Artificial Boundaries Between Epidermis and Neural Plate Produce Neural Folds in the Axolotl

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The process of neurulation comprises raising neural folds and subsequently fusing them into an unbroken epidermis and a hollow neural tube. During or shortly after neurulation, cells migrate out of the epithelium to form the neural crest. The formation of neural folds and neural crest cells occurs at a boundary between two distinct tissue types—the neural plate and the epidermis—and we are currently examining the relationship between this boundary and the buckling of the epithelium into the neural folds.

A number of mechanisms for raising neural folds have been proposed (Karfunkel, 1974; Gordon, 1985, for reviews). These mechanisms are not mutually exclusive, and any or all of them may be acting during neurulation. We divide these mechanisms into three types based on the role they propose for the boundary during fold formation: 1) the boundary has no direct relationship to fold formation, 2) the boundary acts as a line at which buckling can occur, 3) cell behavior is organized at the boundary.

Mechanisms that invoke perpendicular pressure generated by columnarization of the mesoderm and/or expansion of the extracellular matrix below the folds (Morriss and Solursh, 1978; Schroeder, 1970) do not require the presence of a boundary. Anywhere underlying materials exert this pressure, the epithelium should buckle. Likewise, the model that invokes Poisson buckling of an elastic sheet when stretched along a line (Jacobson, 1981; Jacobson and Tam, 1982) does not require that the sheet have a boundary at the point of folding, but does propose that intercalation of cells at a boundary generates the stretching.

In mechanisms that invoke "pushing" by the epidermis (Jacobson and Jacobson, 1973; Brun and Garson, 1983), or "pulling" by the neural plate (Burnside, 1973; Odell, *et al.*, 1981), forces are generated in cells throughout one or both of these tissues. Forces generated in a cell can be transmitted to neighboring cells as stress at apical junctions. Basal portions of neural plate cells at the boundary lie below the junctions with the adjacent epidermal cells, and should be relatively inactive. Because the juxtaposition of different cell types at the boundary represents an abrupt change in resistance to these stresses, buckling is likely to occur here. Cells at the boundary do not change behavior, but continue to generate forces and transmit stresses produced elsewhere, while the boundary focuses and redirects these forces so that the epithelium buckles.

A model that emphasizes the motility of cells in epithelia, called the cortical tractor model (Jacobson, *et al.*, 1985; Jacobson, *et al.*, 1986) implies that a boundary provides not only a focus at which the epithelium can buckle, but also alters and organizes the behavior of cells at the boundary. This model proposes that the cyclical movement of cortical cytoplasm (tractoring) that can power locomotion in motile mesenchymal cells is also present, but restricted, in epithelial cells. Because neural plate and epidermal cells tractor on one another differently than they tractor on cells of their own kind, a boundary can organize these movements to produce columnarization of the cells and a bending moment that helps to form the fold. This model proposes that the lateral and basal surfaces, as well as the apical ends of the cells, are active in raising the fold.

Urodele embryos make excellent subjects for the study of neurulation because they have distinct neural folds and a monolayered epithelium. An added advantage of the axolotl, *Ambystoma mexicanum* (Shaw), is the availability of albino mutants whose eggs and embryos contain no maternally produced pigment. Our current research creates new boundaries in axolotl embryos by juxtaposing pieces of tissue (neural plate and epidermis) that would not normally participate in fold formation. We do this by removing a piece of

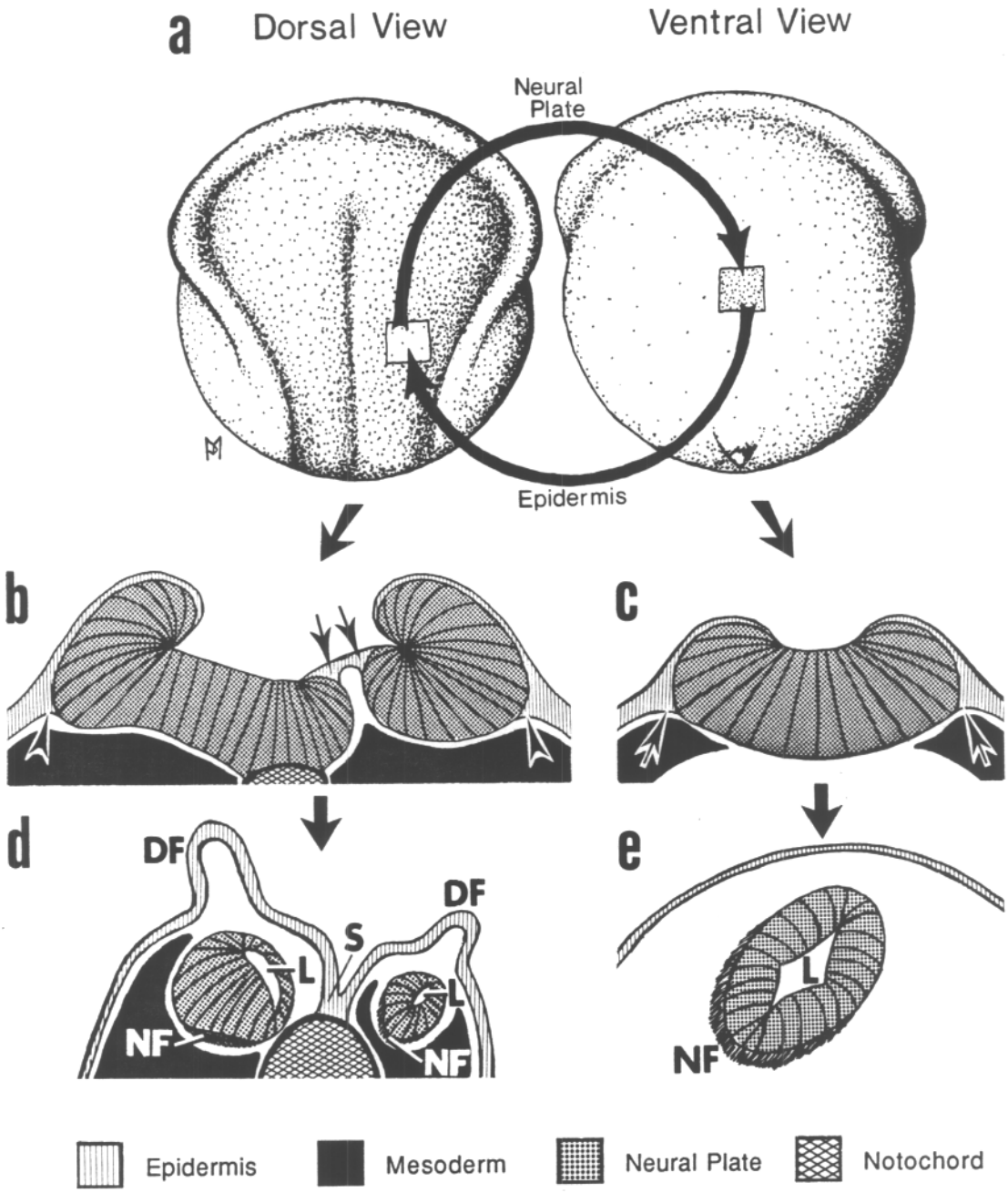

FIG. 1. Diagrams illustrating epidermis-to-neural plate, and neural plate-to-epidermis transplantations and their results. Diagrams are not to scale. DF, dorsal fin; L, lumen; NF, nerve fibers; S, Epidermal septum. a. Surface view of operations used in experiments. b. Cross section through epidermis-to-neural plate transplant 16 hours after operation. Note tabs of epidermis extending beneath the neural plate on either side of epidermal implant (arrows), and their counterparts in the natural folds (arrowheads). c. Cross section through neural plate-to-epidermis transplant 16 hours after operation. Note tabs of epidermis extending beneath neural plate (arrows). d. Cross section through epidermis-to-neural plate transplant at hatching. e. Cross section through neural plate-to-epidermis transplant at hatching.

neural plate (excluding both the notoplate [neural plate above the notochord], and the neural fold) from a donor, and transplanting it into the ventral epidermis of a host (embryos at stages 14-16, Schreckenberg and Jacobson, 1975). We also perform the reciprocal experiment by transplanting epidermis into the neural plate (Fig. 1a). As they develop, embryos are examined for the presence of folds, and, at various points of development, they are fixed (Karnovsky's fluid, Karnovsky, 1965; or Kahle's fluid, Jones, 1966), and processed for examination by light or scanning electron microscopy.

Folds form at each newly created boundary, although the new folds that form when epidermis is transplanted to the neural plate are small (Figs. 1b, c). When neural plate is transplanted into epidermis, the mesodermal cells below the fold show no evidence of columnarization. The newly created folds behave like natural folds—they roll across the neural plate and eventually fuse. When neural plate is transplanted into epidermis, the resulting "neural tube" appears as a nodule of neural tissue with a lumen in the center, and nerve fibers around the periphery (Fig. 1e). Transplanting epidermis into the neural plate causes the neural tissue (and the dorsal fin) to bifurcate in the region of the graft, and sections reveal that the neural tissue branches into two hollow tubes (Fig. 1d). Tabs of epidermal cells that are seen beneath the basal regions of neural plate cells along the natural boundary, also appear at the new boundaries (Fig. 1b, c). When epidermis is transplanted into the neural plate, these tabs of epidermal cells persist as a septum separating the two branches of the neural tube (Fig. 1d).

Transplanting tissue from a pigmented donor into an albino host allows us to trace the dark cells originating in the transplant. Using this system, we can see that transplanted neural plate tissue does not contribute to the epidermis, nor does transplanted epidermis contribute to the neural plate. The presence of pigment granules also allows us to detect donor tissue that is transplanted homotypically as a control. This tissue integrates into the host tissue with no folds at the boundary. We often encounter cells in the mesenchyme of the host that originated from a neural plate transplant. While these could be mesodermal cells that adhered to the transplant, it is probable that some of them are neural crest cells produced at the new boundary. Melanocytes (which are considered to be neural crest derivatives) are detected if the albino hosts whose epidermis contains transplants of pigmented neural plate are allowed to develop to hatching larvae. Further research into the origin of neural crest cells is in progress.

It appears from our research that the neural plate/epidermis boundary specifies the site of a neural fold, and that the basal portions of cells at this boundary are actively involved in raising the fold. In our experiments, folds form at any boundary between the neural plate and epidermis. Since folds form in ectopic locations on the host embryo, it appears that neural induction of the type described by Nieuwkoop *et al.* (1952) is not necessary for fold formation after the neural plate and the epidermis become different tissues. Our observations and those of others (Boerema, 1929) also indicate that fold formation is to a large extent independent of underlying tissues, especially since folds will continue to rise when isolated from underlying materials (Jacobson and Gordon, 1976). It is likely that a fold forms at this boundary partly because differences between the neural plate and epidermis redirect stresses. Apical constriction of neural plate cells through the contraction of bundles of apical microfilaments (Burnside, 1973; Odell, *et al.*, 1981), or as the result of cortical tractoring (Jacobson, *et al.*, 1986) is likely to generate these stresses, but because the epidermis seems to be under tension throughout neurulation (Karfunkel, 1974; Jacobson and Gordon; 1976), "pushing" by the epidermis is unlikely. The boundary, however, appears to be more than simply a line at which the epithelium can buckle. The basal ends of the neural plate cells and the epidermal cells adhere tightly to one another, as anyone who attempts to separate the fold into epidermis and neural plate soon discovers. The tabs of epidermis seen in both normal and experimental embryos indicate that either the basal ends of the neural plate cells are pulling on the basal ends of the epidermis or that the basal ends of the epidermis are able to move along the basal ends of the neural plate cells. In either case, this is evidence for activity in the basal regions of cells at the boundary.

When epidermis is transplanted into the neural plate, the newly created boundaries lie very close to one another. In these embryos, an epidermal septum results apparently because the basal activity in these two boundaries act synergistically, pulling the epidermis (or allowing it to crawl) between the two forming tubes.

Our work implies that the neural fold (and perhaps the neural crest) forms because of conditions at any boundary between the neural plate and the epidermis, and that cell interactions and behaviors of the types described in the cortical tractor model occur at this boundary. Further studies on the cellular and subcellular levels are necessary, however, to determine how the boundary is affecting cell behavior.

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