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Cell shape change and invagination of the cephalic furrow involves reorganization of F-actin

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ABSTRACT

Invagination of epithelial sheets to form furrows is a fundamental morphogenetic movement and is found in a variety of developmental events including gastrulation and vertebrate neural tube formation. The cephalic furrow is a deep epithelial invagination that forms during Drosophila gastrulation. In the first phase of cephalic furrow formation, the initiator cells that will lead invagination undergo apicobasal shortening and apical constriction in the absence of epithelial invagination. In the second phase of cephalic furrow formation, the epithelium starts to invaginate, accompanied by both basal expansion and continued apicobasal shortening of the initiator cells. The cells adjacent to the initiator cells also adopt wedge shapes, but only after invagination is well underway. Myosin II does not appear to drive apical constriction in cephalic furrow formation. However, cortical F-actin is increased in the apices of the initiator cells and in invaginating cells during both phases of cephalic furrow formation. These findings suggest that a novel mechanism for epithelial invagination is involved in cephalic furrow formation.

Introduction

One of the fundamental morphogenetic processes that shape the embryo during development is the invagination, or inward bending, of epithelial sheets (Davidson et al., 1995; Ettensohn, 1985; Fristrom, 1988; Keller et al., 2003; Lecuit and Lenne, 2007; Martin and Goldstein, 2014; Odell et al., 1981; Sawyer et al., 2010). Differences in cell shape changes and movements can produce a variety of epithelial structures (Davidson, 2012; Quintin et al., 2008). Despite the variety of epithelial structures ultimately produced, in the best-studied epithelial invaginations, invagination is associated with apical constriction (Sawyer et al., 2010). Actomyosin contraction appears to be the predominant mechanism of apical constriction and epithelial invagination (Martin and Goldstein, 2014; Sawyer et al., 2010; Sherrard et al., 2010). However, theoretical and modeling studies suggest other possible mechanisms of epithelial invagination, as have experimental studies (Davidson et al., 1995, 1999; Ettensohn, 1985; Fristrom, 1988; Herman et al., 1999; Keller et al., 2003; Kondo and Hayashi, 2013; Wang et al., 2012).

The cephalic furrow (CF) starts to form at the beginning of gastrulation (stage 6), when the ventral furrow starts to form. The CF first appears as a shallow groove at about 65% egg length (EL, measured from the posterior of the embryo) on the lateral sides of the embryo. The groove extends both postero-dorsally and antero-ventrally, eventually forming a ring around the entire embryo. The ring becomes more canted such that by the end of stage 7, the CF ring extends from 54% EL dorsally to 74% EL ventrally (Campos-Ortega and Hartenstein, 1997; Turner and Mahowald, 1977). As gastrulation progresses, the CF rapidly grows deeper. By stage 8, it becomes very deep on the lateral sides, but the ventral and dorsal regions remain relatively shallow (Campos-Ortega and Hartenstein, 1997; Costa et al., 1993; Underwood et al., 1980). Starting during stage 8, several cell divisions occur in the invaginated furrow and might contribute to further CF deepening (Campos-Ortega and Hartenstein, 1997; Foe, 1989). The CF is transient and by stage 11, after germband extension has completed but before germband retraction begins, the CF has unfolded and all the cells that had invaginated have returned to the surface (Campos-Ortega and Hartenstein, 1997; Costa et al., 1993; Turner and Mahowald, 1977).

Little is known about the cell shape changes that occur during CF formation. CF formation is initiated by the shortening of a row of cells in the mid-lateral region of the embryo. These initiator cells undergo a cell shape change from columnar cells to bottle-shaped cells that includes apical constriction, shortening in the apicobasal axis and shifting of the nuclei to a more basal position during the beginning of CF formation (Costa et al., 1993; Turner and Mahowald, 1977; Vincent et al., 1997). However, the apices of the CF initiator cells narrow less than those of the ventral furrow (Costa et al., 1993). Unlike the cells of the ventral furrow, they do not undergo apical flattening or ruffling (Costa et al., 1993; Turner

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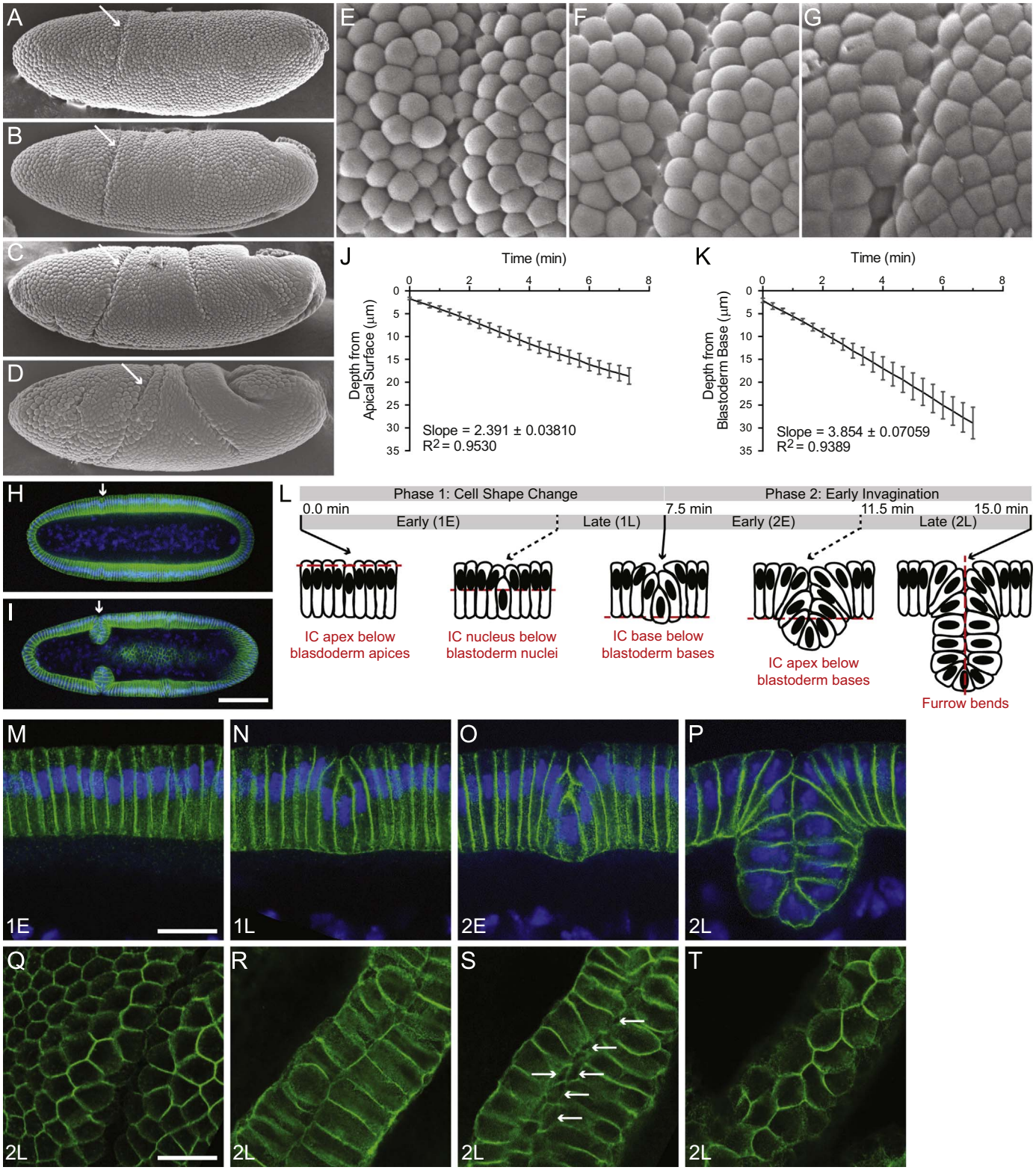

Fig. 1. Morphogenesis of the early cephalic furrow. (A–G) Scanning electron micrographs of gastrulating embryos showing the lateral surface (A–D) and corresponding surface cells in and near the CF (E–G) at early stage 6 (A,E), late stage 6 (B,F), stage 7 (C,G) and stage 8 (D). Arrow: cephalic furrow (CF). (H–I) Confocal images of gastrulating embryos showing cell membranes (Nrt, green) and nuclei (Hoechst, blue) at stage 6 (H) and stage 7 (I). Arrow: CF. Scale bar: 100 μm. (J) Time course analysis of the depth of the CF cleft measured from the apical blastoderm surface. Mean and SD. (K) Later time course analysis of the depth of CF invagination into the yolk sac measured from the blastoderm base. Time point 0 was approximately 7.5 min after time point 0 in (J). Mean and SD. (L) Key morphological features defining the two phases of early CF formation and timeline. (M–P) Confocal images of CF formation in the lateral region of the gastrulating embryo showing cell membranes (Nrt, green) and nuclei (Hoechst, blue) at early phase 1 (1E) (M), late phase 1 (1L) (N), early phase 2 (2E) (O) and late phase 2 (2L) (P). Scale bar: 20 μm. (Q–T) Confocal sagittal images of CF formation from z-stacks of two different embryos at late phase 2 showing cells at the surface of furrow cleft (Q), internalized cells of the CF (R), initiator cell apices (arrows) (S), and basal regions of initiator cells near the base of the CF (T). Scale bar: 20 μm. Anterior: left.

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