New perspectives on the evolution of protochordate sensory and locomotory systems, and the origin of brains and heads*

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Cladistic analyses generally place tunicates close to the base of the chordate lineage, consistent with the assumption that the tunicate tail is primitively simple, not secondarily reduced from a segmented trunk. Cephalochordates (i.e. amphioxus) are segmented and resemble vertebrates in having two distinct locomotory modes, slow for distance swimming and fast for escape, that depend on separate sets of motor neurons and muscle cells. The sense organs of both amphioxus and tunicate larvae serve essentially as navigational aids and, despite some uncertainty as to homologies, current molecular and ultrastructural data imply a close relationship between them. There are far fewer signs of modification and reduction in the amphioxus central nervous system (CNS), however, so it is arguably the closer to the ancestral condition. Similarities between amphioxus and tunicate sense organs are then most easily explained if distance swimming evolved before and escape behaviour after the two lineages diverged, leaving tunicates to adopt more passive means of avoiding predation. Neither group has the kind of sense organs or sensory integration centres an organism would need to monitor predators, yet mobile predators with eyes were probably important in the early Palaeozoic. For a predator, improvements in vision and locomotion are mutually reinforcing. Both features probably evolved rapidly and together, in an 'arms race' of eyes, brains and segments that left protochordates behind, and ultimately produced the vertebrate head.

Keywords: amphioxus; ascidian larvae; CNS evolution; chordate origins; brain architecture

1. INTRODUCTION

The vertebrate head is a complex structure whose constituent features are largely absent in protochordates. The vertebrate brain is likewise complex and of essentially similar design throughout the group, but very little is known about how it first evolved. This is due to the substantial gap that exists between the most primitive vertebrates and their closest protochordate relative, now generally supposed to be amphioxus (Gans 1989; Wada 1998), and the even greater gulf between amphioxus and yet more primitive groups, i.e. tunicates and hemichordates. Interpreting the relationship among these organisms has been a perennial problem for comparative zoologists, the main issue being to determine which characteristics of surviving groups are primitive, if any, and which are derived. The absence of relevant fossils compounds this problem, and there is a history of heated debate on the subject that has tended to discredit the entire enterprise. In part for this reason, living hemichordates and protochordates have been seriously neglected as subjects for research, and key aspects of their physiology, behaviour and general biology are still poorly

understood. The last decade has seen renewed interest in these organisms from molecular biologists using gene sequences and expression patterns to assess homology. At the same time, the shortcomings of classical morphological studies have become increasingly apparent. The nervous system is especially problematic, since much of its structural detail is below the resolving power of traditional microscopy and requires special techniques to render it visible. However, since a number of key developmental control genes are expressed mainly or exclusively in the nervous system, there are good reasons for wanting to know more about neural structure and organization at the cellular level. Ideally, the morphological and molecular data should complement each other in useful ways, and recent studies that apply modern microscopical techniques to the protochordate central nervous system (CNS) are beginning to bear this out, as this paper will illustrate.

Amphioxus, in the words of John Berrill (Berrill 1987), is 'the only surviving prevertebrate segmented chordate and as such has much to answer for', p.6. Yet prevailing opinion for much of the last century was that amphioxus was degenerate, highly specialized, and hence of limited evolutionary importance (see historical accounts by Gee 1994; Holland 2000). This idea has now been convincingly refuted by molecular data, which show that the amphioxus genome lacks any sign of the duplications found in vertebrate genomes (Holland 1996). The locomotory system of amphioxus is nevertheless quite

*Dedicated to the memory of Alfred B. Acton, DPhil (Oxon) and a Professor of Zoology at the University of British Columbia, who taught the author electron microscopy. Alfred died 4 June 2000, just short of his 73rd birthday.

advanced and its body is segmentally organized, with somites much like those of vertebrates.

Among tunicates, ascidian larvae and appendicularians have traditionally been considered the best models for ancestral chordates (for a review, see Gee 1996), but neither show clear signs of segmental organization (Crowther & Whittaker 1994). If tunicates are degenerate but descended from ancestors with somites, then they have lost a great deal of anatomical complexity. Otherwise, they represent a much earlier stage in the evolution of chordate locomotory systems. The nervous system in both ascidian larvae and appendicularians is also reduced and much simplified, which makes comparison with more advanced groups difficult. Nevertheless, molecular and cellular data indicate that the CNS of amphioxus and ascidian larvae have the same basic plan and a similar complement of sense organs. Assuming that these common features were present in stem chordates before the divergence of tunicates and amphioxus, one has a paradox—namely, that the sensory and locomotory control systems in amphioxus are evolutionarily older than the effectors they control. However, amphioxus myotomes are composite structures capable of several distinct locomotory modes, so the paradox can be resolved if one of these is at least as old as the common ancestor of amphioxus and tunicates. This issue is explored further below (§2–4) and leads to a consideration of the role predation has played in chordate evolution. Details aside, the intent is to illustrate the way new data are generating evolutionary hypotheses that can be examined critically, which is evidence in itself for progress.

2. ANCESTRAL CHORDATES: MORE LIKE AMPHIOXUS OR THE ASCIDIAN TADPOLE?

The amphioxus nerve cord has a slight swelling, the cerebral vesicle, at its anterior end but otherwise has few anatomical landmarks that can be used for comparison with the vertebrate CNS. Based on gene expression patterns in embryos and young larvae, however, there are regions in the amphioxus nerve cord that are homologous with vertebrate forebrain and hindbrain, and indications of a rudimentary midbrain region, though without an isthmus (for reviews, see Williams & Holland 1998; Holland & Holland 1999; and see also Kozmik *et al.* 1999). In addition, the larval CNS is patterned on a tiny scale, so despite its small size, there is more than enough diversity of cell type and organization for comparison with vertebrates. The goal of my own research has been to explore cellular architecture of the larval nerve cord as thoroughly as possible using electron microscope (EM)-level reconstructions from serial sections. So far, the morphology accords reasonably well with the molecular data. Briefly, the axial layout of the anterior cord parallels that of the vertebrate brainstem but is much simplified. There are upwards of 300 neurons in the part of the nerve cord that spans the first two somites, but a few cell types account for most of these, including 30 lamellar cells and about 80 preinfundibular sensory-type cells. Plausible counterparts to major features of the ventral brainstem can be tentatively identified, including core limbic structures of the basal diencephalon, tegmentum

and reticulospinal system (Lacalli & Kelly 1999, 2000). However, with the exception of the pineal homologue, represented in amphioxus by the lamellar body, the major dorsal structures used by vertebrates for sensory integration, i.e. the telencephalon and mesencephalic tectum, are missing (Lacalli 1996; Holland & Holland 1999). This is not surprising, since the peripheral sensory organs associated with the major dorsal centres in the brain are also absent in amphioxus. In fact, the dorsal part of the nerve cord in amphioxus larvae is extremely simple, consisting for the most part of simple tracts of bipolar cells and their fibres, and incoming sensory nerves from scattered epithelial sensory cells. In this respect, our results from young larvae largely confirm the descriptions by Bone (1961) of late larvae and adults. There is, therefore, no evidence as yet to support the claim that the dorsal part of the nerve cord is anything other than primitive, or that amphioxus ever had a significantly more elaborate complement of sense organs than it does now.

Comparing the amphioxus CNS with that of ascidian larvae, the molecular and morphological data are again in general agreement. The ascidian homologue of the amphioxus cerebral vesicle is the larval sensory vesicle. Both are forebrain-like in character as indicated by the expression their respective homologues of the *Otx* gene (Wada *et al.* 1998; Williams & Holland 1998) and both contain an assortment of similar sensory structures. The cerebral vesicle in amphioxus larvae (figure 1a) has two photoreceptors: the frontal eye and the lamellar body. Putative photoreceptor cells of the former have simple cilia; cilia of the latter have lateral arrays of parallel lamellae (Ruiz & Anadon 1991; Lacalli *et al.* 1994). The anterior part of the cerebral vesicle contains a variety of other ciliated cells that are probably also sensory in nature, including a cluster of cells with swollen cilia that Lacalli & Kelly (2000) have interpreted as a balance organ. A third set of photoreceptors, the rhabdomic Joseph cells, develops later along the dorsal surface of the cord behind the cerebral vesicle (Welsch 1968; Lacalli & Holland 1998).

The sensory organs of the larval sensory vesicle in ascidians varies a good deal between families (for reviews, see Burighel & Cloney 1997; Sorrentino *et al.* 2000) but there are some common features. Most have a balance organ of some type, e.g. an otolith or statocyte, and frequently an ocellus with a pigment cup. Ciliary bulb cells and various other cells with apical specializations also occur (e.g. Nicol & Meinhertzhagen 1991). Botryllids (figure 1b) are unusual in having a photolith, which functions as a combined light and gravity sensor and incorporates two types of sensory cells with apical and/or ciliary specializations. Though surface microvilli and other apical structures occur, fully developed rhabdomic photoreceptors appear to be absent in ascidians. However, they occur in the adult ganglion of salps (Gorman *et al.* 1971). How these various sensory organs and cell types relate to those in amphioxus is not clear. The range of types is similar, however, suggesting that they are probably homologues in at least some instances. Some ascidian ocelli, for example, have ciliary lamellae, so they could be reduced versions of the amphioxus lamellar body, but they could conceivably also be the remnant of an ancestral frontal eye. The salp eye, in contrast, is structurally closer to amphioxus Joseph

cells (Lacalli & Holland 1998). The amphioxus frontal eye appears to have a role in postural control during feeding (Stokes & Holland 1995), which invites comparison with ascidian balance organs, i.e. the otolith and photolith. In fact, if the sensory vesicle in ancestral ascidians was originally a longer structure, more like the amphioxus cerebral vesicle, reducing its length would bring the pigment and sensory cells of the ancestral frontal eye closer to the ciliary bulb cells. Combining these two could explain the origin of a complex multifunctional structure like the photolith.

Putative hindbrain homologues can be identified in both amphioxus and ascidian larvae on the basis of *Hox* gene expression. In amphioxus, the precise anterior limit of this zone is uncertain, but it extends caudally at least several segments from somite 3 (Holland & Holland 1999; Wada *et al.* 1999). The equivalent region in ascidian larvae is the visceral ganglion, which innervates the tail (Wada *et al.* 1998; Locascio *et al.* 1999). Between the anterior *Otx*-expressing part of the CNS and the *Hox* zone in both groups is a 'middle' zone that probably corresponds, at least approximately, with the vertebrate midbrain + rhombomere 1 of the hindbrain. In amphioxus, this region begins near the back of somite 1 and extends through some or all of somite 2. It contains the anterior-most of the motor neuron series, clusters of large ventral premotor interneurons that coordinate locomotion, and more dorsally positioned transluminal interneurons, an unusual neuronal cell type with apical processes that cross the central canal. Transluminal cells develop from the intermediate zone of the nerve cord, i.e. they lie between the dorsal and ventral neurogenic zones of the neural tube. The expansion of this zone during the mid-to-late larval phase is correlated with increasingly complex locomotory behaviour, including the ability to swim backwards. Many of the nerve cell types found in the anterior hindbrain region of the amphioxus nerve cord occur more caudally as well. Exceptions include the Rohde cells, a subset of transluminal cells with giant axons found only at the anterior and posterior ends of the nerve cord (Bone 1960), and the dorsal compartment motor neurons (see § 3), which are so far only reported from anterior segments.

In ascidians, the putative forebrain and hindbrain homologues (sensory vesicle and visceral ganglion, respectively) are separated by a midpiece or 'neck' from which the adult ganglion develops. The ganglion rudiment buds off the larval nerve cord at its junction with the neurohypophyseal duct. One of the few distinctive landmarks in this region is the auxiliary ganglionic vesicle, a small chamber that connects to the sensory vesicle in some species (Svane 1982; Svane & Young 1991). In some instances, the cells lining the ganglionic vesicle have apical specializations, e.g. folded membranes or lamellar projections, which suggests that they may be either functional or rudimentary sensory cells. *Botryllus* (figure 1b) has recently been the subject of an especially thorough study of ganglion formation by Burighel and co-workers (Manni *et al.* 1999; Sorrentino *et al.* 2000). In this instance, the projections are similar to those of S2 sensory cells in the sensory vesicle. One interpretation is that the two chambers in the larval CNS in *Botryllus* are remnants of an open central canal that has been secondarily

constricted. If the larval CNS of ancestral ascidians had a continuous central canal and a lamellar body like that in amphioxus, the *Botryllus* condition could be explained by distortion due to differential growth, constriction of the canal and reduction of the lamellar cells to remnants in each of the remaining chambers.

The central nervous systems of amphioxus and larval ascidians thus have a number of features in common, but a marked tendency towards reduction and asymmetry is evident in ascidians. Despite uncertainty about exact homologies, the general trend is clear, that amphioxus has a more complete and fully developed complement of sensory structures and cell types, which makes its CNS a better model for the ancestral condition.

3. MULTIPLE LOCOMOTORY SYSTEMS AND THE ANCESTRAL MODE OF LIFE

The previous section has argued that the amphioxus and ascidian larvae are essentially similar in terms of their sense organs and overall CNS organization. If the ascidian tail is primitive rather than degenerate, then the segmented locomotory system of amphioxus is substantially changed from the primitive, presegmental condition. In contrast, neural organization has changed comparatively little. This seems paradoxical until one looks more closely at the locomotory system itself. In amphioxus, the body musculature is innervated by three sets of motor neurons, and the animal has two undulatory swimming modes. As long as one of these modes is primitive, i.e. at least as old as the common ancestor of amphioxus and tunicates, one has a ready explanation for why major parts of the control system might be equally ancient. Taking this as a starting point, it may have been comparatively easy to modify the circuitry in simple ways, to adapt it for more advanced locomotory functions in amphioxus and vertebrates. So, which mode is the primitive one?

The three motor neuron types in amphioxus separately innervate: (i) the oral region and visceral organs (visceral motor neurons); (ii) the superficial fibres of the myotome (DC, or dorsal compartment motor neurons); and (iii) the deep fibres of the myotome (VC, or ventral compartment motor neurons). The visceral motor neurons are arranged segmentally, near the junctions between somites, and are distributed over a large part of the anterior cord (Bone 1961). The VC motor neurons are distributed all along the cord (Bone 1960; Lacalli & Kelly 1999), i.e. they are not segmental, and are thought to control fast, escape swimming, which in vertebrates is a function of the anaerobic deep fibres of the myotome (Bone 1989). The DC motor neurons have only recently been identified from EM reconstructions (Lacalli & Kelly 1999), and are tentatively included among the early *islet* and *neurogenein* expressing neurons in the neurula (Jackman *et al.* 2000; Holland *et al.* 2000). The evidence to date suggests that they are restricted to segments 2–5 of the larva, which means they may be exclusively anterior cells that project caudally to the rest of the nerve cord. The cells differ from other motor neurons in both synaptic morphology and vesicle type, and there are fundamental differences also in the nature of the upstream control circuitry (Lacalli 2001). Amphioxus

Figure 1. A comparison of structures and cell types in the anterior CNS of amphioxus with that of ascidian larvae.

(a) The anterior nerve cord of a young amphioxus larva in dorsal view, based on serial EM reconstruction data (Lacalli *et al.* 1994; Lacalli 1996; Lacalli & Kelly 1999, 2000). Shows the anterior pigment cup, the ciliary bulb cells of the putative preinfundibular balance organ, the lamellar body, which is supposed to be a pineal homologue, and selected motor neurons and interneurons. Symbols refer to specific cell types as indicated, including two types of somatic motor neurons. Zones of expression for selected genes are shown, along with the extent of the Joseph cells, a dorsally positioned series of rhabdomeric photoreceptors that develop later and extend caudally for a number of somites. (b) The larval CNS of the compound ascidian *Botryllus*, modified from Sorrentino *et al.* (2000). Ascidian larvae have an anterior sensory vesicle, usually with an ocellus and a pigmented otolith, but *Botryllus* has a photolith, which combines a pigment-containing cell with a cluster of sensory cell processes. Smaller apical processes extend from two other types of sensory cells into the chamber and processes similar to one of these (the S2 processes) are found in the ganglionic vesicle. The larval ocelli, in species that have them, are ultrastructurally similar to amphioxus lamellar cells and may be their homologues. In *Botryllus*, the S2 cells are better candidates and their presence in both the sensory and ganglionic vesicles implies that these may be remnants of a continuous, axial neural canal that has been constricted and displaced forward on the left side in ascidians, as indicated by the dashed line. The ganglion rudiment probably also belongs to this axial system; it forms at the point where the neural duct and larval CNS fuse and initially shares a lumen with the ganglionic vesicle. The two have separated at the stage shown and the rudiment has not yet begun to differentiate. When it does, a central neuropile will form surrounded by nerve cells of various types. A comparison with salps is useful here; the salp ganglion produces motor neurons, transluminal interneurons and rhabdomeric photoreceptors much like the Joseph cells of amphioxus. Thus, as indicated by the symbols, cell types distributed along the nerve cord in amphioxus are concentrated in a derivative of the 'neck'

larvae are presumed to engage in prolonged periods of slow swimming, during vertical migration (Wickstead & Bone 1959; Webb 1969). The DC system is probably responsible for these, although this has not been confirmed experimentally.

Ascidian larvae develop motor neurons in two locations: in the visceral ganglion and the definitive adult brain. Motor neurons in the latter control the adult pharynx and gut, as well as the rest of the body musculature, so one expects that at least a subset of the cells will prove to be homologues of the visceral motor neurons of amphioxus and vertebrates. Motor neurons in the visceral ganglion, should, in principle, be more closely related to somatic motor neurons in advanced chordates, but it is not obvious, in relation to amphioxus, whether they are more likely to be homologues of DC motor neurons or VC motor neurons. Conceivably, they could be a more primitive precursor related to both. This is something that could be determined with further research, because motor neuron subtypes differ in physiology and synaptic morphology, and in some cases express different molecular markers. If only one of the two subtypes were found to occur in both amphioxus and tunicates, a comparatively strong case could be made as to which locomotory mode evolved first: migration or escape behaviour.

Gans (1989), in an extensive review, argues that stem chordates were pelagic, and evolved a segmental musculature to facilitate escape from predators. Berrill (1955), in contrast, thought its original function was for migration into brackish and freshwater habitats. Berrill's idea may be closer to the mark, in my view, though an equally good or perhaps better case can be made that undulatory swimming evolved for vertical migration in the water column. Amphioxus larvae are thought to migrate vertically as part of a diurnal cycle and in search of richer grazing sites (Wickstead & Bone 1959; Webb 1969). Such tasks require precisely the kind of sense organs that occur in amphioxus and ascidian larvae. Though the function of these sense organs is not known in all cases, the majority appear to be navigational aids of one kind or another. They are probably involved in monitoring light levels, gravity and/or body orientation. Except for surface mechanoreceptors, which make amphioxus quite sensitive to touch, protochordates do not have the kind of sense organs needed to monitor predators or direct an escape. There is also no evidence from the morphology that their current complement of sense cells is secondarily derived from more complex organs that would have had that capability. It is thus more difficult to argue that escape behaviours evolved first, before migratory ones. It may also be significant that the muscle cells responsible for slow swimming are specified early in vertebrates, even before the somites form (Devoto *et al.* 1996; Cinnamon *et al.* 1999). There are numerous precedents for supposing that early events in embryogenesis are evolutionarily

older than late ones, and this may be another example of where this is indeed the case.

In summary, if migratory locomotion did indeed precede escape behaviour in chordate evolution, the visceral ganglion in ascidian larvae should contain neurons homologous with the DC control system of amphioxus. If so, and as long as the absence of segmentation in ascidian larvae is not secondary, there is an implied link between advanced functions, i.e. escape behaviour, which could include burrowing, and advanced structure, i.e. segmental myotomes. In other words, escape behaviour and chordate segmentation may have evolved together in animals that were already capable of using undulatory locomotion for other purposes.

4. A DIGRESSION ON BODY PLAN

It is useful at this point to compare amphioxus and tunicates with respect to overall body plan. In amphioxus, the three neuromuscular systems just described are distributed over varying lengths of the body. To date, only the region homologous with the hindbrain is known to have all three. In ascidians, there are apparently two neuromuscular systems, and these are segregated into separate structures, one for the larva (the visceral ganglion) and one for the adult (the adult ganglion, or 'brain'). It is not clear which of these is closer to the primitive condition: a uniform nerve cord combining multiple systems and cell types, as in amphioxus, or the ascidian pattern that segregates the adult ganglion from the nerve cord. The question is a crucial one because, to the extent that neural organization reflects body plan, one is asking which body plan is primitive. Is it amphioxus, with the visceral organs and locomotory structures integrated in a single body, or ascidians, with a different body and separate nervous system for each stage of life history? I can see no way to decide between these two alternatives using the data currently available.

It is nevertheless remarkable how frequently, when comparing the two groups, the same phenomenon is encountered; for example, structures, cell types or molecules distributed over a range of anterior somites in amphioxus are segregated forward into the adult ganglion in tunicates. Motor neurons have already been mentioned in this regard, but rhabdomeric photoreceptors show the same pattern if one includes salps, which have cells resembling Joseph cells in their ganglia. Finally, *Pax2/5/8*, a gene regionally expressed in vertebrate brain, is expressed in the neck region of ascidian larvae (Wada *et al.* 1998) but over an extended zone of anterior segments in amphioxus, overlapping with the hindbrain homologue (Kozmik *et al.* 1999). Thus, both the molecular and cellular data show that structures and cell types concentrated in distinct centres in ascidians are distributed more broadly along the nerve cord in amphioxus. As with the body plan, however, there is currently no way of deciding

region separating the sensory vesicle and the visceral ganglion in ascidians. Gene expression domains, all from other ascidian species, are also shown. The neck lies between the *Otx* and *Hox* zones and expresses *Pax2/5/8*. The anterior-most *Hox* expression in amphioxus is more caudal than the region illustrated in (a), so the distance between the *Otx* and *Hox* zones is much larger than in ascidians. *Pax2/5/8* expression in amphioxus occurs in this intervening region but also extends well into the *Hox* zone, so it is not clear how useful *Pax* expression is as an indicator of regional homology.

which is the primitive condition. Resolving these questions will probably require more knowledge of yet earlier forms, i.e. hemichordates and echinoderms. If an elongated body and undulatory swimming evolved first in a vermiform ancestor, then tunicates, with their bipartite body plan, may be something of a side issue in chordate evolution, despite being more basal members of the lineage by most accepted criteria.

5. CONTINGENT FEATURES OF HEAD EVOLUTION

The abrupt appearance of shelled or otherwise armoured fossils at the beginning of the Palaeozoic clearly points to the emergence of new types of predators at that time (Vermeij 1990; Conway Morris 1993), including actively swimming predators with eyes. The emergence of such creatures would have substantially increased the selective pressure on benthic invertebrates to evolve escape behaviours or other protective adaptations (Parker 1998). Such changes must also have affected pelagic life, though precisely how is less clear. Signor & Vermeij (1994) have argued that the pelagic realm prior to the Palaeozoic was a comparatively benign environment compared with the benthos, an idea that Brooks (1893) also developed at some length in his classic work on salps. An influx of new predators would have meant major changes in the pelagic fauna. Adopting an armoured benthic habit is an obvious solution, and this may be one reason that the diversity of benthic invertebrates increased so dramatically at the time.

In the case of chordates, the molecular evidence points to a pelagic origin, with appendicularians being the basal group (Wada 1998). If this is true, then ascidians must be a case where stem forms of the lineage escaped very early to the benthos, leaving the brief larval phase as the only clue to the ancestral mode of life. The other solution to increased predation is to adapt and remain in the plankton. This means evolving the locomotory capabilities necessary to evade predators and is evidently the route followed by ancestral cephalochordates.

Any discussion of early Palaeozoic predators is incomplete without reference to the role that the earliest vertebrates may have played. The term 'vertebrate' is used here in the sense of Donoghue *et al.* (1998) to include both living and extinct craniate stem forms, regardless of whether they have vertebrae. It would thus include conodonts, an extremely successful early group of swimming predators. Whether one accepts these as having a definite head, they have cranial sense organs resembling eyes and possibly otic capsules (Donoghue *et al.* 2000). In fact, they appear to have most if not all of the features necessary to make the head a functionally useful predatory device. This is especially apparent by comparison with amphioxus.

First, consider eyes: amphioxus has a frontal 'eye' that has some retina-like features, but the photoreceptor array is strictly one-dimensional (Lacalli *et al.* 1994), with no sign of reduction from something more complex. By contrast, the size of and position of conodont eyes implies that they are capable of forming a proper two-dimensional image. Amphioxus larvae appear to use their frontal eye to monitor body posture while feeding and possibly as a shadow detector (Stokes & Holland 1995). The body

rotates as they swim, however, and the head is thrown rapidly from side-to-side, so it seems unlikely that the eye is used at all during swimming. For a grazing animal using locomotion for escape, this may be enough. Indeed, during blind escape the organisms can move at maximum speed without the need to monitor direction and surroundings, as indeed larval and adult amphioxus appear to do. A predator, in contrast, must continually monitor the movements of its intended prey while it swims. For the eyes to function in this way in a tracking mode, either the displacement of the eyes relative to the rest of the body must be minimal, or there must be a mechanism to compensate for bodily movement by continuously reorientating the eyes. One way to accomplish the former is to fix the eyes to a stable support structure isolated from the locomotory movements of the rest of the body. This essentially means evolving a head with its own skeletal support.

For a predator, a rapid, targeted attack serves no purpose unless the prospective target can be first located from some distance away. Further, without the speed necessary to catch the prey, there is no point in being able to see it at a distance. In this sense, the visual and locomotory systems are inextricably linked; improvements to both are contingent and mutually reinforcing. The two systems thus should evolve together in an 'arms race' that began with the evolution of the first rudimentary image-forming eyes. Other features of head structure can be understood in terms of their contribution to improving the efficiency of this eye/locomotory link. Balance organs, for example, are useful for monitoring the tilt of the head so that appropriate corrective adjustments can be made. They are placed laterally in vertebrates, which is where displacement of the head due to tilt would be maximal. Additionally, so that appropriate adjustments can be made to accommodate or correct for tilt, there are regions in the brain that integrate visual and balance information. Amphioxus has a putative medial balance organ in the cerebral vesicle, but nothing lateral resembling otic capsules. Nor is there evidence from the organization of the nerve cord that such structures might once have existed. Finally, vertebrates typically control all of the oral and facial musculature centrally, which is useful if the opening and closing of the mouth is to be responsive to visual input. By contrast, both amphioxus and tunicates have peripheral plexuses in the oral region, mainly involved in rejection of debris or closing the mouth in response to disturbance (Lacalli *et al.* 1999; Mackie & Wyeth 2000). This is evidently sufficient for the purposes of filter feeding, but not for a predatory mode of life.

By way of summary, figure 2 shows a hypothetical primitive vertebrate, based loosely on reconstructions of the conodont animal. This is not intended to imply anything specific about the actual shape of the head or the position of sense organs, but rather to show the features that make the head functionally useful as a predatory device. The body is elongate with segmented musculature. The eyes are large and supported so as to separate them from the notochord and segmental musculature. There are also paired balance organs and CNS-derived innervation of the oral region. Much of this new construction comes from one tissue, the neural crest, which is both characteristic of vertebrates and restricted

Figure 2. A hypothetical ancestral craniate, drawn to emphasize features essential for the assumption of a predatory mode of life. Image-forming eyes are needed for any kind of visual hunting and these must be fixed on a cranial platform that does not move with the rest of the body during the pursuit of prey. Progressive improvement in the visual system makes sense only if the speed of locomotion also improves. Hence the evolution of the two systems is contingent and mutually reinforcing. Other useful but less essential features are lateral balance organs for monitoring tilt of the head and direct central control of the mouth, integrated with the visual system, for food capture. See text for further discussion.

to them (Northcutt & Gans 1983; Northcutt 1996). *Amphioxus* lacks both crest-derived structures and any sign of neural crest in the embryo (Northcutt 1996; Holland & Holland 1999), although their epithelial sensory cells may be a related cell type, as in vertebrates (Artinger *et al.* 1999). For these reasons, I am inclined to accept the argument of Gans (1989) and others, that the first vertebrates were really predatory organisms and that the structural advances that separate them from *amphioxus* evolved primarily as adaptations for this particular mode of life. *Amphioxus*, by contrast, shows no signs of having evolved from a vertebrate-type predator by reduction. It seems on this basis to be a filter feeder that has always been such, and a relic of a phase in chordate evolution that has since been largely extinguished by more advanced stem vertebrates and their descendants.

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REFERENCES

- Artinger, K. B., Chitnis, A. B., Mercola, M. & Driever, W. 1999 Zebrafish *narrowminded* suggests a genetic link between formation of neural crest and sensory neurons. *Development* **126**, 3969–3979.
- Berrill, N. J. 1955 *The origin of vertebrates*. Oxford: Clarendon Press.
- Berrill, N. J. 1987 Early chordate evolution I. *Amphioxus*, the riddle of the sands. *Int. J. Invert. Reprod. Devel.* **11**, 1–14.
- Bone, Q. 1960 The central nervous system in *amphioxus*. *J. Comp. Neurol.* **115**, 27–64.
- Bone, Q. 1961 The organization of the atrial nervous system of *amphioxus*. *Phil. Trans. R. Soc. Lond. B* **243**, 241–269.
- Bone, Q. 1989 Evolutionary patterns of axial muscle systems in some invertebrates and fish. *Amer. Zool.* **29**, 5–18.
- Brooks, W. K. 1893 *Salpa* in its relation to the evolution of life. *Stud. Johns Hopkins Univ.* **5**, 125–211.
- Burighel, P. & Cloney, R. A. 1997 Urochordata: Ascidiacea. In *Microscopic anatomy of invertebrates*, vol. 15 (ed. F. W. Harrison & E. E. Ruppert), pp. 221–347. New York: Wiley-Liss.
- Cinnamon, Y., Kahane, N. & Kalcheim, C. 1999 Characterization of the early development of specific hypaxial muscles from the ventrolateral myotome. *Development* **126**, 4305–4315.
- Conway Morris, S. 1993 The fossil record and the early evolution of the Metazoa. *Nature* **361**, 219–225.
- Crowther, R. J. & Whittaker, J. R. 1994 Serial repetition of cilia pairs along the tail surface of an ascidian larva. *J. Exp. Zool.* **268**, 9–16.
- Devoto, S. H., Melancon, E., Eisen, J. S. & Westerfield, M. 1996 Identification of separate slow and fast muscle precursor cells *in vivo*, prior to somite formation. *Development* **122**, 3371–3380.
- Donoghue, P. C. J., Purnell, M. A. & Aldridge, R. J. 1998 Conodont anatomy, chordate phylogeny and vertebrate classification. *Lethaia* **31**, 211–219.
- Donoghue, P. C. J., Fortey, P. L. & Aldridge, R. J. 2000 Conodont affinity and chordate phylogeny. *Biol. Rev.* **75**, 191–251.
- Gans, C. 1989 Stages in the origin of vertebrates: analysis by means of scenarios. *Biol. Rev.* **64**, 221–268.
- Gee, H. 1994 Return of the *amphioxus*. *Nature* **370**, 504–505.
- Gee, H. 1996 *Before the backbone: views on the origin of the vertebrates*. London: Chapman & Hall.
- Gorman, A. L. F., McReynolds, J. S. & Barnes, S. N. 1971 Photoreceptors in primitive chordates: fine structure, hyperpolarizing potentials, and evolution. *Science* **172**, 1053–1054.
- Holland, P. W. H. 1996 Molecular biology of lancelets: insights into development and evolution. *Isr. J. Zool.* **42**, S247–S272.
- Holland, P. W. H. 2000 Embryonic development of heads, skeletons and *amphioxus*: Edwin S. Goodrich revisited. *Int. J. Dev. Biol.* **44**, 29–34.
- Holland, L. Z. & Holland, N. D. 1999 Chordate origins of the vertebrate central nervous system. *Curr. Opin. Neurobiol.* **9**, 596–602.
- Holland, L. Z., Schubert, M., Holland, N. D. & Neuman, T. 2000 Evolutionary conservation of the presumptive neural plate markers *AmphiSox1/2/3* and *AmphiNeurogenin* in the invertebrate chordate *amphioxus*. *Devel. Biol.* **226**, 18–33.
- Jackman, W. R., Langeland, J. A. & Kimmel, C. B. 2000 *islet* reveals segmentation in the *amphioxus* hindbrain homolog. *Dev. Biol.* **220**, 16–26.
- Kozmik, Z., Holland, N. D., Kalousova, A., Paces, J., Schubert, M. & Holland, L. Z. 1999 Characterization of an *amphioxus* paired box gene, *AmphiPax2/5/8*: developmental expression in optic support cells, nephridium, thyroid-like

- structures and pharyngeal gill slits, but not in the mindbrain–hindbrain boundary region. *Development* **126**, 1295–1304.
- Lacalli, T. C. 2001 Dorsal compartment locomotory control system in amphioxus larvae. *J. Morph.* (In the press.)
- Lacalli, T. C., Holland, N. D. & West, J. E. 1994 Landmarks in the anterior central nervous system of amphioxus larvae. *Phil. Trans. R. Soc. Lond. B* **344**, 165–185.
- Lacalli, T. C. 1996 Frontal eye circuitry, rostral sensory pathways and brain organization in amphioxus larvae: evidence from 3D reconstructions. *Phil. Trans. R. Soc. Lond. B* **351**, 243–263.
- Lacalli, T. C. & Holland, L. Z. 1998 The developing dorsal ganglion of the salp *Thalia democratica*, and the nature of the ancestral chordate brain. *Phil. Trans. R. Soc. Lond. B* **353**, 1943–1967.
- Lacalli, T. C. & Kelly, S. J. 1999 Somatic motoneurons in amphioxus larvae: cell types, cell position and innervation patterns. *Acta Zool.* **80**, 113–124.
- Lacalli, T. C. & Kelly, S. J. 2000 The infundibular balance organ in amphioxus larvae and related aspects of cerebral vesicle organization. *Acta Zool.* **81**, 37–47.
- Lacalli, T. C., Gilmour, T. H. J. & Kelly, S. J. 1999 The oral nerve plexus in amphioxus larvae: function, cell types and phylogenetic significance. *Proc. R. Soc. Lond. B* **266**, 1461–1470.
- Locascio, A., Ancello, F., Amoroso, A., Manzanares, M., Krumlauf, R. & Branno, M. 1999 Patterning the ascidian nervous system: structure, expression and transgenic analysis of the *CiHox3* gene. *Development* **126**, 4737–4748.
- Mackie, G. O. & Wyeth, R. C. 2000 Conduction and coordination in deganglionated ascidians. *Can. J. Zool.* **78**, 1626–1639.
- Manni, L., Lane, N. J., Sorrentino, M., Zanido, G. & Burighel, P. 1999 Mechanisms of neurogenesis during embryonic development of a tunicate. *J. Comp. Neurol.* **412**, 527–541.
- Nicol, D. & Meinertzhagen, I. A. 1991 Cell counts and maps in the larval nervous system of the ascidian *Ciona intestinalis*. *J. Comp. Neurol.* **309**, 415–429.
- Northcutt, R. G. 1996 The origin of chordates: neural crest, neurogenic placodes, and homeobox genes. *Isr. J. Zool.* **42**, S273–S313.
- Northcutt, R. G. & Gans, C. 1983 The genesis of neural crest and epidermal placodes: a reinterpretation of vertebrate origins. *Q. Rev. Biol.* **58**, 1–28.
- Parker, A. R. 1998 Colour in Burgess Shale animals and the effect of light on evolution in the Cambrian. *Proc. R. Soc. Lond. B* **265**, 967–972.
- Ruiz, S. & Anadon, R. 1991 The fine structure of lamellate cells in the brain of amphioxus. *Cell Tiss. Res.* **263**, 597–600.
- Signor, P. W. & Vermeij, G. 1994 The plankton and the benthos: origins and early history of an evolving relationship. *Paleobiol.* **20**, 297–319.
- Sorrentino, M., Manni, L., Lane, N. J. & Burighel, P. 2000 Evolution of cerebral vesicles and their sensory organs in ascidian larvae. *Acta Zool.* **81**, 243–258.
- Stokes, M. D. & Holland, N. D. 1995 Ciliary hovering in larval lancelets. *Biol. Bull.* **188**, 231–233.
- Svane, I. 1982 Possible ascidian counterpart to the vertebrate saccus vasculosus with reference to *Pyura tessellata* and *Boltenia echinata*. *Acta Zool.* **63**, 85–89.
- Svane, I. & Young, C. M. 1991 Sensory structures in tadpole larvae of the ascidians *Microcosmus exasperatus* and *Herdmania mosmus*. *Acta Zool.* **72**, 129–135.
- Vermeij, G. J. 1990 The origin of skeletons. *Palaios* **4**, 585–589.
- Wada, H. 1998 Evolutionary history of free swimming and sessile lifestyles in urochordates as deduced from 18S rDNA molecular phylogeny. *Mol. Biol. Evol.* **15**, 1189–1194.
- Wada, H., Saiga, H., Satoh, N. & Holland, P. W. H. 1998 Tripartite organization of the ancestral chordate brain and the antiquity of placodes: insights from ascidian *Pax-2/5/8*, *Hox* and *Otx* genes. *Development* **125**, 1113–1122.
- Wada, H., Garcia-Fernandez, J. & Holland, P. W. H. 1999 Colinear and segmental expression of amphioxus *Hox* genes. *Dev. Biol.* **213**, 131–141.
- Wickstead, J. H. & Bone, Q. 1959 Ecology of acraniate larvae. *Nature* **184**, 1849–1851.
- Williams, N. A. & Holland, P. W. H. 1998 Molecular evolution of the brain of chordates. *Brain Behav. Evol.* **52**, 177–185.
- Webb, J. E. 1969 On the feeding and behavior of the larva of *Branchiostoma lanceolatum*. *J. Zool.* **170**, 325–338.
- Welsch, U. 1968 Die Feinstruktur der Josephschen Zellen im Gehirn von Amphioxus. *Z. Zellforsch. Mikros. Anat.* **86**, 252–261.