Roux's Arch Dev Biol (1991) 200:269–276

Roux's Archives of
Developmental
Biology

Role of the cellular environment in interstitial stem cell proliferation in *Hydra*

Thomas C.G. Bosch, Rebecca Rollbühler, Birgit Scheider, and Charles N. David

Zoological Institute, University of Munich, Luisenstrasse 14, W-8000 München 2, Federal Republic of Germany

Received June 20 / Accepted in revised form July 25, 1991

Summary. The role of the cellular environment on hydra stem cell proliferation and differentiation was investigated by introduction of interstitial cells into host tissue of defined cellular composition. In epithelial tissue lacking all non-epithelial cells the interstitial cell population did not grow but differentiated into nerve cells and nematocytes. In host tissue with progressively increased numbers of nerve cells growth of the interstitial cell population was positively correlated to the nerve cell density. In agreement with previous observations (Bode et al. 1976), growth of the interstitial cell population was also found to be negatively correlated to the level of interstitial cells present. The strong correlation between the growth of the interstitial cell population and the presence of interstitial cells and nerve cells implies that interstitial cell proliferation is controlled by a feedback signal from interstitial cells and their derivatives. Our results suggest that the cellular environment of interstitial cells provides cues which are instrumental in stem cell decision making.

Key words: Stem cells – Nerve cells – Differentiation – Microenvironment

Introduction

When stem cells divide, they either replicate to produce more cells of the same kind or become committed to a particular differentiated cell type. Little is known of what determines the decision of such cells to remain a stem cell or to commit to differentiation. Within the last few years, however, it has become evident that diffusible regulatory molecules as well as complex cellular interactions play key roles in survival, proliferation and differentiation of stem cells. Several regulatory factors influencing stem cell decisions in the hematopoietic system have already been identified and characterized (e.g., Whetton and Dexter 1986; Metcalf 1989; Graham et al. 1990; Itoh et al. 1989). Stem cells appear to have receptors for all these growth factors and to lose particular receptors upon commitment and differentiation (reviewed in Hall and Watt 1989). Less is known about the role of the cellular environment in stem cell proliferation and differentiation. However, the in vivo observation of hematopoietic stem cells associated with a complex network of diverse cell types, the different cycling rates of hematopoietic stem cells residing in different locations (Lajtha 1979), the microenvironmental defect known to exist in *steel* mutants of mice (Okamoto et al. 1985) as well as the demonstrated importance of stromal cells in regulation of B-cell differentiation (e.g., Johnson and Dorshkind 1986; Gordon et al. 1987) point to a substantial role for local environmental cues in decisions made by stem cells.

Understanding the role of the cellular environment requires in vivo culture of stem cells under controlled conditions. In the stem cell system of the freshwater polyp *Hydra* such an approach is possible. Stem cells in hydra form a population of multipotent cells which continuously proliferate and differentiate into nerve cells, nematocytes, gland cells and during sexual phases to gametes (Bosch and David 1987). Interstitial stem cells are located primarily in the intracellular spaces between ectodermal epithelial cells and are found throughout the gastric region and in reduced numbers in head and foot region (reviewed in David et al. 1987). In rapidly growing animals, 60% of interstitial cells undergo self-renewal while 40% of stem cell daughters differentiate in each cell generation (David et al. 1987). The low number of interstitial cells in head and foot region as well as the fact that nematocyte differentiation is restricted to the gastric region suggests that positional signals are involved in regulation of differentiation (David et al. 1987).

Another important regulatory factor appears to be the number of interstitial stem cells present. Since decreasing the number of stem cells increases the probability of stem cells for self-renewal (P_S) (Bode et al. 1976; Sproull and David 1979), it was proposed that P_S is regulated by a negative feed-back signal from the neighboring interstitial cells (David and MacWilliams 1978).

Offprint requests to: T.C.G. Bosch

Table 1. Cellular composition of host polyps

Treatment		Strains		
		105	sf-1	B1
Standard-culture	1s+2s/epi	0.25 (0.05)[d]	0.19 (0.03)	0.15 (0.10)
	nv/epi	0.15 (0.02)	0.13 (0.02)	0.08 (0.01)
Nitrogen mustard treatment[a]	1s+2s/epi	≤0.001	≤0.001	≤0.001
	nv/epi	0.09 (0.05)	0.10 (0.02)	0.09 (0.005)
Temperature shift to 26° C[b]	1s+2s/epi	–	0.002 (0.001)	≤0.001
	nv/epi	–	0.071 (0.019)	0.036 (0.008)
Colchicine treatment[c]	1s+2s/epi	≤0.001	–	–
	nv/epi	≤0.001	–	–

Abbreviations: nv = nerve cell; epi = epithelial cell; 1s+2s = interstitial cell

[a] 6 days after nitrogen mustard treatment
[b] 2 days after temperature shift
[c] 4 weeks after colchicine treatment
[d] Numbers in brackets show standard deviation

Heimfeld and Bode (1986) tested some predictions of this model by investigating interstitial cell behavior under reduced densities and found no direct relationship between density and rate of interstitial cell proliferation.

Since the experiments in these apparently conflicting reports were carried out under different conditions, we reinvestigated the issue by addressing the role of the cellular environment. Here we report that in host tissue lacking all non-epithelial cells the interstitial cell population did not grow but differentiated. In contrast, in interstitial cell free tissue which contained nerve cells and differentiating intermediates at progressively increased densities, the interstitial cell population grew rapidly. Since growth of the interstitial cell population was faster in tissue with reduced interstitial cell numbers than in normal tissue, we conclude that growth of the interstitial cell population is governed both by the number of interstitial cells and by their differentiated products.

Material and methods

Strains and culture conditions. The strains of *Hydra magnipapillata* used in this study were wild-type strain 105 (Sugiyama and Fujisawa 1977), mutant strain sf-1 whose interstitial cells are temperature sensitive (Marcum et al. 1980) and chimeric strain B1 whose epithelial cell lineage is derived from strain 105 and interstitial cell lineage from strain sf-1 (Sugiyama and Fujisawa 1978). All strains were kindly provided by T. Sugiyama, Mishima, cultured at 18° C as described previously (Bosch and David 1987) and fed five times per week with freshly hatched *Artemia*.

Cell composition of polyps under normal conditions and production of tissue with defined cell composition. The interstitial cell and nerve cell composition of the strains used is given in Table 1. Cell composition is expressed as the number of a specific cell type relative to the number of epithelial cells, e.g. nerve cell/epithelial cell (nv/epi). This relationship is called the cell density.

To produce polyps with defined cell compositions, we used either standard chemical treatments or temperature-sensitive mutant strains.

"NF" host tissue (nerve free, with epithelial cells only):
Epithelial tissue lacking all non-epithelial cells was produced by double treatment with colchicine as described by Marcum and Campbell (1983) and by incubation of temperature sensitive mutant strains sf-1 and B1 for one week at 26° C (Marcum et al. 1980). After both treatments the interstitial cell level is reduced to nearly zero (Table 1; Wanek et al. 1980; Marcum et al. 1980; Bosch and David 1987). These hydra consisting only of epithelial cells were cultured and fed by hand as described (Bosch and David 1987) for 5–6 weeks prior to use. They were shown previously to grow, multiply by budding and regenerate as normal polyps (Campbell 1976; Wanek et al. 1980).

"IF" host tissue (interstitial cell free, with non-epithelial cells at progressively reduced levels):
Host tissue containing no interstitial cells but all other non-epithelial cells at normal or progressively reduced levels was obtained in two ways. First, polyps were treated with nitrogen mustard following the standard procedure described previously (David and Murphy 1977). Briefly, hydra were incubated in 0.01% freshly prepared nitrogen mustard (NM, Sigma) for 10 min, fed one day after NM treatment and used for experiments 6–7 days later. The cell composition of these animals on day 6 after NM treatment is shown in Table 1. Second, mutant strains sf-1 and B1 were incubated for 2 days at the non-permissive temperature (26° C). This short-term temperature treatment eliminated all interstitial cells whereas nerve cells, nematoblasts, nematocytes and gland cells remained, at least initially, at roughly normal levels (Table 1; Marcum et al. 1980).

Tissue grafting. Donor tissue was vitally stained with Evans blue. For axial grafts lower halves were grafted to upper halves according to standard procedures (see Fig. 1). Following grafting, animals were fed daily with excess numbers of shrimps. Lateral grafting was done by excising a small ring of gastric tissue from the donor polyp, cutting it into 4 equal pieces and implanting these pieces into the same relative position of host polyps (see also Fig. 4).

Cell type analysis and determination of doubling time T_D. Cell types were analyzed by maceration (David 1973). Interstitial cells including stem cells and committed precursors occur as single cells and in pairs (referred to as 1s+2s). The first cell type unique to the nematocyte pathway are clusters of 4 nematoblasts (referred to as 4s) (Sproull and David 1979). The number of cells was determined following standard procedures (e.g. Bosch and David 1984). Doubling time T_D is the number of days required for a 2-fold increase in total number of cells.

Labeling and analysis of DNA synthesis. DNA synthesis was analysed by labeling hydra cells with 5-BrdU following a procedure described previously (Plickert and Kroiher 1988). Polyps were continuously incubated in 5 mM BrdU (Sigma, Deisenhofen) for 72 h. At various time points 5–10 animals were macerated. DNA synthe-

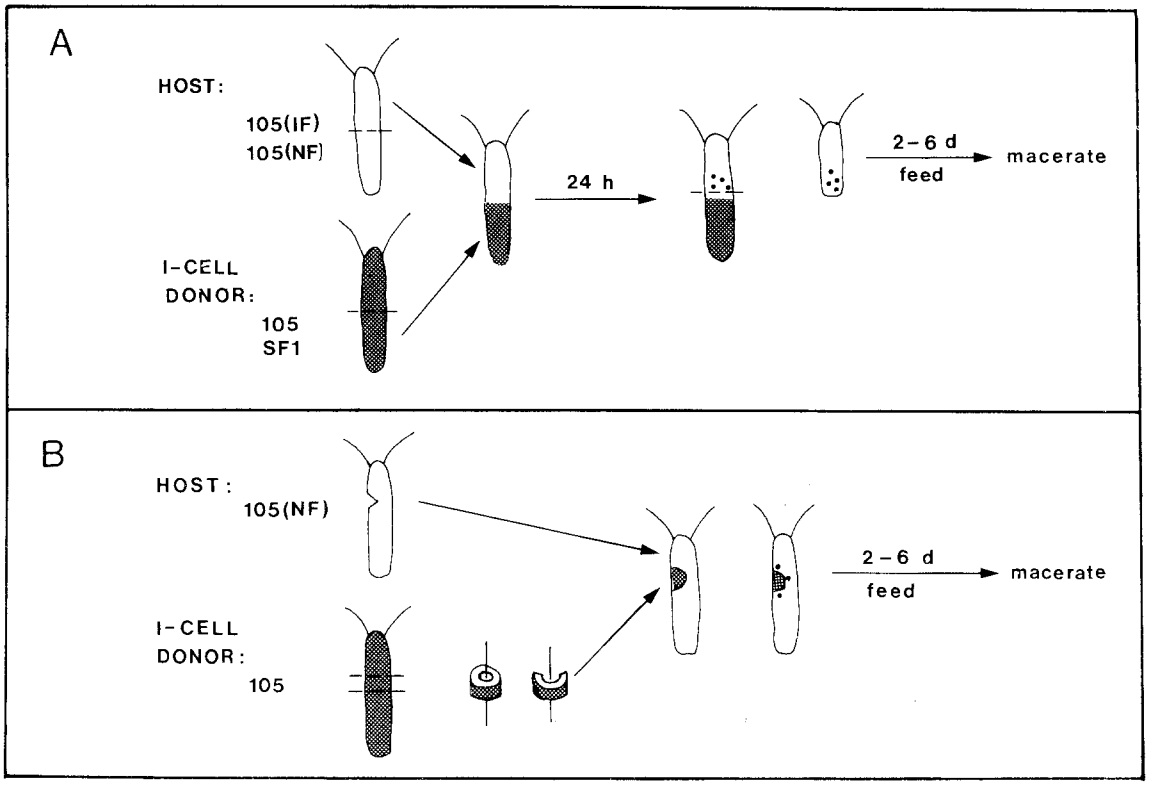

Fig. 1A, B. Scheme for introducing interstitial cells into host tissue of defined cellular composition. In order to distinguish between host and donor tissue, donor tissue had been vitally stained with Evans Blue prior to grafting. **A** axial transplantation; **B** lateral transplantation

sis was monitored by immunostaining cells with an anti-BrdU monoclonal antibody (Becton-Dickinson, Heidelberg, diluted 1:20 in PBS/BSA). Before incubating in anti-BrdU monoclonal antibody overnight, macerates were washed in PBS and exposed to 2N HCl for 45 min. Binding of the antibody was visualized by the anti-mouse Ig-peroxidase method using aminoethylcarbazole as substrate (Harlow and Lane 1988). *Hydra* cells are labeled with BrdU with same efficiency and kinetics as with $^{(3)}$H thymidine (Bosch, pers. observation).

Calculation of fraction of interstitial cells undergoing self-renewal (P_S), nerve differentiation (P_{NV}) and nematocyte differentiation (P_{NM}). P_S of interstitial cells was calculated as described previously (Sproull and David 1979) assuming a cell cycle time of 24 h. The fraction of nerve differentiation (P_{NV}) was calculated according to Fujisawa (pers. comm.) as follows:

$$\frac{(\text{number of nerve cells on day X} - \text{number of nerve cells on day X} - 1)}{(\text{number of 1s} + 2\text{s on day X} - 1) \times 2}$$

The fraction of nematocyte differentiation (P_{NM}) was calculated as follows:

$$\frac{(\text{number of 4s on day X}) \times 1.3}{\text{number of 1s} + 2\text{s on day X} - 1}$$

whereby the factor of 1.3 corrects the observed number of 4s to the total number produced per day (cell cycle of 4s is 18 h; David and Gierer 1974). Since values obtained by this procedure are similar during the first 10 days after grafting (Fujisawa, pers. comm.), values calculated for day X = 6 were taken as representative.

Results

Proliferation of interstitial cells in IF and NF tissue

To investigate the influence of the cellular microenvironment on the growth of interstitial stem cells, interstitial cells were introduced into different host tissues using the axial transplantation procedure shown in Fig. 1A. The growth of interstitial cells in host polyps was followed by counting cells in macerates 2 and 6 days after transplantation. Two types of host tissue were used: "NF" ("nerve free") polyps lacking all interstitial cells, nerve cells and nematoblasts and "IF" ("interstitial cell-free") polyps lacking interstitial cells but containing nearly normal levels of nerve cells and nematocytes. The results are shown in Fig. 2.

When interstitial cells were introduced into NF tissue, their number decreased by a factor of 2 during the first week of culture (Fig. 2A, closed symbols). By contrast, when the same cells were introduced into IF tissue, the cell number doubled every 2 days (Fig. 2A, open symbols). Thus, in contrast to IF host tissue, NF epithelial tissue does not support growth of the interstitial cell population during the first week after introduction. It is important to note, however, that upon prolonged culture in epithelial tissue interstitial cells were able to repopulate the epithelial host (Fujisawa, pers. communication; Bosch, unpublished observation).

The outcome of the experiment in Fig. 2 depended dramatically on the transplantation technique used. In Fig. 2A interstitial cells were introduced into epithelial host tissue by migration after which the donor tissue was removed. In Fig. 2B a roughly similar number of interstitial cells (about 150 cells/polyp) was introduced as a small plug of tissue by "lateral" transplantation as shown schematically in Fig. 1B. Under these conditions donor tissue containing both epithelial cells and non-epithelial cells (nerve cells and nematocytes) re-

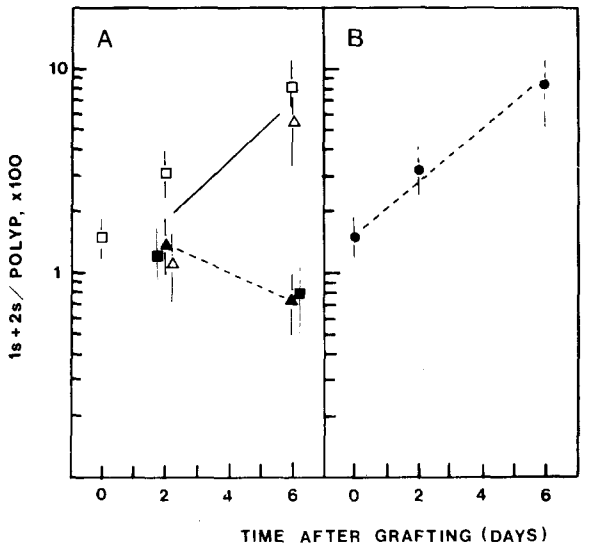

Fig. 2A, B. Increase of sf-1 and 105 interstitial cell numbers in nerve cell free (NF, *closed symbols*) and interstitial cell free host tissue (IF, *open symbols*). Donor interstitial cells were introduced by **A** axial transplantation or **B** lateral transplantation. **A** *triangles*, 105; *squares*, sf-1. **B** 105. Symbols represent mean values (± standard deviation) of 4 independent experiments. 5 polyps were pooled per determination

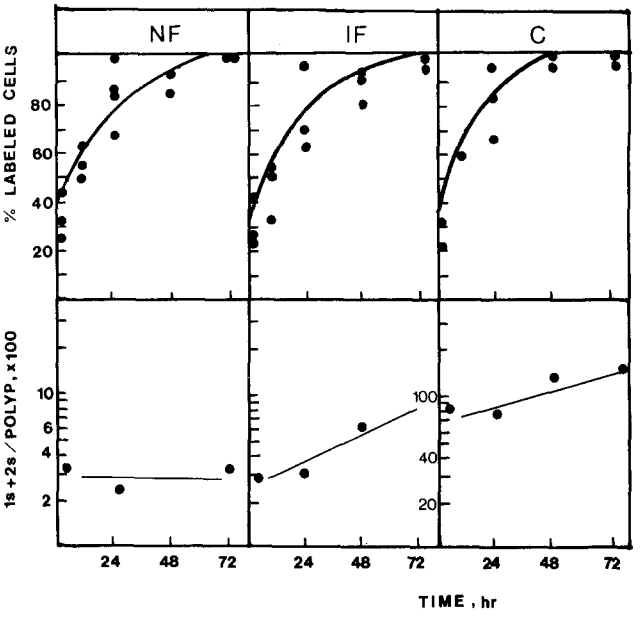

Fig. 3. Labeling index of interstitial cells (*upper panels*) and increase of interstitial cell number (*lower panels*) in NF, IF and control hosts. *Hydra* were continuously labeled with BrdU as described under Material and methods. *Symbols* represent independent determinations. 5 polyps were pooled per determination

mains present in the transplant. The results (Fig. 2B) demonstrate that, under these conditions, interstitial cells grew rapidly with a doubling time of about 2 days. Pulse-labeling with BrdU demonstrated that interstitial cells in the donor plug as well as in the surrounding host tissue were labeled and thus actively proliferating (data not shown). Thus, co-transplantation of interstitial cells with a small number of normal cells (epithelial cells, nerve cells, nematocytes) allowed the interstitial cell population to grow whereas growth of the interstitial cell population in the absence of donor tissue, e.g. following migration into epithelial host tissue, was inhibited.

Cell cycle behavior of interstitial cells in IF and NF tissue

There are two alternative explanations for the failure of interstitial cells which migrated into NF epithelial tissue to increase in number (Fig. 1A): (1) Interstitial cells in epithelial tissue could have a very slow cell cycle or (2) an increased fraction of the interstitial cells could undergo differentiation in each cell generation. To determine which of these explanations is correct, we analyzed the cell cycling behavior of interstitial cells by continuous labeling with the thymidine analog 5-BrdU (see Materials and methods). Interstitial cells were introduced into host tissue by axial transplantation as shown in Fig. 1A. Polyps were continuously labeled with BrdU beginning 4 days after separation of the donor piece and assayed at intervals for the fraction of labeled cells. The results in Fig. 3 indicate no significant differences in the labeling behavior of interstitial cells in all 3 host tissues tested (NF, IF and normal). 30–40% of 1s+2s were labelled immediately; within 48 h virtually all interstitial cells became labeled. Thus, a similar large fraction of interstitial cells was in cycle in tissue with and without non-epithelial cells although in NF tissue no increase in the size of the interstitial cell population was observed (Fig. 3, lower panel). Thus, the lack of growth of the cell population in NF tissue is not due to an altered rate of cycling.

Differentiation behavior of interstitial cells in NF tissue

To analyze the fate of interstitial cells in epithelial host tissue, we calculated the fractions of interstitial cells remaining stem cells (P_S) and differentiating into nerve cells (P_{NV}) and nematocytes (P_{NM}). The results (Table 2) show that P_S decreased from 0.6 in control tissue to about 0.5 in NF tissue, while both P_{NV} and P_{NM} in NF tissue increased. The rate of nerve cell differentiation more than doubled in NF tissue compared to control tissue while the rate of nematocyte differentiation increased by about 40%. Thus, the observed non-growth of the interstitial cell population in NF tissue (Fig. 2; Fig. 3 lower panels) can be accounted for by a shift of interstitial stem cells in the direction of differentiation and away from self-renewal. In Table 2 the sum of P_S, P_{NV} and P_{NM} is less than 1 in both control and experimental animals. Although this could indicate unaccounted-for stem cells, it appears more likely due to discrepancies in cell cycle estimates of the various cell populations.

Table 2. Differentiation behavior of interstitial cells in epithelial tissue[a]

Host		Cells/polyp[b]			P_S	P_{NV}	P_{NM}	Total
		1s+2s	nv	nm				
Normal 105	day 5	10890 (891)	4550 (608)	1572 (297)				
	day 6	13207 (646)	5557 (547)	2234 (151)	0.60	0.05	0.19	0.84
Epithelial 105	day 5	464 (49)	532 (28)	33 (8)				
	day 6	449 (18)	644 635)	91 (10)	0.48	0.12	0.26	0.86

[a] Calculation of P_S, P_{NV} and P_{NM} was done as described in Material and methods
[b] Mean values (± S.D.) of 2 independent experiments. 6–8 polyps were pooled per determination

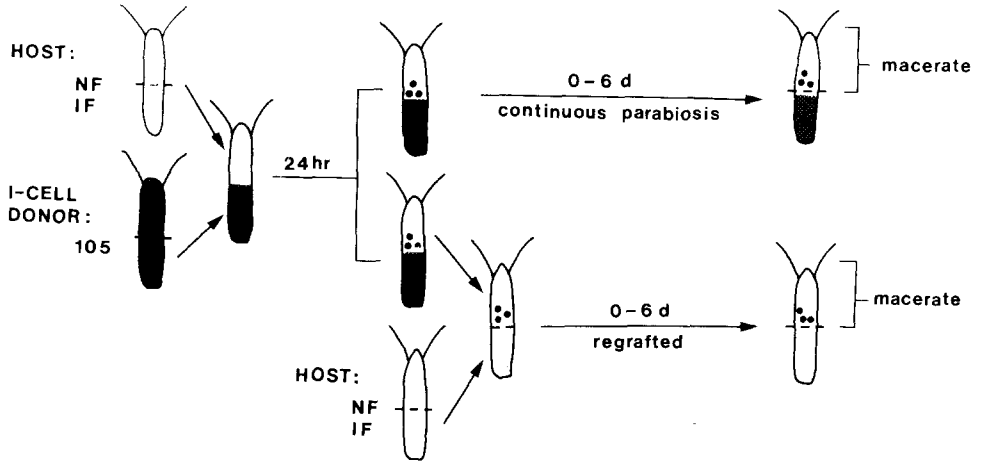

Fig. 4. Grafting procedure for determining the influence of nerve cell density on growth of the interstitial cell population. Details are provided in the text

Role of nerve cells in interstitial cell proliferation

The results in Fig. 2 indicate that stem cells do not grow when introduced into NF hosts by migration but do grow when introduced as a small plug-graft with other normal cells. The principal difference in these two situations is that in the case of migration stem cells are introduced with a minimum of other cells from donor tissue whereas in the case of plug-grafts stem cells are introduced together with several thousand other cells, both epithelial and non-epithelial. It thus seemed likely that some cell types in plug-grafts provided signals stimulating stem cell growth. To identify which cell types were involved we introduced stem cells into a series of host tissues with different cell compositions. Stem cells were introduced by migration in axial transplants so that the number of co-introduced donor cells was minimized and the donor tissue could subsequently be removed.

We examined a series of host tissues which lacked interstitial stem cells but contained differing numbers of differentiation products of the interstitial cell lineage. Because of earlier results suggesting that the nerve cell number could influence stem cell proliferation (Heimfeld and Bode 1985), we deliberately chose IF and NF hosts which have different densities of nerve cells. As IF hosts we used nitrogen mustard treated and temperature treated sf-1 tissue. Nitrogen mustard treated host tissue has higher densities of nerve cells than temperature-treated sf-1 hosts (see Table 1 and Material and methods) because epithelial cell proliferation is not inhibited by temperature treatment. IF hosts made from the chimera B1 have fewer nerve cells than sf-1 host polyps because the endogenous interstitial cell level in B1 is lower than in sf-1.

The grafting procedure is shown schematically in Fig. 4. Host tissue and interstitial cell donor tissue were grafted together for 24 h to permit migration of interstitial cells from donor into the host. Half of the experimental animals were maintained in parabiosis with donor tissue. The other half was regrafted to a proximal half of a host animal.

The results in Fig. 5 show that the proliferation of migrated interstitial stem cells was dramatically different depending on the type of host tissue and on the continuing presence of normal tissue (parabiosis). In the case of continuous parabiosis with normal donor tissue (open symbols, dashed lines), interstitial cells in upper halves increased rapidly, independently of the nerve cell density in the host tissue. The doubling time was similar in all strains tested with a T_D of about 1.7 days. This increase is the result of interstitial cell proliferation and not continuing migration from the donor since removal of the donor tissue in such grafts does not change the result (Fujisawa et al. 1990).

In regrafted animals the growth rate of interstitial cells in the upper halves was generally lower in host tissue containing progressively reduced levels of nerve cells (Fig. 5, solid symbols, solid lines). For example,

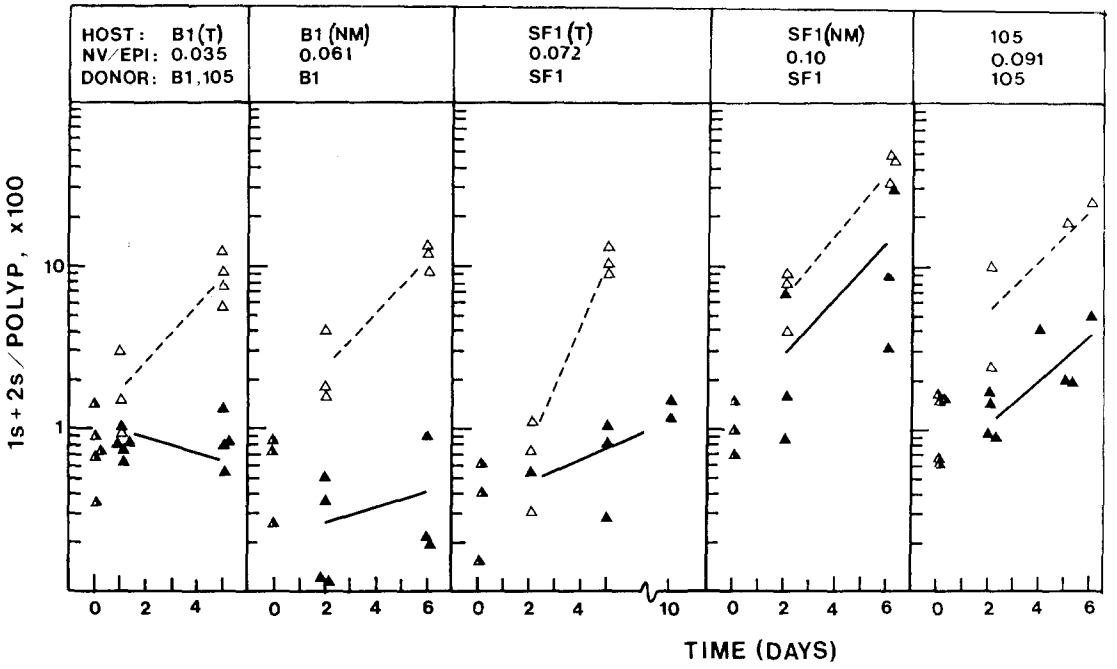

Fig. 5. Increase of interstitial cell numbers in host tissue with varying nerve cell densities (nv/epi). *Open triangles* and *dashed line*, continuous parabiosis; *solid triangles* and *solid line*, regrafted animals. *Symbols* represent independent determinations. 5 polyps were pooled per determination. *NM*, nitrogen mustard treated; *T*, 2 days at 26° C

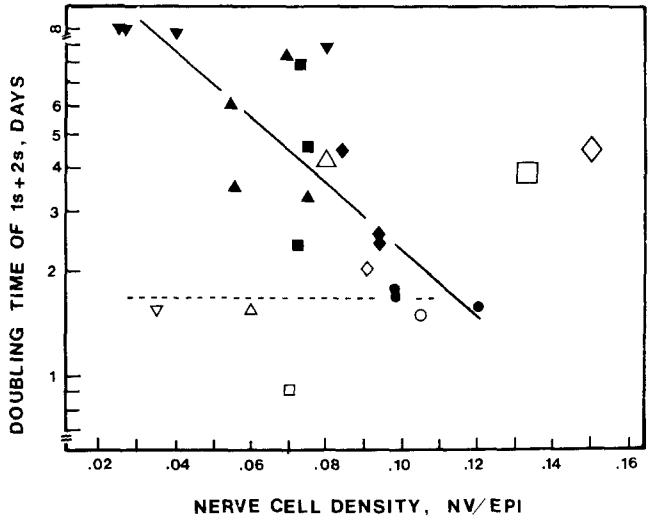

Fig. 6. Correlation between nerve cell density and doubling time of interstitial cells. Data are from Fig. 5. *Closed symbols* and *solid line*, regrafted animals. *Open symbols* and *dashed line*, continuous parabiosis. *Circle*, sf-1 tissue NM treated; *diamond*, 105 tissue NM treated; *square*, sf-1 tissue 2 days at 26° C; *triangles*, B1 tissue NM treated; *upside-down triangle*, B1 tissue 2 days at 26° C. *Large open symbols* indicate doubling time and interstitial cell level in control polyps from our mass culture (*open diamond*, sf-1; *open square*, 105; *open triangle*, B1)

in the temperature treated chimera B1 with a nerve cell density of about 0.035 nv/epi, the interstitial cell population decreased in size similar to the behavior of interstitial cells in tissue lacking all non-epithelial cells (Fig. 2 and Fig. 3). With increasing nerve cell densities in the host tissue, the growth rate of the interstitial cell population increased. Figure 5 also shows that at nerve cell densities of 0.06–0.07 nv/epi, T_D of interstitial cells was about 4 days. At nerve cell densities of 0.9–0.10 nv/epi in NM-treated sf-1 or 105 tissue, T_D of about 1.7 days was observed.

Figure 6 summarizes these results and demonstrates the correlation between nerve cell level and interstitial cell growth: the lower the nerve cell density, the slower the growth rate of the interstitial cell population. Interestingly, this relationship between nerve cell density in the host and doubling time of the interstitial cell population was only observed in experimental animals with reduced interstitial cell levels (Fig. 6, filled symbols). By comparison, normal B1, sf-1 or 105 polyps which contain high levels of both nerve cells and interstitial cells (Fig. 6, large open symbols) exhibited growth rates which were significantly slower than those observed at reduced interstitial cell levels. This dependence of rapid proliferation on reduced interstitial cell density agrees with previous observations (Bode et al. 1976; Sproull and David 1979) and provides further support for the idea that interstitial cell proliferation is negatively regulated by the density of interstitial cells in tissue.

Discussion

Two environmental signals influence stem cell proliferation

The present results demonstrate the influence of two environmental parameters on stem cell proliferation. The first parameter – nerve cell density in host tissue – positively influences proliferation. The second parameter – interstitial cell density – negatively influences proliferation.

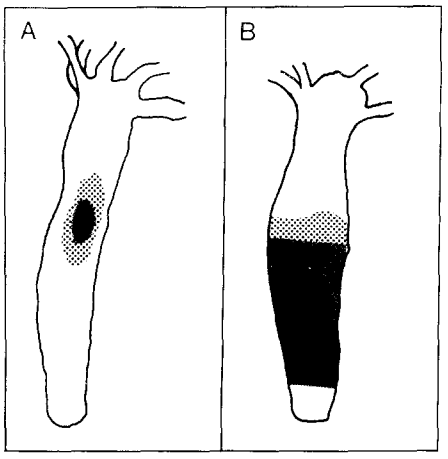

Fig. 7A, B. Effective range of normal donor tissue on interstitial cell behavior in epithelial host tissue. **A** Camera lucida drawings of a typical plug graft and **B** an axial graft 48 h after grafting. The figure is representative for numerous measurements of cell positioning in such grafts. Grafting was done as shown in Figs. 1 and 4 using temperature treated sf-1 as host and normal sf-1 as donor. *Filled area*, transplanted donor tissue; *grey area*, positioning of donor interstitial cells (large 1s+2s)

The influence of nerve cell density on proliferation is shown in Fig. 5: hosts with low nerve cell density inhibit stem cell proliferation; hosts with normal high nerve cell densities support rapid stem cell proliferation. A similar effect of nerve cells on interstitial cell proliferation was observed previously by Heimfeld and Bode (1986) in *Hydra attenuata*. Their experiments, however, focused on the stimulatory effect of nerve cells in head tissue. The present results constitute an extension of these findings to nerve cells located elsewhere in the polyp. The stimulatory effect of nerve cells extends from the proximal half to the distal half in parabiosis experiments (Figs. 4 and 5) and from the plug of grafted tissue (Figs. 1 B and 2 B) into the surrounding host tissue where donor interstitial cells proliferate and spread. Figure 7 shows camera lucida drawings of a typical plug graft and an axial graft indicating the distance over which donor tissue affects interstitial cell proliferation in the host. Because of the distance over which the stimulatory effect is active, it appears to be humoral and could be mediated by an as yet unidentified neuropeptide. In light of recent observations (David et al., manuscript in prep.) it is interesting to note that the influence of nerve cells on the growth rate of the interstitial cell population is not strain specific. Figures 5 and 6 demonstrate that nerve cells of one strain can stimulate proliferation of interstitial cells of another strain.

The influence of stem cell density on proliferation is documented in Fig. 6. Although normal sf-1 and 105 host tissues contain the highest level of nerve cells of any host we tested, the proliferation rate of stem cells is "normal" with a doubling time of about 3–4 days. The stem cell density in such animals is also high. By comparison, host tissue with only slightly fewer nerve cells but very reduced stem cell levels exhibit rapid interstitial cell proliferation. This result is in good agreement with several earlier observations (Bode et al. 1976; Sproull and David 1979) and indicates that stem cell proliferation is negatively regulated by the density of stem cells in hydra tissue. Such a negative feedback model homeostatically regulates the density of interstitial cells in hydra tissue and thus ensures that, despite many generations of asexual growth, the independently proliferating epithelial and interstitial cell lineages remain in balance in hydra tissue. The results of the parabiosis experiments (open symbols and dashed lines in Fig. 5) demonstrate that interstitial cell proliferation in distal halves – where stem cells are at low density – is rapid despite the fact that in proximal tissue the interstitial cell density is high. Thus the negative effect of stem cell density appears to have a relatively short range compared to the stimulatory effect of nerve cells on interstitial cell proliferation.

The idea that specific microenvironments are instrumental in determining stem cell behavior has been current for many years in studies of the hematopoietic system. In addition to diffusible factors, stromal cells and intermediates in the differentiation pathway have been identified as important components influencing the development of hemopoetic cells (Bartocci et al. 1987; for reviews see Lord and Dexter 1988 and Hall and Watt 1989). In *Hydra*, too, there is increasing evidence that the cellular environment influences the behavior of interstitial cells. Female germ cells are inhibited from differentiating when placed in a male polyp (Sugiyama and Sugimoto 1985; Littlefield 1986). This "masculinization" is caused by male interstitial cells either by direct contact with female stem cells or by hormonal action. An influence of epithelial cells on the development of nematocytes has been demonstrated in morphological mutants (Rubin and Bode 1982) and in tissue undergoing head regeneration (Yaross and Bode 1978; Fujisawa and David 1984). A modifying influence of the epithelial environment has also been observed in the development of tentacle-specific nerve cells since these can only differentiate into tentacle battery cells in a complex with epithelial cell precursors (Hobmeyer et al. 1990).

Acknowledgements. We thank Drs. T. Sugiyama and T. Fujisawa for sharing unpublished results, helpful discussion and comments on the manuscript. This work was supported by the Deutsche Forschungsgemeinschaft (DFG).

References

Bartocci A, Mastrogiannis DS, Migliorati G, Stockert RJ, Wolkoff AW, Stanley ER (1987) Macrophages specifically regulate the concentration of their own growth factor in the circulation. Proc Natl Acad Sci USA 84:6179–6183

Bode HR, Flick KM, Smith GS (1976) Regulation of interstitial cell differentiation in *Hydra attenuata*: Relationship between epithelial cell cycle length and growth rate. J Cell Sci 20:29–46

Bosch TCG, David CN (1984) Growth regulation in *Hydra*: Relationship between epithelial cell cycle length and growth rate. Dev Biol 104:161–171

Bosch TCG, David CN (1987) Stem cells of *Hydra magnipapillata* can differentiate into somatic cells and germ line cells. Dev Biol 121:182–191

Campbell RD (1976) Elimination of *Hydra* interstitial and nerve cells by means of colchicine. J Cell Sci 21:1–13
David CN (1973) A quantitative method for maceration of *Hydra* tissue. Roux Arch Entw Mech Org 171:259–268
David CN, Gierer A (1974) Cell cycle Kinetics and development of *Hydra attenuata*. III. Nerve and nematocyte differentiation J Cell Sci 16:359–375
David CN, Murphy S (1977) Characterization of interstitial stem cells in hydra by cloning. Dev Biol 58:372–383
David CN, MacWilliams H (1978) Regulation of the self-renewal capacity in *Hydra* stem cell clones. Proc Natl Acad Sci USA 75:886–890
David CN, Bosch TCG, Hobmeyer E, Holstein T, Schmidt T (1987) Interstitial stem cells in hydra. In: Loomis WF (ed) Genetic regulation of development. Alan Liss, New York, pp 389–408
Fujisawa T, David CN (1984) Loss of differentiating nematocytes induced by regeneration and wound healing in *Hydra*. J Cell Sci 68:243–255
Fujisawa T, David CN, Bosch TCG (1990) Transplantation stimulates interstitial cell migration in hydra. Dev Biol 138:509–512
Gordon MY, Dowding CR, Riley GP, Goldman JM, Graves MF (1987) Altered adhesive interactions with marrow stroma of haematopoietic progenitor cells in chronic myeloid leukaemia. Nature 328:342–344
Graham GJ, Wright EG, Hewick R, Wolpe SD, Wilkie NM, Donaldson D, Lorimore S, Pragnell IB (1990) Identification and characterization of an inhibitor of hemopoietic stem cell proliferation. Nature 344:442–444
Hall PA, Watt FM (1989) Stem cells: The generation and maintenance of cellular diversity. Development 106:619–633
Harlow E, Lane D (1988) Antibodies. A laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
Heimfeld S, Bode HR (1985) Growth regulation of the interstitial cell population in hydra. I. Evidence for global control by nerve cells in the head. Dev Biol 110:297–307
Heimfeld S, Bode HR (1986) Growth regulation of the interstitial cell population in hydra. III. Interstitial cell density does not control stem cell proliferation. Dev Biol 116:51–58
Hobmeyer E, Holstein T, David CN (1990) Tentacle morphogenesis in hydra. II. Formation of a complex between a sensory nerve cell and a battery cell. Development 109:897–904
Itoh K, Tezuka H, Sakoda H, Konno M, Nagata K, Uchiyama T, Uchino H, Mori KJ (1989) Reproducible establishment of hemopoietic supportive stromal cell lines from murine bone marrow. Exp Hematol 17:145–153
Johnson A, Dorshkind K (1986) Stromal cells in myeloid and lymphoid long-term bone marrow cultures can support multiple hemopoietic lineages and modulate their production of hemopoietic growth factors. Blood 68:1348
Lajtha LG (1979) Stem cell concepts. Differentiation 14:1–11
Littlefield LC (1986) Sex determination in hydra: control by a subpopulation of interstitial cells in *Hydra oligactis* males. Dev Biol 117:428–434
Lord BI, Dexter TM (1988) Stem cells. J Cell Sci (Suppl) 10:1–283
Marcum BA, Campbell RD (1983) Eliminating all non-epithelial cells using colchicine. In: Lenhoff HM (ed) *Hydra*: Research methods. Plenum Press, New York, pp 281–286
Marcum BA, Sugiyama T, Fujisawa T (1980) A mutant strain (sf-1) containing temperature-sensitive interstitial cells. In: Tardent P, Tardent R (eds) Developmental and cellular biology of coelenterates. Elsevier/North-Holland, Amsterdam, pp 429–434
Metcalf D (1989) The molecular control of cell division, differentiation commitment and maturation in hemopoietic cells. Nature 329:27–30
Okamoto T, Anthony K, Mintz B (1985) Abnormal development of genetically normal fetal hematopoietic stem cells in *steel* mutant mouse fetuses. Dev Biol 109:251–254
Plickert G, Kroiher M (1988) Proliferation kinetics and cell lineages can be studied in whole mounts and macerates by means of BrdU/anti-BrdU technique. Development 103:791–794
Rubin DI, Bode HR (1982) Nematocyte develoment in *Hydra attenuata* is dependent on both the interstitial cells and the epithelial cells. Dev Biol 90:306–314
Sproull F, David CN (1979) Stem cell growth and differentiation in *Hydra attenuata*. II. Regulation of nerve and nematocyte differentiation in multiclone aggregates. J Cell Sci 38:155–169
Sugiyama T, Sugimoto N (1985) Genetic analysis of developmental mechanisms in hydra. XI. Mechanisms of sex reversal by heterosexual parabiosis. Dev Biol 110:413–421
Sugiyama T, Fujisawa T (1977) Genetic analysis of developmental mechanisms in hydra. I. Sexual reproduction of *Hydra magnipapillata* and isolation of mutants. Dev Growth Diff 19:187–200
Sugiyama T, Fujisawa T (1978) Genetic analysis of developmental mechanisms in hydra. II. Isolation and characterization of an interstitial cell deficient strain. J Cell Sci 29:35–52
Wanek N, Marcum BA, Campbell RD (1980) Histological structure of epithelial hydra and evidence for the complete absence of interstitial cells and nerve cells. J Exp Zool 212:1–11
Whetton AD, Dexter TM (1986) Haemopoietic growth factors. TIBS 11:207–211
Yaross MS, Bode HR (1978) Regulation of interstitial cell differentiation in *Hydra attenuata*. III. Effects of i-cell and nerve cell densities. J Cell Sci 34:1–25