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Short Communication

Cell morphology in amphioxus nerve cord may reflect the time course of cell differentiation

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ABSTRACT Amphioxus embryos elongate following neurulation, and this lengthens the developing nerve cord. Most neurons and support cells remain attached at their apices to the neuroepithelium, and the apices themselves become correspondingly longer. In consequence, apex length can be used in some instances as a measure of whether a given cell last divided before elongation or after, and approximately when. The data indicate that most floorplate, ependymoglial and infundibular cells are generated comparatively early, before most neurons. Among the neurons, the segmentally arranged DC (dorsal compartment) motoneurons appear to be among the first to develop, which accords with molecular data on the time course of neural development, using *neurogenin* and *islet* as markers.

KEY WORDS: *amphioxus, neurogenesis, neurulation floor plate.*

Neuroepithelial cells in vertebrates are oriented so their apical surfaces face inwards, towards the central canal of the nerve cord. Neuroblasts, following their final division, detach from the epithelium and migrate basally before differentiating. In contrast, in cephalochordates (i.e. amphioxus), only a small subset of neurons, mostly dorsal in position, migrate in this fashion. The majority remain attached to neighboring cells by subapical junctions. Such contacts are evidently very stable, judging from the morphology of cells in the adult nerve cord. Adult motoneurons have narrow, fan-shaped apices extending 10μm or more along the surface of the neural canal, and apices as long as 50μm have been observed among the other cells of the cord (Bone, 1960). The mechanism responsible for apex elongation is not known, but the simplest explanation is that the apices are passively stretched as the somites and nerve cord lengthen during postmetamorphic growth. The quantitative data are consistent with this supposition: adult somites are longer than those of young larvae by about a factor of 5, while the apex length for cells in the post-infundibular part of the larval cord (see below) falls roughly between 1.8μm (neurons) and 9μm (ependyma), a range approximately one-fifth to one-sixth of that seen in the adult.

In the ventral half of the larval cord, in particular, all of the motoneurons and large interneurons of the locomotory control system remain attached to the neuroepithelium, and each retains its cilium. Dividing cells also remain attached at their apical surface. This means that positional relationships between cell apices should be preserved during development, and it may thus be possible to infer something of the developmental history of a given group of cells (e.g. lineage relationships) from the pattern of apical contacts.

As in most lower vertebrates, amphioxus embryos begin to elongate during the neurula stage. The notochord, the anterior-most somites, and the adjacent nerve cord all lengthen at this time (Fig. 1). The body continues to elongate in young larvae, but this is largely by addition of new somites caudally rather than by lengthening those already established. The initial period of elongation has two consequences for the developmental biologist. (1) Key neurogenic genes are expressed in neural plate cells in the neurula (e.g. Jackman *et al.*, 2000; Holland *et al.*, 2000), but tracing such cells through the elongation phase to the larva is difficult. Early expression patterns typically fade during elongation, so the precise position a given neural plate cell or its progeny will occupy in the larva relative to known landmarks, such as the neuropore, can only be estimated. This is seldom sufficient to match it unambiguously with an identified larval cell or group of cells. (2) On the other hand, if apex length can be used as a reliable measure of how much elongation a cell has experienced since its last division, it should be possible to distinguish between early- and late-developing cells by comparing their apices. There are complications, since a long apex can be long for various reasons. The dimensions of any two adjacent apices arising from a single division will depend on the

Abbreviations used in this paper: DC, dorsal compartment; EM, electron microscopy; LPN, large paired neurons; VC, ventral compartment.

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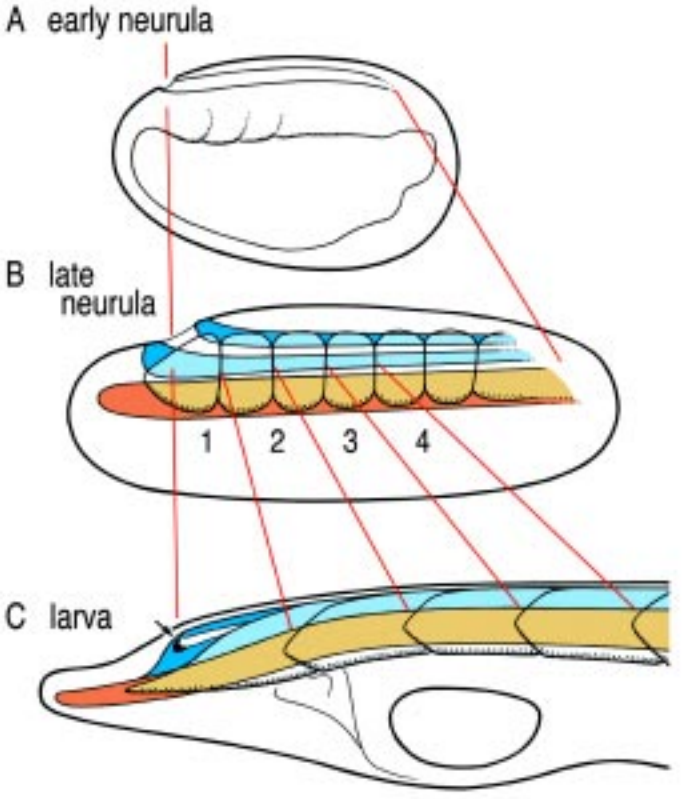

Fig. 1. Three stages in the development of an amphioxus (*Branchiostoma floridae*). *The progressive lengthening of the nerve cord (blue) and notochord (orange) is evident in the changing length (red lines) of the neural plate* **(A to B)** *and numbered somites* **(B to C)**. *The two neurula stages are 10 and 16 hours respectively at 25ºC; see Holland and Holland (1993) for staging. The larva is a 6-day stage with two functional gill slits, by which time the anterior somites have temporarily ceased elongating. In fact, somite length and nerve cord dimensions change very little between 3 and 12.5 days, the stage mapped in Fig. 3. The arrow indicates the anterior pigment spot, which is part of the frontal eye.*

angle and position of the division plane as it passes through the parent apex. If the parent apex is long to start with, dividing it lengthwise will produce side-by-side apices of equal length, while transverse division will generate a pair of shorter apices oriented end-to-end. More active processes may also be a factor in apex elongation (e.g. extension-convergence), but we have no way of monitoring this in fixed specimens. Short apices are less subject to conflicting interpretations, and are most easily explained as belonging to cells whose last division occurred towards the end of nerve cord elongation, or later. Unfortunately, nothing is currently known for certain about how amphioxus neuroepithelial cell apices subdivide during cell division, and whether this occurs in a regular and predictable fashion. While this further complicates the job of interpretation, it is still possible to make some useful inferences regarding the timing of cell differentiation from apex morphology, as this note illustrates.

Figure 2 shows the nerve cord in the late neurula and, for comparison, in a young larva. There are few overt signs of cellular differentiation in the neurula. By the larval stage, however, a variety of support cell types can be identified (e.g. ependyma, ependymoglia, floorplate and midline glia), and these are easily distinguished from neurons. The ventral-most neurons, located adjacent to the floorplate, are largely responsible for locomotory control, and it is mainly these cells that concern us here.

A map of cell apices projecting into the ventral part of the canal in the 12.5-day larva, constructed from serial EM sections, is shown in Fig. 3. It is first evident that there are marked differences in the axial extent of the cell apices depending on cell type. Support cells, especially ependyma and ependymoglial cells, tend to have long apices. The longest are just under 9μm; more typically they fall within the 5 - 7μm range, though more than half are shorter. Often the shorter ones form chains or lie side-by-side (e.g. sections 900-980 on the left; 670-710 on the right); presumably they arise by equal division of the apices of larger cells, and the side-by-side arrangement suggests that the division plane in these instances is oriented parallel to the axis of the cord. Floorplate and midline glial apices are generally arranged end-to-end, implying division is predominantly in the transverse plane, but there are exceptions where floorplate cells lie side-by-side (e.g. sections 1350-1450). Some of the floorplate cell apices in somite 2 are as long as ependymal cells, a few are quite short, but most are within the 3.5

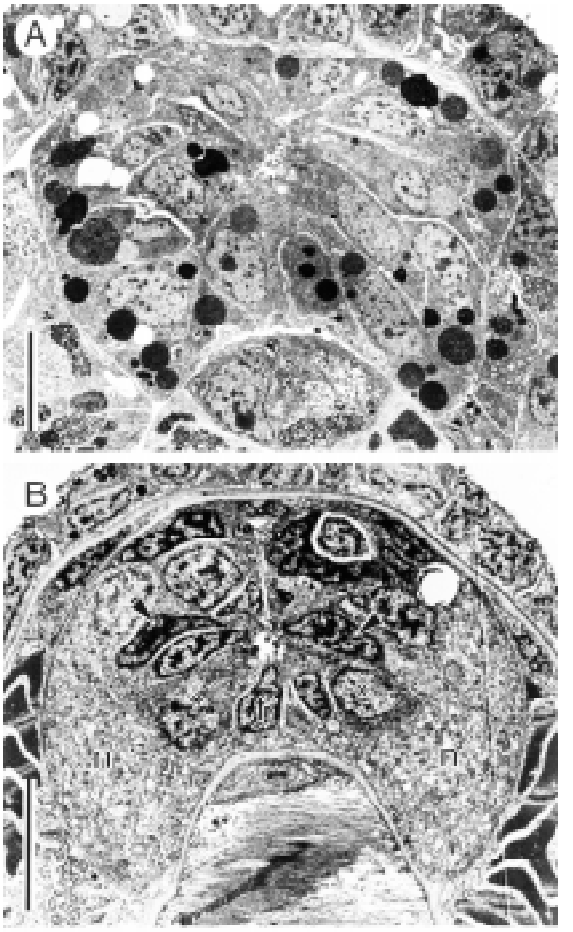

Fig. 2. Transverse sections through the nerve cord. *The sections are taken at approximately the middle of somite 2 of* **(A)** *a late neurula, and* **(B)** *a 12.5-day larva. The locomotory control system in the latter consists mainly of ventral neurons (asterisks) positioned between the floorplate (f) and axial rows of lateral ependymoglial cells (arrows). The ventrolateral fibre tracts (n) are also well developed by this stage. Scale bars 5 μm.*

- 5.2μm range, with a mean of about 4μm. Infundibular cell apices are shorter still, ca. 1.7μm, but six of these are arranged as end-to-end pairs, which suggests that an equal transverse division of each parent apex has occurred.

In quantitative terms, the dimensions of the longest ependymoglial apices can be accounted for by simple stretching of the apices of undivided neural plate cells matching that experienced by the cord as a whole. Measuring from the photographs on which Fig. 1 is based, the anterior cord and adjacent somites elongate by a factor of 1.6 from 10 - 16 hours, and 2.35 from 16 - 72 hours, for a total of 3.75 times by the end of the initial period of somite elongation. Cell apices in the early neurula, as measured from sections, are 1.5 - 2μm across and approximately isodiametric (i.e. similar length and width). Multiplying these values, a neurula cell that does not divide further would have an apex 5.6 - 7.5μm long after elongation, a range that includes the majority of large ependymal and ependymoglial apices in our specimen. Those support cells that have are shorter apices, i.e. infundibular cells, some ependyma and most of the anterior floorplate, must have divided later. Half maximal length (2.8 - 3.7μm) is consistent with the final division occurring in the late neurula.

In contrast to the ependymal and ependymoglial cells, neuronal apices are mostly short (less than 1.8μm) and close to isodiametric. The pre-infundibular neurons have the smallest apices, and these pack so closely that there is insufficient space in the junctional contact zone to accommodate the basal body/rootlet complex. The latter is instead borne on knobs that project into the central canal. These particular cells are probably, therefore, produced quite late, after elongation. This is supported by the observation that the pre-infundibular zone in 6-day larvae contains relatively few cells. Other neuron types are less closely packed, but only a few have apices that are significantly longer than they are wide. Exceptions are: certain interneurons adjacent to floorplate just behind the infundibular cells (parainfundibular neurons, 2.5 - 4.5μm long, indicated in Fig. 3), a few interneurons and VC motoneurons in the primary motor center (3.0 - 3.4μm) and the first two pairs of DC motoneurons (2.8 - 4.4μm, only the first pair is shown in Fig. 3). The latter, as a class, all have comparatively long apices for neurons. Five pairs of DC motoneurons occur in the first six somites, and these are arranged in an approximately segmental pattern (Lacalli and Kelly, 1999). Mean apex length for pairs 3-5, measured in an 8-day larva, are 3.3, 5.9 and 4.4μm respectively, with the right and left members of each pair being essentially identical.

The predominance of small apices among neurons in the 12.5-day larva suggests that when neurons are generated, the postmitotic cell always has an apex of minimal size. This cannot be proven conclusively from the data presented here, but it is the case that solitary neuronal apices are often completely surrounded by much longer apices belonging to non-neural cells. Presumably one of these is the daughter from the preceding division, during which the parent apex has been divided in a highly unequal way. If so, then any neurons whose apices show a significant degree of elongation must have divided early enough to have experienced a degree of post-mitotic stretching. There is some independent evidence that this may be the case, because the first neurons to differentiate, as indicated by molecular markers, correspond approximately in position with neurons known to have long apices. The gene *engrailed*, for example, is expressed during mid-elongation in cells on either side of the midline in the infundibular region (Holland *et al.*, 1997), approximately where the parainfundibular cells will develop. Both *neurogenin* and *islet* are expressed during neurula stages in ventral cells near the junctions between the first five somites (Jackman *et al.*, 2000; Holland *et al.*, 2000), approximately

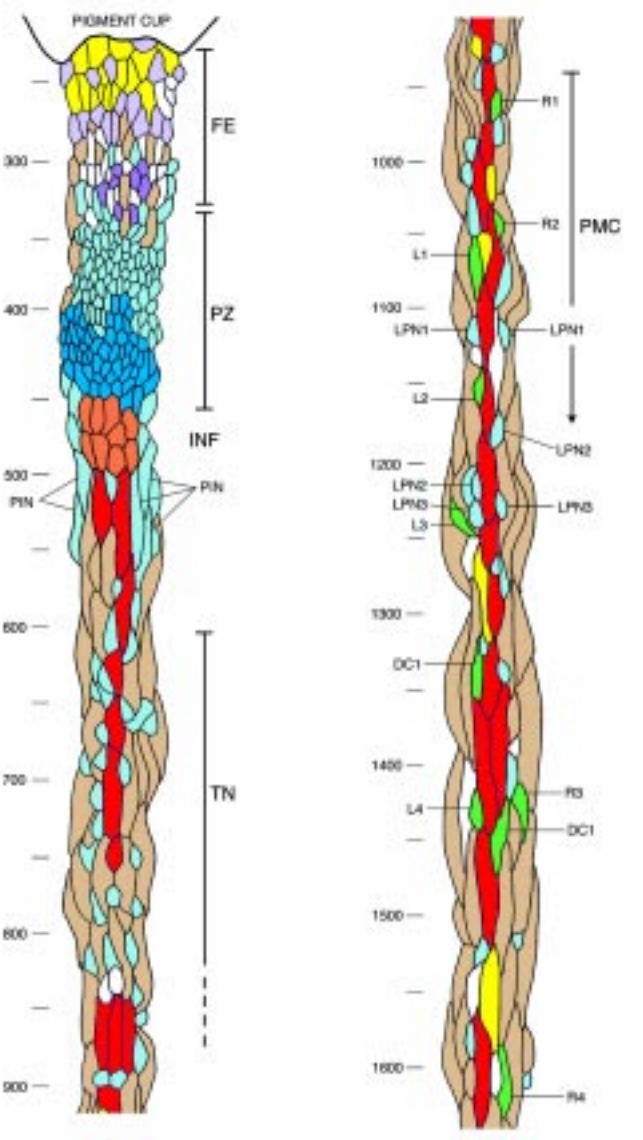

Fig. 3. The midventral lumenal surface of the larval nerve cord. *This figure maps the cell apices of ventrally-positioned cells, i.e. those that project into the lower half of the neural canal. The surface of the latter has been flattened out and is drawn as seen from above. The boundaries of each cell apex are traced from serial EM sections of the anterior nerve cord of a 12.5-day larva. The map extends from the back of the anterior pigment spot to just beyond the middle of somite 2. The sections are numbered (100 sections = 6.8 μm, the somite 1/2 overlap is on sections 1150-1300); see Lacalli and Kelly (1999, 2000) for details on cell types. Regions labelled: frontal eye (FE), pre-infundibular zone (PZ), infundibular cells (INF), tegmental neuropile (TN), primary motor centre (PMC). Cell types, by color: ependymal and ependymoglial cells (tan), floorplate (red), glial or glial-like cells (yellow), photoreceptors and neurons of the frontal eye (light and dark violet, respectively), motoneurons (green), other neurons, including all interneurons (blue, darker for the balance organ); undifferentiated cells are not colored, and there are instances where these are not clearly distinguishable from ependyma or differentiating glial cells. Specific neurons: parainfundibular interneurons (PIN, includes two types), ventral compartment motoneurons (R1-R4; L1-L4), dorsal compartment motoneurons (DC1, paired), large paired neurons (LPN1-LPN3); the two LPN3 cells are the "giant" cells described by Lacalli (1996).*

where the DC motoneurons are located. The genes are expressed in small cell clusters, rather than individual cells. Nevertheless, the DC motoneurons are the only cell type so far found in our studies to be restricted to these sites, which suggests that one or both of these genes may play a role in specifying this particular neuronal lineage.

A further question concerns whether apex position can be used to identify lineage relationships between neurons of different types. This is possible in principle if the progeny of a given cell remain in apical contact. If lineage is important, patterns of contact should then be repeated wherever cells of similar type are generated. The data, however, do not show this. Instead the patterns are varied, quite irregular, and largely asymmetric. In some cases neuronal apices are clustered together, suggesting that the cells may all have arisen from one precursor, while their counterparts on the opposite side of the cord are solitary and surrounded by ependymal or ependymoglial cells (e.g. compare LPN2 and 3 on the right and left sides, sections 1200-1250, in Fig. 3). The apex data therefore is not consistent with there being a simple lineage relationships between identified neuronal types. However, the cell bodies of most neurons are large, and lateral contacts are common between them, even when their apices are not adjacent. This suggests that subsequent contacts and signals exchanged between cells may be more important than lineage in the specification of neuronal cell types in this system.

Experimental Procedures

This is part of a larger study of neural circuitry in the nerve cord of *Branchiostoma floridae*; the methods used have been previously described (Lacalli *et al.*, 1994). Apical maps in this study were prepared from a single serial EM series of 2100 sections through the anterior cord of a 12.5-day larva, with additional observations on the DC motoneurons identified by Lacalli and Kelly (1999) in similar series from an 8-day larva.

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References

BONE, Q. (1960). The central nervous system in amphioxus. *J. Comp. Neurol.* 115: 27-51.

HOLLAND, L.Z., KENE, M., WILLIAMS, N.A. and HOLLAND, N.D. (1997). Sequence and embryonic expression of the amphioxus *engrailed* gene: the metameric pattern of transcription resembles that of its segment-polarity homolog in *Drosophila. Development* 124: 1723-1732.

HOLLAND, L.Z., SCHUBERT, M., HOLLAND, N.D. and NEUMAN, T. (2000). Evolutionary conservation of the presumptive neural plate markers *AmphiSox1/2/3* and *AmphiNeurogenin* in the invertebrate chordate amphioxus. *Dev. Biol.* 226: 18-33.

HOLLAND, N.D. and HOLLAND, L.Z. (1993). Embryos and larvae of invertebrate deuterostomes. In *Essential developmental biology* (Eds. C.D. Stern and P.W.H. Holland). IRL Press, Oxford, pp. 21-32.

JACKMAN, W.R., LANGELAND, J.A. and KIMMEL, C.B. (2000). *islet* reveals segmentation in the amphioxus hindbrain homolog. *Devl. Biol.* 220: 16-26.

LACALLI, T.C. (1996). Frontal eye circuitry, rostral sensory pathways and brain organization in amphioxus larvae: evidence from 3D reconstructions. *Phil. Trans. Roy. Soc. B* 351: 243-263.

LACALLI, T.C. and KELLY, S.J. (1999). Somatic motoneurones in amphioxus larvae: cell types, cell position and innervation patterns. *Acta Zool.* 80: 113-124.

LACALLI, T.C. and KELLY, S.J. (2000). The infundibular balance organ in amphioxus larvae and related aspects of cerebral vesicle organization. *Acta Zool.* 81: 37-47.

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