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Nature and Origin of Patterns of Changes in Cell Shape in Embryos

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Spatial patterns of the future elongation of cells exist in the early embryo. In the newt, such a pattern of changes of cell shape contributes to the formation of the neural plate. Regardless of where neural plate cells are transplanted, they change shape as prescribed by the pattern. Embryonic induction has a role in establishing this pattern.

Key words: cell shape, pattern, neural plate shaping, simulation

INTRODUCTION

A pattern of future changes in cell shape is established in the neural ectoderm of the late gastrula of the newt. Cells of the forming neural plate elongate to a degree that is prescribed by the original positions of the cells in this pattern, even when they are transplanted elsewhere on the embryo. The normal expression of this "shrinkage pattern"* is one important driving force in shaping the neural plate†. The role of these changes in cell shape in shaping the neural plate was deduced from observation, experiments, and a computer simulation (1).

Embryonic induction, and possibly predetermination, appear to be responsible for establishing the shrinkage pattern in the neural plate.

The existence of such patterns implies that cells become individually instructed as to how long they are to make themselves. Mechanisms must exist that direct the organized activities of actin filaments and microtubules in reshaping each cell, and cells may have some means of determining when they are long enough.

METHODS

Experiments and observations were made on embryos of the California newt, Taricha torosa. Staging of the embryos was in accordance with the staged series of Twitty and Bodenstein (2). The main stages discussed here are stage 13, a late gastrula

*Called "shrinkage pattern" because the cells shrink their visible apical surfaces while elongating perpendicular to the surface. The volume of the cells remains constant (1).

†The second force of displacement is caused by the elongation of the notochord and notochordal region of the neural plate in the anteroposterior direction (1).

stage with a small yolk plug, and stage 14, an open neural plate stage just before neural tube closure commences (Fig. 1).

Methods for handling and operating on these embryos are described in detail elsewhere (3). Transplantation and rotation of tissues was done under a dissecting microscope using iridectomy scissors for microsurgery and hair loops for moving the tissues. Methods for time-lapse cinematography are described in (4).

RESULTS

Because cells move in the developing embryo, it is necessary to map cell positions over time before one can measure the shape changes in individual cells. (Many papers that discuss changes of cell shape have not dealt with mapped systems.)

The positions of the cells in the forming neural plate of the California newt were mapped by Burnside and Jacobson (4) using time-lapse cinematography (Fig. 2). Between stages 13 and 15, the forming neural plate is greatly distorted, and some cells are displaced nearly half the length of the embryo at rates of 5–95 μm/hr at 17°C. The changes of positions of cells mapped in 3 different embryos were so similar that they were hardly distinguishable from one another, implying that the process is tightly programmed.

Cells were followed at the intersections of grid lines of a superimposed coordinate grid on the stage 13 neural ectoderm. By stage 15 the grid was greatly distorted (Fig. 2). Local changes in area were measured and an inverse correlation between change in cell height and change in local area was found. This correlation was shown to be true in greater detail by Burnside (5,6), who measured heights, apical surfaces, and volumes of groups of 20 cells at stage 13, and again of the same cell groups, as displaced, at

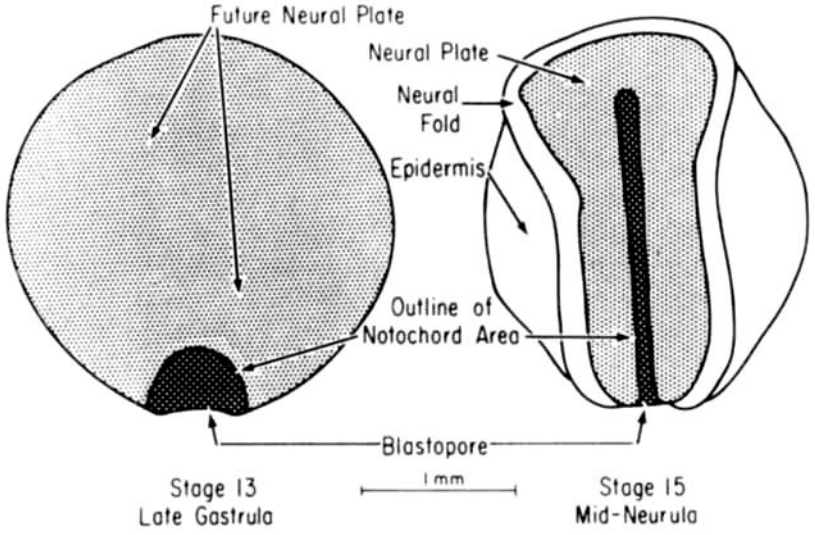

Fig. 1. At stage 13, the future neural plate of the newt occupies the entire dorsal hemisphere of the embryo. By stage 15, the neural plate has contracted and assumed a keyhole shape. These dorsal views are from projected movie frames of the same embryo. The notochordal region of the neural plate overlies the notochord cells. The neural plate is 1 cell thick. The cells are columnar. Their apical ends form the plate surface and their basal ends abut on the underlying mesoderm. [From (1), with permission of J. Exp. Zool. © The Wistar Institute Press.]

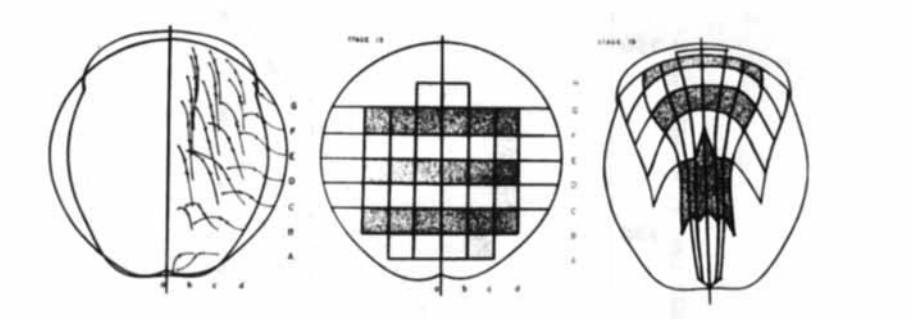

Fig. 2. Left: Pathways of cell displacement traced from time-lapse films. Cells followed were at the origins of the arrows at stage 13, and at the points at stage 15. The solid circular outline represents the contour of the embryo at stage 13, the other solid outline is the contour at stage 15. Cells on the midline moved directly anterior (toward top of figure). Middle: A coordinate grid superimposed on a stage 13 embryo. Cells at the intersections of the grid were traced by time-lapse cinematography. Right: Coordinate grid as distorted by cell movements by stage 15. [Adapted from (4), with permission of Dev. Biol. © Academic Press.]

stage 15. The change in apical surface area of the cells closely matched the measured local area change, and there was no significant increase in cell volumes; if anything, there was a slight decrease. Change in cell heights correlated inversely with apical surface decrease.

To define the pattern of changes of cell shape in the neural plate, we have constructed an empirical map of cell height changes using data from Burnside and Jacobson (4), augmented with many measurements from sectioned material, and from analyses of additional time-lapse movies (1).

The heights of cells at 24 lateral points on each side, and 9 points along the midline, of the neural plate were measured at stage 13, and the same points, as displaced, were measured again at stage 15. Heights of cells between measured points were interpolated from observed cell pathways. We normalized beginning cell heights to 100 μm, and the change in cell height was made proportionate. Cells were placed in 1 of 9 groups, according to their relative height changes (Fig. 3). The dynamics of the cell shape change depends on the ratio of the final height of a cell to its initial height, and is thus unaffected by this normalization (1).

The index numbers 1–9 indicate different amounts of height change between stages 13 and 15 (Fig. 3). This shrinkage pattern can be mapped on a stage 13 embryo (Fig. 4). Cells that will elongate most, hence shrink their apical surfaces most, are located along the anterior margin of the neural plate and in a tongue of cells that extends down the midline to the anterior tip of the notochordal area. This tongue of greatly shrinking cells is important to shrink the cells away anteriorly as the notochordal region elongates.

Burnside (5,6) has demonstrated a contractile ring of microfilaments in a purse-string arrangement just beneath the apical surface of the neural plate cells. When a ring contracts, thereby shrinking a cell surface, the neighboring cells are pulled toward the contracting cell because the rings of filaments are interconnected among the cells through the desmosomes. While the apical surface contracts, the cell elongates. Burnside (5) has shown that microtubules are oriented parallel to the long axes of the elongating cells, and discusses mechanisms of elongation.

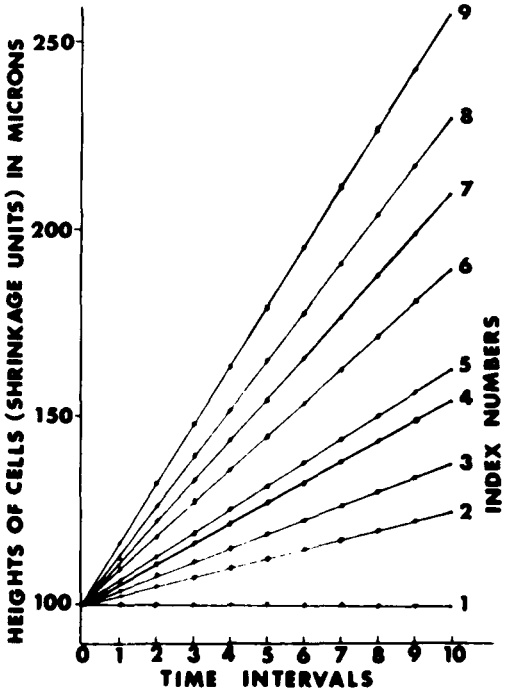

Fig. 3. Change in heights of different groups (index numbers) of neural ectoderm cells from stage 13 (time 0) to stage 15 (time 10). Grouping and normalization of data is explained in text. Intermediate heights are obtained by linear interpolation. These groups (index numbers) are mapped onto the neural plate in Fig. 4. [From (1), with permission of J. Exp. Zool. © The Wistar Institute Press.]

Evidence for the Early Existence of a Fixed Pattern of Future Cell Elongation

We will now give several lines of evidence that the shrinkage pattern is already established at stage 13.

In the neural plate isolated without notochord, the shrinkage pattern alone is played out. The pattern of shrinkage gets painted onto the surface of the neural plate. Regions where shrinkage is greatest are darker because egg pigment is concentrated in the smaller apical ends of the cells. Also, there are more apical ends of cells per unit of plate surface area in regions that shrink more. A neural plate isolated at stage 13 and photographed when control embryos had reached stage 15 is shown in Fig. 5. The more darkly pigmented areas, representing cells that most shrink their apical surfaces and most elongate, compares well with the empirical map of potential cell elongation and shrinkage (Fig. 4).

A second line of evidence that the change of shape of prospective neural plate cells is already programmed at stage 13 comes from observations of Holtfreter (7,8), repeated by Burnside (9), that show that neural plate cells isolated individually at these early stages will continue to elongate in culture.

A third line of evidence comes from reciprocal transplantations at stage 13. When pieces of prospective neural plate and epidermis are exchanged, the transplanted epidermis flattens while surrounded by shrinking neural plate, and the transplanted neural plate cells shrink in a flattening epidermis (1).

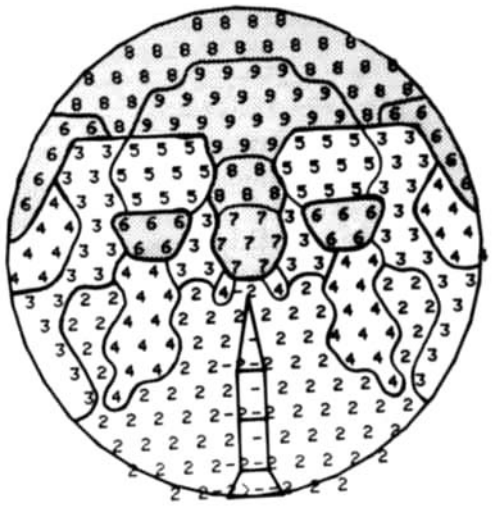

Fig. 4. A map of prospective changes of cell shape or shrinkage pattern on the neural ectoderm of a stage 13 embryo. The numbers are the index numbers illustrated in Fig. 3. Higher numbers represent groups of cells that elongate more and that thus shrink their apical surfaces more. Areas that shrink most are shaded. The posterior or blastoporal end of the embryo is at the bottom of the figure. The neural plate cells that overlie notochord are outlined near the blastopore, and units preceded by a minus sign are on the perimeter of the notochordal area. The outline of the embryo is 2.4 mm in diameter. The map was empirically derived as explained in the text. [From (1), with permission of J. Exp. Zool. © The Wistar Institute Press.]

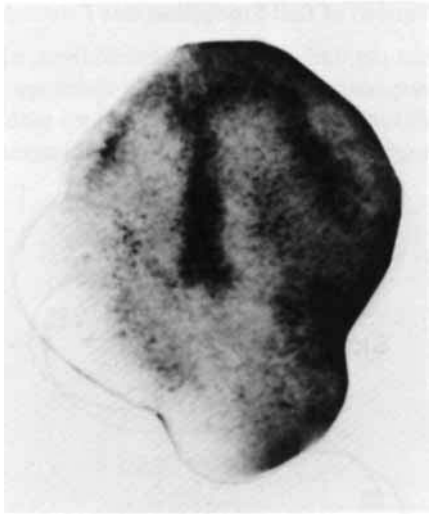

Fig. 5. A prospective neural plate with no other tissue was explanted onto neutral agar at stage 13 and held flat by a piece of cover slip with beaded edges. The pigment concentrated in those cells that shrank (elongated) the most. The photo shows the explant at a time when control embryos had reached stage 15. The concentrated pigment reveals a pattern of cell shrinkage that is only slightly distorted from the shrinkage map shown for the stage 13 embryo in Fig. 4. The magnification of this photograph is to the same scale as the map in Fig. 4. [From (1), with permission of J. Exp. Zool. © The Wistar Institute Press.]

That the shrinkage pattern is programmed in detail is strongly suggested by transplanting anterior neural plate into posterior neural plate regions (Fig. 6). The anterior neural plate cells normally elongate much more (and shrink their apical surfaces much more) than posterior neural plate cells. The expected cell height changes of the anterior plate cells compared to the posterior plate cells can be deduced from the area changes of the distorted grids (Fig. 2). We would expect a 40% difference between the cell heights of the 2 regions. The implanted anterior cells measured from 33–39% taller than their local posterior neighbors of the implant site (Fig. 7), so the data suggests there is detailed local programming by stage 13. Results were similar in 4 cases.

How the Patterned Changes in Cell Shape Help Drive the Distortion of the Neural Plate

Cells are capable of many complex behaviors. One cannot eliminate numerous possible cell activities just by manipulating or observing groups of living cells. For this and other reasons we have modeled the shape conversion of the neural plate from stage 13 to stage 15 using a computer simulation (1).

We explain the details of our computer simulation, mathematical analysis, and experiments and observations elsewhere (1). To briefly summarize, 2 forces drive the shape change of the neural plate: 1) the programmed pattern of changes of cell shape shown on our empirical map (Fig. 4); and 2) a change in position of the neural plate cells overlying the notochord (Figs. 1, 4). The latter force – a convergence of cells to the midline and extension along the midline – is similar to movements seen in formation of the primitive streak of bird embryos.

The computer simulation plays out the pattern of changes of cell shape and notochordal area transformations, and produces a distortion of the shape of the neural plate essentially identical to that seen in a normal embryo (Fig. 8).

When and How Does the Pattern of Cell Elongation Get Established?

One might expect that the underlying chordamesoderm, which induces the neural plate, may also be responsible for imposing the shrinkage pattern.

The neural plate is about half induced by interactions with underlying tissues, and about half predetermined by events that occur during oogenesis and at and around

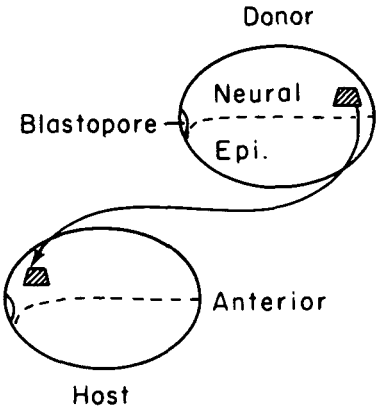

Fig. 6. Diagram illustrating the transplantation of a group of neural plate cells into the posterior neural plate region at stage 13. The cells in the anterior plate normally elongate much more than posterior plate cells (see map, Fig. 4).

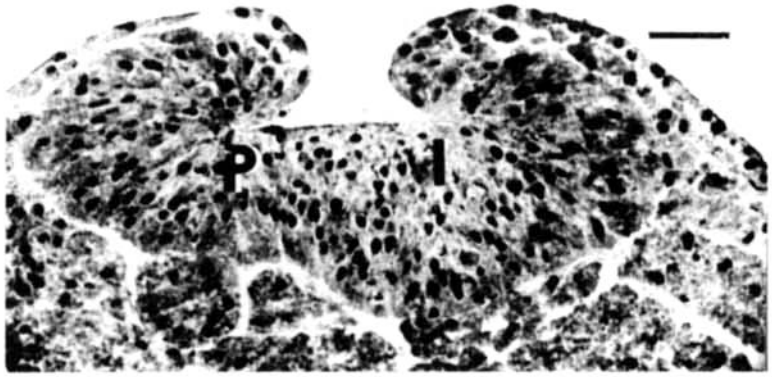

Fig. 7. Section through the implanted (I) anterior neural plate cells in the posterior plate (P) at stage 15. The anterior cells have elongated their normal amount in this foreign site, indicating that the elongation pattern is programmed by stage 13. Bar = 0.1 mm.

the time of fertilization (10). The whole ectoderm is well along toward becoming neural plate before the inductors localize and complete the process.

At stage 12 (a gastrula stage with a large yolk plug), the inductor tissues have just underlain the neural ectoderm. We severed the neural ectoderm at its future boundary with the epidermis (the equator of the embryo), and rotated only the neural plate by 90°. We reasoned that if induction from stage 12 on established the shrinkage pattern, then a neural plate would form in its normal (unrotated) axis since the inductor tissues were undisturbed by the operation. If a pattern already existed in the neural ectoderm at stage 12, but was normally augmented by continued induction, then rotation with respect to the inductor might produce chaotic results. The latter is what occurs. Time-lapse movies of such operated embryos showed highly abnormal movements in the prospective neural plate. No normal plate or neural tube ever formed. In 20 cases the embryos distorted themselves into grotesque shapes (Fig. 9) which, when sectioned, proved to have no brain or spinal cord tissue at all (1). Rather than elongating, the prospective neural cells stratified. The absence of neural tissue is quite remarkable in an embryo in which the inductor and responding tissues remain in contact.

These results suggest that both induction and predetermination have a role in establishing the cell shrinkage pattern in the neural plate, but other possibilities are not eliminated.

When the neural plate is rotated at stages 12+, 13, and 14, induction has had a greater chance before the rotation to augment the effects of predetermination, and embryos with rotated neural plates are obtained.

A Pattern of Change of Cell Shape in the Epidermis: Positioning of Nose, Lens, and Ear by Induction

The nose, lens, and inner ear begin as placodes in the epidermis. The positions of these placodes constitutes a pattern of differential cell elongation in the epidermis. The induction history of these 3 organs, and the role of induction in positioning these organs,

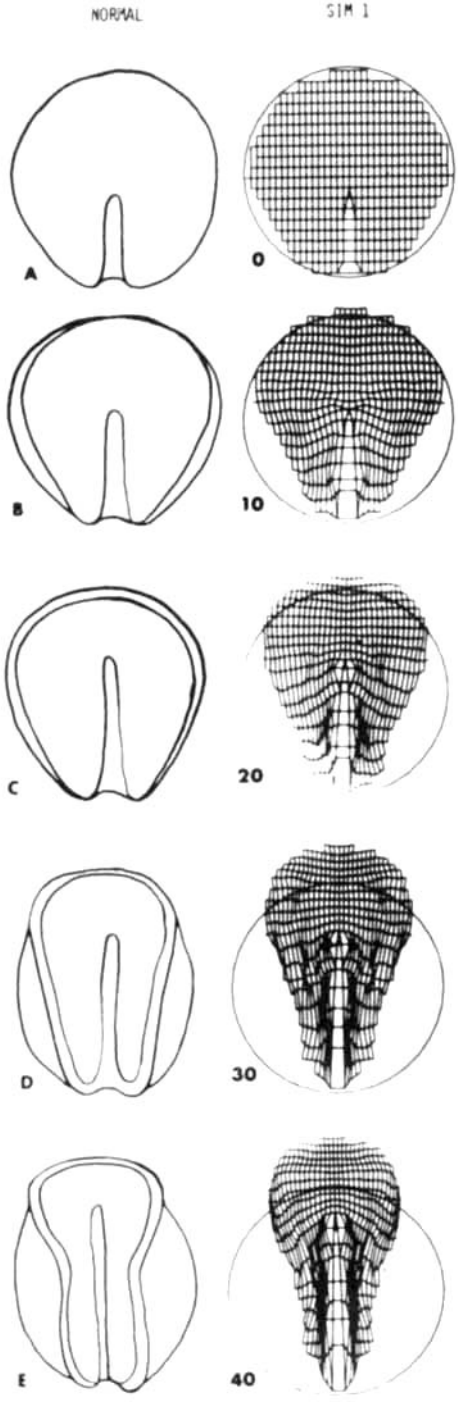

Fig. 8. Normal development of the neural plate from stage 13 (top) to stage 15 (bottom) is indicated (A–E) by outline drawings from projected frames of a time-lapse movie. A computer simulation of neural plate development (right) is indicated with photographs from a computer graphics terminal. The simulation proceeded in 40 steps with every 10 steps shown here. Normal development is shown at equivalent stages. The simulation is driven by 2 forces, the shrinkage pattern and the displacement of cells in the notochordal region. [Adapted from (1), with permission of J. Exp. Zool. © The Wistar Institute Press.]

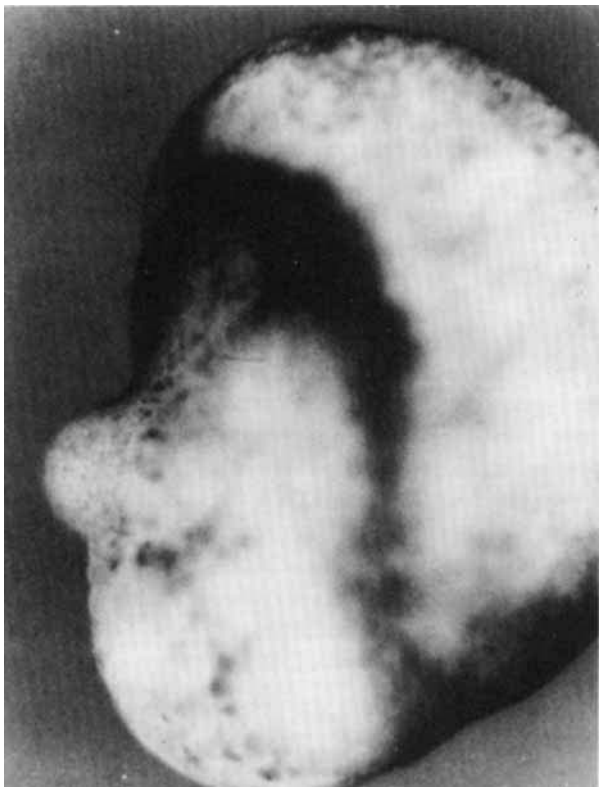

Fig. 9. External view of an embryo whose neural hemisphere was rotated 90° at gastrula stage 12. The picture was taken when control embryos had reached tail bud stage 21. This grotesque embryo completely lacks a brain and spinal cord. X 33. [From (1) with permission of J. Exp. Zool. © The Wistar Institute Press.]

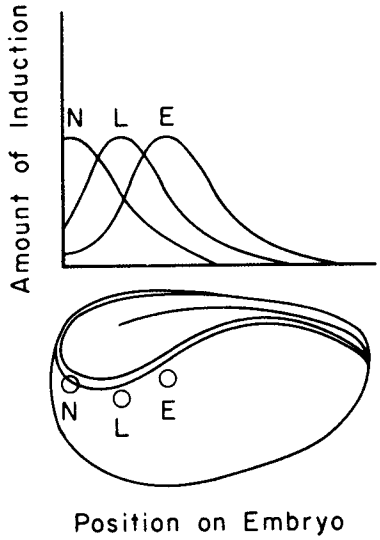

Fig. 10. The ability to induce 3 organs (N, nose; L, lens; E, inner ear) is widely, but unequally distributed in the embryo. The peak of induction for each organ fixes its position (10–13). Induction capability is plotted against position in the neurula of the newt. This is the stage when most induction of these organs occurs.

have been explored in detail (10–13). The positions of specific inductor tissues determine the positions of cells that will elongate to form the 3 sorts of placodes (Fig. 10). As their positions are changed by morphogenetic movements, a sequence of inductor tissues is brought into proximity to the responding tissue areas. These studies demonstrated that some organs are positioned by the cumulative effects of inductive interactions with a sequence of inductor tissues through a fairly long time period. The first visible indications that induction has occurred are the changes in cell shape in the placodes.

CONCLUSIONS

Programmed patterns of future cell elongation are established in embryos, probably by induction and predetermination. The expression of these patterned changes of cell shape contributes to morphogenetic movements and the shaping of organs.

In the prospective neural plate, the pattern of future elongation of cells is established before the plate begins to form. Thus, organized activities of actin filaments and microtubules, which are destined later to drive the shape changes, are prescribed long before they are executed. Programming is probably preserved through more than one cell cycle in some cases since plate cells may divide during the period studied (1).

The challenge now is to find the mechanisms by which the shrinkage pattern is established, and to find how these instructions are executed.

ACKNOWLEDGMENTS

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