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# **Genetic Regulation of Development**

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## 19. Interstitial Stem Cells in Hydra

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Interstitial stem cells in hydra constitute a population of multipotent stem cells that proliferate continuously during asexual growth of the organism while giving rise to a variety of differentiated somatic cell types (nerve cells, nematocytes, and gland cells). When asexual polyps initiate sexual differentiation, interstitial cells also give rise to germ cells, either sperm or eggs.

During the last decade there has been renewed interest in interstitial stem cells as a model system for analyzing the control of stem cell proliferation and differentiation. Of particular interest is the fact that stem cells exhibit strong patterns of cell differentiation within the organism: Nematocytes differentiate exclusively in the gastric region, while nerve cells differentiate primarily in the head and foot regions. Differentiation of germ cells is also spatially patterned: spermatogenesis in the distal gastric region; oogenesis in the proximal gastric region.

### DYNAMICS AND COMPOSITION OF THE INTERSTITIAL STEM CELL SYSTEM

Figure 1 outlines schematically cell proliferation and differentiation in the interstitial stem cell system. Figure 2 shows micrographs of interstitial stem cells and several differentiation products of stem cells as they appear in macerations of hydra tissue [David, 1973]. Interstitial cells occur in macerations as single cells or in larger clusters held together by cytoplasmic bridges [Slautterback and Fawcett, 1959]. We refer to these cells by their cluster size as 1s, 2s, 4s, and so forth. The 1s+2s class includes stem cells and early differentiating precursors.

The interstitial stem cell system constitutes an independent cell lineage in hydra that contributes about 75% of all cells in the organism. The remaining cells are contributed by two further cell types: ectodermal and endodermal epithelial cells. Recent experiments suggest that these two cell populations each constitute an independent cell lineage, since no evidence for intercon-

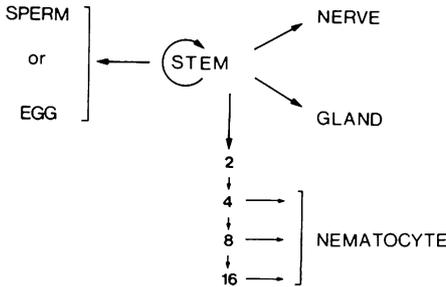


Fig. 1. The interstitial stem cell system.

version of ectodermal and endodermal cells has been observed (Gonzales and Tardent, personal communication). Thus, hydra consists of three independent cell lineages that appear to be related to the ectodermal, endodermal, and mesodermal lineages characteristic of metazoan animal tissue.

Hydra tissue grows primarily by asexual budding. The doubling time of well-fed cultures is about 3 days. Both ectodermal and endodermal epithelial cells proliferate with a cell cycle of about 3 days coincident with the tissue growth rate [David and Campbell, 1972; Bosch and David, 1984]. Interstitial cells, by comparison, have a cell cycle of about 1 day [Campbell and David, 1974] and thus proliferate more rapidly than epithelial cells. The interstitial cells, however, do not outgrow the epithelial cells, since, per interstitial cell generation, only about 60% of daughter cells remain stem cells, the remainder differentiate into nerve cells, nematocytes, and gland cells [David and Gierer, 1974; Schmidt and David, 1986] (also, Bode, Fujisawa, personal communication). With a self-renewal fraction of 60% ( $P_s = 0.6$ ) and a cell cycle of 1 day, the interstitial stem cell population and its differentiating products double in about 3 days, the same rate as the epithelial cells.

Interstitial cells are located primarily in the ectoderm of hydra where they are homogeneously distributed throughout the gastric region (Fig. 3). In the hypostome and subhypostomal region, as well as in a region adjacent to the basal disc, interstitial cells are present in very low levels. A sharp boundary separates these "empty" zones from the main population of interstitial cells. A small number of interstitial cells are located in the endoderm and have been termed *basal reserve cells*. Recent experiments, however, indicate that these cells, which have no capacity for self-renewal, are intermediates in

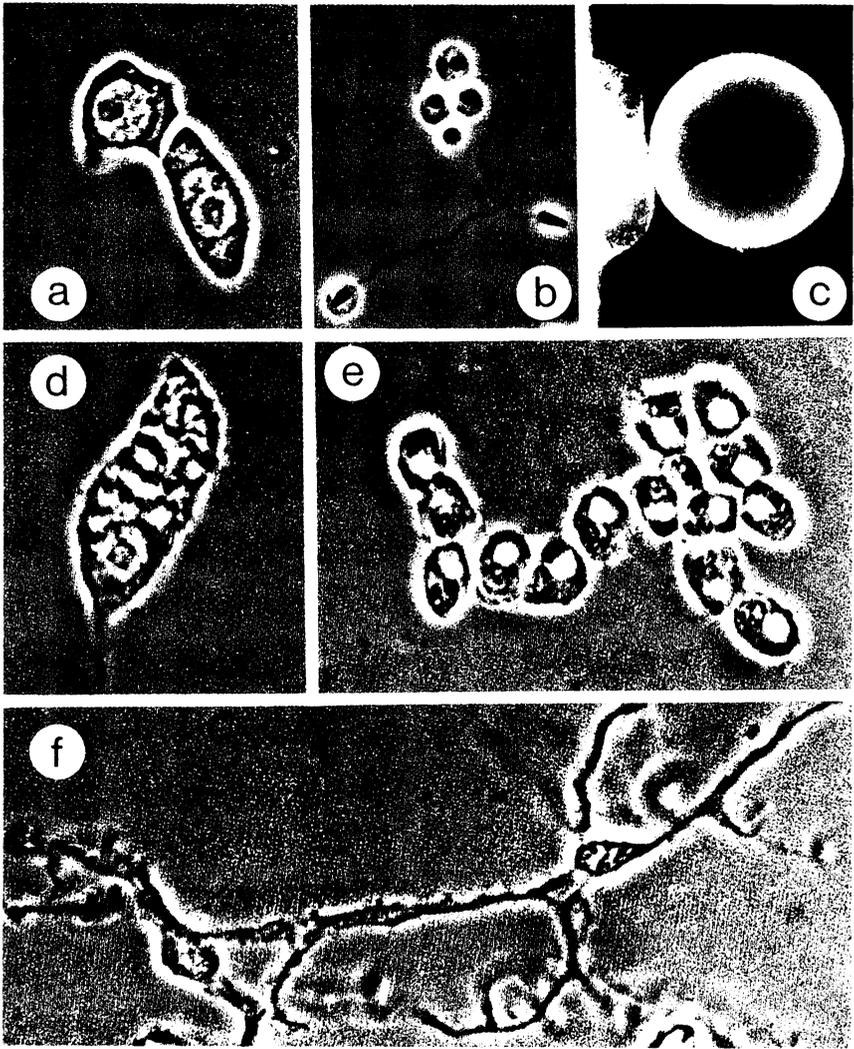


Fig. 2. Morphology of interstitial stem cells and differentiated derivatives. a: nest of two interstitial cells. b: Two sperm. c: Unfertilized egg (darkfield,  $\times 25$ ). d: Gland cells. e: Nest of 16 nematoblasts. f: Two nerve cells. Cells (a, b, d-f) were prepared by maceration of hydra tissue [David, 1973], (phase-contrast,  $\times 900$ ).

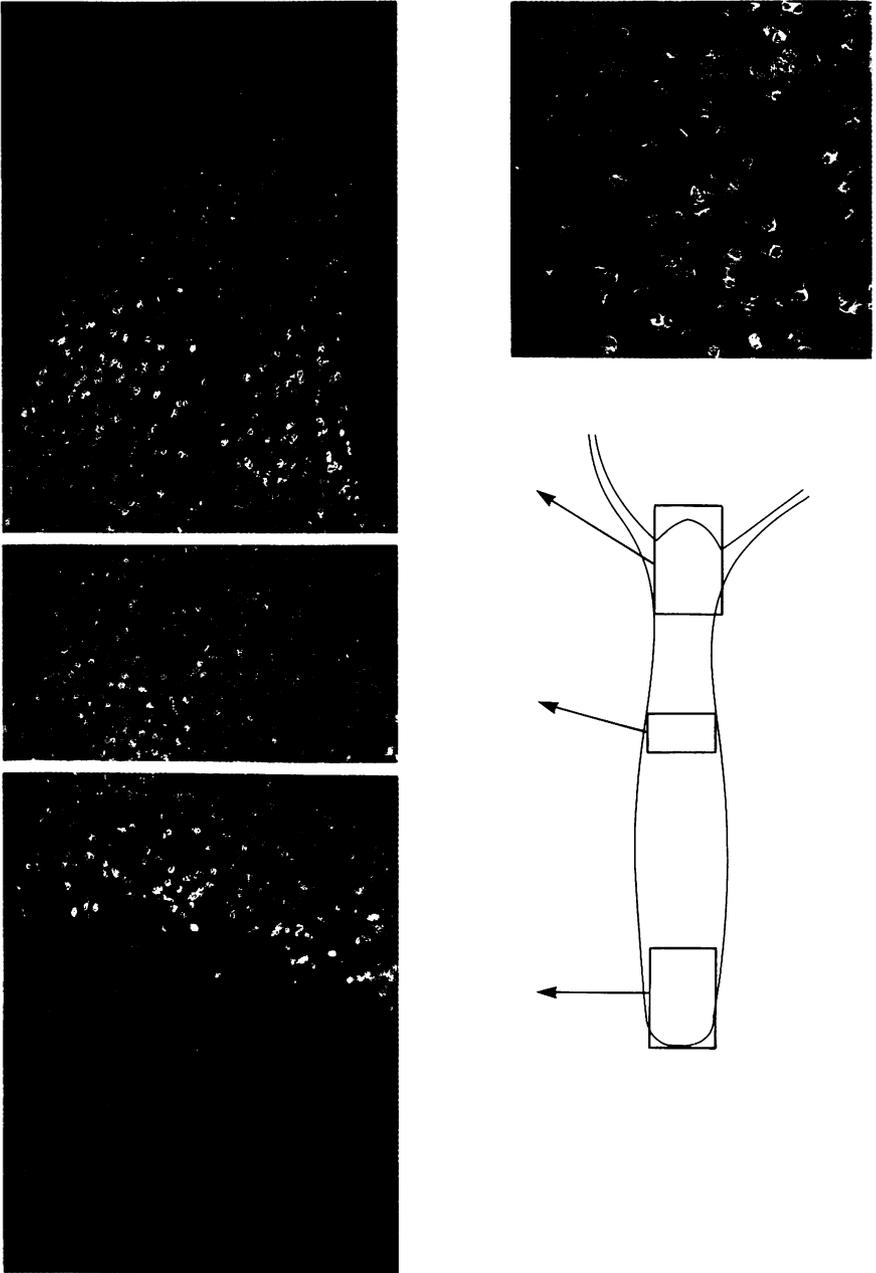


Fig. 3. Distribution of interstitial stem cells (1s+2s) in hydra. Stem cells were stained with a monoclonal antibody. Micrographs on left side show regions indicated in schematic outline of hydra ( $\times 50$ ). Micrograph at top right shows higher magnification view of interstitial cells in gastric region ( $\times 100$ ).

differentiation of nerve cells in the endoderm [Smid and Tardent, 1984, 1986].

Nematocyte differentiation is initiated by stem cell proliferation to form nests of four, eight, or 16 precursor cells, which are connected to each other by cytoplasmic bridges (Figs. 1, 2). Subsequently, all cells in a nest undergo synchronous differentiation into one type of nematocyte [Lehn, 1951; Rich and Tardent, 1969; David and Challoner, 1974]. Depending on nematocyte type, differentiation takes 2–3 days, after which nests break up into single cells that migrate from the gastric region to the tentacles. Mature nematocytes are mounted in battery cells of the tentacles. In large hydra containing 3,500 stem cells, about 1,750 stem cells per day enter the nematocyte pathway [David and Gierer, 1974].

Nerve cell differentiation occurs directly from the stem cell compartment (Fig. 1); there is no evidence of proliferation of nests of precursors prior to differentiation. Stem cells, which become committed to nerve differentiation, complete the cell cycle, divide, and the daughter cells differentiate as nerve cells [Venugopal and David, 1981a]. In large hydra containing 3,500 stem cells and 5,000 nerve cells, about 550 stem cells per day initiate nerve cell differentiation.

There is now clear evidence that interstitial cells in the ectoderm are capable of crossing to the endoderm and differentiating gland cells [Schmidt and David, 1986] (also, Bode, Fujisawa, personal communication). The number of interstitial cells differentiating gland cells is about 75 per day in polyps containing 1,300 gland cells and 2,200 stem cells. Hence, the level of differentiation represents only a small fraction of the stem cell population.

Differentiation of male and female gametes (Fig. 2) also occurs from the interstitial cell population. In both cases stem cells undergo a series of synchronous divisions to produce clusters of 16, 32, and 64 large precursor cells, which then undergo meiosis and differentiate sperm in male animals [Munck and David, 1985; Littlefield et al., 1985] or fuse to form the egg cell in female animals [Zihler, 1972].

### CLONING INTERSTITIAL STEM CELLS

Stem cells are defined by their capacity for extensive self-renewal. Under suitable growth conditions such cells form clones from single cells. Using cloning procedures, it has been possible to identify interstitial stem cells in hydra tissue. Because no tissue culture medium is available for hydra cells, the cloning experiments were carried out *in vivo* in host animals that lacked endogenous interstitial cells as a result of chemical treatment [David and

Murphy, 1977; Heimfeld and Bode, 1984a; Littlefield, 1985] or as a result of mutation [Bosch and David, 1986a]. Single stem cells added to such host tissue by a disaggregation-reaggregation technique grow rapidly and form clones containing stem cells as well as differentiating nerve cells and nematocytes (gland cells have not been analyzed in such clones).

Figure 4 shows schematically a cloning procedure designed to analyze germline and somatic differentiation in stem cell clones. Low numbers of wild type stem cells were added to host tissue of a mutant strain with temperature-sensitive interstitial cells. After reaggregation, the host interstitial cells were eliminated by incubation at the nonpermissive temperature (24°C) and the regenerated polyps analyzed with respect to their ability to self-feed (indicative of nerve cell and nematocyte differentiation) and to their sex (male, female, or asexual).

In 87 out of 92 cases cloned stem cells differentiated both somatic products and gametes. Thus, most interstitial stem cells are multipotent in regard to somatic as well as germline differentiation. Despite this broad multipotency, however, stem cells are restricted in terms of sexual phenotype; individual stem cells give rise to clones that differentiate either male or female gametes [Bosch and David, 1986b]. Female stem cells retain their female phenotype during prolonged clonal growth; male stem cells, however, give rise to female stem cells at a frequency of  $10^{-2}$  per cell per generation. Thus, male clones after several hundred generations contain significant numbers of female stem cells. These female cells do not differentiate because of an inhibitor produced by male cells [Bosch and David, 1986b; Sugiyama and Sugimoto, 1985; Littlefield, 1984], and thus clones of male cells exhibit only male gametogenesis.

The cloning experiment shown in Figure 4 not only identified the presence of multipotent stem cells in hydra but also demonstrated that stem cells with restricted differentiation capacity are rare, if present at all. In this experiment aggregates develop either as self-feeders or nonfeeders (Fig.4). Self-feeders, by virtue of their behavior, contain interstitial cells that differentiate nematocytes and nerve cells. Nonfeeders, by comparison, could in principle contain either stem cell clones with restricted differentiation capacity (and therefore aberrant feeding behavior) or no stem cells at all. Analysis of 140 aggregates with nonfeeder behavior indicated that 116 contained no stem cells. Of 24 aggregates with stem cells, seven recovered self-feeding behavior while 17 did not recover self-feeding behavior during a period of force-feeding to stimulate stem cell proliferation. These latter clones, however, did contain nematoblasts and hence were capable of somatic differentiation. (For technical reasons nerve cells in such aggregates could not be scored.) Al-

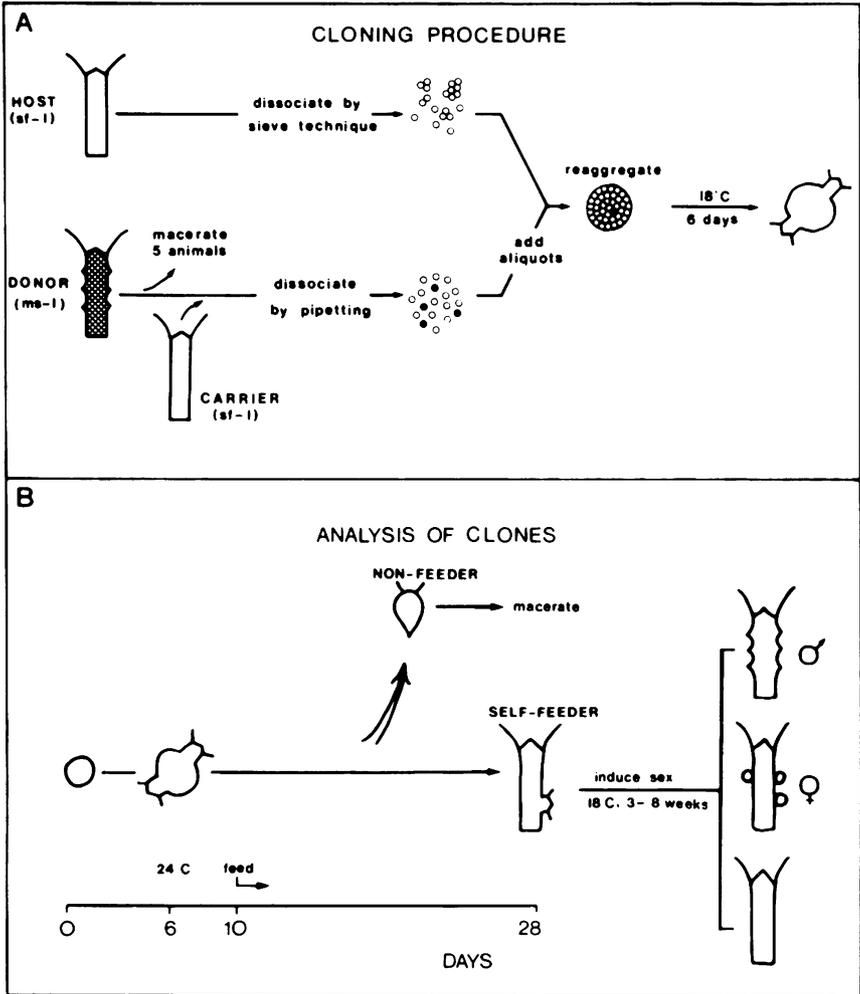


Fig. 4. Procedures for preparation (A) and analysis (B) of stem cell clones. Mutant strain *sf-1* containing temperature-sensitive interstitial cells was used as host; strain *ms-1* contains temperature-resistant interstitial cells but differentiates aberrant sperm. The mutant strains were isolated by Sugiyama and Fujisawa [1978].

though such aggregates could contain stem cell clones that are restricted to nematoblast differentiation, it seems unlikely that these clones are different from the seven "nonfeeder" clones that did recover self-feeding behavior and hence differentiated nerve cells and nematocytes. Thus, these "nonfeeder" clones also appear to consist of multipotent stem cells that, for unknown reasons, grow slowly.

Among 106 stem cell clones that were analyzed by the procedure in Figure 4, 92 developed self-feeder characteristics and thus contained stems capable of nerve and nematocyte differentiation. The remaining 14 clones occurred in aggregates that exhibited nonfeeder behavior. As discussed above, these clones appear to be, for unknown reasons, slow growers but otherwise multipotent. Thus, the cloning results indicate that most stem cells are multipotent. If stems cells with restricted differentiation potential exist, they occur at less than 1% of the frequency of multipotent stem cells.

One exception to the above rule has been observed by Littlefield [1985]. She "cloned" cells by treating hydra with hydroxyurea and then analyzed the properties of clones that grew out from the rare cells that survived the hydroxyurea treatment. Applying this technique to male polyps of *Hydra oligactis*, Littlefield observed numerous clones of stem cells capable of spermatogenesis and incapable of somatic differentiation. These cells were four to five times more numerous than multipotent stem cells capable of somatic as well as germline differentiation. Although the sperm-restricted stem cells have extensive self-renewal capacity under cloning conditions [Littlefield and Bode, 1986], these cells appear to be generated continuously in asexual animals [Littlefield, 1986], presumably from the multipotent stem cell population. They are thus part of a population that is turning over normally. Why such cells have retained the capacity for self-renewal is unclear at present; it is also unclear whether these cells express this capacity for self-renewal under normal conditions in vivo.

### STEM CELL PROLIFERATION: CELL CYCLE AND P<sub>s</sub>

Stem cell proliferation occurs throughout the body column of hydra. The rate of stem cell growth is closely matched to that of tissue (epithelial) growth so that over many generations of asexual growth the tissue has a constant number of stem cells. In asexual animals this equilibrium concentration is about 0.3–0.4 1s+2s per epithelial cell in the body column.

Because the stem cell population continuously gives rise to precursors for nerve cell, nematocyte, and gland cell differentiation, it must proliferate more rapidly, i.e., have a shorter cell cycle, than the epithelial cells. The

average epithelial cell cycle is about 3 to 4 days [David and Campbell, 1972; Bosch and David, 1984]. By comparison, the stem cell cycle is about 1 day [Campbell and David, 1974]. However, since only about 60% of stem cell daughters divide to yield stem cells (self-renewal), the stem cell population doubles in cell number at the same rate as epithelial cell population [David and Gierer, 1974] and thus maintains a constant ratio of stem cells to epithelial cells in tissue.

Because of the striking constancy of the stem cell density (ratio of stem cells to epithelial cells) in tissue during extensive asexual growth, it seems clear that the density must be regulated, most likely by stem cells themselves. Indeed, if the density of stem cells is reduced, e.g., by inactivation of a portion of the population [Bode et al., 1976; Heimfeld, 1985] or by reconstituting animals with experimentally lowered stem cell densities [Sproull and David, 1979a], it is possible to observe faster stem cell proliferation and thus restoration of normal levels of stem cells. Regulation appears to occur by changes in the rate of stem cell proliferation relative to that of epithelial cells.

In principle there are two mechanisms by which stem cell proliferation can be regulated: regulation of the cell cycle and regulation of the fraction of daughter cells that remain stem cells (the probability of self-renewal,  $P_s$ ). While doubling the rate of proliferation requires a twofold reduction of cell cycle, the same result can be achieved by increasing  $P_s$  from 60 to 70%.

Increases in stem cell growth rate of two- to threefold are commonly observed when stem cells proliferate at a stem cell densities five- to 50-fold lower than normal [Bode et al., 1976; David and MacWilliams, 1978; Sproull and David, 1979a]. To achieve such growth rates by changing the stem cell cycle would require shortening the cell cycle from 24 hr to less than 12 hr. Although cell cycle lengths do vary for hydra cells, such a large effect appears unreasonable, and, indeed, in the early experiments, no detectable changes in cell cycle occurred during recovery [Bode et al., 1976; David and MacWilliams, 1978; Sproull and David, 1979a]. Hence, it appeared that growth regulation occurred primarily by variation in  $P_s$ . Indeed, the expected decrease in the ratio of differentiating cells to stem cells was observed in such populations.

Although previous work in *Hydra attenuata* failed to show changes in the cell cycle of stem cells, recent experiments with *H. oligactis* have revealed clearly detectable changes in cell cycle associated with rapid stem cell proliferation (Holstein and David, unpublished results). When pieces of gastric tissue from normal animals are transplanted into the body column of hydra treated with nitrogen mustard to eliminate host interstitial cells [Diehl and Burnett, 1964], the donor interstitial cells spread out into the host tissue

and proliferate rapidly. The growth rate of the population is about twice the normal growth rate until the interstitial cell population recovers to normal levels. When the stem cell density is further reduced by grafting peduncle tissue in place of gastric tissue into the nitrogen mustard hosts, then the stem cell growth rate is even higher. Combining the results of a variety of such experiments it is possible to demonstrate that population doubling times vary from 1.3 to 3.5 days over a range of stem cell densities from 0.02 to 0.35  $1s + 2s$  per epithelial cell (Fig. 5). Higher or lower stem cell densities do not appear to have further effects on the doubling time.

Based on earlier results with *H. attenuata*, it appeared likely that these changes in growth rate were due to changes in  $P_s$ . However,  $^3\text{H}$ -thymidine labeling experiments indicated that the cell cycle also changes under these conditions. The labeling index following a  $^3\text{H}$ -thymidine pulse is 0.75 in rapidly proliferating cells and decreases to about 0.5 as the stem cell density increases and the growth rate decreases. This increased proportion of cell in S phase is also clearly detected in measurements of nuclear DNA content. Figure 6 shows a major shift of cells from  $G_2$  phase to S phase DNA levels in populations that are rapidly proliferating. Thus, the  $G_2$  phase is clearly decreased in length relative to S phase under conditions of rapid proliferation. From the results of continuous labeling experiments with  $^3\text{H}$ -thymidine it is possible to estimate that  $G_2$  phase is decreased from about 8 hr to about 3 hr in the rapidly proliferating stem cell population. (The slowly proliferating population consists primarily of committed precursors to gametogenesis and to nematocyte differentiation; Holstein and David, unpublished observa-

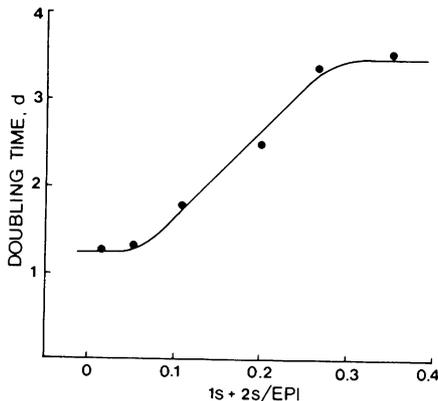


Fig. 5. Dependence of interstitial cell growth rate on the concentration of interstitial cells in tissue. Growth rate is expressed as the population doubling time (in days) of interstitial cells. Interstitial cell concentration is expressed as  $1s + 2s$  per epithelial cell.

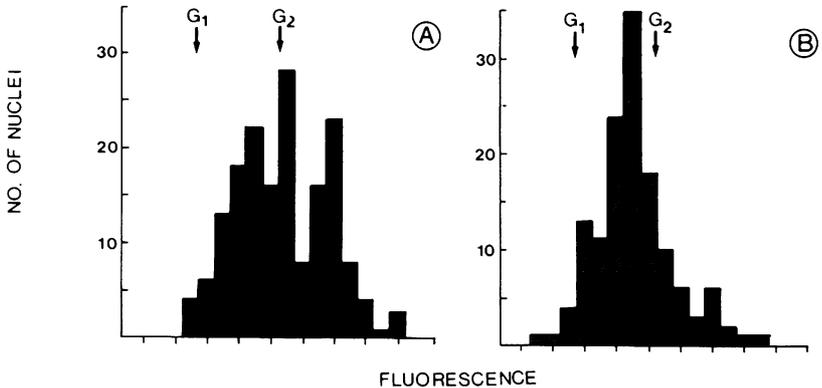


Fig. 6. Distribution of nuclear DNA contents in interstitial cells (1s+2s). DNA was determined microfluorometrically using the DNA-specific fluorochrome DAPI. **A:** Slowly proliferating cells. **B:** Rapidly proliferating cells.

tions). Assuming an S phase duration of about 12 hr [Campbell and David, 1974], then the cell cycle is about 16 hr under conditions of rapid proliferation and 21 hr under normal conditions.

The magnitude of the decrease in the cell cycle is too small to account for the two- to threefold increase in the growth rate of the stem cell population. Thus, as in earlier experiments with *H. attenuata*, it is necessary to postulate changes in  $P_s$  to explain the increase in growth rate of the stem cell population. Consistent with this expectation, the ratio of differentiating nematocyte presursors to stem cells (expressed as 4s/1s+2s) decreases in rapidly proliferating populations and increases with recovery of normal stem cell levels and lower stem cell growth rates. The magnitude of the decrease is consistent with similar observations in *H. attenuata* [Sproull and David, 1979b].

## NERVE CELL AND NEMATOCYTE DIFFERENTIATION

### Spatial Pattern of Differentiation

The most striking feature of nerve cell and nematocyte differentiation is the strong pattern of localization in the body column of hydra. Intermediates in the nematocyte pathway are homogeneously distributed throughout the gastric region but are absent in the regions subjacent to the hypostome and basal disc (Fig. 7). Intermediates in nerve cell pathway cannot be visualized at present, and thus it is not possible to localize the sites of nerve cell differentiation directly. Indirectly, however, it is possible to localize nerve differentiation by labeling the precursor population with  $^3\text{H}$ -thymidine and

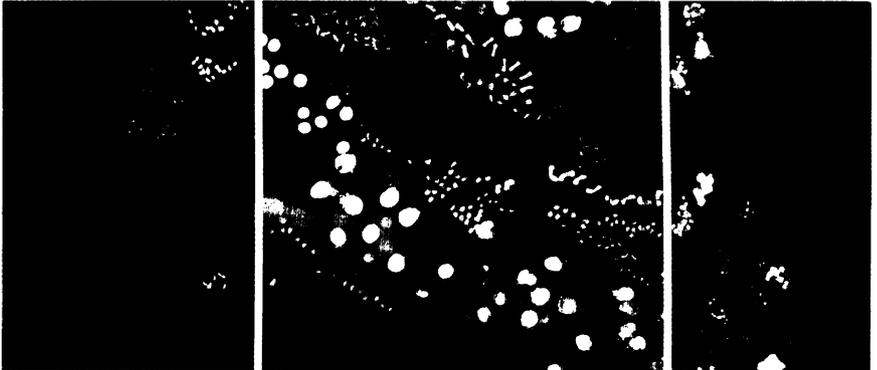
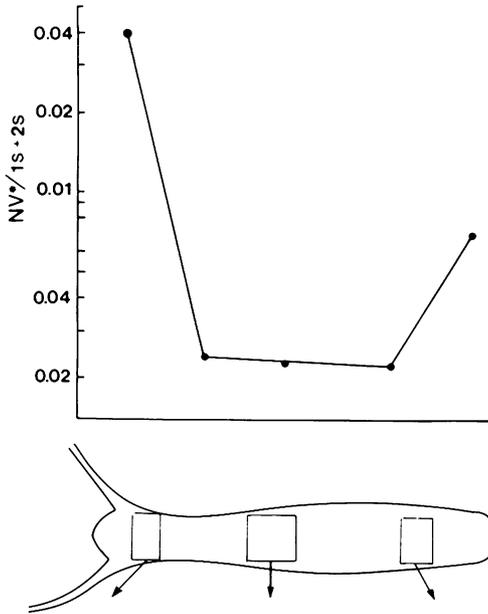


Fig. 7. Localization of differentiating nematoblasts (bottom) and differentiating nerve cells (top) in hydra. Nematoblasts were stained with a monoclonal antibody specific for differentiating capsules at a stage just prior to invagination of the tube. The antibody stains all four types of nematoblasts (stenoteles, desmonemes, holotrichous isorhizas, and atrichous isorhizas) at this stage. Such nematoblasts occur as clusters of  $2^n$  cells. Nerve cell differentiation is expressed as labeled nerves (NV\*) per interstitial cell (1s+2s) 24 hr after pulse labeling hydra with  $^3\text{H}$ -thymidine. Micrographs  $\times 150$ .

scoring the appearance of newly differentiated (labeled) nerve cells. Figure 7 shows the rate of nerve cell differentiation per interstitial cell (expressed as  $^3\text{H}$ -thymidine labeled nerve cells) at various positions along the body column.

### Role of Precursor Migration in Pattern of Nerve Cell Differentiation

Interstitial cell migration along the body column of hydra has been well documented [Tardent and Morgenthaler, 1966; Heimfeld and Bode, 1984b]. There is, however, as yet no clear evidence of the functional properties of the migrating cells, i.e., whether they are stem cells or differentiating precursors. The absence of such information has led to considerable speculation about the nature of migrating cells and their potential contribution to the pattern of cell differentiation. In particular, Heimfeld and Bode [1984a] have proposed that migrating cells include committed nerve cell precursors and thus that the observed pattern of nerve differentiation is the result of precursor migration rather than a pattern of nerve cell commitment.

In an attempt to investigate the role of migrating cells in nerve cell differentiation, Venugopal and David [1981a] labeled nerve precursors with  $^3\text{H}$ -thymidine and then compared the pattern of newly differentiated labeled nerve cells along the body column in intact animals and in animals that had been cut into pieces immediately following labeling. If nerve precursors migrate between S phase and time of differentiation then the two patterns will not be the same. The results showed quite clearly that the pattern of newly differentiated nerve cells was identical in both experiments. Hence no redistribution (migration) of nerve precursors occurs between S phase and differentiation of the precursors to nerve cells. Since additional experiments show that commitment to nerve cell differentiation occurs in S phase [Berking, 1979; Venugopal and David, 1981b; Yaross et al., 1982; Holstein and David, 1986], it appears that the pattern of nerve cell differentiation represents the pattern of nerve cell commitment in uncommitted stem cells.

The discrepancy between the above results and results indicating a contribution of migrating cells to the pattern of nerve cell differentiation [Heimfeld and Bode, 1984a] led us to carry out another experiment to assay the contribution of cell populations from various positions in the body column to nerve cell differentiation in the head. To do this we prepared grafts in which various parts of body column of *H. attenuata* were labeled with  $^3\text{H}$ -thymidine (see Fig. 8) and scored the differentiation of labeled nerve cells in the head at defined intervals thereafter. The results in Figure 8 show that after 1 day all newly differentiated nerve cells in the head are derived from labeled precursors in the distal half; labeled precursors in the proximal half make no contribution to new nerve cells in the head. However, the increase in labeled

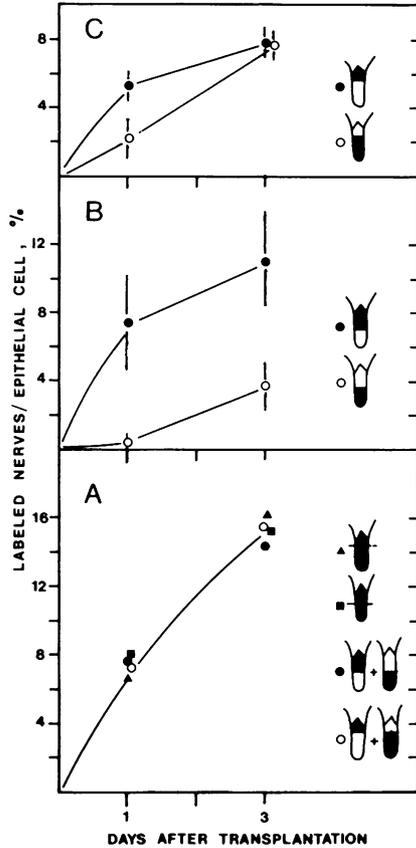


Fig. 8. Migration of nerve cell precursors. Hydra were labeled with  $^3\text{H}$ -thymidine (filled symbols) and then grafted to unlabeled hydra (open symbols) as shown schematically. The head tissue was excised from grafts 1 and 3 days after grafting, macerated, autoradiographed, and scored for labeled nerve cells. **A:** Total number of labeled nerve cells in heads of grafted and control animals. **B:** Distal half grafted to proximal half; **C:** Distal one-quarter grafted to proximal three-quarters.

nerve cells in heads is the same between 1 and 3 days in graft combination irrespective of whether the distal or proximal half is labeled. Thus, the precursor pool in *H. attenuata* must be completely mixed throughout the body column after about 1 day in graft combination. Grafts of the distal quarter to the proximal three quarters support this conclusion, since the increase in labeled nerve cells between 1 and 3 days is directly proportional to the amount of labeled tissue. Since there is no migration of precursors

between the times of commitment (in S phase) and differentiation [Venugopal and David, 1981c], the migrating population appears to consist of uncommitted stem cells.

**Signals Controlling Nerve Cell Differentiation**

The striking localization of nerve cell and nematocyte differentiation together with the evidence that stem cell precursors are locally committed to nerve or nematocyte differentiation has stimulated attempts to identify endogenous factors that control stem cell commitment and that are localized in particular regions of hydra tissue. To date our efforts have concentrated on nerve cell commitment. To assay for committed nerve cell precursors, we have used the procedures outlined in Figure 9. The assays, which are modifications of the stem cell cloning procedure (Fig. 4), are based on the premise that committed nerve precursors will continue differentiation when removed from an environment causing nerve cell differentiation. Under such conditions, uncommitted cells will not differentiate nerve cells; rather, they will proliferate to form more stem cells.

To assay for putative committed nerve cells, tissue was dissociated and the cells were transferred to a culture system consisting of reaggregated hydra cells (Fig. 9A). Donor tissue was labeled with <sup>3</sup>H-thymidine to distinguish it from host tissue. Most of the transferred cells end up in areas of the aggregate that form gastric tissue during regeneration. Since gastric tissue does not stimulate nerve cell differentiation, any precursors that continue

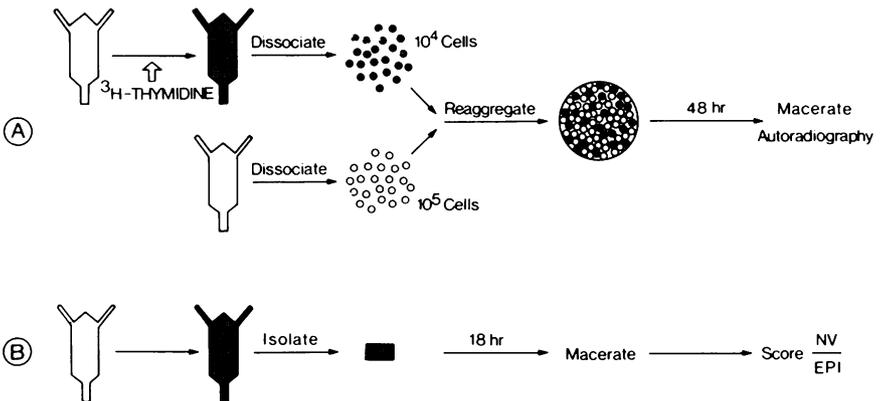


Fig. 9. Procedures for assaying committed nerve cell precursors by reaggregation (A) and by explanation (B). Hydra were treated with extracts or head activator and analyzed for committed nerve cell precursors. Before treatment open symbols; after treatment, closed symbols.

differentiation under these conditions are defined as *committed* [Venugopal and David, 1981a; Yaross et al., 1982]. Uncommitted stem cells form clones under these conditions (cf. Fig. 4).

Because the dissociation/reaggregation procedure is time consuming, we have also developed a simplified explantation technique that achieves the same effect (Fig. 9B). Tissue pieces are explanted from hydra and simply incubated in hydra medium. Such pieces round up and regenerate. Since most of the tissue in the regenerate is gastric region (as in the aggregates), this procedure also tests the ability of nerve cell precursors to differentiate in the absence of signals stimulating nerve cell differentiation. Committed nerve precursors differentiate to nerve cells in such explants in about 18 hr and can be scored.

To identify factors that stimulate nerve cell commitment in hydra, animals were treated with extracts of hydra tissue and then cells or tissue pieces were isolated from treated animals and incubated to permit differentiation of committed precursors. Figure 10B shows that methanol extracts (containing peptides and low molecular weight molecules) of hydra tissue at concentrations of 0.25–0.5 hydra/ml contain a factor that stimulates nerve cell differentiation. Further purification of this activity led to its identification as a peptide that had been purified previously from hydra tissue by Schaller [1973] on the basis of its ability to stimulate head regeneration. The peptide is called the *head activator*. Its sequence has been determined (pGlu-Pro-Pro-Gly-Gly-Ser-Lys-Val-Ile-Leu-Phe), and synthetically prepared peptide has been shown to stimulate head formation [Schaller and Bodenmüller, 1981].

Synthetic head activator stimulates nerve cell commitment in the assay system at a concentration of  $10^{-13}$  M (Fig. 10A). Higher concentrations are also effective but do not increase the yield of committed precursors. Determination of the head activator concentration in methanol extracts indicated that such extracts, at concentrations at which they are maximally active, also contain  $10^{-13}$  M head activator. Thus, the activity of methanol extracts can be completely accounted for by their content of head activator. As expected, treatment of methanol extracts with anti-head-activator antibody quantitatively removed the differentiation stimulating activity [Holstein et al., 1986].

The high rate of nerve cell differentiation in head tissue (Fig. 7) and the ability of head activator to induce nerve differentiation (Fig. 10A) suggest that head activator is concentrated in head tissue and that it may be the endogenous signal that induces nerve differentiation in this tissue. Although this simple model is attractive, recent experiments indicate that it is probably too simple. Using a monoclonal antibody (Nv-1) it is possible to identify a specific subpopulation of nerve cells in tentacles (Schmidt et al., unpublished

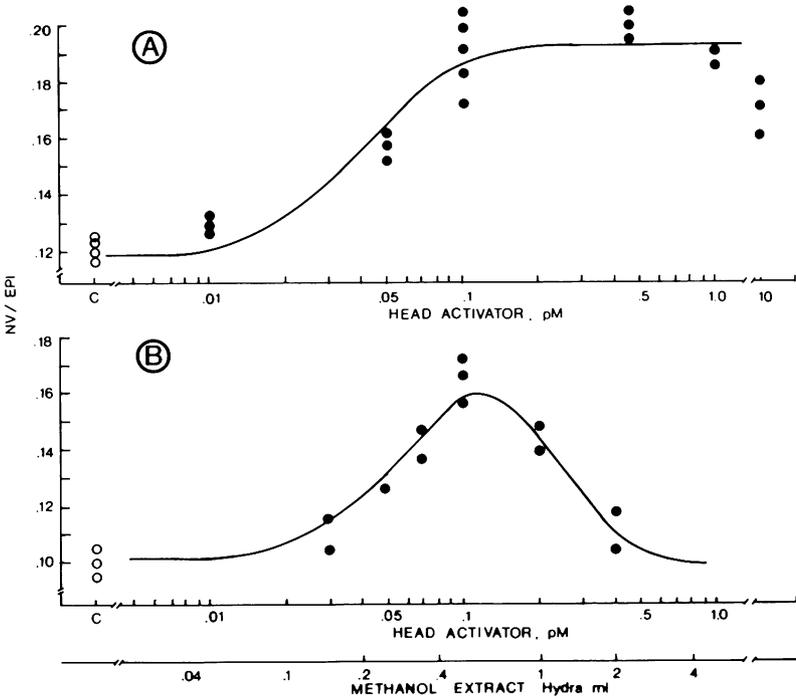


Fig. 10. Stimulation of nerve cell commitment by synthetic head activator (A) and methanol extract (B). Hydra were treated with methanol extract or head activator for 18 hr and then pieces of gastric tissue explanted to hydra medium for 18 hr. Explants were macerated and scored for nerve cells and epithelial cells (NV/EPI). The concentration of head activator in methanol extract was determined by radioimmunoassay [Bodenmüller and Zachmann, 1983].

observations). Nv-1 nerve cells are not present in the hypostome or body column, while there is small concentration of such cells near the basal disc. We have used Nv-1 to test for tentacle-specific nerve differentiation in tissue that was treated with head activator. Hydra were incubated with head activator and isolated pieces assayed for Nv-1-positive nerve cells using the standard procedure shown in Figure 9B. Although increased numbers of nerve cells differentiate in such pieces 12 hr after isolation, none of them are Nv-1 positive. Nv-1-positive cells appear in treated pieces after 2-3 days at about the same time as they appear, as a result of regeneration, in untreated control pieces. Thus, although head activator induces nerve cell differentiation, it is not the signal that determines the tentacle-specific quality of nerve cells. What the signal is remains unclear at present. However, it appears to be

associated with tentacles, since Nv-1 cells are only present in tentacles and not in other parts of head tissue.

## PROSPECTS FOR THE FUTURE

Interstitial stem cells are of particular interest to developmental biologists because of their complicated repertoire of possible differentiation pathways and because of the fact that at least some of these differentiation decisions are controlled by positional signals. The clear demonstration that stem cells in adult hydra are multipotent means that stem cell decisions (commitment events) for particular differentiation pathways are accessible to the experimenter and not hidden in the depths of early ontogeny. The fact that molecular signals, at least in the case of nerve cell commitment, have now been identified encourages the hope that more can be learned soon about signals and signal-processing systems involved in commitment events. Finally, the fact that nerve cell commitment appears to be controlled by the same positional signals that control other features of hydra morphogenesis suggests that learning more about nerve cell commitment may advance our knowledge of positional information in hydra.

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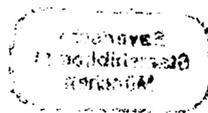
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