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How to observe surface contraction waves on axolotl embryos

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Contraction waves have been observed on the surface of the axolotl (*Ambystoma mexicanum*) embryo (Björklund and Gordon, 1993, 1994; Brodland *et al.*, 1994; Gordon *et al.*, 1994), and waves of comparable speed have been observed in *Drosophila* eye imaginal disc (Suzuki, 1974; Ready *et al.*, 1976), *Paramecium* (Iftode *et al.*, 1989), and sunflower (Hernández and Green, 1993).

In the axolotl, the ectoderm contraction wave leaves the neural plate in its wake. It starts about 45 degrees above the dorsal lip of the blastopore and consists of a single furrow, about 0.1 mm wide and deep on the 2 mm spherical embryo, traveling about 3 μm/min. At the dorsal lip it breaks into an arc. The ends travel faster than the middle, so the arc straightens out, switches from convex to concave, closes on itself, and "self-annihilates" (typical of waves in active media: Luchka and Gordon, 1996). The whole process lasts precisely the duration of gastrulation and is followed by the appearance of the neural plate.

The keys to observing a contraction wave are: 1) use of embryos of intermediate pigmentation (as in axolotl), so that a contraction of the surface produces a change in contrast; 2) a time lapse speedup of 10,000 to 20,000 times; 3) an awareness that, when observing a wave in time lapse, it typically vanishes from sight when the moving image is paused. Waves on a spherical surface have an annoying habit of going past the viewing equator, and the center of gravity of the embryo shifts as invagination proceeds, rotating the whole region under observation. It is sometimes necessary to piece together the full wave trajectory from multiple views and embryos.

For viewing ultraslow waves (Jaffe, 1995), embryos are dejellied and staged. They are mounted in a Petri dish with part of the bottom replaced with a large microscope slide coverslip. Embryos are spaced from one another with autoclaved 1 mm glass beads to allow access to O_2 and loose constraint. A peristaltic pump provides fresh 25% Holtfreter's (Frost *et al.*, 1989) at 300 ml/h, sweeping away dust, mold spores, and bacteria, and prevents both adherence of the vitelline membrane to the dish and changes in media concentration due to evaporation. Medium depth is kept at 5 mm by an open overflow tube. Water immersion objectives give higher magnification from the top.

Embryos are videotaped (with two standard VHS VCRs and high quality 8 h tapes) dorsally and ventrally, simultaneously using two black/white video cameras mounted on two stereomicroscopes (Hara, 1970). Lighting is via heat filtered fiber optic illuminators and temperature is monitored by a miniature thermistor placed near the embryos. (Wide temperature variation and hot spots are created by standard microscope lighting.) We keep the entire apparatus in a thermostatically air conditioned room at 20°C so that the embryo develops normally and the observer is comfortable over the extended period of observation. After videotaping embryos are kept and monitored for normal development.

Digitization can be done with any good digitization software. We recommend the free NIH Image program by Wayne Rasband available by anonymous FTP at zippy.nimh.nih.gov for Macintosh and PC computers. First round digitization rates should be at 1 frame per 15 min giving a 9000x speedup when shown at 15 frames/sec. The capability to digitize and play back at full video frame rates is available in modern desktop computers.

Differentiation waves subdivide the embryo into tissues, and thus their trajectories tend to be ever smaller. The smallest contraction wave we have observed correlates with differentiation of the eye anlagen. Thus it is necessary to adjust the digitization interval to the wave being studied. Video tape, with its 15 frames/sec, allows one to do this after the fact. One 8 h tape stores 432,000 images to select from. Digitizing the whole tape would require about 100 GB.

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References

BJÖRKLUND, N.K. and GORDON, R. . (1993). Nuclear state splitting: a working model for the mechanochemical coupling of differentiation waves to master genes. *Russian J. Dev. Biol. 24:* 79-95.

BJÖRKLUND, N.K. and GORDON, R. (1994). Surface contraction and expansion waves correlated with differentiation in axolotl embryos. I. Prolegomenon and differentiation during the plunge through the blastopore, as shown by the fate map. *Computers Chem. 18:* 333-345.

BRODLAND, G.W., GORDON, R., SCOTT, M.J., BJÖRKLUND, N.K., LUCHKA, K.B., MARTIN, C.C., MATUGA, C., GLOBUS, M., VETHAMANY-GLOBUS, S. and SHU, D. (1994). Furrowing surface contraction wave coincident with primary neural induction in amphibian embryos. *J. Morphol. 219:* 131-142.

FROST, S.K., NEFF, A.W. and MALACINSKI, G.M. (1989). Primary and long-term culture of axolotl cells. In *Developmental Biology of the Axolotl* (Eds. J.B. Armstrong and G.M. Malacinski). Oxford University Press, New York, pp. 244-254.

GORDON, R. and BRODLAND, G.W. (1987). The cytoskeletal mechanics of brain morphogenesis: cell state splitters cause primary neural induction. *Cell Biophys. 11:* 177-238.

GORDON, R., BJÖRKLUND, N.K. and NIEUWKOOP, P.D. (1994). Dialogue on embryonic induction and differentiation waves. *Int. Rev. Cytol. 150:* 373-420.

HARA, K. (1970). "Double camera" time-lapse micro-cinematography; simultaneous filming of both poles of the amphibian egg. *Mikroskopie 26:* 181-184.

HERNÁNDEZ, L.F. and GREEN, P.B. (1993). Transductions for the expression of structural pattern: analysis in sunflower. *Plant Cell 5:* 1725-1738.

IFTODE, F., COHEN, J., RUIZ, F., RUEDA, A.T., CHEN-SHAN, L., ADOUTTE, A. and BEISSON, J. (1989). Development of surface pattern during division in *Paramecium* I. Mapping of duplication and reorganization of cortical cytoskeletal structures in the wild type. *Development 105:* 191-211.

JAFFE, L.F. (1995). Calcium waves and development. *Ciba Found. Symp. 188:* 4-17.

LUCHKA, K.B. and GORDON, R. (1996). Solitary mechanical waves during early embryonic development. In *A Mathematical Approach to Biology* (Ed. R.V. Jean). Steven Wuerz Publishing, Winnipeg. (In press).

READY, D.F., HANSON, T.E. and BENZER, S. (1976). Development of the *Drosophila* retina, a neurocrystalline lattice. *Dev. Biol. 53:* 217-240.

SUZUKI, D.T. (1974). Behavior in *Drosophila melanogaster*: a geneticist's view. *Can. J. Genet. Cytol. 16:* 713-735.