Evolutionary biology

The ancestry of segmentation

E. M. De Robertis

Until recently, most evolutionary biologists would have agreed that the segments of an arthropod, such as an insect, and of the backbone of a vertebrate were independent ('convergent') solutions to a functional need for building bilaterally symmetrical body plans using modular units. The work reported by Linda Holland and colleagues in the May issue of *Development*[1] is bound to change that view.

Holland and colleagues find that a homologue of the *engrailed* gene is expressed in the posterior half of each of the first eight segments (somites) of amphioxus (Fig. 1). In *Drosophila*, the *engrailed* gene encodes a DNA-binding protein that specifies the posterior compartment of each segment. Amphioxus has a notochord but not vertebrae, and is very useful in evolutionary studies because it appears to have retained many of the characteristics of the archetypal chordate ancestor that gave rise to the vertebrates[2]. The fact that *engrailed* is expressed in both *Drosophila* and chordate metameres[1] tells us that segmentation was present in the common ancestor from which the insect and chordate lineages diverged 500 million years ago,

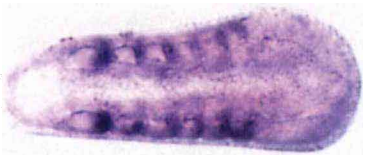


Figure 1 **Expression of *engrailed* mRNA in the posterior portion of each of the five somites that have been formed in a 12-hour amphioxus embryo. The sixth stripe is in the somite that is about to be formed in the posterior (to the right). (Photo courtesy of Linda Holland, from ref. 1.)**

the Urbilateria (Ur, primaeval; Bilateria, bilateral animal)[3]. The report on amphioxus *engrailed* follows soon after the discovery of *her1*, a homologue of the hairy DNA-binding protein that is expressed in every other forming somite in the zebrafish[4]. In *Drosophila*, *hairy* is a 'pair-rule' gene required for the formation of alternating segments. The pair-rule expression of *her1* in zebrafish led Kimmel to propose that Urbilateria was segmented[5].

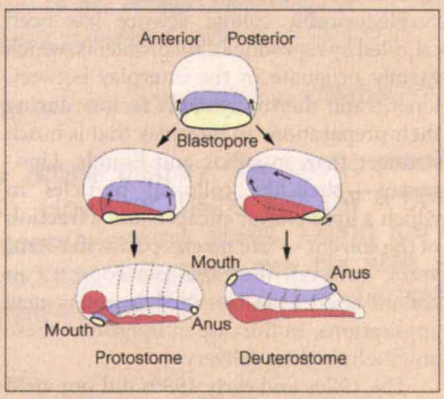
The Nobel prize-winning screen for *Drosophila* embryonic lethal mutations[6] provided researchers with many pair-rule and segment-polarity genes, but their homologues were not implicated in the formation of vertebrate somites. In fact, many homologues of *Drosophila* segment-polarity genes (such as *engrailed, wingless, armadillo, hedgehog* and *patched*) have been found to have other functions in vertebrates, such as the formation of the midbrain–hindbrain border and dorsoventral patterning of the neural tube. The new work[1] will undoubtedly stimulate a second, hard look for expression of these homologues in forming somites in regions such as the tailbud of the vertebrate embryo.

In zebrafish, chick and mouse, *engrailed* homologues are expressed in subsets of cells in the somites[7,8], but only after somites have been formed. This is different from amphioxus *engrailed* or zebrafish *her1*, which form bands of expression before morphological signs of segmentation are detectable[1,4]. There are three *engrailed* genes in zebrafish and only one in amphioxus[1,7]. Although one could argue that an as yet undiscovered *engrailed* homologue might explain the failure to detect early expression, it is more likely that the differences from amphioxus are due to the mode of somite formation. Amphioxus has 50 or more somites but the *engrailed* bands are observed only in the first eight, which are formed by a primitive mechanism by which epithelial outpockets are pinched off the primitive gut ('enterocoely'), whereas more posterior somites are formed from mesenchymal cell blocks which are then subdivided into segments.

Evolving protostomes and deuterostomes

Zoologists classify bilateral animals that possess a coelomic cavity into two fundamental groups: the protostomes – such as arthropods, annelids and molluscs; and the deuterostomes – such as echinoderms and chordates (chordates include ascidians, amphioxus and vertebrates). In protostomes, the mouth is formed at or close to the initial site (proto, first; stomo, mouth) of the blastopore, the site at which the endomesoderm, shown here in mauve, involutes into the interior of the embryo. In many protostomes the anus is formed at the blastopore as well. In deuterostomes, the mouth is formed secondarily (deutero, second) by a perforation of the ectoderm, and the anus is formed at or close to the site of the original blastopore. In addition, most protostomes have a ventral nerve cord which is traversed by the mouth and connects to a supraoesophageal ganglion (brain) in the anterior. In most deuterostomes the central nervous system (CNS, in red) is dorsal and is not traversed by the gut. There are other differences, but these suffice for the present discussion.

Developmental studies suggest that protostomes and deuterostomes had a common ancestor that was complex and segmented, raising the question of how such different body plans evolved. We of course do not know how the adult Urbilateria looked; it could have had either an open gut/blastopore (not shown) or it could have resembled an adult protostome or deuterostome (bottom diagrams). However, by modifying the blastopore during gastrulation it is possible to envisage how the transition could have happened, provided one assumes that the CNS is formed near the blastopore[13].



As shown in the figure, in protostomes such as annelids the slit-like ventral blastopore gives rise to the mouth and anus by closing along its central portion. The fusion of the lateral blastopore lips results in the dorsoventral inversion of the CNS, generating posteriorly a nerve cord ventral to the gut, and anteriorly a supraoesophageal ganglion (bottom left). In deuterostomes the anterior part of the blastopore, corresponding to the mouth, does not form at all and endomesodermal involution takes place from the posterior, eventually leading to formation of the anus. The CNS is induced in nearby ectoderm by proteins secreted by the invaginating endomesoderm, and by the end of gastrulation the deuterostome mouth is perforated secondarily, with the gut and CNS remaining in different sides of the animal throughout their length. The deuterostome depicted here is a frog tadpole, which if inverted would adopt its normal dorsoventral position. Thus, although the Urbilateria ancestor was complex in its adult form (presumably having segments, heart, eyes and appendages), the potential to give rise to widely divergent body plans resided in its mechanisms of embryonic development. **E. M. De R.**

ments. The latter mode, formation of somites from a block of mesenchyme, is observed throughout the vertebrates[9] and may explain the lack of early *engrailed* expression[1].

Even before the recent gene-marker studies[1,4], another similarity should have alerted us to the possibility of a common origin of insect and vertebrate segmentation. In 1855 Remak described the 'resegmentation' process by which vertebrae are formed[10]. In mammalian somites a subset of cells (the sclerotome) forms the vertebrae, whereas the rest gives rise to muscle and dermis (dermomyotome). Each mature vertebra is formed by the posterior half of one sclerotome which fuses to the anterior half of the next one. The end result is a phase shift of the vertebra with respect to the muscle, so that the segmental muscles can span, and move, adjoining vertebrae.

Although sometimes disputed[9], vertebral resegmentation has recently been confirmed by cell-lineage studies using the chick–quail system (L.-M. Bourcheix and N. Le Douarin, personal communication). In an analogous fashion, in *Drosophila* the initial segmentation unit is the parasegment which subsequently subdivides and forms the posterior compartment of one segment and the anterior compartment of the next definitive segment. Pair-rule genes such as *hairy* are required to regulate this two-step segmentation process[11]. It seems improbable that such a complicated way of making individual metameres would have arisen independently twice in evolution.

So three lines of evidence — *engrailed* in amphioxus, a pair-rule *hairy* homologue in zebrafish, and resegmentation in *Drosophila* and vertebrates — support the notion that segmentation in insects and chordates is homologous (that is, derived by descent from a common ancestor). In addition to segmentation, studies with conserved developmental control genes (indicated in parentheses; see ref. 3 for details) suggest that Urbilateria also had the following characteristics: anteroposterior polarity (*Hox* gene complexes), dorsoventral patterning (*sog/chd* and *dpp/BMP-4*), a primitive photoreceptor (*Pax6/eyeless*), a contractile blood vessel or heart (*Tinman/Nkx 2.5* and *DMEF2*), and perhaps a humble appendage or antenna-like outgrowth (*fringe*, *serrate* and other genes[12]).

The reader might rightly ask how it was possible to evolve the enormous variety of bilateral animals that surround us in our daily lives from such a complex ancestor. The answer lies not in the adult forms but in the embryo, in particular in the modifications of embryonic development that gave rise to the protostomes and the deuterostomes[13]. This divergence into two main subdivisions of animals (see box on page 25) took place early in evolutionary time, as indicated by ribosomal DNA phylogenetic trees[14]. The realization that all Bilateria are derived from a complex ancestor represents a major change in evolutionary thinking, suggesting that the constraints imposed by the previous history of species played a greater role in the outcome of animal evolution than anyone would have predicted until recently. □

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Colloid chemistry

Liposomes within liposomes

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Living nature performs many of its tasks using the self-assembling organization of polar lipid molecules. Each eukaryotic cell is a complex multicompartment colloidal system. The paper by Walker *et al.*, on page 61 of this issue[1], describes the artificial preparation of similar structures: small vesicles are aggregated and then wrapped by a larger membrane, using the property that some lipids have of being able to change from an open, rolled-up bilayer into a large vesicle when electrostatic conditions change in the solution[2]. This is an example of the rational construction of complex liposomes, using the interaction between surface-attached ligands and soluble receptors, and a new method for wrapping the aggregates.

Historically, colloid science has been crippled by reproducibility problems, which mainly originate in the interplay between kinetic and thermodynamic factors during their preparation, an interplay that is much stronger than in solids and liquids. Liposomes — shell-like colloidal particles in which a lipid bilayer encapsulates a fraction of the solvent — are no exception. In recent years, however, the rapid development in colloid science has resulted in many new applications. In the case of liposomes these are chiefly in drug delivery.

The 1970s and early 1980s did not yield any viable products, but the fundamental research, which followed a mostly trial-and-error approach, revealed many basic characteristics of such systems, both in the test tube and in multicompartment structures in living organisms[3]. The improved understanding of their stability and interaction characteristics paved the way for a scientifically based rational approach in liposome design. Now we can understand liposomes and their properties via several physically measurable quantities, such as the bending and stretching elasticity of the lipid bilayer, and attractive and repulsive interactions on a molecular and a colloid level[4].

The development of classical liposomes and other lipid-based drug carriers culminated in three formulations of a very insoluble drug (amphotericin B, for fungal infections) embedded in the bilayer upon specific interaction with a particular lipid in well-defined conditions. But it was only the synergistic action of polymers associated with liposomes that revived liposomal drug delivery. A polymer coating results in sterically stabilized liposomes, which have proved to be very effective in cancer chemotherapy. Additionally, the stability and interaction characteristics of these liposomes are well understood. Recently, for instance, it was shown that by breaking the symmetry of polymer distribution in each leaflet of the bilayer, a spontaneous curvature can be generated, and vesicles of extremely narrow size distribution have been formed simply by increasing the salinity of special micellar mixtures[5].

Walker *et al.*[1] have now achieved self-assembly of many-compartment liposomes, with relatively high encapsulation efficiency, by attaching lipid rolls to vesicle aggregates by biotin–streptavidin linkages[6]. Why is this important? Although the main goal was to decrease the leakage of encapsulated agents, their work is also an elegant way to encapsulate larger particles such as proteins or nucleic acids into liposomes — a major problem for many applications. Moreover, vesicles with different functionalities can be combined in this structure: the larger liposome might deliver a load of highly active, smaller liposomes, perhaps containing highly toxic drugs, to a specific site, thus sparing other tissues from any side effects. Such a system could be important in the treatment of cancer, but first the liposomes will have to be made smaller than 250 nm (so far, the smallest are about 300 nm across) and coated in polymers to increase their survival time in the blood.

Other liposomes have been successfully coated in this way. The obvious next step is to attach various ligands to them — ligands can carry out various functions, such as specifically targeting appropriate receptors *in vivo*, and thus choosing which membranes to fuse with. Although natural barriers and defence