

EMBRYOGENESIS

Ultrastructural Analysis of the Cell State Splitter in Ectoderm Cells Differentiating to Neural Plate and Epidermis during Gastrulation in Embryos of the Axolotl *Ambystoma mexicanum*

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Abstract—We have confirmed and elaborated the ultrastructure of the “cell state splitter” (CSS), a “morphogenetic organelle” that we think may be responsible for the differentiation of ectoderm cells into neural plate and epidermis, in the embryonic cells of the axolotl, *Ambystoma mexicanum* (a urodele amphibian). The CSS consists of an apical microfilament (MF) ring, a lower intermediate filament (IF) ring, and a subtending, annular, nearly coplanar mat of apical microtubules (MT), all within 1 μm of the apical end of the 50–100 μm tall cells. The IF ring is attached to the membrane via desmosomes, and the MF ring and apical MTs may be so attached. The CSS is constructed in ectoderm cells between early and mid-gastrula, coincident with: (1) high concentrations of ribosomes that are membrane associated and apically localized, (2) an increase in global tension of the embryo, and (3) a furrowing wave of surface contraction that passes over the presumptive neural plate during gastrulation (“ectoderm contraction wave”). We have quantified the spatial distribution and concentrations of the contributing cytoskeletal elements in these cells at early gastrula and early neural plate stages. We further show evidence (1) suggesting that desmosomes may act as microtubule organizing centers (MTOCs) and (2) the mechanical support of microtubules by intermediate filaments.

Key words: neural induction, neurulation, cytoskeleton, differentiation, urodele

INTRODUCTION

It has been suggested by computer simulation and direct observations (Jacobson and Gordon, 1976; Gordon and Jacobson, 1978) that formation of the neural plate into a neural tube occurs as a function of apical contraction and elongation (perpendicular to the neural plate) of neural plate cells and the elongation of either the notochord or the overlying neural plate cells (called “notoplate”—Gordon, 1985) in the anterior-posterior direction (however, cf. Schoenwolf, 1992). Burnside (1971, 1973), in the California newt *Taricha torosa*, and Schroeder (1973), in the African clawed frog *Xenopus laevis*, attributed neural plate cell constriction and elongation to the contraction of an apical microfilament ring and to paraxial microtubule (MT) elongation, respectively. Further, it was observed (Burnside, 1971, 1973) and is apparent in the micrographs (Schroeder, 1973) that there is additionally an apical mat of microtubules that is coplanar with the microfilament ring. Burnside (1973) suggested that these “apical microtubules,” as we call them, stabilize the apical end of the cell. A similar microtubule/microfilament structure was noted (Burnside, 1971) in ventral ectoderm cells during gastrulation. However, Burnside stated that the

microfilament ring in these cells was replaced by larger tonofilaments after gastrulation. Gordon and Brodland (1987) have proposed that the mechanically opposing forces of microfilament ring contraction and apical microtubule growth via polymerization establish a state of cellular mechanical instability during gastrulation. They further suggested that this ultrastructural combination be termed the “cell state splitter” (CSS), and that it is responsible for differentiation of the ectoderm into neural plate (= “neuroepithelium” or “neural epithelium”) and epidermis. The embryological concept of competence (Nieuwkoop *et al.*, 1985) is explained by assuming that the opposing forces are initially in a mechanically metastable balance. If this theory is correct, then the CSS may be regarded as a “morphogenetic organelle” with a standing similar to the spindle apparatus. One consequence of this model was the prediction of a wave of contraction of the microfilament rings (Gordon and Brodland, 1987), which has been confirmed (Brodland *et al.*, 1993; Gordon, 1993; Björklund and Gordon, 1993), where we called it the “gastrula wave” or “G-wave,” and similar waves at other stages of differentiation have been observed (Gordon *et al.*, 1994). The latter necessitated a clearer

