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Surface contraction and expansion waves correlated with differentiation in axolotl embryos. II. In contrast to urodeles, the anuran *Xenopus laevis* does not show furrowing surface contraction waves

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ABSTRACT We have observed a number of contraction waves traversing the axolotl *(Ambystoma mexicanum)* embryo (a urodelan amphibian) from the midblastula transition up to at least neural tube closure, and wished to learn if similar "differentiation waves" appear on the popular laboratory anuran amphibian, the South African clawed toad, *Xenopus laevis*. Time lapse video microscopy showed that no contraction waves are visible on the surface of *Xenopus* from gastrulation through neurulation. It is possible that cell intercalations in the double-layered ectoderm of the *Xenopus* embryo are homologous to the surface waves in the single layered ectoderm of the axolotl embryo. In any case, a simple, universal correspondence between surface waves and induction phenomena and differentiation does not exist.

KEY WORDS: *Xenopus, no surface contraction waves*

We predicted the existence of contraction waves on the surface of eukaryotic embryos that could correspond to and initiate steps of differentiation (Gordon and Brodland, 1987). The first such wave found by us was a furrow 0.1 mm wide and deep that traverses the dorsal hemisphere of the 2 mm diameter axolotl (*Ambystoma mexicanum*) embryo's ectoderm at a speed about 3 µm/min, leaving in its wake the neural plate (Brodland *et al.*, 1994). Its duration corresponds to axolotl gastrulation. These results were followed by the discovery of numerous expansion and contraction waves on the surface of the axolotl embryo that correlate with steps of differentiation between the midblastula transition and neural tube closure (Gordon *et al.*, 1994; Gordon and Björklund, 1996) and correspond with the shape of Vogt's (1929) fate map (Björklund and Gordon, 1994). We formulated a working model for the relationship between these physical waves and the triggering of master genes for differentiation (Björklund and Gordon, 1993) and gave ultrastructural details of the cytoskeletal apparatus, the "cell state splitter", that may carry these waves from cell to cell (Martin and Gordon, 1996). In our 1994 "Dialogue..." (Gordon *et al.*, 1994), Nieuwkoop suggested that the ectodermal contraction wave may be correlated with the neural induction process, since it seems to follow more or less the spatial extension of the spreading induction process during gastrulation (Eyal-Giladi, 1954). Jaffe (1995) has used the ectoderm contraction wave to propose a new class of "ultraslow" waves characterized by mechanochemical wave propagation, presumably with a calcium wave component. (As the expansion waves have yet to be studied closely, and their relationship to classical epiboly movements determined, we will not consider them further here.)

The surface contraction waves in the axolotl seem to be borne by cells forming part of the original surface coat of the egg and embryo (Holtfreter, 1943). The double-layered *Xenopus laevis* embryo (Nieuwkoop and Florschütz, 1950; Keller, 1975, 1976) may, according to the first author, give us some further insight into the apparent correlation, since in *Xenopus* neural induction seems to occur exclusively in the inner, sensorial layer of the ectodermal moiety, not visibly affecting the epithelial layer before the initiation of neural plate formation. Thus in *Xenopus* we have the opportunity to observe separation of ectodermal from inductive properties. *Xenopus* is an extremal amphibian test of our model for embryogenesis via differentiation waves, because it is least like urodeles in its development (Nieuwkoop and Florschütz, 1950; Nieuwkoop, 1996). It is the most commonly used experimental amphibian and has been widely studied for biochemical analysis and gene expression. Therefore it seemed critical to attempt to generalize the wave phenomenon to *Xenopus*.

It thus came as quite a surprise to us when we could not find homologous waves on *Xenopus* embryos. Here we report on our methods and the possible meanings of this negative result.

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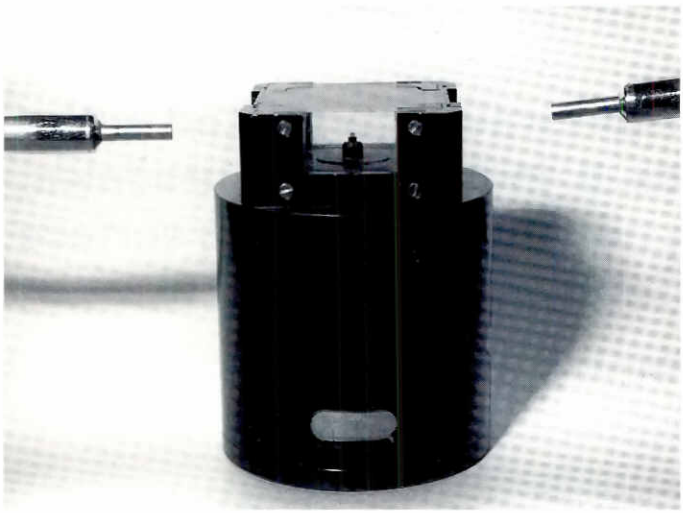

Fig. 1. Photograph of chamber for viewing embryos on a rotatable pedestal.

Results

In the animal view, cleavage of surface cells was observed over the entire animal hemisphere of the *Xenopus* embryo. Epibolic extension was clearly visible in animal and lateral views, while gastrulation could be followed fully in vegetal view. The formation of bottle cells usually started at the dorsal side with irregular, more or less incidental isolated bottle cell formation at the circumference of the future yolk plug before or during initial dorsal blastoporal groove formation. Subsequently the still very shallow dorsal blastoporal groove extended rapidly along the lateral and ventral sides of the future yolk plug before any invagination, started at the dorsal side, took place (Nieuwkoop and Florschütz, 1950; Nieuwkoop and Koster, 1995). According to Keller and Danilchik (1988) mesodermal involution is completed in *Xenopus* at stage 10.5.

In the double-layered *Xenopus* embryo, with a pigmented outer epithelial layer (one cell thick) and a pluricellular inner sensorial layer, mesoderm formation is restricted to the inner layer (internal marginal zone), whereas embryonic pharynx endoderm formation occurs in the outer epithelial layer (Sudarwati and Nieuwkoop, 1971). Involution of the internal marginal zone apparently starts very early, even before dorsal blastopore groove formation, and takes place independently of archenteron invagination (Nieuwkoop and Florschütz, 1950; Nieuwkoop and Koster, 1995). We could clearly observe the early extension of the involuted mesoderm underneath the double-layered ectoderm in the form of a slight bulging of the egg surface. There are rather clear indications of a cleavage wave moving from the equator towards the animal pole in the ectodermal cells overlying the advancing boundary of the involuted mesoderm.

No surface contraction waves were observed in the outer epithelial layer of the ectoderm. The latter remained a smooth cell layer until the initiation of neural plate formation.

Like the mesoderm, the neural plate develops in the inner sensorial layer of the ectoderm at the end of the gastrulation process (Nieuwkoop and Florschütz, 1950; Keller and Danilchik, 1988) by means of vertical induction by the underlying mesoderm (Nieuwkoop and Koster, 1995).

Neural tube formation is initiated by a firm attachment of the thickening neural plate (by means of dorsal convergence and cranial caudal extension) and the overlying epithelial layer (Nieuwkoop and Florschütz, 1950; Keller and Danilchik, 1988). No wave-like surface contraction phenomena were observed in the outer epithelial layer before or during the attachment of the two layers.

Neural tube formation followed the general amphibian pattern of brain formation but showed tube formation over the full length of the neural plate including the neurenteric canal, connecting the neural tube with the ectodermal proctodeum (Nieuwkoop and Florschütz, 1950). Eye vesicle evagination in the anterolateral corners of the neural plate started to be clearly visible before anterior neural tube closure.

Discussion

No systematic waves were observable under our imaging conditions on the surface of *Xenopus laevis* embryos from stages 8 to 10+ through to stage 25 (Nieuwkoop and Faber, 1994). Those movements that were seen concerned smooth gastrulation movements and "shadows" of the blastocoele as it changed size, visible due to the slight translucency of the ectoderm, and similarly visible mesoderm movements underneath the ectoderm. A few irregular contractions were seen that may be similar to, though smaller in extent than, the surface "puckerings" observed in the urodele *Taricha torosa* (A.G. Jacobson in Segel, 1984). At later stages (approximately stage 20), irregular local movements would seem attributable to twitchings of muscle tissue formed out of the underlying mesoderm.

As we see it, we can interpret this negative result in the following ways:

A. *Xenopus* does not use surface contraction waves for early development and does not make use of them in neural induction. Surface contraction waves and inductive interactions thus seem to be independent phenomena. The surface contraction waves, observed in axolotl embryos, must be accompanying phenomena, the significance of which is not yet understood.

B. *Xenopus* uses differentiation waves for neural and other inductions, but they have:

- a different form;
- and/or a different location, below the surface; but nothing is known about their existence.

In terms of the theory that surface contraction waves cause differentiation, we can conclude:

A. The theory is wrong, and should be discarded.

B. The theory may be right, but requires some sort of generalization to encompass *Xenopus.*

The surface layer of the axolotl is able to support visible expansion and contraction phenomena because of the presence of an apical cell state splitter structure (Gordon and Brodland, 1987; Martin and Gordon, 1996). *Xenopus* may have a different type of cell state splitter that results in expansion and contraction phenomena that are visible only as radial and mediolateral intercalations respectively, as described by Keller *et al.* (1992). The spatial propagation of these intercalations may be homologous to differentiation wave propagation in the axolotl.

The concept of the cell state splitter seems only to be applicable to the outer cell layer of the embryo and its epithelial derivatives that retain a portion of the original egg surface coat. In *Xenopus* it may only be applicable from an early neurula stage, when neural tube formation begins. At that stage a firm attachment of the epithelial and sensorial layers begins. The concept may therefore not be applicable to internal organ formation, including neural anlage formation in the inner, sensorial layer.

We must take care not to make the definition of differentiation waves too wide. The nice thing about the surface contraction waves in axolotls is that they are not correlated with invagination movements, and therefore represent a separate phenomenon. They may be related to the spatial extension of inductions. The open question is which causes the other. Are these waves a general phenomenon, characteristic of neural induction in the amphibians? The cell state splitter clearly occurs in the ectodermal sheet of axolotls, and may cause the waves. But this would seem to be, then, a property stemming from the original cortical layer of the egg. In *Xenopus* we have the opportunity to segregate the processes occurring in the outer layer from the inductive phenomena occurring in the inner layer. The lack of surface contraction waves in the outer layer in *Xenopus* indicates that contraction waves are not a universal phenomenon. The mesoderm involution and subsequent neural induction in *Xenopus* must be based on a different phenomenon than the surface contraction wave phenomena in axolotls. The vertical, heterogenetic induction of the neural anlage by the underlying mesoderm can, nevertheless, be the same phenomenon in both animals.

We also saw no contraction waves in the neural plate nor during neural tube closure, such as we described in the axolotl (Gordon *et al.*, 1994). There may be cell state splitters or at least contractile microfilaments involved in the outer layer lying over the neural plate in *Xenopus,* at least from about neural plate stage 13, from which time these two layers become firmly attached to each other. From this time the two layers act as one, and the situation in *Xenopus* may be comparable to that of the axolotl.

Materials and Methods

Xenopus eggs were obtained by stripping females injected with gonadotropin hormone into the dorsal lymph sacs 48 hours earlier. They were artificially fertilized with a sperm suspension made from macerated testes.

The gelatinous outer membranes were carefully dissolved up to the vitelline membrane with the pH 7.8 adjusted 2% cysteine solution in tap water, either at the one cell stage for marking the dorsal side (opposite the sperm entry point) with a Nile blue sulfate crystal, or at the advanced blastulae stage (stage 8 to 9) (Nieuwkoop and Faber, 1994), in case a batch of embryos showed a clear dorsoventral pigment pattern (dorsal lighter, ventral darker).

Time lapse video pictures were taken first from the animal side, then from the vegetal side (with an inverted dissecting microscope) and later in a side view with a horizontally directed video camera. Animal view pictures were taken with a Wild MS stereomicroscope and two fiber optics light sources; the vegetal view pictures with an inverted Zeiss Axtovert 35 microscope and a cold ring illumination set and the lateral view pictures with a 40 mm luminar, an illuminator and an extendible tube. The pictures were taken with a Sony color video camera, type DXC 151P, and a laser disc recorder, type TVR 6000.

The eggs were kept either in tap water or in a 5% ficol solution in order to minimize unpredictable turning of the embryo inside the vitelline membrane during the gastrulation and neurulation processes. The eggs were placed in a specially constructed 5 cm square cuvette (Hengst and Reitsma, 1976) with four side windows 2 cm deep and a turnable central column with a shallow depression in the upper surface fitting the curvature of the egg (Fig. 1). The cuvette was covered by a large thin cover slip in order to prevent evaporation. The cuvette contained about 50 ml fluid with enough oxygen for normal development up to tailbud stages. The magnification was so adjusted that the embryo (1.3-1.5 mm diameter) covered half to the entire field of the color video camera, allowing maximal observations of surface phenomena.

The time lapse was varied from a 1 min interval down to 30 and 20 sec intervals and lasted from late blastula (stage 8 to 9) or early gastrula (stage 10 to 10+) until the closed neural tube stage, approximately stage 20 (or stage 25 or older when taken overnight). Single frames were recorded on a video disk unit. Playback of a PAL video tape copy was at the standard rate of 25 full frames/sec, giving a speedup of 1500 to 750 and 600 for the 1 min, 30 sec and 20 sec intervals, respectively. While this speedup is substantially less than we used for the axolotl (Brodland *et al.*, 1994), gastrulation and neurulation are much faster in *Xenopus*.

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