were not excluded as causes of variation. Chromosome segregation can occur at high rates in hyperdiploid cells (*15*), and conclusions based on studies of variant formation in such cells may not be applicable to diploid cells. Heterozygous markers linked to the selected marker are helpful in distinguishing between chromosomal segregation and intrachromosomal mechanisms of variation and are essential for detailed characterization of intrachromosomal mechanisms of variation. HLA markers and selective systems are particularly useful in this regard because there are four closely linked HLA alloantigenic loci which in turn are linked to the structural loci for the polymorphic enzymes PGM3 and glyoxalase I.

The mutation rate we have obtained for HLA B-27, approximately 1×10^{-6} per cell per generation is close to that found for HGPRT in diploid human fibroblasts (*13*). The gametic mutation rate for an H-2 gene in mice (H-2 is the mouse homolog of HLA) obtained by a grafting procedure was 5×10^{-1} per gene per generation (*16*). For the mutation rates in gametes and somatic cells to be compared, the rate for gametes must be divided by the number of doublings preceding formation of mature gametes, which DeMars has estimated to be 10^2 to 10^3 (*17*). Application of this correction factor results in good agreement between the mutation rate in vivo for H-2 and the rate in vitro for HLA. This agreement may be fortuitous, however; it could reflect offsetting differences related to the different methods of mutant detection, errors in the assumptions made in calculating the correction factor, and possible intrinsic differences in the mutability of germinal as opposed to somatic cells or of particular genes. These questions should be resolvable by further studies of somatic and germinal mutation rates. Systems such as the HLA system which allow for evaluation of mutagenesis and for quantitation and characterization of mechanisms of variation in diploid somatic cells should be valuable in this regard as well as in assessing the risks of environmental agents to humans.

Note added in proof: Since submission of this manuscript we have isolated two variants of T5-1 with B8 antiserum in which both B8 and A1 are affected. It thus appears that not all HLA variants of T5-1 are single gene mutants.

DONALD PIOUS
CARL SODERLAND
Departments of Pediatrics and *Genetics, School of Medicine, University of Washington, Seattle 98195*

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Polarity Reversal in Nerve-Free Hydra

Abstract. *Hydra experimentally depleted of all nerve cells and nerve stem cells (interstitial cells) demonstrate normal polarity reversal. Thus hydra epithelial cells are capable of controlling complex developmental patterning phenomena.*

The control over developmental patterning in hydra is frequently ascribed to neurosecretory cells (*1*). Hydra's developmental gradients, for example, have been postulated to be due to gradients in nerve cell density along the column (*2*) as well as to the diffusion gradients of the neurosecretory materials that act as developmental hormones (*3*). Regeneration, cell determination, budding, cell cycle duration, and cell differentiation are all considered to be influenced by neurosecretory cells (*4*). However, we are finding that experimentally denervated hydra grow, bud, and develop rather normally (*5, 6*). To determine the developmental capabilities of denervated hydra we have analyzed the kinetics of polarity reversal in these nerve-free animals. Polarity reversal is one of the most complex developmental traits analyzed in hydra and therefore represents the most severe test of developmental capability in nerve-free hydra.

An isolated segment of hydra's column regenerates with strict polarity. A hydranth (hypostome and tentacle whorl) regenerates from the end that was

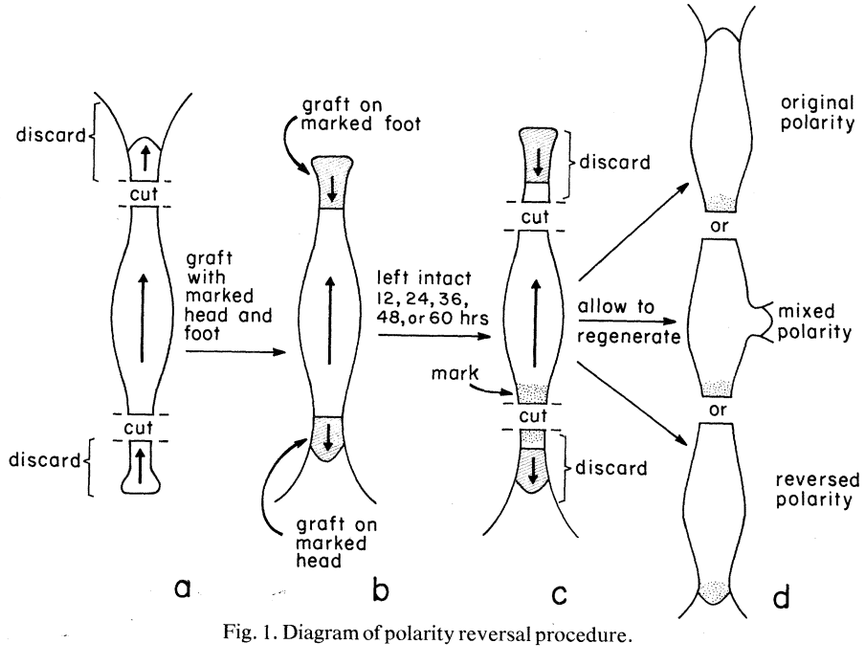

Fig. 1. Diagram of polarity reversal procedure.

originally closer to the hydranth, and a basal disc regenerates from that end that was originally closer to the basal disc.

The polarity of hydra tissue may be reversed by cutting a column segment out and grafting it back into the hydra in reverse orientation (*7, 8*). The polarity of this segment changes after a period of time in accord with its new orientation in the animal. In the experiments reported here we cut the column out of one animal and grafted a vitally marked hydranth and basal disc, from another hydra, onto the wrong ends of this column. This operation (*9*) is diagramed in Fig. 1. Then at 12-hour intervals we cut out the reversed column, marking one end of it vitally to keep track of its polarity, and observed whether this isolated column regenerated according to its original polarity or to the polarity appropriate to its recent reversed orientation.

In a control experiment with normal hydra (Table 1) polarity reversal occurred in 24 to 36 hours (*10*). Tissue left grafted in a reversed orientation for less than 24 hours regenerated according to its original polarity. Tissue left in reversed orientation for more than 36 hours regenerated according to its new, reversed polarity. Tissue left grafted in a reversed orientation for intermediate periods of time, 24 to 36 hours, regenerated in a mixed fashion (head structures forming either at both ends or in the middle), possibly indicating (*7*) that polarity was in the process of reversing.

We then studied polarity reversal in what we term nerve-free hydra. These are hydra lacking all nonepithelial cell types: nerve, interstitial, nematocyst, and gland cells. These hydra, which consisted entirely of ectodermal epitheliomuscular cells and endodermal epithelial digestive cells, were produced by soaking polyps of *Hydra attenuata* in 0.4 percent colchicine for 8 hours on two occasions spaced 14 days apart (*11*). Three treated polyps that were found to be lacking all nonepithelial cell types were cultured by pressing food into the gastric cavity daily. Although lacking spontaneous behavior, they budded in a normal fashion and gave rise to three asexually propagated clones (*12*). The cellular compositions of these hydra were monitored over the course of a year before experimentation. Both the quantitative maceration method (*13*) and serial paraffin histological sections were used. Counts on 35,000 cells verified that the hydra of these three clones contained only epithelial cells (*6*). Polyps used in these experiments represented approximately the 25th asexual generation of these clones.

Table 1. Polarity of regeneration in normal and nerve-free hydra.

Time in reversed orientation (hours)	Hydra (No.)	Polarity of regeneration		
		Original	Mixed	Reversed
Normal hydra				
12	11	11	0	0
24	11	0	10	1
36	9	0	3	6
48	12	0	2	10
60	15	0	1	14
Nerve-free hydra				
12	12	12	0	0
24	13	8	1	4
36	8	1	2	5
48	11	0	0	11
60	10	0	0	10

Nerve-free regenerates were similar in appearance to normal regenerates, and their polarity was easily identifiable (Fig. 2). The kinetics of polarity reversal in nerve-free hydra, as shown in Table 1, have two notable features: First, the time required for full polarity reversal is indistinguishable from that in normal hydra. Reversal begins after the tissue has been in reversed orientation for 24 hours and is complete by 48 hours. Second, nerve-free hydra only rarely showed "mixed" regeneration during the period of polarity reversal, while most normal hydra showed these intermediate states.

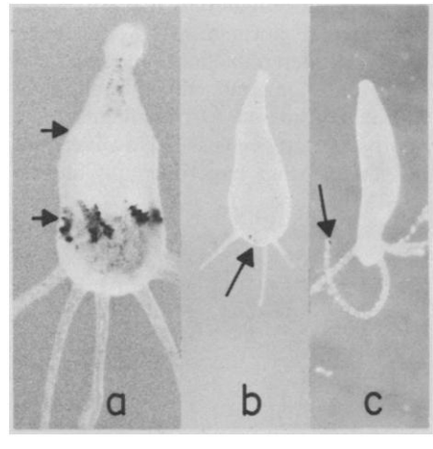

Fig. 2. Example of polarity reversal. (a) Grafted (36 hours) nerve-free hydra. Arrows indicate junctions between the grayish, endodermally marked head and foot tissue (crosshatching in Fig. 1b) and the unmarked, inverted gastric region. The three darker spots are ectodermal marks (stippling in Fig. 1c) that show the original proximal (foot) end of the column segment (×34). (b) Nerve-free regenerate arising from the gastric region cut out of the polyp shown in (a) (cuts were made as shown in Fig. 1c, 36 hours after grafting). The arrow points to ectodermal marks in the hydranth, demonstrating that the originally proximal end regenerated a hydranth (×17). (c) Control regenerate arising from the gastric region of a normal hydra, which was treated just as the polyp in (b). The arrow points to an ectodermal mark in the tentacle (×17).

These experiments indicate that epithelial cells are capable of determining complex polarity phenomena in hydra. Not only is polarity retained and reversed with kinetics typical for normal hydra, but in addition a hydranth and basal disc of pure epithelial cells are sufficient to induce polarity reversal in intervening tissue. The lack of mixed polarity during reversal in nerve-free tissue suggests that some aspects of polarity require involvement of other cell types. This agrees with other recent observations that nerve-free hydra are capable of nearly, but not precisely, normal development in all ways we have so far tested (*6*).

These experiments immediately suggest that hydra polarity is determined by epithelial cells, as proposed previously (*7*), and might be due to either a metabolic and transport polarity (*7*) or to a structural asymmetry. The ectodermal epithelial cells do have axially aligned muscle processes (*14*) and show polarized motions (*15*), which might provide a structural basis for polarity.

An alternative interpretation of these data is that nerve cells normally control developmental patterns, but that in their absence the epithelial cells can take over nerve cell functions. Epithelial cells could conceivably become neurosecretory, for example. This interpretation has the advantage that it fully reconciles our data with the evidence implicating nerve cells in hydra development, and it has precedence in vertebrate regeneration for which nerve cells perform essential trophic functions that can be taken over by nonnervous tissue (*16*).

BEVERLY A. MARCUM
RICHARD D. CAMPBELL, JOSÉ ROMERO
Department of Developmental and Cell Biology and *Center for Pathobiology, University of California, Irvine 92717*

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Correlation Between Lipid Synthesis in Tumor Cells and Their Sensitivity to Humoral Immune Attack

Abstract. *Prolonged incubation of two antigenically distinct, chemically induced guinea pig hepatomas with relatively high concentrations of chemotherapeutic drugs or metabolic inhibitors increases their susceptibility to killing by antibody and complement. This effect is reversible when the cells are cultured in the absence of the drugs. The drug-induced sensitivity and the ability of the cells to recover their resistance to killing are directly correlated to their ability to synthesize complex lipids.*

Most nucleated cells are more resistant than nonnucleated cells to killing by antibody and complement (*1*). Different nucleated cells also differ in their susceptibility to immune killing. Susceptibility or resistance of nucleated cells to killing could not be explained by differences in antigen concentration on the cell surface (*2, 3*), by the mobility of antigen on the cell surface (*4*), by the immunoglobulin class used to sensitize the cells (*1*), or by the amount of early acting complement components fixed to the cell (*1, 2, 5*). Some resistant nucleated cells, however, become susceptible to killing at certain stages in their cell cycle (*6*), or can be rendered susceptible to immune attack by treatment with certain metabolic inhibitors and chemotherapeutic drugs used in the treatment of cancer (*7*). Resistance of nucleated cells to killing by immune attack mechanisms may be an intrinsic property of the target cell under metabolic control. Since the cytotoxic action of antibody and complement occurs on or in the cell membrane (*8*), we were prompted to investigate cellular metabolic pathways associated with membrane structure and function which might be affected by treatments which increase the sensitivity of tumor cells to humoral immune attack. In this report, we present evidence that the ability of tumor cells to resist killing by antibody and complement is related to their ability to synthesize lipids.

The ascitic forms of two antigenically distinct guinea pig hepatomas (line 1 and line 10) induced by diethylnitrosamine were collected as previously described (*9*). These cells are resistant to killing by specific rabbit antitumor antibodies plus guinea pig complement (GPC) (*3*). In addition, cells of line 10, but not of line 1, are resistant to killing by rabbit IgM antibody to Forssman antigen plus GPC (*3*). However, cells of both line 1 and line 10 sensitized either with antibody to specific tumor antigens or with IgM antibody to Forssman antigen are susceptible to killing by human complement (*3*).

Tumor cells of line 1 and line 10 were incubated for various lengths of time with selected metabolic inhibitors and chemotherapeutic agents (see Fig. 1). Those drugs effective in increasing sensitivity to killing were used at the lowest concentrations giving a maximum effect, whereas the drugs ineffective in increasing sensitivity were used at the highest concentrations which were not toxic to the cells (*7*). Control suspensions were prepared concurrently consisting of cells incubated in tissue culture medium alone. At several time intervals during the incubation, drug-treated and control cells were tested for their sensitivity to killing by antibody and GPC and for their ability to synthesize DNA, RNA, protein, and complex carbohydrates (*10*); in addition, the cells were tested for their ability to synthesize complex lipids as measured by following their incorporation of fatty acids into extractable cellular lipids (*11*).

The data presented in Fig. 1 are the summary of results obtained with line 1 tumor cells; similar results were obtained with line 10 cells. Increased susceptibility of the drug-treated tumor cells to killing by IgM antibody to Forssman antigen or antibody to specific tumor antigens plus GPC as compared to nondrug-treated cells similarly tested was noted only after 17 hours of incubation with actinomycin D, adriamycin, mitomycin C, or puromycin (see Fig. 1A). These drugs will be referred to as "effective." Cells incubated with 5-fluorouracil, cytosine arabinoside, cyclophosphamide, vincristine sulfate, 6-mercaptopurine, or hydroxyurea remained resistant to killing at all time intervals tested (see Fig. 1B). These drugs will be referred to as "ineffective." Tumor cells which had been incubated 17 hours with the effective drugs, washed free of drug, and reincubated in drug-free medium recovered their resistance to killing by antibody plus GPC within 4 hours in culture (see Fig. 1A). Those

Table 1. Effect of Atromid-S on the macromolecular synthesis and the susceptibility to antibody-complement mediated killing of line 1 tumor cells.

Concentration of Atromid-S*	Percentage inhibition of incorporation of				Cells stained with trypan blue (%)	
	[^{3}H]Thymidine	[^{3}H]Uridine	^{14}C-Labeled amino acids	[^{14}C] Palmitic acid	IgM antibody to Forssman antigen (1:80) plus GPC (1:8)	Antibody to line 1 antigens (1:10) plus GPC (1:8)
None†	0	0	0	0	6	1
$1 \times 10^{-4}M$	14	3	34	37	8	3
$5 \times 10^{-4}M$	24	19	35	70	34	27
$1 \times 10^{-3}M$	56	62	52	68	37	29

*Line 1 cells (5×10^5/ml) were incubated with Atromid-S for 60 minutes at 37°C. †Untreated control cells incorporated, in terms of counts per minute, approximately 10,000 [^{3}H]thymidine, 7500 [^{3}H]uridine, 6000 ^{14}C-labeled amino acids, and 25,000 [^{14}C]palmitic acid.