Early Differences Between Alternate n Blast Cells in Leech Embryo

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SUMMARY

Segmentally iterated tissues of the mature leech comprise five distinct sets of definitive progeny that arise from chains of *blast cells* (m, n, o, p, and q *bandlets*) produced by five bilateral pairs of stem cells (M, N, O/P, O/P, and Q *teloblasts*). In each n and q bandlet, two blast cells are needed to generate one set of hemisegmental progeny, and two alternating classes of blast cells (nf and ns, qf and qs) can be distinguished after their first divisions. Furthermore, two distinct subsets of definitive N and Q progeny exist within each hemisegment. Here we first show that there is a fixed correspondence between the class of blast cell and the subset of final progeny: ns cells contribute mainly anterior ganglionic neurons and epidermal cells; nf cells contribute mainly posterior ganglionic neurons, peripheral neurons and neuropil glia; qs cells contribute both ventral and dorsal progeny; and qf cells contribute only dorsal progeny. Second, ablation studies indicate that the two classes of n blast cells do not behave as an equivalence group in the germinal band. Finally, we show that the cycles giving rise to nf and ns blast cells differ. These data suggest that cellular interactions within the germinal band may not be critical in establishing the distinct nf and ns cell fates and that, conversely, differences between the two classes of n blast cells may be established at birth.

INTRODUCTION

During development, cells follow different fates as determined by combinations of intrinsic and extrinsic factors. Leech embryonic development is highly determinate, meaning that cell fates are highly predictable (Weisblat et al., 1980, 1984; Stent et al., 1982; Stent, 1985), even though extrinsic factors are critical in establishing the fates of certain cells (Weisblat and Blair, 1984; Shankland and Weisblat, 1984; Ho and Weisblat, 1987). For that reason, leech embryos should be useful for elucidating mechanisms by which cell fates are determined. Here we examine a particular kind of stem cell in the leech embryo that gives rise to two different classes of progeny in exact alternation. We show that the two classes of progeny give rise to distinct subsets of definitive progeny in the segmental nervous system of the leech, and, furthermore, that differences between the two classes of progeny may be specified at the time of their births.

After a series of stereotyped cleavages, the early leech embryo contains,

along with various other cells, five bilateral pairs of stem cells or *teloblasts:* one mesodermal pair (M) and four ectodermal pairs (N, O/P, O/P, and Q; Fig. 1). Each teloblast produces a chain or *bandlet* of several dozen much smaller segmental founder cells called primary *blast cells.* Blast cells are born at the rate of one per hour (Wordeman, 1982); within each bandlet, the firstborn blast cells contribute progeny to the anterior segments and lie distal to the progressively younger ones that contribute progeny to the posterior segments. On both sides of the embryo the bandlets merge into *germinal bands,* which then lengthen and move over the surface of the embryo as more blast cells are produced. The blast cell bandlets are designated by the lower case letter corresponding to the teloblast of origin and their relative positions in the germinal band. Eventually the right and left germinal bands coalesce along the ventral midline to form the *germinal plate.* Blast cell proliferation within the germinal bands and germinal plate ultimately gives rise to the segmentally iterated tissues of the mature leech.

Each right and left half segment (hemisegment) is composed of five distinct sets of definitive progeny, the M, N, O, P, and Q *kinship groups* (Weisblat et al., 1984). A kinship group comprises the cells that lie in one segment and are derived from a given bandlet; each kinship group is named according to its bandlet of origin (Kramer and Weisblat, 1985). Each primary blast cell in the m, o, and p bandlets gives rise to a clone comprising one hemi-
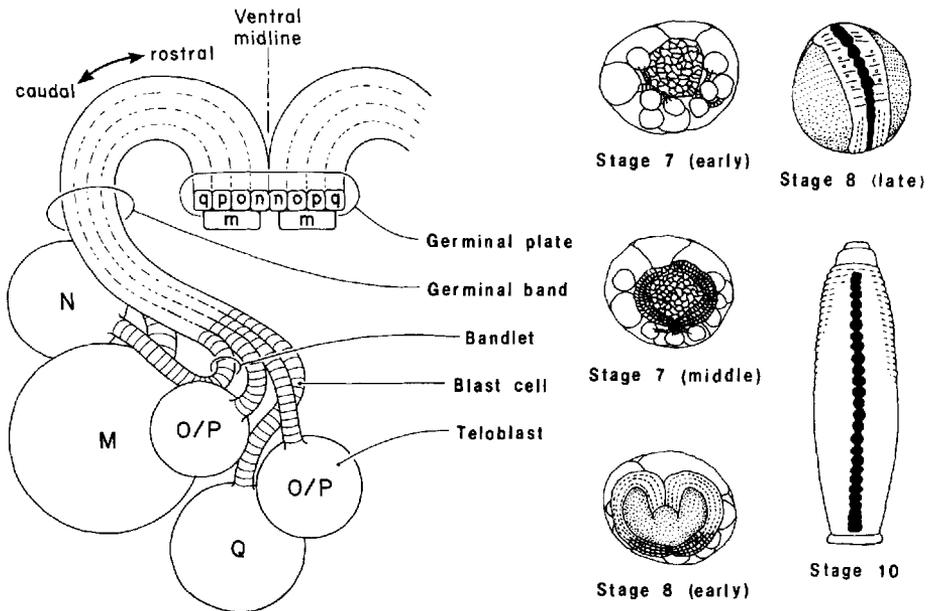

Fig. 1.   Schematic representation of embryonic development in *Helobdella triserialis.* Left: hemilateral arrangement of teloblasts and their primary blast cell bandlets within the germinal band and germinal plate. Near right: dorsal views of three embryos. Early stage 7 embryo in which the teloblasts have begun to produce blast cell bandlets; middle stage 7 embryo in which the bandlets have merged to form germinal bands; early stage 8 embryo showing the heart-shaped germinal bands that have begun to coalesce into the germinal plate. Far right: ventral views of two embryos. Late stage 8 embryo showing the germinal plate on the ventral midline, with the nascent ventral nerve cord and its ganglia and ganglionic primordia indicated in black; stage 10 embryo in which the chain of ganglia, shown in black, closely resembles the adult nerve cord.

segmental complement of progeny. [Because the clones of m, o, and p blast cell progeny interdigitate across segment borders, the kinship group is not a clone, however (Weisblat and Shankland, 1985).] Accordingly, each primary blast cell in the m, o, and p bandlets divides with a symmetry and timing that is characteristic of its bandlet (Zackson, 1984). In contrast, two consecutive primary blast cells in each n and q bandlet are required to generate one hemisegmental complement of progeny. Furthermore, there are two distinct subsets of definitive progeny within each N and Q kinship group (Weisblat et al., 1980; Weisblat and Shankland, 1985), and there are two alternating symmetries and timings of primary blast cell division in each n and q bandlet (Zackson, 1984).

In the leech embryo, therefore, there are two alternating classes of primary blast cells in each n and q bandlet and two distinct subsets of definitive progeny in each N and Q kinship group. The present studies were undertaken to determine whether or not there is a fixed correspondence between the classes of primary blast cells and the subsets of definitive progeny in the N and Q cell lines and to begin to elucidate the mechanisms by which the two alternating classes of primary blast cells in the n and q bandlets assume different identities.

METHODS

Animals

Helobdella triserialis embryos were obtained from a laboratory breeding colony and cultured as previously described (Blair and Weisblat, 1984). The developmental staging system and cell lineage nomenclature are those of Stent et al. (1982) and Weisblat et al. (1984). A segment is defined as the anatomical unit associated with an individual ganglion of the ventral nerve cord (Mann, 1953), and segments are numbered by the system of Kristan et al. (1974).

Lineage Tracers

Teloblasts in early stage 7 embryos were pressure-injected with lineage tracers as previously described (Weisblat et al., 1978, 1980). Horseradish peroxidase (HRP; Sigma, type IX), fluorescein-dextran (FDX; Sigma), tetramethylrhodamine-dextran-amine (RDA; Gimlich and Braun, 1985), and biotinylated-fixable-dextran (BFD; Ho and Weisblat, 1987) were used as lineage tracers.

After development to stage 10, HRP-injected embryos were processed as described by Weisblat et al. (1984), and BFD-injected embryos were processed according to Ho and Weisblat (1987). The distribution of the reaction product was observed in whole mounted or microdissected embryos or in serial thick (3 μm) sections of Epon-embedded specimens. Fluorescently labeled dextrans were viewed with epifluorescence in living stage 7 embryos. Alternatively, at the desired stage of development, RDA-labeled embryos were fixed with formaldehyde (3.5% in 0.1 M Tris-HCl, pH 7.4), counterstained with the fluorescent DNA stain, Hoechst 33258, and cleared in 70% glycerol. RDA-labeled cells were viewed in whole mounted or microdissected embryos.

Ablations

FDX-labeled primary blast cells were selectively ablated by exciting the
fluorescent dye in the living cells with a brief (1–2 s) pulse of a laser mi-
crobeam at the fluorescein excitation maximum of 485 nm through a 40X
water immersion objective (Zeiss Plan-Neofluar) (Shankland, 1984; Braun,
1985).

RESULTS AND DISCUSSION

Correspondence Between Primary n Blast Cells and Definitive N Progeny

Nearly all the elements of the N kinship group are found within the central
nervous system. This group includes an estimated 130–160 neurons and
one giant neuropil glia in the ganglion, in addition to three peripheral
neurons (nz1, nz2, and nz3) that lie outside the posterior half of the ganglion
and at least one squamous epidermal cell that is located in the ventral body
wall (Weisblat et al., 1984; Kramer and Weisblat, 1985; Braun, 1985).

When an N teloblast is injected with lineage tracer after the onset of blast
cell production, the resultant embryos fall, with equal probability, into either
of two groups with respect to the distribution of labeled cells in the first
labeled hemisegment. One group has labeled cells throughout the entire
first labeled hemisegment, whereas the other has fewer labeled cells and
only in the posterior part of the first labeled hemisegment (Weisblat et al.,
1980, 1984). These experiments indicate that the N kinship group is com-
posed of two distinct subsets of cells: one comprises mainly the progeny in
the anterior portion of the hemiganglion, whereas the other comprises mainly
the progeny in the posterior part of the hemiganglion and the peripheral
neurons (Weisblat and Shankland, 1985).

Earlier in development, two alternating classes of primary blast cells in
the n bandlet can be distinguished after their first divisions (Zackson, 1984).
Cells in one class (called nf) undergo an asymmetric division (large anterior
daughter and small posterior daughter) approximately 22 h after they are
born, whereas those in the other class (called ns) undergo a symmetric
division approximately 28 h after birth.

To determine whether there is a fixed correspondence between the two
classes of primary blast cells in the n bandlet and the two subsets of definitive
N progeny, a mixture of FDX and HRP was microinjected into N teloblasts
that had already begun making blast cells (early stage 7). The embryos were
allowed to develop for approximately 24 h (middle stage 7), at which time
the eldest labeled nf, but none of the labeled ns, blast cells had divided.
Thus, incipient nf cell clones, consisting of a large anterior and a small
posterior cell, could be distinguished from ns cells, which had not yet divided
[Fig. 2(A, B, D, and E)].

At this time, the embryos were separated into two groups on the basis of
whether the first labeled blast cell was identified as nf or ns. Fluorescent
light (at low levels to minimize photodamage to the labeled cells) was used
to locate the first FDX-labeled cells, and then transmitted light was used
to identify the cells on the basis of size and shape. The tendency of dividing
cells to round up was an additional cue for identifying nf cells. Embryos in
which the first labeled cell contained less tracer than the remaining cells
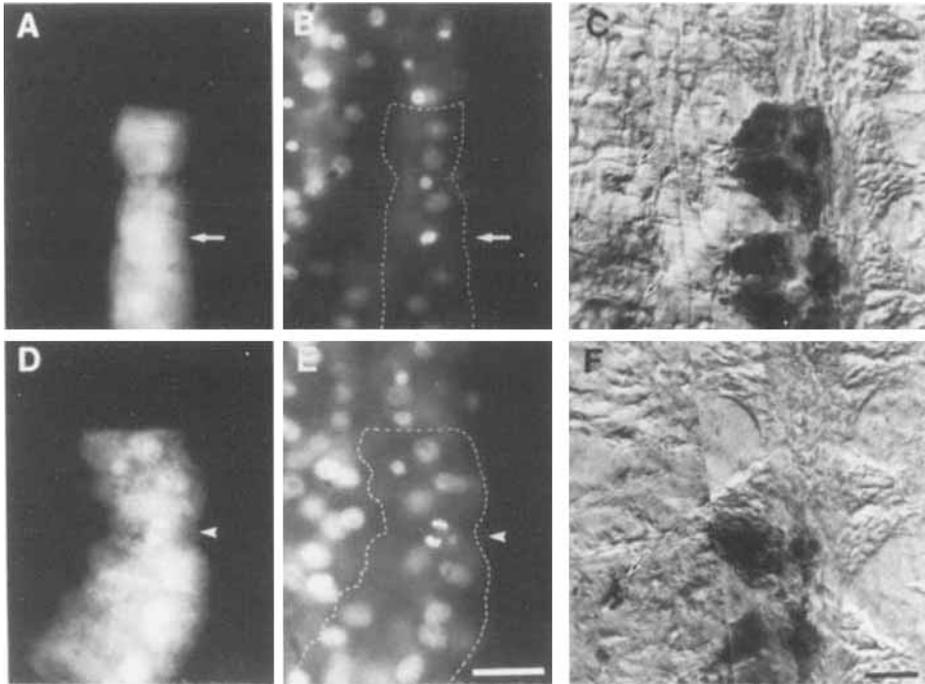in the bandlet (see below) were not included.

Fig. 2.   Two classes of n blast cells and two subsets of definitive N progeny. In this and all subsequent figures, anterior is uppermost. (A and D) Fluorescence photomicrographs of RDA-labeled n bandlets in which the first labeled blast cell is ns (A) or nf (D). (B and E) Corresponding photomicrographs showing Hoechst-stained nuclei of the germinal band cells; the n bandlet is outlined with dashed lines. Arrows designate an nf blast cell in prophase, and arrowheads denote an nf blast cell in anaphase. (D and E) The germinal band is twisted so that the n bandlet is lying above several other bandlets, whose nuclei are evident by Hoechst fluorescence. (C and F) Nomarski photomicrographs of ventral nerve cords and the surrounding body wall in microdissected stage 10 embryos. In each panel three segmental ganglia are shown; one with unlabeled and two with BFD-labeled cells of the N kinship group in the left sides. The ventral midline runs along the center of the ganglia. (C) BFD-labeled, definitive N progeny in an embryo whose first labeled blast cell was ns. (D) BFD-labeled, definitive N progeny in an embryo whose first labeled blast cell was nf. The peripheral neurons (nz1, nz2, and nz3) are indicated by the arrows. Note that the fluorescence photomicrographs are of germinal bands that were removed from fixed and glycerol-cleared embryos. For the actual correspondence experiments, the first labeled blast cells were identified in living embryos. Scale bars = 20 μm.

The two groups of embryos were allowed to develop to stage 10 (4–5 days), at which time the segmental ganglia were well differentiated. They were then fixed, stained for HRP, and the first labeled subset of definitive N progeny was identified. The frontmost labeled cells were generally found within body segments 3 or 4. The trauma of microinjection occasionally disrupted the development of the first labeled blast cell. This problem was exacerbated by the handling and illumination needed to score and sort the living stage 7 embryos. For these reasons, the first labeled subset of N progeny could be identified in only 73% of the stage 10 embryos.

Among the embryos in which the first labeled blast cell had been identified as ns, 27 (82%) had labeled cells throughout the frontmost labeled hemisegment, and 6 (18%) had labeled cells only in the posterior region of the frontmost labeled hemisegment. In the majority of these embryos, therefore,

the first labeled blast cell gave rise to progeny located mainly in the anterior part of the hemiganglion [Fig. 2(C)]. Among the embryos in which the first labeled blast cell had been identified as nf, 23 (88%) contained label only in the posterior part of the first labeled hemisegment, and three (12%) had label throughout the frontmost labeled hemisegment. Thus, in the majority of embryos in this group, the first labeled blast cell gave rise to progeny located mainly in the posterior part of the hemiganglion, in addition to the peripheral neurons [Fig. 2(F)]. The three peripheral neurons were descended from nf blast cells because they were always labeled in the frontmost labeled hemisegment, whether that hemisegment contained label throughout or only in the posterior part.

Identification of the first labeled primary blast cell is subject to error because of the difficulties encountered in visualizing the living blast cells without damaging them. For this reason we attribute the small percentage of nonconforming cases in each group to misidentified first labeled blast cells, rather than to developmental variability. Thus, despite the fact that these correlations are not perfect, we take these results to indicate that there is a fixed correspondence between the two classes of primary blast cells and the two subsets of definitive progeny. Specifically, ns blast cells give rise to cells that lie mainly in the anterior part of the ganglia, and nf blast cells give rise to cells that lie mainly in the posterior part of the ganglia and the peripheral neurons. Furthermore, the ns cell that contributes progeny to a given hemiganglion is older than the nf cell that contributes to the same hemiganglion.

Cell Migration and Specific Blast Cell Precursor of Identified Cells in the N Kinship Group

In partially labeled hemisegments the border is quite distinct between unlabeled, anterior and labeled, posterior cells, indicating that there is little anteriorly directed migration of nf-derived cells (but see below). The amount of posteriorly directed migration of ns-derived cells is more difficult to assess, however, for two reasons. First, the techniques used here do not allow us to label only ns-derived cells in a ganglion. And second, the large number of N-derived cells in a ganglion precludes the reliable identification of individual labeled cells by position alone.

Many cells in the leech have been identified by various other (i.e., morphological, physiological, and biochemical) criteria, however (Muller et al., 1981). Developmental studies suggest that each identified cell invariably arises from a particular teloblast (Kramer and Weisblat, 1985; Stuart et al., 1987). Within each segment, the N teloblasts give rise to a pair of giant neuropil glia whose somata lie just ventral to the neuropil in the ganglion, at least two squamous epidermal cells located in the ventral body wall, all of the serotonin-containing neurons, and numerous other identified neurons (Weisblat et al., 1980, 1984; Blair and Weisblat, 1982; Kramer and Weisblat, 1985; Stuart et al., 1987). To further distinguish between the nf and ns blast cell fates, the specific blast cell precursor of some of these identified cells was determined.

Both N teloblasts in early stage 7 embryos were injected with HRP or BFD, and after development to stage 10, the embryos were fixed and stained. The first labeled segment in the resultant embryos was either completely

labeled (ns- and nf-derived cells on both sides contained stain), or only the posterior half was labeled (only the nf-derived cells on each side contained stain). (Those few embryos in which the borders between labeled and unlabeled cells on the two sides were not in register were discarded.) In these experiments the first labeled cells were generally found in body segments 7 or 8.

The giant neuropil glia were examined in six microdissected or sectioned embryos whose first labeled ganglion had stain in only the nf-derived (posterior) cells. In these ganglia, both neuropil glia contained label [Fig. 3(A)], which indicates that they are derived from nf blast cells. Since the bulk of the nf-derived cells in each segment occupy the posterior half of the ganglion and yet the pair of neuropil glia are arranged anteroposteriorly along the ganglionic midline, one of these glia or its precursor must move somewhat anteriorly during gangliogenesis.

There were no labeled epidermal cells in the ventral body wall of the first partially labeled segment of these embryos, but there were two labeled epidermal cells in the ventral body wall of the remaining labeled segments. The N-derived epidermal cells were, therefore, examined in four embryos whose first labeled segment had stain in both nf- and ns-derived cells. The first labeled segment in these embryos contained two labeled epidermal cells, one of which is shown in Fig. 3(B). These data indicate that the squamous epidermal cells descend from ns blast cells.

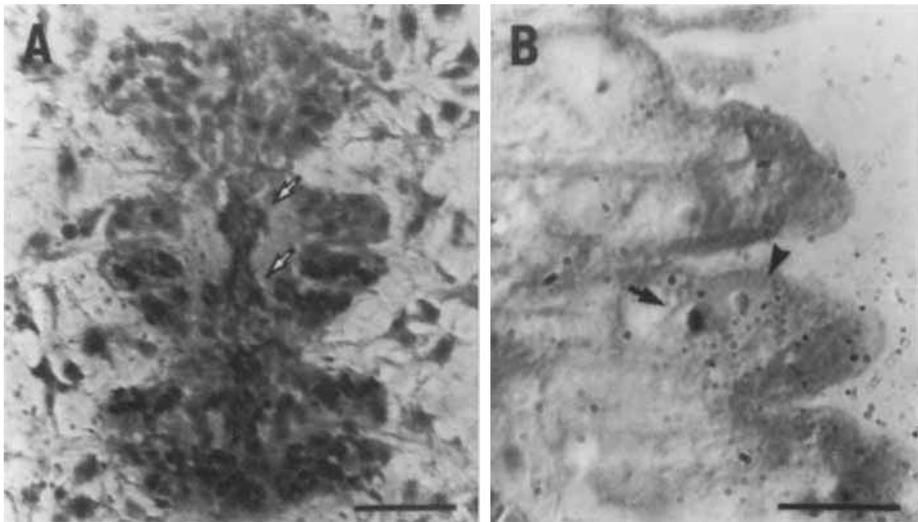The developmental origin of the serotonin-containing neurons has been

Fig. 3. The giant neuropil glia and squamous epidermal cells of the N kinship group. Nomarski photomicrographs of horizontal sections through stage 10 embryos in which both N teloblasts had been injected with BFD; sections were counterstained with toluidine blue. (A) Both neuropil glia (arrows) are labeled in the first labeled ganglion in which only nf-derived (posterior) cells contained label. (B) Epidermal cells in the ventral body wall of the first labeled segment that contained both nf- and ns-derived, BFD-labeled cells. One labeled N-derived epidermal cell in which the label is concentrated in the nucleolus and an unlabeled non-N-derived epidermal cell are designated by the arrow and arrowhead, respectively. The corresponding ganglion does not appear in this section. Scale bars = 20 μm in A and 10 μm in B.

studied by Stuart et al. (1987). They found that the dorsolateral and the ventrolateral neurons (also called cells 21 and 61, respectively) arise with the posterior cluster of ganglionic neurons. Thus, we conclude that these cells are derived from nf blast cells and that the remaining serotonin-containing neurons, namely the Retzius, the anteromedial neurons, and the (unpaired) posteromedial neuron are descended from ns blast cells. These results are interesting because of the implication that the posteromedial neuron, which lies in the posterior part of the ganglion, arises with the anterior cluster of cells and migrates posteriorly to its final position. Thus, within the nascent ganglion, there seems to be migration of specific cells in both the anterior and posterior direction.

Correspondence Between Primary q Blast Cells and Definitive Q Progeny

Most of the cells in the Q kinship group are epidermal cells and peripheral neurons that lie in the dorsal body wall. There are, however, several peripheral neurons in the ventral body wall as well as several central neurons and connective glia (Kramer and Weisblat, 1985; Weisblat and Shankland, 1985).

Embryos, in which a Q teloblast has been injected after the onset of blast cell production, also have two different types of borders between unlabeled and labeled cells. In some embryos the anterior edge of the labeled dorsal epidermis is in register with the first labeled hemiganglion. In the remaining embryos the distal edge of the labeled dorsal epidermis extends into the next anterior (unlabeled) hemisegment (Weisblat and Shankland, 1985). These findings indicate that the Q kinship group is also composed of two distinct subsets of cells: one consists of all the ventral progeny, one dorsal neuron, and a small patch of dorsal epidermis that includes two epidermal specializations called cell florets; the other contains the remaining dorsal neurons and a large patch of dorsal epidermis that extends into the next anterior hemisegment and includes one cell floret (Weisblat and Shankland, 1985).

Earlier in development two alternating classes of primary blast cells in the q bandlet can be distinguished by the timing and geometry of their first divisions (Zackson, 1984). Cells in one class (called qf) divide about 28 h after they are born, yielding a small anterior daughter and a large posterior daughter. Cells in the other class (called qs) divide about 33 h after birth, yielding a large anterior daughter and a small posterior daughter (Zackson, 1984).

To determine whether or not each class of primary q blast cells generates a distinct subset of definitive Q progeny, embryos were treated in a manner similar to those used for the N cell line analysis. Briefly, Q teloblasts in early stage 7 embryos were injected with RDA (or a mixture of FDX and BFD), and about 30 h later the embryos were examined and separated into two groups on the basis of whether the first labeled blast cell was identified qf or qs. After development to stage 10, the two groups of embryos were fixed and stained.

To facilitate the identification of the frontmost labeled subset of definitive Q progeny, which was in body segment 7, 8, or 9, the embryos were slit along the dorsal midline and flattened between coverslips so that both dorsal

and ventral cells could be seen in the same plane of focus. Identified neurons and cell florets could reliably be located in these preparations. The frontmost labeled subset of Q progeny was identified in only those embryos in which the six cell florets near the border were clearly visible (unlabeled as well as labeled). Because of the disruptive effects of injecting and handling that were described above, only 31% of the stage 10 embryos met this criterion.

In all of the embryos (5/5) whose first labeled blast cell had been identified as qs, the anterior edge of labeled dorsal epidermis was in register with the frontmost labeled hemiganglion, i.e., the first labeled blast cell gave rise to the mixed dorsal and ventral subset of progeny [Fig. 4(A)]. Among the embryos whose first labeled blast cell had been identified as qf, 9 (90%) had a patch of labeled dorsal epidermis that extended into the next anterior hemisegment, i.e., the first labeled blast cell gave rise to the purely dorsal subset of progeny [Fig. 4(B)]. In the remaining embryo (10%), the first labeled subset of progeny was of the other type.

The identification of q blast cells is subject to the same difficulties as described earlier for n blast cells. Thus, even though these correlations are not perfect (namely, one nonconforming case), we take these results to indicate that here, too, there is a fixed correspondence between the class of primary blast cell and the subset of definitive progeny. Specifically, qs blast cells give rise to both dorsal and ventral progeny: the anteroventral cluster of central neurons, the connective glia, the isolated neuron qz1, the cluster of ventrally located peripheral neurons (which includes the medial dopamine-containing neuron), the peripheral neuron qz4, cell floret 4, cell floret 5, and a patch of dorsal epidermis. And qf blast cells give rise to only dorsal progeny: the peripheral neurons qz5, qz6 and qz7; cell floret 6; and a large patch of dorsal epidermis. Although it takes one qs and one qf blast cell to generate a hemisegmental complement of progeny, qf blast cell clones cross segment boundaries. Hence, one hemisegment contains the entire clone of one qs blast cell and parts of the clones of the next older and the next younger qf blast cells.

The n Blast Cells in the Germinal Band Do Not Constitute an Equivalence Group

Having concluded that each class of primary n and q blast cell invariably gives rise to a distinct subset of definitive progeny during normal development, it is of interest to know the mechanisms by which the two alternating classes of blast cells take on distinct fates. Models for this process are generally either of two basic kinds: mosaic or plastic. According to mosaic models, the developmental potential of alternate blast cells would be restricted to one class or the other from birth (i.e., by the inheritance of different intracellular determinants). According to plastic models, the alternate cells would be initially equipotent and assume distinct fates only later as the result of interactions with their environment.

Although mosaicism is a prominent feature of leech development, an example of plasticity is provided by the O/P teloblasts, whose blast cells constitute an *equivalence group*. That is, o/p-derived blast cells are initially equipotent, but during normal development hierarchical interactions cause the blast cells in one bandlet to follow the O fate and those in the other to follow the P fate (Weisblat and Blair, 1984; Shankland and Weisblat, 1984).
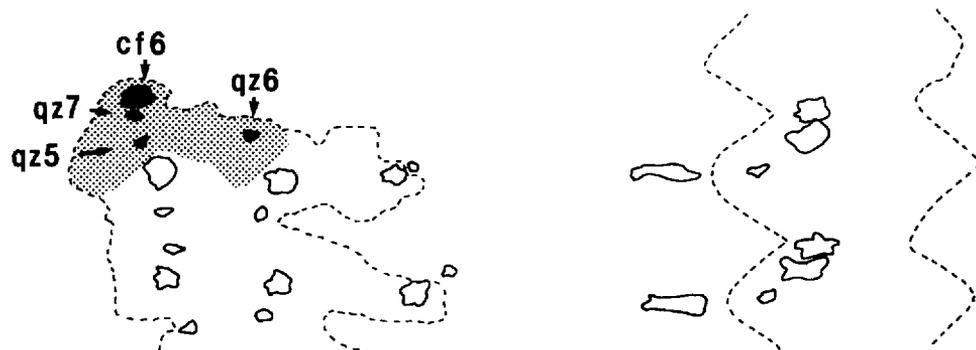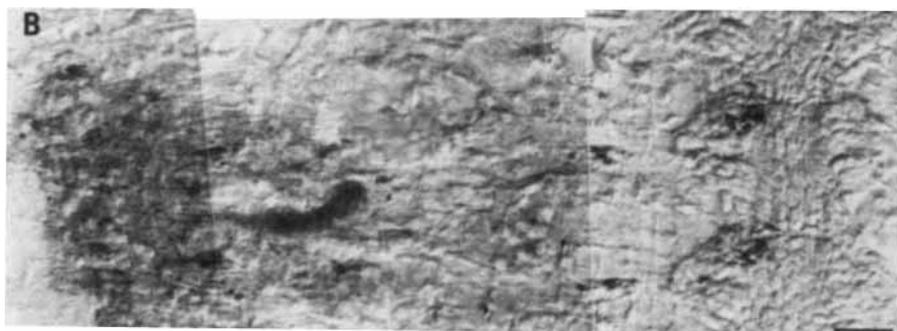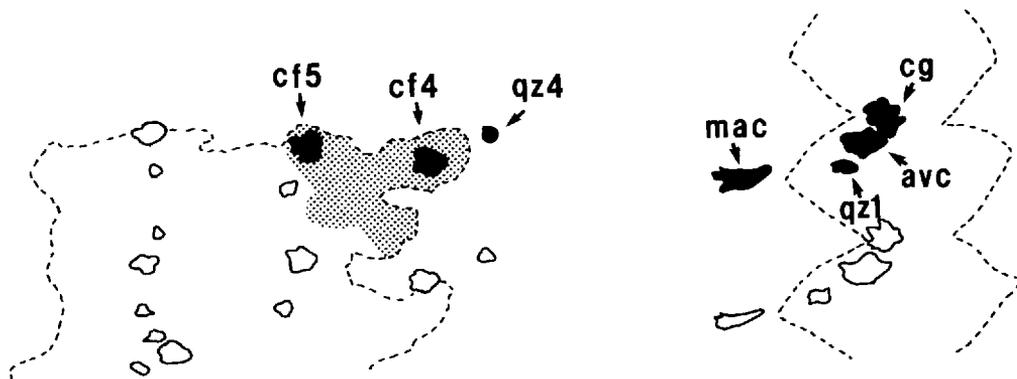
If only one O/P-derived bandlet is present, its blast cells follow the primary P fate, and the O-derived cells are absent. Positionally defined o and p blast cells exhibit different mitotic patterns but are still capable of assuming either fate after their first divisions (Shankland and Weisblat, 1984). Cells outside the equivalence group may also be important in the fate determining interactions (Ho and Weisblat, 1987).

In examining different models for the establishment of the alternate blast cell fates, we chose to use the N cell line because it generates most of the leech nervous system, and because its two sets of definitive progeny are easier to distinguish. One plastic model that could account for the differences between nf and ns blast cells is based on the equivalence group formalism. In this model n blast cells would be initially equipotent and assume distinct nf and ns fates on the basis of hierarchical interactions between adjacent blast cells within the n bandlet, with either the nf or the ns fate being primary. For example, at some point in development the first born n blast cell would automatically take on the primary fate and cause the cell directly behind it to adopt the secondary fate; the third cell in the bandlet would then be able to follow the primary fate, and so forth. To test this model, a lesion was made in an n bandlet so that cellular interactions between the n blast cells would be interrupted, and the fate of the first surviving n blast cell posterior to the lesion was examined.

A mixture of FDX and HRP was injected into N teloblasts during early stage 7. After 28–32 h of subsequent development, one FDX-labeled primary blast cell that had just entered the germinal band was ablated by laser microbeam irradiation. Since the two classes of n blast cells undergo their first divisions at different relative ages and with different symmetries, their first divisions could be evidence that they had already assumed different identities. For this reason, cells that had not yet divided were photoablated. The photolesioned cell was approximately 5–6 cells behind the most recently divided nf blast cell; thus, the cell directly behind the lesioned cell was isolated from putative anterior influences passing through the n bandlet more than 6 h before its first division.

The fluorescence of the illuminated cell was rapidly reduced, and 6–12 h later the area previously occupied by that cell was devoid of fluorescence,

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Fig. 4.   Two subsets of definitive Q progeny. Top: Nomarski photomicrographs of the left sides of microdissected stage 10 embryos. Three segments are shown: one with unlabeled and two with BFD-labeled cells of the Q kinship group. Dorsal midline is at the left edge of the figure; ventral midline runs along the center of the ganglia at right. Bottom: Tracings of the micrographs showing the labeled cells of the Q kinship group. On the left, labeled squamous epidermis is outlined with dashed lines and the other labeled cells are outlined with solid lines. On the right, the contours of the segmental ganglia are indicated with dashed lines. (A) BFD-labeled, definitive Q progeny in an embryo whose first labeled blast cell was qs. The anterior edge of the labeled dorsal epidermis is in register with the first labeled hemiganglion. In the tracing, the neural, glial and special epidermal cells that are descended from the first labeled (qs) blast cell are shown in black. The approximate domain of the squamous epidermis derived from this cell is stippled. (B) BFD-labeled, definitive Q progeny in an embryo whose first labeled blast cell was qf. The distal end of the labeled dorsal epidermis extends into the next anterior segment. In the tracing, the cells derived from the first labeled (qf) blast cell are shown in black or are stippled. Abbreviations: avc, anteroventral cluster of central neurons; cf, cell floret; cg, connective glia; mac, cluster of ventral neurons along the MA nerve; qz, neurons of the Q kinship group. Scale bars = 20 μm.

presumably because it had completely disintegrated and all debris was removed. The ablation physically disrupted the bandlet and sundered it into labeled anterior and posterior fragments; the possibility of further direct communication between the two fragments was minimal. The posterior fragment was temporarily retarded from entering the band, and its cells slipped out of their normal register as described previously by Shankland (1984).

The embryos were grown to stage 10, at which time they were fixed, stained for HRP, and the first labeled subset of definitive N progeny posterior to the lesion was identified. Frequently, the labeled cells adjacent to the illuminated cell were also affected during the ablation procedure and developed abnormally; such embryos contained aberrantly positioned clumps of labeled cells near the lesions. Since it was impossible to classify these embryos, the subset of N-derived cells directly behind the lesion was identified in only 48% of the stage 10 embryos.

If the premitotic n blast cells were developmentally equipotent and assumed primary or secondary fates because of positional interactions between adjacent blast cells, then the first surviving cell posterior to the lesion should always assume the same (primary) fate. It was found, however, that the first subset of progeny posterior to the lesion was the ns-type in 9 and the nf-type in 13 of the 22 embryos. Thus, these data contradict the hypothesis that n blast cells assume distinct fates via an equivalence group mechanism within the germinal band.

They do not eliminate the possibility that n blast cells are developmentally plastic, however. For instance, it is possible that the n blast cells do constitute an equivalence group but assume primary and secondary fates earlier in development. The effects of ablating younger blast cells in the n bandlet cannot be studied, however, because in order for the bandlet cells to enter the germinal band, they must be in physical contact with the more anterior cells that are already in the band (Shankland, 1984). Alternatively, other types of plastic models could be operative, such as the assignment of fate to alternate cells by diffusible molecules, electrical potentials, or interactions with other kinds of cells. Previous work has demonstrated no fate change in the N cell line following the ablation of other teloblasts (Blair and Weisblat, 1982) or of the overlying epithelium (R. K. Ho, personal communication), which suggests that these other cells are not critical in the establishment of n blast cell fates. The other possible plastic models are, unfortunately, untestable at this time. Evidence presented below, however, lends circumstantial support to the hypothesis that differences between the two classes of n blast cells could be imposed at birth, i.e., according to a mosaic model.

Alternate n Blast Cells May Differ from Birth

Following the injection of a teloblast with lineage tracer, the first produced blast cell frequently (~50%) receives markedly less tracer than the subsequently produced blast cells (DAW, unpublished). This is manifested by reduced fluorescence or less HRP reaction product in the first labeled blast cell and its progeny relative to the rest of the labeled blast cells and their progeny (Fig. 5). We shall refer to the first labeled blast cells that inherit about the same amount of tracer as the following cells in the bandlet as "bright" and those that receive less tracer than the following cells as "faint."
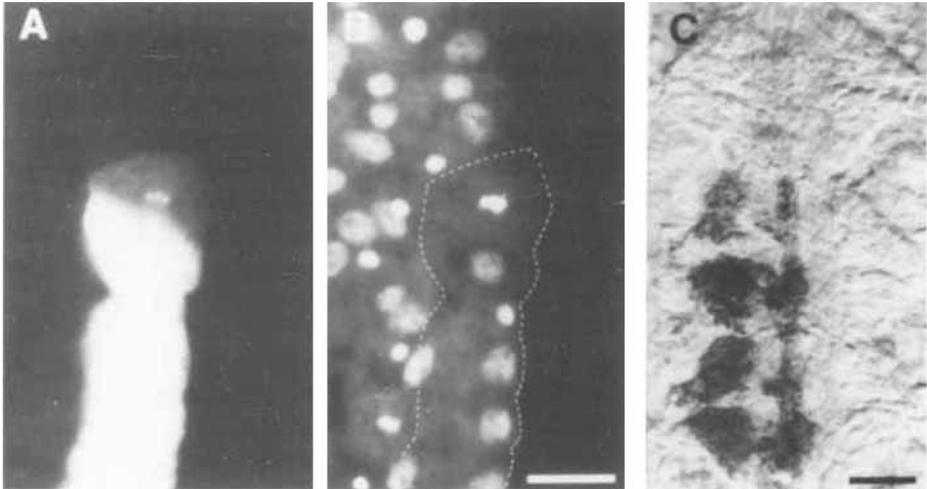
Fig. 5.   Faint, first labeled ns blast cell and faint, first labeled (ns) definitive progeny. (A) Fluorescence photomicrograph of a RDA-labeled n bandlet whose first labeled cell contains less tracer than the rest of the cells in the bandlet. (B) Corresponding fluorescence photomicrograph of Hoechst-stained nuclei of the germinal band cells; the n bandlet is outlined with dashed lines. The faint, first labeled cell is ns and has entered prophase. (C) Nomarski photomicrograph of a stage 10 embryo in which the most anterior BFD-labeled, ns-derived cells contain less stain than subsequent BFD-labeled cells of the N kinship group. Scale bars = 20 μm.

One possible explanation for this phenomenon is that the injection occurred near the end of the cell cycle and cytokinesis was completed before the tracer reached equilibrium concentration in the teloblast and the nascent blast cell. The long duration of the cell cycle and the speed with which the tracers diffuse, however, suggest that this interpretation is inadequate to account for the high frequency of faint, first labeled cells.

Another explanation is that cytokinesis is a relatively extended process; thus, blast cell production can be divided into two periods. During one, the *unrestricted period*, large molecules pass freely between the teloblast and the nascent blast cell, while during the other, the *restricted period*, large molecules cannot readily pass between the two cells. If a teloblast is injected during the restricted period, the first labeled blast cell is faint because relatively few molecules of tracer are able to enter the nascent blast cell. The restriction may result from a physical barrier in the form of a midbody (Fig. 6), the intercellular bridge formed between sister cells by the constriction of the cytokinetic furrow around the remnants of the mitotic spindle (Fry, 1937; Byers and Abramson, 1968). According to this explanation, the proportion of faint, first labeled cells would reflect the relative lengths of the unrestricted and restricted periods. Furthermore, these periods could differ between the cycles that give rise to the two alternate classes of n blast cells.

To test this hypothesis, N teloblasts in stage 7 embryos were injected with RDA. To ensure that teloblasts would be injected at different times during the production of nf and ns cells, the injections were staggered over two complete cell cycles (~2 h). The embryos were fixed and counterstained with Hoechst 33258 after 24–26 h of subsequent development, at which time the eldest labeled nf, but none of the labeled ns, cells had undergone their first
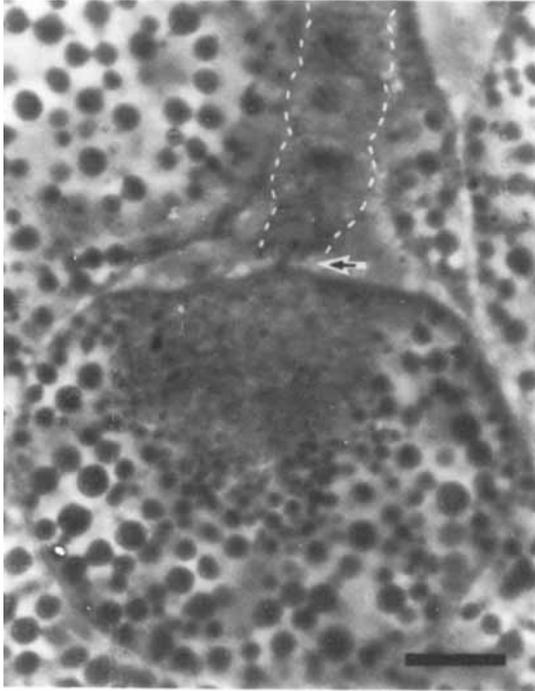
Fig. 6.   Putative midbody between a teloblast and an emerging blast cell. Phase-contrast photomicrograph of a section through a stage 7 embryo. A Q teloblast occupies the lower half of the photograph; its chain of blast cells (outlined with dashed lines) extends upward. Four blast cells are visible; the youngest is still connected to the parent teloblast via a thin strand of material, the putative midbody (arrow). The dark spots in the teloblast and adjacent cells are yolk particles. The part of teloblast from which the blast cells emerge is filled with yolk-free cytoplasm. Scale bar = 20 μm.

divisions. The embryos were examined with epifluorescence to determine (1) whether the first labeled cell was nf or ns and (2) whether it was bright or faint.

The first labeled cell was nf in 51% and ns in 49% of the 176 embryos examined. Since the probability of the first labeled cell being nf was essentially the same as it was for being ns, we conclude that the length of the cycle producing the nf cell is approximately the same as that producing the ns cell. The percentage of faint, first labeled cells, however, differed significantly between the two classes of blast cells; 54% (47/87) of the first labeled nf cells, but only 39% (35/89) of the first labeled ns cells were faint ($p <$ 0.05, test of the difference between two proportions). These data suggest that, although the overall cycles giving rise to the two classes of n blast cells are of the same length, the relative lengths of the restricted and unrestricted periods differ during the two cell cycles. Specifically, the unrestricted period is longer during the production of ns blast cells that during the production of nf blast cells.

The percentage of faint, first labeled cells was also determined in the other ectodermal bandlets. In the q bandlet the first labeled cell was qf in 53% and qs in 47% of the 217 embryos, which indicates that the cycles giving rise to qf and qs cells are also about the same length. The percentage of faint, first labeled cells, however, did not differ significantly between the

two classes of q blast cells; 46% (53/115) of the first labeled qf cells and 51% (52/102) of the first labeled qs cells were faint ($p = 0.50$, test of the difference between two proportions). In the o and p bandlets, the percentage of faint, first labeled cells was also approximately 50% (20/37 of the first labeled o blast cells and 26/46 of the first labeled p blast cells were faint). These analyses suggest that, during the generation of nf, o/p, qf, and qs blast cells, the periods of unrestricted and restricted access between the parent teloblast and nascent blast cell are about the same length. In contrast, the unrestricted period is longer than the restricted period during the production of ns blast cells.

The foregoing analysis suggests that the cycles giving rise to the two classes of n blast cells are different and is, therefore, congruent with the possibility that nf and ns blast cells themselves differ from the time they are born. It should be noted, however, that even if such differences exist, the issue of the *commitment* of these cells to different fates can only be addressed by ablation or other experimental pertubations.

CONCLUSIONS

Each Class of n and q Blast Cells Gives Rise to a Distinct and Reproducible Subset of Final Progeny

Previous work has shown that there are two distinct subsets of progeny within each N and Q kinship group, and that two consecutive primary blast cells in each n and q bandlet are required to generate one set of hemisegmental progeny (Weisblat et al., 1980; Weisblat and Shankland, 1985). Additionally, two classes of n blast cells (nf and ns) and two classes of q blast cells (qf and qs) can be distinguished much earlier in development by differences in their first divisions (Zackson, 1984). The experiments presented here demonstrate that, in normal development and within the limits of our ability to identify live blast cells unambiguously, each class of primary blast cell invariably gives rise to a distinct subset of final progeny.

In particular, it was found that nf blast cells, which divide unequally about 22 h after they are born, give rise to neurons that lie in the posterior region of the ganglion, the giant neuropil glia, and the peripheral neurons (nz1, nz2, and nz3). The ns blast cells, which divide equally about 28 h after they are born, give rise to neurons that lie mainly in the anterior region of the ganglion and the squamous epidermal cells. The qs blast cells, which divide approximately 33 h after they are born, give rise to both ventral and dorsal progeny, whereas the qf blast cells, which divide approximately 28 h after they are born, give rise to only dorsal progeny.

Determination of n Blast Cell Fates

Since alternate cells in the n bandlet give rise to distinct subsets of progeny in the leech nervous system, it is of interest to know when and how these blast cells take on their different fates. The alternate n blast cells could be intrinsically different from birth. Or, the alternate n blast cells could be initially equipotent and follow different fates after responding to intercellular signals during development.

Two lines of evidence suggest that cellular interactions in the germinal

band are not critical in establishing the distinct nf and ns cell fates. First, the ablation of other teloblasts (Blair and Weisblat, 1982) or overlying epithelium (R. K. Ho, personal communication) and the concomitant changes of neighboring cells within the germinal band do not affect the fates of the n blast cells. Second, studies presented here show that the n blast cells do not invariably assume a primary fate after the disruption of cellular interactions within the n bandlet via n blast cell ablations. These results rule out certain models, but not others, that invoke developmental plasticity in establishing the differences between nf and ns blast cells.

Analysis of the phenomenon of faint, first labeled blast cells, however, suggests that the two classes of n blast cells may be different from the time
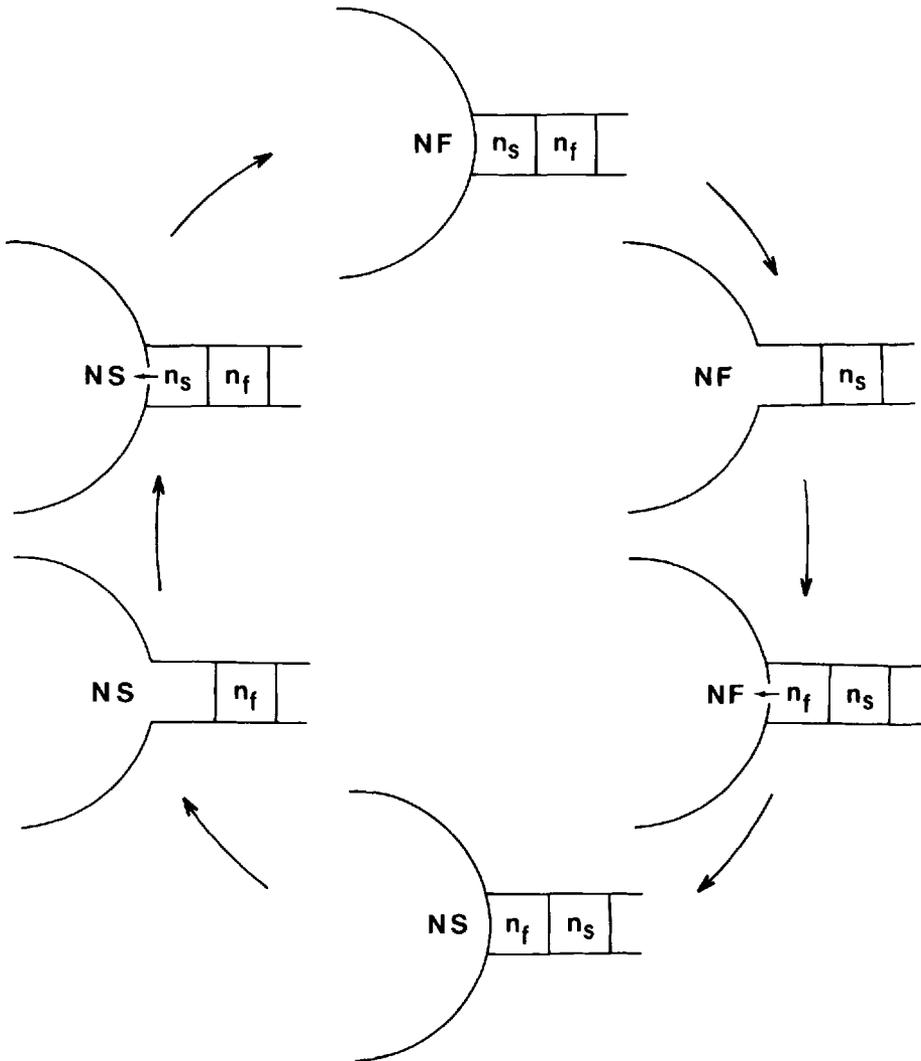
Fig. 7.   Proposed syncytial feedback model between an N teloblast and a nascent blast cell. According to this model the teloblast alternates between two states, NF and NS, that determine the class of blast cell, nf or ns, to be produced next. An emerging n blast cell sends a signal back to the teloblast that causes it to switch states, so that the other class of n blast cell is produced during the next cycle.

they emerge from the parent teloblast. The observed morphogenetic differences between the two classes of n blast cells, namely, that the cycles giving rise to nf and ns blast cells differ, are merely suggestive and do not demonstrate that these cells differ in developmental potency. For the purpose of this discussion, however, we wish to pursue the possibility that these differences may reflect the process by which different cell fates are assigned. Thus, during these different cycles the teloblast could bestow different intracellular determinants to each of the two classes of n blast cells. This differential partitioning could be controlled by mechanisms that are either intrinsic or extrinsic to the teloblast.

Syncytial Feedback Model

One model that could account for the sequential production of the two classes of n blast cells invokes syncytial feedback between the N teloblast and the nascent n blast cell (Fig. 7). According to this model the N teloblast alternates between two states, NF and NS, that determine the next class of blast cell, nf or ns, to be made. A newly born n blast cell sends a signal back to the teloblast that causes it to switch states, so that the other class of n blast cell is produced during the next cell cycle. This feedback results in a temporal flip-flop in the state of the teloblast and the production of two classes of n blast cells in exact alternation.

Since the q blast cells appear to be similar to the n blast cells in many respects, we find it surprising that there were no differences between the cycles of the Q teloblast as revealed by the proportion of faint, first labeled blast cells. If the postulated syncytium between the blast cell and the teloblast is sensitive to mechanical trauma, it is possible that differences between qf and qs would not be detected in these experiments because the Q teloblasts are situated somewhat awkwardly in the embryo and are more difficult to inject. Alternatively, the fate of the q blast cells could be controlled by different mechanisms than the n blast cells.

An important heuristic point, however, is the following. The discovery that a teloblast and its nascent blast cell pass through two periods of differential access and that the length of these periods differ in the cycles by which nf and ns blast cells arise is important because it suggests that nf and ns *may be* different from the time they are born, and also because it led us to realize that these differences *could* arise by a mechanism involving syncytial feedback. Nonetheless, the hypothesis that differences between alternate blast cells in stem cell lines may arise via syncytial feedback requires neither any differences between the restricted and unrestricted periods nor even the existence of the two periods themselves. The same sort of feedback mechanisms could be operating to establish differences between blast cells in the Q cell line, and even to establish regional (i.e., segment specific) differences in any of the five cell lines, without being detectable by the sort of analysis we have employed here.

A Possible Homology Between Leeches and Insects

The postulated binucleate syncytium comprising a teloblast and its nascent blast cell suggests a possible homology between the development of leeches and insects. In insects, such as *Drosophila,* the zygote undergoes

numerous nuclear divisions without cytokinesis, and the nuclei, each accompanied by a little cytoplasm, migrate to the surface of the embryo to form the syncytial blastoderm. Eventually, cell membranes envelop the nuclei, and the cellular blastoderm is formed. The syncytial nuclei are initially equipotent and assume a particular fate depending upon the area of cortex to which they migrate (Garcia-Bellido and Merriam, 1969; Illmensee, 1976). To account for segmentation in the insect, Meinhardt and Gierer (1980) have proposed a gradient model that involves excitatory and inhibitory interactions resulting in the formation of periodicity across an initially homogeneous field. Additionally, certain genes are expressed in a striped pattern (i.e., a spatial flip-flop) prior to cellularization in the *Drosophila* blastoderm (Hafen et al., 1984).

This local accumulation of transcript may represent the same sort of syncytial interaction that is postulated for a teloblast and its nascent blast cell. Although the cellular aspects of embryogenesis differ dramatically between insect and leech, the syncytial nuclear interactions that have been postulated for the insect blastoderm and the leech stem cells and their progeny might represent a fundamental evolutionary homology between these groups.

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