Expression of the cell cycle control gene, *cdc25*, is constitutive in the segmental founder cells but is cell-cycle-regulated in the micromeres of leech embryos

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SUMMARY

The identifiable cells of leech embryos exhibit characteristic differences in the timing of cell division. To elucidate the mechanisms underlying these cell-specific differences in cell cycle timing, the leech *cdc25* gene was isolated because Cdc25 phosphatase regulates the asynchronous cell divisions of postblastoderm *Drosophila* embryos. Examination of the distribution of *cdc25* RNA and the zygotic expression of *cdc25* in identified cells of leech embryos revealed lineage-dependent mechanisms of regulation. The early blastomeres, macromeres and teloblasts have steady levels of maternal *cdc25* RNA throughout their cell cycles.

The levels of *cdc25* RNA remain constant throughout the cell cycles of the segmental founder cells, but the majority of these transcripts are zygotically produced. *Cdc25* RNA levels fluctuate during the cell cycles of the micromeres. The levels peak during early G2 due to a burst of zygotic transcription, and then decline as the cell cycles progress. These data suggest that cells of different lineages employ different strategies of cell cycle control.

Key words: Cdc25 phosphatase, G2/M transition, cell cycle, cell lineage

INTRODUCTION

Cell division must be temporally and spatially regulated during the development of multicellular organisms. While the mechanisms of cell cycle control have been examined in cell-free extracts of *Xenopus* eggs and in *Drosophila* embryos, these embryos are unique in that they display asynchronous or position-specific differences in cell cycle timing after an earlier period of very rapid, synchronous cycles (Newport and Kirschner, 1982; Foe and Alberts, 1983). In contrast, many other embryos, e.g. mollusk, nematode, and leech embryos, exhibit cell-specific or lineage-specific differences in the timing of cell division from the beginning of development (van den Biggelaar, 1971; Deppe et al., 1978; Weisblat et al., 1984). Little is known about how the cell-specific differences in cell cycle timing are regulated in these embryos.

The onset of mitosis in all eukaryotic cells is triggered by the activation of the Cdc2 protein kinase. This activation requires the physical association with a mitotic cyclin regulatory subunit, as well as the removal of inhibitory phosphate groups by the phosphatase, Cdc25 (see Nurse, 1990; Solomon, 1993; Dunphy, 1994, for reviews). The activated Cdc2 kinase initiates mitosis through the phosphorylation of specific target proteins, while degradation of the cyclin subunit leads to exit from mitosis.

Different components of this network appear to play key regulatory roles in embryos of different organisms or at different stages of development. For example, the accumulation of Cyclin B from maternally supplied RNA is rate limiting for mitosis in extracts of *Xenopus* eggs (Minshull et al., 1989; Murray and Kirschner, 1989) and in some of the early mitotic cycles of *Drosophila* embryos (Edgar et al., 1994a). The levels of Cdc2 and Cdc25 remain constant during these rapid cycles (Gautier et al., 1989; Jessus and Beach, 1992; Lehner and O’Farrell, 1990a; Edgar et al., 1994a). In contrast, the accumulation of Cyclin B is not rate limiting during the later asynchronous cell cycles of *Drosophila* embryos; rather, zygotically expressed Cdc25 (string) is the dose-dependent regulator of mitosis (Edgar and O’Farrell, 1989, 1990; Edgar et al., 1994a). Cdc2 is continuously present and the mitotic cyclins gradually accumulate during these cell cycles of postblastoderm *Drosophila* embryos (Lehner and O’Farrell, 1989, 1990a,b; Edgar et al., 1994a).

Since Cdc25 regulates the timing of the asynchronous cell divisions of postblastoderm *Drosophila* embryos (Edgar and O’Farrell, 1990), the leech *cdc25* gene was isolated so that the mechanisms regulating the cell-specific differences in cell cycle timing could begin to be unraveled. Leech embryos provide an opportunity to examine the mechanisms of cell cycle control at the level of individual cells because each embryo undergoes the same sequence of invariant cleavages to yield identifiable cells whose patterns of cell division, cell lineages, cell cycle compositions, and developmental fates have been characterized extensively (Whitman, 1878; Weisblat et al., 1984; Zackson, 1984; Weisblat and Shankland, 1985; Shankland, 1987a,b; Ho and Weisblat, 1987; Bissen and...
A fragment of leech cdc25 was amplified using a reverse transcriptase/PCR-based strategy. Total RNA was purified from stage 9-10 embryos using guanidine thiocyanate extraction and CsCl centrifugation (Sambrook et al., 1989). First strand cDNA was synthesized and used immediately for PCR as described by Doherty et al. (1982). As modified by Bissen and Weisblat (1989), PCR was performed with degenerate primers corresponding to the amino acid sequence GYKEFF (5'-A/GAAA/GAAC/TTCC/TTTA/GTANCC-3'). Amplification was carried out for 30 cycles of 1 minute at 43°C, 2 minutes at 72°C, and 30 seconds at 94°C. The reaction products were separated by polyacrylamide gel electrophoresis, and fragments of the expected size (280 bp) were purified and sequenced directly (Cyclist kit; Stratagene). The cdc25-like PCR fragments were used to screen an H. robusta stage 10 embryo cDNA library constructed in λZAP (kindly provided by M. Shankland, Harvard Medical School). Four positive clones were purified and phBluescript SK(−) phagemids were rescued by in vivo excision. Partial restriction mapping revealed that all cDNA clones were identical. The clone (pS5.2) with the longest insert (1.45 kb) was sequenced on both strands (Sequenase kit, US Biochemical).

Although there were extensive similarities between the predicted protein product of this cDNA and other Cdc25 proteins, the similarity abruptly ended in the middle of the highly conserved C-terminal region. Near the point of departure there was a stretch of 9 A residues in clone pS5.2 (starting at position 954), whereas there were only 8 A residues at this position in the PCR-generated fragments. Partial sequence analysis of the other 3 cDNA clones revealed that they also had 8 A residues starting at this position. Since the correct reading frame was maintained with 8 A residues, it appears that the extra A in clone pS5.2 could have been artifically generated during the synthesis of the cDNA used to construct the library. Thus, a complete and correct cdc25 clone (pSB151) was generated by replacing the 693 bp Psrl (position 838)-Kpn1 fragment from clone pS5.2 with the 692 bp Psrl-Kpn1 fragment from clone pS5.1.

**In situ hybridization**

Most of the hybridization experiments were performed using digoxigenin-labeled RNA probes as described by Nardelli-Haefliger and Shankland (1992), with some modifications. Sense and antisense digoxigenin-labeled RNAs were synthesized with T3 RNA polymerase (MEGAscript kit, Ambion) using linearized pSB151 and pSB153, respectively. (pSB153 was constructed by inserting the cdc25 coding region of pSB151 into restricted phBluescript KS(+)) (Stratagene). Embryos were fixed for 1.5 hours in 4% formaldehyde and 0.25x PBS (130 mM NaCl, 7 mM Na2HPO4, 3 mM NaH2PO4), and their vitelline membranes were manually removed in Pbs (Pbs and 0.1% Tween-20). Stage 1-6 embryos were permeabilized by a 5 minute incubation with 50 µg/ml proteinase K (US Biochemical); stage 7-8 embryos were not treated with proteinase K. Embryos were hybridized overnight at 55°C with approximately 1 ng/µl riboprobe, rinsed, treated with RNase A, and incubated in a 1:2500 dilution of alkaline phosphatase (AP)-conjugated anti-digoxigenin antibodies (Boehringer-Mannheim) according to the method of Nardelli-Haefliger and Shankland (1992). The AP color reaction was allowed to proceed for 2-8 hours. The embryos were dehydrated in ethanol and cleared in uncatalyzed Poly/Bed 812 embedding medium (Polysciences, Inc.).

The specificity of the hybridization was tested using PCR-generated, strand-specific, digoxigenin-labeled DNA probes (Kostriken and Weisblat, 1992). In brief, an internal primer was used to drive synthesis from linearized plasmid DNA. Antisense probes specific to the highly conserved carboxy-terminal region were generated using the 3' PCR primer described above. Antisense probes specific to the divergent amino-terminal region were generated using a 3' primer (sb2; 5'-CTGAAATCCGGAATCAGG-3') corresponding to amino acid positions 205-210. Embryos were fixed as described above and hybridized according to Kostriken and Weisblat (1992).

**Silver staining**

The boundaries of superficial cells in stage 5-6 embryos were revealed by staining with silver methenamine according to the method of Arnolds (1979), except that embryos were fixed for 7 minutes in 0.8% formaldehyde and 0.1 M Na cacodylate, pH 7.4, and were immediately exposed to bright light after being introduced to the silver methenamine solution. After the second fixation, the embryos were rehydrated into 0.1 M Tris-HCl, pH 7.5, stained with 1 µg/ml of Hoechst 33258 (Sigma), and cleared in 80% glycerol/0.1 M Tris-HCl, pH 7.5.

**Inhibition of RNA synthesis**

RNA synthesis was blocked by either of two methods. (1) Embryos were pressure-injected with 75 µg/ml or 7.5 mg/ml of α-amanitin (Sigma), as described by Bissen and Weisblat (1991). Sibling control embryos were sham injected. (2) Embryos were incubated (in the dark) in embryo medium containing 100 µg/ml of actinomycin D-mannitol (Sigma). Control embryos were incubated with an equivalent concentration of mannitol (5 mg/ml).
RESULTS

Isolation of leech cdc25 cDNA

Since the carboxy-terminal region is highly conserved among all Cdc25 proteins, a PCR-based strategy was used to amplify a fragment of the leech cdc25 gene. The amplified fragment was used to isolate cDNA clones from an H. robusta stage 9-10 embryo cDNA library. Clone pSB151 (see Materials and Methods), which had a 1.45 kb insert, appears to be nearly full-length because northern blots revealed a 1.5 kb transcript in leech embryos (not shown). The complete DNA sequence of Hro-cdc25 and its deduced amino acid sequence are presented in Fig. 2. The three in-frame stop codons upstream of the presumed initiation codon suggest that this cDNA clone contains the complete protein coding sequence. Hro-cdc25 is predicted to encode a 422 amino acid protein with a Mr of approximately 46,000.

Sequence comparisons

The protein encoded by Hro-cdc25 shares the highly conserved C-terminal region with other known Cdc25 proteins. Within the ~170 amino acid region shown in Fig. 3, Hro-Cdc25 has the greatest identity (56.8%) with the Drosophila string protein (Edgar and O’Farrell, 1989). Leech Cdc25 contains the amino acid sequence HCEFSSE, which corresponds to the HC motif present in all protein phosphatases, including Cdc25 proteins (Gautier et al., 1991; Millar et al., 1991; Strausfeld et al., 1991). Hro-Cdc25 shares no significant sequence similarity with any of the other Cdc25 proteins in the N-terminal region.

Drosophila also contains a second cdc25 homolog called twine (Alphey et al., 1992; Courtot et al., 1992). Not only does Hro-Cdc25 share more sequence identity with string (57%) than with twine (48%) in this conserved C-terminal region, but the distribution patterns of Hro-cdc25 RNA resemble those of string mRNA in that they are present in all actively dividing cells of leech embryos. In contrast, twine appears to function during meiotic cell cycles (Alphey et al., 1992; Courtot et al., 1992). Additional support for the mitotic role of Hro-Cdc25 is that the introduction of in vitro-transcribed Hro-cdc25 mRNA into specific cells in leech embryos causes them to undergo premature cell divisions (K. Hayes and S.T.B., unpublished). Thus, on the basis of sequence identity, patterns of expression, and function, it appears that this leech gene is a cdc25/string homolog.

Leech may contain additional cdc25-related gene(s). Low stringency Southern hybridizations using Hro-cdc25 probes revealed the presence of additional weakly hybridizing bands (not shown).

Distribution of Hro-cdc25 RNA in identified cells of leech embryos

The in situ patterns of RNA localization were examined by hybridizing fixed, permeabilized Helobdella embryos to digoxigenin-labeled antisense Hro-cdc25 RNA. Hybridized probe was detected histochemically using AP-conjugated anti-digoxigenin antibodies. There was no staining in embryos incubated with digoxigenin-labeled sense Hro-cdc25 RNA (Fig. 4B) or in embryos processed without either digoxigenin-labeled RNA or anti-digoxigenin antibodies (not shown). The distribution of Hro-cdc25 RNA was examined in more than 800 embryos fixed at regular intervals during the first eight stages of development.

To determine that the observed staining was due to hybridization with Hro-cdc25 transcripts and not with any related gene products, the staining patterns of probes corresponding to the divergent 5’ region of Hro-cdc25 were compared to those of probes corresponding to the conserved 3’ region of Hro-cdc25. It is assumed that additional leech cdc25-like gene(s) would contain the highly conserved 3’ region(s), but would have divergent 5’ region(s). It was found that both 5’-unique and 3’-unique PCR-generated, digoxigenin-labeled antisense Hro-cdc25 cDNA probes gave identical patterns of staining. These findings suggest that the observed staining was due to hybridization of the probes to only Hro-cdc25 transcripts. Although the staining patterns obtained with DNA and RNA probes were similar, RNA probes were used throughout the study because of their increased sensitivity.

Levels of maternal Hro-cdc25 RNA remained constant throughout the cell cycles of the blastomeres, macromeres and teloblasts

One cell (stage 1) embryos contained Hro-cdc25 mRNA in the pools of yolk-deficient cytoplasm, or teloplasm, located at the animal and vegetal poles. The pools of teloplasm, which arise during the first cell cycle as a consequence of cytoplasmic rearrangements, are enriched in mitochondria, endoplasmic reticulum, and poly(A)+ RNA (Whitman, 1878; Fernandez and Stent, 1980; Holton et al., 1994). These localized Hro-cdc25 transcripts were maternally supplied because zygotic transcription has not been initiated (Bissen and Weisblat, 1991). Hro-cdc25 RNA was partitioned to each cell during the subsequent cleavages. After three divisions, the embryos contain four macromeres (A’, B’, C’ and D’), and four micromeres (a’, b’, c’ and d’). Macromere D’ undergoes a series of invariant cleavages (stages 4-6) to produce five bilateral pairs of embryonic stem cells called teloblasts. The Hro-cdc25 tran-
scripts were always associated with the pools of teloplasm in the large, yolk-filled blastomeres, macromeres and teloblasts. There were no noticeable differences in the intensity of staining in any of these large yolky cells in embryos fixed at 15 minute intervals during stages 4-6. These data suggest that the levels of \textit{cdc25} RNA remained constant during the 60-140 minute cell cycles of these cells. Furthermore, the levels of Hro-cdc25 RNA were not decreased in these cells following the inhibition of RNA synthesis with low concentrations of either actinomycin D or α-amanitin (as an example, see Fig. 4D), suggesting that these transcripts were of maternal origin. Levels of Hro-cdc25 RNA were invariant throughout the cell cycles of the blast cells

Each teloblast divides at the rate of once per hour to generate a chain, or \textit{bandlet}, of several dozen much smaller segmental founder cells, or \textit{primary blast cells}. The blast cells are spatially arranged in the bandlets according to their order of birth, i.e. the first-produced blast cells lie furthest (future anterior) from the teloblast and each blast cell is one hour older than its next posterior neighbor. The cell cycles of the primary blast cells are longer in duration (i.e. 9-32 hours) than those of the early blastomeres and teloblasts (Zackson, 1984). The blast cells undergo stereotyped divisions during stages 7-8 (Zackson, 1984; Shankland, 1987a,b; Bissen and Weisblat, 1989) to yield distinct sets of segmentally repeated progeny (Weisblat and Shankland, 1985). Each set of segmental progeny is brought into proper register through a complex series of morphogenetic movements (Shankland, 1991). As part of this, the five bandlets on each side of the embryos converge to create the right and left \textit{germinal bands} (see Fig. 1).

Hybridization of stage 7 embryos revealed that Hro-cdc25 transcripts were present in every blast cell (Fig. 4A,C). The blast cells are small yolk-free cells and the transcripts were distributed uniformly throughout their cell bodies. Hro-cdc25 RNA was present in the most recently-produced blast cells that lie adjacent to the parental teloblasts (arrowheads in Fig. 4A,C), as well as in the older blast cells that lie in the germinal bands (thick arrows in Fig. 4C). Although the cells in the germinal bands of the embryo shown in Fig. 4C appear to be more intensely stained than the bandlet cells that have not entered the germinal bands, manual dissection of individual bandlets away from the rest of the germinal band revealed that all of the cells had the same intensity of staining (not shown). The cells in the germinal band appear to be more heavily stained only because the germinal bands are composed of several layers of cells.

Since the position of a blast cell in a bandlet or germinal band correlates with the age of the cell, its phase of the cell cycle can be inferred. For example, the 4-5 ectodermal blast cells closest to the parental teloblast were in S phase, those in the germinal band located near the thick arrows in Fig. 4C were in (or near) M phase, and the intervening 16-28 cells were in...
G2 phase (Bissen and Weisblat, 1989). It appears, therefore, that the levels of Hro-cdc25 RNA remained unchanged throughout the long cell cycles of the primary blast cells. Furthermore, the progeny of the primary blast cells, i.e. the secondary blast cells, located anterior to the mitotic cells in the germline bands had entered new cell cycles, and they too appeared to have similar levels of Hro-cdc25 RNA. All these data suggest that the levels of Hro-cdc25 mRNA remained constant throughout the cell cycles of these cells.

**Zygotic expression of Hro-cdc25 was constitutive in the blast cells**

To determine whether any of the Hro-cdc25 RNA in the blast cells was of zygotic origin, RNA synthesis was blocked in early stage 7 embryos with low concentrations of either α-amanitin or actinomycin D. After 24 hours of further development, the distribution of Hro-cdc25 mRNA was examined in the control and inhibitor-treated embryos (Fig. 4C,D). These embryos were overstained to ensure that low levels of message would be detected. Since inhibition of RNA synthesis blocks cell division in blast cells but not teloblasts, the inhibitor-treated embryos contained long bandlets of undivided blast cells between the teloblasts and the nascent germinal bands (see Fig. 4 in Bissen and Weisblat, 1991).

After treatment with either α-amanitin or actinomycin D, the bandlets of blast cells contained little or no Hro-cdc25 mRNA (Fig. 4D). In the few bandlets that had residual staining, there was no difference in the intensity of staining in blast cells of different ages. The intensity of staining in the teloblasts (thin arrows) and the cells at the animal pole was not reduced after inhibitor treatment, however. The levels of Hro-cdc25 RNA in these cells were similar to those in the corresponding cells of control embryos (Fig. 4C). These data suggest that the bulk of the Hro-cdc25 RNA that is normally present in the blast cells is zygotically produced. Furthermore, if zygotic Hro-cdc25 mRNA, like zygotic string (cdc25) mRNA in Drosophila embryos (Edgar et al., 1994b), has a short half-life, then the apparently invariant transcript levels throughout the cell cycles of the blast cells would be due to the continuous expression of Hro-cdc25 in these cells.

**Levels of Hro-cdc25 RNA peaked during early G2 of the cell cycles of the micromeres**

In addition to the macromeres and teloblasts, the early cleavages also generate 25 micromeres, whose descendant cells contribute to the prostomium of the adult, as well as the epithelial layer of a provisional body wall of the embryo. The first four micromeres (the primary quartet) arise sequentially at the third cleavage; micromere d' arises first, then micromere c' and lastly micromeres a' and b'. Six additional micromeres arise from the A, B and C quadrants of the embryo, and 15 micromeres arise from the D quadrant during stages 4-6 (Sandig and Dohle, 1988; Bissen and Weisblat, 1989). Hro-cdc25 RNA was partitioned to each micromere at the time of its generation and, since these are small yolk-free cells, these transcripts were distributed uniformly. In contrast to the cells described above, however, the levels of Hro-cdc25 RNA fluctuated throughout the cell cycles of the micromeres.

In the primary quartet of micromeres, the levels of Hro-cdc25 RNA increased 10-20 minutes after each was generated and then declined 20-30 minutes later. For example, the intensity of staining was similar in micromere d' and its sister cell, macromere D', immediately (Fig. 5A,B) and 10 minutes (not shown) after they were produced. Ten minutes later, the intensity of Hro-cdc25 RNA staining increased in micromere d' (Fig. 5C,D) and remained elevated for 10 minutes (not shown). After another 10 minutes (or 40 minutes after micromere d' was produced), the intensity of its staining declined (Fig. 5E,F). The levels of Hro-cdc25 mRNA fluctuated with a similar time course in the other three primary quartet micromeres. Since the cell cycles of the primary quartet of micromeres lack G1 phases, have 15 hour G2 phases and ~0.5 hour M phases (Bissen and Weisblat, 1989; Sandig and Dohle, 1988), Hro-cdc25 RNA during stages 4-6 of the primary quartet of micromeres is likely to be translated during late G2 phases early during the G2 phases of their cell cycles.

The levels of Hro-cdc25 RNA also fluctuated during the cell cycles of the later-produced micromeres, which lie at the animal pole in a mass of cells that is sometimes called the micromere cap. There was a mottled pattern of micromere RNA localization in the micromere cap during stages 5-6 (Fig. 6). Some cells had high levels of Hro-cdc25 mRNA (double or triple arrows), other cells had intermediate levels (single arrows), while still other cells had low levels (arrowheads). While many of the cells in the micromere cap are difficult to identify because these cells have not been mapped very well during stages 4-6, some of the most recently-produced
micromeres such as, dnopq’, (single arrow), dnopq” (double arrow), dnopq’’’ (triple arrow), nopq’L and nopq’R (the two tiny cells below cell dnopq’’’), can be identified in these stage 5 embryos (see Fig. 6B,D). Examination of identifiable micromeres in 160 embryos fixed at various times during these stages of development revealed that the levels of Hro-cdc25 mRNA were low at the time of their generation, increased soon after they were produced, and then fell as they progressed through their cell cycles. For example, micromeres nopq’L and nopq’R had just been produced when the embryos shown in Fig. 6A-D were fixed and these cells contained low levels of Hro-cdc25 transcripts. Micromere dnopq” (double arrow) and dnopq’’’ (triple arrow) were 4.0 and 2.8 hours old, respectively, at the time of fixation and they contained high levels of Hro-cdc25 transcripts. Micromere dnopq’ (single arrow) was 5.3 hours old at the time of fixation and had lower levels of Hro-cdc25 RNA. This cell had already passed through its period of high cdc25 since it was stained heavily in embryos fixed at earlier time points (not shown). Although the lengths of these cell cycles have not been determined, it is known that they lack G1 phases and have 45 minute S phases (Bissen and Weisblat, 1989). It appears, therefore, that the levels of Hro-cdc25 RNA
were elevated during early G2 and then declined as these micromeres advanced through their cell cycles.

**Zygotic expression of Hro-cdc25 was transient in the micromeres**

The elevated levels of *Hro-cdc25* RNA during the G2 phases of the cell cycles of the micromeres suggests that *Hro-cdc25* was transcribed in these cells. To test this, stage 1 embryos were collected immediately after egg deposition and incubated in the absence or presence of actinomycin D. The embryos were developmentally synchronized and fixed at intervals following the generation of micromere d’. The intensity of staining was not increased in the micromeres of the actinomycin D-treated embryos. The embryos shown in Fig. 5G,H were fixed 20 minutes after the generation of micromere d’. *Hro-cdc25* transcript levels were elevated in micromere d’ of the control embryos (Fig. 5G), but were similar between micromeres d’ and c’ in the actinomycin D-treated embryos (Fig. 5H). These data indicate that the elevated levels of *Hro-cdc25* RNA in the micromeres was due to the zygotic expression of *Hro-cdc25*. Incidentally, the microinjection of concentrations of α-amanitin up to 7.5 mg/ml had no effect on the intensity of staining in the micromeres. Lastly, since these transcript levels later declined and since zygotic *Hro-cdc25* RNA, like zygotic *string (cdc25)* RNA of *Drosophila* (Edgar et al., 1994b), may be unstable, it appears that the transcription of *Hro-cdc25* in the micromeres was transient.

**DISCUSSION**

Leech embryos display cell-specific or lineage-specific differences in cell cycle timing from the onset of development. The data presented here, along with that of previous studies, suggest that the cells of leech embryos employ different strategies of cell cycle control. The early blastomeres, macromeres and teloblasts contain steady levels of maternal *Hro-cdc25* RNA, and their cell-specific timings of cell division appear to be controlled by maternal cell cycle regulators. In contrast, the majority of *cdc25* RNA in the blast cells and the micromeres is zygotically produced, and their cell-specific timings of cell division appear to be regulated by zygotic Cdc25. The levels of *Hro-cdc25* RNA remain constant during the cell cycles of the blast cells, but fluctuate during the cell cycle of the micromeres. If zygotic *Hro-cdc25* mRNA is short-lived, like zygotic *string (cdc25)* mRNA in *Drosophila*, this suggests that the transcription of *Hro-cdc25* is constitutive in the blast cells but is cell cycle-regulated in the micromeres.

**The cell cycles of the blast cells and micromeres require zygotic Cdc25**

*Hro-cdc25* is expressed in a cell cycle-dependent manner in the micromeres and constitutively in the blast cells. Although both of these modes of expression have been described in other organisms, they have not been described in the same organism. The G2-dependent expression of *Hro-cdc25* in the micromeres is similar to that observed in fission yeast and the postblastoderm cells of *Drosophila* embryos. In these cells, the levels of *cdc25* mRNA and protein increase during the cell cycle and reach a maximum at mitosis (Moreno et al., 1990; Ducommun et al., 1990; Edgar and O’Farrell, 1989, 1990). The cell cycle-independent expression of *Hro-cdc25* in the blast cells is similar to that observed in mammalian cells. In these cells, the levels of Cdc25C protein and *cdc25B* RNA and protein are relatively constant throughout the cell cycle (Millar et al., 1991b; Nagata et al., 1991). It should be noted that while Cdc25 is essential for mitosis in all these cells, its activity is also regulated by phosphorylation (Kumagai and Dunphy, 1992; Izumi et al., 1992; Clarke et al., 1993; Hoffman et al., 1993; Izumi and Maller, 1993).

While it is not known why leech blast cells and micromeres display different modes of *cdc25* expression, it is known that these two types of cells have quite different developmental fates. The blast cells give rise to all the segmentally iterated...
ectodermal and mesodermal tissues of the body of the mature leech (Whitman, 1878; Weisblat et al., 1984; Weisblat and Shankland, 1985). In contrast, the micromeres contribute to the nonsegmental head structures and a provisional layer of epithelial cells that partially covers the embryo during epiboly (Weisblat et al., 1984; Ho and Weisblat, 1987; Smith and Weisblat, 1994). These different modes of cdc25 expression appear to be early indicators that the cells have embarked down different developmental pathways. The mechanisms by which cdc25 transcription is activated in these cells remain to be elucidated.

It appears that Hro-Cdc25 is the mitotic regulator in the blast cells and the micromeres. The cell cycles of these cells are much longer in duration than those of the early blastomeres and teloblasts, and the increased durations are mainly due to lengthened G2 phases (Bissen and Weisblat, 1989). It appears, therefore, that their divisions must be coordinated with other aspects of embryogenesis, and as a consequence, these cells are held in extended G2 phases until the appropriate time to divide. Thus, if Hro-Cdc25 was the mitotic inducer in these cells, their active transcription of this regulator may help ensure that they divided at the appropriate time. Since the divisions of the blast cells and the micromeres are blocked in the presence of RNA synthesis inhibitors (Bissen and Weisblat, 1991), and since these cells undergo premature cell divisions upon the elevation of Hro-cdc25 mRNA levels (K. Hayes and S.T.B., unpublished), it appears that zygotic Hro-Cdc25 regulates the timing of cell division in these cells.

The cell cycles of the teloblasts and the early blastomeres are controlled by maternal cell cycle regulators

The teloblasts do not synthesize Hro-cdc25 RNA, but rather appear to rely on maternally supplied messages. Since the divisions of the teloblasts proceed normally in the presence of RNA synthesis inhibitors (Bissen and Weisblat, 1991), it appears that their cell cycles are driven by maternal cell cycle regulators. Presumably the accumulation and destruction of Cyclin B from maternal RNA drives these repeated, rapid cell cycles. My colleagues and I have isolated leech cyclin cDNA clones and are examining their roles in the control of these cell cycles.

Similarly, the early blastomeres do not express Hro-cdc25, and it appears that the timings of their divisions are controlled by maternal messages. If these cell cycles are maternally controlled, however, it remains to be determined why these blastomeres divide asynchronously rather than synchronously. Although the mechanisms of cell cycle control appear to be similar in the early blastomeres and the teloblasts, there is one important difference. Namely, the divisions of some of the early blastomeres require zygotic RNA synthesis. The cleavages of the D'-derived blastomeres are perturbed after the inhibition of RNA synthesis (Bissen and Weisblat, 1991). The data presented here show that this requisite zygotic transcript cannot be cdc25 because these cells apparently do not transcribe Hro-cdc25. Furthermore, it is unlikely the essential zygotic RNA is cyclin because the D'-derived cells do divide, albeit incorrectly. Thus, it appears that the divisions of these D'-derived blastomeres are regulated by maternal cell cycle regulators, as well as some unidentified zygotic transcript(s).

Different strategies of cell cycle control can operate concurrently

While it has been shown that different mechanisms of cell cycle control are utilized during successive stages of Drosophila development (Edgar et al., 1994a), the present work reveals that different mechanisms can be utilized in different cells during the same stage of development. At any given time in leech embryos, some cells are using maternal regulators and others zygotic regulators. For example, the teloblasts are still producing blast cells when the first-produced blast cells undergo their first divisions. The cell cycles of the teloblasts appear to be controlled by maternal cell cycle regulators, while the cell cycles of the blast cells appear to be driven by zygotic Cdc25. Thus, the lineage of a cell and not the progression of development determines the strategy of cell cycle control in leech embryos.

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