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Hemichordates and the origin of chordates

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Hemichordates, the phylum of bilateral animals most closely related to chordates, could reveal the evolutionary origins of chordate traits such as the nerve cord, notochord, gill slits and tail. The anteroposterior maps of gene expression domains for 38 genes of chordate neural patterning are highly similar for hemichordates and chordates, even though hemichordates have a diffuse nerve-net. About 40% of the domains are not present in protostome maps. We propose that this map, the gill slits and the tail date to the deuterostome ancestor. The map of dorsoventral expression domains, centered on a Bmp–Chordin axis, differs between the two groups; hemichordates resemble protostomes more than they do chordates. The dorsoventral axis might have undergone extensive modification in the chordate line, including centralization of the nervous system, segregation of epidermis, derivation of the notochord, and an inversion of organization.

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Introduction

The notochord, dorsal hollow nerve cord, gill slits and a post-anal tail are phylotypic traits of chordates. Less prominent are the endostyle/thyroid, the pituitary, left–right asymmetries, and the inverse dorsoventral organization of chordates relative to that of protostomes. In chordate development, Spemann's organizer is distinctive not only as a key signaling center of the chordate gastrula but also as the precursor of the notochord, gill slit endoderm, and prechordal endomesoderm. Did these originate entirely within the chordate lineage or were some already present in non-chordate ancestors?

Hemichordates should offer the best opportunity to discern the evolutionary origins of these traits. However, beyond their gill slits, they bear little resemblance to chordates [1,2,3]. The phylum contains two classes: enteropneusts ('acorn worms') and pterobranchs. Enteropneusts are worm-like, solitary animals, a few centimetres to two metres in length, with up to several hundred pairs of gill slits. They dwell in burrows or under objects in intertidal zones worldwide. The body has three parts (prosome, mesosome and metasome), each with a coelomic cavity or paired cavities, whereas chordates have but one coelom pair. The prosome is the proboscis, the mesosome is the collar, and the metasome contains the pharynx, gonads and gut (Figure 1). Enteropneusts burrow with the muscular proboscis, and move within the burrow by the action of cilia and muscles of the body wall. The mouth is positioned ventrally, between the prosome and the mesosome. As suspension and detritus feeders, they sweep particles into the mouth by cilia, or ingest sand coated with organic materials. Of the 70 hemichordate species, some develop directly from an egg to a juvenile, and others develop indirectly, with a planktonic tornaria larva as an intermediate. Pterobranchs, the other class, are minute (1mm), sessile, stalked, deep-ocean animals that live in colonies. They too have a three-part body, but only one pair of gill slits, or none. Ciliated tentacles of the mesosome pass food particles to the mouth. All 10 or so species are direct developers.

In the 1880s, William Bateson [4,5,6] first compared hemichordate and chordate anatomy. Studying the direct-developing enteropneust *Saccoglossus kowalevskii*, he perceived major chordate traits and placed hemichordates in the chordate phylum. To him, a short, stiff rod of cells, projecting from the anterior gut into the proboscis, was a notochord (see 'stomochord' in Figure 1). Nerves of the dorsal midline looked like a dorsal hollow nerve cord of a centralized nervous system. Gill slits were obviously present, and he judged them to resemble those of amphioxus. Although Bateson didn't dwell on it, Burdon-Jones [7] later examined the post-anal tail of the juvenile and found it to resemble the chordate tail. Bateson and, later, Goodrich [8] saw a possible pituitary homolog in the proboscis pore region of hemichordates and a possible homolog of the endostyle/thyroid in the pharynx.

However, by the 1940s, biologists became skeptical of homologies, except for gill slits, and hemichordates were relegated to a phylum of 'half chordates' [1,2]. Their development was largely unstudied for 50 years, with a few exceptions [9,10,11]. In this review, we focus on recent comparisons of hemichordates and chordates

Figure 1
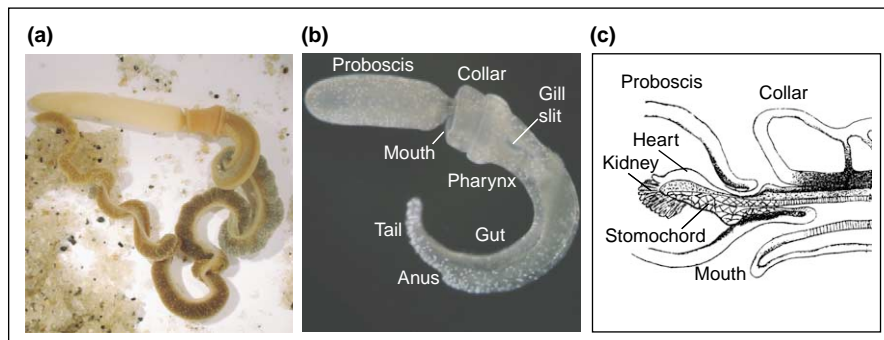
Saccoglossus kowalevskii, a direct-developing enteropneust hemichordate of the US Atlantic coast. **(a)** Adult female. Note the white proboscis, orange collar, tan pharynx and gut, and grey ovaries. Length: 10–18 cm. **(b)** Juvenile, a week after hatching, two weeks after fertilization of the egg. Length: 1 mm **(c)** The 'notochord', so-called by Bateson (see text), now called the stomochord, located between the proboscis and collar. Shown in sagittal section. Redrawn from Bateson [5].

regarding their gene sequences and expression domains. We discuss the updated deductions about their common ancestor and, hence, about the origin of chordates.

Modern phylogenies

Recent DNA phylogenies place hemichordates as the sister group of echinoderms [12–14]. Together, these two are the sister group of chordates (Figure 2). The three phyla constitute the supertaxon of deuterostomes (*Xenoturbella* might be a fourth [15]). The lineage from the ancestor of deuterostomes to the ancestor of chordates bore no branches to extant groups. Paleontology of the past decade has uncovered a profusion of Cambrian deuterostomes (e.g. vetulicolians, yunnanozoans) that has still to be reconciled with this simple phylogeny [16].

Other bilateral animals, comprising approximately 25 phyla, are protostomes. The last common ancestor of deuterostomes and protostomes is the ancestor of all bilateral animals, one node before the deuterostome ancestor.

Four venerable hypotheses of chordate origins

Consistent with the modern phylogeny are four hypotheses for the origin of chordates from a deuterostome ancestor. We present these and comment on them in light of recent results.

1. Hemichordate hypothesis: for Bateson [17], chordates evolved by the exaggeration of structures of a hemichordate-like ancestor that had a dorsal central nervous system. Goodrich [8] proposed that the two anterior coelom pairs shrank to preotic somites in chordates, and dorsal anterior structures were displaced around the front end to ventral locations.
2. Auricularian hypothesis: for Garstang [18], the chordate ancestor was a motile, ciliated larva of a sessile,

pterobranch-like adult. Rows of cilia, within a diffuse nervous system, moved dorsally by a series of evolutionary intermediates, and eventually internalized at the midline as a new, centralized, dorsal nerve-cord. From the sessile adult, gill slits, a notochord, and sexual maturity were transferred to the larva by neoteny, forming a motile protochordate adult.
3. Bilaterial ancestor hypothesis: in the 1990s, chordates (mice, frogs and fish) were found to share many domains of gene expression with protostomes (mainly *Drosophila*), such as Hox domains in the posterior head and trunk; *emx* and *otx* domains in the anterior head; *pax6* and *six* expression in light receptive organs of the head; many genes of neuron identity and differentiation; *nkx2.5* in the heart; and *hh/bmp* in the gut and visceral mesoderm [19,20]. All these domains were ascribed to the ancestor of bilateral animals. Given the existence of such a complex ancestor, the evolution of deuterostomes and chordates entailed less innovation than was previously thought.
4. Inversion hypothesis [21,22]: the chordate ancestor had a protostome-like arrangement of organs in the dorsoventral dimension: a ventral centralized nervous system, ventral musculature and a dorsal heart. A descendent in the chordate line inverted its body dorsoventrally and evolved a mouth on the new ventral surface, making chordate anatomy the inverse to that of protostomes. As recent support, *bmp* was found to be expressed dorsally in protostome embryos but ventrally in chordate embryos, whereas expression of *chordin*, which encodes a Bmp antagonist, is the reverse. This Bmp–Chordin axis, which underlies dorsoventral patterning in all bilateral animals, is inversely oriented. Motoneurons and interneurons develop in the Bmp-free ectoderm, and sensory neurons develop in the Bmp area. Although not logically required, the hypothesis usually starts with an ancestor having a centralized nervous system. Hemi-

Figure 2
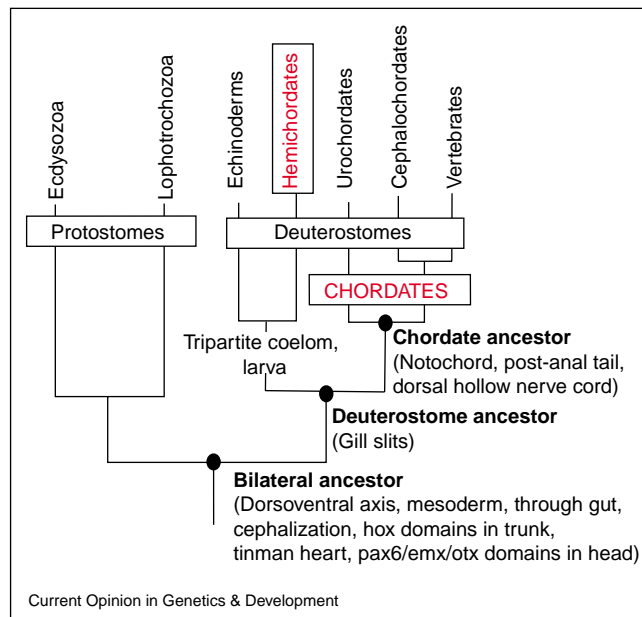
Phylogeny of hemichordates and chordates, from 18S rDNA comparisons [12–14]. Note that the chordate ancestor, in addition to the ancestor of hemichordates and echinoderms, descends from the deuterostome ancestor, which descends from the bilateral ancestor.

chordates might be uninverted, implying that inversion is a trait exclusive to the chordate line [23].

Updating the comparisons

Ambiguities of morphology have impeded comparisons between hemichordates and chordates. Gene expression domains offer an alternative of a more conserved kind of anatomy. We update the traits chosen by Bateson and then compare body plans.

1. Gill slits: in addition to anatomical similarities, the endoderm of the gill slit in both hemichordates and chordates expresses *pax1/9* and *six 1* genes [24,25••]. Furthermore, gill slits occur at the same body level in organisms of both phyla (see 'domain map' below). The deuterostome ancestor probably had gill slits, which were later lost in echinoderms.

2. The post-anal tail of hemichordates expresses the *hox11/13a*, *b* and *c* genes, and the chordate tail expresses *hox11*, *12* and *13* [25,26••]. The two groups probably have homologous post-anal body regions, brought forward from the deuterostome ancestor. The hemichordate tail doesn't function for chordate-like swimming, but it is contractile. Motility functions have probably diverged.

3. The hemichordate notochord of Bateson was later called the stomochord or buccal diverticulum to avoid the implication of homology. Like the notochord, the stomochord contains large, vacuolated cells [27].

Genes that are expressed in the chordate notochord and in its precursor cells of Spemann's organizer — namely, *bra* [28], *chordin*, *hnf3b/foxA1*, *admp*, *hh*, *nodal* and *noggin* (J Gerhart *et al*., unpublished) — are not expressed in the stomochord but are expressed elsewhere (see below). The stomochord and nearby endoderm express *otx*, *dmbx*, *gsc*, *hex* and *dkk*, as does the prechordal endomesoderm of chordates, which itself has precursors in Spemann's organizer. The stomochord also expresses *ttf2* strongly, as do the enigmatic club-shaped organ of amphioxus and the vertebrate thyroid [29]. Prechordal endomesoderm, which was unknown to Bateson, and the stomochord have the same location in the body plan (see 'domain map' below).

4. Hemichordates have no dorsal hollow nerve cord. Bullock [30] and Knight-Jones [31] silver-stained the nervous system and found an epidermal nerve net. Nerves are finely mixed with epidermal cells over the entire body surface. Pan-neural genes *sox2/3*, *elav* and *musashi/nrp* are expressed pan-ectodermally [25••]. Axons extend into a dense basiepithelial mat, some synapsing locally and some extending into thick axon tracts at the dorsal or ventral midlines [30,31,32]. These tracts do not constitute a central nervous system; they lack neural cell bodies and hence neurogenesis, and they lack interneurons and hence information processing. Bateson mistook the dorsal tract for a hollow cord. We favor the interpretation that the deuterostome ancestor had no central nervous

system or notochord, rather than the alternative explanation that these traits were present but that both were lost in hemichordates and echinoderms.
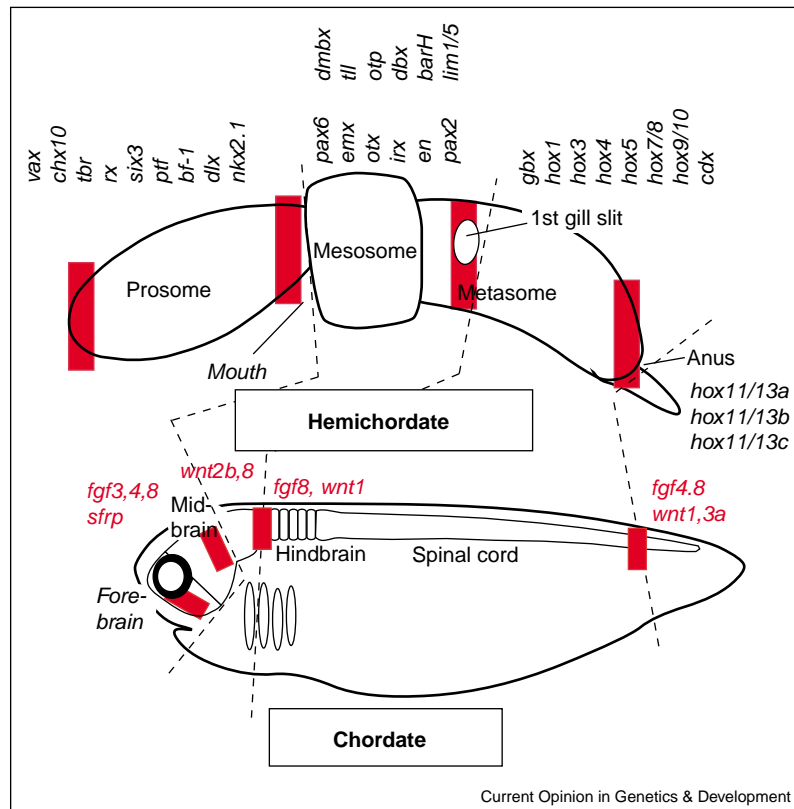
The anteroposterior domain map

Although hemichordates and chordates differ anatomically, their body plans are similar in the anteroposterior dimension regarding the topology of the domains of expression of 32 genes [25**,29], chosen for their importance in neural patterning. They encode transcription factors and signaling proteins. Genes expressed in the forebrain of chordates are expressed in the prosome of hemichordates (Figure 3). Those in the midbrain of chordates are expressed in the mesosome and anterior metasome of hemichordates, stopping at the first gill slit. Those in the chordate hindbrain and spinal cord are expressed in the hemichordate metasome, entirely posterior to the first gill slit [25**]. Finally, those in the chordate tail are expressed in the hemichordate post-anal extension, mentioned above. For the first time, the body plans of chordates and hemichordates can be aligned on this shared map. The first gill slit of hemichordates and

first branchial arch of chordates — ventral to the midbrain–hindbrain boundary — fall at the same domain intersections. Presumably, the deuterostome ancestor had this detailed map.

About 60% of the domains are shared with protostomes, indicating their likely presence in the bilateral ancestor. The others are unique to deuterostomes, particularly those encoding signaling proteins. For example, *fgf8* and *wnt1* are expressed in neurectoderm at the midbrain–hindbrain boundary of chordates, which is just dorsal to the first branchial arch (Figure 3), and genes for similar signals are expressed in the ectoderm of hemichordates at the level of the first gill slit (C Lowe, unpublished) [33]. In the posterior forebrain of chordates, *wnt2b* and *wnt8* are expressed, and similar genes are expressed at the base of the hemichordate prosome. In the anterior forebrain of chordates, *sfrp* and various *fgfs* are expressed, and similar genes are expressed at the anterior tip of the hemichordate prosome (C Lowe, unpublished) [33]. Protostomes have no counterparts of these centers.

Figure 3

The anteroposterior map of expression domains for genes important in chordate neural patterning, encoding transcription factors (in black) and signaling proteins (in red). Note the alignment of the bodies: prosome with ventral forebrain; mesosome and anterior metasome with dorsal forebrain and midbrain; posterior metasome with hindbrain and spinal cord; and post-anal tails together. The gill slits of both chordates and hemichordates develop at the same domain intersections. Signaling centers (red bars), which are important in patterning the chordate nervous system, are similar in hemichordates.

Although hemichordates and chordates share this map, they develop different morphologies: a diffuse versus a centralized nervous system. The map is clearly more ancient and conserved than are the particular anatomies and physiologies that develop from particular domains in different lineages of animals.

Two differences stand out. First, domains encircle the hemichordate body, each as a band, whereas in chordates they are patches within the neural ectoderm. This correlates with the nervous systems; in hemichordates it encircles the body, whereas in chordates it is centralized within a subregion of ectoderm. Second, most of these genes are expressed only in the hemichordate ectoderm, whereas in chordates they are also expressed in the mesoderm, such as Hox genes in somites.

Our data, combined with those on larvae, by others, disfavor Garstang's hypothesis. *S. kowalevskii* has no larval stage, but another hemichordate, *Ptychodera flava*, does. Of the few domains investigated, the larva lacks some and places others (*otx*, *nkx2.1*) at locations unlike those in the map of *S. kowalevskii* or chordates [34,35]. Assuming that the *P. flava* adult map resembles that of *S. kowalevskii*, and assuming that the deuterostome ancestor already had modern larval and adult expression patterns, the ancestral larva would seem to be a poor candidate for the evolution of the chordate nervous system, compared with the ancestral adult. Furthermore, echinoderm larvae lack Hox domains, whereas metamorphosing adults have them [36] although not in neurogenic ectoderm. Garstang's hypothesis differs from others in starting with an ancestral diffuse nervous system and proposing centralization in the chordate line. Unknown to him, the hemichordate adult, not just the larva, has such a system.

Dorsoventral organization in hemichordates

Dorsal and ventral positions are hard to define because many hemichordates live vertically in burrows, in uniform surroundings. If the mouth is defined as ventral, a differentiated dorsoventral dimension can be designated (Figures 1,4), for example, with dorsolateral gill slits.

In this dimension, hemichordates and chordates differ considerably in their domain maps. Hemichordates express *bmp2/4* and *bmp7* in an ectodermal stripe at the dorsal midline (Figure 4), and also genes (*xolloid*, *twg*, *crossveinless* and *bambi*) encoding agents for transmitting and regulating Bmp signals. In a ventral midline ectodermal stripe, *chordin* is expressed, encoding a Bmp antagonist (C Lowe et al., unpublished). *admp* is also there, encoding a Bmp complement. Hemichordates clearly have a Bmp–Chordin axis, as do *Drosophila* and chordates, but, when referred to the location of the mouth, it is oriented like that of *Drosophila* and the inverse of chordates. In hemichordates, several dorsoventral domains are placed relative to Bmp (Figure 4). *tbx2/3*, *dlx*, *slit*, *unc5* and *dcc/neogenin* span the dorsal midline, and *mnh*, *sim* and *netrin* span the ventral midline. Further resemblance to protostomes is revealed by *sim* and *netrin*, which are expressed at the *Drosophila* ventral midline, and by *slit*, which is expressed at the dorsal midline in *Caenorhabditis elegans*. Netrin and Slit might repel and attract axons to the dorsal and ventral tracts in hemichordates. Table 1 summarizes the organs and domains that are inversely located in hemichordates and chordates. The

Figure 4
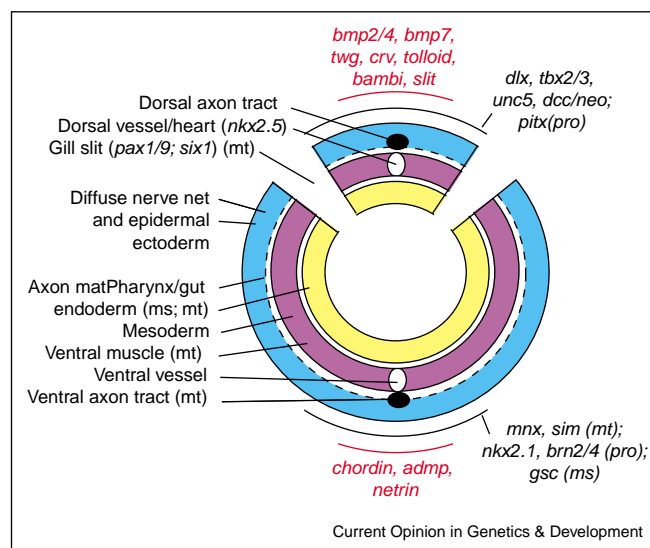
Anatomy of the dorsoventral axis of hemichordates, and, superimposed, the map of expression domains of genes encoding transcription factors (black) and signaling proteins (red). Ventral is defined by the location of the mouth. The section crosses the pharynx in the metasome (mt), but dorsoventral domains have been included from the prosome (pro) and mesosome (ms).

nervous system must be omitted because it is diffuse in hemichordates, although sensory and motoneurons might differ dorsoventrally.

Body inversion can explain the inverse relationship, as long as centralization of the nervous system is kept a separate question. Holland [37•] recently summarized arguments that the bilateral and deuterostome ancestors were diffuse, and that centralization occurred independently in chordates, arthropods and several other protostome lines. Chordate ancestors had to segregate neurogenic ectoderm from epidermis for centralization. This could have happened after body inversion. Centralization might have been an easy morphological modification to achieve once a diffuse ancestor had a rich domain map and the means to segregate axon tracts, as the deuterostome ancestor probably had.

Alternatively, perhaps the Bmp–Chordin axis, and not the 'body', inverted, and the mouth stayed put. In chordates, Chordin is produced, not by ectoderm but by the notochord and Spemann's organizer, which derive from endomesoderm of the archenteron roof. Given that the origin of the chordate notochord is still unknown, who can say whether it arose dorsally or ventrally? If it arose on the old dorsal side, it would reverse the Bmp–Chordin axis and, with it, the development of the anatomy of all three germ layers. Another alternative, not yet made explicit, is the Bateson–Goodrich [8] hypothesis that various parts of an uninverted ancestor moved around the anterior and posterior tips to give chordate organization.

Conclusions

Chordate evolution, we suggest, entailed little or no change of domain organization from that already present in the anteroposterior axis of the deuterostome ancestor. Gill slits and the post-anal tail might be ancestral deuterostome traits of this conserved dimension. Considerable change from the ancestor has occurred in the chordate line in the dorsoventral dimension, particularly in the centralization of the nervous system and the origination of

the notochord; an inversion of the Bmp–Chordin axis might also have occurred. Although the hemichordate nervous system is diffuse, it is extensively patterned. None of the old hypotheses of chordate origins seems wholly apt, but all have elements worth pursuing. Further studies of hemichordate development will help to assess these suggestions and to devise new ones. Of particular interest are the means by which the embryo establishes six signaling centers important in its patterning: dorsal and ventral midlines; anterior and posterior termini; the prosome base; and the first gill slit.

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