

## Apical Organs, Epithelial Domains, and the Origin of the Chordate Central Nervous System<sup>1</sup>

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**SYNOPSIS.** The apical organ is a key structural landmark in a wide range of invertebrate larvae, but its homolog in chordates has not been identified. A widely accepted explanation of chordate origins, Garstang's auricularia hypothesis, suggests several possibilities. Structural and biochemical evidence both support the idea that the apical organ, in amphioxus, has been incorporated into the frontal eye complex. Structural and positional similarities between this eye-like structure and paired eyes in vertebrates suggest the two may be homologous. The implication is that the cells of the neural retina in vertebrates may be derivatives of the primitive apical organ. Other implications of Garstang's hypothesis are discussed: that it provides (1) an evolutionary rationale for the restriction of Hox expression in ectoderm to neural tissue, (2) grounds for doubting that the CNS in chordates and that of protostome invertebrates can in any way be homologous, and a starting point for exploring (3) whether the eyes of ancestral chordates were single or paired, and (4) the origin of the vertebrate telencephalon.

### INTRODUCTION

The origin of chordates from non-chordate invertebrates remains one of the major unsolved problems of animal phylogeny. Many zoologists accept, in general terms, the supposition that chordates probably arose by neoteny from an invertebrate larva of some type. The obvious chordate credentials of the tunicate tadpole larva make it a possible candidate, but tunicates themselves are increasingly seen as specialized and rather distant from the vertebrate lineage (Bone, 1960; Jollie, 1973). This means the tadpole larva is probably not a very good model for the ancestral chordate. A more fruitful approach to the problem would appear to be that taken by Walter Garstang in his auricularia hypothesis (Garstang, 1894), not to be confused with his later, rather more complicated ideas concerning vertebrate origins from tunicates (Garstang, 1928). The auricularia hypothesis takes as starting point the dipleurula larva, basically

an idealized version of the modern auricularia larva of holothurian echinoderms. The hypothesis then accounts for the chordate nerve cord in an ingenious way, deriving it by dorsal convergence from the lateral loops of the larval system of ciliary bands (Fig. 1). The hypothesis does not account for the origin of either the notochord or myotomes. It is nevertheless useful conceptually, and is widely cited in texts and reviews, indicating a degree of acceptance. The hypothesis has not, however, been explored in any systematic way, and our knowledge of the surviving dipleurula-type larvae closest to the supposed ancestral form is still incomplete.

My own research, over the past decade, has involved me in a series of detailed EM-level studies of larval nervous systems in a variety of invertebrates, including work on ciliary band innervation in echinoderms and hemichordates. While there are cell types and organizational features that appear to be common to both ciliary bands and neural tube (*e.g.*, Lacalli *et al.*, 1990; Lacalli and West, 1993), few obvious structural landmarks can be identified that could be used for a more precise comparison of the two systems. The apical organ is one such struc-

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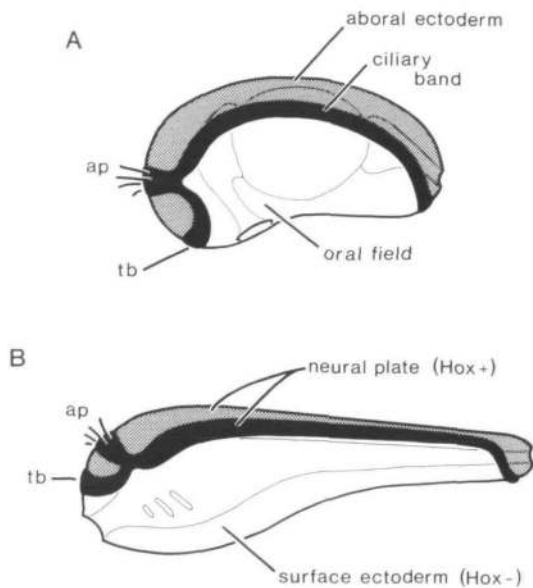


FIG. 1. The auricularia hypothesis, schematic, based on Garstang (1894). (A) A dipleurula or auricularia-type larva showing the division of the surface ectoderm into three domains: oral field, ciliary band, and the aboral ectoderm. Two anterior transverse elements, the apical organ (ap) and the preoral transverse band (tb), bridge between the lateral parts of the ciliary band system. (B) A hypothetical ancestral chordate prior to neurulation. The neural plate is presumed to derive from the aboral epithelium and the ciliary band with the latter providing some or all of the neurogenic tissue. A key issue concerns the fate of the preoral transverse structures, *i.e.*, whether they are included within the neural tube or left outside. As indicated, the shaded domains produce structures that typically express Hox genes during development. Derivatives of the oral field, which forms the external body surface, do not.

ture, however. Apical organs are widespread in invertebrate embryos and larvae, where they mark the extreme anterior pole of the organism. Neither their role in development nor their larval function is fully understood, but they are assumed to be sensory structures of some type. Most include neural elements, and they are clearly involved in the establishment of early neuronal tracts in some phyla (*e.g.*, in polychaetes, Lacalli, 1984). The chordate counterpart of the apical organ, if one exists, has not previously been identified. Before examining the relevant evidence, however, some of the implications of the auricularia hypothesis need to be considered.

#### THE AURICULARIA HYPOTHESIS: SOME IMPLICATIONS

##### *Epithelial domains and the external body surface*

Garstang's hypothesis emphasises the division of the body surface into three epithelial domains: the oral field, a dorsal strip of aboral epithelium, and the ciliary band. Neurogenesis is then assumed to be a property of the ciliary band and its derivatives, possibly an exclusive property of them. If the band and aboral ectoderm are then internalized at neurulation, the definitive body surface is necessarily derived from the oral field.

The organisms in question here are deuterostomes. The deuterostome mouth characteristically lies considerably forward of the primitive position it is assumed to have once occupied, and which it still occupies in some protostomes. This means either that the mouth has moved forward from an originally more posterior position, indicating a degree of positional flexibility, or alternatively, that it is a totally new orifice imposed secondarily on the original body plan. Whichever view is correct, there are functional and developmental arguments for treating the oral field and mouth together, as an organized unit. The situation in chordates is thus fundamentally different from that in protostomes. In chordates, an expanding ventral ectodermal domain, originally associated with the mouth, appears to have taken over the whole of the body surface. This could involve, for example, a shift in the positional reference system controlling ectodermal cell fate. However it is achieved, the result is that the ectodermal domain that originally covered the body appears to have been displaced dorsally, where it is internalized as neural tube. In contrast, the original ectoderm of protostome embryos remains as the definitive body surface. Various ectodermal structures differentiate in association with the protostome mouth, as in the case of the trochophore food groove and stomodeum, but these are basically ventral structures that are not, in any obvious sense, counterparts to the deuterostome oral field.

Now consider some of the molecular data

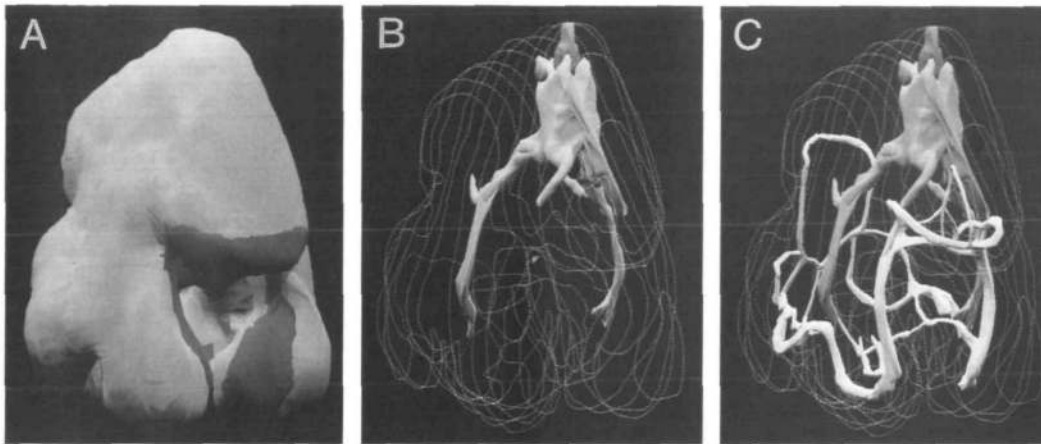


FIG. 2. Müller's larva, 3D reconstruction from EM data displayed as computer images, data from Lacalli (1982). (A) The external surface of a hatching larva showing the ciliary band (shaded). (B) The brain and internal nerve tracts of the same larva with, in (C), the ciliary nerves added. A thick basement membrane separates the internal tracts from the ciliary system, which is intraepithelial, showing clearly that they have separate developmental origins.

available on expression patterns of a key category of developmentally important genes, the antennapedia-class Hox genes. These occur in all metazoa so far examined, and are typically expressed in a series of domains along the anteroposterior axis of the body during embryogenesis. They are thought to be involved in specifying the basic axial body plan (McGinnis and Krumlauf, 1992; Holland, 1992). In vertebrates, their expression after gastrulation in the ectoderm is restricted chiefly to neural tissue, namely the developing neural tube, neural crest and some placodes. They are not expressed in the generalized ectoderm of the body surface. This is in striking contrast with the situation in protostomes like *Drosophila*, in which the ectoderm as a whole expresses such genes, which are thought to control segment identity in part through their effect on ectoderm. In this respect, the body surface, *i.e.*, the ectodermal part of the "primitive body" in insects, finds its closest counterpart in vertebrates, in terms of gene expression, in the internalized neural tube. Garstang's hypothesis provides an evolutionary rationale for why this may be so, assuming the oral field does indeed represent a new and separate epithelial domain that characteristically fails to express Hox genes. Interpreting the molecular evidence

in this way immediately raises the question of whether the external body surface of protostomes and that of chordates should be considered homologous.

#### *The central nervous system (CNS)*

A second issue concerns the nature and origin of the CNS. Garstang's hypothesis derives the tubular, internalized CNS of chordates from a simple external set of ciliary bands, which are presumed to be neurogenic. EM and immunohistochemical evidence show that larval ciliary bands in a wide range of invertebrate phyla are indeed neurogenic, producing nerve cells of diverse types from which the ciliary nerves themselves arise. Protostome larvae typically also have an internal system of ganglia and nerve cords, *i.e.*, a larval CNS, that develops quite separately from the intraepithelial nerves supplying the ciliary bands. It is therefore difficult to escape the conclusion that the CNS of protostomes and chordates cannot be homologous. The Müller's larva of polyclad flatworms (Fig. 2) provides a particularly clear example of separation between the deeper CNS elements and the more superficial system of ciliary nerves: the two are anatomically separate and lie on opposite sides of a thick basement membrane. Polychaetes show what appears to be a more

advanced condition; both systems are present, but the internal one dominates, while the ciliary system serves only as a provisional source of nerves for the larval metatroch and neurotroch (Lacalli, 1984). This suppression is carried further in mollusks, so that even the velar innervation mainly originates from the internal ganglia of the CNS (Mackie *et al.*, 1976). In contrast, invertebrate deuterostome larvae like those of echinoderms and hemichordates lack any sign of an internal, centralized system of ganglia. They instead have only ciliary nerves, and no indication that these have any primitive association with or derivation from any more centralized system. Clearly, the two groups may have similar types of nerve cells and subcellular mechanisms, derived from common ancestral cell types. But the organizational characteristics of the central nervous system as a whole, and that of its constituent parts, must have originated and evolved independently in the two groups.

#### THE APICAL ORGAN

A number of invertebrate larval types have simple, external apical tufts of cilia that mark their anterior pole. Even when such cilia are absent, a characteristic cluster of apical cells or a distinct apical plate can usually be identified. In the polychaete and mollusk larvae so far examined, the apical organ sinks inwards as development proceeds, but continues to occupy a central position at the midpoint of the cerebral commissure (Lacalli, 1984; Page, 1992; see Fig. 3A), and the plexus it forms appears to act as a target for fibres from the paired cerebral ganglia and the larval eyes.

The molecular marker most reliably associated with the apical organ in a wide range of phyla, including deuterostomes, is the neurotransmitter serotonin. It is found in apical organs in polychaetes (Hay-Schmidt, 1984), mollusks (Marois and Carew, 1990; Saini *et al.*, 1991), phoronids (Hay-Schmidt, 1990), and all echinoderm larvae so far examined (Bisgrove and Burke, 1987; Burke *et al.*, 1986; Nakajima, 1988; Moss *et al.*, 1993; see Fig. 3B). In amphioxus, the earliest developing and most anterior of the

serotonin-containing cells in the larva are associated with the anterior pigment spot, which lies at the extreme anterior end of the nerve cord, beneath the neuropore (Holland and Holland, 1993; see Fig. 3C).

My most recent research on amphioxus has included a detailed examination of this region by serial EM reconstruction. The arrangement of cells associated with the pigment spot is highly ordered, but on a minute scale involving too few cells for it to have been evident to earlier workers. The cells form a compact and highly structured assemblage (Fig. 4). Our interpretation is that these are the receptor and nerve cells belonging to a rudimentary photoreceptor, the frontal "eye." As described by Lacalli *et al.* (1994), we find a sequence of cell types, from anterior receptor-type cells to more posterior nerve-like cells, arranged in transverse rows (three such rows are shown in Fig. 4). The arrangement of cells in transverse rows is suggestive, as is the forward projection of their cilia toward the neuropore. Neither is true of cells elsewhere in the cord. Both features can be explained if the anterior part of the cord, including the frontal eye, were derived from an apical plate having its own ciliary orientation, and positioned as shown in Figure 1B. The apical cells would be rolled up with the neural tube to form its anterior margin as shown in Figure 5. The argument is developed at greater length by Lacalli *et al.* (1994), but basically suggests that the embryonic apical pole in amphioxus lies at the front of the nerve cord, and that preoral elements of the ancestral band system lying forward of (ventral to) the apical pole are left outside the CNS at neurulation. The latter represent potentially neurogenic tissue forward of the CNS that could conceivably provide a source of placodal tissue (*e.g.*, the olfactory placodes). Whether or not this conjecture is eventually proven correct, the possibility suggests that one goal of future molecular studies should be to trace neurogenic centers through a phylogenetic series that includes both the most primitive extant chordates and prechordate larvae like the auricularia.

There is little direct evidence regarding function of the frontal eye in amphioxus. The arrangement of cells in relation to the

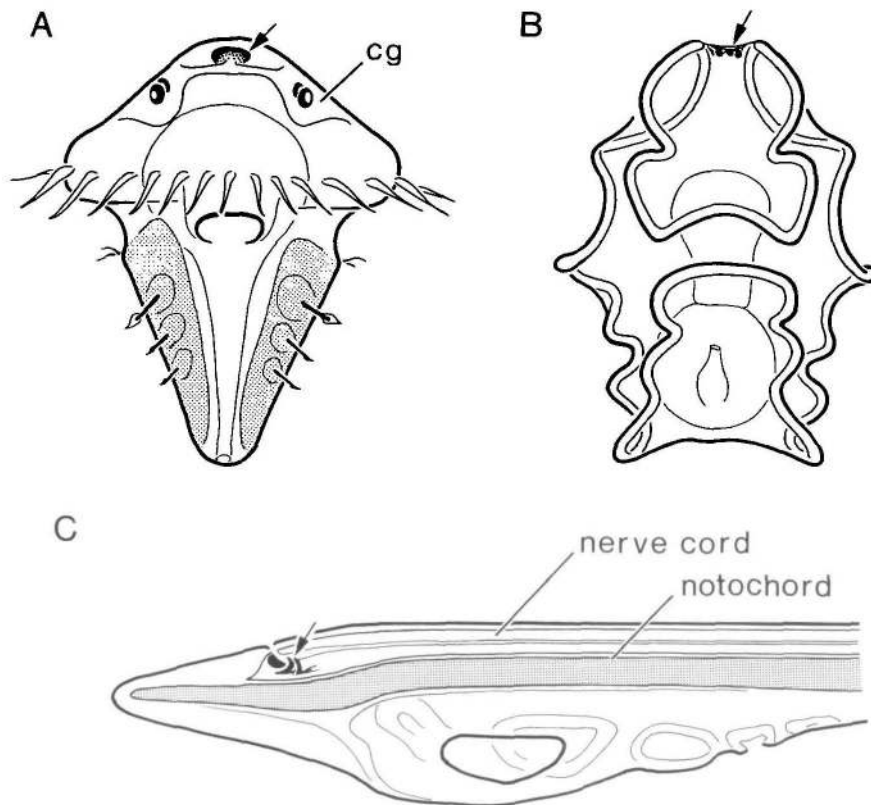


FIG. 3. A selection of invertebrate larvae showing their apical organs and associated serotonergic cells (arrows). (A) A late trochophore, after Lacalli (1984). The apical organ sinks inward and forms a central element of the cerebral commissure. The latter links the paired cerebral ganglia (cg) in which the larval ocelli are located. (B) A holothurian auricularia larva. The serotonin-containing apical cells, identified by Burke *et al.* (1986), form a loose plexus across the apex of the larva bridging the two sides of the band. (C) An amphioxus larva showing the serotonergic anterior cells reported by Holland and Holland (1993).

pigment cup is at least consistent with its being a simple, directional photoreceptor, but this is still an open question. A related question concerns the relation between this putative "eye" and the lateral eyes of vertebrates. This has been the subject of considerable past debate. Vertebrates also have epiphyseal (or pineal) photoreceptors, but there is a separate dorsally positioned photoreceptor complex in amphioxus that is the more likely homolog of these structures. The cells we have identified in the frontal eye are similar in type and relative position to the receptor cells and interneurons of the retina, which strengthens the case for homology with the lateral eyes. Also of interest is the positional similarity between vertebrate eyes and the frontal eye in

amphioxus: a single fused eye can occur in vertebrates due to mutation or the action of certain teratogens, a condition known as cyclopea (Adelmann, 1936). The single eye invariably lies near the ventral anterior margin of the forebrain (Fig. 6) in essentially the same position as the frontal eye in amphioxus. This is also the position of the initial eye rudiment in vertebrate embryos in the early neurula (Eagleson and Harris, 1990). The above supports the idea that receptor and nerve cells in the vertebrate retina may ultimately derive from the sensory cells of the primitive apical organ. These cells may thus be evolutionarily much older than chordates themselves, and some features of their organization may have been fixed very early in evolution. This could

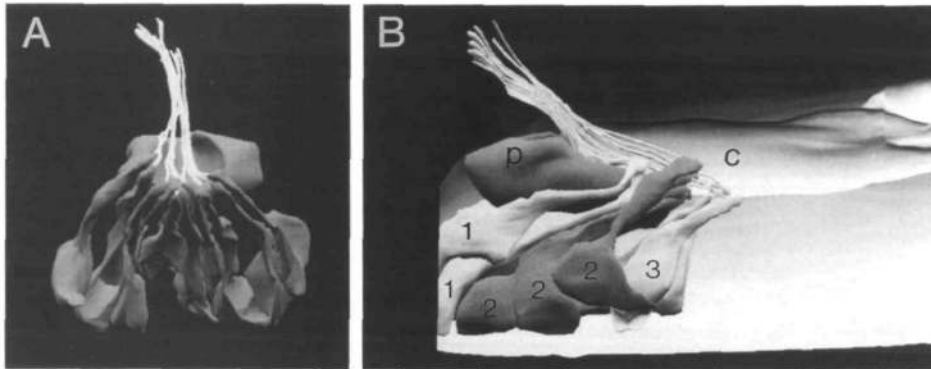


FIG. 4. Sample reconstructions from a serial EM analysis of the frontal eye of an amphioxus larva, data from Lacalli *et al.* (1994). Three rows of receptor and nerve-like cells lie just behind the pigment cup. (A) A view of the row 2 cells from behind. These cells have cilia that exit the neuropore; their morphology suggests they are receptor cells. (B) The cord and central canal (c) in cut-away side view with the pigment cup (p) and cells of all three rows (numbered).

explain why, of various CNS derivatives in vertebrates, the retina is among the most conservative in structure and organization.

*The original condition: Single or paired?*

The above links the apical organ with the amphioxus frontal eye, and the frontal eye with paired eyes in vertebrates. If amphioxus and vertebrate eyes are homologous, there would still be two possible evolutionary scenarios: the paired condition seen in vertebrates could be primitive, so the amphioxus eye would be secondarily fused; or the medial eye in amphioxus could represent the primitive condition, with paired eyes being advanced. Comparing the chordate situation with that in related invertebrate larvae does not resolve the question, but is suggestive. Specifically, just as there is evidence within chordates for transitions between the single and paired condition, the apical organ in echinoderm larvae occurs also in single and paired versions. This can be illustrated by comparing the auricularia with the starfish bipinnaria larva (Fig. 7). The apical organ of the former is similar to that of amphioxus in being a single, anterior structure. In starfish, in contrast, the apical plate broadens with development so that an extended anterior process is produced from its center, splitting the serotonin-containing region in two. This produces a pair of dorsolateral cell clusters, the "dorsal ganglia" of Nakajima (1988). These clusters have

been the subject of a more recent study (Moss *et al.*, 1994, see Figs. 7D and E), which reveals ordered clusters of peptide-containing cells closely associated with the serotonergic cells, at the same paired sites. The

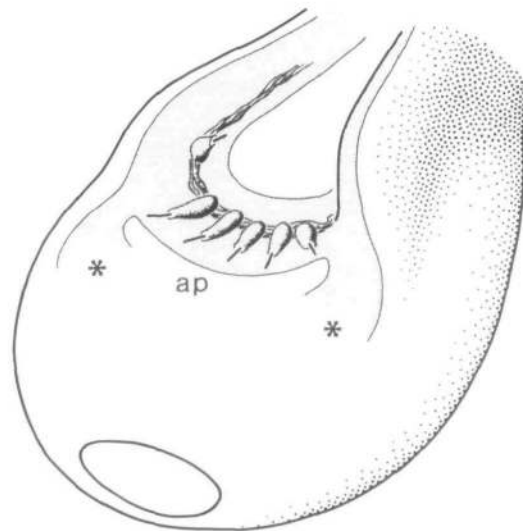


FIG. 5. A proposal for how the apical organ (ap) may be incorporated into the neural tube in amphioxus. If the apical organ were to form the anterior margin of the tube at neurulation, as shown, its cells would lie in transverse rows with their cilia projecting forward. This is precisely the arrangement found in the anterior-most cells in the nerve cord, including cells of the frontal eye complex. Band elements lying forward of (ventral to) the apical organ, *e.g.*, the preoral transverse band (\*) would, in this scheme, remain outside the neural tube.

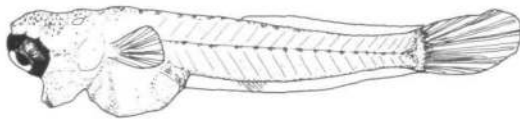
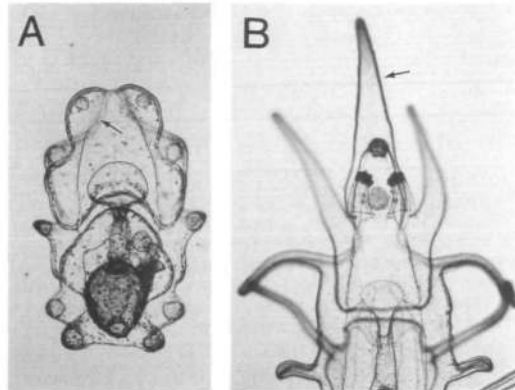


FIG. 6. Cycloopia in a *Fundulus* larva, a result of magnesium treatment, from Stockard (1909). Apparently, in such larvae, the eye rudiments have failed to separate normally resulting in a single, fused medial eye, located at the anterior base of the forebrain. The telencephalon is much reduced. Compare with the position of the frontal eye in amphioxus (Fig. 4B).



function of these cell clusters is not known, but they are distinctive and remarkably well organized. Further, as they develop, one sees the same process of medial outgrowth and lateral splitting that, in vertebrates, is responsible for producing paired eye vesicles from an initial medial rudiment. The ability of such rudiments to modulate their development in a similar fashion in both groups may be more than just coincidence. In addition, if the starfish larva were taken as a starting point for the chordate, rolling up the dorsal band would produce an anterior extension to the neural tube, forward of the serotonergic region, from which the most anterior part of the CNS, the telencephalon, could be derived. This suggests that the latter may derive from the medial-most part of the apical organ or adjacent midline cells associated with it.

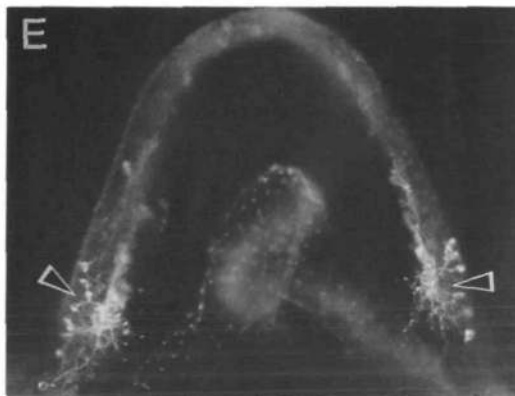
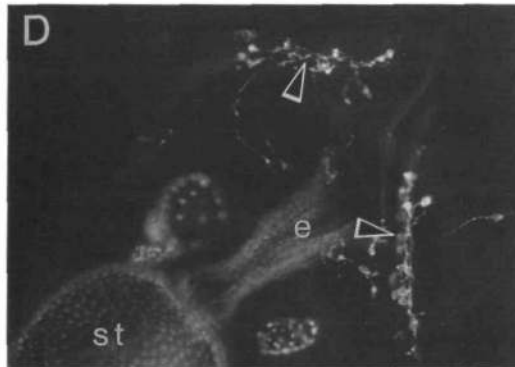
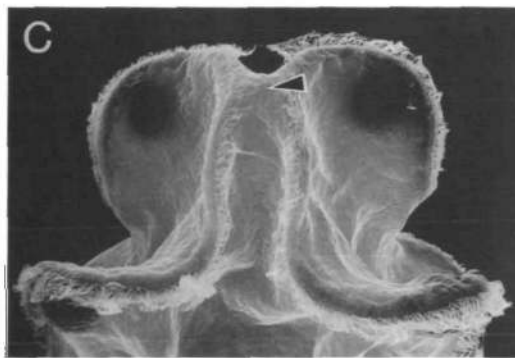


FIG. 7. Single and paired apical organs in echinoderm larvae. The auricularia larvae are *Stichopus californicus*; the bipinnaria are *Pisaster ochraceus*. (A) Auricularia larva. The arrow indicates the anterodorsal parts of the band shown in dorsal view in (C), seen here through the transparent tissues. (B) Late bipinnaria larva showing (arrow) the slender apical extension of the anterior pole of the larva. (C) Dorsal view of an auricularia; the arrow shows the location of the apical organ and the serotonergic cells. The lateral parts of the band system fold together behind this point in a fashion reminiscent of neurulation. (D) Oblique dorsal view of a 7-day bipinnaria stained immunohistochemically for serotonin. The apical pole of the larva is to the upper right; paired clusters of serotonergic cells (arrows) lie on either side of the apex. (E) Dorsal view of the apical region of a 34-day bipinnaria stained for the neuropeptide GFNSALMFamide, showing clusters of peptidergic cells in roughly the same positions as the serotonergic clusters. (D) and (E) courtesy of JMBA; see Moss *et al.* (1994) for details.

## CONCLUDING REMARK

Our understanding of chordate origins is limited because there are so few identifiable antecedents for the structural features characteristic of the group. The explosion of molecular data on expression patterns of highly conserved genes, *e.g.*, Hox genes, promises a wealth of new information on regional homology at the molecular level, and a very real prospect of resolving some major phylogenetic issues. Ideas like Garstang's cease being merely interesting in this situation, and become, instead, working hypotheses. As such, they need careful reexamination and updating. Structural evidence for homology, of the type discussed here, though not always conclusive in itself, provides a basic framework for hypothesis building and for identifying key organisms and structures for further study. The work discussed here illustrates this last point very clearly, in showing the importance of the comparatively poorly understood larvae of the dipleurula type for what they may yet tell us about the origin of chordates.

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