



Cell Lineage Analysis by Intracellular Injection of a Tracer Enzyme

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5×10^6 cells. Activity of peritoneal cells remained unchanged after the cells were treated with antiserum to T and antiserum to mouse immunoglobulin in the presence of complement (Table 1).

Results of experiments with human peripheral blood monocytes, lymphocytes, and polymorphonuclear leukocytes were similar to those obtained with the corresponding murine cells.

The other formed elements of peripheral blood assayed for NADase activity were erythrocytes and platelets (Table 2). Both human and murine red blood cells had negligible activity as compared with that of peritoneal, alveolar, and spleen macrophages—approximately 0.1 unit per 500 μg of erythrocyte protein, as compared to 100 units per 500 μg of macrophage protein. Thus, activity of red blood cells was approximately one-thousandth that of macrophages. Platelets, on the other hand, showed a relatively high activity, 11 to 12 units per 500 μg of protein, a value surpassed only by the activity of macrophages.

Platelets share several properties with macrophages, such as the property to adhere to foreign surfaces, and the capacity to engulf inert particles, which represents true phagocytosis. Although bactericidal and lytic properties have been ascribed to platelets, their antixenic function has not been proved (4).

Two or possibly three enzymes split NAD at the nicotinamide riboside linkage. The single protein NADase (E.C. 3.2.2.5) also has two activities: NAD glycohydrolase and NAD transglycosidase. The common intermediate is ADP-ribosylated (ADPR) enzyme, which promotes transfer of the ADPR moiety of NAD to water (glycohydrolase activity) or to various pyridine derivatives in place of nicotinamide (transglycosidase activity). The two other enzymes are ADP-ribosyl transferase, which catalyzes the transfer of ADPR moiety of NAD to a macromolecular acceptor, and poly(ADPR) synthetase, which promotes polymerization of ADPR. The formation of poly(ADPR) seems to be restricted to eukaryotic nuclei having chromatin structure (5).

The activity of all three enzymes can be followed by the cyanide addition reaction (6). The quaternary nitrogen of the β -N-glycosidic linkage is essential for the reaction. When NAD is split at the pyrophosphate bond, there is no change in the cyanide reaction. Poly(ADPR) synthetase can be distinguished from NADase by the formation of an acid-precipitable ADPR polymer.

We do not yet know which of the above enzymes is responsible for the

cleavage of NAD in murine macrophages. Throughout the text we have chosen to refer to the NAD splitting enzyme of macrophages and platelets as NADase, because the spleen enzyme was reported to have transglycosidase activity (2).

The biological function (or functions) of the enzymes which split NAD are unknown, with the notable exception of NAD glycohydrolase of toxigenic strains of *Corynebacterium diphtheriae*, where the enzyme was identified as the diphtheria toxin (7). The existing data on the possible role of NAD splitting enzymes in eukaryotes are both fragmentary and contradictory (8).

It would be of interest to ascertain whether the NAD splitting activity of macrophages (and platelets) is related to their function and whether the high NADase activity of lungs and liver, like that of spleen, is the property of lung and liver macrophages.

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Cell Lineage Analysis by

Intracellular Injection of a Tracer Enzyme

Abstract. *Cell lineages during development of leeches can be ascertained by injection of horseradish peroxidase as a tracer into identified cells at early stages of embryogenesis. The injected embryos continue their normal development, in the course of which horseradish peroxidase is passed on in catalytically active form to the descendants of the injected cell. The distribution of the tracer enzyme and hence of the progeny of the injected cell can then be observed at a later stage of development by staining the preparation for horseradish peroxidase.*

Knowledge of the lines of descent of the cells that eventually compose the mature organism is fundamental for understanding embryonic development. Regulative mechanisms, such as differentiation controlled by position within an external gradient, and determinant mechanisms, such as differentiation controlled by inherited cytoplasmic factors, may be involved to varying degrees at different developmental stages; learning the fate of clonally related cells can aid in discerning the relative importance of these mechanisms.

In the past, cell lineages have been established by the direct observation of dividing cells or of extracellular marker particles, by selective ablation, and by induction of genetic mosaics (1). Each of these techniques has disadvantages that limit its scope: direct observation of living embryos becomes progressively

References and Notes

1. Abbreviations used: NAD, nicotinamide adenine dinucleotide; ADPR, adenosine diphosphate ribose; poly(ADPR), polymer of adenosine diphosphate ribose; C, complement; BSA, bovine serum albumin; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid. Antiserum to mouse immunoglobulin and antiserum to T cells (that is, to theta antigen) rabbit complement (absorbed with agarose and mouse tissues) were obtained from Cedarline Laboratory, London, Ontario, Canada, and from Miles Laboratory, Elkhart, Ind.
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least three conditions be met: (i) after injection of HRP, embryonic development must continue normally; (ii) the injected HRP must remain catalytically active and not be diluted too much in the devel-

oping embryo; and (iii) HRP must not pass through the gap junctions that commonly link embryonic cells.

This technique was successfully applied in the leech *Helobdella triserialis*,

whose early embryonic development is shown in abbreviated schematic form in Fig. 1 (2). *Helobdella triserialis* lays yolky eggs about 0.5 mm in diameter, whose early cleavages can be observed

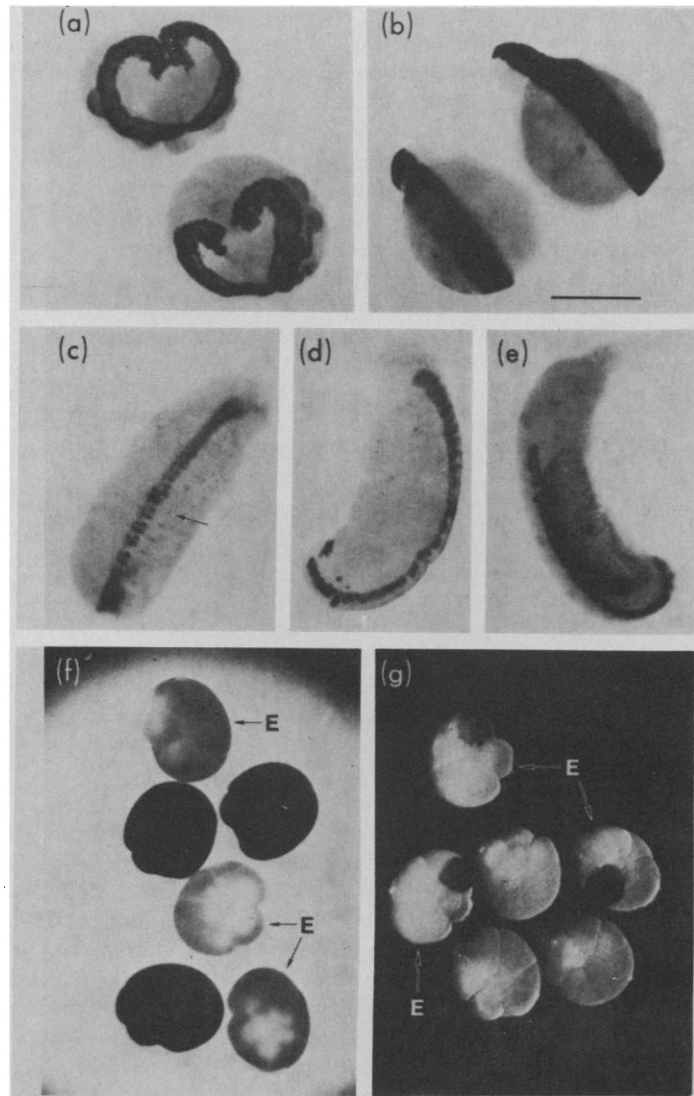
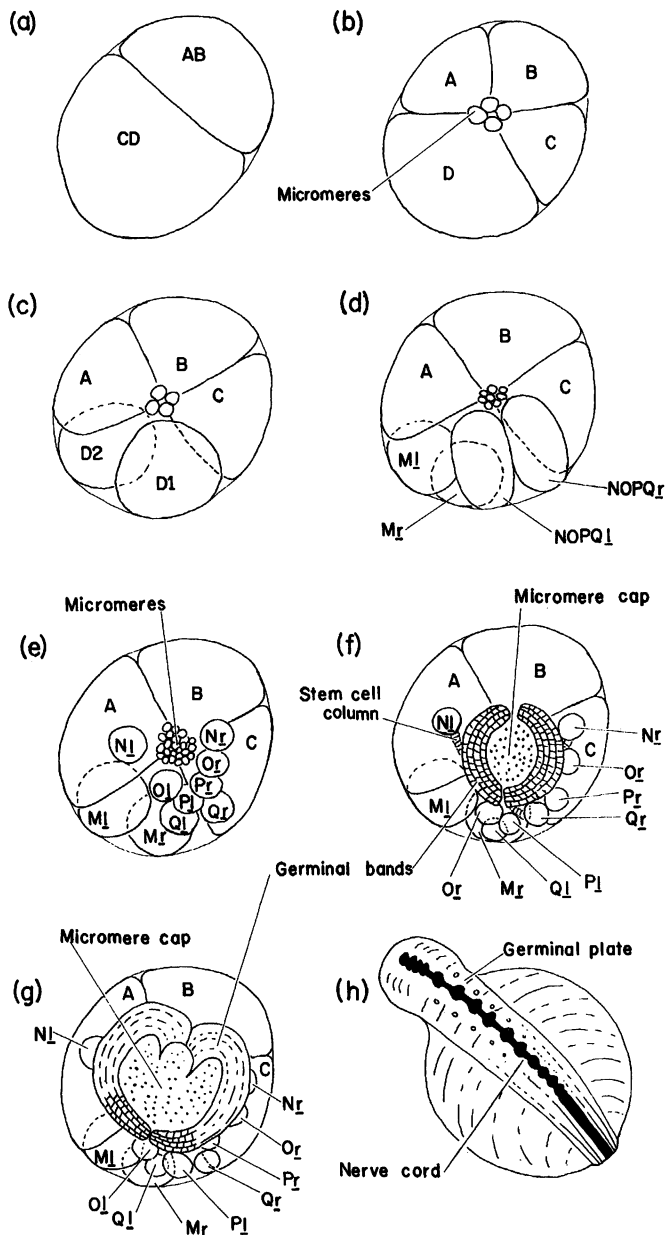


Fig. 1 (left). Schematic representation of the initial stages of development of the leech *Helobdella triserialis*. Times refer to development at about 23°C. (a) First cleavage of the egg into cells AB and CD (4½ hours). (b) Cleavage of AB into macromeres A and B and of CD into macromeres C and D, and cleavage of A, B, C, and D to produce one micromere each (10 hours). (c) Cleavage of macromere D into the ectoteloblast progenitor D1 and the mesoteloblast progenitor D2 (11 hours). (d) Cleavage of D1 into the ectoteloblast progenitors NOPQl and NOPQr and of D2 into the mesoteloblasts Ml and Mr. Increase in the number of micromeres (16 hours). (e) Formation of all five pairs of teloblasts, after sequential cleavage of each NOPQ into N and OPQ; cleavage of OPQ into O and PQ; and cleavage of PQ into P and Q (28 hours). (f) Production of stem cell columns by teloblasts. Stem cell columns from left and right teloblasts forming left and right germinal bands, respectively. Stem cell column produced by M lying under the other four columns. Development of micromere cap lying between germinal bands (2 days). (g) Right and left germinal bands joined at future head and beginning rostro-caudal coalescence along future ventral midline. Further expansion of micromere cap (2½ days). (h) Completion of germinal band coalescence and formation of germinal plate. Ventral view of embryo with head lying at upper left. [This view is the obverse of that shown in (a) to (g).] Coalescence of the rostral ganglionic mass and of the rostral segmental ganglia (6 days).

Fig. 2 (right). Photomicrographs of HRP-injected leech embryos. (a) Two embryos whose cell D1 had been injected with HRP and which were fixed and stained 4 days later. Individual columns of stained stem cells and ectoteloblasts can be seen. (b) Two embryos in each of which an NOPQ cell had been injected with HRP, and which were fixed and stained 5 days later. In each specimen, the germinal plate is stained hemilaterally. (c) Ventral aspect of an embryo whose Nl ectoteloblast was injected with HRP prior to the production of any stem cells, and which was fixed and stained 6 days later. The left hemiganglia (apparent right) are stained as are the putative sensillae (arrow) on the left side. (d) Lateral aspect of the embryo shown in (c). (e) Lateral aspect of embryo whose Nl ectoteloblast had been injected after some stem cells had already been produced and which was fixed and stained 6 days later. Only the caudal but not the rostral hemiganglia are stained. (In this photograph the presumptive gut appears dark, as a result of suboptimal dehydration of the embryo.) (f) Fluorescence micrograph taken within 30 minutes after cell NOPQl or NOPQr in three embryos had been injected with a mixture of HRP and lucifer yellow. Three uninjected control embryos are dark. The stage of development of these embryos corresponds to that shown in Fig. 1d. All cells of the three injected embryos (E) are brightly fluorescent. (g) The same injected (E) and control embryos as in (f), after fixation and staining for HRP, photographed under white light. Only the injected cells show the HRP stain. Scale bar, 0.25 mm in (a) through (e); 0.5 mm in (f) and (g).

under the dissecting microscope. Normal embryonic development proceeds in a simple saline solution. At the developmental stage shown in Fig. 1e, the cleavage process has resulted in three macromeres, A, B, and C, a cluster of small cells (micromeres), and five pairs of teloblasts: one pair of left and right mesoteloblasts *Ml* and *Mr* and four pairs of left and right ectoteloblasts *Nl* and *Nr*, *Ol* and *Or*, *Pl* and *Pr*, and *Ql* and *Qr*. An individual teloblast can be identified visually by its position in the embryo and by the particular cleavage sequence under which it arose from its progenitors (Fig. 1, a to d). Each teloblast gives rise to a column of several dozen small stem cells. The five columns of stem cells on both sides form a pair of germinal bands on the surface of the embryo. Each germinal band consists of four superficial stem cell columns produced by the ectoteloblasts N, O, P, and Q and an underlying fifth stem cell column produced by the mesoteloblast M (Fig. 1f). Right and left germinal bands then migrate laterally into ventral territory (Fig. 1g) and eventually coalesce zipperlike on the future ventral midline, starting at the future head of the embryo, to form the germinal plate (3) (Fig. 1h). Later divisions of the stem cells and growth of the germinal plate eventually give rise to the ectodermal and mesodermal tissues of the embryo (4).

To identify the progeny of a particular teloblast or of its progenitor, a cell of an early stage embryo is penetrated with a micropipette filled with a 2 percent solution of HRP (Sigma, type VI) in 0.2M KCl, and a small volume of the solution is forced into the cell by pressure. The HRP-injected embryo is then allowed to develop to some later stage. At this point it is fixed and stained for HRP by standard histochemical techniques with benzidine as the substrate (5). The stained embryos can be examined under the microscope, either as whole mounts or, after embedding them in plastic (Epon), as serial thick sections. The examination of sections is aided by counterstaining them with toluidine blue, which causes the yellow HRP-stained cells (that do not take on toluidine blue) to contrast with the blue background of the other cells.

The embryos shown (Fig. 2a) are preparations whose cell D1 (compare with Fig. 1c) had been injected with HRP and which were fixed and stained after four more days of development, by which time the germinal plate was beginning to form. As can be seen, on both sides of the embryo, the four ectoteloblasts N, O, P, and Q and the four superficial stem cell columns of the germinal bands were

stained, whereas the mesoteloblast M and the stem cells issuing from it were not stained. Moreover, in thick sections of a similar preparation (not shown) the deeper column of stem cells was not stained. In other preparations (not shown) cell D2 had been injected with HRP, with the results that both mesoteloblasts M and the deeper stem cell columns were stained, whereas the ectoteloblasts and their stem cells were not stained. These results confirm the cell lineage scheme of Fig. 1, according to which the four bilateral ectoteloblast pairs are derived from a single progenitor (D1) that forms a line of descent different from that founded by the progenitor (D2) of the bilateral mesoteloblast pair. Moreover, these results confirm the earlier finding, based on direct visual observation, that the germinal bands are formed by the merger of columns of stem cells derived from the teloblasts (4).

In the embryos shown (Fig. 2b) the right or left NOPQ cell (see Fig. 1d) was injected with HRP; the embryos were fixed and stained after allowing six more days of development. By then formation of the germinal plate had proceeded to the caudal end and the embryos had begun peristaltic movements associated with hatching from the egg membrane. Only the right or left half of the embryo was stained. This result thus shows that each NOPQ cell is the progenitor of only the ipsilateral half of the germinal plate; that is, that by the time of the division of cell D1 into the sister pair NOPQr and NOPQl the bilateral symmetry of the animal has been established.

The embryo shown in Fig. 2, c and d, is one whose left ectoteloblast *Nl* (see Fig. 1e) had been injected with HRP before production of its stem cell column had begun. Development was then allowed to proceed for six more days, by which time formation of the ventral nerve cord, with its rostral and caudal ganglionic masses and the intervening chain of 21 segmental ganglia on the ventral midline of the germinal plate was complete. Evidently, the left half of the whole ventral nerve cord was stained. This finding confirms an earlier indirect inference (6) that the N ectoteloblast on one side and the stem cell column to which it gives rise is the major, if not exclusive, precursor of the ipsilateral half of the leech nervous system. The embryo shown in Fig. 2e is a preparation similar to that shown in Fig. 2, c and d, except that in this case the *Nl* ectoteloblast had been injected with HRP at a somewhat later stage, after production of the stem cell column had already begun. Here the caudal but not the rostral part

of the left half of the ventral nerve cord was stained. This shows that the caudal part of the hemilateral nervous system develops from the younger stem cells in the column produced by the N ectoteloblast. The sharpness of the boundary between stained caudal and unstained rostral portions of the cord, and the sharpness of the boundary along the midline between stained left and unstained right hemiganglia, suggests that up to this stage of development of the nerve cord there occurs little migration of cells either longitudinally or across the midline of the embryo. Nevertheless, some cells do seem to migrate circumferentially; in Fig. 2c, regularly spaced spots of stained tissue appear outside of, and ipsilateral to, the stained hemiganglia. These spots may be primordial sensillae, the peripheral segmental sensory structures that lie circumferentially in the central annulus of the segment (7). Thus neurons might establish the sensillae by migration from the developing nerve cord.

The foregoing results demonstrate that, in satisfaction of the first two conditions for the success of the HRP tracer technique, embryonic development does continue normally after enzyme injection and HRP remains catalytically active in the developing embryo. To demonstrate that this technique satisfies the third condition of cellular localization of HRP, the results of a control experiment (Fig. 2, f and g) are shown. Individual NOPQ cells of several embryos were injected with a mixture of HRP and the fluorescent dye, lucifer yellow (8), as indicated in Fig. 1d. Without allowing further development, the injected embryos, as well as some uninjected embryos, were first photographed under conditions of fluorescence microscopy. The embryos were then fixed, stained for HRP, and rephotographed under standard illumination. Injection of a single NOPQ cell caused the whole embryo to fluoresce (Fig. 2b). Thus, we infer that, at this stage, all cells of the leech embryo are linked by junctions that permit intercellular passage of lucifer yellow (500 daltons) (9). By contrast, the larger HRP molecules (40,000 daltons) remained confined to the injected single cell. Hence, the HRP tracer does not move between cells of separate lineages, despite an extensive network of intercellular junctions. The presence of HRP in a cell at later stages of development can therefore be taken to indicate a direct descent from the injected cell (10).

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11. We thank Walter W. Stewart for the gift of lucifer yellow, Georgia Harper and Ellis N. Story for technical assistance, and Juan Fernandez and Eduardo Macagno for helpful suggestions and discussions. Supported by NIH postdoctoral fellowship NS05445, by NIH research grant NS 12818, and by NSF grant BN 574-2437 A02.

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Social Plasticity in the Acorn Woodpecker

Abstract. *Acorn woodpeckers (Melanerpes formicivorus) in southeastern Arizona exhibited two different types of social organization: one of highly cooperative and resident groups and another of birds that migrated and formed only temporary male-female pairs during reproduction. The occurrence of both patterns in the same population indicates a high degree of social flexibility in this species.*

The acorn woodpecker (*Melanerpes formicivorus*) has one of the most unusual social organizations of any North American bird. Individuals live in permanent groups containing as many as 12 adult members (1). The groups occupy and defend year-round territories, and they breed communally; most or all of the adult members help to feed the young of a single nest. Another distinctive feature of this species' behavior is its extensive dependence upon stored mast. Acorns and other nuts are collectively harvested by the groups in the fall and stored in individual holes that have been made by the woodpeckers. The storage holes usually are concentrated in one or two trees on the group's territory, and the stored mast forms an important food resource during the winter. This type of group social organization and mast storage behavior has been reported for all locations in which the acorn woodpecker

has been observed, including a number of areas in California (1, 2), in New Mexico and Arizona (3), as well as in Mexico (4) and parts of Central America (5). We now describe a population of acorn woodpeckers in southeastern Arizona in which most of the woodpeckers exhibited an entirely different form of social organization and ecology than that described earlier. Some individuals in this area, however, behaved like the acorn woodpeckers observed previously. The coexistence of two different types of social organization within the same population indicates that the acorn woodpecker can be extremely flexible in its social behavior.

We observed the acorn woodpeckers at the Research Ranch, Elgin, Arizona, 85 km southeast of Tucson. The main study area was in an oak savanna and oak woodland (6). Additional observations were made in a number of lo-

cations in the nearby Huachuca Mountains. The study was conducted in January 1975, May through August 1975, January and March 1976, and May 1976 through March 1978. During this period 63 adults and juveniles were marked with individually color-coded leg bands and wing tags.

Unlike the acorn woodpeckers studied elsewhere, the majority of birds at the Research Ranch did not construct acorn storage holes and their territories did not contain storage trees. The woodpeckers did store acorns, but the nuts were placed under loose bark and in natural crevices of oak trees and power poles throughout their territories. In most years, these stores were exhausted soon after the oaks ceased acorn production. The woodpeckers then abandoned their summer territories and briefly wandered over the study area before they disappeared. At the same time, a number of transient and solitary individuals moved through the area from other locations. By early winter, almost all of the acorn woodpeckers had left the study site and surrounding areas. The abandoned territories remained unoccupied during the winter. In the spring, birds returned and established new breeding territories. During the winter of 1976-1977, for example, only one of 12 summer territories was occupied between December and March. Ten of these territories were reoccupied by woodpeckers in the following spring.

Some of the acorn woodpeckers that established breeding territories on the study site during the spring had been present in previous summers (Table 1); most of the adults occupied the same territories that they had held the year before. In general, territories were established in the same locations each year, although often by new birds.

Although we do not know where the acorn woodpeckers that leave the Research Ranch spend the winter (7), the regular abandonment of summer breeding territories over large areas, absence during the winter, and reoccupation during the spring suggest that these individuals do indeed migrate. The pattern of behavior observed during this study is not a recent occurrence; similar seasonal movements of acorn woodpeckers were reported in the adjacent Huachuca Mountains in 1904 (8).

The migratory acorn woodpeckers exhibited a radically different form of social organization than that previously described for this species. Many of the differences seem to be the result of a lack of stable, long-term bonds between individ-

Table 1. The behavior of acorn woodpeckers in the winter and summer that followed the breeding season in which they were color-banded. The table includes individuals banded during 1975 and 1976; it does not include six birds captured in the summer of 1977.

Behavior	Adults	Juveniles	Transients*
Migrated and returned to same territory the following year	7	1	
Migrated and returned to different territory the following year	2	0	
Winter resident in resident group	2	0	
Solitary winter resident	0	0	1
Did not return in first year but returned in second year	1	0	0
Transients that migrated and then returned to breed in area			2
Disappeared and were not seen again	18	13	10
Total	30	14	13

*Transients are individuals that did not breed in the study site but moved into the area and were captured prior to the fall migration.