GENETIC ANALYSIS OF DEVELOPMENTAL MECHANISMS IN HYDRA

V. CELL LINEAGE AND DEVELOPMENT OF CHIMERA HYDRA

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SUMMARY

Chimeric hydra were produced by making use of a strain (nf-i) which lacks interstitial cells, nerve cells and nematocytes. This strain arises by spontaneous loss of interstitial cells from its parental strain (sf-i) (Sugiyama & Fujisawa, 1978). Reintroduction of interstitial cells from other strains into nf-i leads to the creation of chimeric strains that consisted of epithelial cells derived from strain sf-i and interstitial cells and their derivatives (nerves and nematocytes) from other strains.

In chimeras, interstitial or epithelial cells apparently maintain very stable cell lineages; no indication was obtained that suggested interstitial cell differentiation into epithelial cells or dedifferentiation in the opposite direction during the long courses of chimera cultures (up to one year).

Developmental characters of chimeras were examined and compared to those of the epithelial cell (sf-i) and the interstitial cell donors. Almost all of the chimera’s characters examined (growth rate, budding rate, tentacle numbers, polyp size, regenerative capacity, etc.) closely resembled those of the epithelial cell donor, but not of the interstitial cell donors. This suggests that epithelial cells, rather than interstitial or nerve cells, are the primary determinant of most, if not all, of hydra developmental characters.

INTRODUCTION

The hydra tissue consists of 6 basic types of cells: epitheliomuscular, digestive, gland, interstitial, nerve cells and nematocytes. Strains lacking the last 3 cell types have been isolated, first by Campbell (1976) by means of colchicine-treatment of hydra, and then by Sugiyama & Fujisawa (1978) by genetic inbreeding. These strains which lack interstitial cells and their differentiation products (nerves and nematocytes) are unable to feed by themselves. When force-fed, however, they can grow and propagate by asexual budding.

Availability of the interstitial cell-deficient strains has opened up new ways to examine the roles that interstitial cells and their derivatives or epithelial cells play in hydra development. One way is to compare directly various properties between interstitial cell-deficient and normal strains (Marcum & Campbell, 1978; Sugiyama & Fujisawa, 1978). Such studies have revealed that hydra lacking interstitial cells and their derivatives possessed most, if not all, of the developmental capabilities found in

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normal hydra strains. This suggested that, although long implicated as crucial to hydra development, interstitial or nerve cells may play rather limited roles in hydra development.

Another way of examining the roles of different cell types is through chimeric hydra that can be produced using the interstitial cell-deficient strains (Sugiyama & Fujisawa, 1978). When an upper half polyp of the interstitial cell-deficient strain is temporarily grafted to the lower half polyp of a normal strain, massive migration of interstitial cells and nematocytes occurs from the normal tissue into the interstitial cell-deficient tissue. No migration, however, of epithelial (epitheliomuscular and digestive) cells occurs under the same condition. Thus, upon graft separation, a new chimeric strain is obtained that has the epithelial cells from one origin and interstitial cells and their derivatives from another.

Using the same procedure, a variety of chimeric strains were produced in the present work. Strain nf-i, a genetically produced interstitial cell-deficient strain, was used as the epithelial cell host in all cases. For interstitial cell donors, we used various wild type and mutant strains most of which were described previously (Sugiyama & Fujisawa, 1977a). We then examined various characters of chimeras to see whether they resemble those of interstitial or epithelial cell donors. Resemblance to the former or the latter should reveal characteristics that are primarily determined by interstitial cells and their derivatives or by epithelial cells. The results to be presented show that almost all of the chimera's developmental characters examined closely resemble those of the epithelial cell donor, supporting the previous suggestion that interstitial cells or nerve cells play limited roles in hydra development.

Another aspect studied in the present work was the cell lineage changes in chimeric strains. Epitheliomuscular, digestive and interstitial cells are mitotic cells and each apparently maintains its population by self-proliferation (David & Campbell, 1972; Campbell & David, 1974). However, cell type changes among them have been reported (or suggested) to occur under certain regenerating conditions (Davis, 1973a; Lowell & Burnett, 1973). Chimeras provided an excellent opportunity to examine whether cell lineage changes may occur between epithelial and interstitial cells in 'normally' growing hydra. No indication, however, of such changes has been obtained with chimeric strains cultured for up to one year.

MATERIALS AND METHODS

Culture conditions

Self-feeding hydra strains were cultured according to Loomis & Lenhoff (1956) in the modified 'M' solution described by Muscatine & Lenhoff (1965) (1 mM NaCl, 1 mM CaCl₂, 0.1 mM KCl, 0.1 mM MgSO₄ and 1 mM Tris (hydroxymethyl) aminomethane, adjusted to pH 7.6 with HCl). Freshly hatched Artemia salinas nauplii were used as food.

Non-feeding animals were cultured according to Marcum & Campbell (1978). They were kept in modified M solution containing an antibiotic rifampicin (Boehringer-Mannheim, 50 μg/ml), and fed artificially by hand feeding.

All experiments were done in a constant temperature room maintained at 18 ± 1 °C.
Strains

All strains used belonged to *Hydra magnipapillata* (Ito, 1947), and they are listed in Table 1.

The origin and properties of the interstitial cell-deficient strain, nf(non-feeder)−1, and its parental strain, sf(self-feeder)−1, were previously described (Sugiyama & Fujisawa, 1978). Sf-1 is a sexually inbred clonal line (F6) which appears normal in every respect except in one: its mass cultures sometimes spontaneously produce animals that cannot feed by themselves. When these non-feeders are force-fed, some revert back to self-feeders but some remain as non-feeders and produce non-feeding buds that, upon continued forced-feeding, eventually form populations of non-feeders. These subclonal lines are all alike and they are collectively called nf−1. The nf−1 tissue consists of only epitheliomuscular, digestive and gland cells, lacking interstitial cells, nerves and nematocytes. Nf−1 cannot feed by itself. However, when force-fed by the procedure of Campbell (1976), it can grow and reproduce by budding.

The isolation and characters of some of the other strains listed in Table 1 were previously described (Sugiyama & Fujisawa, 1977a).

Table 1. List of strains used

<table>
<thead>
<tr>
<th>Strain</th>
<th>Major characteristic</th>
<th>Origin</th>
<th>Reference*</th>
</tr>
</thead>
<tbody>
<tr>
<td>nf−1</td>
<td>Deficient in interstitial cells, nerves and nematocytes</td>
<td>By spontaneous segregation</td>
<td>(2)</td>
</tr>
<tr>
<td>sf−1</td>
<td>Morphologically normal, yield nf−1 by spontaneous segregation</td>
<td>Sexual inbreeding (F6)</td>
<td>(2)</td>
</tr>
<tr>
<td>sf−21</td>
<td>Morphologically normal, yield an interstitial cell-deficient strain (nf−21) by spontaneous segregation</td>
<td>Sexual cross (F1)</td>
<td>—</td>
</tr>
<tr>
<td>105(?)</td>
<td>Normal (wild type)</td>
<td>Field</td>
<td>(1)</td>
</tr>
<tr>
<td>L2(?) &amp; L4(?)</td>
<td>Normal (wild type), original parental strains from which sf−1 was derived by sexual crosses</td>
<td>Field</td>
<td>—</td>
</tr>
<tr>
<td>SSC(?)</td>
<td>Normal</td>
<td>Sexual crosses</td>
<td>—</td>
</tr>
<tr>
<td>SSE(?)</td>
<td>Normal</td>
<td>Sexual crosses</td>
<td>—</td>
</tr>
<tr>
<td>tc</td>
<td>Twisted column</td>
<td>Field</td>
<td>(1)</td>
</tr>
<tr>
<td>mh−1</td>
<td>Multi-head</td>
<td>Sexual cross (F1)</td>
<td>(1)</td>
</tr>
<tr>
<td>maxi−1</td>
<td>Large size</td>
<td>Sexual cross (F1)</td>
<td>(1)</td>
</tr>
<tr>
<td>mini−4</td>
<td>Small size</td>
<td>Sexual cross (F1)</td>
<td>(1)</td>
</tr>
<tr>
<td>nem−1</td>
<td>Deformed holotrichous isorhiza</td>
<td>Sexual cross (F1)</td>
<td>(1)</td>
</tr>
<tr>
<td>nem−3</td>
<td>Holotrichous isorhiza deficient</td>
<td>Sexual cross (F1)</td>
<td>(1)</td>
</tr>
<tr>
<td>nem−4</td>
<td>Stenotele migration deficient</td>
<td>Sexual crosses</td>
<td>(1), (3)</td>
</tr>
<tr>
<td>nem−10</td>
<td>Holotrichous isorhiza deficient</td>
<td>Sexual crosses</td>
<td>(1)</td>
</tr>
<tr>
<td>reg−16</td>
<td>Regeneration deficient</td>
<td>Sexual crosses (F1)</td>
<td>(1), (4)</td>
</tr>
<tr>
<td>nf−17</td>
<td>Food ingestion deficient</td>
<td>Sexual cross (F1)</td>
<td>—</td>
</tr>
</tbody>
</table>


Chimera production

Fig. 1 shows the temporary axial grafting procedure for chimera production which was described previously (Sugiyama & Fujisawa, 1978). The interstitial cell-deficient strain nf−1 was used as the epithelial cell host while various wild type and mutant strains were used as the donors of interstitial cells and their derivatives. An upper half of an nf−1 polyp and a lower half of an interstitial cell donor polyp which had been vitally stained with Evans blue (Wilby & Webster, 1970) were axially grafted together by threading a piece of nylon fishing line (0.20 mm
in diameter) through them, and they were left to heal for overnight before removing the line. One or two days after the grafting, the grafted animal was cut transversely above the graft zone to obtain the upper half, making sure that it contained no stained tissue. No feeding was done during the grafting. Daily forced-feeding was started 1 day after the graft separation, and continued for 3 to 4 weeks or until the polyps gained self-feeding capacity.

**Parameters of growth**

Growth parameters were determined using populations of individually cultured animals. From an actively growing culture, 3 or 4 small polyps bearing no buds (presumably recently detached from parents) were selected and they were individually placed in 50-mm plastic Petri dishes containing about 10 ml of modified M solution. Each animal was fed heavily once daily, allowing it to reproduce asexually by budding. Culture water was changed a few hours after each feeding. Buds produced and detached from parents were individually transferred to new Petri dishes and cultured individually as described above, keeping track of the parental history of each member. Within a week or so, exponentially growing populations were established each originating from the single polyps. From the culture record of such populations, the following 3 parameters of growth were obtained.

**Population growth rate.** Population growth rate ($\alpha$) is defined as

$$\frac{dN}{dt} = \alpha N,$$

where $N$ is the total number of hypostomes of an exponentially growing culture at time $t$, and $t$ is the time measured in days.
Chimeric hydra

To obtain it experimentally, the total hypostome numbers of an exponentially growing population are plotted on a logarithmic scale against the day of culture (see Fig. 2A, p. 222), and from the slope $\alpha$ is calculated as

$$\alpha = \frac{\log N_B - \log N_A}{t_B - t_A},$$

where, $N_A$ and $N_B$ are the total numbers of hypostomes at time $t_A$ and $t_B$, respectively.

Population growth rate $\alpha$ is proportional to the reciprocal of the doubling time ($d.t.$) of the culture as shown by

$$\alpha = \frac{\log 2}{d.t.}.$$

Budding rate. Budding rate ($\lambda$) is defined as the average number of buds produced per day per hydra. To obtain it experimentally, the number of buds produced by the original polyp which was used to start an exponentially growing population was plotted against the day of culture (see Fig. 2B), and budding rate is calculated from the slope of the line.

Bud developmental time and bud developmental rate. Bud developmental time ($K$) is defined as the number of days required by a newly formed bud to produce its own first bud, and its reciprocal is defined as bud developmental rate ($k = 1/K$). Completion of a basal disk (while the bud is still attached to the parent) is used as the criterion of formation of a new bud. Bud developmental rates were measured for several individual polyps that were produced consecutively by the original polyp used to start an exponentially growing population (Fig. 2C), and their average value is used as the bud developmental rate of the population.

Standard polyp

Size or tentacle numbers of hydra vary according to the stage of polyp development and also to the culture conditions. To compare polyps in the same stage of development and cultured under the same conditions, we therefore chose 'standard polyps' obtained in the following manner. Standard polyps are young polyps that are just beginning to produce their first buds. They are obtained from parental polyps cultured individually in 50-mm plastic Petri dishes containing about 10 ml of culture solution. Small polyps detached from parents were collected daily, and then cultured at a low density (3 or less per 10-ml per dish). Both parental and small polyps were fed heavily once daily, changing culture water a few hours after each feeding. Small polyps were examined daily just prior to feeding, and any that had produced since the preceding examination a small protrusion in the budding region (indicating the initiation of the first budding) were harvested as standard polyps.

Protein content

Protein content per standard polyp was used as a measure of the size of a polyp. Individual standard polyps were dissolved in 0.2 ml of 0.5 N NaOH-7 % glycerol solution and the protein content was determined by the method of Lowry, Rosebrough, Farr & Randall (1951) using bovine serum albumin as standard.

Nematocyst examination

Nematocysts present in the tentacles were examined as described previously (Sugiyama & Fujisawa, 1977d). Tentacles cut from an animal were placed in a drop of 0.25 % phenol-50 % glycerol solution on a microscopic slide. A coverglass was then gently pressed against the sample and photographs were taken using a Nikon microscope (type Biophoto) with 400 x differential interference optics.
RESULTS

Chimera production

Fig. 1 shows the temporal grafting procedure used to produce chimeric hydra. We have previously shown that when an upper half of an nf-1 polyp (deficient in interstitial cells and their derivatives) and a lower half of a wild type (strain 105) polyp were grafted together for 24 to 48 h, interstitial cells and nematocytes migrated massively from the wild type tissue into the nf-1 tissue (Sugiyama & Fujisawa, 1978). Epithelial cells, however, did not migrate under the same conditions. Thus, when the grafted animal was separated again, we obtained an upper half which contained the epithelial cells from nf-1 origin and the interstitial cells and their derivatives from the wild type origin. This animal was originally unable to feed by itself. When force-fed, however, it gradually regained ability to capture and ingest feed by itself, indicating the re-establishment of interstitial cells and their derivatives within it. The progenies produced by this animal by budding were all self-feeders and they eventually formed a new chimeric clone named chim-1.

Using the same procedure, we produced various chimeric strains in the present work. Strain nf-1 was used as the epithelial cell host in all cases, and different wild type and mutant strains were used as interstitial cell donors (Table 1).

In total we made 62 grafts using 17 different interstitial cell donor strains, and obtained 18 chimeric strains that were successfully established (Table 2). An average of 17.8 (± s.d. of 5.2) days of forced-feeding culture was needed before these chimeras were established.

<table>
<thead>
<tr>
<th>Interstitial cell donor strain</th>
<th>No. of grafts made</th>
<th>No. of chimeras established (%)</th>
<th>Code name of chimeras</th>
</tr>
</thead>
<tbody>
<tr>
<td>sf-1</td>
<td>8</td>
<td>0 (0)</td>
<td>chim-1, chim-2</td>
</tr>
<tr>
<td>sf-2I</td>
<td>3</td>
<td>0 (0)</td>
<td>chim-3</td>
</tr>
<tr>
<td>105</td>
<td>4</td>
<td>2 (50)</td>
<td>chim-6, chim-7</td>
</tr>
<tr>
<td>reg-16</td>
<td>1</td>
<td>1 (100)</td>
<td>chim-8, chim-9, chim-23</td>
</tr>
<tr>
<td>nem-3</td>
<td>3</td>
<td>1 (33)</td>
<td></td>
</tr>
<tr>
<td>nf-17</td>
<td>4</td>
<td>2 (50)</td>
<td></td>
</tr>
<tr>
<td>L2</td>
<td>3</td>
<td>3 (100)</td>
<td></td>
</tr>
<tr>
<td>L4</td>
<td>2</td>
<td>2 (100)</td>
<td>chim-10, chim-11</td>
</tr>
<tr>
<td>SSE</td>
<td>1</td>
<td>1 (100)</td>
<td>chim-12</td>
</tr>
<tr>
<td>mh-1</td>
<td>5</td>
<td>1 (20)</td>
<td>chim-14</td>
</tr>
<tr>
<td>SSC</td>
<td>1</td>
<td>1 (100)</td>
<td>chim-15</td>
</tr>
<tr>
<td>nem-10</td>
<td>1</td>
<td>1 (100)</td>
<td>chim-16</td>
</tr>
<tr>
<td>maxi-1</td>
<td>4</td>
<td>1 (25)</td>
<td>chim-17</td>
</tr>
<tr>
<td>nem-1</td>
<td>5</td>
<td>2 (40)</td>
<td>chim-21, chim-22</td>
</tr>
<tr>
<td>tc</td>
<td>5</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>mini-4</td>
<td>7</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>nem-4</td>
<td>5</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>(Total)</td>
<td>62</td>
<td>18 (29)</td>
<td></td>
</tr>
</tbody>
</table>
Chimeric hydra

In the case of the other 44 grafts, interstitial cells apparently failed to re-establish themselves in their new hosts. The tentacles of such polyps soon after graft separation usually appeared rough, indicating the presence of nematocysts which presumably migrated from the interstitial cell donor strains during grafting. Within several days of forced-feeding culture, however, their tentacles gradually turned smooth, indicating the loss of nematocysts from them. The polyps remained non-feeders and buds, when produced by them, were also all non-feeders.

Success or failure of chimera production apparently depends greatly on the origins of interstitial cells. For example, 8 grafts made with sf-i or 7 grafts made with mini-4 as interstitial cell donors all failed to produce self-feeding chimeras, while 3 grafts made with strain L2 or 2 grafts made with strain L4 as interstitial cell donors all succeeded. L2 and L4 are the original wild type strains from which strain sf-i (and hence also nf-i) was derived by sexual inbreeding.

Parameters of growth

Out of the 18 chimeric strains produced, 8 were selected and these and their interstitial cell donor strains were subjected to detailed examination of their characters. Strain sf-i was also examined as a control.

Three parameters of growth were examined: population growth rate, budding rate and bud developmental rate (see Materials and methods). An example of determining these parameters for a culture of chim-i is shown in Fig. 2. Fig. 2A shows that starting from a single polyp, this culture after an initial lag time grew exponentially with a population growth rate of 0.17 animal per day per animal. Fig. 2B shows that the original polyp that started this culture produced buds at a rate of 0.49 bud per day, and Fig. 2C shows that the average developmental rate of these buds was 0.12 day⁻¹.

Similar analyses were repeated on at least 3, and often 4 to 6, independently started cultures for each strain, and the results obtained from 8 pairs of interstitial cell donor strains and corresponding chimeras (plus strain sf-i) are summarized in Fig. 3.

Fig. 3A shows the correlation between the population growth rates of the interstitial cell donor strains and those of the chimeras strains. Each point in the figure shows the growth rate of an interstitial cell donor strain on the abscissa and the growth rate of the corresponding chimera on the ordinate. It can be noted that the growth rates of the interstitial cell donor strains varied rather greatly, ranging from 0.05 to 0.23. In contrast, the growth rates of the chimeras showed a much narrower range, and they were all very close to the growth rate of strain sf-i (0.15). No statistically significant correlation was found between the population growth rates of the chimeras and their interstitial cell donor strains.

Similar studies were also carried out on budding rate and bud developmental rate, and the results obtained are shown in Fig. 3B and C, respectively. In these cases, chimeras exhibited somewhat larger variations than in the previous case. In essential points, however, the results are very similar to the results of the preceding study. Both budding rates and bud developmental rates of chimeras were all similar to those of strain sf-i regardless of the rates of the interstitial cell donors. Also, no statistically signifi-
Fig. 2. Determination of growth parameters. A culture of chim-1 was started from a single small animal which was placed in a 50-mm plastic Petri dish containing about 10 ml of modified M solution. It was heavily fed once daily, and buds detached from it were individually transferred to new Petri dishes to be cultured individually in the same way. A shows the rate of increase of the total number of hypostomes as a function of days in culture. The line drawn gives a population growth rate of 0.17 animal per day per animal, corresponding to a doubling time of 4.0 days. B shows the number of buds produced by the original polyp that started the culture. The line drawn gives a budding rate of 0.49 bud per day per polyp. C shows the bud developmental rate (reciprocal of the number of days required by a newly produced bud to produce its own first bud) for the buds produced consecutively by the original polyp. The line drawn shows an average developmental rate of 0.12 day\(^{-1}\) for the first 8 buds. (For detail, see Materials and methods.)
cant correlations were found between the rates of chimeras and those of interstitial cell donors.

These results show that population growth rates, budding rates and bud developmental rates of chimeras are influenced by the origin of epithelial cells but not by the origin of interstitial cells.

**Morphological properties**

Relationship between the morphological properties of interstitial cell donors and chimeras were also examined. Fig. 4A shows correlation between the tentacle numbers of interstitial cell donors and those of corresponding chimeras. As in the preceding experiments, the chimeras exhibited a much narrower range of variation than the interstitial cell donors, and their tentacle numbers were all very close to the tentacle number of strain sf-i. No statistically significant correlation was found between the tentacle number of the interstitial cell donors and the chimeras. These results indicate that the tentacle numbers of chimeras are influenced by the origin of epithelial cells, but not by the origin of interstitial cells.

Essentially the same result was also obtained for the protein content (Fig. 4B).

These experiments were carried out using the 'standard' polyps which are the young polyps just beginning to produce their first buds (see Materials and methods). Experiments were also done using polyps in other stages of development (polyps newly detached from parents and polyps actively producing buds). The results obtained with them were essentially identical to those obtained with the standard polyps.

**Other properties**

Some chimeric strains obtained in the present work were produced using various types of developmental mutants as interstitial cell donors. These chimera can be divided into 2 groups; those that show the same mutant phenotypes as their interstitial cell donors and those that do not.

Chim-14 is an example belonging to the latter group. Its interstitial cell donor, mh-i, is a multi-headed strain (Sugiyama & Fujisawa, 1977a), but this character is not exhibited by chim-14. Chim-3 also belongs to the same group. We previously reported that its interstitial cell donor, reg-16, is a regeneration-deficient strain, but that chim-3 shows normal regenerative capacity (Sugiyama & Fujisawa, 1977b). These results suggest that mutant characters of mh-i or reg-16 are not associated with interstitial cells or their derivatives.

In contrast, mutant characters are transferred to the chimeras when nematocyst-deficient or nerve-deficient strains are used as interstitial cell donors. Strain nem-i contains deformed holotrichous isorhizas while strains nem-3 and nem-10 contain virtually no holotrichous isorhizas in their tentacles (Sugiyama & Fujisawa, 1977a). The same nematocyst deficiencies are expressed by the chimeras that are produced using these strains as interstitial cell donors. Fig. 5 shows representative examples.

Strain nf-17 is unable to feed by itself. It therefore requires forced feeding like the interstitial cell deficient strain, nf-i. Strain nf-17, however, is different from nf-i in
Fig. 3.
important ways; It contains interstitial cells and their derivatives. It can also capture and paralyse *A. salinas* nauplii, but it fails to ingest them. Chim-6 and chim-7, both produced using this strain as the interstitial cell donor, exhibit the same feeding deficiency as nf-17, suggesting that the defect in these strains is probably located in the nerve cells involved in the coordinated movement for food ingestion.

**DISCUSSION**

**Chimera production**

Chimera hydra are produced by making use of a strain (nf-1) that lacks interstitial cells and their derivatives (nerves and nematocytes) in its tissues. Reintroduction of interstitial cells from other strains into nf-1 results in establishment of chimeric strains in some cases, but not in others.

Success or failure of chimera production apparently depends greatly on the origin of interstitial cells. For example, all attempts to repopulate interstitial cells failed when sf-1, the parental strain of nf-1, was used as the interstitial cell donor strain. This is rather surprising since epithelial cells of sf-1 origin failed to reaccept the interstitial cells of the same origin. In contrast, attempts were always successful when the original wild strains (L2 and L4), from which sf-1 was derived by repeated inbreeding, were used as the interstitial cell donors. With certain strains, some attempts succeeded while others failed.

Several alternative mechanisms can be considered for failure of chimera production. They are discussed below.

*Cell migration may not occur from the interstitial cell donor tissue into the nf-1 tissue during temporary grafting.* This, however, can be ruled out in the majority of cases since almost always a large number of nematocysts appeared in the tentacles of nf-1 during, or soon after, the temporary grafting. This shows that migration of nematocytes occurred. In addition, when tissues were macerated and cells examined (David, 2003).
Immediately following graft separation, interstitial cells were usually found in nf-i tissues.

*Interstitial cells may not multiply in nf-i tissues.* Interstitial cells from certain origins may not be able to multiply in the new environment of nf-i tissue due to incompatibility between them and epithelial cells.

*Interstitial cells may be eliminated in nf-i tissues.* Interstitial cells are phagocytosed by epithelial cells in colchicine-treated animals (Campbell, 1976). Elimination by phagocytosis or by other mechanisms may occur spontaneously for the newly introduced interstitial cells in nf-i tissues.

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**Fig. 4.** Correlation of morphological properties between interstitial cell donors and chimeras. Tentacle number and protein content per standard polyp were examined as described in Materials and methods, and the results are expressed in the same way as in Fig. 3. A, correlation between the average tentacle numbers of interstitial cell donors (t) and those of chimeras (t'). \(t' = 7.29 - 0.085 \cdot t, r = 0.26\). B, correlation between the average protein contents of interstitial cell donors (p) and that of chimeras (p'). \(p' = 65.6 + 0.015 \cdot p, r = 0.035\). The regression coefficient and correlation coefficient are not statistically significant in both cases.
Figure 5. Nematocysts in the tentacles. A, strain 105 (wild type); B, chim-i (produced with strain 105 as interstitial cell donor); C, nem-1; D, chim-21 (produced with nem-1 as interstitial cell donor); E, nem-3; and F, chim-4 (produced with nem-3 as interstitial cell donor). Four types of nematocysts present in the wild type are marked: s, stenoteles; h, holotrichous isorhizas; a, atrichous isorhizas; and d, desmonemes. Note that holotrichous isorhizas in nem-1 (C) and its chimera (D) are deformed, while nem-3 (E) and its chimera (F) lack holotrichous isorhizas.
All interstitial cells may differentiate into other cell types. In normal hydra, some proportion of interstitial cells always remains as stem interstitial cells while the rest differentiate into other cell types (David & Gierer, 1974). The mechanism(s) involved in keeping the balance between the 2 proportions may not function properly in chimeras and all the interstitial cells introduced into nf-1 may differentiate into other cell types, leaving none for stem cells.

Strain nf-1 is produced from strain sf-1 by spontaneous loss of interstitial cells (Sugiyama & Fujisawa, 1978). This loss and the loss that takes place when interstitial cells are reintroduced into nf-1 tissues are probably related to each other. Studies on this subject will be reported later elsewhere.

Cell lineages in chimeras

When first produced, chimera's tissue consists of cells derived from 2 different origins: epithelial (epitheliomuscular and digestive) cells from strain sf-1 (via strain nf-1) and interstitial cells and their derivatives (nerves and nematocytes) from the interstitial cell donor strains. The origin of gland cells in chimera is obscure at present. These points have been discussed previously (Sugiyama & Fujisawa, 1978).

During long courses of culture, however, cell lineages may change gradually in chimeras. Epithelial cells are mitotic cells, and they normally maintain their populations by self-proliferation (David & Campbell, 1972). This, however, does not rule out the possibility that new epithelial cells sometimes arise by differentiation from interstitial cells. If this happens in a chimera, epithelial cells derived originally from strain sf-1 may eventually be replaced with ones derived from the interstitial donor strain.

Lineage change may also occur for interstitial cells. It could occur through 'dedifferentiation' of epithelial cells into interstitial cells. It could also occur through a different mechanism. We previously reported that nf-1 tissues contain no detectable levels (less than 0-02%) of interstitial cells (Sugiyama & Fujisawa, 1978). This, however, does not rule out the possibility that a few of them exist in nf-1 polyps which contain nearly 10^6 cells. A colchicine-derived interstitial cell-deficient strain, which is very similar to nf-1, sometimes contains a very low number of interstitial cells (Marcum & Campbell, 1978). In chimera tissues, such interstitial cells, if present, may somehow be activated to multiply vigorously to eventually become predominant there.

Evidence from the present study, however, speaks strongly against such cell lineage change in chimeras. Two types of evidence exist against interstitial cell differentiation into epithelial cells.

On a diet of A. salinas nauplii, H. magnipapillata strains are either pink or yellow. Pigments responsible for the colour are located in the food vacuoles of digestive cells. Strains sf-1 and nf-1 are yellow while strain 105 is pink. A chimera strain, chim-i, which was produced between strain 105 and strain nf-1 was yellow when it was initially produced (Sugiyama & Fujisawa, 1978). After more than a year of culture its colour still remains yellow (data not shown), indicating the persistent presence of the digestive cells of nf-1 origin (yellow). Gradual colour change from yellow to pink should have
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If interstitial cell differentiation into epithelial cells occurs in a chimera, its tissues eventually may be occupied by interstitial and epithelial cells both originating from the interstitial cell donor strain. Chimera's various properties then should naturally become identical, or very similar, to those of the interstitial cell donor strain. This, however, has not been observed to occur in any of the chimeras produced. As shown in Figs. 3 and 4, all developmental and morphological characters of the chimeras examined are similar to those of the epithelial cell donor (strain sf-1) rather than the interstitial cell donors.

Evidence against dedifferentiation of epithelial cells into interstitial cells is also multifold. When nematocyst-deficient strains are used as interstitial cell donors, the resultant chimeras all showed the same types of nematocyst deficiencies (Fig. 5). This shows that the defects responsible for the nematocyst deficiencies are located in the stem cells for the nematocytes (i.e. interstitial cells) themselves, and that the chimeras also contain these latently defective interstitial cells. Nematocyst deficiencies should have disappeared from the chimeras, had the interstitial cells changed their lineages in them.

A similar observation is also made when a strain with defective nerve cells (nf-17) is used as the interstitial cell donor strain. Both this strain and the chimeras produced with it (chim-6 and chim-7) are unable to feed by themselves, indicating that the latently defective interstitial cells (stem cells for nerves) are shared by them. The chimeras should have gained self-feeding capacity if their latently defective interstitial cells had been replaced with the normal ones originating from strain sf-1.

The evidence presented above all speaks strongly against cell lineage changes in the chimeras. Apparently no differentiation of interstitial cells into epithelial cells or dedifferentiation in the opposite direction occurs. Lowell & Burnett (1973) reported complete hydra regeneration from isolated epidermis, suggesting interstitial cell differentiation into gastrodermal cells. This type of differentiation may occur under unusual conditions such as in the isolated epidermis, but not under more normal conditions of chimera tissues. The present study has produced no evidence that has bearings on dedifferentiation of gland cells or interconversion between epithelio-muscular and digestive cells (Davis, 1973a).

Developmental characters

In the present work, we examined various characters of chimeras and compared them to those of the epithelial and interstitial cell donors. The rationale behind doing this is as follows: If a particular character of hydra is primarily determined by epithelial cells, that character of chimeras should resemble the epithelial cell donor strain since both share the same epithelial cells. Similarly, if the character is controlled by interstitial cells or by its derivatives, the chimera's character should be similar to that of the interstitial cell donor strains.

The present study showed that almost all of the chimera's characters examined closely resembled those of the epithelial cell donor and not those of the interstitial cell
donors. The only characters found by the present study to be transferable from the interstitial cell donors to the chimeras are the feeding deficiency and nematocyst deficiencies, characters known to be directly associated with the derivatives of interstitial cells (nerves and nematocytes, respectively). All the other characters examined are apparently not transferable with the interstitial cells. These results suggest that epithelial cells, rather than interstitial cells or their derivatives, are the primary determinant of most, if not all, of hydra developmental processes.

This conclusion is in a sharp contrast to the generally held view that nerve cells play major roles in hydra (and other animals) development. Nerve cells are abundant in those hydra regions (hypostome, basal disk, bud tip and regenerating tip) that are morphogenetically active (Bode et al. 1973). Some nerve cells contain neurosecretory granules (Lentz, 1966; Davis, 1973b), and low-molecular-weight substances having properties expected for morphogens have been isolated from fractions rich in such granules (Schaller, 1973; Schaller & Gierer, 1973; Berking, 1977).

Isolation of nerve-free hydra, however, casts some doubt on the roles of nerve cells in morphogenesis and development. Strains lacking interstitial cells and their derivatives (nerves and nematocytes), obtained either by means of colchicine or by genetic inbreeding, were shown to possess extensive developmental capabilities comparable to normal hydra strains (Marcum & Campbell, 1978; Sugiyama & Fujisawa, 1978).

To reconcile the 2 lines of conflicting evidence discussed above, Marcum & Campbell (1978) have provided 4 alternative suggestions for the roles that nerve cells play in hydra development; (1) nerve cells are not involved in hydra development; (2) nerve cells play a role in ‘fine tuning’ developmental patterns which are basically established by the epithelial cells; (3) nerve cells play essential roles in patterning hydra, but in their absence compensatory activities can be displayed by the epithelial cells; and (4) nerve cells and epithelial cells may both be exerting identical or overlapping controls over hydra development.

In our previous paper (Sugiyama & Fujisawa, 1978), we presented evidence that supported the second suggestion (fine tuning roles). Morphogenetic processes such as budding or regeneration occur in hydra lacking nerve cells, showing that nerve cells are not essential for such processes. The rates, however, of such processes are strongly influenced by the presence or absence of nerve cells, suggesting that these cells may be involved in fine tuning the rates of these processes. Interstitial cell-deficient hydra also carry a significantly greater number of tentacles per polyp than normal hydra, suggesting that morphogenesis of tentacles proceeds in the absence of nerve cells but that the process is fine tuned by them.

We thought that a limited number of experiments carried out using a chimeric strain also supported the same view (Sugiyama & Fujisawa, 1978). The growth rate of a chimera strain was apparently closer to the growth rate of its interstitial cell donor strain than to that of the epithelial cell donor strain. This appeared to suggest that nerve cells regulated the chimera's growth rate.

This suggestion, however, must now be retracted on the basis of more extensive works with chimeras. Growth rate determination shows a considerable degree of variation from one analysis to another. Therefore, statistical methods were used in
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the present work to treat the results obtained from many experiments involving various chimera and interstitial cell donor strains. The results thus analysed clearly established that no significant correlation exists between the population growth rates of the chimeras and those of the interstitial cell donors (Fig. 3A). Instead, the chimera's growth rates are all very similar to that of their epithelial donor strain. Essentially the same conclusions are also obtained for budding rate, bud developmental rate, tentacle numbers per polyp and protein content per polyp (Figs. 3, 4). It was also found that morphogenetic mutant characters such as regeneration deficiency or multi-headedness are not transferable to the chimeras.

This evidence all strongly suggests that nerve cells (and interstitial cells) play little, if any, role in hydra development. Four suggestions made by Marcum & Campbell (1978) on the roles of nerve cells have been described already. Of them, only the first one is compatible with the present evidence. Had nerve cells played such roles as described in the alternatives, chimeras should have reflected the characters of interstitial cell donors much more strongly than observed. It is suggested that epithelial cells, and not interstitial or nerve cells, are the primary determinant of most, if not all, of hydra developmental characters.

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