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Competition-based head versus foot decision in chimeric hydras

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ABSTRACT The decision head versus foot in a regenerating fragment of *Hydra* has been proposed to result from long-range competition for resources such as head-specific precursor cells and hormonal factors, with the winning end of the body column forming the head and the losing end forming the foot. The present study presents new experimental support for this hypothesis. Chimeras prepared from two strains of *Hydra magnipapillata* with high and low capacity for head regeneration reveal that the low capacity of reg-16 to regenerate a head resides in a low ability to recruit head-promoting resources. When confronted with competing wt105 tissue taken from the same body region, reg-16 tissue is caused to form feet while wt105 is enabled to form enlarged heads which sometimes split into two. Triplet chimeras prepared with labeled donor animals and two competing unlabeled recipients indicate that head-forming wt105 tissue incorporates migrating cells more effectively than head-forming reg-16 tissue.

KEY WORDS: *pattern formation, regeneration, transplantation, chimera, Hydra magnipapillata*

Introduction

What are the basic activities of the organizing center "head" in a hydra? This question is the background to the present study, which aims at finding a partial answer by preparing chimeras of *Hydra* strains known to have high or low capacities to regenerate a head. The mutant strain reg-16 of *Hydra magnipapillata* is known to exhibit a low capacity when compared with strain wt105 of the same species in a standard test (Sugiyama and Fujisawa, 1977; Achermann and Sugiyama, 1985; Nishimiya *et al.*, 1986; Wanek *et al.*, 1986; Kobatake and Sugiyama, 1989).

Two explanations for this low ability of strain reg-16 to regenerate a head have been proposed: (1) polyps of reg-16 were supposed to generate low levels of "head activation" but high levels of "head inhibition" by producing small amounts of a supposed head-activating morphogen while releasing large amounts of a hypothetical head-inhibiting morphogen (Sugiyama and Wanek, 1993). (2) Alternatively, the high level of head inhibition in reg-16 was interpreted as a low level of resources, in particular of head-promoting factors, and the low level of head activation as low ability to make use of resources (Müller, 1995a). The present study uses chimeras to facilitate a decision between these alternative explanations and to elucidate fundamental organizing activities of the hydra's head.

Results

In chimeric animals the head of the regeneration-deficient strain reg-16 did not have a high but a low capacity to suppress budding and competitive head formation

Chimeric hydras were prepared with a reg-16 head and a wt105 trunk, and vice versa (Fig. 1). The chimeras were fed daily, and the rate of budding was scored over a period of 2-3 weeks. In addition, any abnormalities which appeared in this time period were observed and scored. The reg-16 head allowed the wt105 trunk to increase its budding activity (Fig. 1) and even allowed it to form a competitive head (Fig. 2). In the vicinity of such supernumerary wt105 heads the reg-16 tissue often formed a foot. This effect was even more conspicuous in the following experiment.

When 'strings of beads' are made with alternating wt105 and reg-16 rings, the wt105 rings form heads, while the reg-16 rings form feet

In an elegant experiment, Ando *et al.* (1989) prepared long body columns without developmental gradients by joining together ring-shaped pieces of the body column taken from the same body region of several donors. Using this experimental principle rings taken from the zone subjacent to the tentacle whorl of reg-16 and wt105 polyps were joined in an alternating manner. First an unstained reg-16 ring was strung onto the needle, then a vitally stained wt105 ring, then an unstained reg-16 ring and so forth. Six such tandems were prepared

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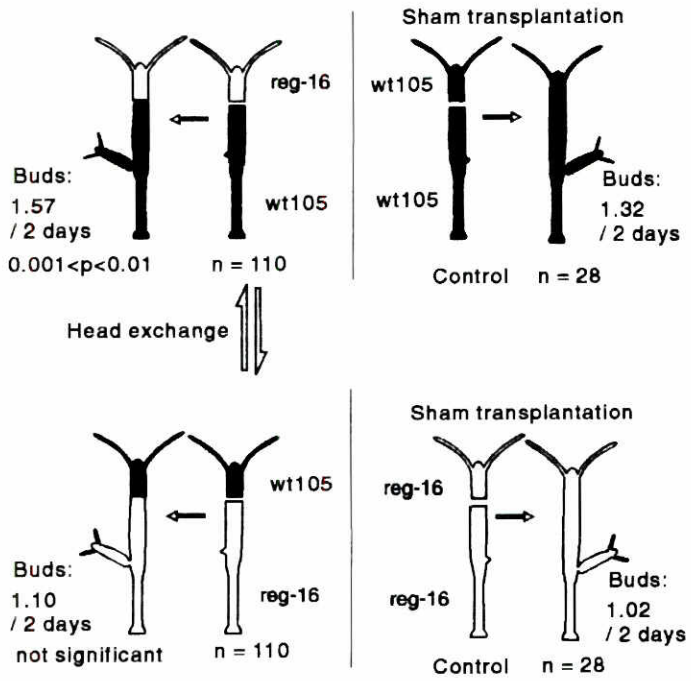

Fig. 1. Grafting procedure, here exemplified for the examination of budding activities under the influence of an alien head. *Controls were subjected to sham transplantations.*

with 3-5 wt rings and 3-5 reg-16 rings each.

In sum, 24 reg-16 rings were apposed to 24 wt rings. In the course of a week all wt105 rings developed tentacles while the reg-16 rings formed one or more feet (Fig. 3). About half of the reg-16 rings in addition formed a few tentacles as indicated in Figure 3. In such tandem grafts often the foot was ring-shaped and eventually caused the 'beads' in the string to separate at several positions.

Within a wt105 body a reg-16 ring is caused to form a foot as if it were taken from a lower position in the body

Hydra transplants form feet if their own positional value is substantially lower than that of their new surroundings (for references see accompanying paper). When rings were taken from a reg-16 body and inserted into a wt105 body column at a position corresponding to the same relative distance along the body as that from which they were excised, their effective positional value was lower than that of the host at this position: the reg-16 rings formed feet (Fig. 4). Below a foot-bearing ring, the host frequently formed an ectopic head. In control transplantations (n= 14) with reg-16 donors and reg-16 hosts the rings integrated unobtrusively without forming feet or causing morphological aberrations in the host when the relative position of the ring along the body column was not changed.

Downstream from a reg-16 ring, the basal end of a cut wild-type animal frequently forms a head instead of regenerating a foot

Twenty-eight grafts were performed with rings taken from the region subjacent to the budding zone of reg-16 donors and inserted into the midgastric region of wild type animals. One to two hours after grafting, the trunk of the host was bisected at a point half-way between the transplant and the budding zone. The lower body part of the host was discarded, so that the chimera consisted of wt105 head + upper gastric region, a reg-16 ring of low positional value and wt105 middle gastric region. If we subdivide the body column of a hydra in 10 positional values (p values), the entire chimera (Fig. 5) comprised the p values 10...7/4,3/76. In all 28 grafts a head instead of a foot was formed at the lower body end of the host, while the reg-16 ring formed a foot. Similar observations were made in *Hydra vulgaris*, which also formed a head instead of regenerating a foot downstream from a foot-bearing ring (see accompanying paper).

Upstream of a foot-forming ring reg-16 regenerated a head well

The mutant strain reg-16 is characterized by its low ability to regenerate a head in a standard assay (Sugiyama and Fujisawa, 1977). In this standard assay animals bearing a just-emerging bud are chosen. It has been shown that the presence of this emerging bud is the main cause for the reduced regenerative ability of the parental individual (Müller 1995a). A regenerating head and a developing bud compete for common resources. To reduce competitive interaction in the following experiments a ring with low positional value was inserted into the gastric region above the budding zone one to two days before decapitation. In this situation, reg-16 individuals regenerated a head well. By day 8 after decapitation the control heads had formed 3.7±2.1 tentacles in the presence of a competing bud, while the heads above the ring-shaped transplant had formed 7.9±1.8 tentacles ($p \leq 0.001$). Also the graft-bearing specimens produced buds, but these formed below the transplant. Apparently, the transplant formed a barrier preventing competition by buds.

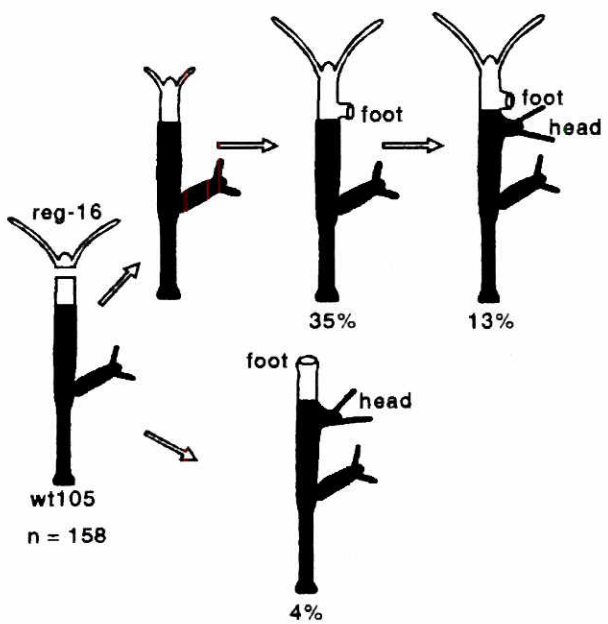

Fig. 2. Development of chimeras with a mutant head and a wild-type trunk. *Two hours after the head was grafted it was cut to initiate regeneration. The number of tentacles regenerated by the mutant head was highly variable (data not shown) in contrast to Figure 6.*

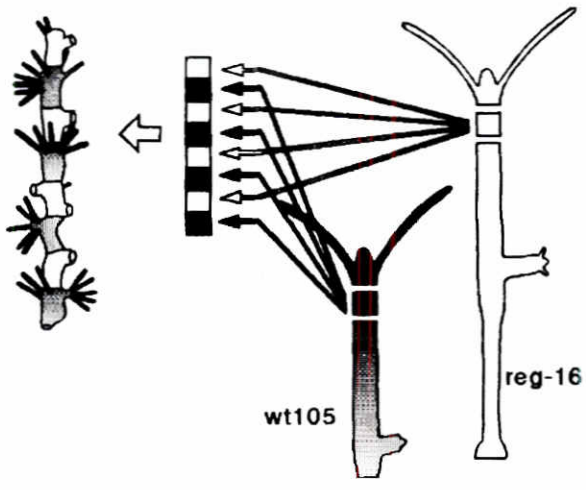

Fig. 3. 'Strings of beads' prepared from rings excised from the subtentacular zone of several reg-16 and wt105 donors.

In the following modification of the experiment not a young bud but a regenerating wild-type head plays the role of the competitor. A head plus upper gastric region (positional values 10...7) of a mutant was grafted onto the upper end of a mutant peduncle /3/, and a wild-type head onto its lower end. The complete combination was 10...7/3/7...10 . The following day, 15 to 18 h after grafting, the two heads were removed (remaining sequence 8,7/3/7,8) to start regeneration. For a control, the combination 10...7/7...10 was prepared and decapitated (final sequence 8,7/7,8).

In such combinations reg-16 inevitably forms a foot. However, the ring /3/ formed a foot very quickly within 12 to 36 h, while region 7 in the control grafts developed a foot only after 48 h. The results shown in Figure 6 document that the reg-16 trunk regenerates a head rapidly and with a normal number of tentacles even in the presence of a competing wild-type head, provided the competition is eliminated early by an inserted zone of low positional value (8,7/3/7,8 →10...1...10 in 1-2 days). If competition is interrupted only late by a foot-forming zone (8,7/7,8 →10...1...10 in 3-5 days), the quality of the head in the mutant partner improves with a corresponding delay.

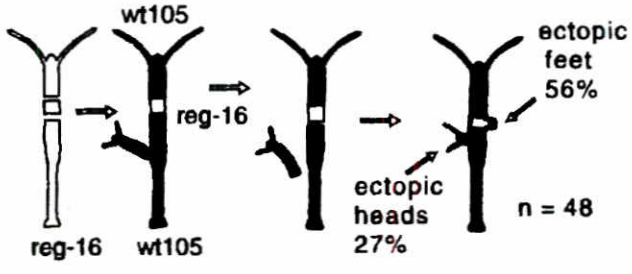

Fig. 4. A ring excised from reg-16 and inserted into a wild-type animal at the same relative distance along the body frequently forms a foot, while the wild-type trunk may form an ectopic head.

A wild-type head on top of a reg-16 trunk can increase its number of tentacles, enlarge and split into two heads

Interactions between reg-16 and wild-type tissue are mutual. While reg-16 tissue adjacent to wild-type tissue often forms a foot, the wild-type tissue can improve the quality of its head in terms of tentacle number (Fig. 7). In a significant number of cases the wild-type head enlarged and split into two or three (Fig. 8). Such an effect has previously been observed in diacylglycerol-treated *H. vulgaris* (Müller, 1995a) but not in *Hydra magnipapillata*, wt105.

Wild-type heads appear to have a greater ability to attract and incorporate movable cells than reg-16 heads

Upper body columns (10...7) were joined to a vitally labeled gastric region /6/; an unlabeled reg-16 column was linked to the one end of the labeled zone /6/, and an unlabeled wt105 column to the other end, yielding the graft 10...7/6/7...10. After the pieces healed together, the heads (region 10,9) were removed to initiate head regeneration on both sides of the labeled gastric region. The final combination thus was 8,7/6/7,8 (Fig. 9). In the following days the spread of blue pigments, indicating the movement of endodermal cells, or of fluorescent latex beads, indicating the movement of ectodermal cells, was observed.

The experiment was varied in two ways: in 50% of all cases the labeled ring /6/ was taken from labeled wt105 polyps, in the other 50% of cases the ring /6/ was taken from labeled reg-16 polyps.

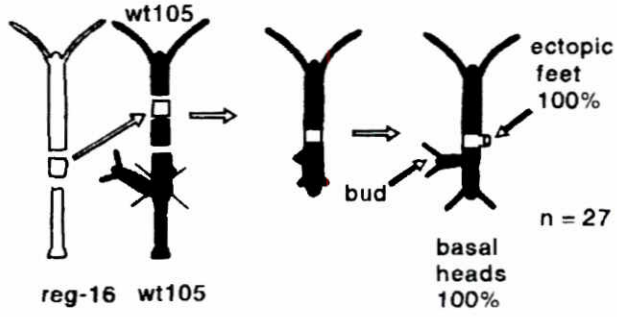

Fig. 5. Head formation at the basal end of wt105 polyps downstream from a foot-forming reg-16 ring.

Both these two groups were subdivided into two subgroups. In the first subgroup (25% of all grafts) the unlabeled reg-16 head was grafted at the upper end and the unlabeled wt105 head was grafted at the lower end of the labeled ring; in the other subgroup the positions were reversed (Fig. 9). About 24 h after grafting, cells began to emigrate from the labeled donor into the unlabeled partners. Endodermal cells and occasionally also fluorescent ectodermal cells moved as a coherent patch (Fig. 10A). More frequently, the fluorescent spots indicating ectodermal cells with phagocytosed fluorescent beads moved individually (Figs. 10C, 11) and were found even in tentacles as early as 1-3 days after grafting. In macerated cell preparations the vast majority of latex beads was seen incorporated in epithelial cells but occasionally fluorescent beads were also detected in nematoblasts and within battery cells apparently attached onto a nematocyst capsule (photos on request).

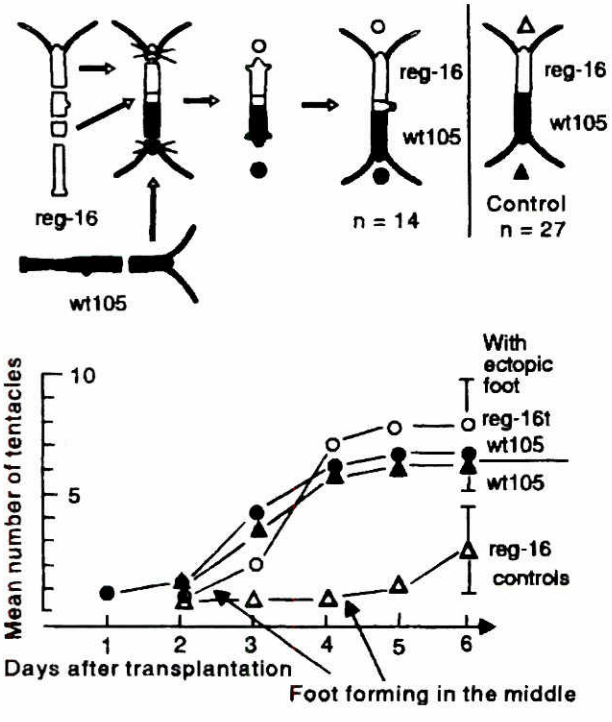

Fig. 6. Improvement of the capacity of reg-16 to regenerate a head by implanting a ring with low positional value to shield it from competition by the wild-type head.

The spread of labeled cells was directed preferentially toward the wild-type tissue, especially in the combination wt/wt/reg-16. However, two causes must be envisaged for this bias: (1) the two competing partners, wt105 and reg-16, might take up the labels at different rates, or (2) the accessibility of the two partners might be different.

Where it contacts wt105, reg-16 forms feet. If the labeled middle ring was wt105 (complete combination wt/wt/reg-16 or reg-16/wt/wt), a foot formed in the reg-16 partner frequently at, or close to, the wt105/reg-16 boundary. A zone developing a foot generally prevents or impedes the immigration of labels into the unlabeled partner, even if only a small lateral foot forms (Fig. 10B, and accompanying paper). If some labeled spots cross the boundary before a foot develops, these spots may quickly spread and even reach the tentacles within 1-2 days (Fig. 10C). Thus, the barrier built up by a foot-forming zone rather than an inferior ability to incorporate the labels might be the cause of the observed imbalance in the spread of labels in the wt/wt/reg-16 grafts. Therefore, the triplet chimeras were fixed with ethanol and examined as early as 48 h after grafting, and only those chimeras which were still footless were evaluated. The experimental design in these experiments was similar to that shown in Figure 9. However, the donor of the labeled cells was wt105 (previously labeled for 48 h) and the number of emigrated cells was as follows:

9.44 ± 8.6 fluorescent cells were found in the wt tissue,

3.08 ± 3.65 were found in the reg-16 tissue ($0.001 < p \leq 0.01$).

In the triplets reg-16/reg-16/wt or wt/reg-16/reg-16 the situation was much clearer. In most grafts a foot formed only after 48 h and the (lateral) foot appeared frequently in the middle of the labeled ring. Only those grafts with the foot forming in the middle of the labeled donor were evaluated (Fig. 9). Although both the reg-16 trunk and the wild-type trunk regenerated heads almost equally fast and with almost equal quality (because the foot-forming graft in the middle prevented direct competition) the spread of labeled spots, marking epithelial cells, was biased in direction of the wild-type head (Figs. 9, 11), even against the inherent polarity of the middle piece (Fig. 9).

Thus, under the same experimental conditions, the wild-type tissue incorporated more label (Fig. 11A) than the mutant tissue (Fig. 11B), irrespective whether the source of the label was wild-type or mutant tissue, and irrespective of the inherent polarity of the donor tissue.

Discussion

The low capacity of the mutant strain reg-16 of *Hydra magnipapillata* to regenerate a head has previously been ascribed to a high level of a putative head inhibition gradient (Achermann and Sugiyama, 1985; Nishimiya *et al.*, 1986; Wanek *et al.*, 1986; Sugiyama and Wanek, 1993). However, the present study with chimeras clearly shows that the head region of reg-16 is not a source of a particularly strong head-inhibiting activity. On the contrary, reg-16 even allowed ectopic competitive head formation by a wild-type trunk. In the vicinity of the reg-16 tissue, the wild-type was able to improve the quality of its head in terms of tentacle number and even split its enlarged head into two, while the reg-16

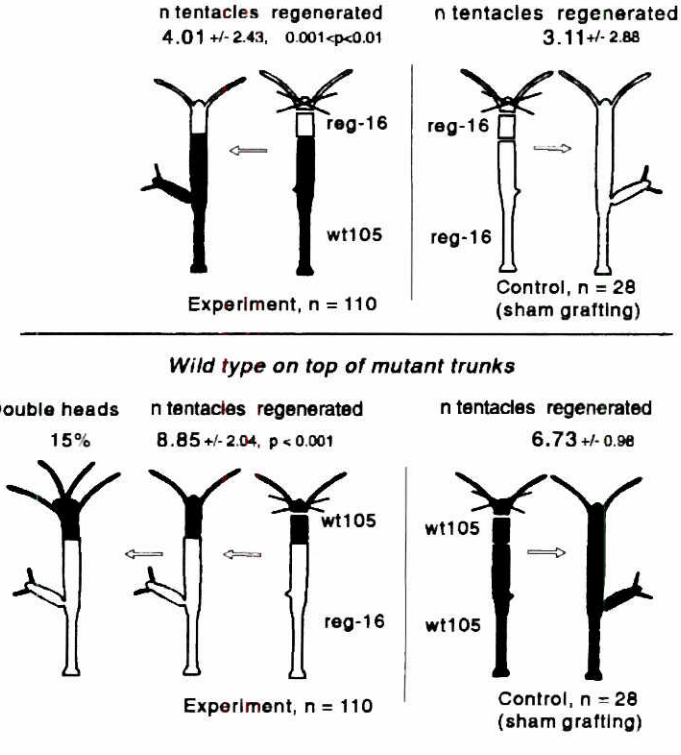

Fig. 7. Improved quality of a regenerating wild-type head on top of a reg-16 trunk. *Criterion of quality is the average number of tentacles per head. Note: even duplication of the head occurs, by the splitting of enlarged heads.*

Fig. 8. Wild-type head on top of a mutant trunk, being about to split into three heads.

tissue was caused to form feet. The results are well explained in terms of the "competition for resources" model (Müller, 1995a,b): the wild-type has a higher ability to attract moving precursor cells and to bind hormonal head-promoting factors than reg-16 tissue. Impoverished reg-16 tissue in turn is caused to form feet.

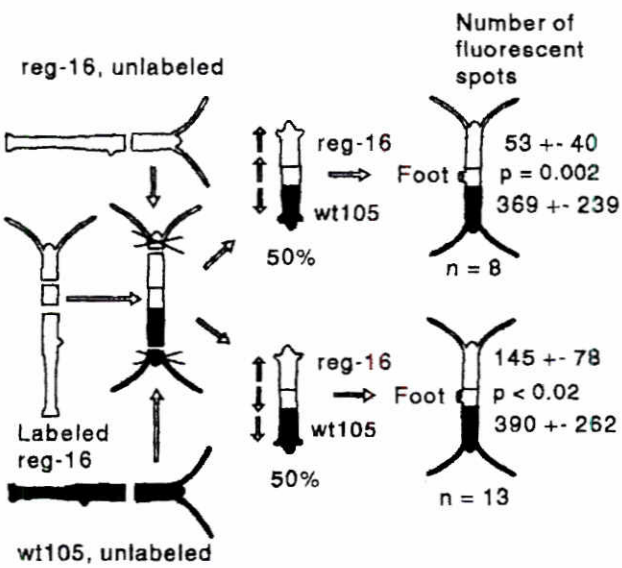

Fig. 9. Shift of label in chimeras. *Donor reg-16 polyps were prelabeled by incubating them in a suspension of fluorescent beads for 72 h (Materials and Methods). In a second experiment (not shown here) the donor was wt105. Triplet chimeras were prepared with a labeled donor gastric region in the middle and unlabeled head-bearing recipients at the ends. The day after, the heads of the two recipients were removed to initiate head regeneration and thus speed up the incorporation of precursor cells. Data shown concern the number of fluorescent spots found in the recipients 3 days after grafting.*

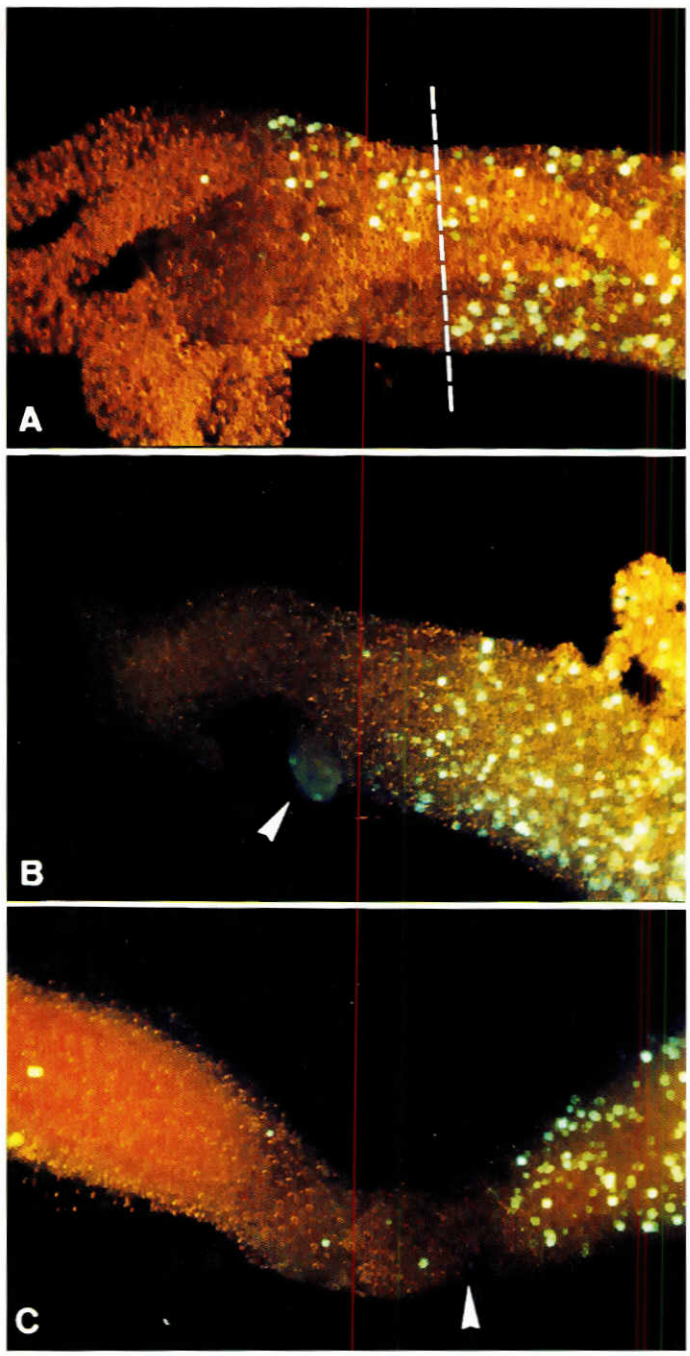

Fig. 10. Movement of label into unlabeled grafts. (A) *Example of a chimera in which labeled cells (initially) moved as a cohort. The broken line indicates the boundary between the originally labeled donor (wt) tissue and the originally unlabeled recipient (wt).* **(B)** *A foot-forming zone as a barrier to migrating cells. No labeled cells are present in the regenerating recipient, although only a small lateral foot (arrow) is present.* **(C)** *When the foot forms late, some immigration may occur. In the case shown, imminent foot formation (arrow) is indicated by constrictions of the body column.*

This interpretation has been proposed previously (Müller, 1995a). Nonetheless, this study provided some unexpected results and additional information. In particular, the enlargement and duplication of the wt105 head on the top of a reg-16 trunk and the formation

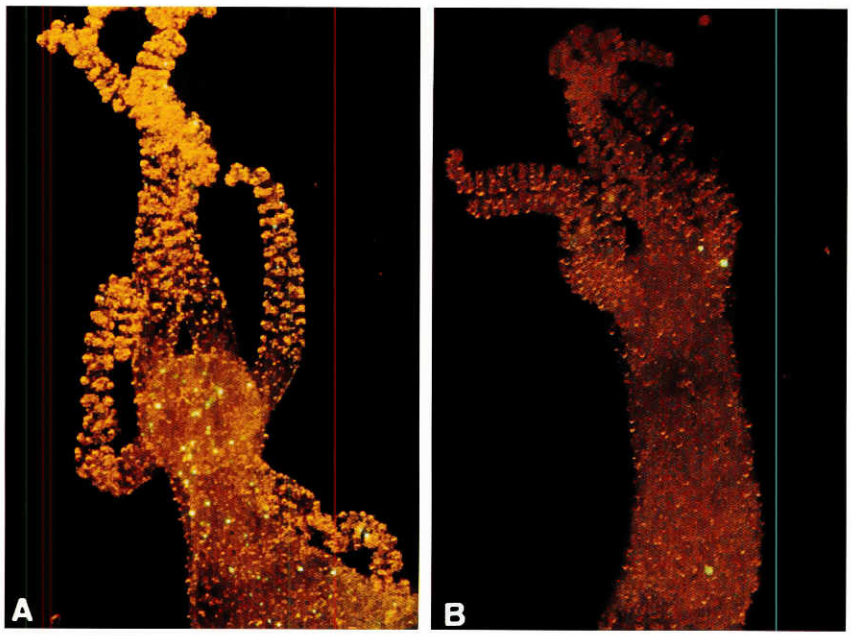

Fig. 11. Details of a triplet chimera showing the wild-type recipient (A) and the mutant recipient (B).

of heads at the lower body end, downstream from the (ring-shaped) reg-16 transplant, were not predicted. In hindsight, the appearance of heads at the basal end downstream from foot-bearing transplants and the resulting mirror-image body duplication of the entire body can readily be interpreted: like a ring of low positional value intercalated into the gastric region of *Hydra vulgaris* (see accompanying paper), a reg-16 ring inserted into the body column of wt105 acts as a physiological barrier or ligature. Downstream from this ligature, competitive head formation is no longer suppressed by the original head. On the other hand, upstream of such a barrier which separates the regenerating apical end from competing buds or a competing second head, reg-16 polyps form heads well.

The enlargement of the wild-type head on top of the reg-16 column is tentatively explained as follows: when a head was grafted onto a foreign trunk not only the head but also the subjacent zone was transferred. Thus, on top of the mutant trunk the wild-type head was in immediate contact with a short wild-type gastric segment. Perhaps the reg-16 trunk below bound and consumed less of the head-promoting factor produced in this wild-type segment. Factor not used by the trunk may have accumulated in the producing zone itself. The augmentation of factor might in turn have caused the commitment of more cells to head-specific differentiation. In the subtentacular zone precursors of head-specific cells are prepared (Hobmayer *et al.*, 1990; Javois, 1990).

A differential binding and uptake of factors is an essential feature of a model of pattern control in *Hydra* that is based on receptor-mediated competition (Sherratt *et al.*, 1995). By contrast, the extensive movement and migration of cells has not been taken into account in any of the hitherto proposed theories on pattern formation.

Ever since the basic features of cell movements in *Hydra* were described (Campbell, 1967a,b, 1973), most studies on cell movements have focused on cells of the interstitial cell lineages (Teragawa and Bode, 1990, 1991), with emphasis on the migration behavior of nerve cell precursors (David and Hager, 1994; Teragawa and Bode, 1995). Interstitial cells from the midgastric region preferentially migrate into the head, and it was proposed that the directional cue is provided by a chemical attractant (Teragawa and Bode, 1991).

The present study was designed to monitor the movement of epithelial cells. The labeling particles are incorporated by endodermal (Evans blue) or ectodermal (latex beads) epithelial cells through phagocytosis. However, a critical evaluation of the data must take several causes for the observed spread of labeled spots into account. Potentially, spread of the label might have one of three different causes: (1) particles might have been released from cells, in particular from cells undergoing apoptosis or necrosis; the particles might have been distributed in the extracellular spaces and taken up by other cells. (2) Although the beads are normally incorporated into epithelial cells (Technau and Holstein, 1992), occasionally migratory cells of the interstitial lineage also incorporate the label. In a few cases, the labeled cells were identified as nematoblasts (this study). (3) Occasionally, epithelial cells might transiently release themselves from contact with their neighbors and migrate individually as do metastasizing tumor cells.

Irrespective of the actual mechanisms by which the labels are transferred, the bias in the redistribution remains noteworthy. Cells moved preferentially in the direction of the wild-type tissue, or the wild-type tissue displayed a higher ability to incorporate released particles. Apparently, the wild-type head had a higher ability to recruit resources, either by attracting more migratory cells or by incorporating non-cellular resources at a higher rate.

Materials and Methods

Hydra magnipapillata, wild-type strain wt105, and the mutant strain reg-16, were kindly provided by Tsutomu Sugiyama (Mishima, Japan) and cultured under standard conditions (Müller, 1995a). The transplantation procedure is described in the accompanying paper. The subdivision of the body column in positional values ranging form 10 (mouth) to 1 (foot) is arbitrary and the scale is relative. For example, positional values 8 or 6 indicate the position at 80% or 60% body length with the foot end as point of reference.

Labeling of donor or host tissue

Labeling of donor or host tissue was performed (a) by feeding the animals with *Artemia* nauplii which in turn had been fed with particles of Evans blue (suspension of 5 mg Evans blue in 100 ml of salt water). Evans blue particles are taken up by endodermal epithelial cells through phagocytosis. (b) Alternatively, animals were labeled by incubation in a suspension of fluorescent latex beads (protocol by Technau and Holstein, 1992). The beads chosen were microspheres, 1 µm diameter, FITC-labeled (from Polysciences, Inc., Warrington, PA, USA). The suspension was 0.025% (w/v); incubation time was 48 h in the first and 72 h in the second experiment. The beads are also taken up through phagocytosis but predominantly by ectodermal epithelial cells.

Cell migration and tissue displacement

Cell migration and tissue displacement were inferred from the shift of vital stain or fluorescent spots from one partner across the boundary into the unstained partner in chimeras. Chimeras between labeled and unlabeled animals were no longer fed. Three to five days after grafting, the chimeras were anesthetized with 2% (w/w) urethane for 30 sec, fixed with 70% ethanol, embedded DABCO-glycerol and analyzed microscopically. The number of spatially separate labeled spots roughly reflects the number of migrated cells and does not coincide with the number of endocytosed beads because a single cell can engulf several beads simultaneously.

Identification of cell types

Types of cells labeled with fluorescent latex beads were determined in macerated cell preparations (David, 1973).

Statistics

Data were statistically evaluated using the Mann-Whitney test for means and the Fisher-Yates test for ratios.

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