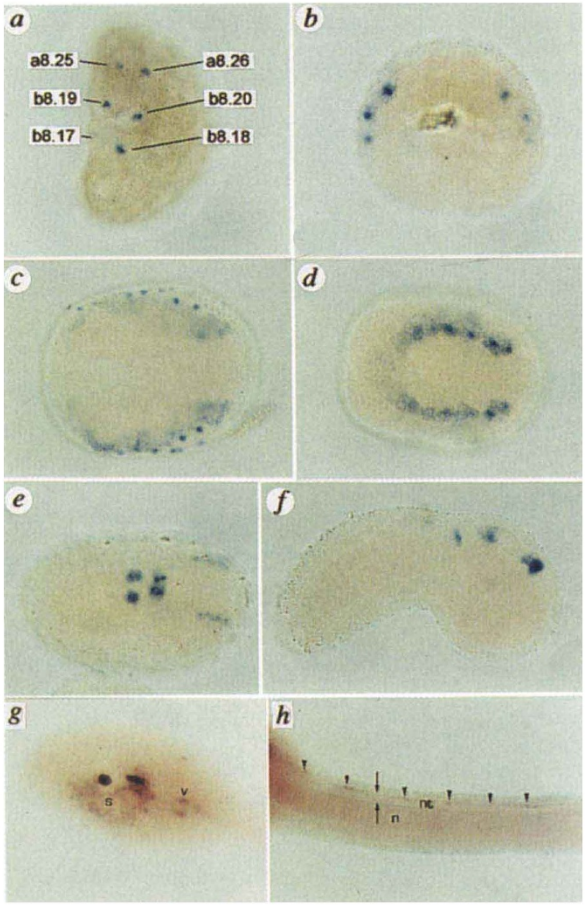
Origin of patterning in neural tubes

SIR — A notochord and dorsal tubular nerve cord are essential features of chordates, and must have evolved in the most ancient members of our phylum. How complex was the primitive chordate neural tube? Ascidians, as members of the urochordate subphylum, derived from the most basal extant lineage within Chordata[1]; anatomically, the nerve cord of ascidian tadpole larvae is simpler than that of vertebrates or cephalochordates, with less than 100 rostral neurons and just axons and ependymal cells in the tail[2]. It is unclear whether this reflects a simple ancestral pattern, or whether it masks hidden developmental complexity.

Here we report the expression of an ascidian (*Halocynthia roretzi*) *Pax* gene, *HrPax-37*, descendant from the precursor of *Pax-3* and *Pax-7* in vertebrates. The last two are expressed dorsally in both neural tube and somitic mesoderm; in addition, mouse *Pax-3* mutations reveal a function in differentiation of the dorsal neural tube[3–5]. Expression of *HrPax-37* is first detected at the early gastrula stage (*a* in the figure) in the nuclei of six bilateral pairs of cells, destined to form the dorsal part of the neural tube (a8.25, b8.19 and b8.17; the last two also yield muscle cells) or dorsal epidermis (a8.26, b8.20 and b8.18)[6,7]. By the mid-gastrula stage, expression is maintained only in the first three cells (*b* in the figure). In contrast, at the later neural-plate stage the expression becomes downregulated in the primordial dorsal nerve cells and reactivated in the dorsal epidermis (*c* in the figure). At the neurula stage, just a single row of epidermal cells flanking the presumptive neural tube continues to express the gene (*d* in the figure).



Spatial and temporal expression of *HrPax-37* as deduced from *in situ* hybridization. *a*, Lateral view of embryo at early gastrula stage. The signal of b8.17 is out of the plate of focus. *b*, Dorsal view of mid-gastrula embryo. Anterior is to the top in *a* and *b*. *c*, *d*, Dorsal view of embryo at neural-plate stage (*c*) and neurula stage (*d*). *e*, Dorsal view of the embryo at initiating tailbud stage. The neural tube is closing from the posterior region; in more anterior regions, the expression of *HrPax-37* remains in epidermis. *f*, Lateral view of the embryo at mid-tailbud stage. Anterior is to the right in *c*–*f*. *g*, Dorsal view of the trunk region of larva. s: sensory vesicle, v: visceral ganglion. *h*, Lateral view of larval tail. The segmental expression of *HrPax-37* in the nerve cord is indicated by arrowheads. Arrows, dorsal and ventral boundary of the neural tube; n, notochord; nt, neural tube. Anterior is to the left in *g* and *h*.

Expression during these early embryonic stages indicates that *HrPax-37* might have a similar function to vertebrate *Pax-3* and *Pax-7* in the differentiation of dorsal neural tube, although *HrPax-37* is also expressed in dorsal epidermis. Epidermal sensory neurons of ascidian larvae are derived from the dorsal epidermal cells[7,8], raising the possibility that the expression in this region is comparable with *Pax-3* in vertebrate neural crest[3].

During closure of the neural tube, expression fades from dorsal epidermal cells, but is reactivated in the three populations of cells in the neural tube (*e*, *f* in the figure). By the tadpole larva stage, expression in the most rostral cell population disappears, although extensive expression persists in the sensory vesicle and visceral ganglion (*g* in the figure). At this stage, a striking pattern of *HrPax-37* expression emerges more posteriorly, in the nerve cord of the tail. Expression is detected in roughly 15 reiterated spots, within a dorsal row of cells in the nerve cord (*h* in the figure). To our knowledge, no segmental structures have previously been reported in the ascidian neural tube. Because this expression must derive from ependymal cells (there are no neuronal cell bodies in the nerve cord[2]), we suggest this reiterated distribution of *HrPax-37* is an evolutionary remnant reflecting ancestry from a more elaborate segmental organization. This suggestion is compatible with the existence of a segmental ancestor for all urochordates, and would resolve the paradox that appendicularians (also urochordates) have motor neurons segmentally distributed in the tail neural tube[9,10].

In conclusion, we suggest that dorsal specification by genes of the *Pax-3/7* subfamily, and segmental organization of neural tube, were established in the ancestors of extant chordates during emergence of the dorsal tubular nervous system.

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1. Wada, H. & Satoh, N. *Proc. Natl Acad. Sci. USA* **91**, 1801–1804 (1994).
2. Nicol, D. & Meinaertzhagen, I. A. *J. Comp. Neurol.* **309**, 415–429 (1991).
3. Goulding, M. D., Chalepakis, G., Deutsch, U., Erselius, J. R. & Gruss, P. *EMBO J.* **10**, 1135–1147 (1991).
4. Jostes, B., Walther, C. & Gruss, P. *Mech. Dev.* **33**, 27–38 (1991).
5. St-Onge, L., Pituello, F. & Gruss, P. *Semin. Dev. Biol.* **6**, 285–292 (1995).
6. Conklin, E. G. *J. Acad. Nat. Sci. (Philadelphia)* **13**, 1–119 (1905).
7. Nishida, H. *Dev. Biol.* **121**, 526–541 (1987).
8. Torrence, S. A. & Cloney, R. A. *Zoomorphology* **99**, 103–115 (1982).
9. Flood, P. R. *J. Microsc.* **18**, 317–326 (1973).
10. Bone, Q. *Am. Zool.* **29**, 5–18 (1989).

Neuronal cell death and tPA

SIR — The serine protease tissue plasminogen activator (tPA) has been implicated in brain hippocampal function, as its messenger RNA is present[1] and is rapidly induced in the rat and mouse hippocampus on pharmacological or electrical induction of neuronal activity[2,3]. In addition, mice deficient in tPA ($tPA^{-/-}$)[4] are resistant to neuronal destruction[5] after intra-hippocampal injection of high concentrations of glutamate analogues (excitotoxins[6,7]). Here we investigate whether $tPA^{-/-}$ mice are resistant to neuronal degeneration because of pre- and/or postnatal development in the absence of tPA, or whether tPA is necessary for neuronal death in the adult mouse.

An adult requirement for tPA would be significant for two reasons. First, tPA has recently been approved for treatment