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ORIGINAL ARTICLE

Morphogenetic mechanisms of coelom formation in the direct-developing sea urchin *Heliocidaris erythrogramma*

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Abstract Indirect development via a feeding pluteus larva represents the ancestral mode of sea urchin development. However, some sea urchin species exhibit a derived form of development, called direct development, in which features of the feeding larva are replaced by accelerated development of the adult. A major difference between these two developmental modes is the timing of the formation of the left coelom and initiation of adult development. These processes occur much earlier in developmental and absolute time in direct developers and may be underlain by changes in morphogenetic processes. In this study, we explore whether differences in the cellular mechanisms responsible for the development of the left coelom and adult structures are associated with the change in the timing of their formation in the direct-developing sea urchin *Heliocidaris erythrogramma*. We present evidence that left coelom formation in *H. erythrogramma*, which differs in major aspects of coelom formation in indirect developers, is not a result of cell division. Further, we demonstrate that subsequent development of adult structures requires cell division.

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Introduction

Among sea urchin species, there are two major modes of development that differ in many aspects, including the source of larval nutrition, cell lineage, larval morphology, and the timing of initiation of adult development. The ancestral form of sea urchin development, indirect development, is characterized by the production of many small eggs that develop into elaborate pluteus larvae (Fig. 1; Wray 1996). The pluteus larvae of indirect developers are obligate planktotrophs, meaning that they are required to feed in the water column, generally for a few weeks, in order to amass the resources needed for growth and adult development.

In sea urchins, adult development is initiated within the left side of the larva by contact between the left coelom, a mesodermal structure, and the overlying vestibular ectoderm. During indirect development, right and left coelomic pouches form before the end of gastrulation. Gastrulation has been observed to occur in two phases in indirect developers, although there are some species-specific exceptions (Kominami and Takata 2004). The first phase involves the symmetric involution of cells at the vegetal end of the embryo. This involution causes a buckling of the vegetal plate, which results in the formation of a short, shallow archenteron (Burke et al. 1991; Ettensohn 1984). During the second phase of gastrulation, the cells comprising the archenteron undergo the process of convergent extension. The intercalation and rearrangement of cells during this process result in the extension of the archenteron across the blastocoel (Ettensohn 1985; Hardin and

Cheng 1986; Hardin 1989). Gastrulation in indirect developers does not require concurrent cell division. Treatment with aphidicolin, an inhibitor of cell division, after the vegetal plate stage results in normal, albeit delayed, morphogenetic events, including gastrulation and formation of a pluteus larva (Stephens et al. 1986).

Towards the end of gastrulation during indirect development, small left and right coelomic pouches pinch off from their respective sides of the archenteron (Fig. 1a; Pehrson and Cohen 1986; Ferkowicz and Raff 2001; Smith et al. 2008). The archenteron then differentiates into a tripartite gut. By 12–20 days after fertilization (at 15°C), during the eight-arm pluteus stage, each coelom divides into three compartments, the right and left axocoel, hydrocoel, and somatocoel, which extend along the length of the esophagus (Smith et al. 2008). On the left side of the larva, a small patch of oral ectoderm then differentiates into vestibular ectoderm, and the left hydrocoel enlarges (Smith et al. 2008). The vestibular ectoderm ingresses to contact the left hydrocoel, and this contact initiates development of the pentameral adult form (Fig. 1b).

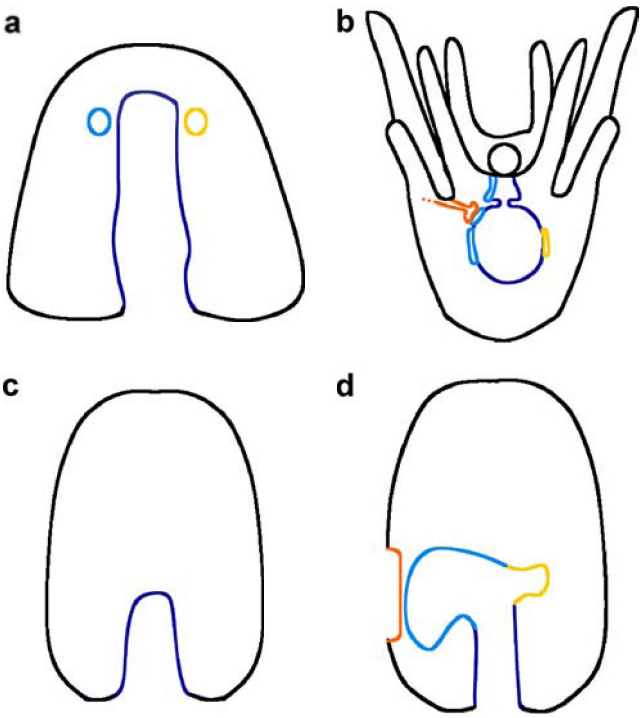

Fig. 1 A schematic comparison of early larval development between indirect developers (**a**, **b**) and the direct developer, *H. erythrogramma* (**c**, **d**). During indirect development, the archenteron elongates by the convergent extension of cells. The left and right coelomic precursors pinch off from the archenteron towards the end of gastrulation (**a**), and the left coelom does not enlarge until weeks after fertilization (**b**). In contrast, during *H. erythrogramma* development, there is no full extension of the archenteron across the blastocoel, and no coelomic anlagen are formed (**c**). Instead, a relatively large left coelom forms hours after fertilization from the tip of the archenteron followed soon by the slower formation of the right coelom (**d**). Tissues are color coded as follows: *light blue*, left coelom; *yellow*, right coelom; *dark blue*, archenteron and gut (pluteus only); *orange*, vestibular ectoderm

In contrast, a derived form of sea urchin development, direct development, has evolved multiple independent times among sea urchin clades (Strathman 1978; Wray 1996; Jeffery et al. 2003). Direct development is characterized by the production of fewer, substantially larger eggs that develop into ovoid larvae. These larvae do not feed but instead rely solely on maternal provisioning for the energy needed for development of the larval and adult forms. Direct-developing larvae metamorphose after a few days in the water column.

Cell lineage studies reveal that in the direct-developing sea urchin *Heliocidaris erythrogramma*, there have been major changes in gastrulation and coelom formation associated with the evolution of developmental mode. First, a disproportionately large number of cells ingress over the left lip of the blastopore, rather than symmetrical involution of cells over the blastopore as occurs in indirect developers (Wray and Raff 1991). Secondly, there is continuous involution of cells throughout gastrulation, rather than just in the initial phase (Wray and Raff 1991).

A third key difference associated with these two types of developmental modes lies in the formation of the left coelom. In the direct developer *H. erythrogramma*, the archenteron does not fully extend across the blastocoel or differentiate into a functional larval gut or mouth (Fig. 1c). Instead, a relatively large left coelom forms directly from the tip of the archenteron before the end of gastrulation, at approximately 24 h after fertilization. The right coelom forms shortly thereafter (Fig. 1d; Ferkowicz and Raff 2001). Soon after formation, the left coelom divides into three compartments, the axocoel, hydrocoel, and left somatocoel, followed by enlargement of the left hydrocoel. The hydrocoel forms only on the left side of the larva and eventually forms the pentameral water vascular system of the adult. As in pluteus larvae, contact between the left hydrocoel and overlying vestibular ectoderm, coupled with growth of both of these compartments, initiates development of the adult rudiment in *H. erythrogramma* (Fig. 1b,d; Ferkowicz and Raff 2001).

An important and striking difference between indirect and direct development is the timing of events associated with the formation and development of the left coelom and left-coelom-derived hydrocoel (Table 1). *H. erythrogramma* forms a left hydrocoel much earlier in developmental (late gastrulation/early larva) and absolute time (hours after fertilization), relative to indirect developers where the hydrocoel forms during the eight-arm larval stage several days to weeks after fertilization (Smith et al. 2008). However, regardless of the shift in timing of formation, gene expression in the left coelom is conserved across developmental modes (Ferkowicz and Raff 2001).

Table 1 Contrasts the absolute timing of developmental events associated with left coelom and adult development for the indirect developer *S. purpuratus* and the direct developer *H. erythrogramma*

Stage	Events	Time in *S. purpuratus*	Time in *H. erythrogramma*
Gastrulation	Formation and extension of the archenteron	24–34 h	16–22 h
Early larva	Coelomic pouches form	48–72 h	22–25 h
Four-arm pluteus	Coelomic extension	4–13 days	24–28 h
Eight-arm pluteus	Coelomic compartments (axocoel, hydrocoel, somatocoel) form	12–20 days	28–36 h
Vestibular invagination	Vestibular ectoderm ingression	18–28 days	28–36 h
Rudiment initiation	Vestibule and hydrocoel contact	25–35 days	34–36 h
Pentagonal disc stage	Adult pentamary obvious	29–43 days	36 h
Advanced rudiment stage	Adult and juvenile spines form	37–46 days	48 h
Tube foot protrusion/metamorphosis	Tube feet out of rudiment and metamorphosis into juvenile	45–50 days	72–96 h

The timing of the *S. purpuratus* gastrula and early larva stages is based on Ferkowicz (1997) from embryos reared at 12–14°C. All other timings of stages of *S. purpuratus* are based on Smith et al. (2008) from embryos reared at 15°C. The timing of developmental events for *H. erythrogramma* are based on Ferkowicz and Raff (2001), Ferkowicz (1997), Haag (1997), and personal observations of the authors. The times reported for *H. erythrogramma* are from embryos reared at 21–25°C. The times reported for *H. erythrogramma* indicate initiation of developmental events. Ranges of times are presented to reflect variation among cultures, variation in culture temperatures, and persistence of the developmental events for some duration of time. Because *H. erythrogramma* lacks many of the larval features used by Smith et al. (2008) to characterize specific stages in *S. purpuratus*, we rely heavily on adult features for assignment of each stage and the basis of our comparison. The events used to characterize each stage are reported in the 'Event' column. *H. tuberculata*, the indirect-developing sister species to *H. erythrogramma* that shares an overlapping range of the eastern coast of Australia, develops via a typical pluteus larva, roughly on the timescale as does *S. purpuratus* if adjusted for differences in natural temperature at which these species develop (E. Popodi, personal communication). The drastic differences in timing of the developmental events between *S. purpuratus* and *H. erythrogramma* cannot be attributed to differences in culture temperature

The shift to early development of the left coelom in direct developers was likely an early and important step in the evolution of direct development, driven by selection for reduced time to metamorphosis (Snoke Smith et al. 2007). Developmental events associated with growth and development of the left coelom, hydrocoel, and adult occur rapidly in direct developers from only the resources provisioned in the egg. This scenario contrasts with indirect development. In pluteus larvae, the left hydrocoel and adult development occurs later in development, and late larval development does not occur in starved larvae (Strathman et al. 1992; our observations). The source of larval nutrition (maternal provisioning versus larval feeding) affects larval growth and development, possibly through changes in the underlying cellular developmental mechanisms (Hoegh-Guldberg and Emlet 1997; McEdward 1996; Strathman et al. 1992). Based on the differences in timing of left coelom development and the basis of larval resources, we hypothesize that the evolution of direct development in *H. erythrogramma* may involve changes in the cellular mechanism underlying left coelom and adult development.

The rapid generation of a new tissue, such as the left coelom or adult structures in sea urchins, can be the result of several possible morphogenetic processes, such as movement of cells from elsewhere in the embryo, formation of new cells through cell division, changes in cell shape, or a combination of these processes. In indirect developers, the left coelom arises from derivatives of the small micromeres and macromeres, but the cellular processes underlying development of the left coelom have not been extensively studied (Pehrson and Cohen 1986; Tokuoka et al. 2002). However, there is evidence from a study of the small micromeres that cell division occurs in the small micromere descendents during left coelom formation.

Pehrson and Cohen (1986) fluorescently labeled small micromeres of *Strongylocentrotus purpuratus* embryos using an antibody to histone type H1cs. They first showed that small micromeres and their descendent cells contribute to formation of the right and left coelomic pouches and, secondly, that over time, the number of fluorescently labeled cells within the coeloms increases and that the intensity of fluorescence in the labeled cells diminishes. The increase in cell number and dilution of histone type H1cs as indicated by fluorescence intensity both suggest that cell division of small micromere descendents occurs in the developing left coeloms of pluteus larvae. However, it remains unreported whether divisions of macromere descendents similarly undergo cell division or if other cellular processes are also occurring.

It was unknown whether evolution of the early onset and acceleration of left coelomic growth in *H. erythrogramma* larvae was based on cell division, cell migration and rearrangement, or differences in cell shape. The cellular processes underlying the later patterning and development of

adult structures in sea urchins are also not well known. *H. erythrogramma* is a particularly useful species in which to study adult structures because adult development begins hours after fertilization and metamorphosis occurs by about 4 days (Williams and Anderson 1975; Ferkowicz and Raff 2001).

To begin to understand the cellular mechanism contributing to left coelom formation and adult development in *H. erythrogramma*, we looked for evidence that sufficient cell division occurs to generate the left coelom and adult structures. Cell division in normal embryos was assessed using bromodeoxyuridine (BrdU). BrdU is a modified uracil, which incorporates into DNA during the S phase of the cell cycle and serves as a proxy for cell division. Its incorporation into DNA can be detected using commercially available antibodies, thus marking DNA replication and also likely cell division over intervals longer than a cell cycle (Dolbeare et al. 1983). We also experimentally restricted cell division using aphidicolin, which inhibits DNA synthetase-α, thus halting the cell cycle and cell division (Stephens et al. 1986). In this paper, we present results indicating that in *H. erythrogramma*, left coelom formation is not largely the result of cell division but proper patterning of adult tube feet requires this process.

Materials and methods

BrdU treatment

BrdU (Sigma) was resuspended to 100 mg/ml stock solution in dimethyl sulfoxide (DMSO). This stock was diluted to 0.1 mg/ml directly into the filtered sea water (FSW) in which embryos were cultured. An equivalent volume of DMSO (30 μl) was added to control cultures. Embryos were cultured in 30 ml of FSW at 22°C. All embryos were fixed in 2% paraformaldehyde for at least 1 h, rinsed twice in 0.56 M sodium chloride (NaCl), and dehydrated to 70% ethanol.

BrdU immunocytology

Treated and control embryos were embedded in paraffin wax following previously described methods (Angerer and Angerer 1991), cut into 7-μm-thick sections, and mounted on Superfrost/Plus slides (Fisher). BrdU incorporation was detected with an anti-BrdU primary antibody (BD Pharmingen) and donkey anti-rabbit rhodamine-conjugated secondary antibody (Jackson ImmunoResearch) following the protocol of Stander (1999). Briefly, embryo sections were dewaxed in xylene and rehydrated. The slides were then washed three times in pH 7 phosphate-buffered saline (PBS) and blocked for 1–3 h in 10% normal goat serum (NGS) in PBS. The anti-BrdU primary antibody was diluted 1:200 in 2% NGS in PBS and hybridized to the sections overnight at 4°C. The primary antibody was removed with three PBS washes. The secondary antibody was diluted 1:100 in PBS, and Hoechst dye 33258 (Sigma) was added to a final concentration of 5 μg/ml to stain DNA. Sections were exposed to the secondary antibody plus Hoechst dye for 1–3 h at room temperature. The secondary antibody was removed with three PBS washes, and the slides were mounted with Aquapolymount (Polysciences).

Counts of nuclei

To calculate the percentage of nuclei incorporating BrdU in embryos at 5 and 28 h, the number of cells incorporating BrdU and the total number of cells were counted from a subset of the sections treated as described above in "BrdU immunocytology". One section from each of four different embryos was counted for 5 h embryos and five sections total among three different embryos were counted for 28 h embryos. Comparable sections were compared across embryos. In 28 h embryos, only sections in which the left coelom was clearly defined and of comparable size were counted. The percentage of nuclei for each stage that incorporated BrdU was calculated as the average, across sections, of the number of nuclei incorporating BrdU divided by the average, across all sections, of the total number of nuclei in the embryos, times 100%.

Aphidicolin treatment

Aphidicolin was resuspended to a concentration of 10 mg/ml in DMSO. This aphidicolin stock was further diluted to 0.5 μg/ml in the FSW in which the embryos were cultured. Control embryos were treated with an equivalent concentration of DMSO. Embryos were cultured in the same manner as embryos treated with BrdU. These embryos were fixed in 2% paraformaldehyde or 2% glutaraldehyde following the protocol outlined by Angerer and Angerer (1991).

Aphidicolin histology

Embryos were embedded and sectioned following the same procedure used for immunocytology (Angerer and Angerer 1991). Sections were then stained with erichrome cyanin (Chapman 1977). Erichrome cyanin stains nuclei dark blue and cytoplasm magenta. All photographs were taken using a SPOT RT digital camera and SPOT software.

Statistical analysis

To test whether 24 h embryos exposed to aphidicolin from 14 to 24 h have significantly fewer cells then control

embryos not exposed to aphidicolin, we counted the nuclei of echrichrome-cyanin-stained sections. Nuclei from three comparable sections per embryo in which both the coelom and archenteron were present were counted. Counts were averaged across all three sections for each embryo. Sections were counted for nine aphidicolin-treated embryos and ten control (DMSO only) embryos. Ratios were taken as the number of cells in each tissue type (ectoderm, coelom, or archenteron) over the total number of cells counted for all tissue types combined. All data was natural log (ln)-transformed, and we performed a *t* test in SPSS v14.0.1 (SPSS, Chicago IL, USA) to determine if the medians were significantly different.

Results

Embryos were exposed to BrdU following fourth cleavage (~3 h) for 2 h to examine whether BrdU could be incorporated into developing embryos and detected using an anti-BrdU antibody. The presence of intense red staining in the nuclei in BrdU-treated embryos (Fig. 2f) and lacking in DMSO control embryos (Fig. 2c) indicates that *H. erythrogramma* embryos incorporate BrdU and that it can be detected. Most (~88%) of the nuclei in these cleavage stage embryos were labeled with BrdU, as expected in embryos exposed to BrdU during early cleavage stage when rapid cell division is occurring (Parks et al. 1988). Additionally, embryos were exposed to both BrdU and aphidicolin at the same time to verify that aphidicolin inhibits DNA synthesis in *H. erythrogramma*. The presence of few nuclei and none that incorporated BrdU in early aphidicolin-treated embryos supports the conclusion that aphidicolin inhibits DNA synthesis (Fig. 2i–k and data not shown). The reduction in cell number and irregularity in cell size and shape in aphidicolin-treated embryos (Fig. 2h–k) relative to the cells of control embryos (Fig. 2a–d) indicates that the halting of DNA synthesis substantially

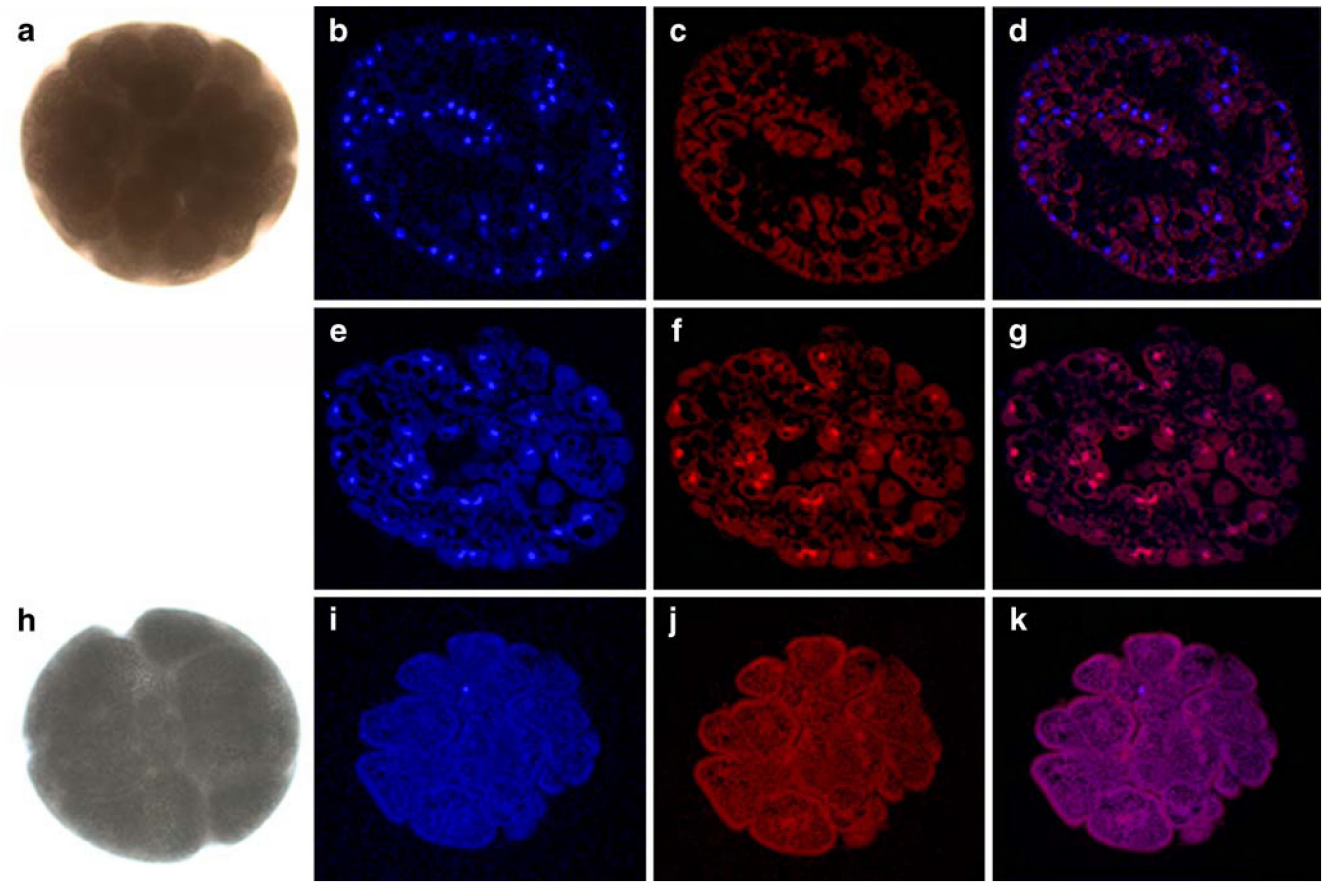

Fig. 2 Effects of blocking cell division in cleavage stage embryos on BrdU incorporation. *H. erythrogramma* embryos were exposed to only DMSO as controls (**a**–**d**), BrdU (**e**–**g**), or BrdU and aphidicollin (**h**–**k**). **a**, **h** Pictures of whole embryos, and all other images are of 7 μm sections of embryos. Hoechst dye 33258 highlights in *blue* all nuclei in an embryo (**b**, **e**, **i**); Nuclei that incorporated BrdU are in red (**f**, **j**); and the merge of these images shows in *pink* the nuclei of cells that replicated their DNA and likely divided during BrdU exposure (**g**, **k**). Embryos treated with 0.5 μg/ml of aphidicolin after first (**h**) and fourth cleavage (**i**–**k**) have fewer cells overall, and their cells are of abnormal shape and size relative to control embryos (**a**–**d**). Embryos (5 h) exposed to 0.5 μg/ml aphidicolin and 0.1 mg/ml BrdU at 3 h also have fewer nuclei (**i**) and cease DNA synthesis (**j**) based on BrdU incorporation

reduces cell division in *H. erythrogramma*. These cleavage stage embryos show that BrdU is useful for detecting cell division in *H. erythrogramma*.

We detected practically no incorporation of BrdU in the nuclei of cells in the developing left coelom, relative to nuclei of cells in the rest of the embryo, at 28 h in embryos

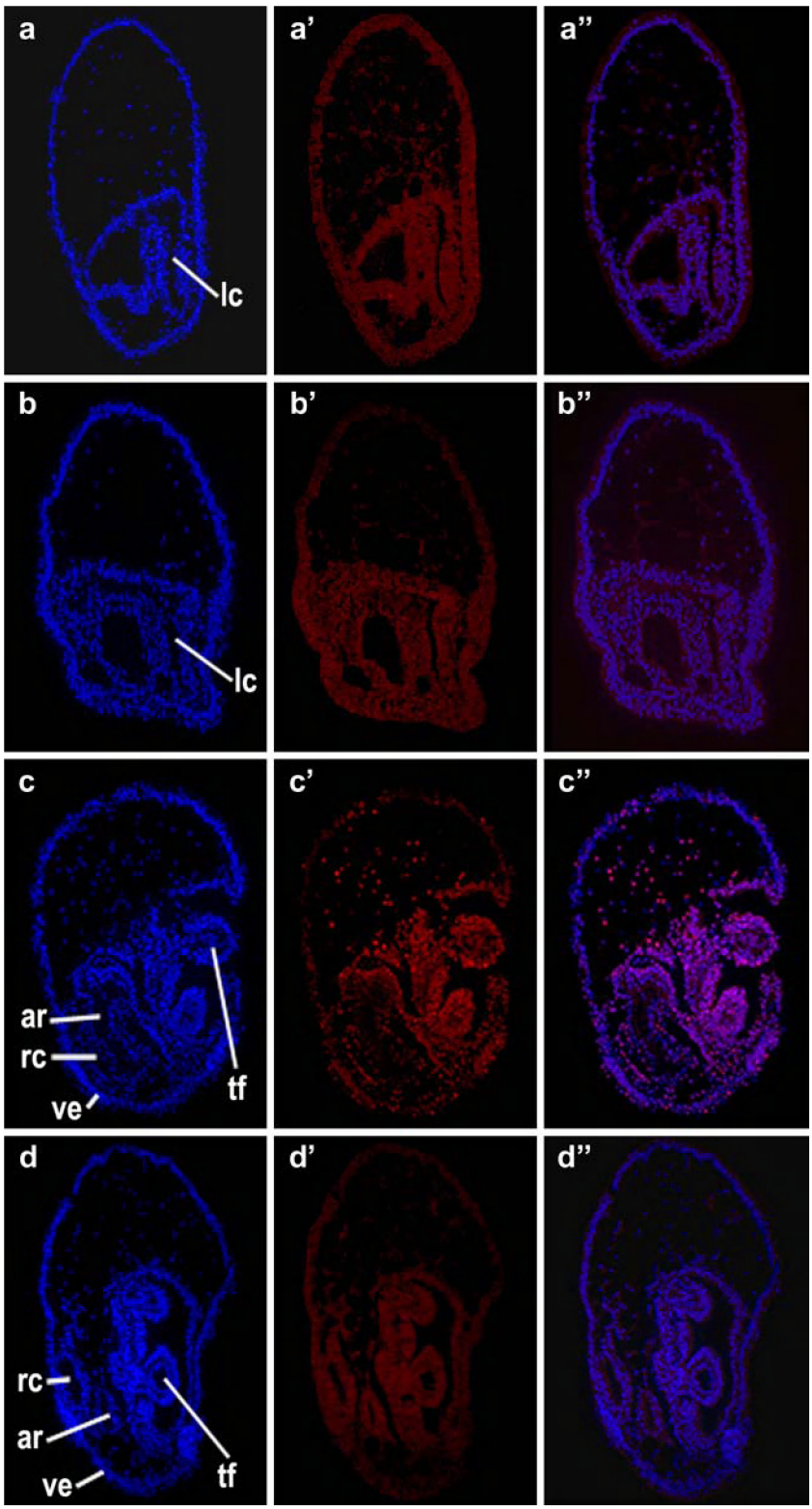

Fig. 3 BrdU incorporation by early and late larvae. Row **a** A representative section of a 28 h *H. erythrogramma* larva exposed to BrdU for 18 h. All nuclei, as stained by Hoechst dye, are stained in blue (**a**); BrdU incorporation is indicated in red (**a'**); and the merge of **a** and **a'** shows that no nuclei are pink, indicating that effectively no cells underwent cell division during exposure of this larva to BrdU (**a"**). Row **b** A section of a 28 h larva that was exposed to BrdU. All nuclei are stained in blue (**b**). Because this larva was not exposed to BrdU, the background level of secondary antibody staining is indicated in (**b'**), and no intense red staining overlaps with nuclei (**b"**). Row **c** A representative section of a 51 h larva exposed to BrdU for 33 h. All nuclei are stained in blue (**c**). Nuclei that incorporated BrdU are stained in red (**c'**). The merge of **c** and **c'** (**c"**) shows in *pink* the nuclei of cells that underwent DNA replication/cell division while this embryo was exposed to BrdU. DNA replication/cell division occurred in the adult rudiment, including tube feet (*to*), the right coelom (*arc*), and the vegetal ectoderm (*vet*), but not in the archenteron (*are*) or remaining ectoderm. Row **d** A section of a 51 h larva not exposed to BrdU where all nuclei are stained in blue (**d**), the background level of red fluorescence is in *red* (**d'**), and **d"** is the merge of **d** and **d'**. *lc* left coelom, *tf* tube foot, *rc* right coelom, *vg* vegetal ectoderm, *ar* archenteron

Table 2 Reports the counts of nuclei labeled with BrdU, the total number of nuclei in the embryo, and the percentage of BrdU labeled nuclei in *H. erythrogramma* for both cleavage stage embryos (5 h) and embryos exposed to BrdU during the time when the left coelom was forming (28 h)

Embryo stages	Embryos (sections) counted	Average BrdU labeled nuclei per section	Average total nuclei per section	Average percent (%) BrdU labeled nuclei per section
5 h embryos	4 (4)	15 (±4)	16 (±4)	88.6 (±5.2)
28 h embryos	3 (5)	7 (±3)	832 (±22)	0.9 (±0.4)

The numbers of nuclei reported in the second, third, and fourth columns are averages (±standard error) per section across all sections counted. Counts of nuclei for 5 h embryos are based on four sections total, one section from each of four different embryos. Counts of nuclei for 28 h embryos are based on a total of five sections from among three different embryos

exposed to BrdU for 18 h (Fig. 3, row a). The percentage of cells incorporating BrdU in these embryos (0.9%) is close to two orders of magnitude less then cleavage stage embryos (Table 2). These observations suggest that there is little cell division occurring when the large left coelom is forming and are consistent with the results of studies with aphidicolin.

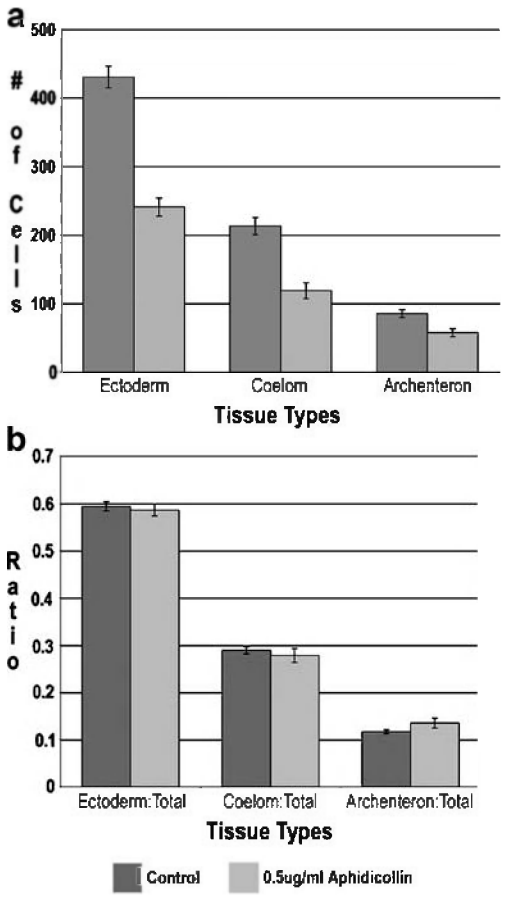

Fig. 4 In *H. erythrogramma*, embryos exposed to aphidicolin at 14 h have significantly fewer cells then control embryos across all tissue types present at 24 h ($p<0.001$) (**a**). The reduction in the number of cells in larva exposed to aphidicolin is consistent across all cell types counted because there is no significant difference between the ratios of cells in each tissue type to the total number of cells (**b**). Standard error bars are presented on both graphs

The 24 h embryos exposed to aphidicolin at 14 h have significantly fewer cells then control embryos ($p<0.001$; Fig. 4a), but they are still capable of forming a left coelom (Fig. 5b,c). This observation indicates that cell division during this time is not required for left coelom formation. The timing differences between those exposed to BrdU (28 h) and those exposed to aphidicolin (24 h) reflect different cultures across different years, but are of a comparable developmental stage.

Exposure to aphidicolin reduces cell division evenly across all tissue types because the ratio of cells of each individual tissue to the total number of cells in the embryo does not significantly differ between control and aphidicolin-treated embryos (coelom: total $p=0.157$; archenteron: total $p=0.124$; ectoderm: total $p=0.300$; Fig. 4b). Taken together, these lines of evidence indicate that the cell division is not an important mechanism for left coelom formation.

In contrast to left coelom formation, proper development of adult structures requires cell division. A high level of BrdU incorporation was detected in developing adult structures at 51 h (Fig. 3, row c). However, cell division appears to occur at a low rate over a long period of time because these embryos were exposed to BrdU for 33 h before obvious signal was apparent. Detection of BrdU in adult structures after this period of time is not an artifact of prolonged exposure to BrdU because this is roughly the same fraction of the life of the embryo for which the 28 h embryos were exposed with no evidence of cell division in the left coelom.

Treatment with aphidicolin also supports the observation that cell division is important for adult development. Inhibition of cell division following left coelom formation by exposure to aphidicolin results in overall smaller larvae (Fig. 5e,f) with reduced, poorly organized adult tube feet (Fig. 5h,i). Notably, a reduction in cell number does not affect overall adult patterning because pentameral symmetry is maintained. All larvae examined initiated development of five tube feet.

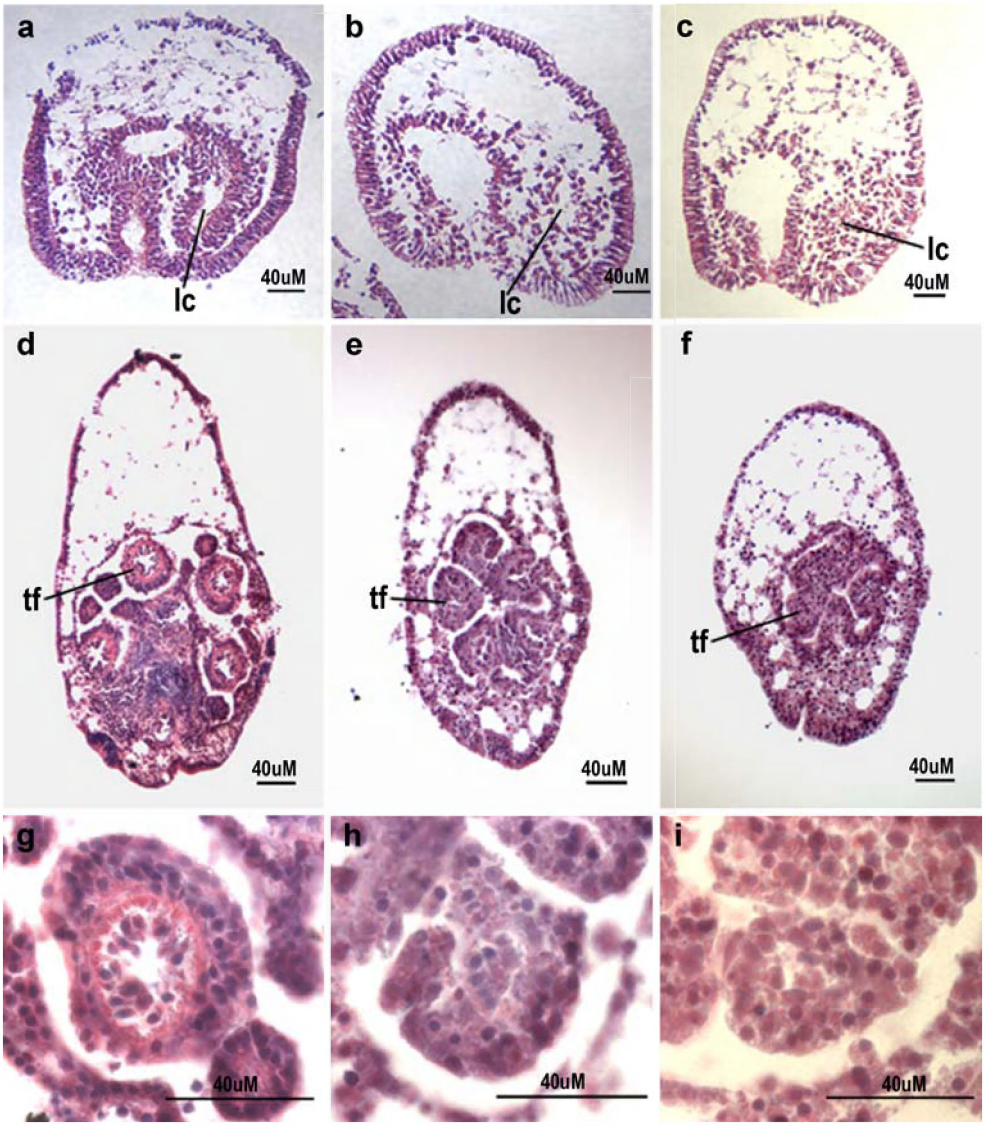

Fig. 5 Treatment of *H. erythrogramma* embryos with 0.5 μg/ml aphidicolin at 14 h still results in the formation of a left coelom (*lc*) (**b**, **c**) of a similar shape and size, although more loosely cohering, to that of control larvae (**a**) at 24 h. Treatment with aphidicolin at 26 h (**f**, **i**) or 30 h (**e**, **h**) results in an overall smaller larvae at 72 h (**f**, **e**) with poorly organized tube feet (*tf*) (**h**, **i**) relative to the control (**d**, **g**). **g**–**i** Magnified pictures of a tube foot consistent with those of the embryos presented above them (**d**–**f**, respectively). *lc* left coelom, *tf* tube foot

Conclusion

Cell division does not appear to play a large role in left coelom formation in *H. erythrogramma*, indicating that another morphogenetic process is responsible for the formation of this structure. Although the cellular mechanisms underlying formation of the left coelom in indirect developers have not been well studied, the lack of importance of cell division in *H. erythrogramma* contrasts what is currently known from the behavior of small micromeres, which divide during the development of the left coelom of indirect-developing larvae. *H. erythrogramma* produces no micromeres and instead dedicates a large proportion of its blastomere mass to gastrulation (Wray and Raff 1991). These observations strongly hint that changes in the cellular mechanism underlying left coelom formation are associated with its early developmental appearance and the evolution of direct development in *H. erythrogramma*. We hypothesize that these changes result from the co-option of the cell movement mechanisms responsible for elongating the archenteron during the second phase of gastrulation as seen in indirect developers. These cell movement processes could be extended in time and incorporated into the early development of the left coelom during *H. erythrogramma* development. This hypothesis is consistent with the observation that in *H. erythrogramma*, the left coelom forms before the end of gastrulation from the tip of the archenteron.

Additional evidence on the developmental processes underlying left coelom formation in both direct- and indirect-developing species are needed to further evaluate this hypothesis. Smith et al. (2008) have described in detail formation of the coeloms and subsequent division into and development of the coelomic derivatives (axocoel, hydrocoel, and somatocoel). However, data are not yet available

on the cellular processes underling coelom development in late pluteus larvae. Testing for cell division during the development of the left coelom in pluteus larvae should be possible and would yield important comparative data on evolution of coelomic development.

Overall, shifts in the timing of importance of cell division versus cell movement could be important for the evolution of direct development. For example, *H. erythrogramma* exhibits increased rounds of early cell division relative to indirect developers (Parks et al. 1988). Gastrulation involves cell movement across both developmental modes, but cell division is not important again in *H. erythrogramma* until formation of the adult structures, which is likely later than in the pluteus. Execution of events underlying adult development also differs in timing. The adult rudiment develops strikingly earlier *H. erythrogramma* relative to pluteus larvae. However, despite this difference in timing, development of the adult rudiment, once initiated, proceeds in the same way regardless of developmental mode, resulting in a conserved pentamerally symmetric adult form.

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