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RAPID COMMUNICATION

NEURAL TUBE (CANAL) MORPHOGENESIS IN NOTOCHORDLESS AMPHIBIAN (XENOPUS LAEVIS) EMBRYOS

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Neural tube (canal) morphogenesis was examined in embryos which exhibited notochord defects. Embryos which displayed a range of notochord defects were produced by either ultraviolet irradiation or cold shock treatments. Both treatments produced similar results. The neural canal appeared normal in morphology and internal ciliation in many of the embryos which contained severe notochord defects.

Introduction

Neural morphogenesis is controlled, to a large extent, by forces within the neural plate cells themselves. As early as 1895, Roux (1) demonstrated that an isolated amphibian neural plate maintains the capacity to fold and transform into a partially complete neural tube as an organ culture. The complete development of the morphogenetic features of neural tube formation have been discussed by various earlier investigators as depending--to one degree or another--on the interactions which occur between the neural tube and its surrounding tissues. For example, the morphology of the neural canal was originally observed by Lehmann (2) to be strongly influenced by the presence of the notochord which normally lies directly under the neural tube. Holtfreter (3, 4) presented evidence that the somites also influence the development of the neural canal. He suggested that the somites play a role in the thickening of the lateral walls of the neural tube.

Once formed, the neural canal appears to depend on the turgor pressure of the fluid in the lumen for its structural rigidity. Cells which line the neural canal are believed to be the source of the fluid which fills the canal (5, 6). The cilia which eventually line the canal may play a role in the agitation of that fluid.

Recent studies from our laboratory initially appeared to confirm the classical view that the morphology of the amphibian neural canal depends upon the presence of appropriate interactions with surrounding tissues such as the underlying notochord (7). However, upon further examination of larger numbers of irradiated embryos which displayed notochord defects, a substantial number of cases were observed in which the neural canal morphology was completely normal, even in the absence of an intact notochord. Those findings prompted a thorough analysis of the neural canal in "notochord defective" embryos. The studies described in the present report were designed to ask 2 questions: 1) Is there any correlation between the morphology (e.g., size, shape) of the neural canal and the structural integrity (e.g., presence or absence) of the underlying notochord?; and 2) Are the alterations in axial structure morphology which are observed in irradiated embryos a unique feature of the irradiation treatment?

Both questions were answered by preparing for scanning electron microscope (SEM) observation sets of Xenopus embryos which were either ultraviolet irradiated or cold shocked in order to generate axial structure

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defects. The results reveal that the neural canal can develop normally in the absence of a notochord and that the morphological features of axial structures in irradiated embryos are grossly similar to those in notochord defective embryos produced by the cold shock treatment.

Methods and Materials

Xenopus laevis eggs were either ultraviolet irradiated (UV'd) at 254 nm shortly after fertilization (7) or cold shocked (CS'd) at 0-2° C within 40 min after artificial insemination (8). They were then permitted to develop to the tailbud stage and were fixed and prepared for SEM examination (9). Figure 1 summarizes the procedures employed to generate the data described in this report.

Each specimen was fractured (broken) at the level of the 5th-7th somite so that precise comparisons between embryos could be made. During the course of these studies, which extended over a period of approximately three years, the neural canal was observed in over 100 embryos which were either UV'd or CS'd. From a large number of photographs, typical views were selected and included in Figs. 2-4. Each specimen is labeled as either UV'd or CS'd. From a large number of photographs, typical views were selected and included in Figs. 2-4. Each specimen is labeled as either "UV" or "CS," according to the treatment employed to generate the notochord defect.

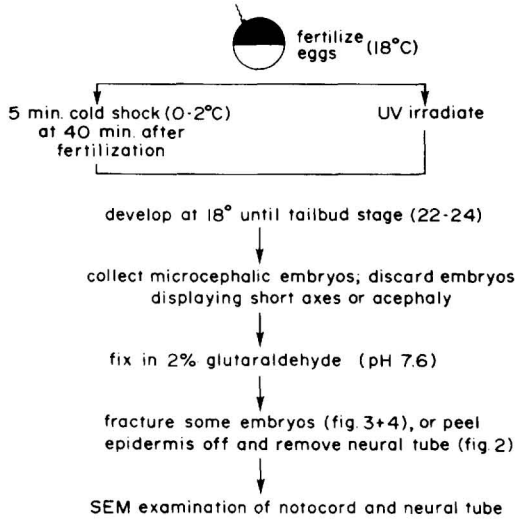

Figure 1. Manipulations which were employed to produce and examine with the SEM embryos which displayed defects in their notochord structure. Embryos previously scored (7) as 0 to +1 were studied.

Results and Discussion

Typically, either treatment (UV or CS) generated a wide spectrum of notochord defects. Some embryos were apparently unaffected while others were completely notochordless. Several embryos displayed a notochord which was diminished in width and/or interrupted along its length. Figure 2 contains photographs of CS'd embryos which exhibited the typical range of notochord morphologies. In those embryos which completely lacked a notochord the somites fused across the midline. Previous analysis revealed that those UV'd embryos which completely lacked a notochord had indeed never developed one. It did not develop early and regress later (7). A similar situation is presumed for the CS'd embryos displayed in Fig. 2. The similarity between the effects of CS shown in Fig. 2 and the effects of UV described earlier (9) is striking.

Detailed SEM analysis of the structure of the neural canal was carried out for both UV'd and CS'd embryos which displayed the types of notochord morphologies seen in Fig. 2.

The structure of the neural tube and the morphology of the canal in embryos with a normal sized notochord is shown in Fig. 3a and b. Also shown in that figure are photographs of embryos which contained a notochord of somewhat diminished size (Fig. 3c-e) and embryos which completely lack a notochord (Fig. 3f-h). In approximately 75% of the embryos which displayed a defective notochord the neural canal was normal in size. Its shape was also normal and, as will be mentioned below, it contained the cilia which line the canal.

Embryos which completely lacked a notochord exhibited a complete spectrum of neural canal morphologies.

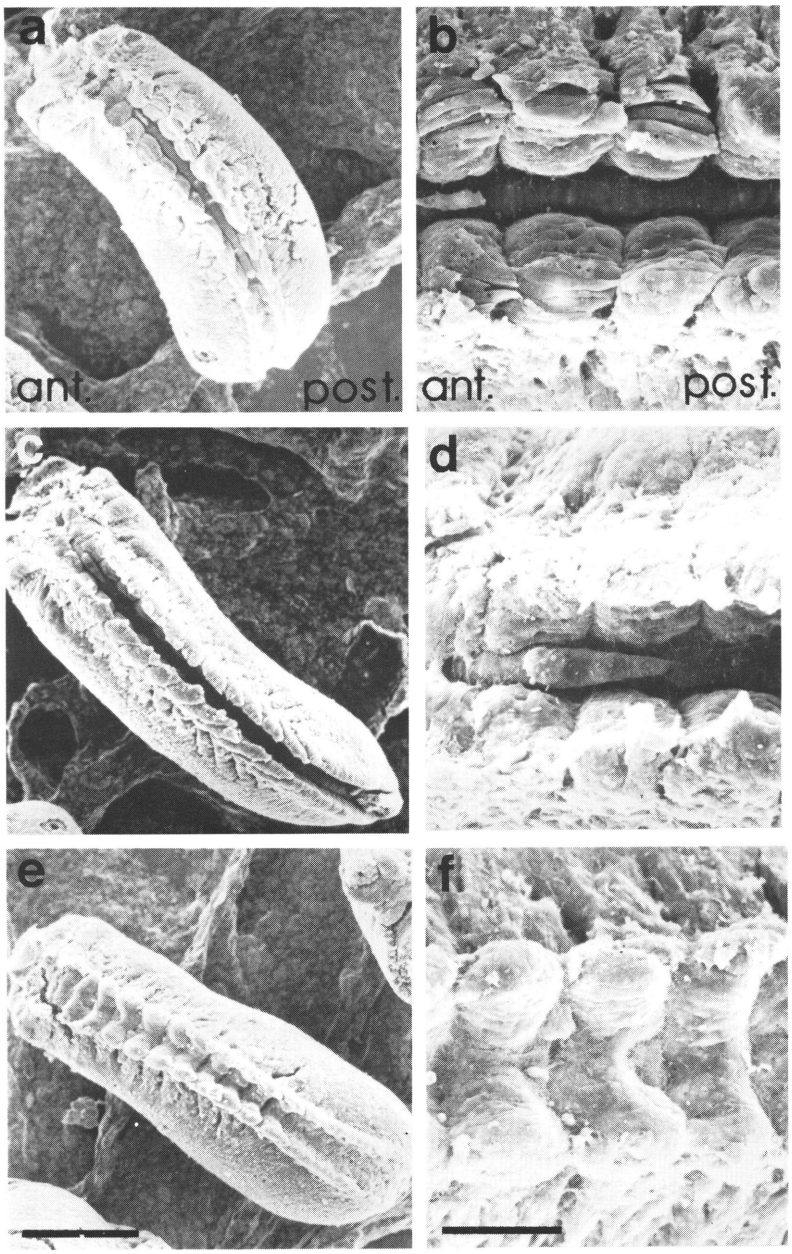

Figure 2. Typical notochord morphologies in CS'd embryos. The epidermis was peeled off and the neural tube removed to reveal the underlying somites and notochord. (a) contains a normal notochord which can be seen more easily at higher magnification (b). (c) displays a notochord which is somewhat reduced in diameter in the anterior region and is also severely constricted in one region (arrow). (d) shows a higher magnification view of the incomplete notochord. (e) completely lacks a notochord as the view in (f) clearly demonstrates. Additional photographs of UV'd embryos have been published previously (7). (a), (c), (e) = 44X; bar = 400 μm. (b), (d), (f) = 180 X; bar = 100 μm.

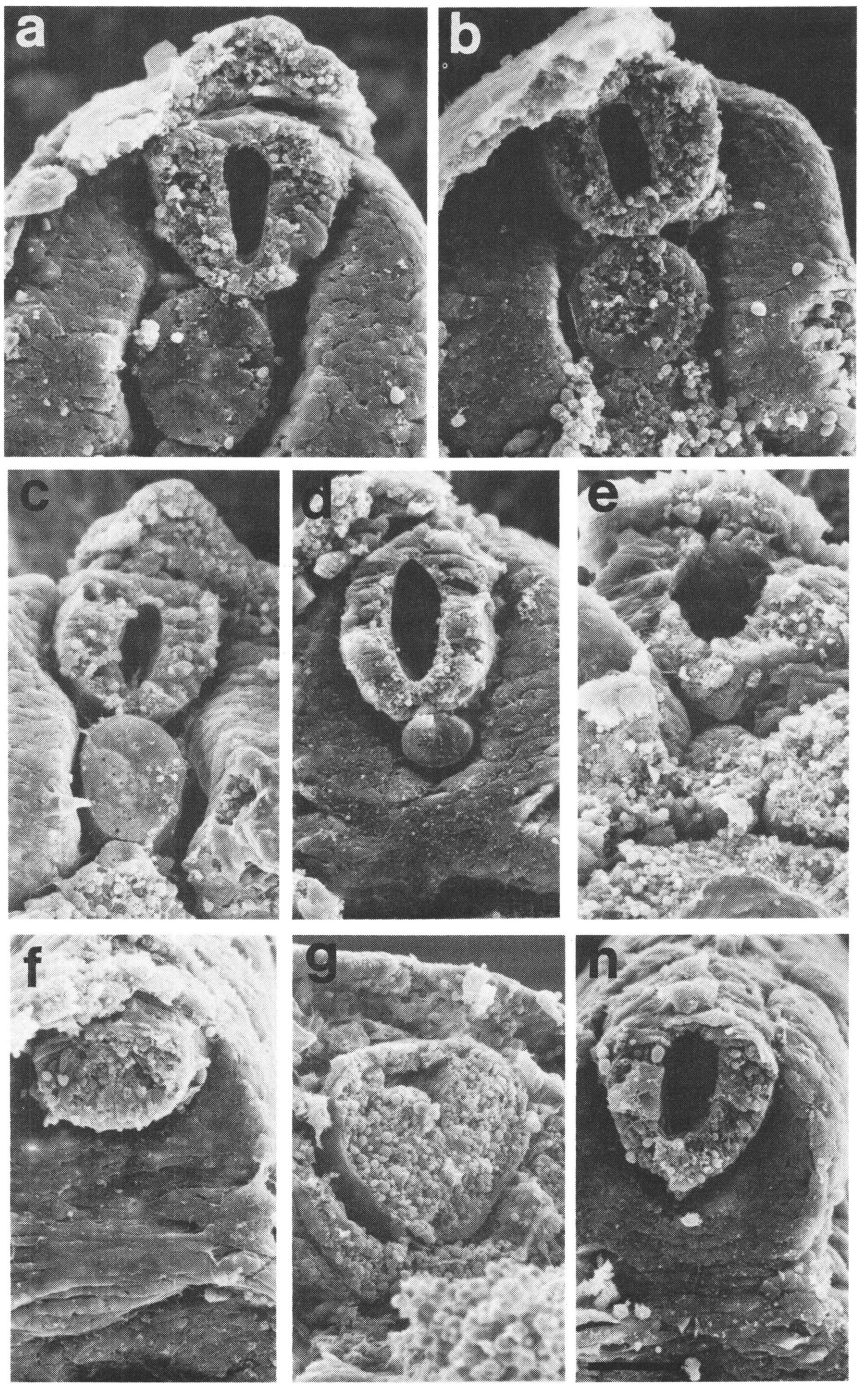

Figure 3. Views of broken sections of the level of the 5-7th somite of normal embryos (a, b). Embryos which displayed a notochord which was slightly (c), substantially (d), or severely (e) reduced in size are also shown. Notochordless embryos are shown in (f-h). nc = notochord; s = somite; fs = fused somite. All taken at 260X; bar = 50 μm.

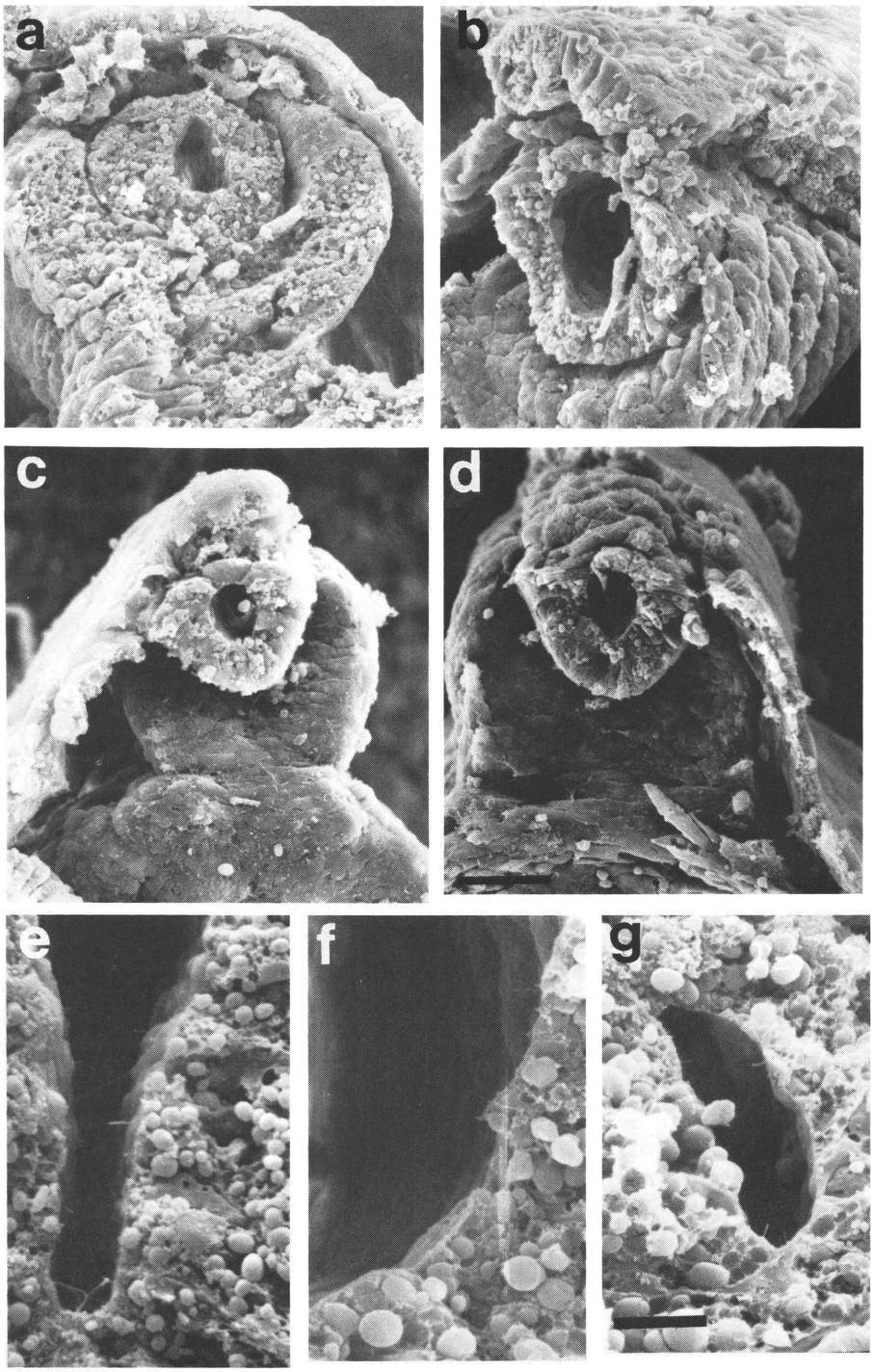

Figure 4. Additional photographs of notochordless embryos which displayed a normal neural canal (a-d). The neural canal of a normal embryo is lined with cilia (e) as is the canal of the neural tube of notochordless embryos (f, g). (a-d) = 260 X; bar = 50 µm, (e-g) = 1400 X; bar = 10 µm.

In some embryos the neural canal was completely obliterated (e.g., Fig. 3f), while in others (Fig. 3g) it was slightly diminished in size. Approximately 60% of the notochordless embryos, however, displayed a neural canal which was normally proportioned (e.g., Fig. 3b). Figure 4 contains additional photographs of broken sections of either UV'd or CS'd notochordless embryos. Clearly, the presence or absence of the neural canal is not related to the state of the notochord. Likewise, the neural canal size and shape do not depend upon the presence of the notochord. The neural canal of normal embryos contains cilia which line the canal (Fig. 4e). The canal of neural tubes which developed in notochordless embryos was also routinely observed to contain cilia (Figs. 4f and g).

From the observations included in this study, several conclusions can be drawn: 1) UV and CS produce similar effects on notochord development; 2) the presence of an intact fully developed notochord is not a prerequisite for the formation of the neural canal; and 3) the differentiation of the neural canal lining, including the elaboration of cilia, proceeds in the absence of the notochord.

The pattern for the formation of neural tube canal appears to be intrinsic to the cells which actually develop into the neural tube. One aspect of the morphogenesis of the neural tube--canal formation--does not, therefore, as previously suggested (see Introduction), depend upon tissue interactions with the underlying notochord.

Acknowledgements

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