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Localization of polyadenylated RNAs during teloplasm formation and cleavage in leech embryos

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Abstract In the embryos of glossiphoniid leeches, as in many annelids, cytoplasmic reorganization prior to first cleavage generates domains of yolk-deficient cytoplasm (called teloplasm) that are sequestered during the first three cell divisions to the D' macromere. Subsequently, the D' macromere generates a set of embryonic stem cells (teloblasts) that are the progenitors of the definitive segmental tissues. The hypothesis that fate-determining substances are localized within the teloplasm and segregated to the D' macromere during cleavage is supported by experiments in which a redistribution of yolk-deficient cytoplasm changes the fate of blastomeres that inherit it (Astrow et al. 1987; Devries 1973; Nelson and Weisblat 1992). As a step toward identifying fate-determining factors in teloplasm, we describe the distribution of polyadenylated RNAs (polyA+ RNA) in the early embryo of the leech, *Helobdella triserialis*, as inferred from in situ hybridization using tritiated polyuridylic acid (3H-polyU). Our results indicate that polyA+ RNA colocalizes with teloplasm during cytoplasmic rearrangements resulting in teloplasm formation, and that it remains concentrated in the teloplasm during the cell divisions and a second cytoplasmic rearrangement during early embryogenesis. Lesser amounts of polyA+ RNA appear to be localized in cortical cytoplasm at most stages.

Key words Leech · Annelid · Maternal RNA

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Introduction

Cytoplasmic rearrangements that generate anisotropic distributions of cytoplasmic constituents have been described in the early development of many species (reviewed in Davidson 1986; Wilson 1925). It is postulated that these cytoplasmic rearrangements localize fate-determining substances (determinants) to specific regions within a fertilized egg that are inherited by specific blastomeres during cleavage, and determine the ultimate fate of the blastomeres containing them. Although the identity of putative determinants has yet to be established for many systems where they are believed to play a role, experiments in amphibians (Melton 1987) ascidians (Jeffery 1983; Jeffery and Meier 1983) and insects (Anderson and Nüsslein-Volhard 1984; Kalthoff 1983) support the notion that mRNAs act as developmental determinants. In particular, maternally-derived *bicoid* mRNA has been clearly shown to act as a developmental determinant of the anterior-posterior axis in *Drosophila* (Berleth et al. 1988; Driever and Nüsslein-Volhard 1988a, b).

In embryos of the glossiphoniid leech, *Helobdella triserialis*, as in many other unequally cleaving annelids and molluscs, cytoplasmic rearrangements prior to first cleavage generate domains of yolk-deficient cytoplasm (called *teloplasm*) that are sequestered during the first three cell divisions to the D' macromere. Subsequently, a set of embryonic stem cells (*teloblasts*) arise from the D' macromere; teloblasts are the progenitors of the definitive segmental tissues. The formation of teloplasm has been examined previously in glossiphoniid leeches (Whitman 1878; Schleip 1914; Fernandez 1980; Fernandez and Olea 1982; Fernandez et al. 1987; Astrow et al. 1989) and a homologous process has also been studied in the oligochaete *Tubifex hattai* (Shimizu 1982, 1984, 1986). The inheritance of teloplasm by the D' macromere in the 8-cell embryo has been associated with the unique developmental fate of this cell, i.e. to give rise to the segmental tissues. This correlation has been elevated to a causal relationship by the finding that cell fates in centrifuged or compressed leech embryos are

altered predictably on the basis of the amount of teloplasm they inherit (Astrow et al. 1987; Nelson and Weisblat 1992).

As a step toward identifying components of the teloplasm that may act to direct developmental fate, we sought to determine the subcellular distribution of messenger RNA during early development. In situ hybridization of tritiated polyuridylic acid (3H-polyU) to intact embryos (Mahoney and Lengyel 1987) was used to infer the location of polyadenylated RNAs. This procedure permits the use of plastic embedding resins, which improves tissue preservation, without the problems inherent in hybridizing probes to tissues that are already infiltrated with embedding medium.

Using in situ hybridization, we found changes in the distribution of polyadenylated RNAs during early development in *Helobdella*. Our results indicate that, initially, cytoplasmic polyA+ RNA is homogeneously distributed. It colocalizes to a significant extent with yolk-deficient cytoplasm during cytoplasmic rearrangements leading to teloplasm formation, and remains concentrated in the teloplasm throughout the cell divisions and further cytoplasmic rearrangements during stages 1–7.

Materials and methods

Embryos

Embryos of the glossiphoniid leech *Helobdella triserialis* were obtained from a laboratory breeding colony (Weisblat et al. 1980). The staging system and nomenclature used is that of Fernandez (1980) as amended (Stent et al. 1992). This nomenclature is a modified version of that traditionally applied to spiralian embryos; macromeres A'–D' and micromeres a'–d' in this terminology correspond to cells 1A–1D and 1a–1d in the traditional spiralian nomenclature, and blastomeres DM and DNOPQ correspond to cells 2D and 2d, respectively. The timing of developmental events is given as minutes after deposition of the fertilized zygote at 23° C.

Localization of polyadenylated nucleic acids by in situ hybridization

In situ hybridization was carried out on fixed, intact specimens. All the results reported here reflect observations on a minimum of four embryos for each condition. (Unfertilized) eggs and some (fertilized) zygotes were obtained by dissection from gravid leeches; the elevation of the vitelline membrane above the surface of the embryo was used to distinguish between the unfertilized and fertilized states. More advanced zygotes and embryos were removed from cocoons attached to the ventral surface of the parent. Eggs and embryos were fixed for 15 min in a biphasic permeabilization buffer consisting of one part buffered formalin (100 mM MES, pH 7.6, 2 mM EDTA, 2 mM MgCl₂, 3.7% formalin) and one part heptane. The tube containing the embryos in the biphasic solution was inverted gently about once per second throughout fixation.

Fixed embryos were transferred to TNE buffer (10 mM Tris, pH 7.6, 100 mM NaCl, 1 mM EDTA) and the vitelline membranes were removed with fine pins. The embryos were extracted for 15–24 hr with constant shaking in TNE containing 1.2% Triton X-100 (TNET buffer). After extraction with TNET, embryos were prehybridized for 24–48 hr at 33° C in hybridization buffer [(0.15 M NaCl, 10 mM Tris, pH 7.6, 5 mM MgCl₂, 40% form-

amide, 500 µg/ml tRNA or herring sperm DNA, 0.01% pyrophosphate, and 1% Denhardt's solution (2% Ficoll, 2% polyvinylpyrrolidone, 2% BSA)]. After prehybridization, tritiated polyuridylic acid [3H-polyU; 3.6 million cpm/µg, synthesized by the method of Bishop et al. (1974)] was diluted to 5 ng/µl, i.e. about 18 thousand cpm/µl in hybridization buffer. 100 µl of the resultant hybridization solution was added to 2–5 embryos in a 250 or 400 µl polypropylene tube, cut down to reduce the volume and sealed with parafilm to prevent evaporation. After hybridizing for four days at 33° C, embryos were washed in hybridization buffer containing 1.2% Triton X-100 at 33° C for 24 hr. [The temperatures used for the hybridization and washes were 8.5° C below the T_m calculated for DNA-DNA hybrids. These conditions are at least moderately stringent for RNA-RNA hybrids, since hybridizations carried out using the same buffer at 37° C instead of 33° C gave very little binding.] Embryos were then washed once with RNase buffer (0.5 N NaCl, 10 mM Tris 7.5, 1 mM EDTA), and half of the embryos of each stage were incubated 1 hr at 33° C with RNase A (20 µg/ml) to digest any unhybridized probe. All embryos were then washed with RNase buffer, dehydrated in a graded series of ethanol solutions and embedded in glycol methacrylate, cut into serial 4 micron thick sections and mounted onto gelatin-subbed glass slides. Slides were dipped in NTB emulsion (Kodak) and exposed at –80° C for 2–6 weeks, then developed in Kodak Dektol (50 mg/ml) at 16° C for 4 min., washed briefly in water at 16° C, fixed with Kodak Fixative at 16° C and washed for 30 min in 16° C H₂O that was brought slowly to room temperature. The tissue was counterstained with 1% toluidine blue (0.1 M Na₂B₄O₇), cleared with Permount and mounted with glass coverslips for examination under phase contrast, bright field, or dark field optics.

The following procedures served as controls for the hybridization experiments:

1) As internal controls for the quality of the hybridization, one or more stage 7 embryos were hybridized in the same tube with each batch of younger embryos. Moreover, embryos of different stages were embedded in each block so that direct comparisons between stages could be made from single sections.

2) To control for non-specific binding of homopolymeric RNA to the embryos, some embryos were prehybridized with 0.3 mg/ml polycytidylic acid (polyC) or polyguanidylic acid (polyG) instead of tRNA. The hybridization patterns obtained with 3H-polyU after prehybridization with polyC or polyG were equivalent to those obtained after prehybridization with tRNA. Other embryos were hybridized with tritiated polyadenylic acid (3H-polyA; 20–70 Curies/mmol, Amersham). The specific activity of the 3H-polyA probe was 4 fold lower than that of the synthesized 3H-polyU probe, but even after greater than 10 fold longer exposure times, sections of embryos incubated with 3H-polyA showed no hybridization signal above background levels.

3) To test the dependence of the hybridization signal on polyA sequences, some embryos were pretreated with RNase A, using conditions that promote polyA digestion (Capco and Jeffery 1978). Specimens were incubated with 50 µg/ml RNase A at 37° C for 2 hr in a buffer composed of 10 mM TrisHCl (pH 7.6), 10 mM KCl, 1 mM MgCl₂, then washed several times in a solution of diethylpyrocabonate (an RNase inhibitor). 3H-polyU was then hybridized to the embryos as above; no signal above background levels was detected.

4) To control for differential extraction of RNAs by the detergent treatment, some embryos were prepared for in situ hybridization using a technique that does not entail permeabilization with Triton X-100. For this purpose, embryos were fixed for 15 hr in 4% paraformaldehyde in 75 mM Hepes buffer, pH 7.4. They were then rinsed in Hepes buffered saline (HBS; 75 mM Hepes pH 7.4, 130 mM NaCl), treated for 12 hr in 0.2 mg/ml chitinase in HBS at room temperature, and rinsed in buffer. The rest of the hybridization was carried out following the standard procedure, starting with the prehybridization step. This protocol yielded the same results as the one involving Triton X-100 permeabilization, indicating that the non-uniform binding observed is not a result of detergent treatment.

5) To test the possibility that unhybridized probe might be binding nonspecifically, but still anisotropically (e.g. to some unevenly distributed subcellular component), embryos were treated with RNase *after* hybridization to digest unhybridized (i.e. single-stranded) probe. These embryos showed the same distribution of silver grains as embryos that were not treated with RNase, indicating that the observed signal arises from RNA-RNA hybrids. This RNase treatment did reduce the intensity of the hybridization signal, however; this is to be expected if the some of the bound 3H-polyU molecules overhung the polyA tails to which they hybridized and therefore contained some single-stranded sequence.

Results

The formation of teloplasm in *Helobdella triserialis* (Astrow et al. 1989) resembles the homologous processes termed "ooplasm" formation described for the glossiphoniid leech, *Theromyzon rude* (Fernandez et al. 1987)

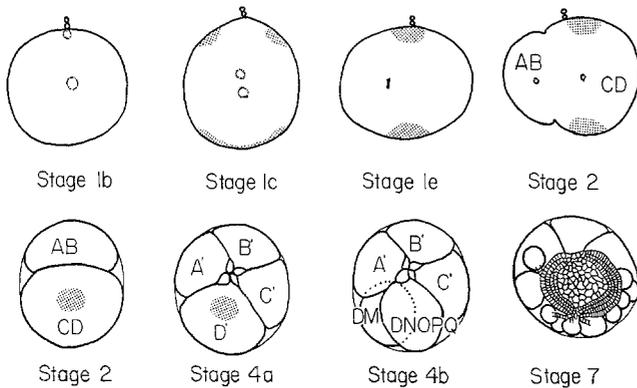


Fig. 1 Partial summary of *Helobdella* development. Diagrammatic views of embryos at progressively later stages, as viewed in meridional sections approximately through the center of the embryo (top four drawings) or from the animal pole (bottom four drawings); polar bodies are indicated by small circles at the animal pole, and pronuclei and nuclei by dashed circles in the first four panels. Teloplasm (stippling) begins to accumulate shortly after the second polar body is extruded (stage 1b), and is evident as two ringed domains of clear cytoplasm (stage 1c) visible as profiles in meridional section. Compact domains of teloplasm form as the rings of teloplasm move poleward. The embryo elongates along the future dorsal/ventral axis; by late metaphase (stage 1e), the chromatin (black bar) is located eccentrically and the first cleavage is unequal, segregating most of both pools of teloplasm into the larger daughter cell, CD, at stage 2, and thence into cell D of the 4-cell embryo (stage 3, not shown). During stages 3–4a, further cytoplasmic arrangements result in the translocation of the vegetal teloplasm toward the animal pole, where it becomes coextensive with the animal teloplasm (Holton et al. 1989) and is inherited by macromere D' in stage 4a. Cleavage of D' divides the teloplasm again so that both daughter cells, proteloblasts DM and DNOPQ inherit some. Partial circles in the lower half of the depiction of the stage 7 embryo represent the five pairs of teloblasts (one descended from DM and four from DNOPQ). These produce bandlets of blast cells which join to form the germinal bands (stage 7), which will ultimately generate the segmental tissues of the leech. The primary quartet of micromeres is depicted by small, unlabeled contours at the animal pole in the drawings of the stage 4a and 4b embryos. By stage 7, these, along with additional micromeres descended from cell D', have generated a cluster of cells that separate the germinal bands and cover them with a squamous epithelium. Drawings are roughly to scale; the diameter of the uncleaved egg is about 400 microns

and "pole plasm" formation described for the oligochaete, *Tubifex hattai* (Shimizu 1982, 1984, 1986, 1989). *Helobdella* eggs are fertilized internally and remain arrested in meiosis until they are laid. Polar bodies are extruded approximately one and two hours after egg deposition, at 23° C (stage 1b, Fig. 1). Soon thereafter, two domains of yolk-deficient cytoplasm (teloplasm) begin to form near the surface of the zygote in the animal and vegetal hemispheres, centered at the animal and vegetal poles respectively. The animal teloplasm is initially toroidal, while the vegetal teloplasm arises as a disk that covers the pole (stage 1c, Fig. 1). The outer limits of both domains of teloplasm initially lie about two thirds of the way from the equator to the pole. Each domain compacts toward its respective pole prior to the initiation of first cleavage (stage 1e, Fig. 1).

Teloplasm is sequestered unequally during the cleavages comprising the early stages of embryogenesis. The 8-cell embryo (stage 4a) consists of 4 macromeres and 4 micromeres; macromere D' contains most of the teloplasm. At the fourth cleavage (stage 4b, Fig. 1), D' divides into a mesodermal precursor, proteloblast DM (the vegetal daughter of D') and an ectodermal precursor, proteloblast DNOPQ (the animal daughter of D'). DM and DNOPQ cleave further to make teloblasts and additional micromeres (Sandig and Dohle 1988; Bissen and Weisblat 1989). Teloblasts are embryonic stem cells whose iterated divisions give rise to coherent, age-ranked columns of segmental founder cells (blast cells) (stage 7, Fig. 1; e.g., see Weisblat and Shankland 1985).

As a first step in testing the hypothesis that inherited RNAs serve as cytoplasmic determinants, we wished to examine the distribution of polyadenylic acid (polyA) moieties, as an indicator of polyadenylated RNAs, during teloplasm formation and in early embryogenesis. For this purpose, in situ hybridizations with 3H-polyuridylic acid (3H-polyU) were performed on embryos of different stages. Some stage 7 embryos were included in each experiment, to monitor the success of the technique. In stage 7 embryos, blast cells are actively synthesizing mRNAs (Bissen and Weisblat 1991); thus the nuclei and cytoplasm in the blast cells should hybridize 3H-polyU.

Figure 2a shows a brightfield photomicrograph of a toluidine blue-stained section from a 3H-polyU-hybridized stage 7 embryo, showing teloblasts, columns of blast cells, and some blast cell nuclei. Figure 2b shows the same section photographed with darkfield illumination to reveal the silver grains of the autoradiograph. Silver grains are at background levels above the yolky regions of the macromeres and teloblasts but are significantly above background levels over the cortex of these cells and over the yolk-deficient cytoplasm of the teloblasts and blast cells. Even greater densities of silver grains are seen over the blast cell nuclei, consistent with the previous findings that these cells are transcriptionally active (Bissen and Weisblat 1991) and that many polyadenylated RNAs never leave the nucleus of eukaryotic cells (Brandhorst and McConkey 1974). Other stage 7 embryos processed for wholemount in situ hy-

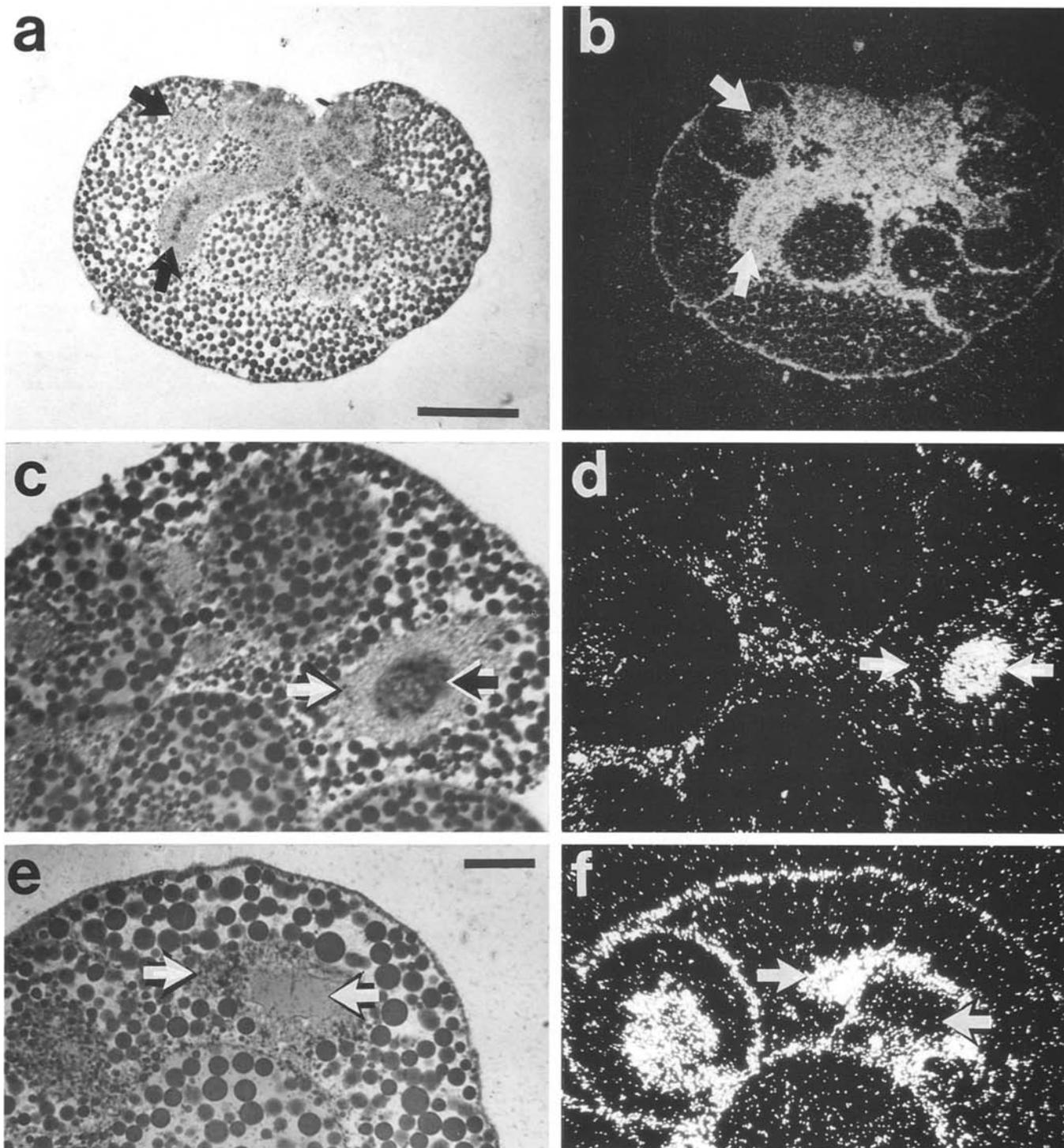
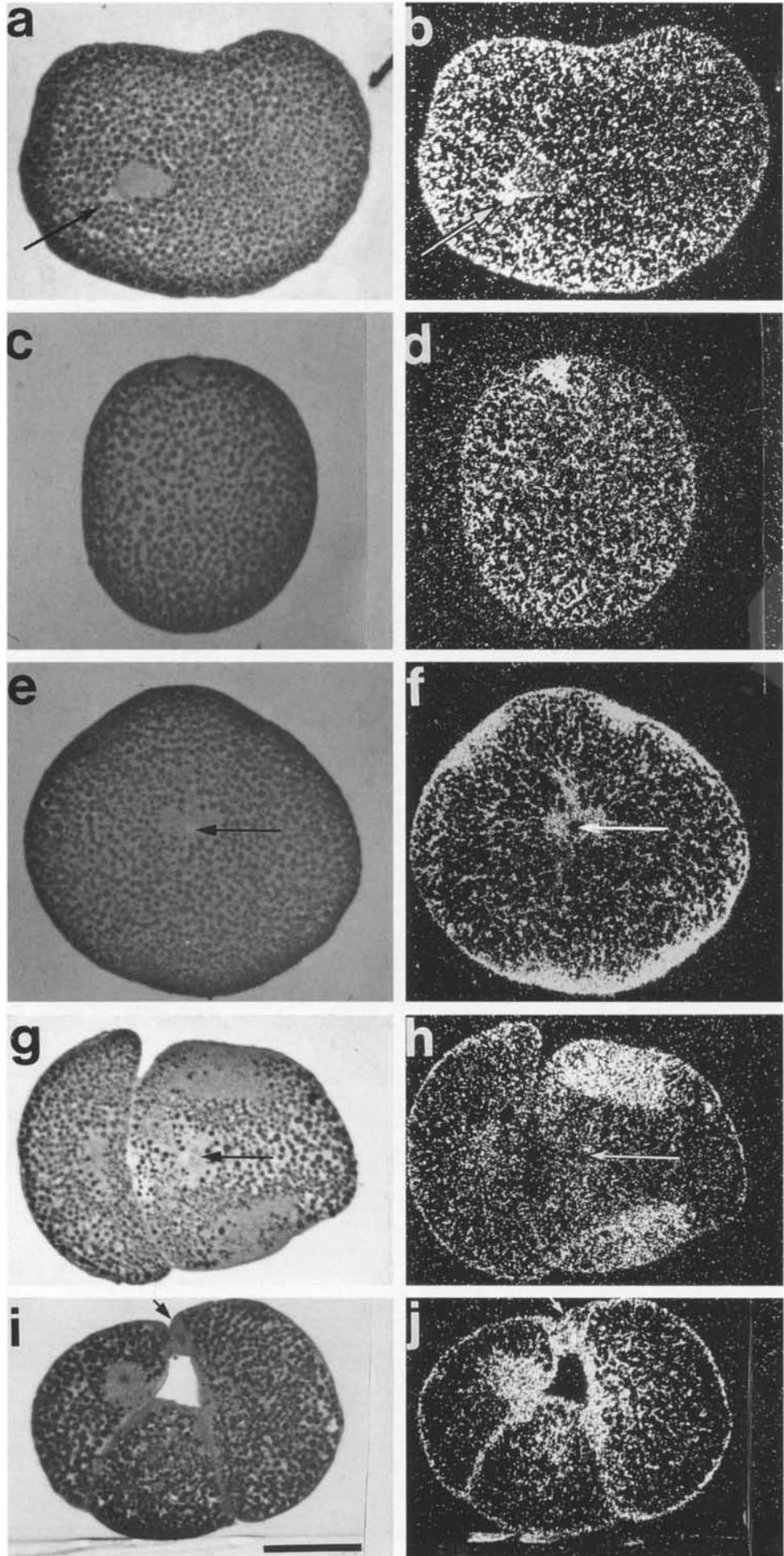


Fig. 2a-f Whole embryo in situ hybridization. Photomicrographs of sections from *Helobdella* embryos that were hybridized with 3H-polyU, and processed for autoradiography. The toluidine blue-stained sections were photographed under brightfield illumination for the lefthand panels and under darkfield for the righthand panels, so that silver grains, which mark areas of 3H-polyU binding, stand out as white spots. **a** In a stage 7 embryo, darkly stained, yolk-filled teloblasts and macromeres occupy most of the section, but their boundaries are difficult to distinguish. Light grey areas extending from the upper center of the section represent the bandlets of blast cells and the cytoplasm around the teloblast nuclei (*upper arrow*). In the leftmost bandlet (*lower arrow*) is a row of darkly stained blast cell nuclei. **b** Viewing the same section under darkfield illumination, the boundaries of the teloblasts and

macromeres are highlighted by concentrations of silver grains, while the yolkly portions of these cells are at background levels. Yolk-free cytoplasm in the perinuclear regions of the teloblasts and in the blast cells is extensively labeled, and the blast cell nuclei are even more so. **c** A higher magnification of a section from a similar embryo, showing the enlarged nucleus (*right arrow*) and perinuclear cytoplasm (*left arrow*) of one of the macromeres, along with the profiles of several teloblasts. **d** The darkfield view of the same section reveals that the nucleus is much more extensively labeled than the perinuclear region. **e** Another macromere nucleus (*right arrow*) and perinuclear cytoplasm (*left arrow*) from a different embryo. **f** In this macromere, the nucleus is much less extensively labeled than the perinuclear cytoplasm. Scale bar, 50 μ m in (a) and (b), 10 μ m in (c-f)

Fig. 3a-j Localization of polyA during early development. Brightfield (*left*) and darkfield (*right*) photomicrographs of sections made from embryos fixed at various stages and treated as described in the legend to Fig. 2. (**a** and **b**) Unfertilized egg. Both the cytoplasm and the germinal vesicle are labeled at about the same level. The cell cortex and a small zone of perinuclear cytoplasm (*arrows*) are labeled at higher levels. The apparent increase from right to left in the extent of the labeling results from a gradation in the thickness of the photographic emulsion over the section, since the density of grains outside the section vary in a similar manner. (**c** and **d**) In a newly laid (fertilized) embryo, the entire region of the female pronucleus is extensively labeled, perhaps because the germinal vesicle has broken down in preparation for polar body formation. The extent of cortical labeling is reduced relative to the unfertilized egg. (**e** and **f**) During teloplasm formation (*stage 1e*) both animal and vegetal domains of yolk-deficient cytoplasm label extensively. Although the nucleus itself (*arrows*) is unlabeled, the perinuclear cytoplasm is densely labeled. Within the yolky cytoplasm, silver grains are not distributed uniformly among the yolk platelets, but are organized into strands between yolk platelets. The equatorial cortex of the egg is not labeled to any greater extent than the interior of the cell, in contrast to the polar regions. (**g** and **h**) In the stage 2 embryo, animal and vegetal teloplasm are clearly labeled in cell CD (*righthand cell*), and the extent of cortical labeling is relatively slight, especially in the region of contact between cells AB and CD. The cell nucleus (*arrows*) remains unlabeled. (**i** and **j**) By stage 4b, when micromeres (*arrows*) and cells DM and DNOPQ have been formed, cell cortices are clearly labeled, even at their interior faces. Yolky cytoplasm is no longer significantly labeled, whereas yolk-deficient cytoplasm remains labeled both in large cells and in micromeres. Animal pole is up in each panel (except **a** and **b**, which are before this axis is evident); scale bar, 50 μ m



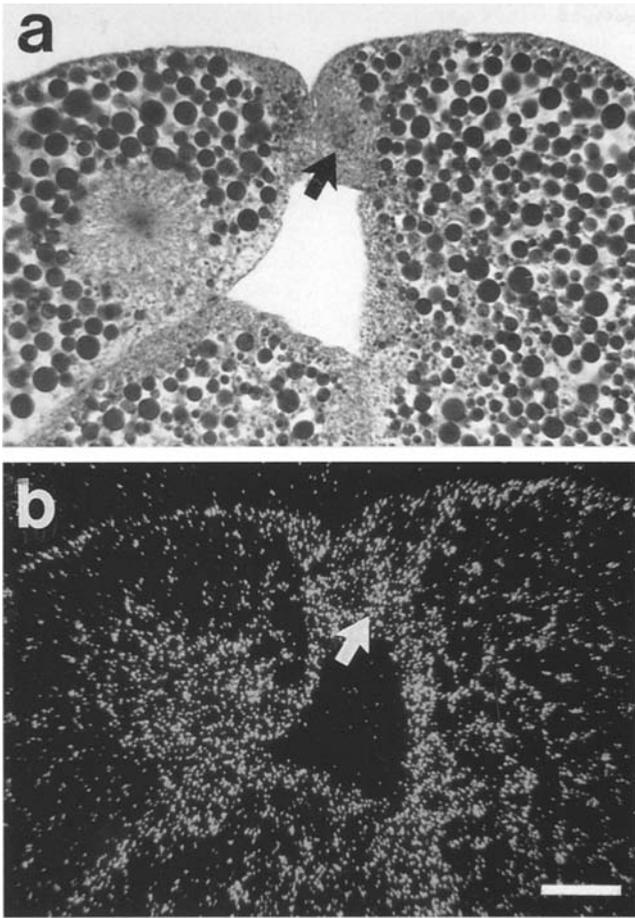


Fig. 4a–b Localization of polyA during early development (continued). Higher magnification photomicrographs of portions of the sections in panels i) and j) in Fig. 3. (a and b) In stage 4b, micro-mere nucleus (arrows) is labeled, as are cell cortices. Animal pole is up; scale bar, 10 μ m

bridization (Fig. 2c–f) reproduce the result that silver grains are above background levels in the cortex and yolk-deficient cytoplasm of the teloblasts and macromeres. In contrast to this general uniformity, however, we found that the large macromere nuclei (Fig. 2c, e) sometimes are heavily labeled relative to their surrounding cytoplasm (Fig. 2d) and sometimes much more lightly labeled than the surrounding cytoplasm (Fig. 2f). We conclude that these localized differences in the labeling pattern reflect systematic temporal variation in the transcriptional activity of the macromere nuclei. Such activity may in turn reflect the cell cycles associated with the generation of syncytial nuclei in the macromeres (Anderson 1973; Weisblat et al. 1984) that appear to contribute to endodermal tissues (Nardelli-Haefliger and Shankland 1993).

Given the positive results obtained from the stage 7 embryos and the negative results obtained from the various controls described in the Materials and Methods (data not shown), we interpret the 3H-polyU obtained in oocytes and cleavage stage embryos as an accurate indication of the distribution patterns of polyA+ RNAs in

these stages. In general, silver grains were observed at higher density over cortex and regions of granular cytoplasm, and at lower density over yolk cytoplasm in all stages examined.

In unfertilized eggs (Fig. 3a, b) the probe was bound between the yolk platelets, in the germinal vesicle and in the cortex. After fertilization (Fig. 3c, d) the probe bound in the region corresponding to the breakdown product of the germinal vesicle and in between yolk platelets as before; however, the relative level of cortical binding was reduced relative to that observed in unfertilized eggs.

During teloplasm formation (Fig. 3e, f) 3H-polyU hybridization was detected in the teloplasm, in the cytoplasm surrounding the male pronucleus, and in the channel leading from the male pronucleus to the female pronucleus beneath the animal pole. Within the yolk-rich regions of the cytoplasm, the probe was not bound uniformly in the spaces between yolk platelets, but rather in a stellate pattern of strands extending outward from the center of the zygote toward the cortex.

At the 2-cell stage (Fig. 3g, h), this stellate pattern of cytoplasmic labeling was no longer obvious. 3H-polyU was extensively bound within the teloplasm of cell CD. Labeling of perinuclear cytoplasm in the 2-cell embryo was markedly reduced relative to the teloplasm, whereas in earlier stages perinuclear cytoplasm and teloplasm bound probe equally (cf. Figs. 3f and 3h). Cortical labeling still appeared somewhat lower than in the unfertilized egg, except in those regions overlying teloplasm.

3H-polyU probe was not detected over the nucleus in either the zygote (Fig. 3e, f) or the 2-cell embryo (Fig. 3g, h), indicating that little polyA was accumulating at these stages. However, by the time macromere D' had cleaved to form DM and DNOPQ (stage 4b), 3H-polyU was frequently bound over nuclei (Fig. 4a, b). The sudden accumulation of polyA sequences in nuclei at stage 4b suggests that zygotic transcription was well underway by this stage. The teloplasm in cells DM and DNOPQ was heavily labeled, whereas the spaces between yolk platelets contained relatively fewer grains than at previous stages. Moreover, the entire cortices of cells were uniformly heavily labeled for the first time since fertilization (Fig. 3i, j).

Discussion

Domains of yolk-deficient cytoplasm (teloplasm) act as classical developmental determinants by conferring a particular developmental potential (teloblast formation) to recipient cells in embryos of the leech *Helobdella triserialis* (Astrow et al. 1987; Nelson and Weisblat 1992). Maternally derived mRNAs have been implicated as developmental determinants in a number of other organisms. Previous studies have shown that teloplasm contains ribosomes, mitochondria and other membranous organelles, cytoskeletal elements and soluble proteins (Fernandez and Stent 1980; Fernandez et al. 1987; Astrow et al. 1989). Here, using in situ hybridization of 3H-

polyU to whole, fixed and extracted embryos, we have demonstrated that teloplasm is also enriched for polyA sequences. Given the low levels of zygotic transcription prior to stage 5 (Bissen and Weisblat 1991; Kostriken and Weisblat 1992), we believe that the distribution patterns reported here reflect the distribution of maternally inherited polyA+ mRNAs in the early cleavage stages. It should be noted, however, that the hybridization patterns obtained by this technique will also reflect any zygotic polyadenylation of maternally inherited polyA- RNAs. Zygotic polyadenylation of maternal RNAs has been observed in both invertebrates and vertebrates (e.g. Wilt 1973; Clegg and Piko 1983) and may be occurring in *Helobdella* (M Dixon, personal communication).

Prior to teloplasm accumulation, polyA+ RNAs are distributed uniformly throughout the cytoplasm between the yolk platelets, with slightly higher levels at the cortex of the cell and in the germinal vesicle. During teloplasm formation, polyA+ RNAs are enriched in the teloplasm from the time it first starts to accumulate (soon after formation of the second polar body) and remain so throughout the early cleavage divisions. The distribution of polyA+ RNA within the yolky cytoplasm also changes during teloplasm formation; they are no longer uniformly distributed between yolk platelets, but rather, appear in strands coursing between yolk platelets, as does the granular cytoplasm itself. It is unlikely that these strands are an artifactual result of the fixation procedures used, since the strands occur with a variety of fixation protocols and since they become evident only after teloplasm accumulation has commenced. We suggest that they may represent cytoskeletal pathways along which granular cytoplasm and associated polyA+ RNAs are moved either from deep cytoplasm to the cell cortex and/or to the center of the cell as yolk-deficient cytoplasm accumulates. To test this suggestion, it will be necessary to study the dynamics of cytoplasmic rearrangements with better resolution and to examine the cytoskeletal basis of these rearrangements.

Between first cleavage and the cleavage of macromere D' into mesodermal and ectodermal precursor cells, two changes in the distribution of polyA+ RNAs are seen. First, hybridization signal can be seen over the nuclei of cells in the stage 4b embryo, whereas in the earlier stages the nuclei are conspicuous by the absence of overlying silver grains. These results corroborate previous reports that zygotic transcription is initiated or increased in these cells (Bissen and Weisblat 1991) and that some micromeres have initiated zygotic expression (of a *wnt* gene) by late stage 4a (Kostriken and Weisblat 1992). A second change in the distribution of polyA+ RNAs by stage 4b is that the cortex of the blastomeres in the stage 4b embryo appears to contain a much higher level of polyA+ RNAs than in the precleavage or stage 2 embryo.

PolyA+ RNAs are enriched in the teloplasm at all the stages examined. Thus, we conclude that polyA+ RNAs segregate to and translocate with the teloplasm during all the cytoplasmic rearrangements and cell divisions of early embryogenesis. This result extends our previous re-

sults, in which acridine orange fluorescence was used as an indicator of total nucleic acids (Astrow et al. 1987) and suggests that the intense staining of teloplasm by acridine orange at least partly reflects a large maternal polyA+ RNA content.

Regarding the role of teloplasm in specifying cell fate, various lines of evidence suggest that the animal and vegetal teloplasms are developmentally equipotent: first, the two domains of teloplasm mix shortly before cell D of the 4-cell embryo cleaves to form macromere D' and micromere d' (late stage 3), and then are redistributed to both DM and DNOPQ at stage 4b by the oblique cleavage of macromere D' (Holton et al. 1989); second, DM and DNOPQ can assume distinct mesodermal and ectodermal fates even when the animal and vegetal teloplasms are combined prematurely by centrifugation of the 2-cell embryo (Astrow et al. 1987); third, in the sibling species *H. robusta*, cell DNOPQ can still follow its normal ectodermal fate even when animal teloplasm has been extruded and replaced with vegetal pole teloplasm by centrifugation (Nelson and Weisblat 1991); moreover, the assumption of specific ectodermal fates by prospective DNOPQ cells depends on whether or not the teloplasm interacts with factors in the animal cortex (Nelson and Weisblat 1992).

Our present results provide no evidence that animal and vegetal teloplasm differ in terms of their total polyA+ RNA content. They are therefore consistent with the notion that equipotent animal and vegetal domains of teloplasm are equipotent and act only *instructively* during early cleavage (stages 1–3), by diverting the recipient blastomere from the presumptive endodermal fates of the A, B and C macromeres (Nardelli-Haefliger and Shankland 1993) to being the progenitor of the teloblasts that generate segmental ectoderm and mesoderm; the polyA+ RNAs associated with teloplasm may be important in dictating this fate decision. In seeking determinants for the subsequent distinction between mesodermal and ectodermal fates, cortically localized factors, including the cortically localized polyA+ RNAs described here, should be considered. The description provided here for the overall distribution pattern of polyA+ RNAs provides the basis for future studies of the localization of specific maternal and early zygotic mRNAs.

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