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The durations and compositions of cell cycles in embryos of the leech, *Helobdella triserialis*

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Summary

When tritiated thymidine triphosphate ([^{3}H]TTP) or its immunohistochemically detectable analogue, bromodeoxyuridine triphosphate (BrdUTP), is injected into blastomeres of leech embryos it passes throughout the entire embryo and is rapidly incorporated (within 2 min after injection) into nuclei of cells synthesizing DNA (S phase). In the same embryos a DNA-specific stain can be used to identify cells in mitosis (M phase) or nonreplicative interphase (G_1 or G_2 phase) on the basis of nuclear or chromosomal morphology. Using this procedure, we have determined the lengths and compositions of the mitotic cell cycles of identifiable cells in early embryos of the leech, *Helobdella triserialis*, and have analysed how the cell cycles change during the first seven stages of development. The relatively short cell cycles of the early blastomeres comprise not only phases of M and S, but also postreplicative gap (G_2) phases. The lengthening of the cell cycles that occurs as development progresses is primarily accomplished by an increase in the length of G_2 and secondarily by an increase in the length of S and, in some instances, the addition of a prereplicative gap (G_1) phase; M phase remains relatively constant. These data suggest that the durations of the cell cycles of embryonic cells are regulated by a variety of mechanisms.

Key words: bromodeoxyuridine, DNA synthesis, mitosis, interphase, leech, cell cycle, *Helobdella triserialis*.

Introduction

The development of a complex multicellular organism from a single cell requires an orchestrated series of cell divisions, macromolecular syntheses and cell movements. During the development of most organisms, an early period of rapid, synchronous cell divisions is followed by a period of slower, asynchronous divisions. The lengthening of the cell cycle represents a major transition in the development of *Xenopus* (Newport & Kirschner, 1982*a*; Kimelman *et al.* 1987) and *Drosophila* (Edgar *et al.* 1986; Edgar & Schubiger, 1986).

Similar changes in cell cycle duration occur during the development of glossiphoniid leeches; an early period of relatively fast, fairly synchronous cell divisions is followed by a period of slower, asynchronous cell divisions (Whitman, 1878; Fernandez, 1980; Wordeman, 1983; Zackson, 1984). The early divisions subdivide the embryo into numerous identifiable cells, the most prominent of which are five bilateral pairs of stem cells, called *teloblasts*. Each teloblast undergoes a series of rapid, iterative divisions to generate a rostrocaudally arrayed chain, or *bandlet*, of segmental founder cells, the *primary blast cells*. Each class of primary blast cell gives rise to a distinct set of definitive progeny (Weisblat & Shankland, 1985; Bissen & Weisblat, 1987*a*), and each has a characteristic cell cycle duration that is much longer than those of the parent teloblasts and early large blastomeres (Zackson, 1984).

Studies of the control of cell cycle duration in cultured mammalian cells have shown that the phases of DNA synthesis (S), postreplicative gap (G_2) and mitosis (M) are quite constant in length, whereas the prereplicative gap (G_1) phases are variable (Prescott, 1976). There is a commitment event during G_1 at which a cell either pauses or proceeds through the rest of the cell cycle (Pardee, 1974). Similarly, the budding yeast, *S. cerevisiae*, has a commitment point for controlling division in G_1 (Hartwell *et al.* 1974), but the fission yeast, *S. pombe*, has commitment events in both G_1 and G_2 phases (Fantes & Nurse, 1977). By contrast, it is not clear how the durations of embryonic cell cycles are regulated. In *Xenopus* embryos the early, rapid cell cycles are primarily composed of S and M phases, with negligible G_2 phases, while the longer cell cycles of blastulae adopt G_1 phases, as well as lengthen their S and G_2 phases (Graham & Morgan, 1966). In *Drosophila* embryos the early, abbreviated nuclear divisions are composed of back-to-back M and S phases; the mitotic cycles are lengthened after cellularization by the addition of G_2 phases (Foe & Alberts, 1983; Edgar & Schubiger, 1986). Similarly, the early, rapid cell cycles of *Caenorhabditis* embryos comprise only M and S phases; the cycles of the cells in the gut lineage are

lengthened after the fourth cleavage by the acquisition of G_2 phases (Edgar & McGhee, 1988).

In the research reported here, the durations and compositions of the cell cycles of identifiable cells in embryos of the leech, *Helobdella triserialis*, were analysed to determine: (1) what accounts for the lengthening of the cell cycles in the later embryo; (2) whether or not the early cell cycles are composed exclusively of alternating M and S phases; (3) what accounts for the differences in cell cycle duration among the different classes of primary blast cells; (4) whether or not there are class-specific differences in cell cycle composition among the classes of primary blast cells and their progeny.

It was found that the relatively short cell cycles of the early large blastomeres comprise not only M and S phases, but also G_2 phases of significant duration. Similarly, the longer cell cycles of the primary blast cells are also composed of S, G_2 and M phases. The transition from the shorter cell cycles of the early blastomeres to the much longer cell cycles of the primary blast cells is primarily accomplished by an increase in the proportion of time spent in the G_2 phase of the cell cycle. The cell cycles of the progeny of the primary blast cells vary widely in duration and are composed of S, G_2, M and, in some instances, prereplicative gap (G_1) phases; differences in the lengths of their cell cycles are due to differences in the lengths of their G_1, S and G_2 phases. The only part of the cell cycle that remains constant during development is M phase.

Materials and methods

Embryos

Embryos of the glossiphoniid leech, *Helobdella triserialis*, were obtained from a laboratory breeding colony and kept at 24°C as previously described (Blair & Weisblat, 1984). The developmental staging system and cell lineage nomenclature are based on that of Fernandez (1980) as amended and extended by various authors. Micromeres are designated by the lowercase letter(s) corresponding to the parent blastomere (Ho & Weisblat, 1987); the names of the first micromere and large blastomere descended from a particular cell are followed by one prime (′), the names of the second set are followed by two primes (″), and so forth. Newly identified secondary blast cells are named according to the system of Zackson (1984), as expanded by Shankland (1987*a*).

Injections

The procedures for microinjecting blastomeres with lineage tracers or other compounds have been presented elsewhere (Weisblat *et al.* 1984). Teloblasts and their blast cell bandlets were labelled with either fluorescein- or tetramethylrhodamine-dextran-amine, FDA and RDA, respectively (Gimlich & Braun, 1985).

Autoradiography

Cells in S phase were identified by autoradiographic detection of thymidine incorporated into their nuclei. For this purpose, a sample of tritiated deoxythymidine 5′-triphosphate ([^{3}H]-TTP; 109·6 Ci mmol^{-1}, New England Nuclear) was taken to dryness under a stream of N_2 and resuspended at a concentration of 0·9 mM in a solution of 0·2 M-KCl and 0·5% fast green FCF (Sigma). The resulting solution was microinjected into blastomeres of embryos, in which a specific cell line had previously been labelled with FDA or RDA. The phosphorylated nucleotide passed throughout the entire embryo resulting in a dilution of several hundredfold. (Preliminary studies using bath-applied or microinjected tritiated thymidine resulted in no incorporation.) After a period of incubation (15–30 min), the embryos were fixed with 3·5% formaldehyde (in 0·1 M-Tris–HCl, pH 7·4) for 16 h at 4°C. The embryos were rinsed in 0·1 M-Tris–HCl, stained with 1 μg ml^{-1} Hoechst 33258 (Sigma), dehydrated in graded alcohols (30–95%), embedded in glycol methacrylate (JB-4; Polysciences, Inc.) and sectioned. Slides bearing 4 μm sections were coated with NTB2 emulsion (Kodak), exposed for 6–7 wk at −70°C, developed (5% Dektol; Kodak) and viewed with epifluorescent and bright-field optics. Silver grains were found almost exclusively over nuclei.

Immunohistochemistry

Cells in S phase were also identified by immunohistochemical detection of bromodeoxyuridine incorporated into their nuclei. Embryos, some of which contained FDA- or RDA-labelled cells, were microinjected with 5-bromo-2′-deoxyuridine 5′-triphosphate (BrdUTP; Sigma, 50 mM in 0·2 M-KCl, 0·5% fast green FCF) and incubated for 15 min. During the incubation period, the injected embryos were exposed to 0·15% pronase E (protease type XIV, Sigma) for 10 min; this treatment sufficiently permeabilized the vitelline membrane and eliminated the need for its removal. Embryos were fixed with 2% formaldehyde (in 50 mM-cacodylate buffer, pH 7·3, 1 mM-$CaCl_2$) for 1 h at 4°C, rinsed with cacodylate buffer and transferred to phosphate-buffered saline (PBS; 137 mM-NaCl, 2·7 mM-KCl, 4·3 mM-Na_2HPO_4, 1·4 mM-KH_2PO_4). They were then treated with 2 N-HCl in PBS for 1·5 h to partially denature the DNA and briefly rinsed in 0·1 M-sodium borate, pH 8·5, to neutralize the acid. After being rinsed in PBS, they were incubated in 1% Triton X-100, 2% bovine serum albumin in PBS (TBP) for 6–8 h at room temperature.

All subsequent incubations were for 16 h at room temperature with constant agitation, and all rinses were for 6–8 h with several changes of solution. Embryos were incubated with a mouse monoclonal anti-BrdU antibody (Becton-Dickinson, diluted 1:25 in TBP), rinsed, incubated with rhodamine- or fluorescein-conjugated rabbit anti-mouse IgG (ICN, diluted 1:500 in TBP), rinsed with PBS and stained with 1 μg ml^{-1} Hoechst 33258 in H_2O. After the primary antibody incubation and rinse, some embryos were incubated with goat anti-mouse IgG (Polysciences, Inc., diluted 1:50 in TBP), rinsed, incubated with 50 μg ml^{-1} peroxidase–anti-peroxidase complex (PAP; Polysciences, Inc.), rinsed with PBS and processed with H_2O_2 in the presence of 0·5 mg ml^{-1} diaminobenzidine to yield a dark brown reaction product. Labelled nuclei were viewed in either 14 μm sections of plastic-embedded embryos (see above) or whole-mounted embryos that were cleared in 70% glycerol. The following modifications were made for uncleaved zygotes: they were fixed with 4% formaldehyde, the vitelline membranes were removed with fine dissecting pins and the antibody incubations were for 4–5 days (S. H. Astrow, personal communication).

In a separate series of experiments, embryos were injected with BrdUTP and incubated for varying lengths of time (1–15 min) prior to fixation. These embryos were not exposed to pronase E; vitelline membranes were removed with fine pins and then the embryos were processed as described above.

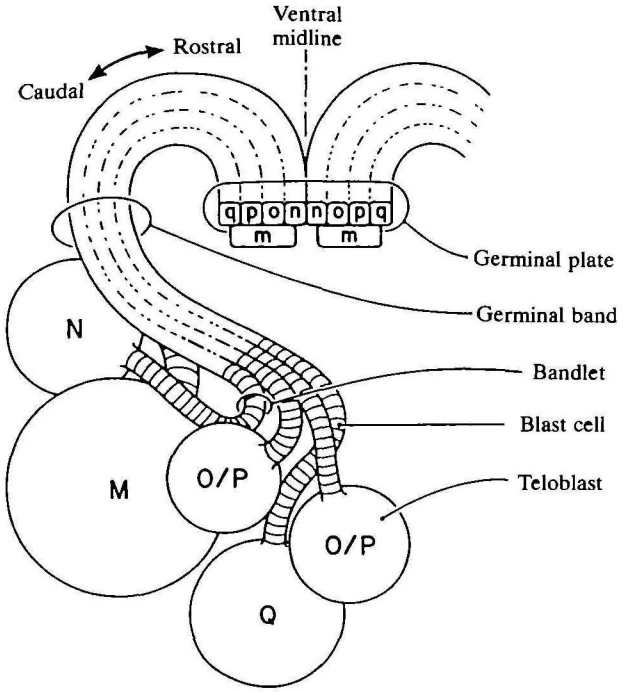


Fig. 1. Schematic representation of the hemilateral arrangement of the teloblasts and their bandlets of blast cells within the germinal band and germinal plate in *Helobdella* embryos.

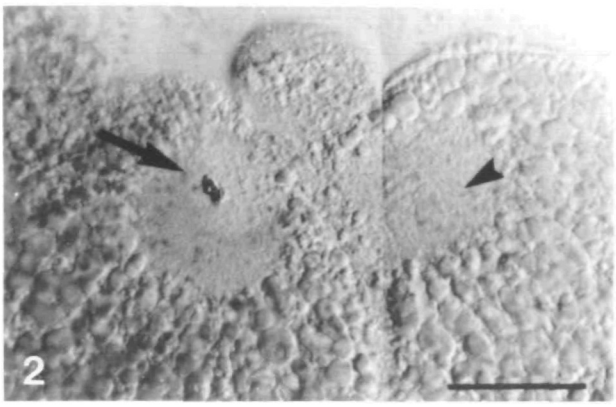


Fig. 2. Photomicrograph of a 14 μm section through an embryo in which BrdU was incorporated into the nucleus of an S phase cell. The nucleus (arrow) of macromere B, on the left of this stage 4a embryo (see Fig. 3), bound the anti-BrdU antibody, as visualized with a PAP secondary antibody complex, indicating that this cell was in S phase during some or all of the pulse of BrdUTP. Macromere C, on the right, was in G_2 phase throughout the BrdUTP pulse because its interphase nucleus (arrowhead) was not labelled. Each nucleus is surrounded by an area of yolk-free cytoplasm; the circular profiles in the rest of the cell are yolk platelets. Micromere b′ is visible between the two macromeres at the top of the figure. Scale bar, 50 μm.

These experiments revealed that detectable amounts of BrdU were incorporated into S phase nuclei by 2 min after injection.

Results

Summary of leech development

Each hemisegment of the leech is composed of five cell lines (M, N, O, P and Q) that are descended from the (m, n, o, p and q) bandlets of blast cells. Each primary blast cell in the m, o and p bandlets gives rise to one hemisegmental set of definitive progeny. In contrast, two consecutive primary blast cells in each n and q bandlet are needed to generate a complete hemisegmental set of definitive progeny, and there are two distinct subsets of progeny in each N and Q cell line (Weisblat & Shankland, 1985). Moreover, each primary blast cell in the m, o and p bandlets divides with a timing and symmetry characteristic of its bandlet, whereas there are two alternating symmetries and timings of division within each n and q bandlet (Zackson, 1984). There are, therefore, seven classes of primary blast cells; each m, o and p bandlet contains one class of primary blast cell (m, o or p), whereas each n and q bandlet contains two alternating classes of blast cells (nf and ns; qf and qs) (Weisblat & Shankland, 1985).

On each side of the embryo the bandlets come together to form right and left *germinal bands*, which lengthen and migrate over the surface of the embryo and eventually coalesce along the ventral midline into a structure called the *germinal plate* from which the segmental tissues of the leech arise (Fig. 1). In the germinal bands and plate each class of blast cell undergoes a stereotyped sequence of divisions to give rise to its definitive progeny (Zackson, 1984; Torrence & Stuart, 1986; Shankland, 1987*a*,*b*; Braun & Stent, in press). The primary blast cells are produced by five bilateral pairs of stem cells, the M, N, O/P, O/P and Q teloblasts, which are themselves generated through a series of stereotyped cleavages from cell D′, the largest of the four *macromeres* of the eight-cell embryo. The four *micromeres* of the eight-cell embryo, as well as additional micromeres generated during the early cleavages, give rise to various nonsegmentally distributed progeny in the embryo.

Cell cycles of cells in the cleavage stage embryo

Glossiphoniid leech eggs are fertilized internally; the zygotes are released from meiotic arrest upon laying, a process that occurs over a period of many minutes (Fernandez, 1980). To circumvent the resultant developmental asynchrony, individual batches were sorted into subgroups of at least two, but usually three or more, developmentally synchronous embryos at the time of a specific cytokinetic event. Sequential time points were obtained by injecting subgroups of synchronous embryos with BrdUTP at progressively later times (30 min intervals relative to the synchronization event). Injected embryos were incubated for 15 min, processed as described in Materials and methods and examined in serial thick sections. Fig. 2 presents a photomicrograph of a section of an embryo in which one cell had incorporated BrdU during the pulse of BrdUTP, as evidenced by its labelled nucleus,

and another cell had not, as evidenced by its unlabelled nucleus. Although this embryo was incubated with a PAP secondary antibody complex, most embryos were incubated with fluorescent secondary antibodies so that the fluorescent DNA-specific dye, Hoechst 33258, could be used to monitor nuclear and chromosomal morphology.

Cells were classified as being in S phase on the basis of their nuclear incorporation of BrdU. Cells were judged to be in M phase on the basis of their condensed chromosomes and the absence of BrdU incorporation. It was frequently observed that the nuclei of nascent large blastomeres incorporated BrdU before the chromosomes had decondensed; such cells were categorized as being in S phase rather than telophase of mitosis. Cells were judged to be in G phase on the basis of their unlabelled interphase nuclei; such cells were in G_2 phase because the direct transition from M to S phase mentioned above indicates that these cell cycles lack G_1 phases and because the observed G phases always followed, never preceded, S phases in these timed experiments. Identified cells in 285 cleavage-stage embryos (stages 1–6c) were examined; the lengths and compositions of their cell cycles are presented in Figs 3 and 4.

Limitations to the accuracy of our data are as follows: (1) Embryos were fixed at 30 min intervals; thus, if homologous cells in synchronous embryos fixed at successive time points were classified as having been in two different phases, there is a 30 min period of uncertainty regarding the actual transition from one phase to the next. (2) Whereas cells were judged to have been in G_2 or M phase on the basis of criteria that reflect the state of the cell at the time of fixation, cells were judged to have been in S phase on the basis of a criterion that could have been met during any of the last 13 min of the 15 min BrdUTP pulse preceding fixation. Thus, if homologous cells in synchronous embryos, fixed at times t and t+30, were judged to have been in G_2 and M phases, respectively, the transition from G_2 to M occurred at some point during the 30 min interval between t and t+30. A similar argument applies to establishing the timing of the M-to-S transition. In contrast, if homologous cells in synchronous embryos, fixed at times t and t+30, were judged to have been in S and G_2 phases, respectively, the transition from S to G_2 occurred at some point during the 30 min interval between the last 13 min of the BrdUTP pulse given to the first subgroup of embryos and the first 2 min of the BrdUTP pulse given to the second subgroup of embryos, i.e. between t−13 and t+17. The period of uncertainty for the S-to-G_2 transition, therefore, is phase shifted relative to the times of fixation. In presenting the data, the intervals of uncertainty have been divided evenly between the two relevant phases, unless additional information was available (such as whether the M phase cells were near the beginning (prophase) or end (telophase) of mitosis) that could be used to assign the transition more precisely. Estimates were rounded off to the nearest five minutes. The variance in our results was minimal; homologous cells in the precisely staged embryos within each subgroup were always in the same phase of the cell cycle.

The first three cell cycles

The first mitotic cell cycle is about 140 min in length and comprises 10 min S, 90 min G_2 and 40 min M phases. S phase begins soon after the release of the second polar body (110 min after egg deposition), and the female pronucleus approaches the male pronucleus in the centre of the zygote. During G_2 phase, pools of yolk-deficient cytoplasm called *teloplasm* accumulate at the animal and vegetal poles (Whitman, 1878; Schleip, 1914; Fernandez, 1980, 1987; Astrow *et al.* in preparation). The zygote cleaves unequally into a larger daughter, cell CD, which inherits the bulk of the teloplasm, and a smaller daughter, cell AB.

Cell CD has a cell cycle of about 110 min that comprises a 10 min S phase, a 70 min G_2 phase and a 30 min M phase. Cell CD cleaves to yield cells C and D; cell D is the larger of the two and inherits the teloplasm. Cell AB has a 125 min cell cycle composed of a 15 min S phase, an 80 min G_2 phase and a 30 min M phase. Cell AB cleaves equally into cells A and B.

The length of the cell cycle of cell D is 85 min, whereas those of cells A, B and C are 120 min. All four cells have S phases of 15 min and M phases of 25–30 min; cell D has a G_2 phase of 45 min, whereas cells A, B and C have G_2 phases of 75 min. Differences in the lengths of these cell cycles, therefore, are due to differences in the lengths of their G_2 phases. Each cell undergoes a highly unequal division to generate a macromere and a micromere, which lies at the animal pole of the embryo. Cell D divides first to yield macromere D′ and micromere d′, cell C divides next into macromere C′ and micromere c′ and, lastly, cells A and B divide to yield macromeres A′ and B′ and micromeres a′ and b′.

Cell cycles in the A, B and C cell lines

The cell cycles of macromeres A′, B′ and C′ are 135–140 min in length and are composed of 15–20 min S phases, 80–90 min G_2 phases and 30–35 min M phases. These macromeres divide unequally, generating a secondary trio of micromeres, a″, b″ and c″, that lie under the primary quartet of micromeres at the animal pole, and a trio of macromeres, A″, B″ and C″.

The cell cycles of macromeres A″, B″ and C″ are 130–135 min in length and are composed of 25–30 min S phases, 70–75 min G_2 phases and 30–35 min M phases. These macromeres divide unequally giving rise to a tertiary trio of micromeres, a‴, b‴ and c‴, at the animal pole and a trio of macromeres, A‴, B‴ and C‴.

The complete cell cycles of macromeres A‴, B‴ and C‴ are unknown; each has a 30–40 min S phase and an extended G_2 phase. These cells have not been observed to divide again, but rather undergo a series of nuclear divisions and become multinucleated (Weisblat *et al.* 1984); they are ultimately incorporated into the gut (Weisblat *et al.* 1980*a*). Although it has been reported that in *Theromyzon* these cells commence karyokinesis during stage 5 (Fernandez & Olea, 1982), our analysis

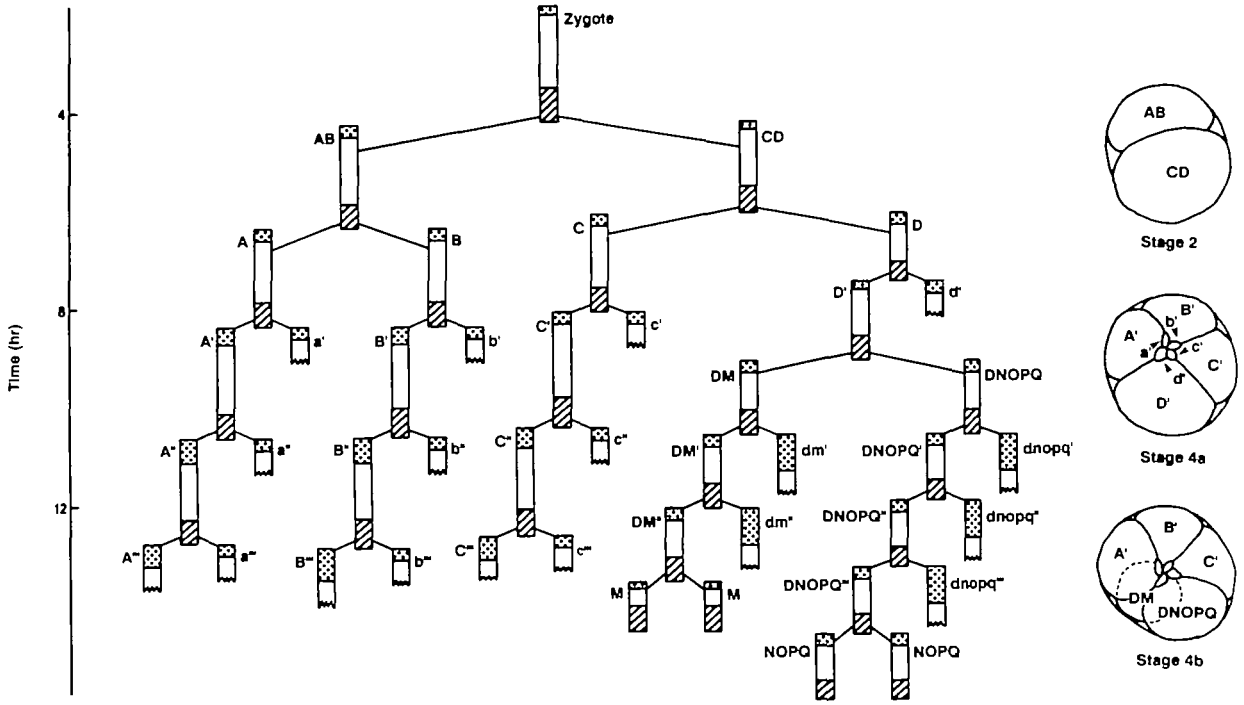


Fig. 3. Cell cycles of cells during stages 1 through 4 of *Helobdella* development. The cell lineage tree presents the divisions, as well as the composition of the cell cycle of each cell. Each vertical bar represents the cell cycle of an identified cell; S phase is shown in stippling, G_2 phase in white, M phase in diagonal stripes. Time was measured from the time of egg deposition. The sloping horizontal lines represent cytokinesis. Note that nascent large blastomeres entered S phase before cytokinesis was completed. Since the complete cell cycle of the micromeres and macromeres A‴–C‴ are not known, their bars end in jagged lines. On the right are schematic drawings of embryos at representative stages, with each cell identified. Approximate diameter of an embryo is 400 μm.

has revealed that in *Helobdella*, through early stage 7, they undergo neither karyokinesis nor additional DNA synthesis.

Cell cycles in the D cell line

The 95 min cell cycle of cell D′ is characterized by a 10 min S phase and a 55 min G_2 phase during which the nucleus moves from near the animal pole to the centre of the cell and a 30 min M phase. Cell D′ undergoes a nearly equal cleavage to give rise to a mesodermal precursor, cell DM, that lies toward the vegetal pole and an ectodermal precursor, cell DNOPQ, that lies toward the animal pole.

Cell DM and its large blastomere progeny, cells DM′ and DM″, have 90 min cell cycles composed of 15 min S phases, 45 min G_2 phases and 30 min M phases. Cell DM divides unequally into cell DM′ and micromere dm′; cell DM′ divides to yield cell DM″ and micromere dm″; cell DM″ cleaves nearly equally into the left and right mesodermal teloblasts, cells M_l and M_r.

Cells DNOPQ, DNOPQ′, DNOPQ″ and DNOPQ‴ have 80–90 min cell cycles composed of 15 min S phases, 40–45 min G_2 phases and 25–30 min M phases. Cells DNOPQ, DNOPQ′ and DNOPQ″ each divide to yield a large cell (cells DNOPQ′, DNOPQ″ and DNOPQ‴, respectively) and a small cell (micromeres dnopq′, dnopq″ and dnopq‴, respectively). Cell DNOPQ‴ cleaves equally into the left and right ectodermal proteloblasts, cells $NOPQ_l$ and $NOPQ_r$. After this division the bilateral symmetry of the embryo has been established; subsequently, equivalent divisions occur on both sides of the embryo.

The cell cycles of cells NOPQ, NOPQ′ and NOPQ″ are about 70–80 min in length and are composed of 15 min S phases, 30–40 min G_2 phases and 25–30 min M phases. Cells NOPQ and NOPQ′ divide unequally into cells NOPQ′ and nopq′ and cells NOPQ″ and nopq″, respectively. Cell NOPQ″ cleaves to give rise to a smaller N teloblast and a larger OPQ proteloblast.

Cells OPQ, OPQ′ and OPQ″ have 70–90 min cell cycles composed of 15 min S, 25–45 min G_2 and 30 min M phases. Cell OPQ divides into cell OPQ′ and micromere opq′; cell OPQ′ divides to yield cell OPQ″ and micromere opq″; cell OPQ″ cleaves nearly equally into a Q teloblast and an OP teloblast.

The N teloblast generates three n blast cells that lie near the animal pole and then produces micromere n′, which lies in the cleavage furrow between the OP and N teloblasts. The cell cycles leading to the production of these four cells are 70–90 min in length and contain 15 min S, 25–45 min G_2 and 25–30 min M phases. The N'_3 teloblast (hereafter referred to as N) then resumes production of primary n blast cells, which are contiguous with the first three n blast cells and form a bandlet (Fernandez & Stent, 1980).

Cell OP produces four op blast cells before cleaving

into two O/P teloblasts. The cell cycles leading to the generation of the op blast cells range from 75 to 90 min in length and are composed of 15 min S, 30–45 min G_2 and 30 min M phases. There are four op blast cells produced in *Helobdella*, whereas five op blast cells are produced in *Theromyzon* (Sandig & Dohle, 1988). The op blast cells constitute a short bandlet immediately anterior to the o and p bandlets in the germinal band (Fernandez & Stent, 1980).

Large blastomeres of D cell line have shorter cell cycles than those of A, B and C cell lines

The cell cycles of the macromere and proteloblasts of the D cell line average about 84 min in length and comprise 15 min S phases, 41 min G_2 phases and 28 min M phases. In contrast, the cell cycles of the macromeres of the A, B and C cell lines average about 130 min in length and contain 21 min S phases, 77 min G_2 phases and 32 min M phases. The lengths of the S and M phases are quite similar between these two groups of cells, but the G_2 phases of the A–C cell line macromeres are nearly twice as long as those of the D-derived cells. The differences in the lengths of these cell cycles, therefore, are due to differences in the lengths of their G_2 phases.

Cell cycles of micromeres

The total lengths of the cell cycles of the micromeres generated during the early cleavages are not known because these small cells are hard to follow in progressively older embryos without the use of lineage tracers. It is known, however, that these cell cycles lack G_1 phases because each micromere enters S phase immediately after it is born. The primary quartet, micromeres a′–d′, the secondary trio, micromeres a″–c″, and the tertiary trio, micromeres a‴–c‴, have 15 min S phases, as do micromeres opq′, opq″ and n′. In contrast, micromeres dm′, dm″, dnopq′, dnopq″, dnopq‴, nopq′ and nopq″ have 45 min S phases (Figs 3 and 4). Each micromere then enters a G_2 phase of unknown length.

Cell cycles of teloblasts

The cell divisions become more asynchronous as development proceeds. Although the teloblasts divide at about the same rate, those in one embryo do not divide at the same time and, furthermore, homologous teloblasts on each side of the embryo do not divide synchronously (Wordeman, 1983). For this reason, the experimental protocol used to analyse the cell cycles of the early blastomeres could not be used to analyse the cell cycles of the teloblasts. Since each teloblast undergoes an extensively iterated series of divisions, however, it was possible to determine the composition of its cell cycle by determining the proportion of teloblasts in each phase of the cell cycle within a population of embryos. For this purpose, early-stage-7 embryos were injected with BrdUTP, incubated for 15 min, processed for immunohistochemistry and viewed in section. Individual teloblasts were identified on the basis of size or through the prior injection of lineage tracer. The best available estimates of the length of the teloblasts' cell cycles range from 0·9 to 1·2 h at 25°C (Wordeman, 1983). For the present study, we have assumed a 1 h cell cycle of a teloblast as a reasonable value on which to

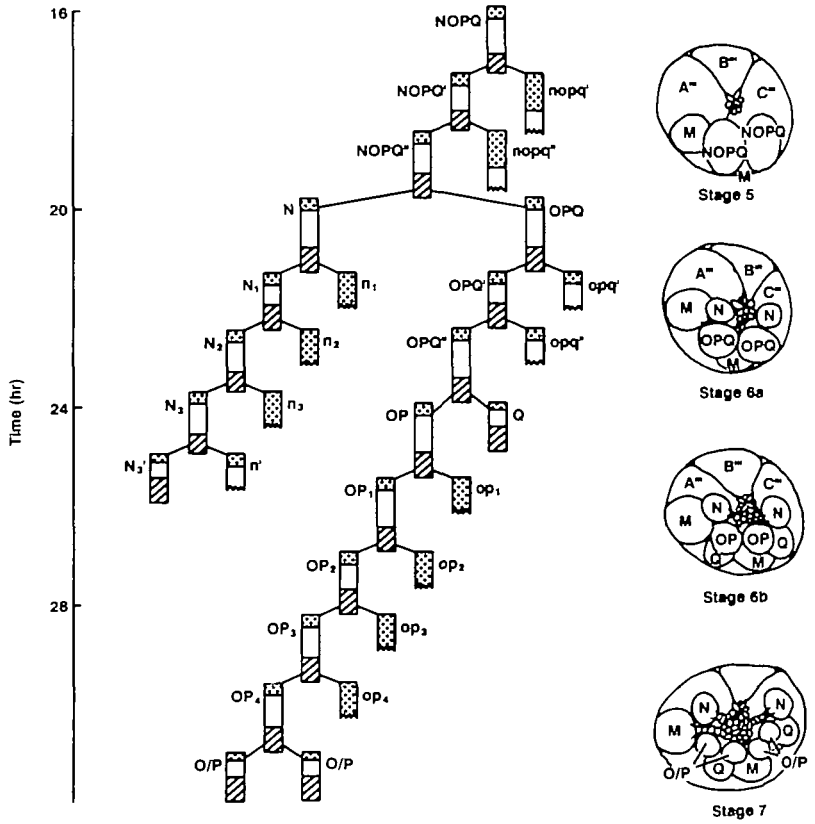


Fig. 4. Cell cycles of cells during stages 5 to 7 of *Helobdella* development. The lineage tree presents the divisions leading to the generation of the ectodermal teloblasts, on one side of the embryo, and the cell cycles of each of these cells. Phases of the cell cycle are designated as in Fig. 3. Schematics of embryos at representative stages are presented at the right.

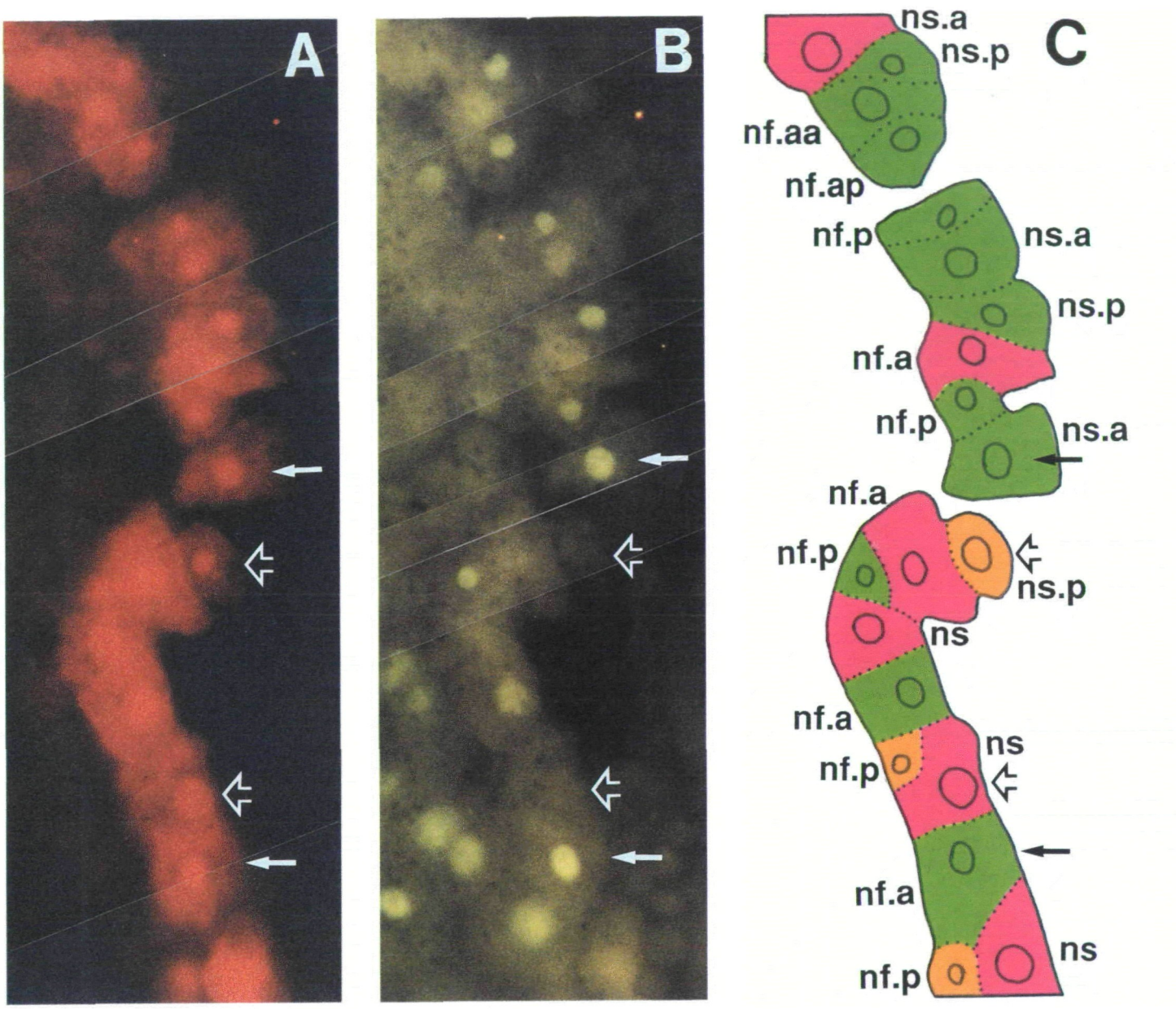


Fig. 5. Analysis of the cell cycles of n blast cells. (A and B) Montage of photomicrographs of an isolated right germinal band in which the n blast cells were labelled with RDA (A) and the nuclei of cells in S phase during the BrdUTP pulse were labelled by indirect immunofluorescence with the antibody to BrdU (B). The germinal band was dissected from a fixed, glycerol-cleared embryo and mounted between coverslips, which resulted in breaks between recently divided cells; anterior is up. (C) Tracing of the RDA-labelled n blast cells from (A) in which each cell is identified by name and each is colour-coded with respect to phase of the cell cycle: orange = G_1 phase, green = S phase, pink = G_2 phase. Solid arrows point to cells in S phase, as evidenced by their anti-BrdU nuclear labelling in B. Open arrows point to cells in G phases; their interphase nuclei (A) have not bound the antibody (B). The lower open arrow points to cell ns that has not yet divided, and, therefore, is in G_2 phase. The upper open arrow points to cell ns.p, the posterior daughter of cell ns, which has been recently born but has not yet entered S phase, and, therefore, is in G_1 phase. In the next anterior clone of cells, cell ns.p is in S phase.

base our analysis of the relative lengths of the different phases of the cell cycle.

Since no lineage-specific differences in cell cycle composition were detected, the data for all the teloblasts were pooled. Of the 90 teloblasts examined, 47 ± 9% (90% confidence interval) contained condensed chromosomes and, thus, were in M phase at the time of fixation. Unlabelled interphase nuclei were observed in 14 ± 6% of the teloblasts, which indicates that these teloblasts were in G phase at the time of fixation. These teloblasts were judged to be in G_2 phase because we observed several cases in which the nucleus of a teloblast had incorporated BrdU, while the newly born blast cell was still in telophase. This indicates that, like the earlier blastomeres, teloblasts begin DNA synthesis immediately after mitosis and, therefore, lack G_1 phases. The rest of the teloblasts (39 ± 9%) contained nuclei that had incorporated BrdU, which indicates that they were in S phase during part or all of the BrdUTP pulse. Assessing the cell cycles of a population of cells cycling at random using the present protocol will overestimate the length of S at the expense of G_2 because cells in G_2 phase at the time of fixation will be classified as having been in S phase if they had made the S-to-G_2 transition during the last 13 min of the BrdUTP pulse. This bias was corrected by adjusting the apparent lengths of the S and G_2 phases. The teloblasts' approximate 60 min cell cycles, therefore, comprise 11 ± 5 min S phases, 21 ± 5 min G_2 phases and 28 ± 5 min M phases.

Cell cycles of blast cells

Primary blast cells, born at the approximate rate of one per hour, are spatially arranged in strict birth order within the bandlets; the first-born blast cells lie in the anteriormost parts of the bandlets, while later-born blast cells occupy more posterior positions in the bandlets. Moreover, each class of blast cell undergoes a similar sequence of stereotyped divisions to give rise to *secondary blast cells* (Zackson, 1984; Shankland, 1987*a*,*b*). In the first approximation, therefore, it is possible to infer the cycle of a specific blast cell by examining progressively older blast cells or blast cell clones, previously labelled with a lineage tracer, along the length of a bandlet. Cells in S phase were identified by their nuclear incorporation of BrdU (or [^{3}H]thymidine). Cells with unlabelled interphase nuclei were classified as being in either G_1 or G_2 phase by comparing them with adjacent blast cells or blast cell clones in that bandlet (i.e. newly born cells not yet in S phase were in G_1 phase and post-S, pre-M phase cells were in G_2 phase). Divisions were inferred by the observation of mitotic figures and/or an increase in cell number in the next anterior (older) cell clone. This method is illustrated in Fig. 5, which shows part of an n bandlet that is composed of five nf and six ns blast cell clones. The youngest nf blast cell clone, in the lower part of the figure, contains two cells, nf.p and nf.a, which have been recently born. In this and the next older (next anterior) nf clone, cell nf.a is in S phase because its nucleus is labelled with the antibody. In the third and fourth nf clones, however, cell nf.a is in G_2 phase because its nucleus is no longer labelled with the antibody. And in the fifth nf clone cell, nf.a has divided into cells nf.ap and nf.aa, which are both in S phase. In this bandlet, therefore, cell nf.a was observed in four cell clones; cell nf.a was in S phase in two clones and in G_2 phase in two clones.

Since the embryos used for these experiments were asynchronous, the exact age of any given cell varied randomly at the time of fixation. Thus, the length of each phase of a cell cycle was estimated by dividing the frequency with which that phase was observed in the appropriate blast cell clones in a population of embryos by the frequency with which the parent class of primary blast cell arose. For example, cell nf.a was observed to be in S phase in an average of 1·3 ± 0·6 clones/bandlet (32 clones observed in 25 bandlets). From this observation, and because one nf clone was born every two hours, we estimated that the length of the S phase of cell nf.a was 2·6 ± 1·2 h (Table 1). It should be noted that the lengths of each phase of the cell cycles of the blast cells, as for the teloblasts, were calculated using the approximation that blast cells are produced at the rate of about one per hour. The blast cells examined in these experiments were those that would have given rise to segmental structures in the midportion of the body. We have not addressed the issue of segment-specific differences in cell cycle length or composition.

Ectodermal blast cells

To analyse the cell cycles of the ectodermal primary and secondary blast cells, a specific bandlet of blast cells was labelled by injecting its parent teloblast with RDA or FDA in early-stage-7 embryos. After 18–54 h of development, the embryos were injected with BrdUTP (or [^{3}H]TTP), incubated for 15 min (or 15–30 min) and processed accordingly. The most recently born blast cells were viewed in section because they lie deep within the embryo, whereas the older primary and the secondary blast cells were viewed in wholemount because they lie on the surface of the embryo. A total of 33 bandlets were viewed in sectioned embryos, and 133 bandlets were viewed in wholemounted embryos.

The cell cycles of each class of ectodermal primary blast cell have characteristic lengths; those of o and p blast cells are 21 h, those of nf blast cells are 22 h, those of ns and qf blast cells are 28 h, and those of qs blast cells are 33 h (Zackson, 1984). The cell cycles of the primary blast cells lack G_1 phases because each enters an S phase of 4·7 ± 0·8 h immediately after birth. The lengths of their M phases range from about 0·3 to 0·8 h. These data, in combination with those of Zackson (1984), indicate that o and p blast cells have G_2 phases of about 16 h, nf blast cells have G_2 phases of about 17 h, ns and qf blast cells have G_2 phases of about 23 h and qs blast cells have G_2 phases of about 28 h. Thus, the class-specific differences in cell cycle duration among the six classes of ectodermal primary blast cells are due to differences in the lengths of their G_2 phases (Figs 6–9).

The cell cycles of the ectodermal secondary blast cells

Table 1. *The lengths and compositions of the cell cycles of secondary blast cells*

Cell	G_1 phase (h)*	S phase (h)	G_2 phase (h)	M phase (h)	Total (h)
nf.a	0	2·6 ± 1·2	3·7 ± 0·9	0·4 ± 1·3	6·6 ± 1·1
nf.p	2·7 ± 2·1	7·5 ± 2·2	–	–	–
nf.aa	0	2·3 ± 0·8	2·9 ± 1·2	0·6 ± 0·9	5·8 ± 1·4
nf.ap	0·4 ± 1·3	4·4 ± 1·3	–	–	–
nf.aaa	0·6 ± 1·2	2·3 ± 0·4	–	–	–
ns.a	0	2·8 ± 1·2	4·6 ± 1·6	0·8 ± 1·0	8·1 ± 1·5
ns.p	0·6 ± 0·9	4·9 ± 1·7	–	–	–
ns.aa	0	2·2 ± 1·0	–	–	–
ns.ap	0·3 ± 0·7	–	–	–	–
o.a	0	1·6 ± 1·0	2·8 ± 1·0	0·2 ± 0·4	4·6 ± 1·1
o.p	12·4 ± 1·3	–	–	–	–
o.aa	0·2 ± 0·4	3·0 ± 1·2	3·0 ± 0·6	0·4 ± 0·6	6·8 ± 1·3
o.ap	0	1·9 ± 0·7	3·3 ± 0·9	0·3 ± 0·5	5·4 ± 0·7
o.apa	0·1 ± 0·2	1·9 ± 0·6	2·6 ± 0·6	0·1 ± 0·3	4·8 ± 0·7
o.app	0·1 ± 0·3	3·1 ± 1·1	–	–	–
o.aaa	0·2 ± 0·4	3·7 ± 1·1	–	–	–
o.aap	0·2 ± 0·4	3·7 ± 1·1	–	–	–
o.apaa	0·6 ± 0·6	–	–	–	–
o.apap	0	1·9 ± 0·6	–	–	–
p.a	0·1 ± 0·3	2·1 ± 0·8	3·1 ± 1·1	0·4 ± 0·5	5·6 ± 1·3
p.p	0·2 ± 0·4	2·5 ± 0·9	4·9 ± 1·3	0·4 ± 0·6	7·9 ± 1·5
p.aa	0·1 ± 0·4	2·6 ± 0·7	2·7 ± 0·8	0·4 ± 0·6	5·8 ± 1·0
p.ap	0·1 ± 0·4	2·9 ± 0·8	5·0 ± 0·8	0·5 ± 0·5	8·6 ± 1·1
p.pa	0·1 ± 0·2	3·7 ± 0·9	2·0 ± 0·5	0·5 ± 0·5	6·3 ± 1·0
p.pp	0·2 ± 0·5	4·5 ± 1·1	3·6 ± 1·0	0·5 ± 0·5	9·2 ± 1·2
p.aal	0·6 ± 0·7	3·5 ± 1·1	–	–	–
p.aam	0·1 ± 0·3	3·1 ± 0·8	–	–	–
p.apl	0·3 ± 0·5	2·2 ± 0·6	–	–	–
p.apm	2·7 ± 1·5	–	–	–	–
p.paa	0·2 ± 0·4	3·0 ± 0·5	–	–	–
p.pap	0·1 ± 0·3	2·9 ± 0·6	–	–	–
p.ppl	0·1 ± 0·3	–	–	–	–
p.ppm	0·2 ± 0·4	–	–	–	–
qf.a	2·5 ± 1·7	7·2 ± 1·7	–	–	–
qf.p	0	2·8 ± 1·0	3·5 ± 1·2	0·7 ± 0·9	7·0 ± 1·3
qf.pa	0·2 ± 0·6	3·2 ± 1·2	3·2 ± 1·5	0·5 ± 0·9	7·0 ± 1·6
qf.pp	0·3 ± 0·8	4·0 ± 1·3	4·5 ± 1·3	0·3 ± 0·7	9·2 ± 1·4
qf.pal	0·3 ± 0·7	3·7 ± 0·9	–	–	–
qf.pam	0·3 ± 0·7	3·5 ± 0·9	–	–	–
qf.ppl	0·7 ± 1·4	3·4 ± 1·4	–	–	–
qf.ppm	3·0 ± 2·6	–	–	–	–
qs.a	0·1 ± 0·5	2·7 ± 1·5	5·0 ± 1·5	0·3 ± 0·7	7·9 ± 1·2
qs.p	0·7 ± 0·9	5·3 ± 1·8	–	–	–
qs.aa	0	3·5 ± 1·2	–	–	–
qs.ap	0	3·5 ± 1·2	–	–	–
m.l	0	0·9 ± 0·8	3·8 ± 0·7	0·4 ± 0·5	5·1 ± 0·6
m.m	0·1 ± 0·4	0·8 ± 0·7	5·3 ± 1·4	0·6 ± 0·5	7·0 ± 0·9

* Data are given as mean ± S.D. The length of each phase was calculated upon examination of the appropriate blast cell clones in at least 7 bandlets.

range from 4·6 h to more than 22 h in length and are composed of phases of S, G_2, M and, in some cases, G_1 (Figs 6–9; Table 1). The lengths of the G_1, S and G_2 phases of these cell cycles range widely; G_1 phases range from 0 to 12·4 h, S phases range from 1·6 to 7·5 h and G_2 phases range from 2·0 to more than 15 h. The lengths of the M phases remain quite constant; they range from 0·1 to 0·8 h.

There were no apparent class-specific differences among the six classes of ectodermal secondary blast cells with regard to cell cycle length or composition. Several properties of the cell cycle, however, are correlated with the size of the cell. First, the presence or absence of a G_1 phase is correlated with cell size. All of the smallest secondary blast cells (i.e. those similar in size to cells nf.p and o.p, with nuclear diameters of less than 4 μm) have G_1 phases, most of which are longer than 2 h. In contrast, 69 % of the larger secondary blast cells have G_1 phases, all of which are less than 1 h. Second, cell cycle length is also correlated with cell size. Smaller cells have longer cell cycles than larger cells, and the average lengths of the G_1, S and G_2 phases of the smaller cells are longer than those of larger cells (Table 2). Differences in the lengths of the cell cycles of the secondary ectodermal blast cells, therefore, are due to differences in the lengths of their G_1, S and G_2 phases. The length of M phase remains quite constant.

In the region of the q bandlet where both cells qf and qs have divided, there is a repeating pattern of four cells; a large cell (qf.p), two small cells that lie side by

Table 2. *Length of the phases of the cell cycle as a function of cell size*

Diameter of nucleus (μm)*	G_1 phase (h)†	S phase (h)	G_2 phase (h)	Total (h)
<4	3·1 ± 4·7 (n = 8)	6·2 ± 1·1 (n = 5)	11+ (n = 2)	17+ (n = 4)
>4	0·2 ± 0·2 (n = 37)	3·0 ± 0·8 (n = 35)	3·6 ± 0·9 (n = 17)	6·9 ± 1·4 (n = 17)

* Index of cell size.
† Data are presented as mean ± S.D.

side (qf.a and qs.p) and a large cell (qs.a) (see Fig. 9B). Further anterior in the bandlet, in the region where cells qs.a and qf.pa have divided, one of the small cells (qf.a or qs.p) was frequently missing from the bandlet. By examining the anteriormost-labelled blast cell clones in various embryos, the cell that was sometimes absent from the bandlet was identified as cell qf.a. In the same embryos in which cell qf.a was absent from the bandlet, there often were small lineage tracer-labelled cells in the area between the germinal bands, which we take to be the missing qf.a cell(s). Frequently cell qf.a was missing in several blast cell clones, but present in more anterior blast cells clones in the same bandlet; examination of 92 qf clones (near or anterior to the region where cell qf.pa had divided) revealed that this apparently errant cell was inside the bandlet 33 % of the time, outside the bandlet 43 % of the time and could not be found anywhere 24 % of the time. Zackson (1984) reported the existence of lineage tracer-labelled cells outside the q bandlet and suggested that they represented dying cells. Although this interpretation may be correct, it is also possible that this cell migrates from the bandlet to give rise to progeny elsewhere.

Mesodermal blast cells

The mesodermal (m) bandlets contain one class of primary blast cell (Zackson, 1982; Weisblat & Shankland, 1985) and each undergoes its first division about 10 h after its birth (Weisblat *et al.* 1980*b*). The cell cycles of the primary, and some of the secondary, m blast cells were estimated upon examination of 16 RDA-labelled m bandlets in embryos previously injected with BrdUTP, incubated for 15 min and processed for immunohistochemistry. The m bandlets were viewed in sectioned embryos or after being dissected from the rest of the embryo and mounted between coverslips.

The primary m blast cells have 9·3 ± 0·6 h cell cycles composed of 0·7 ± 0·5 h S phases, 8·0 ± 0·5 h G_2 phases and 0·8 ± 0·4 h M phases (Fig. 10). Although the cell cycles of the mesodermal primary blast cells are shorter than those of the ectodermal primary blast cells, all are similar in composition in that G_2 occupies about 80–90 % of the total cell cycle. The cell cycles of cells m.m and m.l are similar to those of the primary m blast cells in that the G_2 phases are much longer than the S phases (Fig. 10; Table 1). The complete cell cycles of subsequently produced m blast cells have not been determined because it is difficult to follow these cells in the three-dimensional array of secondary m blast cells.

Changes in the cell cycle throughout development

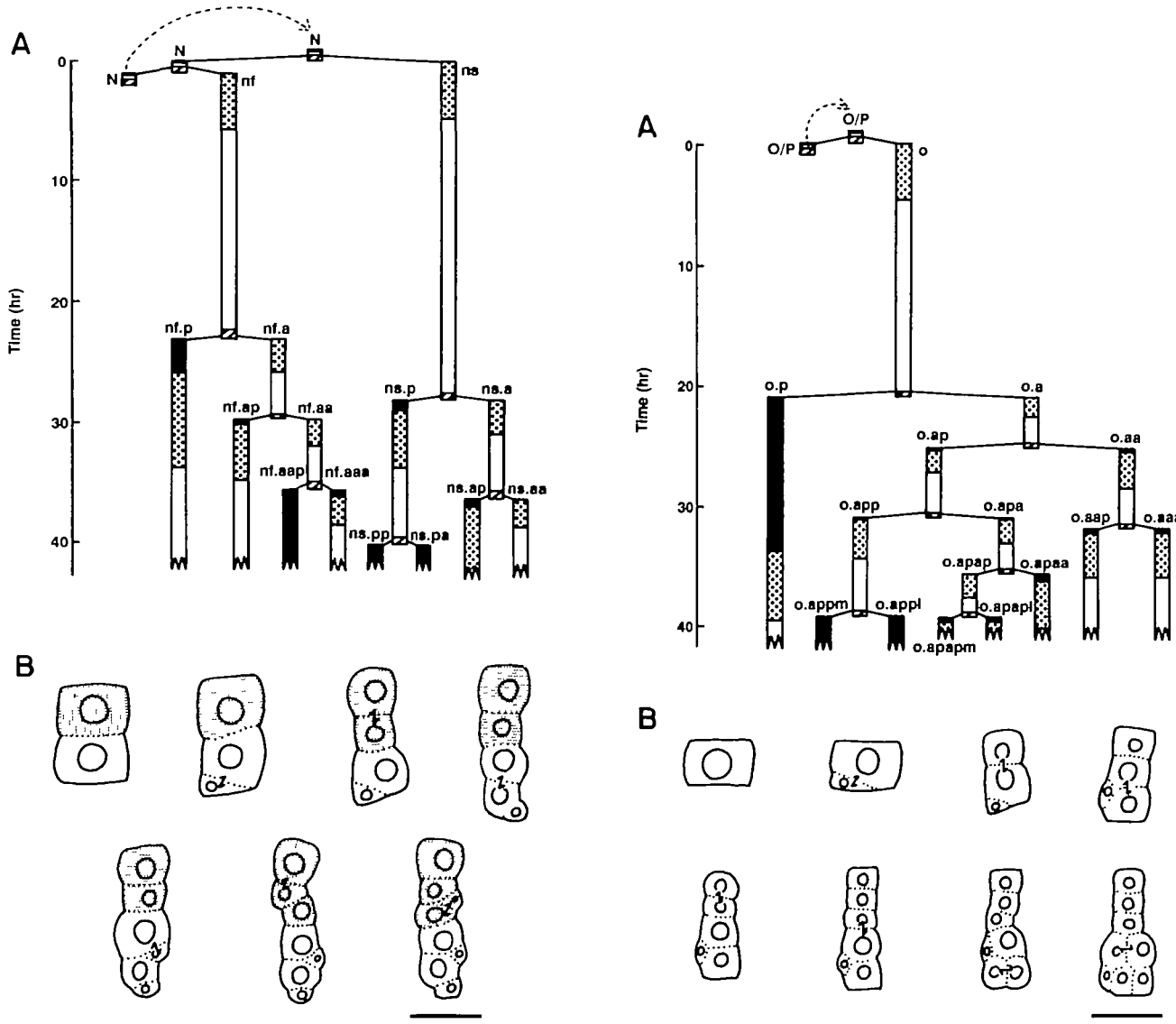
During the earliest period of leech development a series of relatively rapid divisions gives rise to 10 teloblasts, 3 macromeres and 20 micromeres. Excluding the micromeres, the blastomeres arising during these divisions have about 1·6 h cell cycles composed of 0·3 h S phases, 0·9 h G_2 phases and 0·5 h M phases. During the next period of development the teloblasts undergo iterative stem cell divisions to generate bandlets of primary blast cells. The cell cycles of the teloblasts average about 1 h in length and contain 0·2 h S phases, 0·3 h G_2 phases and 0·5 h M phases.

The blast cells divide during the next period of development and, at this time, there is a transition in cell cycle duration and composition. The cell cycles of the ectodermal and mesodermal primary blast cells are much longer in duration than those of the early macromeres, proteloblasts and teloblasts. The primary blast cells have 23·2 ± 7·0 h cell cycles composed of 4·1 ± 1·4 h S phases, 18·5 ± 6·0 h G_2 phases and 0·6 ± 0·2 h M phases. Although both the S and G_2 phases increase in duration in the cell cycles of the primary blast cells relative to those of their parent teloblasts, there is a larger increase in the length of G_2; S phases increase about 20-fold, whereas G_2 phases increase about 60-fold. The length of M phase remains constant. As a consequence, the proportion of time spent in G_2 phase is greater among the primary blast cells than among the earlier cells.

The cell cycles of the secondary blast cells differ from those of cells in the earlier embryo in that some contain G_1 phases, as well as S, G_2 and M phases. Each secondary blast cell has a cycle of characteristic duration and composition, but the range of values for various aspects of their cell cycles is much wider than for the earlier cells.

Discussion

We have presented here a thorough survey of the lengths and compositions of the cell cycles of identified cells in early *Helobdella* embryos, and have analysed how the cell cycles change during this time. Our experiments revealed that the lengths of some of the cell cycles in the cleavage-stage embryo were shorter than expected on the basis of previous studies of leech cell lineages (Whitman, 1878; Schliep, 1914; Muller, 1932; Fernandez, 1980; Ho & Weisblat, 1987). The reason for these discrepancies is that the earlier studies failed to detect two cell divisions, viz. that of cell DM′ into cell DM″ and micromere dm″ and that of cell NOPQ′ into cell NOPQ″ and micromere nopq″. Micromeres dm″ and nopq″ are extremely small and lie beneath earlier produced micromeres at the animal pole. The formation of these two micromeres has also been observed by Sandig & Dohle (1988) in embryos of *Theromyzon*. Additionally, Fernandez (1980) and Ho



Figs 6–10. Cell cycles of primary and secondary blast cells. (A) Cell lineage trees present the divisions and cell cycles of each class of blast cell. Each vertical bar represents the cell cycle of an identified cell; G_1 phase is shown in black, S phase in stippling, G_2 phase in white and M phase in diagonal stripes. Cells whose cell cycles are not known in entirety have bars ending with jagged lines. Time is measured from the birth of each class of primary blast cell; in Figs 6 and 9, time is measured from the birth of the ns and qs blast cells, respectively. Thus, the time scale is consequently shifted by one hour for the nf and qf blast cells and their progeny. Abbreviations are as follows: a, anterior; p, posterior; l, lateral; m, medial; d, deep; s, superficial. (B) Schematic drawings of the primary blast cell clones after each division, arranged in two rows that read from left to right. The daughters of the most recent division are indicated by the double arrow. The clones are drawn as viewed in the left germinal band; anterior is up, lateral (which refers to the future position of the cell in the germinal plate) is to the right. Scale bar, 20 μm.

Fig. 6. Cell cycles of ns and nf blast cells. The three divisions of the nf blast cells and the first division of the ns blast cells have been previously described by Zackson (1984); the next two divisions of the ns blast cells are newly described. A total of 25 n bandlets were examined. In B ns blast cell clones are presented in stippling and nf blast cell clones in white.

Fig. 7. Cell cycles of o blast cells. The first three divisions have been described by Zackson (1984) and the next three divisions have been described by Shankland (1987*a*); the division of cell o.apap is newly described. A total of 35 o bandlets were examined.

& Weisblat (1987) referred to only one OPQ-derived micromere, which they called (OPQ)′ or opq′, respectively. On the basis of size, position and the timing of division, the micromere they described is really micromere opq″, however.

Furthermore, our experiments demonstrate that, during the early stages of development, differences in the lengths of the cell cycles are due to differences in the lengths of the G_2 phases whereas, later in development, they are due to differences in the lengths of the G_1, S and G_2 phases. In cells as diverse as yeast and mammalian cells in culture the length of the cell cycle is

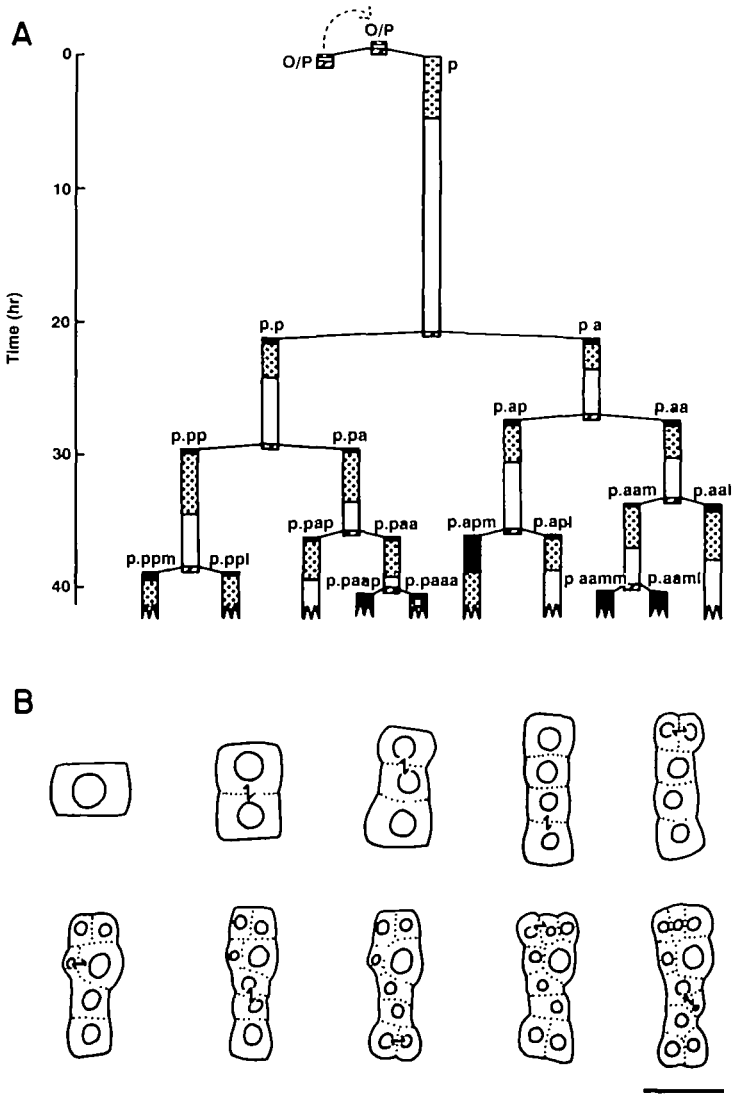


Fig. 8. Cell cycles of p blast cells. The first three divisions have been described by Zackson (1984) and the next four divisions have been described by Shankland (1987*b*); the next two divisions are newly described. A total of 20 p bandlets were examined.

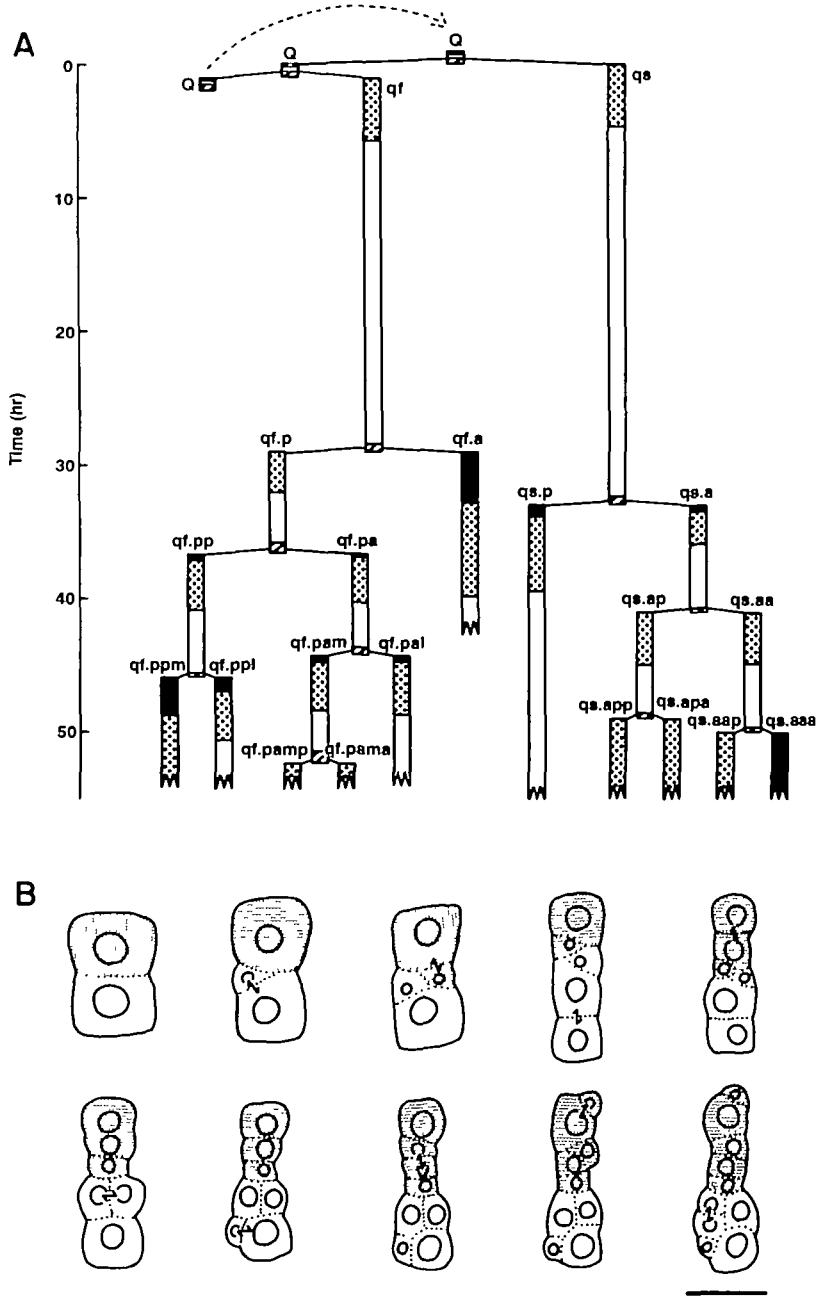
largely regulated during G_1, when the cell monitors whether or not conditions (cell size, availability of external nutrients, etc.) are favourable for cell division. The cells of most early embryos (i.e. fruit fly, frog, nematode) are exempt from this sort of regulation because they have inherited sufficient maternal components to allow them to divide at extremely rapid rates, with limited synthetic activity; consequently, their cell cycles are composed of back-to-back phases of M and S. In contrast, the cell cycles of the blastomeres of the early leech embryo comprise not only S and M phases, but also G_2 phases of significant duration. The cell cycles of the early leech embryo are similar in composition to the first several cycles of mouse, sea urchin and snail embryos (Dalq & Pasteels, 1955; Hinegardner *et al.* 1964; van den Biggelaar, 1971).

In early leech embryos, the lengths of the G_2 phases differ among the large blastomeres, while the lengths of the S and M phases remain constant. In snail embryos it has also been observed that differences in the lengths of the cell cycles of the early blastomeres are due to differences in the lengths of the G_2 phases (van den Biggelaar, 1971). In leech embryos, the large blastomeres of the D cell line, which have shorter G_2 phases, differ from those of the A–C cell lines in that they contain more yolk-free cytoplasm, or teloplasm. Teloplasm is enriched with mitochondria and ribosomes (Fernandez, 1980), as well as polyadenylated RNAs (B. Holton, S. H. Astrow & D. A. Weisblat, unpublished results), and plays a role in determining the future pattern of cleavages (Astrow *et al.* 1987). The cell cycles of the large blastomeres of the D cell line may be shorter because these cells are enriched with teloplasm, which enables them to more efficiently manufacture the components needed for mitosis to commence.

The first point for the control of cell cycle duration in many embryos occurs when the cell cycles lengthen. In *Xenopus* embryos this happens after the twelfth cleavage, and it has been assumed that the lengthening of the cell cycles is due to the acquisition of G_1 phases (Newport & Kirschner, 1982*a*) because the cell cycles of later blastulae contain G_1, S, G_2 and M phases

(Graham & Morgan, 1966). In *Drosophila* embryos, the cycles are lengthened after the thirteenth division by the addition of G_2 phases (Foe & Alberts, 1983; Edgar & Schubiger, 1986). Likewise, in *Caenorhabditis* embryos, the cell cycles of the gut lineage lengthen after the fourth cleavage by the addition of G_2 phases (Edgar & McGhee, 1988). In *Helobdella* embryos, the cell cycles of the primary blast cells are much longer than those of the cleavage-stage embryo; these cell cycles are lengthened by a large increase in the length of their G_2 phases, with a smaller increase in the length of their S phases. Additionally, differences in cell cycle duration among the six classes of ectodermal primary blast cells are due to differences in the lengths of their G_2 phases. It appears, therefore, that during early development some aspect of the G_2 phase, rather than the G_1 phase, is the limiting factor in governing progress through the cell cycle.

In *Xenopus* embryos, the transition after the twelfth cleavage from rapid, synchronous cell divisions to slower, asynchronous cell divisions, with the concomitant onset of transcription and cell motility, has been termed the midblastula transition (MBT) (Gerhart, 1980; Newport & Kirschner, 1982*a*). A similar increase in mitotic cycle duration and desynchronization, as well as transcription activation, occurs in *Drosophila* embryos after the thirteenth division (Edgar *et al.* 1986). The timing of this transition is determined by the ratio of nuclear to cytoplasmic material (Newport & Kirschner, 1982*a*,*b*; Mita, 1983; Mita & Obata, 1984; Edgar *et al.* 1986). It appears that the primary event of this transition is the lengthening of the cell cycle and



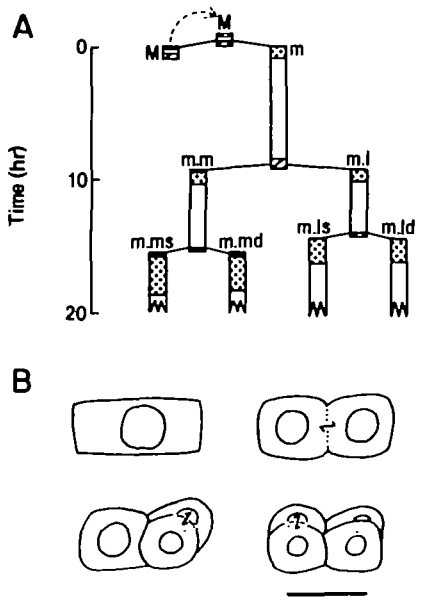


Fig. 9. Cell cycles of qs and qf blast cells. The first two divisions of qf blast cells and the first division of qs blast cells have been previously described by Zackson (1984); all subsequent divisions are newly described. A total of 53 q bandlets were examined. In B qs blast cell clones are shown in stippling and qf blast cell clones in white. Cell qf.a, the errant cell that sometimes leaves the bandlet, has not been included in the second row of drawings.

Fig. 10. Cell cycles of m blast cells. The first division has been previously described by Weisblat *et al.* (1980*b*) and the next two divisions have been described by Schimmerling (1986). A total of 16 m bandlets were examined.

that the other processes are secondary; the longer cell cycles permit transcription to proceed (Edgar *et al.* 1986; Kimelman *et al.* 1987).

In *Helobdella* embryos the transition from the relatively short cell cycles of the early large blastomeres and teloblasts to the much longer cell cycles of the primary blast cells could be considered analogous to the MBT of *Xenopus*. The primary blast cells are much smaller than the earlier cells and, thus, have a much higher nucleocytoplasmic ratio. Furthermore, preliminary autoradiographic studies of incorporated tritiated uridine have shown that RNA synthesis by primary blast cells, but not teloblasts, is inhibited by low concentrations of α-amanitin, which suggests that the primary blast cells are synthesizing mRNAs (Bissen & Weisblat, 1987*b*). It is possible that (as for other embryonic cells undergoing MBT) the primary blast cells of the leech embryo may have reached a critical ratio of nuclear to cytoplasmic material that triggers an increase in cell cycle duration and, consequently, transcription is initiated during the extended G_2 phases.

Differences in cell cycle duration among the secondary blast cells of leech embryos are due to differences in the lengths of their G_1, S and G_2 phases. Differences in the lengths of the cell cycles of cells in the frog blastula and embryonic mouse neural tube are also due to differences in the lengths of the G_1, S and G_2 phases (Graham & Morgan, 1966; Kauffman, 1968). These data suggest that aspects of G_1 and S, as well as G_2, are limiting progress through the cell cycle during later stages of development. In leech embryos, the correlation between the size of a cell and the length of its G_1 phase suggests that smaller cells may have to pause in G_1 to synthesize essential components needed for cell cycle progression (i.e. the initiation of DNA synthesis), presumably because they inherited less from the mother cell than their larger sister. Although there is no obvious growth of these small cells as they get older, there appears to be some correlation between the size of a cell and the time required before division can occur; such control during the G_1 phase of the cell cycle is similar to that observed in cultured mammalian cells and yeast. The fact that the S and G_2 phases of the cell cycles of the leech secondary blast cells also differ in length, however, suggests that additional mechanisms of cell cycle regulation prevail during later stages of embryonic development.

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