Cell proliferation and early differentiation during embryonic development and metamorphosis of *Hydractinia echinata*

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Summary

The early embryonic development of *Hydractinia* lasts about 2-5 days until the developing planula larva acquires competence for metamorphosis. Most embryonic cells stop cycling on reaching the larval stage. In older larvae of *Hydractinia*, cells that are still proliferating occur exclusively in the endoderm in a typical distribution along the longitudinal axis. During metamorphosis, proliferation activity begins again. The number of S-phase cells has increased by the 9th hour after induction of metamorphosis. Proliferative activity starts in the middle gastric region and in basal parts of primary polyps. Tentacles and stolon tips are always free of replicating cells.

Key words: proliferation kinetics, metamorphosis, *Hydractinia echinata*, cell proliferation.

Introduction

Hydroids often are chosen as model organisms for studies that are aimed at understanding how cell proliferation and differentiation are controlled and how cells are governed to assemble into a complex pattern. One reason for such a choice is that the animals have a simple architecture and consist of only a few cell types. Also, they have so-called interstitial cells (I-cells) which are true pluripotent stem cells for several different cell lines. Furthermore, pattern formation in hydroids is a continuous process and is not restricted to embryogenesis. The control mechanisms must be present all the time.

*Hydractinia echinata* is a colonial hydroid. The early embryonic development is transiently terminated by reaching the stage of a planula larva. The planula represents an already complex pattern of different cell types. The larva undergoes metamorphosis (only upon an exogenous stimulus), and the rapid reshaping process leads to the definite morphology of the adult polyp. For the first time, a head with tentacles and a stolon region develops in the animal. This rapid morphogenetic process has been the subject of investigations which were aimed at understanding the control mechanisms of pattern formation. The morphology of the adult polyp can be deduced from the polar organization of the larva. The anterior blunt end of the planula gives rise to the basal part of the polyp while the head always develops from the tapered posterior tail of the larva. In this respect, the planula appears to be a developmental mosaic. On the other hand, exogenous compounds as well as factors isolated from coelenterate tissue have been demonstrated to affect patterning during metamorphosis (Müller et al. 1977; Berking, 1984, 1986, 1987; Plickert, 1987). Thus, it appeared that part of the larval tissue may be capable of regulation by external signals.

The early development of *Hydractinia echinata* has been studied already by histological methods (Bunting, 1894; Van de Veyver, 1964) and by electron microscopy (Weis et al. 1985). However, a quantitative analysis of the cell composition of the developing and the mature larva and of early cell differentiation during development has not yet been performed. This paper intends to provide these data including information on changes in the cellular composition during metamorphosis and, in particular, on the developmental fate of proliferating cells in the planula.

Materials and methods

*Animal culture and induction of metamorphosis*

Natural colonies of *Hydractinia echinata* were purchased
from the Biologische Anstalt Helgoland, FRG. They were kept in artificial sea water (Tropic Marin Neu) and fed daily with brine shrimp nauplii. Eggs were collected 2 h after light-induced spawning and reared at 18°C in Millipore-filtered sea water. Metamorphosis was induced according to Müller & Buchal (1973) by incubation in 56 mM-CsCl in sea water for 3 h.

Labelling of S-phase cells
Animals were labelled by immersing them in a solution of 200 µM-BrdU (Serva, Heidelberg, FRG) in sea water for 1 or 2 h. For details see Plickert & Kroicher (1988).

Cell maceration
*Hydractinia echinata* was exposed to a heat shock (39°C) for 10–25 min in Ca^2+/-free sea water, pH 6-7, to which 0·1 i.u. ml^-1 protease from *Streptomyces griseus*, type VI (Sigma) had been added. Subsequently, maceration fluid (water–glycerol–acetic acid, 6:1:1) was added to a final concentration 13:1:1, and the animals were stored overnight at 4°C. Cells were separated by aspirating the macerated animals gently up and down in a microcapillary pipette several times (modified from David, 1973; Schmid et al. 1981). Macerated cells were fixed by addition of 37% formaldehyde and spread on gelatine-coated slides. They were dried overnight at 40°C.

Cell counting
Cell numbers in developing embryos were either determined in whole-mount preparations (up to 2000-cell stage) or in macerates by counting stained nuclei. In order to prepare whole mounts, embryos were fixed in 4% paraformaldehyde in 0·1 M-phosphate buffer at 4°C, overnight. After fixation, the specimens were rinsed in 0·1 M-glycine, pH 7·2, for 1 h and then in phosphate-buffered saline (PBS, 0·15 M-NaCl, 0·01 M-Na₂HPO₄) for 0·5 h. Subsequently, the specimens were incubated in diamidino-2-phenylindole (DAPI, Serva, Heidelberg) in PBS at a final concentration of 200 ng ml^-1. The specimens were transferred to slides. They were mounted in glycerol by squeezing them in order to bring all nuclei in almost the same focal plane. For cell counts in macerates, slides that were covered with the fixed cells were immersed into the DAPI solution. Comparative countings in whole mounts and macerates indicated that cells were lost during the preparation of tissue macerates. From the 500-cell stage onwards, values for cell numbers in macerates were in the range of the standard deviation of values counted in whole mounts. Cell counting and the identification of cell types in macerates of larvae or primary polyps were performed using phase-contrast or Nomarski optics. DAPI-stained nuclei were counted by means of u.v.-excitation fluorescence microscopy.

Results

Proliferation kinetics and early differentiation during embryonic development of *Hydractinia echinata*

In *Hydractinia echinata*, spawning of gametes is light-induced and commences at 18°C about 30 min after exposure to light. Oocytes complete meiotic divisions prior to spawning (Freeman & Miller, 1982). Approximately 60 min after spawning, the fertilized egg starts to divide. Until the 9th cleavage (512-cell stage), all blastomeres divide nearly synchronously every 50 min. Subsequently, division becomes asynchronous (Fig. 1) and slows down further according to the proliferation kinetics shown in Fig. 2. The labelling index (as determined by 1 h pulse labelling) decreases from 100% to 45% (Fig. 4) during the time period from 8 to 12 h after fertilization (Table 1). This presumably is due to an increase in the cycle length of some of the cells. In addition, some cells that had begun differentiation earlier completely stopped proliferation. To show this, embryos were labelled continuously from the 12th until the 28th h and were examined immediately and 30 h after the end of the treatment. Nerve cells and epithelial cells (Fig. 3) were found to be the only unlabelled cells in the embryo (data not shown). Accordingly, nerve cells are the first terminally differentiated cells that can be identified in the developing larva (Table 1).

![Fig. 1. (A) Synchronous mitosis in a 64-cell-stage embryo (DAPI-stained). (B) Asynchronous mitosis in 512- to 1000-cell-stage embryo (DAPI-stained). an, anaphase; in, interphase; me, metaphase; pr, prophase. Bar, 50 µm.](image-url)
Proliferation in Hydractinia

Fig. 2. Semilogarithmic plot of the mean number of cells per developing embryo of *Hydractinia echinata* versus time after fertilization. Mean values calculated from countings in 8–15 animals. The bars indicate one standard deviation of the mean.

By the 12th h after fertilization, the embryos consist of two populations of differently shaped blastomeres. 47% of the cells closely resemble the later epithelial cells of the larva. They have a characteristic elongated cell body with a somewhat rectangular cell shape. Some of them have already developed processes at their bases, oriented perpendicularly to the cell body. The nucleus is located off-centre near the base of the cell. The other cells are still typical embryonic cells with compact and round shape and without any cell processes. They resemble the later I-cells (Fig. 3) of the animal. The cytoplasm of these cells appears to be homogeneous in consistency. Any inclusions or cell vacuoles are absent. The overall labelling index has decreased to 45% at this time (determined by means of 1 h pulse labelling). This decrease is due predominantly to a decrease in the labelling index of the interstitial-like cells. Only 31% of them become labelled by 1 h pulse, whereas the majority (60%) of the epithelial-like cells is still labelled (Table 1).

In the larva, three different types of nematocytes occur (Fig. 3). One is of the acrophore type (Werner, 1965; Mariscal, 1974). It exclusively occurs in the larva. Microbasic euryteles (Mariscal, 1974) are found in planulae and in polyps of *Hydractinia echinata*. It is the most abundant stinging cell in the catching tentacle. The third type is yet unclear in identity and function. It has a slim capsule 12 μm long containing a helically coiled thread. It occurs in both the larva and in the polyp.

Nematoblasts can be identified first about 30 h after fertilization. In *Hydractinia*, the very first nematocytes at least appear to develop directly from blastomeres and thus not as usual from interstitial cells.

### Table 1. Proliferative activity and cellular composition of embryonic stages of *Hydractinia echinata*

<table>
<thead>
<tr>
<th>Age of embryo (h)</th>
<th>Total number of cells</th>
<th>Epithelial and gland cells</th>
<th>Embryonic/I-cells†</th>
<th>Nerve</th>
<th>Nemato-</th>
<th>Nematoblasts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (s.d.)</td>
<td>n (s.d.)</td>
<td>n (s.d.)</td>
<td>n (s.d.)</td>
<td>n (s.d.)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1 (±101)</td>
<td>0 (±1)</td>
<td>1 (±100)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>8-5</td>
<td>411 (±100)</td>
<td>0 (±96)</td>
<td>411 (±100)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>12.5</td>
<td>1780 (±332)</td>
<td>460 (±3-7)</td>
<td>920 (±106)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>28.5</td>
<td>6890 (±509)</td>
<td>4640 (±226)</td>
<td>2215 (±239)</td>
<td>35</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>53.5</td>
<td>7995 (±293)</td>
<td>5500 (±329)</td>
<td>2430 (±290)</td>
<td>65</td>
<td>some</td>
<td></td>
</tr>
<tr>
<td>78</td>
<td>1030 (±778)</td>
<td>7830 (±368)</td>
<td>1520 (±378)</td>
<td>310</td>
<td>352</td>
<td></td>
</tr>
</tbody>
</table>

* Cell numbers were determined in whole-mount preparations (up to about 2000-cell stage) and in macerates. Cell types were determined in macerates. For each stage, 8–40 animals were scored. Numbers are mean values: s.d., one standard deviation.

† Embryonic cells means cells of early developmental stages having still the morphology of blastomeres. I-cells which have already the typical morphology of coelenterate interstitial cells, i.e. spindle shape, homogeneous cytoplasm, appear only in later developmental stages, i.e. 3 days after fertilization.

n.d. not determined.
mode of differentiation is remarkable. Cells can be observed that consist of two interconnected compartments. One relatively large part contains the nucleus while, in a smaller denuded satellite, the nematocyst develops (Fig. 3). 53.5 h after fertilization, 3.7% of all larval cells are either nematoblasts or nematocytes. Most early developing nematocytes are of the larva-specific acrophore type. Later microbasic euryteles also differentiate (Table 2).

3 days after fertilization, the larva consists of epithelial cells, typical I-cells, nematocytes, nerve cells and gland cells (Table 1, 2; Fig. 3). Most of the gland cells of Hydractinia echinata were reported to be ectodermal and to occur concentrated in the blunt anterior end of the larva (Van de Vyver, 1964). Differentiating gland cells are not easy to distinguish from larval epithelial cells because the densely packed yolk platelets of the embryonic epithelial cells look similar to the granules of gland cells. Accordingly, both cell types are included within one group (Table 1).

At this time (3 days), exclusively endodermal cells take up label (see Fig. 7A which shows this characteristic distribution in a 4-day-old larva). These labelled

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**Table 2. Mature nematocytes in early larvae of Hydractinia echinata**

<table>
<thead>
<tr>
<th>Age of embryo (h)</th>
<th>Acrophores ( n \uparrow ) (s.d.)</th>
<th>Microbasic euryteles ( n \uparrow ) (s.d.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>53.5</td>
<td>18 (±7)</td>
<td>0</td>
</tr>
<tr>
<td>57.0</td>
<td>33 (±2)</td>
<td>20 (±8)</td>
</tr>
<tr>
<td>60.0</td>
<td>66 (±8)</td>
<td>40 (±7)</td>
</tr>
<tr>
<td>78.0</td>
<td>71 (±17)</td>
<td>74 (±19)</td>
</tr>
</tbody>
</table>

* Nematocytes with discharged nematocyst (after treatment with 5% acetic acid).
† \( n \), no. per larva.
Proliferation in Hydractinia

Fig. 4. Decrease of proliferative activity in aging planulae of Hydractinia echinata. S-phase nuclei were screened in whole-mount preparations immediately after 2 h pulse labelling in BrdU. The plot shows mean numbers of labelled nuclei per animal versus the age of the larva. The bars indicate one standard deviation of the mean. 10–20 animals were analysed for each point.

Development of competence for metamorphosis
The earliest evidence that embryos become competent to undergo metamorphosis is found 33 h after fertilization. Using the standard treatment that usually stimulates 100% of mature larvae, only 10% of the treated preplanulae transformed into primary polyps. In addition, metamorphosis proceeded much more slowly than normal. Whereas metamorphosis is normally completed in about 18–20 h after stimulation, 33 h preplanulae needed up to 45 h (Table 3). 90% of the animals remained larvae. They appeared normal in shape and behaviour. Reexamination of competence for metamorphosis by a later second exposure to CsCl resulted in a normal number of the animals transformed with normal velocity (Table 3).

With increasing age of the developing larvae, increasingly more animals acquired the competence to undergo metamorphosis (Table 3). The duration of metamorphosis was the same as in larvae of several days of age.

Temporal and spatial aspects of S-phase cell distribution during metamorphosis of Hydractinia echinata

Proliferative activity increases soon after the onset of metamorphosis
The planula of Hydractinia represents a resting stage. Induction of metamorphosis not only gives rise to dramatic changes in the morphology of the animal, but also triggers resting cells to reenter the cycle. About 8–9 h after onset of metamorphosis (reception of the inducing stimulus), proliferative activity starts to increase (Fig. 5). At this time, metamorphosis has progressed to a stage where tentacles and stolons can appear just 8 h later (18°C).

Proliferating cells are unevenly distributed in larvae and primary polyps
Until 2 days after fertilization, both the ectoderm and the endoderm of developing planulae contain proliferating cells (as determined by 1 h pulse labelling). Larvae only 1 day older contain proliferating cells exclusively in the endoderm in a typical spatial distribution. The tapered tail of the planula comprises about one third of the total larval length and is void of any S-phase cells (after 2 h pulse labelling). Also in the anterior tenth of the larva no proliferating cells occur (Figs 6, 7A). In macerates of these larvae, 29% of the cells that have typical I-cell morphology are labelled. Of the epithelial cells 1–4% are labelled. Thus, in mature larvae proliferation continues predominantly in I-cells.

Cells that restart proliferation during metamorphosis are also arranged in a peculiar spatial pattern. Along the distoproximal axis of the young hydranth, these proliferating cells are most abundant in the proximal half. At the time that the tentacles become visible (Fig. 7B), the distal part contains only a few S-phase cells.

Only a few additional cells become labelled in distal body regions at later developmental stages, e.g. during the next 24 h. Tentacles generally do not contain cycling cells (Fig. 8A). During metamorphosis, a disc-like structure forms at the base of the primary polyp. In this basal disc, proliferating cells are most abundant along the margin and only a few are located in the centre of the plate (Figs 7B, 8B). However, when stolon tips grow out from the basal disc, the circular distribution pattern of proliferating cells changes. At any site where a tip is beginning to develop and for some time before tip outgrowth is visible, cells no longer can be labelled in S-phase. Stolon tips continue to be void of S-phase cells even when they have grown out from the plate (Fig. 8B).
Table 3. Competence for metamorphosis in early larvae of Hydractinia echinata

<table>
<thead>
<tr>
<th>Age of larvae at time of exposure to CsCl (h)</th>
<th>No. of polyps (% of total) formed after 24 h</th>
<th>No. of polyps (% of total) formed after 45 h</th>
<th>No. of remaining larvae (% of total) after 45 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>33-0</td>
<td>240 (9)</td>
<td>24 (10)</td>
<td>215 (90) (reexposed at 86h)</td>
</tr>
<tr>
<td>49-5</td>
<td>151 (70)</td>
<td>45 (30)</td>
<td></td>
</tr>
<tr>
<td>53-5</td>
<td>139 (86)</td>
<td>22 (14)</td>
<td></td>
</tr>
<tr>
<td>56-5</td>
<td>141 (91)</td>
<td>12 (9)</td>
<td></td>
</tr>
<tr>
<td>57-5</td>
<td>403 (99)</td>
<td>4 (1)</td>
<td></td>
</tr>
<tr>
<td>86-0 (reexposure of non-metamorphosed 33 h animals)</td>
<td>161 (99)</td>
<td>2 (1)</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 5. Increase in proliferative activity in metamorphosing Hydractinia echinata. At indicated times after onset of metamorphosis animals were pulse labelled for 2 h and subsequently prepared for analysis. The numbers of S-phase nuclei were determined in whole-mount preparations. Each mean value has been calculated from analysis of 3–8 animals. Bars indicate one standard deviation.

Fig. 6. Distribution of S-phase nuclei in 4-day-old planulae. Larvae were prepared for whole-mount analysis immediately after 2 h pulse labelling. Prior to analysis the length of each animal was measured and the number of labelled nuclei was determined in each 1/20th of the larva. The bars indicate the size of the fraction of labelled nuclei occurring in each individual segment as the percentage of the total number of labelled nuclei in the larva. The values are mean values calculated from analysis of four animals. Bars indicate one standard deviation. Note that all labelled nuclei occur exclusively in the endoderm of the larva as documented in Fig. 7A.

However, if tips stop stolon elongation and lose typical tip properties – due to starvation, for instance – S-phase cells reappear in the terminal stolon tissue (Fig. 8A).

The velocity of nematocyte differentiation varies in different developmental stages

In initial developmental stages, the first mature nematocytes of the microbasic eurytele type appeared already after 57 h (Table 2). In older planulae differentiation of these nematocytes proceeded much slower. 3 days after pulse labelling (1 h) of 10-day-old planulae, tissue macerates contained labelled epithelial and interstitial cells and labelled nematoblasts. The labelled nematoblasts constituted about 20 % of all larval nematoblasts and nematocytes. In macerates prepared 6 days after labelling, the labelled fraction of differentiating or mature nematocytes increased to 26 %. By this time, half of these labelled cells were mature microbasic eurytele. In a parallel experiment, the fate of the proliferating cells of late planulae was traced in the course of metamorphosis. 10-day-old larvae from the same batch as the above-mentioned animals were induced to undergo metamorphosis immediately after labelling. The developing primary polyps were also examined 3 days after administration of the label. In whole-mount preparations, labelled nematocytes were identified throughout the animal including the tentacles. These nematocytes were dischargeable and therefore fully
Fig. 7. Distribution of S-phase nuclei in planula and primary polyp of *Hydractinia echinata*.
(A) Larva, 4 days after fertilization, immediately prepared after 2 h pulse labelling. Labelled nuclei occur exclusively in the midlarval endoderm (for data see Fig. 6).
(B) Primary polyp, pulse labelled for 1 h about 20 h after induction of metamorphosis. The animals were sacrificed immediately after labelling in order to document the actual distribution of S-phase cells. Nuclei are labelled in the midgastric and basal parts of the polyp. Stolon tips (st, outlined) which grow from the basal plate (bp) do not contain replicating cells. The distal parts of the polyp including tentacle buds (tb) and hypostome (hy) are also void of S-phase cells at this stage. an, anterior; po, posterior. Bar, 100 μm.

Fig. 8. Labelled S-phase nuclei in whole-mount preparations of primary polyps of *Hydractinia echinata*. Animals were labelled for 2 h. (A) Stolon and tentacles of starved primary polyp. Due to starvation, the stolon tip has lost typical morphology. S-phase cells occur throughout the stolon including the extreme terminal apex. Tentacles (one indicated by arrows) do not contain S-phase cells. (B) Normal growing stolon with functional tip. The stolon has grown out from the basal plate of a primary polyp. Note that the extreme tip of the stolon (arrows) is void of labelled nuclei. Bar, 50 μm.

differentiated. This indicates a reacceleration of nematocyte differentiation as a consequence of metamorphosis.

In summary, I-cells which are still proliferating in late developmental stages may either remain stem cells or become polyp-specific nematocytes. The velocity of differentiation depends on whether the animal remains a larva or undergoes metamorphosis into the polyp stage.

Discussion

Proliferation kinetics during embryonic development
of *Hydractinia* reflects the temporal proliferation patterns characteristic of all animals. Rapid divisions at less than 1 h intervals quickly lead to a multicellular embryo. Early embryonic development reaches a plateau at the resting stage of the larva. Proliferationrestarts as soon as metamorphosis is induced. Some interesting additional findings should be pointed out. Early in development, some cells slow down or even leave the cycle. These either become nerve cells, epithelial cells or larval-specific nematocytes. These
cell types are essential for larval life as well as for the preparation for successful metamorphosis. Terminal

differentiation of larval nerve cells and nematocytes may account, at least in part, for the early decline of the labelling index from the 512-cell stage onwards.

Some of the cells that stop or interrupt cycling in the young embryo are larval epithelial cells. Presumably, they are responsible for the reshaping of the spherical gastrula stage into a longitudinally stretched larva. The epithelial cells that first acquired their typical cell morphology may function as a basic epithelial frame for the establishment of the polar organization of the larva. A longitudinally organized matrix certainly could facilitate the generation and maintenance of a signalling gradient such as the distribution of a morphogen.

Early differentiation, however, also occurs in cells that obviously are not essential for form and function of the larva. Polyp-specific nematocytes are produced in substantial amounts. Even mature larvae continuously produce these nematocytes. Thus, for this cell population, at least, it is true that larvae are in fact a developmental mosaic already containing later polyp cells.

All larval cells that are still proliferative are located in the endoderm. Their distribution pattern along the longitudinal axis of the planula is very similar to the distribution of so-called basophilic cells and nemato-
blasts described earlier in *Hydractinia* (Van de Vyver, 1964). Moreover, during metamorphosis these basophilic cells were reported to reappear in the ectoderm in a distribution which is similar to the pattern of S-phase cells we found about 20 h after the onset of metamorphosis. After maceration of labelled larvae, most of the labelled cells were identified to be interstitial cells. Thus it is concluded that proliferating I-cells of late planulae either give rise to nematocytes or remain as stem cells. Both the I-cells and the nematocyte precursors pass from endoderm to ectoderm during metamorphosis. It may be the stem cells, now located in the ectoderm, that are the first cells to recommence proliferative activity after onset of metamorphosis. In the hydrozoan*Pennaria tiarella*, I-cells also arise in the larval endoderm and migrate in the larva as well as during metamorphosis to the ectoderm (Martin & Archer, 1986).

Metamorphosis is based on morphogenetic events that require a minimum level of maturation of the larva. In general, embryos, 33 h old, are not yet able to respond to inducing stimuli by normal metamorphosis. A few of them, however, already have been induced even though metamorphosis proceeds at an abnormally low velocity. This indicates that, in principle, not all of the cells of the mature larva are necessary. Moreover, the capability for receiving the trigger signal apparently develops independently from the ability of the larva to respond to the inducing stimulus.

The external metamorphosis-inducing stimulus appears to trigger the release of at least two internal signals. One signal drives resting cells back into the cycle. It is not yet clear in which part of the cycle cells arrest at the end of the embryonic development. Preliminary observations did not indicate a dramatic increase in mitosis after induction of metamorphosis. Thus cells may be postmitotic when they stop cycling and reenter the cycle by starting in S-phase 8–12 h after the triggering event.

The other signal(s) generated by metamorphosis induction affect(s) cell differentiation and the reshaping of the animal. Our results indicate that substantial amounts of the larval cells proceed directly to post-
metamorphic differentiation without passing through a postmetamorphic S-phase. Some of them have completed differentiation as early as 8 h after the reincrease in proliferative activity. Moreover, labelled nuclei predominantly appear in the middle and basal body parts of primary polyps. At least head and tentacles which are void of S-phase cells in these early metamorphic stages differentiate without passing through a further S-phase.

In *Hydra*, this is the part of the cycle in which under normal conditions stem cells are committed to nerve cell and nematocyte differentiation (Berking, 1979; Venugopal & David, 1981; Yaross et al. 1982; Fujisawa & David, 1982).

Nevertheless, metamorphosis is not a 1:1 transformation of larval into polyp tissue. Larval halves may produce complete primary polyps (Müller et al. 1977). Several low molecular weight factors that have been isolated from coelenterates have been shown to affect pattern formation during metamorphosis (Berking, 1986, 1987; Plickert, 1987). One of these factors substantially increases the formation of new nerve cells (Plickert, 1988). Thus it appears that at least part of the cells become newly committed during metamorphosis in response to a signalling system established in the transforming larva.

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References


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