The molecular ancestry of segmentation mechanisms

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In 1830 a very important debate on natural history took place in the French Academy of Sciences. As retold in enjoyable detail by T. A. Appel (1), the adversaries were Georges Cuvier and Etienne Geoffroy Saint-Hilaire. These were pre-Darwinian times—the Origin of Species was published in 1859—so their arguments sound antiquated today, but their reverberations among biological ideas have continued to the present day. Cuvier, the discoverer of extinction, held the view that animal anatomy was determined by the functional purpose of each organ, which he called the "conditions of existence." Geoffroy, the discoverer of "analogies" (now called homologies), held that animal anatomy had a "unity of plan" upon which thousands of variations were imposed. He compared vertebrates to arthropods, arguing that they shared antero–posterior (A–P) characteristics, such as head, thorax, and abdomen, as well as dorsal–ventral (D–V) landmarks such as a CNS, gastrointestinal tract, and heart, except that a D-V inversion of the body plan had occurred. Geoffroy even had the temerity of comparing the segmentation of arthropods to that of vertebrates. Cuvier presented the better arguments and was considered the victor of the debate. However, modern evolution and development studies have discovered that common genetic networks pattern the A–P and D–V axes of very distantly related bilateral animals (2, 3). This realization has provided a measure of molecular support for Geoffroy's unity of plan hypothesis and for the idea that the last common ancestor of the invertebrate and vertebrate lineages was a rather complex animal, called Urbilateria (which means primeval bilateral animal), which predated the Cambrian explosion that took place 535–525 million years ago (3). In this issue of PNAS, Pueyo *et al.* (4) make an important contribution to the question of whether common mechanisms of segmentation are shared by insects and vertebrates (5, 6).

The Importance of Being a Cockroach Embryo

The key to studying in detail the expression of genes known to regulate vertebrate segmentation was the use of a particularly favorable embryo, the cockroach *Periplaneta americana*. *Drosophila* has a long germ-band type of development, in which the embryo develops very rapidly and all segments develop almost simultaneously under the control of gap, pair-rule, and segment polarity genes that encode transcription factors (7). Most other insects, including the cockroach, develop from a short embryonic germband within a much larger egg. New metameres are added sequentially through the proliferation of a posterior growth zone. This sequential addition of metameres resembles segmentation in the verterbrate posterior paraxial mesoderm. After *Periplaneta* segment borders are formed (and marked by a stripe of the

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homeobox gene *engrailed*), the mesoderm separates from the ectoderm forming somites with an internal cavity. Formation of mesodermal somites represents an additional similarity with vertebrate metamerism. In contrast, *Drosophila* segmentation takes place exclusively in the ectodermal layer. The greatest advantage of using *Periplaneta* is that the female lays daily clutches of eggs encapsulated in an oötheca. Eggs are laid in a gradient of age from one end of the oötheca to the other, representing a time series that differs by less than one segment (a new segment is formed every 6 h in the cockroach). Furthermore, there are two rows of embryos in each oötheca, so that one side can be fixed and the other cultured further.

Through detailed *in situ* hybridization studies, Pueyo *et al.* (4) observed waves of expression of *Delta*, a gene that encodes a transmembrane protein that binds to and activates the receptor Notch in adjoining cells in the posterior growth zone. *Notch* and its target gene *hairy* (a basic-loop–helix transcription factor) were also expressed, leading to the activation of a band of the homeobox gene *engrailed* once each new segment is formed. Only one band was seen for *Delta*, *Notch*, and *hairy*, with their order of expression in a posterior-to-anterior progression consistent with the view that their sequential activation leads to the expression of *engrailed* and a new segment. The most important new observation reported in this study is that the stripes of *Delta* and *hairy* are cyclical and move rhythmically from the posterior to the anterior growth zone every time a new segment is formed in this insect (4).

A Requirement for Notch

In the vertebrates, the cycling behavior of chick *hairy* has been known since the landmark experiment of the Pourquié group (8), in which the paraxial mesoderm was bisected. One half was fixed and the other incubated for variable times, revealing posterior-to-anterior waves or cycles of expression with the same periodicity as somite formation. The *hairy* gene was discovered in *Drosophila* as a pair rule gene (7). However, *Notch* signaling plays no role in *Drosophila* segmentation, whereas in the vertebrates it is essential for the segmentation clock (9).

In the cockroach, RNAi is very effective when injected into the abdominal cavity of virgin females. Many other arthropods, such as beetles, moths, crickets, and spiders, also show potent systemic effects of parental RNAi. In *Drosophila*, however, the effects of RNAi are limited to the cells in which it is expressed (this is believed to be due to the loss of a gene encoding an siRNA transporter in the cell membrane).

In *Periplaneta*, parental injection of RNAi for *Notch* or *hairy* strongly inhibited segmentation in the posterior region of the embryo, causing truncations. Notch is a transmembrane receptor that requires the proteolytic cleavage and nuclear translocation of its intracellular domain for signaling. The last step after activation of Notch by Delta ligand is the cleavage of the transmembrane domain by γ-Secretase, an intramembranous protease for which an effective chemical inhibitor, called DAPT, exists. Both *Notch*-RNAi and immersion in DAPT inhibited segmentation and the cycling behavior of *hairy* (4).

The evolutionary conservation of the segmentation clock mechanism is the important contribution made by this study in *Periplaneta* embryos (4). The new results should be compared with previous work in the spider embryo, in which it was also
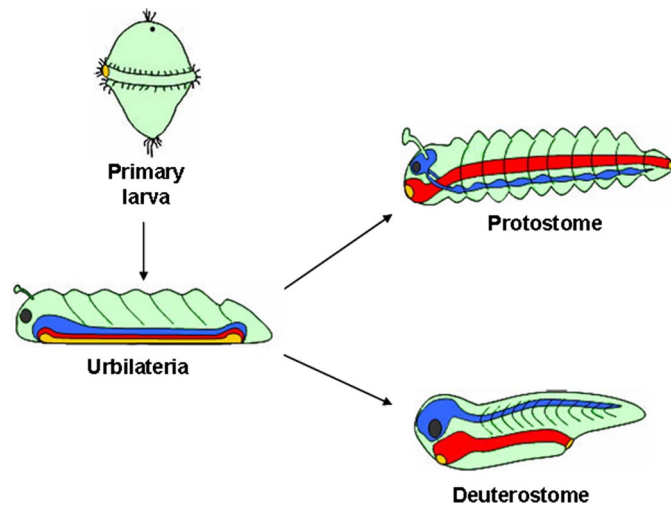

Fig. 1. Diagram showing the two great branches of the Bilateria. Protostomes (proto, first; stomo, mouth) develop the mouth near the initial site of the blastopore. Deuterostomes (deutero, second) develop the anus close to the blastopore and the mouth is perforated secondarily. Urbilateria is the hypothetical last common ancestor of the two bilaterian branches and is depicted here as a complex segmented bottom-dwelling (benthic) animal. Many extant protostome and deuterostome phyla have primary larval forms that are anatomically very similar to each other and are therefore considered ancestral (14–16). Shown here is a trochophore-like larva having ciliary bands that collect food by beating in opposite directions, an apical eye and an apical ciliary tuft. These larvae are free-swimming (pelagic) and have a planktonic existence until they settle in the ocean bottom for their adult life phase. Ectoderm is shown in green, CNS in blue, eye in black, and endoderm in red, with its openings in yellow.

found that *Notch* and *Delta* parental RNAi caused segmental malformations (10, 11). Therefore, strong evidence that the Notch pathway was required for segmentation in both arthropods and vertebrates existed previously. However, the earlier work in the spider embryo had been unable to document the existence of a cycling mechanism in the posterior growth zone, a demonstration for which the cockroach oötheca was better suited. The fact that spiders and a primitive insect require Notch for segmentation suggests that this mechanism was ancestral to the arthropod lineage. When discussing the ancestry of segmentation, mention should also be made of an earlier study in the cephalochordate amphioxus, in which the first eight somites had a stripe of *engrailed* in the posterior half (12). It has been argued that this discovery affected only 8 of a total of 50 somites formed in amphioxus and that *engrailed* bands are

not seen in vertebrate somites. However, it is worth keeping in mind that a classical arthropod segmentation gene does display the expected metameric expression pattern in the primitive chordate amphioxus.

Evolutionary Implications

The fact that an insect and the vertebrates share rhythmic cycling of the Notch pathway genes raises the question of whether the Urbilaterian ancestor was a segmented animal (Fig. 1). If this were the case, it would have very important evolutionary implications. Thirty-five animal phyla exist, of which 30 correspond to bilaterians (13). A segmented ancestor would represent a bottom-dwelling (called benthic) ancestor with a body suited for an existence burrowing in the ocean floor. This body plan would correspond to the adult form of the animal. It is very likely that Urbilateria had a complex life cycle with an initial ciliated free-swimming (pelagic)

larval phase, because many phyla of the two main branches of the bilateral animals (Fig. 1)—such as for example annelids, mollusks, hemichordates, and echinoderms—have retained the ancestral primary pelagic larval phase (14, 15). These larval forms share many anatomical similarities (Fig. 1). However, the ancestral pelagic larval phase of the life cycle has been lost repeatedly in many marine animal lineages (16).

It is interesting to reflect on how contingent the future body plans of most animals that surround us today were on the survival of the urbilaterian lineage. Present-day fauna would probably look very different if the lineage of this complex ancestor had become extinct. The finding of cycling of the Notch pathway in *Periplaneta* and vertebrates certainly strengthens the idea of a segmented common ancestor. It should be mentioned that other authors are of the opinion that the last common ancestor could not have been a complex creature because of the lack of a fossil record (17). As Pueyo *et al.* (4) discuss, it always remains a possibility that the Notch pathway was recruited by convergent evolution rather than by descent from a common ancestor. For example, it could be that Notch was selected because it was particularly well suited for the establishment of boundaries. However, the rhythmic segmentation clock would have had to be constructed independently as well.

There are many genes that oscillate rhythmically during vertebrate somite formation, in particular components of the Wnt, FGF/MAPK, and Notch pathways (18). The ancestry of segmentation will be resolved, and perhaps Geoffroy vindicated, when additional genes are found to play conserved roles in segment formation in multiple phyla. In the ideal case, new homologies might be found between the segmentation of vertebrates and that of *Drosophila*, the species that initiated the molecular dissection of metamerism (7).

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