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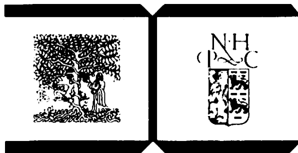

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CONTROL OF STEM CELL PROLIFERATION IN HYDRA ATTENUATA

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INTRODUCTION

Interstitial stem cells in Hydra constitute a rapidly proliferating population of cells which continuously gives rise to differentiated nerves and nematocytes.[1] Under conditions of assexual growth 60% of stem cell daughters divide to yield more stem cells (self-renew), 30% differentiate nematocytes and 10% differentiate nerves per stem cell generation.[2] The interstitial cell system makes up 70% of total Hydra cells;[3] about 4% are stem cells.[2]

Analysis of the stem cell system has been facilitated by the maceration technique[4] for dissociating tissue quantitatively into single cells or small clusters in the case of interstitial cells and nematoblasts held together by cytoplasmic bridges.[5] By combining appropriate pulse and continuous ^3H-thymidine labeling techniques with tissue maceration it has been possible to determine the cell cycle and differentiation kinetics of stem cells, differentiating nematoblasts and differentiating nerves.[3,6] The results are shown schematically in Fig. 1. Stem cells proliferate with a 24 hr cell cycle. Stem cells committed to nematocyte pathway divide several times (18 hr cell cycle) to yield nests of 4,8 or 16 nematoblasts; each cell in a nest differentiates

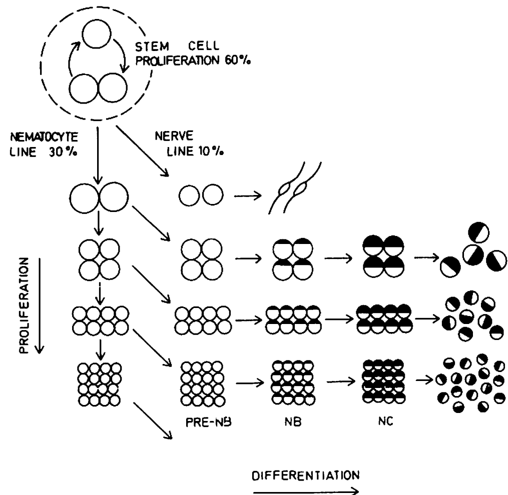


Fig. 1. Schematic representation of stem cell proliferation and nerve and nematocyte differentiation in Hydra.

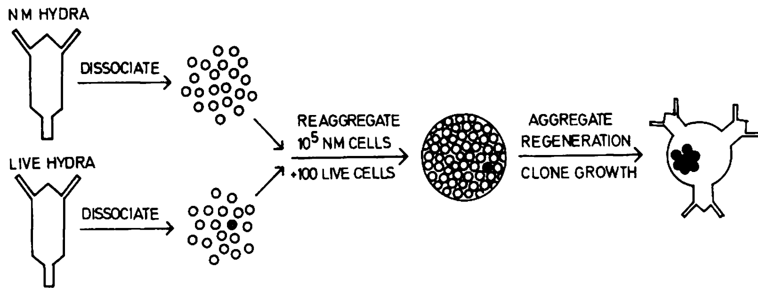


Fig. 2. Procedure for cloning stem cells in (●) in NM aggregates.

a nematocyte capsule in 2-3 days. Stem cells committed to nerve pathway divide once and both daughters cells differentiate as nerves in about 6 hours.

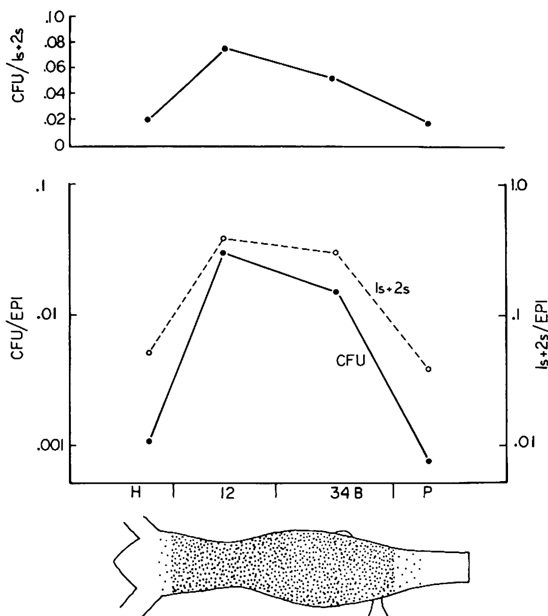
CULTURE OF STEM CELLS IN FEEDER LAYERS

To investigate factors affecting stem cell proliferation and differentiation we have developed a technique for culturing stem cells in feeder layers of nitrogen mustard (NM) inactivated Hydra tissue.[7] NM treatment eliminates interstitial cells from Hydra tissue[8] and thus provides an empty host in which growth and differentiation of added interstitial cells can be followed. Interstitial cells are added to feeder layers using the aggregation technique[9] shown in Fig. 2. Normal Hydra and NM treated Hydra are dissociated to single cell suspensions in cell culture medium. Aliquots containing 10^5 NM cells and a small number of normal cells are mixed together and centrifuged. The cell pellets are then incubated during which time they regenerate normal Hydra structures. Stem cells added to the NM aggregates proliferate and differentiate normally. Individual stem cells form clones which grow to contain several hundred cells after 1-2 weeks.[7] NM aggregates are easily manipulated and provide a versatile technique for culturing and analyzing stem cells and stem cell differentiation. Several examples are given below.

MULTIPOTENCY OF STEM CELLS

The ability to clone stem cells in NM aggregates has allowed a rigorous test of the differentiation potential of individual stem cells. When clones derived from single stem cells were examined, all were found to contain both differentiated nerves and differentiated nematocytes.[7] No clones were found which contained only nerves or nematocytes. Thus, the stem cell population is homogeneous and multipotent with regard to nerve and nematocyte differentiation.

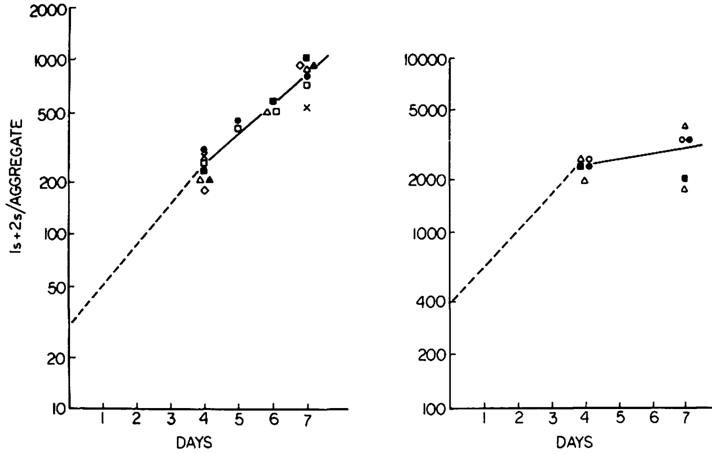
Fig. 3. Distribution of stem cells and ls+2s in Hydra. Stem cells were determined as clone-forming units (CFU) in NM aggregates. About 13% of stem cells yield clones under these conditions. ls+2s are a morphological class of large interstitial cells occurring as single cells or in pairs. ls+2s consist of stem cells and early committed precursors to nerve and nematocyte differentiation (Fig. 1). The concentration of stem cells and ls+2s is expressed per epithelial cell (EPl).



DISTRIBUTION OF STEM CELLS IN HYDRA

Using the NM culture system we have found that stem cells are uniformly distributed along the body column in the gastric region and upper peduncle (Fig. 3).[10] The concentration of stem cells, expressed as clone-forming units (CFU) per epithelial cell, is about 0.02. In the hypostome and basal disk, however, the concentration of stem cells is 20-fold lower consituting only about 0.001 CFU/epithelial cell.

Stem cells and early committed nerve and nematocyte precursors constitute a morphologically distinct class of interstitial cells which occur as single cells and in pairs (see Fig. 1). We refer to this class as ls+2s. The distribution of ls+2s in Hydra is similar but not identical to that of stem cells. In particular, the ratio of stem cells/ls+2s drops in the hypostome and basal disk compared to the gastric region (Fig. 3) indicating an increase in these regions in early committed cells. Changes in the proportions of stem cells and early committed cells result from changes in the proportion of stem cells which self-renew versus differentiate. From the observed decrease in the CFU/ls+2s ratio (Fig. 3) it is possible to estimate that the fraction of stem cells undergoing self-renewal in the hypostome and basal disk has decreased to $<10\%$ compared to 60% in the gastric region (see below).



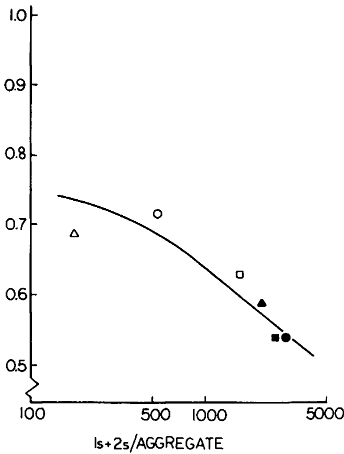


Fig. 4. Growth of interstitial cells in NM aggregates. NM aggregates were seeded with 30 or 400 CFU. After 4-7 days incubation, aggregates were macerated and scored for 1s+2s.

Fig. 5. Dependence of self-renewal probability (P_s) on interstitial cell density in NM aggregates. P_s was calculated from the growth rate of interstitial cell populations as shown in Fig. 4.

CONTROL OF STEM CELL POPULATION GROWTH

The growth of stem cell populations depends on both the cell generation time and the fraction of daughter cells remaining stem cells (the self-renewal fraction, P_s). Several independent experiments have now made it clear that regulation of stem cell growth in Hydra occurs by changing P_s.[11,12,13] In all these experiments the length of the stem cell generation was not observed to change significantly. These experiments also suggested that the parameter controlling P_s was the density of stem cells in tissue. We have now confirmed this directly using the NM culture system. Fig. 4 shows that the growth rate of stem cell populations (scored as 1s+2s) in NM aggregates depends on the

number of stem cells seeded.[13] When 30 stem cells are seeded per aggregate, the growth rate is 4-fold faster than when 400 stem cells are seeded per aggregate. The value of P_s can be directly calculated from the doubling time. Fig. 5 shows the dependence of P_s on stem cell density in NM aggregates. As stem cell density increases in feeder layers, P_s decreases from 0.7 to 0.5.[13]

MODEL FOR CONTROL OF STEM CELL PROLIFERATION

A model for the control of stem cell proliferation must explain the growth as well as the distribution of stem cells in *Hydra*. The observed dependence of P_s on stem cell density in tissue (Fig. 5) indicates that P_s is regulated by negative feedback from neighboring stem cells. Because stem cells are spread out in tissue at some distance from each other, the feedback signal appears to be mediated by a diffusible factor secreted by stem cells and to which stem cells are also sensitive.[12] Low stem cell concentration leads to low factor concentration and high P_s; high stem cell concentration leads to high factor concentration and low P_s. Such a model will regulate the stem cell concentration to a specific level since low concentrations will raise P_s and increase the population growth rate while high concentrations will lower P_s and decrease the population growth rate. Thus, the model explains the observed homeostasis of stem cell population density in *Hydra*.[3,14]

The negative feedback model predicts that stem cells should fill all available ectodermal space uniformly. Any areas of low stem cell density will have locally higher P_s and tend to fill up. This prediction agrees well with the observed uniform stem cell concentration throughout the gastric region. It does not, however, explain the depletion of stem cells in hypostome and basal disk (Fig. 3). Thus, in hypostome and basal disk other factors in addition to stem cell density must affect P_s.

Nerve differentiation is localized in the hypostome and basal disk.[2,15,16] These same regions are depleted in stem cells and enriched in early committed cells. The simplest interpretation of these observations is, therefore, that locally enhanced nerve differentiation effectively removes stem cells from the self-renewal pathway. If this interpretation is correct, it is the first direct evidence that self-renewal and differentiation compete for the *same* target stem cell population.

In summary, the results indicate that stem cell growth in the gastric region is regulated by negative feedback from neighboring stem cells. Expansion of the epithelium due to proliferation of epithelial cells spreads stem cells apart, thereby lowering stem cell density and increasing P_s. Growth of the stem cell population then fills in the gaps to maintain a uniform density of

stem cells. Stem cells carried into hypostome and basal disk by epithelial tissue movements[17] are forced to differentiate nerves by morphogenetic signals localized in these regions.[1,15] Extensive nerve differentiation essentially eliminates the stem cell population from the epithelium as it moves into the hypostome and basal disk, thereby creating the empty zones observed there.

ACKNOWLEDGEMENTS

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