophan or phenylalanine) in at least 65 of the 75 cyclins (see Fig. 2). Pairwise protein sequence comparisons were made by aligning two sequences with the Wisconsin Package BESTFIT program and counting the number of identical amino acids and dividing by the shorter of the two sequences to get percent identities (see Table 1).

Complementation of Yeast Cyclin (*cln*) Mutants. Epitope-tagged Cdis were expressed from the 2-μm plasmid pJG4-6 (J. Gyuris and R.B., unpublished data). *Saccharomyces cerevisiae* strain 3c-1AX (*MAT***a** *bar1* Δ*cln1* Δ*cln2* Δ*cln3 cyh2* [pLEU2-CYH2-CLN3] *trp1 leu2 ura3 ade1 his2*) (provided by J. Roberts and F. Cross, Rockefeller University) was transformed with pJG4-6 or derivatives expressing Cdis, and transformants were grown at 30°C in liquid galactose/raffinose Trp^- medium (23) to saturation, diluted 1:10, grown for another 6 hr, and then plated on galactose/raffinose Trp^- medium containing 10 mg of cyclohexamide per ml to select for loss of the pLEU2-CYH2-CLN3 plasmid. On these plates colonies formed only from cells that contained pJG4-6-Cdi3, pJG4-6-Cdi3N, pJG4-6-Cdi5, or pJG4-6-Cdi7. Several colonies from each were tested and were shown to be Leu^-, indicating loss of the *CLN3*-expressing plasmids, and were galactose-dependent for growth, indicating complementation of the *cln* defect due to Cdi expression from the *GAL1* promoter.

RNA Blot Hybridization Analyses (Northern Analyses). Total RNA from the sources shown in Fig. 4 was isolated as described (24) and enriched for poly(A)$^+$ RNA on oligo(dT)-cellulose columns. Poly(A)$^+$ RNA (3 μg) was loaded on each lane of a 1% agarose-formaldehyde gel. After electrophoresis the RNA was transferred, UV-photo-crosslinked to a nylon membrane, probed with ^{32}P-labeled Cdi3 and Cdi5 cDNAs, and, with a ^{32}P-labeled PCR product corresponding to codons 3–195 of *Drosophila ras64B*, autoradiographed as described (25).

***In Situ* Hybridizations.** Third-instar eye imaginal discs were incubated in *Drosophila* Schneider's medium containing 75 μg of BrdUrd per ml for 30 min and then processed for *in situ* hybridization essentially as described (26) except that the tissue was fixed in phosphate-buffered saline (PBS) containing 4% formaldehyde and 0.6% Triton X-100 for 30 min and then postfixed after proteinase K treatment in PBS containing 4% formaldehyde and 0.2% glutaraldehyde for 15 min at room temperature. Discs were then probed with random-primed digoxygenin-labeled DNA probes (500 ng/ml; BMB). After signal detection, the samples were processed for detection of BrdUrd incorporation. Briefly, tissue was permeabilized in 0.6% Triton X-100 and then transferred to PBS containing 2 M HCl for hydrolysis of DNA for 30 min. After extensive washing, BrdUrd was detected by using a monoclonal antibody (Beckton-Dickinson) and the ABC detection kit (Vector Labs).

RESULTS

To isolate *Drosophila* Cdis, we made *Drosophila* cDNA libraries (see *Materials and Methods*) for the interaction trap two-hybrid system (20). We used the 0- to 12-hour embryo library in hunts for cDNAs that encode proteins that interact with the two known *Drosophila* Cdks, Dmcdc2 and Dmcdc2c (27, 28). We isolated seven Cdis that interacted with one or both of the fly Cdks but not with unrelated proteins (Fig. 1). Two of the Cdis (Cdi11 and Cdi12) have no striking sequence similarity to previously identified proteins in data bases and will be described elsewhere. Cdi4 has similarity to the p21/p27 family of Cdk inhibitors (R.L.F. and R.B., unpublished data). Cdi2 is 78% identical to human Cks1, a homolog of *S. cerevisiae* Cks1 and *Schizosaccharomyces pombe* Suc1, essential proteins that interact with Cdks but whose function is unclear (29–31). Another, Cdi7, is nearly identical to *Drosophila* cyclin E type II (16) (R.L.F. and R.B., unpublished data). The remaining two proteins, Cdi3 and Cdi5, have significant sequence similarity to cyclins (Table 1).

The Cdi3 cDNA encodes a protein of 452 residues with strongest similarity to cyclins in a 135-amino-acid region from residue 157 to 292, which contains 20 matches to the 20 residues conserved in most cyclins (Fig. 2 *Upper*). The first 97 amino acids contain PEST and sequences (Fig. 2 *Upper*) found in many cyclins and thought to be involved in rapid protein turnover (33). From amino acid 98 to 407, Cdi3 shares extensive sequence similarity with the mammalian D cyclins, D1, D2 and D3 (34–37), and is 39% identical to human cyclin D2 (Table 1; Fig. 2 *Lower*). By comparison, the same portion

Table 1. Comparison of cyclin classes

	Dm A	Dm B	Dm C	DmCdi3 (D)	Dm E	DmCdi5 (J)
Hs A	39	28	20	24	26	25
Hs B	28	38	23	20	26	27
Hs C	16	20	72	19	17	19
Hs D1	25	24	19	35	26	21
Hs D2	29	22	24	39	27	23
Hs D3	25	25	18	39	26	21
Hs E	22	23	18	21	41	19
Hs F	24	22	18	18	23	21
Mm G	21	20	19	22	22	21
Hs H	20	18	28	21	20	21

Numbers represent percent amino acid identity as determined using the Wisconsin Package BESTFIT program (see *Materials and Methods*). Hs A through Hs H are human cyclins A through H (Mm G is mouse cyclin G). Dm A, B, etc., are *D. melanogaster* cyclins A, B, etc.

of Cdi3 is only 18–24% identical to the other classes of cyclins identified in humans (Table 1) or to the other *Drosophila* cyclins (types A, B, C, E, and J; see below). These facts indicate that Cdi3 is a D-type cyclin and that this class of cyclins has been independently conserved through evolution. Comparison of Cdi3 with 11 vertebrate D cyclins allowed us to derive a consensus sequence for D cyclins. The consensus sequence (Fig. 2 *Lower*) consists of 65 residues, of which 24 are unique to D cyclins. These residues may be necessary for the specific function of D cyclins (for example, interaction with specific Cdks). A recently isolated *Arabidopsis* cyclin with similarity to human cyclin D1 lacks 22 of the 24 cyclin D-specific residues and is only 24% identical with Cdi3, significantly less similar than *Arabidopsis* and *Drosophila* A cyclins (31% identity) or B cyclins (32% identity).

Cdi5 (Fig. 3) also has strong similarity to cyclins but defines a protein of a new sequence type. From residue 74 to 223, Cdi5 contains 16 matches to the 20 residues conserved in most

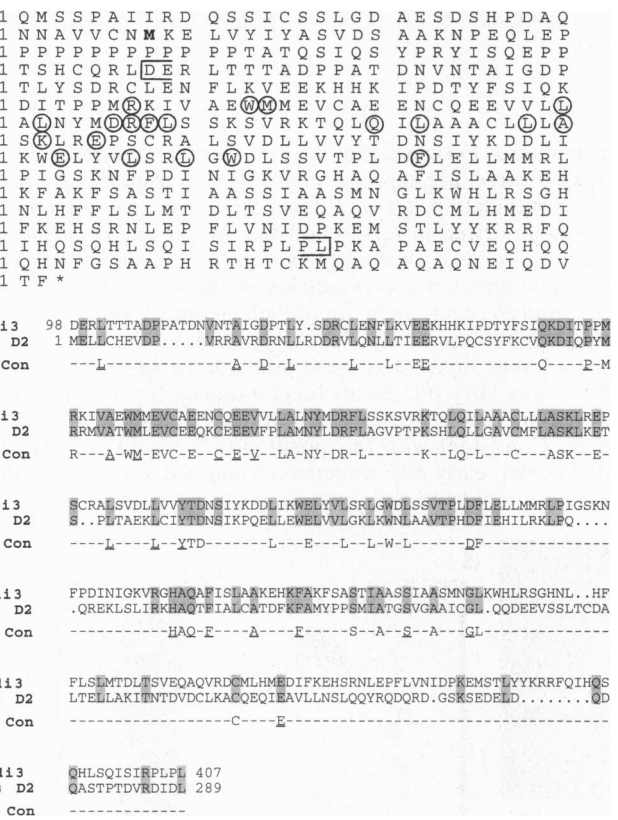

FIG. 2. (*Upper*) Cdi3 predicted protein. Conceptual translation of Cdi3 cDNA (GenBank accession number U41808). The ATG encoding methionine-38 (boldface M) is within a consensus translational start signal (32); the ATG encoding methionine-2 is not within a consensus translational start signal. Brackets from amino acid 98 to 407 indicate the region with similarity to human cyclin D2 (Fig. 2 *Lower*). Of 20 residues that are conserved in most cyclins, 20 are also conserved in Cdi3 (circled residues). Of the amino-terminal 92 amino acids, 43 are proline, glutamic acid, aspartic acid, or serine; such PEST regions may be involved in rapid turnover of proteins (33) and are common to many cyclins. The carboxyl-terminal 45 amino acids that lie beyond the cyclin D homology region are rich in glutamines (10) and alanines (7). The first amino acid encoded by the Cdi3ΔN cDNA corresponds to proline-89. (*Lower*) Amino acids 98–407 of the predicted Cdi3 protein are aligned with the entire human cyclin D2 protein. Residues identical in the two proteins are shaded. Below the alignment are shown consensus residues (D Con) found in Cdi3 and all 11 previously identified D-type cyclins, including 9 from mammals (D1, D2, and D3 from human, rat, and mouse), 1 from chicken, and 1 from *Xenopus*. Underlined residues are unique to the D-type cyclins; they are found in <7 of 53 other cyclins inspected and are not common to any other class, including types A, B, C, and E.

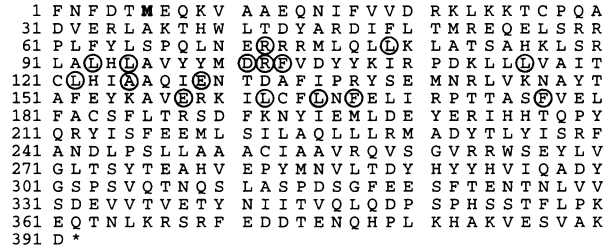

FIG. 3. Cdi5 predicted protein. Conceptual translation of the Cdi5 cDNA (GenBank accession number U41809). The ATG-encoding methionine-6 (boldface M) is within a translational start signal (32). Of 20 residues that are conserved in most cyclins (see Fig. 2 *Upper*), 16 are also conserved in Cdi5 (circled residues).

cyclins (Fig. 3). While Cdi5 is most similar to certain A cyclins and B cyclins (Table 1), it does not fit the consensus for the A or B class; Cdi5 lacks 50 of the 86 residues common to known A cyclins, and 16 of the 35 residues common to known B cyclins (Fig. 3). Cdi5 also lacks a consensus destruction box (38) found in A and B cyclins. These facts suggests that Cdi5 defines a new cyclin class, cyclin J.

Drosophila cyclin D (Cdi3) and cyclin J (Cdi5) are functional; both complemented a yeast strain that lacked Cln1-3 G_1 cyclin activity required for growth (Table 2). An independently isolated amino-terminal truncation of Cdi3 (Cdi3ΔN; Fig. 2*Upper*) also complemented the yeast cyclin mutant (Table 2), indicating that the first 88 amino acids encoded by the Cdi3 cDNA, which share no similarity with cyclins, are dispensable for cyclin function. Cdi7 (cyclin E) also complemented the yeast cyclin mutants, as previously observed (16), whereas the other Cdis failed to complement. The fact that cyclin D and cyclin J can function as cyclins in yeast is consistent with the fact that they can interact with the *S. cerevisiae* Cdk, Cdc28, in two-hybrid assays (22).

Northern analysis showed that cyclin J expression is strictly maternal; its mRNA is present in the newly laid egg and in adult females but is undetectable in the embryo after zygotic transcription begins (Fig. 4). This expression pattern suggests that cyclin J may be involved in the early nuclear division cycles that lack G_1 and G_2 phases. Alternatively, cyclin J may function in the ovary during oogenesis, its mRNA being deposited in the egg along with the nurse cell cytoplasm in the final stages of oogenesis. By contrast, a 2.3-kb cyclin D message is present throughout early embryonic development but is less abundant after 12 hr and is nearly absent in larvae (Fig. 4). An additional lower abundance cyclin D message of about 2.1 kb is present only in females and early embryos (asterisk in Fig. 4). Because few cells are dividing in embryos older than 12 hr and in larvae, these results indicate that, like Dmcdc2 and Dmcdc2c message (27, 28), cyclin D mRNA is most abundant at times of rapid and widespread division. Despite its general absence from larval tissue, cyclin D mRNA is abundant in imaginal discs from third

Table 2. Cdi3 and Cdi5 complement *cln*⁻ yeast

Cdi2	−
Cdi3 (cyclin D)	+
Cdi3ΔN (cyclin D truncation)	+
Cdi4	−
Cdi5 (cyclin J)	+
Cdi7 (cyclin E)	+
Cdi11	−
Cdi12	−
Vector only	−

Cdis were expressed from the *GAL1* promoter with a 9-amino acid hemagglutinin epitope tag (20) at their amino termini. +, Complementation of *cln*⁻ yeast (growth of strain 3c-1AX on galactose but not glucose after loss of the *CLN3* plasmid); −, no complementation (absence of *cln3* segregants).

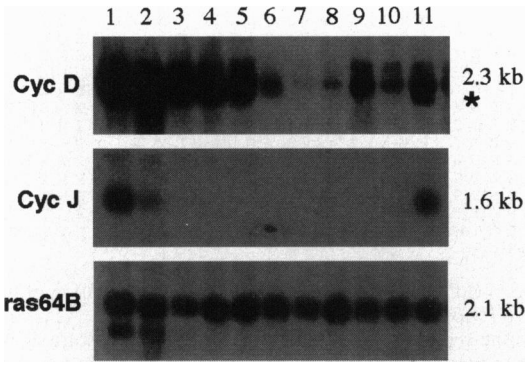

FIG. 4. Expression of Cdi RNAs during development. A Northern blot containing poly(A)$^+$ RNA from staged embryos (lane 1, 0–1.5 hr; lane 2, 1.5–3 hr; lane 3, 3–6 hr; lane 4, 6–9 hr; lane 5, 9–12 hr; lane 6, 12–24 hr), larvae (lane 7, L1 + L2; lane 8, L3), mixed discs (lane 9), and virgin adults (lane 10, males; lane 11, females) was probed with ^{32}P-labeled Cdi3 (cyclin D) and Cdi5 (cyclin J) cDNAs and with a PCR product corresponding to codons 3–195 of *Drosophila ras64B*. The asterisk shows lower abundance cyclin D message at ≈2.1 kb.

instar larvae (Fig. 4), suggesting that it might function in disc development. To explore this possibility, we examined the spatial pattern of cyclin D expression during development of the eye imaginal disc.

The eye disc provides a system to study how developmental signals control cell divisions (39, 40). During the third larval instar, a wave of differentiation (the leading edge of which is marked by the morphogenetic furrow) moves anteriorly across the eye disc; cells within and posterior to the furrow organize into clusters and differentiate (41). Anterior to the furrow, cells divide asynchronously, but at the anterior edge of the furrow, all cells arrest in the G_1 phase of the cell cycle (40). Those cells destined to divide enter S phase in synchrony just posterior to the furrow, while the remaining clustered postmitotic cells begin to differentiate. This precise cell cycle regulation is essential to proper eye development; loss of function mutations in *rux*, a gene essential for the G_1 arrest, result in severe defects in eye morphology (40).

Cyclin D message is expressed uniformly in a band of the G_1 cells just anterior to the furrow (Fig. 5). Double-labeling with cyclin D and BrdUrd showed that cyclin D expression commences well before cells enter S phase behind the morphogenetic furrow (Fig. 5 *a* and *b*). This pattern of expression is similar to the cell cycle regulator "string," although string expression precedes that of cyclin D (40) (B.J.T. and S.L.Z., unpublished observations). Significantly, the band of cyclin D expression is separated from the synchronous band of S-phase cells by a gap, demonstrating that cyclin D expression precedes reentry into the cell cycle posterior to the furrow. In contrast, cyclin E is expressed posterior to the band of cyclin D expression, in a region partially overlapping the synchronous band of S-phase cells (Fig. 5*c*; ref 42). This pattern suggests that cyclin E expression at the posterior edge of the furrow drives cells into S phase, similar to its function in embryonic neurogenesis (16, 17), and that cyclin D functions earlier than cyclin E.

DISCUSSION

In mammals, D-type cyclins are necessary for progression from G_1 to S phase, are expressed during G_1 in response to growth factor stimulation, and are down-regulated upon serum starvation (reviewed in refs. 4 and 18). Moreover, D cyclins are often mutated or aberrantly expressed in proliferative disorders, and directed overexpression in tissue culture cells leads to a reduced requirement for growth factors (4, 18). These facts suggest that the D cyclins may mediate the response to extracellular signals that start and stop progression from G_1 into S. Similarly, our results suggest that cyclin D may mediate the proliferative signals important for *Drosophila* development. Consistent with this possibility, cyclin D expression is high during early embryogenesis when cell and nuclear divisions are rapid and widespread. In this view, the lack of a G_1

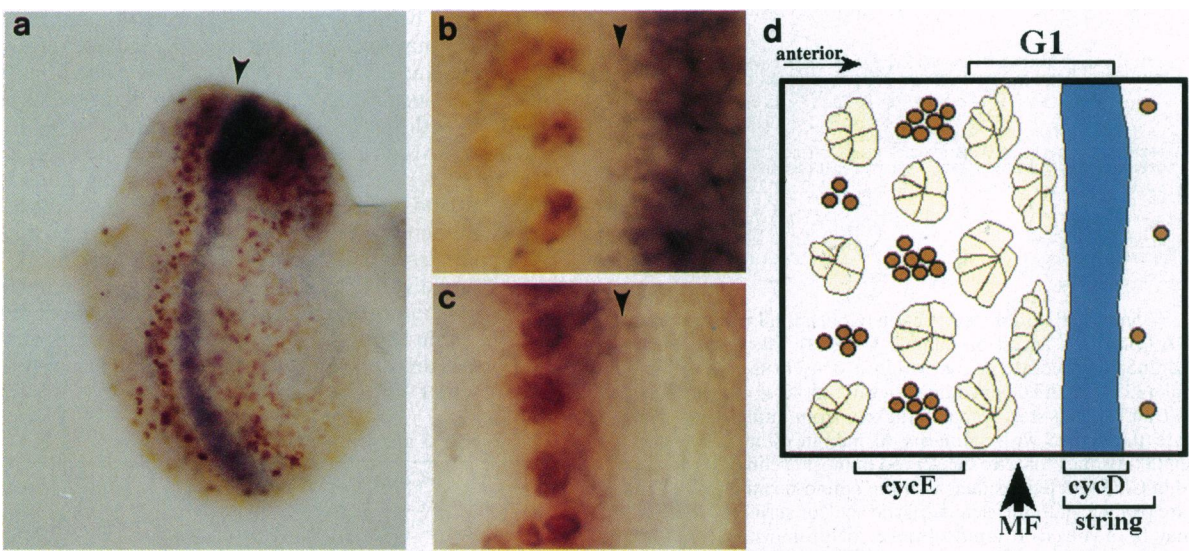

FIG. 5. Cyclin D expression precedes cyclin E expression in the morphogenetic furrow (MF). Third-instar eye imaginal discs were labeled for mRNA expression (blue) and S-phase cells by incorporation of 5-bromo-2-deoxyuridine (BrdUrd; brown). Anterior is to the right and the MF is indicated by an arrowhead. (*a*) Cyclin D is expressed uniformly in a band of cells anterior to the MF. (*b*) High-magnification view of a disc stained for cyclin D mRNA and BrdUrd. The patterned band of S-phase cells behind the MF is separated from the band of cyclin D mRNA expression by an unstained gap. (*c*) High-magnification view of a disc stained for cyclin E mRNA and BrdUrd. Cyclin E expression begins just prior to entry of cells into S phase behind the MF. (*d*) Schematic representation of the high-magnification views shown in *b* and *c*. S-phase cells are in brown, differentiating photoreceptor cell precursors are in pale yellow, and the band of cyclin D mRNA expression is in blue. Cyclin D is expressed to high levels in all cells in a band anterior to the MF (arrowhead). This pattern of expression is very similar to that of the cell cycle regulator string, although string expression precedes that of cyclin D (40) (B.J.T. and S.L.Z., unpublished observations). Cells in this region and in the unstained region immediately posterior are in G_1 phase. Pattern formation initiates in this G_1 domain as photoreceptor cell precursors assemble into groups. Behind the MF, cells between the developing preclusters enter S phase synchronously.

phase during early embryonic cell divisions may be due to constitutive expression of cyclin D or to constitutive proliferation signals that activate it. In the eye disc, cyclin D is expressed in the G_1-arrested cells in the morphogenetic furrow, and expression precedes that of cyclin E and entry into S phase. This timing of cyclin D and cyclin E expression parallels that seen in mammalian cells and is consistent with a model in which entry into the cell cycle involves activation of Cdk–cyclin D complexes, followed by activation of Cdk–cyclin E complexes to drive cells into S phase. Interestingly, the stripe of cyclin D expression in G_1-arrested cells includes both cells about to enter S-phase and cells about to leave the cell cycle and differentiate (Fig. 5 *a* and *b*). These results suggest that in cells not destined to enter S phase, cyclin D may not be active because of the absence of a Cdk partner or because of negative regulation by other developmental signals.

We thank Jenö Gyuris, Erica Golemis, and Steve Hanes for reagents; Gary Ruvkun, Nick Dyson, Barak Cohen, and Anne Hart for critical reading of the manuscript; and members of the Brent and Ruvkun labs for helpful discussions. We also thank Christian Lehner for providing the *Drosophila* Cdks and those mentioned in the text for additional reagents. This work was supported by a Pew Scholar's award to R.B.; R.L.F. was supported by a Public Health Service National Research Service Award, and S.L.Z. is an investigator of the Howard Hughes Medical Institute. R.B. is supported by an American Cancer Society Faculty Research Award.

1. Draetta, G. (1990) *Trends Biochem. Sci.* **15,** 378–382.
2. Nurse, P. (1994) *Cell* **79,** 547–550.
3. Pardee, A. B. (1989) *Science* **246,** 603–608.
4. Sherr, C. J. (1994) *Cell* **79,** 551–555.
5. Glover, D. M. (1991) *Trends Genet.* **7,** 125–132.
6. Orr-Weaver, T. L. (1994) *Trends Genet.* **10,** 321–327.
7. Whitfield, W. G., Gonzalez, C., Sanchez-Herrero, E. & Glover, D. M. (1989) *Nature (London)* **338,** 337–340.
8. Lehner, C. F. & O'Farrell, P. H. (1990) *Cell* **61,** 535–547.
9. Knoblich, J. A. & Lehner, C. F. (1993) *EMBO J.* **12,** 65–74.
10. Lehner, C. F., Yakubovich, N. & O'Farrell, P. H. (1991) *Cold Spring Harbor Symp. Quant. Biol.* **56,** 97–114.
11. Edgar, B. A. & O'Farrell, P. H. (1990) *Cell* **62,** 469–480.
12. Alphey, L., Jimenez, J., White-Cooper, H., Dawson, I., Nurse, P. & Glover, D. (1992) *Cell* **69,** 977–988.
13. Courtot, C., Frankhauser, C., Simanis, V. & Lehner, C. (1992) *Development (Cambridge, U.K.)* **116,** 405–416.
14. Lahue, E. E., Smith, A. V. & Orr-Weaver, T. L. (1991) *Genes Dev.* **5,** 2166–2175.
15. Leopold, P. & O'Farrell, P. H. (1991) *Cell* **66,** 1207–1216.
16. Richardson, H. E., O'Keefe, L. V., Reed, S. I. & Saint, R. (1993) *Development (Cambridge, U.K.)* **119,** 673–690.
17. Knoblich, J. A., Sauer, K., Jones, L., Richardson, H., Saint, R. & Lehner, C. F. (1994) *Cell* **77,** 107–120.
18. Hunter, T. & Pines, J. (1994) *Cell* **79,** 573–582.
19. Estojak, J., Brent, R. & Golemis, E. A. (1995) *Mol. Cell. Biol.* **10,** 5820–5829.
20. Gyuris, J., Golemis, E., Chertkov, H. & Brent, R. (1993) *Cell* **75,** 791–803.
21. Paroush, Z., Finley, R. L., Jr., Kidd, T., Wainwright, S. M., Ingham, P. W., Brent, R. & Ish-Horowicz, D. (1994) *Cell* **79,** 805–815.
22. Finley, R. L., Jr., & Brent, R. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 12980–12984.
23. Finley, R. L., Jr., & Brent, R. (1995) in *Gene Probes: A Practical Approach*, eds. Hames, B. D. & Glover, D. M. (1995) (Oxford Univ. Press, Oxford), pp. 169–203.
24. Ashburner, M. (1989) *Drosophila, A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
25. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K., eds. (1989) *Current Protocols in Molecular Biology* (Greene & Wiley, New York).
26. Tautz, D. & Pfeifle, C. (1989) *Chromosoma* **98,** 81–85.
27. Lehner, C. F. & O'Farrell, P. H. (1990) *EMBO J.* **9,** 3573–3581.
28. Jimenez, J., Alphy, L., Nurse, P. & Glover, D. M. (1990) *EMBO J.* **9,** 3565–3571.
29. Brizuela, L., Draetta, G. & Beach, D. (1987) *EMBO J.* **6,** 3507–3514.
30. Hadwiger, J. A., Wittenberg, C., Mendenhall, M. D. & Reed, S. I. (1989) *Mol. Cell. Biol.* **9,** 2034–2041.
31. Richardson, H. E., Stueland, C. S., Thomas, J., Russell, P. & Reed, S. I. (1990) *Genes Dev.* **4,** 1332–1344.
32. Cavener, D. R. (1987) *Nucleic Acids Res.* **15,** 1353–1361.
33. Rogers, S., Wells, R. & Rechsteiner, M. (1986) *Science* **234,** 364–368.
34. Matsushime, H., Roussell, M. F., Ashmun, R. & Sherr, C. J. (1991) *Cell* **65,** 701–713.
35. Xiong, Y., Connolly, T., Futcher, B. & Beach, D. (1991) *Cell* **65,** 691–699.
36. Lew, D. J., Dulic, V. & Reed, S. I. (1991) *Cell* **66,** 1197–1206.
37. Motokura, T., Bloom, T., Kim, H. G., Jüppner, H., Ruderman, J. V., Kronenberg, H. M. & Arnold, A. (1991) *Nature (London)* **350,** 512–515.
38. Glotzer, M., Murray, A. W. & Kirschner, M. W. (1991) *Nature (London)* **349,** 132–138.
39. Ready, D. F., Hanson, T. E. & Benzer, S. (1976) *Dev. Biol.* **53,** 217–240.
40. Thomas, B. J., Gunning, D. A., Cho, J. & Zipursky, S. L. (1994) *Cell* **77,** 1003–1014.
41. Tomlinson, A. & Ready, D. F. (1987) *Dev. Biol.* **120,** 366–376.
42. Richardson, H., O'Keefe, L. V., Marty, T. & Saint, R. (1995) *Development (Cambridge, U.K.)* **121,** 3371–3379.