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Segmentation and Commitment in the Leech Embryo

Minireview

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Leeches are useful for studying embryogenesis. As members of the phylum Annelida, they are organized into a discrete number of metameric units called segments, akin to those of insects and other members of the closely related phylum Arthropoda. For several reasons, developmental studies of the leech often complement those of insects. Leeches have fewer segment-specific specializations and far fewer cells than do insects. Moreover, many of the cells in the mature animal—most notably the neurons—are individually identifiable and often physiologically accessible. Finally, embryogenesis in the leech proceeds via complete cleavages, so that individual cells can be identified from the start, whereas the insect egg undergoes several rounds of nuclear division without cytokinesis, forming a syncytial blastula with several thousand, apparently uncommitted, nuclei.

The general pathway of events in leech embryogenesis is presented in the figure. Cells comprising the segments arise by stereotyped lineages from ten columns (bandlets) of blasts cells, longitudinally arrayed in an embryonic structure called the germinal plate. The blast cells in each bandlet are the birth-ranked (first born at the rostral end) progeny of a defined embryonic stem cell called a teloblast, which lies at the caudal end of the bandlet. There are five bilateral pairs of teloblasts: one mesodermal pair (M) and four ectodermal pairs (N, O/P, O/P, and Q). The teloblasts themselves arise by stereotyped, holoblastic cleavages from the egg.

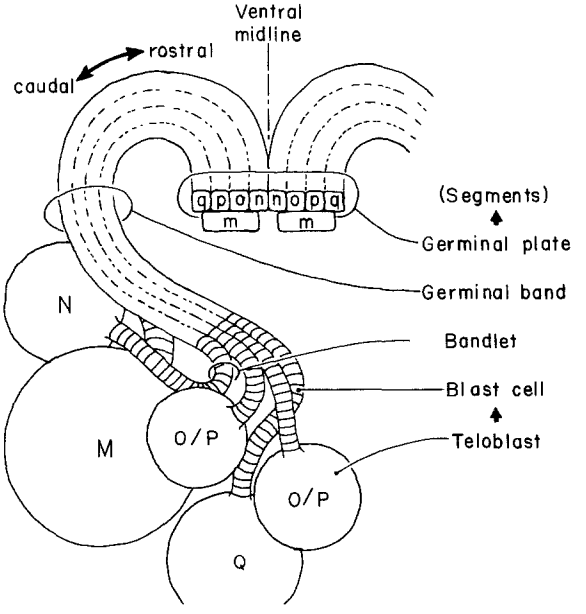
Genesis of Tissue Type

Based on the results of pioneering lineage studies with numerous invertebrates, it was predicted that each bilateral pair of teloblasts in the leech would generate only one tissue type. This hypothesis has recently been disproven (as have analogous dogmas for nematode and ascidian embryos). All leech teloblasts, including the mesodermal precursors, give rise to some neurons (traditionally regarded as ectodermal derivatives); all four ectodermal precursors generate epidermal cells and one even contributes to the ducts of nephridia (traditionally regarded as a mesodermal derivative). However, these contributions to tissue type are not made randomly (Kramer and Weisblat, J. Neurosci. 5, 388–407, 1985; Weisblat et al., Dev. Biol. *104*, 65–85, 1984). Corresponding to the five teloblasts on each side of the early embryo are five distinct groups of cells in each half-segment of the late embryo, called the M, N, O, P, and Q kinship groups. The stereotyped spatial pattern of each kinship group invariably contains certain identified cells and not others. At present, there is no obvious morphological, biochemical, or functional explanation for the assignment of cells to particular kinship groups.

Genesis of Segments

Segmentation in the insect is thought to occur via formation of subsegmental domains called compartments. At the early blastula stage, the syncytial nuclei migrate to the surface of the embryo and are cellularized by invaginations of the limiting membrane. Small groups of cells then become coordinately committed to form particular embryonic compartments. The fate of individual cells depends on prior interactions of the syncytial nuclei with their environment, not on their line of descent from the zygote nucleus. With respect to the epidermis at least, this commitment is such that the polyclone of cells descended from one group of founder cells remains spatially separate from the polyclones generated by other founder cells. Each segment thus appears to be divided into anterior and posterior (and possibly other) compartments.

If segments in the leech were to arise by a similar mechanism, one would expect that the primary blast cells (one or more) from each teloblast would generate all the progeny for the appropriate kinship group in one segment. Each segmental kinship group would thus be a clone (or polyclone). Analysis of the spatial distribution of progeny generated by individual blast cells shows that this is not the case (Weisblat and Shankland, Phil. Trans. Roy. Soc. [Lond.] B, in press). It is true that one blast cell from the M teloblast, for example, gives rise to one of each cell type found in one M kinship group, and that similarly, one blast cell from an O/P teloblast makes one of each cell type for either an O or a P kinship group. But in all these cell lines, the clones of serially adjacent blast cells intermingle, thereby contributing cells to more than one segment. The clone of a single O/P-derived blast cell is distributed longitudinally over a distance of about one and two-thirds morphologically defined segments and the clone of a sin-



gle M-derived blast cell extends across more than two segments.

In spite of this intermingling of blast cell clones, there are still recognizable segments in the leech because individual blast cells in each cell line give rise to spatially stereotyped clones. The spatial repeats that we use to define segments in the leech are thus analogous to those in a hedge formed by the interweaving branches of identical shrubs, whereas the spatial repeats in the insect are analogous to those in a hedge formed by nonidentical shrubs whose branches do not interweave. The disparity is somewhat surprising since the segments of annelids and insects are presumed to be phyletic homologs. This apparent paradox may eventually be resolved by integrating the molecular biological analysis of segmentation in Drosophila with the cellular analysis possible in the leech.

The distinction between segmentation in the leech and in the insect is not absolute. The N and Q teloblasts contribute to segments in a manner that borrows from the insect mode. In these cell lines, two blast cells (rather than just one) are used to make a single segment's worth of cells. Although the clones derived from the pairs of N and Q blast cells are spatially stereotyped, as in the M, O, and P lines, there is little or no overlap between the clones of adjacent pairs. Thus, for the N and Q cell lines, there is a redundancy in the specification of segment boundaries.

Differential Commitment of Equipotent Cells

Which aspects of a cell's fate are determined by its lineage and which by its position? This fundamental question in development is usually addressed by examining cell fates in embryos where normal positional relationships have been perturbed. In the leech embryo, this has been accomplished by injecting selected blastomeres with toxic enzymes (thus ablating them directly) or with nontoxic, but photosensitizing, lineage tracers (which renders their progeny subject to selective photoablation) (Blair, Dev. Biol. *95*, 65–72, 1983; Shankland, Nature *307*, 541–543, 1984).

These techniques have been used to confirm that cell lineage is an important factor in deciding cell fate. But a clear positional effect has been found in the differential commitment of O/P-derived blast cells to generate progeny of either the O or P kinship groups (Weisblat and Blair, Dev. Biol. *101*, 326–335, 1984; Shankland and Weisblat, Dev. Biol. *106*, 326–342, 1984; Shankland and Stent, in *Genes, Molecules, and Evolution*, eds. J. P. Gustafson, G. L. Stebbins and F. J. Ayalya, Plenum Press, 1985). The two O/P teloblasts on each side, sister blastomeres formed by the symmetric cleavage of a precursor blastomere, give rise to blast cells of equal developmental potential. Cells in these bandlets become determined to generate progeny of either an O or a P kinship group on the basis of their relative position in the germinal band of the early embryo. If only one of the bandlets is present, its cells always follow the P fate. If both bandlets are present, cells in the one closest to the dorsal pole of the embryo take on the P fate, while cells in the more ventrally situated bandlet follow the O fate. By analogy with previous descriptions of such hierarchical fate-determining interactions in the nematode (Sulston and White, Dev. Biol. *78*, 577–597, 1980), the interacting O/P-derived blast cells in the leech are said to constitute an "equivalence group." Further evidence for the generality of this phenomenon comes from a recent study of insect neurogenesis (Kuwada and Goodman, Dev. Biol. *110*, 114–126, 1985).

Incipient blast cell clones in the positionally defined o bandlet become committed to the O fate in a stepwise process. Apparently, initial mitoses in the incipient o blast cell clone generate one cell committed to making a particular subset of the O kinship group and one still capable of making either O or P progeny. When the latter cell next divides, again one daughter is committed, now to make a second subset of the O kinship group and the other daughter is again uncommitted. As this process continues, the completeness of the future O kinship group increases stepwise with the birth of cells committed to generating successive subsets of O type progeny, while the capacity of the remaining uncommitted cell to generate P type progeny is concomitantly reduced. Finally, at the fourth mitosis in the positional o clone, both daughters are committed to generating O type progeny.

Conclusions

The work summarized here—representing but a small subset of ongoing research on leech development—serves to illustrate two ways in which the study of this organism may contribute to the field as a whole. First, as in the analysis of the O/P equivalence group, experimental questions of general interest to developmental biologists can be rephrased in terms of the properties and behavior of identified cells. Second, as in the analysis of "same but different" segmentation processes in insects and annelids, experiments can be designed which may explain how evolutionary tinkering with developmental processes contributes to major phylogenetic variation. The integration of diverse approaches in these relatively simple animals seems a promising strategy for eventually understanding the complex interplay of genetic, cytoplasmic, extracellular, and evolutionary factors affecting development.