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CELL REARRANGEMENT AND DIRECTIONAL MIGRATION IN PRONEPHRIC DUCT DEVELOPMENT

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Abstract

The morphology of the directed migration of the pronephric duct rudiment of three vertebrates, the salamander, chick and sturgeon, has been examined by scanning electron microscopy. Of particular interest in this paper are the morphology of the duct tip, the role of cell rearrangement, and the relation of duct extension to somite segmentation. The duct rudiments of all three species have motile cell processes (lamellipodia and filopodia) largely confined to their posterior tips. The salamander and sturgeon embryos extend their duct rudiments by extensive cell rearrangements. A short, wide rudiment is elongated to form a long, thin one. The chick duct rudiment stays about the same width and apparently gains volume by cell proliferation. The salamander duct rudiment's posterior tip is always two somites behind the last formed somite. Both the sturgeon and chick embryo's duct rudiments lie well posterior of the last segmented somite adjacent to segmental plate mesoderm. There is still a close coupling, however, between the posterior progression of the duct rudiments and the advancing wave of somite segmentation.

KEY WORDS: pronephric duct, development, comparative embryology, cell migration, salamander, sturgeon, chick.

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Introduction

Pronephric duct development in several vertebrate species has been characterized by utilizing scanning electron microscopy together with surgical and marking techniques. Most observations have been done on embryos of the axolotl (the salamander, Ambystoma mexicanum). The caudal extension of the axolotl pronephric duct rudiment occurs by cell rearrangement (Poole and Steinberg, 1981). The extension is apparently propelled by the active extension and contraction of motile cell processes at the rudiment's posterior tip. The tip is guided by a gradient of adhesion on its cellular substratum (Poole and Steinberg, 1982). This guidance information is encoded by a trypsin-sensitive protein (Gillespie et al., 1985) and other cell types such as the cranial neural crest are able to use it (Zackson and Steinberg, 1986). The region of lateral mesoderm able to support duct migration (the "active" region) progresses posteriorly at the same rate as somite segmentation and duct elongation (Poole and Steinberg, 1982). At the same time there is an interesting morphological change in the lateral mesoderm from a two-cell to a single-cell layer and back again which coincides with the anterior boundary of the active region (Gillespie and Armstrong, 1986). These studies make the salamander pronephric duct rudiment's morphogenesis one of the best understood examples of directed cell migration in embryology.

The African clawed frog (Xenopus laevis) forms its pronephric duct by segregation from the dorsalmost portion of the lateral mesoderm. Marking experiments and scanning electron microscopy provide no evidence for directed cell migration (Poole and Steinberg, 1984). The chick pronephric (Wolffian) duct forms by directed migration as has been demonstrated by the construction of chick-quail chimeras (Martin, 1976). The posterior tip of the chick duct has fine filopodia in close contact with underlying mesoderm. It apparently elongates by active cell locomotion but the diameter of the duct rudiment does not change markedly with its elongation (Poole and Steinberg, 1984).

Because of the differences between salamander and frog embryos it was of interest to look at fish embryo pronephric duct development.

It has been suggested, based on substantial developmental differences, that urodeles (salamanders) and anurans (frogs) evolved from different fish (Nieuwkoop and Sutasurya, 1976). Here I review some of the major features of salamander and chick duct development whose rudiments form by directed migration and present some new work on the morphology of pronephric duct directed migration in sturgeon embryos.

Materials and Methods

Axolotl (Ambystoma mexicanum) embryos were obtained from laboratory spawnings. Embryos of the white sturgeon (Acipenser transmontanus) were obtained from the University of California at Davis aquaculture facility. Chick embryos were obtained from eggs incubated for 30 to 55 h.

Embryos were fixed in 2.5% glutaraldehyde, 2.0% paraformaldehyde and 5mM calcium chloride in 0.1M sodium cacodylate buffer, pH 7.4. Axolotl and sturgeon embryos had their ectoderm manually peeled off with watchmaker's forceps. Embryos were rinsed in several changes of 0.15M sodium cacodylate buffer and post-fixed in 1% osmium tetroxide. They were dehydrated with ethanol and critical point dried from liquid carbon dioxide. The ectoderm from chick embryos was lifted off after drying with scotch tape. After mounting on stubs, dried embryos were sputter coated with gold-palladium (60:40) and photographed at 15-25 kV.

Results

Figure 1 is a scanning electron micrograph (SEM) of a stage 25 axolotl embryo whose ectoderm has been peeled off the right side. The arrow marks the posterior limit of the pronephric duct rudiment which has extended approximately one third of its way to the cloaca. Note the location of the duct tip two somites behind the last formed somite. Figure 2 is a SEM of a duct tip of an older embryo showing the filopodial contacts with the substratum (somite and lateral mesoderm) and the overlapping of cells within the duct rudiment. The duct tip retains the appearance of actively motile cells as the duct extends by thinning through cell rearrangement to the cloaca.

Figure 3 is a SEM of a 9 somite chick embryo. The pronephric duct rudiment appears as a collection of stellate-shaped cells lateral to the segmental plate and somites. (The larger cells over the lateral mesoderm are prospective endothelial cells.) Figure 4a is the posterior region of a 14 somite embryo. During the active caudal extension of the chick duct rudiment, the duct tip travels in a groove with cells contacting both segmental plate and lateral mesoderm. In more cranial portions as on the right edge of figure 4a, cells within the rudiment are more closely apposed and make fewer contacts with the substratum. Figure 4b is a higher magnification micrograph of this rudiment's tip. The substratum is covered by fine meshwork of extracellular matrix fibers whereas the surface of the duct rudiment is relatively free of such fibers. There is overlapping of cells within the duct rudiment but the tip is less tightly organized then that of axolotl embryos.

Figure 5a shows a 7 somite sturgeon embryo whose ectoderm has been peeled off the left side. The sturgeon embryo possesses a large pronephric duct rudiment. The posterior limit is indicated by an arrow. Figures 5b and 5c show older sturgeon embryos of 9 and 12 somites, respectively. Note that the rudiment extends further posteriorly and has thinned. Also note that in contrast to axolotl embryos the posterior tip lies far caudal to the last segmented somite (by approximately 6 somite widths) adjacent to the segmental plate. Figure 5d is a 17 somite embryo whose pronephric duct rudiments are fully extended caudally. In sturgeon embryos vitally stained with Nile blue sulfate beneath somites 4 to 9, evidence was presented for the directed caudal extension of the pronephric duct rudiment (Ballard and Ginsburg, 1980). Figure 6 shows, at higher magnification, the posterior tip of the duct rudiment of the embryo shown in 5c. The rudiment tip is quite broad, approximately 6 cells across, and in close contact with its mesodermal substrate. Figure 7 shows the extreme posterior tip of the embryo in figure 5b. There are numerous lamellipodia with filopodial extensions contacting the substrate and other cells within the pronephric duct rudiment.

Discussion

The directed migration of the pronephric duct rudiment occurs in salamander, chick and sturgeon embryos. These three examples show interesting differences. Axolotl and sturgeon embryos form their pronephric ducts by active cell migration and cell rearrangement. In the axolotl, experimental manipulations show a critical coupling of duct extension and mesoderm differentiation such that during normal development the duct tip lies approximately two somites behind the last formed somite. In sturgeon and chick embryos, the duct tip lies far in advance of the last segmented somite but similarly passes anterior to posterior at the same rate as somite segmentation.

The chick pronephric duct rudiment does not thin during its caudal extension. There is a region of relatively high cellular proliferation near the level of the duct rudiment's posterior tip (Overton, 1959). This may provide the new cells which are then pulled out to lengthen the duct rudiment. In summary, directed migration occurs as the mode of pronephric duct morphogenesis in a wide variety of vertebrate embryos. This migration is closely coupled to axial morphogenesis but varies among species such that the elongating duct tip may lie in advance of or lag behind the wave of somite segmentation.

Acknowledgements

We thank Marisa Martini for technical assistance and Peter B. Armstrong for sturgeon embryos. Figure 2 is reproduced with permission from Developmental Biology 92: 144-158 (1982).

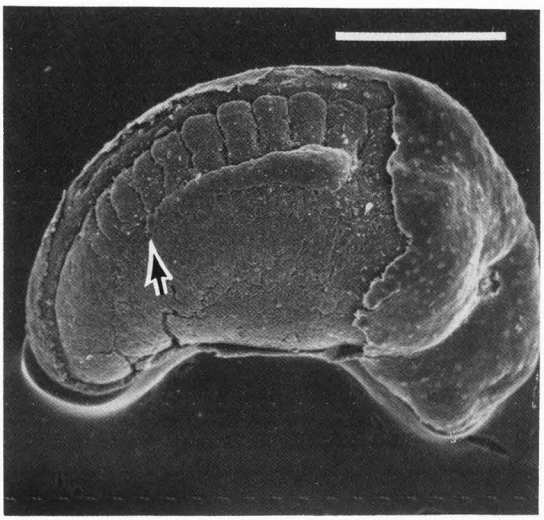


Figure 1. A stage 25 axolotl embryo with ectoderm removed to reveal the surface of the mesoderm. An arrow marks the caudal tip of the pronephric duct rudiment. Bar = 1 mm.

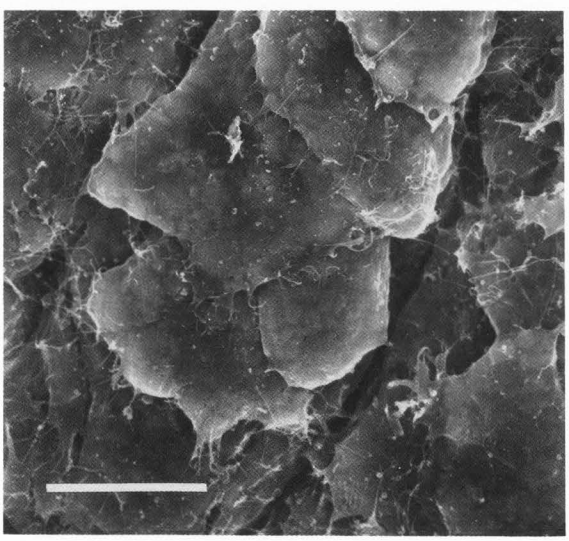
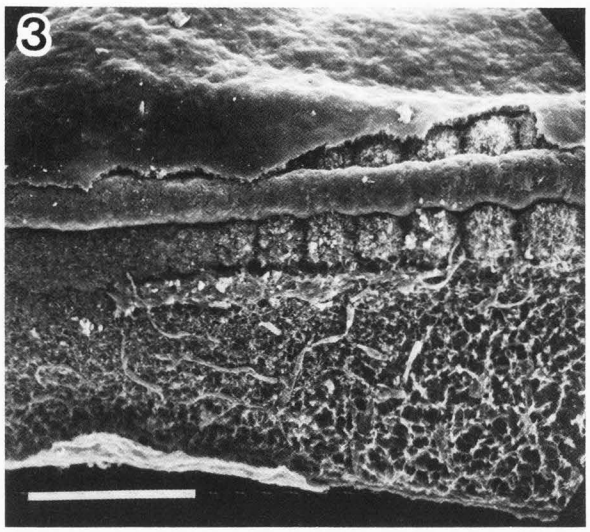
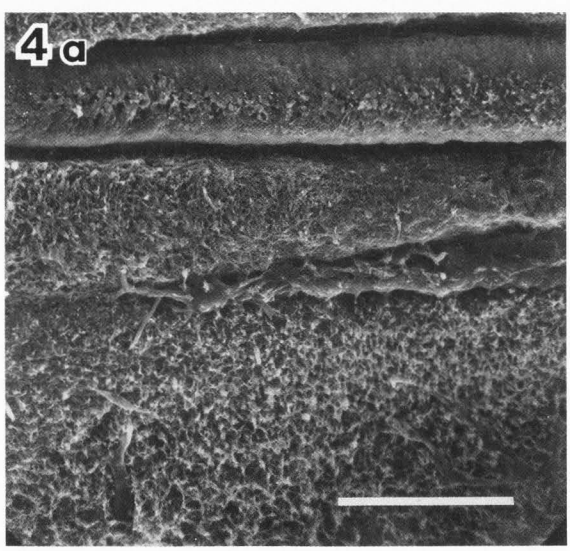


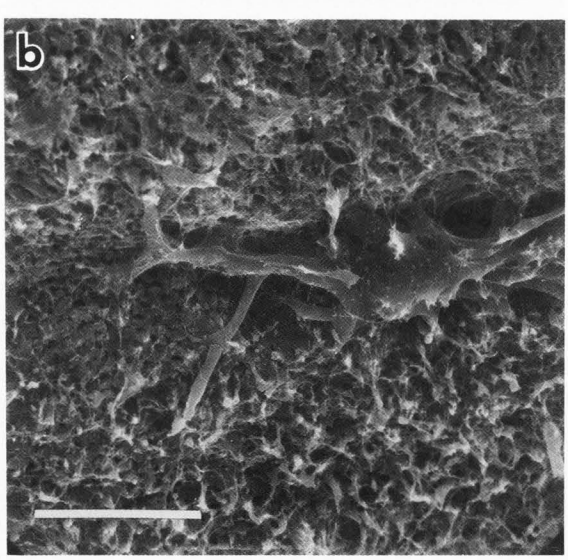
Figure 2. The extreme caudal tip of an older duct rudiment shows flattened lamellipodia and filopodia extending onto the substrate and onto more posterior cells within the rudiment. Note the regular overlap of rudiment cells. Bar = 30 μm.

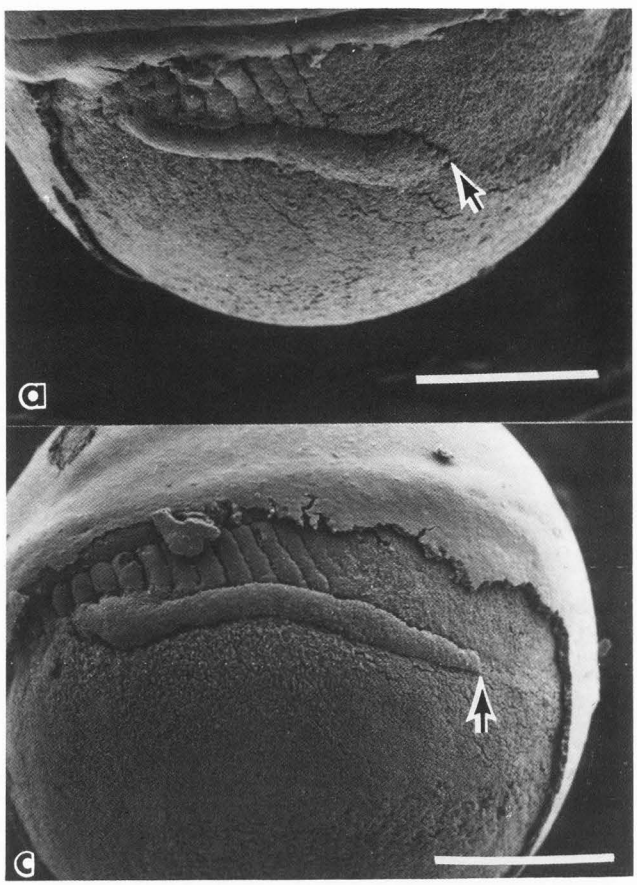
Figure 3. An early stage of chick pronephric duct development. In this 9 somite embryo the duct rudiment is seen as a cord of cells. Bar = 300 μm.

Figure 4a. The posterior tip of a 14 somite chick embryo's duct rudiment. Bar = 150 μm. b. The extreme posterior end showing fine filopodial extensions of duct rudiment cells and extracellular matrix fibers covering the mesodermal substratum. Bar = 50 μm.









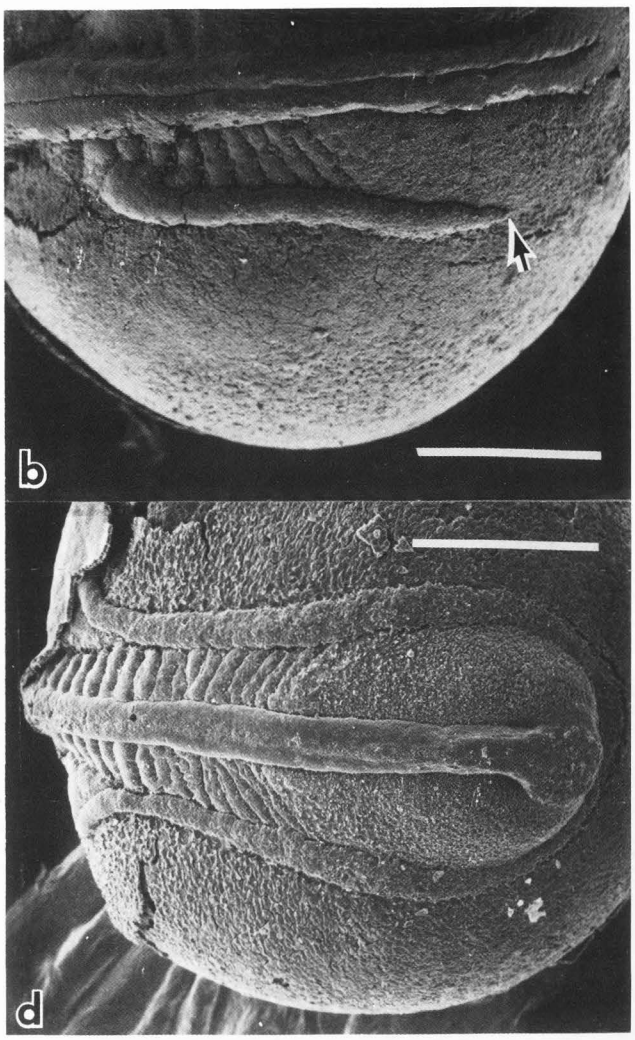


Figure 5a. Scanning electron micrograph of a 7 somite sturgeon embryo whose ectoderm has been peeled off the left side after fixation. An arrow marks its posterior tip. Bars = 750 µm. b. 9 somite sturgeon embryo. c. 12 somite sturgeon embryo. d. 17 somite sturgeon embryo.

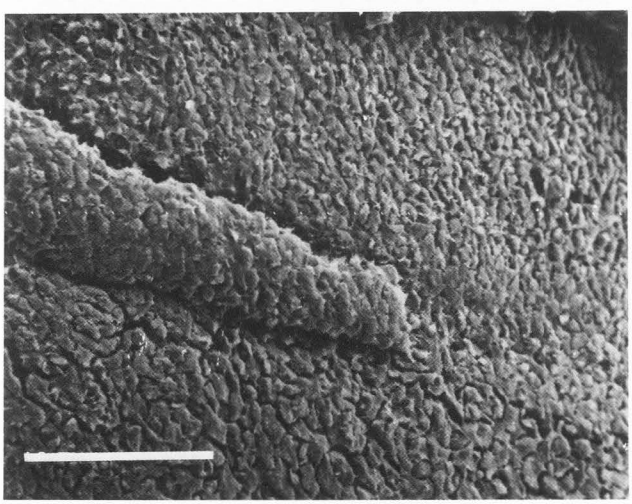


Figure 6. The posterior third of the duct rudiment of the sturgeon embryo in figure 5c. Bar = 200 µm.

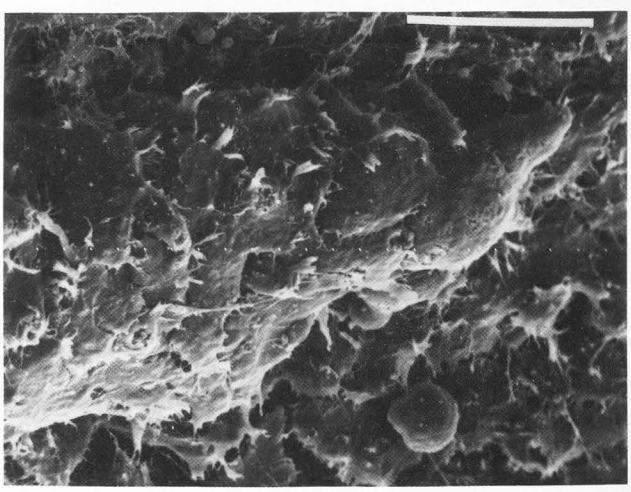


Figure 7. The extreme posterior tip of the duct rudiment of the sturgeon embryo in figure 5b. Note the many filopodial extensions of cells within the pronephric duct rudiment. Bar = 50 µm.

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Discussion with Reviewers

S.L. Zackson: Xenopus is known to use alternative mechanisms for accomplishing several different morphogenetic mechanisms when compared not only with urodeles but also with other anurans. Do all anurans form their pronephric duct by segregation from the lateral mesoderm?
Author: Overton (text reference 1959) treated 20 to 22 somite chick embryos with colchicine for 4 hours and noted a shortening and thickening of the pronephric duct rudiment. She attributed much of the shortening to a rounding up of cells elongated in the anterior-posterior axis (such as the duct's posterior tip). Since this treatment was for such a short time period, it is not as yet clear if the inhibition of cell division will totally prohibit duct elongation in chick embryos.

S.L. Zackson: The chick pronephric duct pathway appears to be a groove, suggesting contact guidance as the mechanism directing the duct. Is there experimental evidence concerning this hypothesis? The photographs included in this paper suggest that a shallow groove might be present along the sturgeon pathway. Do stereo photographs more definitively reveal a groove?
Author: There is evidence that the duct path in bird embryos is bidirectional, a characteristic of direction by contact guidance. Martin (C.R. Acad. Sci. 275: 1075-1077, 1972) placed a band of quail embryo with its cranio-caudal axis reversed behind a chick duct rudiment's caudal tip. The chick duct was able to migrate along the quail path in the caudal to cranial direction. There does seem to be a groove along the sturgeon pathway but we have not yet made any stereo pairs to better reveal such a groove.

A.P. Evan: What is the nature of the extracellular matrix material found in the substratum? If one looks closely at Fig. 4b, there does appear to be a fine meshwork of material on a few cells of the pronephric duct. Could the preparation protocol have removed a portion of this material from some cells and not others?
Author: The extracellular material present consists largely of collagen and fibronectin. In preliminary observations of whole mount preparations and sections of chick embryos immunohistochemically stained for fibronectin, there are extensive fibrillar fibronectin deposits on somite, segmental plate and lateral mesoderm. The pronephric duct is relatively free of fibronectin. This indicates that even though there might have been some selective removal in preparation for SEM, there is some independent evidence for a relative paucity of matrix materials on the surface of the pronephric duct.

A.P. Evan: What is meant by the statement that in the chick "the tip is less tightly organized then that of axolotl embryos?" There is a clear difference in cell shape.
Author: There appears to be more space between cells at the tip of the chick duct rudiment then in the axolotl where cells are closely apposed.