JOURNAL OF MORPHOLOGY 219:131–142 (1994)

Furrowing Surface Contraction Wave Coincident With Primary Neural Induction in Amphibian Embryos

G. WAYNE BRODLAND, RICHARD GORDON, MICHAEL J. SCOTT, NATALIE K. BJÖRKLUND, KURT B. LUCHKA, C. CRISTOFRE MARTIN, CINDY MATUGA, MORTON GLOBUS, SWANI VETHAMANY-GLOBUS, AND DONGWEI SHU
Departments of Civil Engineering (G.W.B.,M.J.S.,D.S.) and Biology (G.W.B.,M.G.,S.V.-G.,C.M.), University of Waterloo, Waterloo, Ontario N2L 3G1 and Departments of Botany (R.G.,N.K.B.,C.C.M.), Radiology (R.G.), Physics (R.G.,K.B.L.), and Electrical and Computer Engineering (R.G.), University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada

ABSTRACT We predicted, and have now observed, a surface contraction wave in axolotl (*Ambystoma mexicanum*) embryos that appears to coincide temporally and spatially with primary neural induction and homoiogenetic induction, and with involution of the chordomesoderm. The wave starts from a focus anterior to the dorsal lip of the blastopore and spreads as an ellipse, until part of it encounters the rim of the blastopore and vanishes there. The remaining arc then continues over the dorsal hemisphere until it reforms an ellipse that decreases in size. About 9 to 12 hours after it begins, the wave vanishes at a focus diametrically opposite its point of origin. The wave involves both local contraction and furrowing in the monolayer ectoderm. To a good approximation, the hemispherical portion of the ectoderm traversed by the wave becomes neuroepithelium, while the ectoderm not transversed by the wave becomes epidermis. The wave might provide a mechanism to determine the time and location at which neuroepithelial differentiation occurs.

Primary neural induction in vertebrates has long been a model system for eukaryotic differentiation (Spemann and Mangold, '24; Spemann, '38). It has been divided into two steps: 1) induction of a small portion of the ectoderm by, presumably, the underlying mesoderm; 2) homoiogenetic induction of one ectoderm cell by the next, in a wave-like sequence that affects one hemisphere of the ectoderm (Nieuwkoop et al., '85). We proposed a model for mechanical causation of both of these phenomena based on the presumed mechanical instability of an observed cytoskeletal structure that we called the "cell state splitter" (Gordon and Brodland, '87, '89). In particular, we predicted that homoiogenetic induction of neuroepithelium would be caused by a wave of stretch-induced contraction of the microfilament rings that occur around the apical perimeter of each ectoderm cell. This model has been supported by our work (Björklund et al., '91; Martin and Gordon, '91) and that of others (Beloussov et al., '90).

Here we report our discovery of a furrowing surface contraction wave in axolotl ectoderm that appears to coincide temporally and spatially with homoiogenetic induction. Its initiation site is anterior to the dorsal lip of the blastopore and in the general location of the organizer (Spemann and Mangold, '24; Hama et al., '85). The part of the wave that might otherwise reach the ventral hemisphere and possibly propagate across it is suppressed when it encounters the edge of the blastopore. Thus, the wave does not traverse the ventral hemisphere.

Mechanical waves are frequently associated with morphogenetic processes (Kirshner et al., '80; Schroeder, '75; Jacobson, '78). This wave appears to coincide spatially and temporally with involution of the prospective chordomesoderm and with primary neural induction. This, and the observed differentiation of cells in the furrow of a surface contraction wave in the eye of *Drosophila* (Ready et al., '76), raise some interesting questions about the possible causal relationship between the wave reported here and primary neural induction.

MATERIALS AND METHODS

Axolotl (*Ambystoma mexicanum*) embryos used in our study were obtained directly from the colony of John B. Armstrong at the University of Ottawa or from our breeding colony in Winnipeg, a colony that was originally derived from the Ottawa colony.

Both naturally and artificially spawned embryos were used. Embryos were staged (Bordzilovskaya et al., '89), dejellied, and then photographed using video cameras in our two laboratories using Wild M8 and M420 macroscopes while being kept in 25% Holtfreter's medium at 20 ± 2°C. Video camera images were taped and subsequently digitized, or were digitized directly using custom time-lapse and focus-collage software running on Macintosh II computers equipped with QuickCapture DT2255 video frame capture boards (Data Translation, Marlboro, MA) and a motor controller board (Electronic Products, Mountain View, CA). Images were collected at 5-minute intervals. Embryos were illuminated using heat-filtered fiber-optic illuminators.

The surface contraction wave was elucidated by using Image (version 1.22) digital image processing software written by Wayne Rasband (U.S. National Institutes of Health, Bethesda, MD) to replay video frames sampled at 30-minute intervals, at approximately 12,000 times faster than real time. Difference images were produced by digitally subtracting one image of the embryo from a similar image collected a short time, typically 5 minutes, later. The result is an image in which moving morphological features are made lighter or darker than the surrounding area.

RESULTS

The wave, seen as a darkened band that travels over the surface of the embryo, begins at approximately stage 10¾ and ends before stage 13. Figures 1 through 4 together show the initiation, progression, and termination of the wave. Each sequence was taken from a different embryo. The first two columns of these figures show, respectively, video and corresponding difference images collected over a period of 5 hours. In the difference images, the wave appears as two parallel, adjacent bands, one light and one dark. The third column of Figures 1 through 3 shows corresponding line drawings indicating the wavefront positions. Computer animation of time-lapse images facilitated accurate location of the wavefront. The first two columns of Figure 4 show video and difference images of the dorsal surface of the embryo, while the third and fourth columns show corresponding ventral views. The wave traverses approximately a hemisphere. It is not possible to observe the entire propagation of the wave from any one fixed viewing position, because substantially less than a hemisphere of the embryo is visible in detail from any one viewing position.

Four embryos were viewed simultaneously from their dorsal and ventral sides, using two cameras. Four other embryos were viewed simultaneously from dorsal and two lateral surfaces, using angled, front surface mirrors, and three were viewed from top, side, and bottom, using silvered prisms. In all cases, there was no evidence of a wave on the ventral surface of these embryos either before, during, or following the occurrence of the wave on the dorsal surface. Although pigment is absent on the ventral surface, it is our opinion that the wave would have been made visible by shadowing from its furrowing component.

Multiple views of the wave provided by data collected from numerous embryos made it possible to reconstruct and verify accurately the complete geometry of the wave as it propagated over the embryo surface, and to establish its relationship to both the blastopore and subsequently formed neural plate. Figure 5a–c are composites of the wavefront positions shown in Figures 1 through 3, respectively, oriented so that the embryo midplane is vertical. Figure 6 is a reconstructed side view of typical wave propagation, and shows the relative viewing angles of Figures 1 through 4. Slight inconsistencies in matching of the views were noted during reconstruction of the wave. These suggest that there are minor variations in the wave shape, and that the time required for the wave to complete its travel may range from 9 to 12 hours.

From the reconstructions, it is apparent that the wave is symmetrical about the midplane of the embryo and that it spreads as an ellipse (Fig. 1) from a focus anterior to the dorsal lip of the blastopore. The part of the wave that propagates into the rim of the blastopore is suppressed, while the remaining wavefront propagates as an open arc (Fig. 2) with its center traveling anteriorly at