The Cortex of *Xenopus laevis* Embryos: Regional Differences in Composition and Biological Activity

(protein composition/induction/blastula/differentiation/cortical implantation/polyacrylamide gel electrophoresis)

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Communicated by Robert Briggs, September 16, 1971

ABSTRACT The cortex of fertilized *Xenopus laevis* eggs undergoes regional changes in its composition and morphogenetic activity during the first three divisions. Gray crescent and animal pole cortex of stage 1 (1-cell) and stage 4 (8-cell) embryos each contain a characteristic array of proteins. Implantation of pieces of cortex into the blastocoel of midblastula embryos was used to assay their inducive capacity. The gray crescent cortex was shown to be the only region that had significant axis-inducing activity. This activity increased from stage 1 to stage 4.

Shortly after fertilization, the dorsal side of the *Xenopus laevis* embryo is marked by the appearance of the gray crescent. Vital staining experiments, with several amphibian species, have shown that the gray crescent demarcates the future site of the dorsal lip of the blastopore, and thus the position of the axial organs. Since centrifugation of fertilized amphibian eggs at low speed, which rearranges most cytoplasmic components, does not affect the position of the axial organs (1), it is likely that the information necessary for the formation of these organs is located in that part of the egg resistant to the centrifugation—the cortex. This idea was supported by the experiments of Curtis (2–4). Using the difficult method of cortical grafting, he showed that grafted bits of cortex had different effects depending upon their origin, the age of the host, and the position on the host embryo. He found that the gray crescent cortex of fertilized eggs (stage 1) or 8-cell embryos (stage 4) would induce the formation of a secondary axis when grafted to the ventral margin of a stage-1 embryo (5). Besides this indication that the gray crescent cortex was sufficient to cause axial differentiation, the failure to gastrulate stage-1 eggs, whose gray crescent cortex had been excised, indicates that the influence of this part of the cortex is necessary for normal axial differentiation. Curtis also found that grafts of the gray crescent cortex from stage-1 or stage-4 embryos to stage-4 embryos were ineffective in inducing secondary axes. This suggests that the egg is only fleetingly susceptible to the influences of the grafted gray crescent cortex.

Hebard and Herold (6) have found that the cortex becomes thickened, particularly in the gray crescent and animal pole regions, shortly after fertilization. They did not find any evidence for qualitative differences in the different regions of the cortex in their electron microscopic study.

The present study began as an attempt to repeat the work of Curtis, but his methods were soon abandoned because of difficulty in performing cortical grafting. A simpler bioassay for the regional morphogenetic activities of the cortex was devised in order to facilitate further work on the nature and mode of action of axis-inducing factors in the egg of *X. laevis*, and temporal and regional differences in cortex composition during the first three divisions were analyzed by acrylamide gel electrophoresis. In this report, we show that some of the regional and temporal differences in morphogenetic activity of the cortex reported by Curtis can be confirmed by a blastocoel implant bioassay, and that changes in cortical protein composition accompany these changes in morphogenetic activity.

MATERIALS AND METHODS

Adult *X. laevis* were obtained from J. Cooke, Cockeysville, Md., and induced to spawn with chorionic gonadotropin. For cortical implantations, fertilized eggs of the appropriate stages were manually dejellied and treated with a solution of 2% cysteine•HCl-0.4% papain (pH 7.0) for 90 sec at 20°C. The purpose of this treatment was to weaken the vitelline membrane so that it could later be removed manually. The eggs were then washed 10 times in 100% Steinberg’s solution (7) (pH 7.4), transferred to a Petri dish, which contained a bottom coat of 1.5% agar in 10% Steinberg’s solution (pH 6.3), containing 100% Steinberg’s solution (pH 6.3), and freed of their vitelline membranes with forceps. The desired piece of cortex was excised with fine tungsten needles, freed from any adhering cytoplasm, and laid aside in the dish while the host was prepared. A midblastula embryo was freed of its vitelline membrane and a small hole was made in the roof of the blastocoel with a ball-tip rod. The rod was then used to maneuver the previously prepared piece of cortex into the blastocoel. The host usually healed within 1 hr, and the solution was gradually exchanged for 10% Steinberg’s solution (pH 6.3) before the onset of gastrulation. Sham-operated embryos were treated in identical fashion, except that no cortex was implanted. The development of the hosts was observed at intervals over the next 3–4 days, and the animals were then fixed in Bouin’s fluid, sectioned at 10 μm transversely to the primary axis, and stained with hema-toxulin-eosin.

Cortex was prepared for electrophoresis by first dejelling and removing the vitelline membrane manually, then excising the desired portions of the cortex with tungsten needles and transferring them to a centrifuge tube held at −60°C. After a sufficient number of samples was obtained, they were thawed and centrifuged at 10,000 × g for 10 min. The supernatants (soluble fraction) were concentrated and electro-
phoresed by the method of Davis (8). The pellets (insoluble fraction) were prepared, without heating, and electrophoresed on 6.9% acrylamide gels by the method of Kiehn and Holland (9). The gels were fixed overnight in 20% sulfosalicylic acid and stained for protein with 0.25% Coomassie Brilliant Blue (10).

**RESULTS**

The use of cysteine-papain to free embryos of their vitelline membranes, and rearing of control demembranated embryos in the same medium as the experimental animals, did not affect their development. The method of cortical implantation was found to be simple and reliable. 75 implants and 17 sham operations were performed, of which only two implants failed to live long enough to provide results. Normal development, or the development of a small lump at the site of the wound, in the host was seen in most implants of material other than gray crescent and shams. In 15 cases, small tail-like structures were seen at the site of the wound. Some were clearly cases of spina bifida, whereas others had only muscle in them. These are classed with the normal embryos for the statistical analysis below. Large secondary axes were at least 70% of the length of the primary axis and contained a neural tube and notochord (Fig. 1). The results of the various implants are shown in Table 1. Gray crescent implants led to large secondary axis formation in significantly more cases than did the other types of operations ($X^2 = 35.3759, P < 0.001$). Stage-4 gray crescent induced significantly more large secondary axes than did stage-4 animal pole cortex ($X^2 = 20.0573, P < 0.001$), or shams ($X^2 = 16.3327, P < 0.001$). The ability of stage-1 gray crescent cortex to induce large secondary axes approaches, but does not attain, statistical significance, as compared with the ability of stage-1 animal pole cortex ($X^2 = 2.0101$). Stage-1 and stage-4 animal pole cortices did not differ significantly from the shams in their ability to induce large axis.

The soluble fraction of the cortical samples did not contain any detectable bands upon gel electrophoresis at the concentrations used. The electrophoretic patterns obtained from 80 samples each of stage-1 and stage-4 animal pole and gray crescent cortex (insoluble fraction) are shown in Fig. 2. A unique pattern of insoluble proteins was found in each type of
The results of the cortical implantation experiments support the conclusion of Curtis that the cortex contains information that causes the formation of axial structures. Furthermore, this activity is localized in the gray crescent, and increases in potency from stage 1 to stage 4. Other regions of the cortex that were tested, from both stage-1 and stage-4 embryos, do not have this property. The results differ from those of Curtis in that the midblastula embryo responds to the influences of the gray crescent cortex, whereas Curtis found that by stage 4, the embryo could not be induced to form a secondary axis with a stage-4 gray crescent grafted to the ventral margin. Stage-4 embryos may be temporarily refractory to the influences of added cortex, or the cortex may be more able to exert its influence from within the blastocoel. An alternative explanation is that the influences of the cortex from the interior of the blastula bear no relation to the influences it has in situ, for although the cortex has been found to be sufficient to induce secondary axes in this study, it has not been shown that the process is identical to the normal one.

If the cortex is indeed necessary for inducing axial structures in *Xenopus*, a further problem arises. Since the mesoderm of this species arises from an internal position (11, 12), and presumably contains none of the cortex, is the mesoderm induced to form notochord and muscle by components of the presumptive ectoderm? There is no precedent for this.

We thank Dr. M. S. Steinberg for his advice and encouragement during the course of this study. This study was supported by an allocation from the Eugene Higgins Trust Fund and by a grant to the Biology Department from Merck, Sharp, and Dohme Research Laboratories.