Hydra viridis: Inhibition by the Basal Disk of Basal Disk Differentiation

Abstract. *When the basal disk of* Hydra viridis *is excised, a new disk is regenerated. A basal disk grafted on an animal regenerating its own disk can suppress this regeneration. The effect is reversed if the grafted basal disk is subsequently excised. For inhibition to occur, the grafted disk must be present for at least 3 hours, beginning no later than 2 hours after amputation. The results indicate that the basal disk participates in the control of its own differentiation.*

The basal disk is the organ by which hydras adhere to the substrate. It consists of a compact group of cells which elaborate and secrete a sticky substance thought to be an acid mucopolysaccharide (*1*). Disk cells are not normally present anywhere in the animal except at the disk itself (*1*). However, differentiation of a disk can be brought about at any level of the peduncle by excision of the more proximal parts; the cut heals, and the animal regenerates a basal disk at the healed site, now the most proximal part of the body. Even in the intact animal, the epidermis of the peduncle is destined to become a basal disk: disk cells are continually sloughed off and are replaced by cells which arrive at the disk site as a result of a continuous migration of peduncle epidermis in the proximal direction (*2*).

Most current theories interpret all differentiation in hydra as being controlled by gradients of one or two factors whose main function is regulation of hypostome differentiation (*1*, *3*). We investigated the possibility that regeneration of the basal disk is induced by a mechanism directly responsive to the absence of the basal disk itself. Under that circumstance, the ease of surgical manipulation and the simplicity of the system might make the basal disk favorable material for the study of induction.

Hydra viridis were obtained from a clone cultured at 18°C according to the method of Loomis and Lenhoff (*4*). Animals were fed in the evening, 6 or 7 days a week. Glass needles (0.1 mm thick with 0.5-mm shanks) glued inside the tips of disposable Pasteur pipettes were used to manipulate hydras for observation and grafting. A ring of Tackiwax (Central Scientific Company) was sealed flat inside a petri dish by heating. Two glass needles of unequal length were positioned horizontally and sealed to the wax by their shanks so that they crossed at right angles, touching one another within the ring. A second short needle was positioned parallel to the first, crossing the long needle at a point 1 mm further toward the tip, but without touching it; its shank was pressed into the wax but not sealed.

The wax ring was first filled with culture medium. Sections of a hydra were threaded onto the long needle and pushed along until blocked by the first short needle. The second short needle was then lowered into contact with the long needle, and the sections were thus confined between and pressed together by the short needles (Fig. 1A). The dish was then placed in an incubator at 18°C for 1 hour, during which time the sections fused together. The second short needle was then removed; the animal was guided off the long needle and transferred to fresh medium. Simple grafts were generally completed in about 5 minutes, with more than 95 percent success.

The basal disk and the proximal half-peduncle of a large budding hydra were excised and discarded. The remaining distal fragment was left undisturbed for 0 to 180 minutes. We shall call such truncated animals "hosts." At the end of intervals longer than 30 minutes, a small amount of tissue was cut from the healing end to expose a fresh surface for grafting. A piece of peduncle was then isolated from a normal hydra and immediately grafted to the host.

Two series of experiments were performed. In series A, the graft was a proximal half-peduncle which included the basal disk. Only a small piece of the disk was removed, to allow passage of the needle and to provide a second cut surface on the proximal end, as in the other series. In series B, by contrast, the graft was a proximal half-peduncle from which the entire basal disk had been excised. The variation in size of the grafts within series A or B was appreciably greater than the systematic difference in size between grafts of the two groups owing to the inclusion of the basal disk in

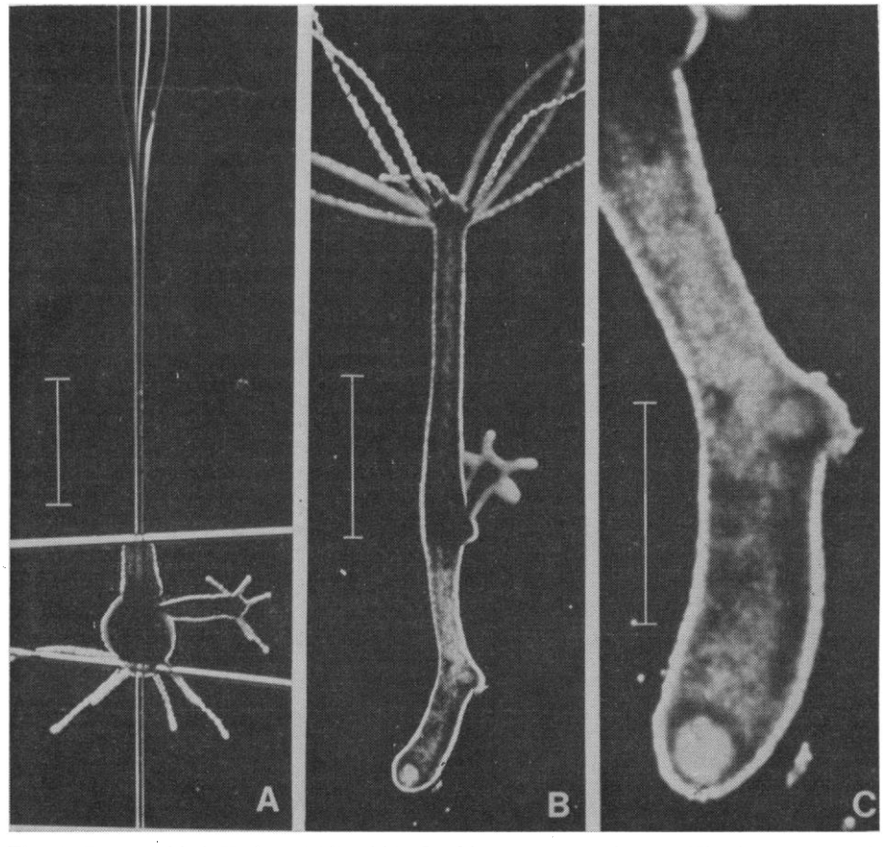

Fig. 1. Reassembled *Hydra viridis*. (A) Grafting apparatus in use. The host and graft have been threaded on the long needle (vertical in photograph) and are retained between two short needles (scale = 1 mm). (B) Series-A experiment (P = 120 minutes) 2 days after grafting. A supernumerary basal disk has differentiated from the proximal end of the host (scale = 1 mm). (C) The two basal disks of the same experiment as in (B) (scale = 0.5 mm).

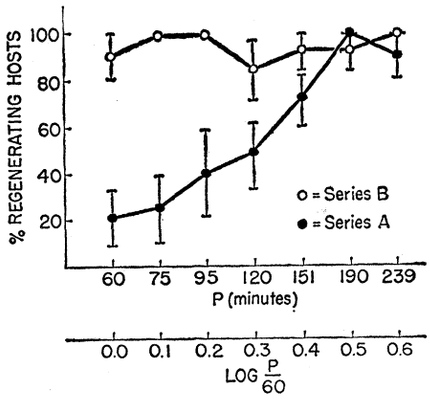

Fig. 2. Percent of hosts regenerating a basal disk (at mid-peduncle of the reassembled animal) as a function of length of the incubation period, P. Each point represents 8 to 17 experiments. Bars show the standard error of the estimate ($\sqrt{r(1-r)/n}$, where r is the proportion regenerating and n is the number of experiments).

A and not in B (*5*). The original polarity of the fragments was maintained in all experiments. The time elapsed between amputation of the host and completion of grafting was recorded, and 60 minutes were added as a correction for the time required for fusion of host and graft. The resulting time interval was used as an estimate of the period during which the host was free of tissue-mediated influence from the graft. This interval was designated the incubation period, P.

In series A, all grafts retained their basal disk. In series B, all grafts regenerated a basal disk at the proximal tip. In addition, disks appeared in the mid-peduncle of many of the reassembled animals (Fig. 1, B and C). Such animals sometimes retained a constriction at the graft line; in these cases the supernumerary basal disk always appeared immediately distal to the constriction, indicating that it had differentiated from the proximal tip of the host. All regenerating basal disks appeared between 24 and 48 hours after grafting. Whether derived from the host or from series-B grafts, they were easily recognized by their form and by their characteristic stickiness.

Nearly all hosts grafted with truncated half-peduncles (series B) regenerated a basal disk, regardless of the duration of P. The experimental treatment was thus sufficient to induce formation of basal disks in the series B hosts. In contrast, the majority of hosts grafted with half-peduncles bearing a basal disk (series A) failed to regenerate a disk, unless the incubation period exceeded 2 hours (Fig. 2). Induction of the basal disk in series-A hosts was thus either blocked or reversed by the disk of the graft (*6*). It is clear that the mechanism of disk induction must be sensitive to the presence of a basal disk.

With increasing duration of P, grafted basal disks were progressively less successful in inhibiting induction of disks in the hosts. Presumably, induction becomes irreversible after a variable period following amputation. Under our conditions, irreversible induction in 50 percent of the cases required that the host remain without a basal disk for a critical period of 2 hours. Three hours of incubation without a disk sufficed for irreversible induction in substantially all cases. This is much less than the time (24 to 48 hours) required for the formation of a morphologically recognizable disk.

We sought to determine how long a grafted disk has to be present to inhibit disk induction. Series-A experiments with P equal to 60 minutes were conducted as previously, except that the basal disk of the graft was excised at various intervals after fusion. If the disk had not been excised, only 20 percent of the hosts would have regenerated disks at mid-peduncle (Fig. 2). An increased incidence of regeneration indicated that there had been insufficient time for complete inhibition. The presence of the grafted disk for 5 hours was sufficient to reduce the incidence of regeneration to the background value of 20 percent, whereas removal of the disk after only 2 hours allowed 70 percent of the hosts to regenerate.

In our system, a grafted basal disk can inhibit the development of another disk if present during a period of induction, which appears to last less than 5 hours. During this period, removal of the grafted disk is sufficient to alleviate the inhibition. We wish to emphasize that we have defined inhibition operationally; the evidence on hand does not establish any particular mechanism. However, it is tempting to speculate that the effect is mediated by an inhibitory substance produced by the basal disk itself. Such a substance may also serve as a negative feedback in the intact animal, regulating the size of the basal disk.

H. K. MacWilliams
F. C. Kafatos

Biological Laboratories,
Harvard University,
Cambridge, Massachusetts

References and Notes

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5. We did not attempt to make A and B grafts equal in size, since this would have resulted in a systematic difference between the groups with respect to the origin of the distal end of the graft. We felt that it was most important to standardize the nature of the graft tissue in direct contact with the assay site.
6. The difference between the two experimental series at P equal to 60 or 75 minutes was highly significant (probability is less than .05). The difference (70 to 75 percent) was large compared to the standard error of the estimate for each result (13 to 15 percent) and thus cannot be attributed to the systematic difference in size between grafts of the two series, which was small compared to the size variability within each group.
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Apparent Production of Two Types of Antibodies by a Single Cell

Abstract. *Immunization of mice with bacterial, sheep red cells coated with antigen resulted in apparent production of antibody-forming plaques with specificities for both hemolysis and bactericidal activity. The secondary response for production of these bifunctional types of plaques could be induced by red blood cells or bacteria alone. A significant mutual suppression of immune response between the two immunizing agents was observed.*

One of the most heated controversies in immunology centers around the potentiality of the antibody-forming cells. The dispute concerns whether a single cell can produce only one type of antibody at a given time. The experimental evidence has been equivocal, and available data support the possibility of production of one (*1*) or several types of antibodies per single cell (*2*). The major factor for the persistence of the controversy is the fact that, although methods utilized in the past for testing the potentiality of antibody-forming cells were elegant and precise, they were also quite complex and cumbersome. Development of simple and rapid tests for detecting antibody-forming cells provided an opportunity to extend and test the suggested alternatives (*3*).