DEVELOPMENTAL BIOLOGY 138, 509–512 (1990)

BRIEF NOTES

Transplantation Stimulates Interstitial Cell Migration in Hydra

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Accepted December 7, 1989

Migration of interstitial cells and nerve cell precursors was analyzed in *Hydra magnipapillata* and *Hydra vulgaris* (formerly *Hydra attenuata*). Axial grafts were made between [^{3}H]thymidine-labeled donor and unlabeled host tissue. Migration of labeled cells into the unlabeled half was followed for 4 days. The results indicate that the rate of migration was initially high and then slowed on Days 2–4. Regrafting fresh donor tissue on Days 2–4 maintained high levels of migration. Thus, migration appears to be stimulated by the grafting procedure itself.

INTRODUCTION

Results in the accompanying paper (Bosch and David, 1990) demonstrate that interstitial cells grow as contiguous patches of cells in undisturbed hydra tissue. Thus, interstitial stem cells do not appear to migrate extensively in hydra tissue under conditions of normal growth. The absence of migration was surprising since good evidence for interstitial cell migration has been presented in several independent reports (Tardent and Morgenthaler, 1966; Vögeli, 1972; Herlands and Bode, 1974; Heimfeld and Bode, 1984a,b). All these experiments involved grafting pieces of labeled (usually [^{3}H]-thymidine) donor tissue to unlabeled host tissue and analyzing migration of labeled cells 1 day after grafting.

One possible explanation for the difference between these two sets of experiments is that the grafting procedure itself stimulated interstitial cell migration. To test this hypothesis we have reexamined the extent of migration in grafting experiments. In particular we have examined migration over longer periods of time than in the previous experiments. Experiments were done both with *Hydra magnipapillata* and *Hydra vulgaris*, the two strains most commonly used in previous experiments. Our results confirm the extensive migration observed previously after 1 day of grafting. However, our results also indicate that this migration activity slows dramatically on Days 2 to 4 after grafting.

MATERIALS AND METHODS

Strains and Culture Conditions

H. magnipapillata (strain 105) and *H. vulgaris* (previously called *Hydra attenuata*) were cultured at 18°C under standard conditions as described (Bosch and David, 1987). The animals were fed daily with excess numbers of shrimps. Large budless animals were used for all experiments.

Radioactive Labeling of Donor Cells and Tissue Grafting

Hydra were radioactively labeled with a single injection of [*methyl*-^{3}H]thymidine (sp act, 44 Ci/m*M*). Animals were injected with 0.2–0.5 μl of the isotope solution at a concentration of 50 μCi/ml. All existing data support the view that this isotope concentration has no effect on the behavior of interstitial cells. One injection labels about 50% of all interstitial cells (Campbell and David, 1974). Grafting of labeled and unlabeled pieces of hydra tissue was done as previously described (Fujisawa, 1989). Labeled donor tissue was vitally stained with Evans blue to identify the graft border and facilitate isolation of unlabeled host tissue. Following grafting animals were fed daily with excess numbers of shrimps.

Analysis of Labeled Cells

At each time point 5–10 pieces of hydra tissue were macerated and processed for autoradiography as previously described (David, 1973). Interstitial cells occur as single cells and in pairs (referred to as 1s + 2s). As described by Heimfeld and Bode (1984a), two classes of interstitial cells were distinguished: large interstitial cells with large nuclei and prominent nucleoli and small interstitial cells with condensed nuclei and no discernible nucleoli. The number of labeled cells was determined using phase-contrast optics. At least 50–100 labeled cells were scored at each time point.

RESULTS AND DISCUSSION

Grafting Stimulates Interstitial Cell Migration for 1 Day

Grafting experiments were performed with both *H. magnipapillata* and *H. vulgaris*. Animals were pulse-la-

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0012-1606/90 $3.00

beled with [^{3}H]thymidine and grafted to unlabeled tissue as shown schematically in Fig. 1. In all experiments labeled lower halves were grafted to unlabeled upper halves. The upper halves were isolated on Days 1 to 4 after grafting, macerated, and analyzed for the presence of labeled interstitial cells and labeled nerve cells.

Figure 2 shows the results using *H. vulgaris*. On Day 1 100 labeled large interstitial cells and 130 labeled small interstitial cells were found in the upper half. There were very few labeled nerve cells present on Day 1. One day later the number of labeled interstitial cells decreased dramatically to about 10 large interstitial cells and 50 small interstitial cells. Thereafter the low level in labeled interstitial cells was constant. The number of labeled nerve cells increased from 10 on Day 1 to 80 on Day 2. From Day 2 to Day 3 this increase slowed to 30 additional labeled nerve cells.

Our finding of about 100 labeled large interstitial cells 1 day after grafting agrees well with previously published results. For example, Heimfeld and Bode (1984a, Table 3) observed in the same experiment with *H. vulgaris* about 90 large interstitial cells and 60 small interstitial cells which had migrated into upper halves 1 day after grafting. These authors also reported extensive nerve cell differentiation from labeled interstitial cells which migrated into the unlabeled upper halves (Heimfeld and Bode, 1984b).

The cloning experiments in the accompanying paper (Bosch and David, 1990), which showed limited interstitial cell migration, were done with *H. magnipapillata* (strain 105). Therefore we repeated the grafting experiment shown in Fig. 1 using this species. Consistent with the results in *H. vulgaris* (Fig. 2), the number of migrating interstitial cells in *H. magnipapillata* was initially high and decreased dramatically on Days 2 to 4. Newly differentiated labeled nerve cells appeared in the upper halves beginning on Day 1. The rate of nerve cell differentiation was rapid between Day 1 and Day 3 and then decreased on Day 4. To exclude the possibility that this decrease in the number of labeled cells was due to dilution of the radioactive label during cell proliferation, we determined the labeling index of interstitial cells in the vitally stained bottom halves which were used as interstitial cell donor tissue. The labeling index was 49.3 on Day 0 and 46.6 on Day 4, indicating no decrease in the labeling index over the course of the experiment. In *H. magnipapillata* the extent of interstitial cell migration on Day 1 is about three- to fivefold lower than that in *H. vulgaris*. This difference is consistently observed with *H. magnipapillata* (Fujisawa, 1989). However, the reason for the difference between these two closely related species is currently not known.

The decrease in the number of migrated cells on Days 2 to 4 in both experiments (Figs. 2 and 3) coincides with the appearance of newly differentiated labeled nerve

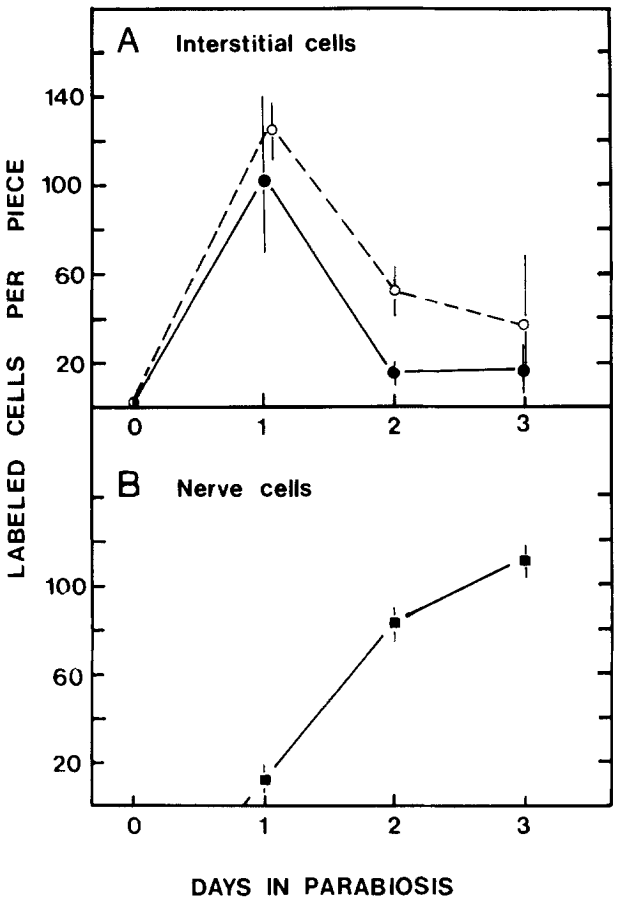

FIG. 2. Kinetics of interstitial cell migration and nerve cell differentiation in distal halves of *H. vulgaris*. (A) Interstitial cells. Closed circles, large 1s + 2s; open circles, small 1s + 2s. (B) -Nerve cells. Each point represents an average of three to four independent experiments (five halves per determination).

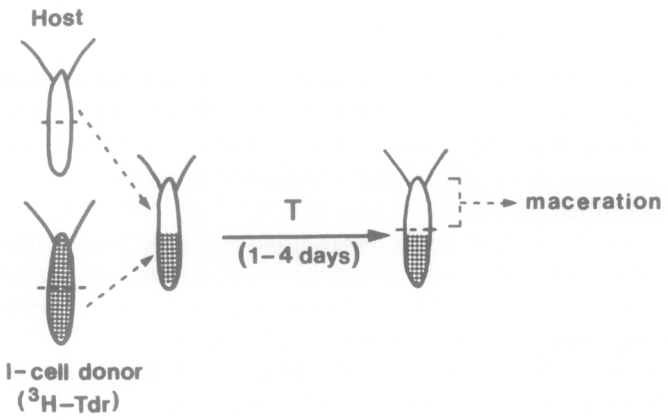

FIG. 1. Grafting procedure used to examine migration and differentiation of interstitial cells. Distal halves of unstained polyps were grafted to proximal halves which had been vitally stained with Evans Blue and pulse-labeled with [^{3}H]thymidine 1 hr before grafting. The two pieces were kept in contact throughout the experiment. At various times during parabiosis the unstained halves were excised and scored for the number of labeled interstitial cells and nerve cells.

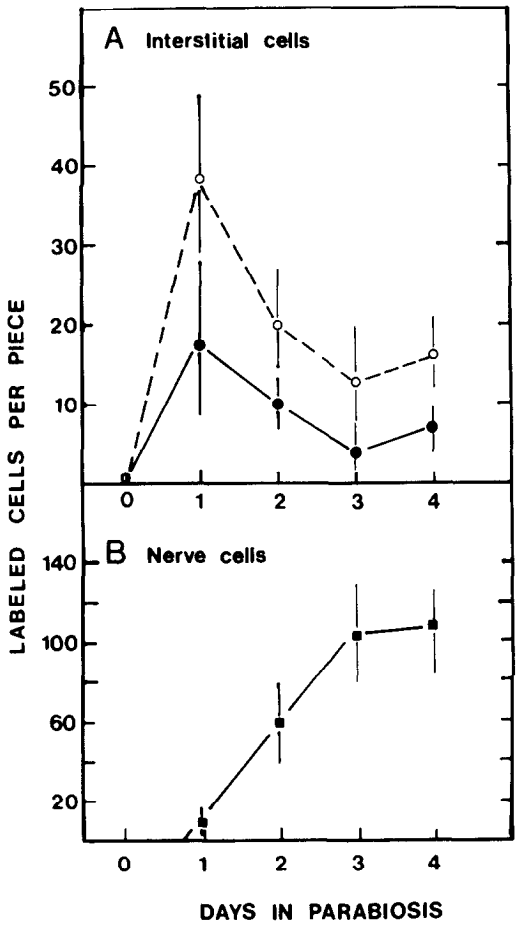

FIG. 3. Kinetics of interstitial cell migration and nerve cell differentiation in distal halves of *H. magnipapillata* strain 105. (A) Interstitial cells. Closed circles, large 1s + 2s; open circles, small 1s + 2s. (B) Nerve cells. Each point represents an average of three to four independent experiments (five halves per determination).

cells. Thus, it appears that many of the migrating cells are precursors to nerve cells as has been demonstrated by others (Heimfeld and Bode, 1984a,b; Fujisawa, 1989).

Maintenance of High Level of Migration in Repeated Grafts

The results with both species demonstrate that interstitial cell migration occurs following grafting. However, the number of migrated cells does not continue to increase after Day 1. Thus the migration observed in grafting experiments is transient and appears to be induced by the grafting procedure itself. To test this hypothesis directly, we attempted to maintain high levels of migration by repeated grafting of labeled donor tissue to unlabeled upper halves. The experiment is shown schematically in Fig. 4 and the results are shown in Fig. 5. The experiments were done with *H. magnipapillata*. The results indicate that the level of about 60 labeled interstitial cells was maintained on Days 2 to 4 when the upper halves were regrafted to labeled donors every day. Thus, regrafting prevents the decrease in the number of labeled interstitial cells observed in Figs. 2 and 3.

The number of labeled nerve cells in upper halves of regrafted tissue increased linearly at a rate of about 40 cells per day for 4 days following the first graft (Fig. 5). Thus the number of labeled nerve precursors migrating into upper halves appears to be constant in regrafting experiments. By comparison, when only one donor graft was made, the rate of nerve cell differentiation declined between Days 2 and 4 (Fig. 2B and 3B), implying that the pool of labeled nerve precursors in the upper halves decreased with time. This suggests that the rate of migration slows down at later times after grafting.

The results of our experiments indicate that both the migration of interstitial cells (Figs. 2A and 3A) and the migration of nerve cell precursors (Figs. 2B and 3B) are high immediately after grafting and slow down thereafter. Thus, the grafting procedure itself appears to stimulate interstitial cell migration. An important consequence of this finding is that the high migration rates observed in short-term grafting experiments cannot be

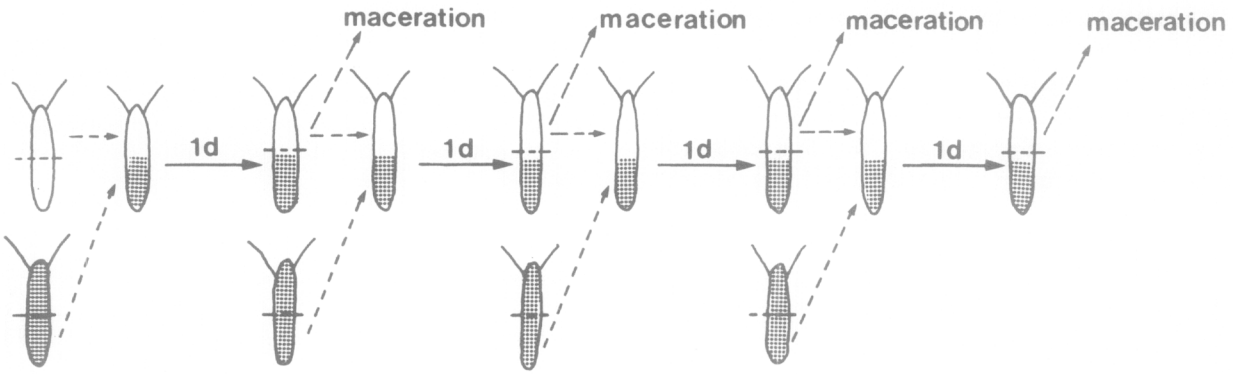

FIG. 4. Scheme for repeated grafting of labeled proximal halves to unlabeled distal halves. Unstained distal halves were grafted to proximal halves of polyps which had been vitally stained with Evans blue and pulse-labeled with [^{3}H]thymidine 1 hr before grafting. After 1 day in contact, the distal halves were removed and either macerated to score for labeled interstitial cells and nerve cells or regrafted to proximal halves of freshly labeled polyps.

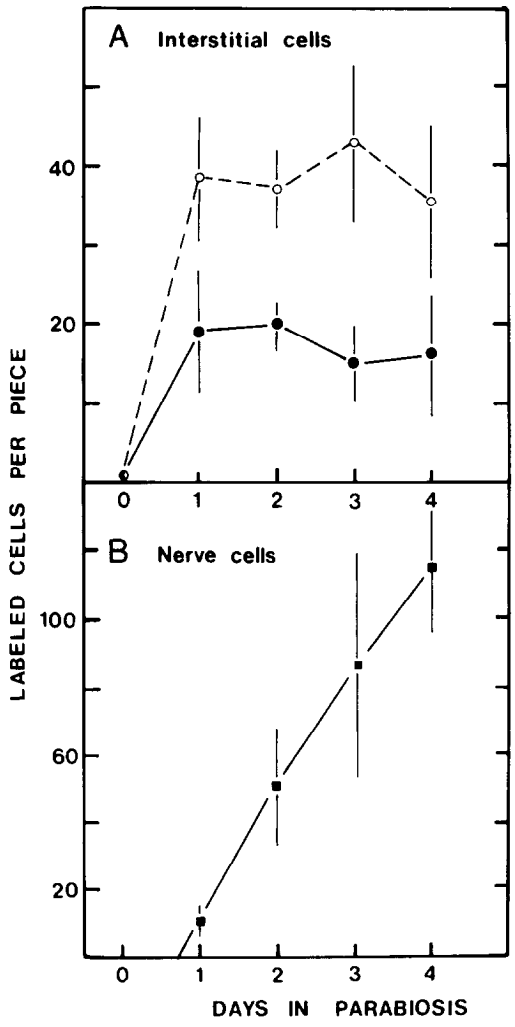

FIG. 5. Kinetics of interstitial cell migration and nerve cell differentiation in repeated grafts of *H. magnipapillata* (strain 105). (A) Interstitial cells. Closed circles, large 1s + 2s; open circles, small 1s + 2s. (B) Nerve cells. Each point represents an average of three to four independent experiments (five halves per determination).

used to estimate the level of migration in intact animals. By comparison, migration rates observed in long-term experiments, such as those reported here, do appear to reflect the *in vivo* behavior of interstitial cells. In agreement with this conclusion, the only other long-term grafting experiment between labeled and unlabeled hydra tissue (*Hydra littoralis;* Campbell, 1967) also gave no evidence for interstitial cell migration.

A similar conclusion has also been obtained by Fujisawa (1989) in experiments designed to analyze the role of migration in the pattern of nerve cell differentiation in hydra. During head regeneration a high level of interstitial cell migration was found during the first 2 days after head removal. Thereafter the number of migrating cells decreased. The number of nerve cells which differentiated from these migrated interstitial cells was also initially high and decreased two- to four-fold thereafter.

We thank N. Sugimoto for technical assistance and T. Holstein for critical reading of the manuscript. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan and by the Deutsche Forschungsgemeinschaft (Da 163/1-3).

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