Developmental Biology 394 (2014) 6–14

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

Review

The Fog signaling pathway: Insights into signaling in morphogenesis

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ARTICLE INFO

Article history:
Received 28 May 2014
Received in revised form
28 July 2014
Accepted 4 August 2014
Available online 12 August 2014

Keywords:
Apical constriction
Morphogenesis
Cell signaling
Folded gastrulation
Actin
Myosin

ABSTRACT

Epithelia form the building blocks of many tissue and organ types. Epithelial cells often form a contiguous 2-dimensional sheet that is held together by strong adhesions. The mechanical properties conferred by these adhesions allow the cells to undergo dramatic three-dimensional morphogenetic movements while maintaining cell–cell contacts during embryogenesis and post-embryonic development. The *Drosophila* Folded gastrulation pathway triggers epithelial cell shape changes that drive gastrulation and tissue folding and is one of the most extensively studied examples of epithelial morphogenesis. This pathway has yielded key insights into the signaling mechanisms and cellular machinery involved in epithelial remodeling. In this review, we discuss principles of morphogenesis and signaling that have been discovered through genetic and cell biological examination of this pathway. We also consider various regulatory mechanisms and the system's relevance to mammalian development. We propose future directions that will continue to broaden our knowledge of morphogenesis across taxa.

Introduction

Epithelial morphogenesis, the process through which simple sheets of cells are rearranged and change shape to form mature structures and organs, is an area of intense focus in the field of developmental biology (Nelson and Gleghorn, 2012; Spear and Erickson, 2012; Suzuki et al., 2012). A key morphogenetic movement, which occurs in almost all multicellular animals, is the folding or bending of flat epithelial sheets to form more complex configurations. These changes are often driven at least in part by actin- and myosin-based apical constriction (Sawyer et al., 2010). One of the best-studied developmental signaling pathways regulating this process is the *Drosophila* Folded gastrulation (Fog) pathway in which many of the crucial molecular events are known, from initiation by transcription factors (TFs) to the mechanics of cell shape changes. This pathway, which drives apical constriction, therefore allows examination of some of the intricacies of cell signaling during development *in vivo*.

Many stereotypical signaling mechanisms are exemplified in the Fog pathway, including patterned induction of gene expression by TFs, G-protein coupled receptor (GPCR) to G-protein signaling, and actin rearrangement induced by Rho GTPase signaling. The Fog pathway also reveals some novel insights, such as how multiple signaling pathways can be integrated into a single outcome and that GPCRs, among their many other functions, have morphogenetic roles. While certain aspects of the Fog pathway have been worked out in great detail, many questions still remain. What mechanisms recruit signaling components apically? How are Fog pathway components spatially and temporally patterned in tissues and time and what role does this patterning play in development? Which mechanisms regulate the attenuation of Fog signaling? We will explore these questions in this review.

Pathway overview

The Fog pathway, diagramed in Fig. 1, begins with the specific expression of Fog in subsets of cells fated for actomyosin-based shape changes. Fog is a large secreted protein that is thought to signal primarily as an autocrine factor (Costa et al., 1994). The Fog

Abbreviations: Fog, Folded gastrulation; TF, transcription factor; GPCR, G-protein coupled receptor; Mist, mesoderm invagination signal transducer; Cta, Concertina; RhoGEF, Rho guanine nucleotide exchange factor; Rok, Rho kinase; PMG, posterior midgut; GBE, germ band extension; VF, ventral furrow; MT, microtubule; GRK, G-protein coupled receptor kinase; Krz, Kurtz; GAP, GTPase activating protein; Dia, Diaphanous; Abl, Abelson kinase

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http://dx.doi.org/10.1016/j.ydbio.2014.08.003

signal is transmitted across the plasma membrane by the GPCR Mesoderm invaginating signal transducer (Mist), a member of the secretin family of GPCRs, to a G-protein of the $G\alpha_{12/13}$ family, Concertina (Cta; Parks and Wieschaus, 1991; Manning et al., 2013). In turn, RhoGEF2, a Dbl family Rho guanine nucleotide exchange factor (RhoGEF), the small GTPase Rho1, and the Rho effector, Rho Kinase (Rok) are all activated (Barrett et al., 1997; Dawes-Hoang et al., 2007). Rok phosphorylates the regulatory light chain of non-muscle myosin II to induce contraction of the apical actomyosin network in the cells that receive the Fog signal. While the ligand, Fog, is not conserved outside of *Drosophila* and the receptor, Mist, is not conserved outside of insects, the axis of signaling from $G\alpha_{12/13}$ proteins through Rho to affect actin rearrangement is highly conserved and is important in human development and disease (Fig. 1; Waterhouse et al., 2011). For example, lysophosphatidic acid and sphingosine 1-phosphate are membrane lipid derivatives known to signal through GPCRs, the $G\alpha_{12/13}$ family, RhoGEFs, RhoA, and various downstream effectors in mammals (Suzuki et al., 2009; Xiang et al., 2013). These pathways modulate cytoskeletal and cell shape changes such as neurite outgrowth and retraction, tumor cell invasion, or angiogenesis.

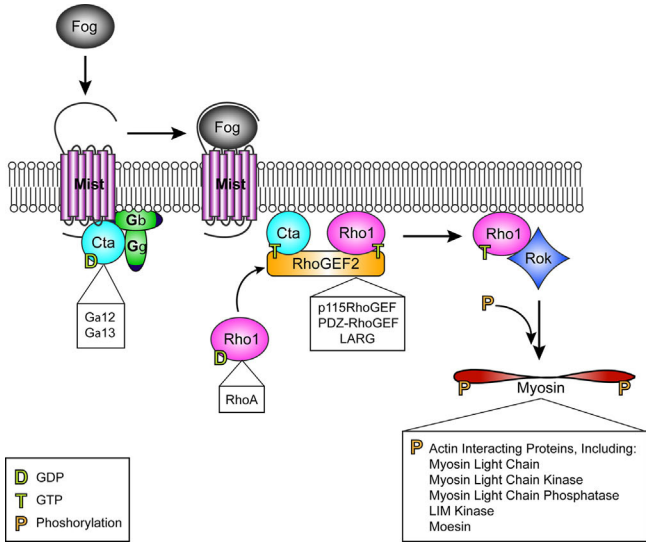

Fig. 1. The Fog Signaling Pathway. Fog is a large secreted protein which acts as a ligand for Mist, a seven pass transmembrane GPCR. In its ligand-free state Mist is predicted to interact with inactive, GDP-bound Cta. Once Fog binds Mist, it likely stimulates Cta's exchange of GTP for GDP, which allows Cta to dissociate from its trimer partners, Gβ and Gγ. Cta-GTP binds to RhoGEF2 which can then act as a GEF for Rho1. In its GTP-bound form Rho1 then activates Rok. Finally, the regulatory light chain of non-muscle myosin II, Spaghetti squash, is phosphorylated by active Rok to induce apical actomyosin network contraction in the cells which receive the Fog signal. Boxed are vertebrate components of Rho axis signaling which act in a similar manner to induce actomyosin cytoskeleton rearrangements. In vertebrates, Rok is known to phosphorylate many proteins which interact with actin, activating some and inactivating others.

The Fog pathway is active in several morphogenetic events in *Drosophila* development, with known roles in ventral mesoderm and posterior midgut (PMG) invagination during gastrulation, salivary gland internalization in mid-embryogenesis, and imaginal disc folding during larval development (Fig. 2A–D; Costa et al., 1994; Nikolaidou and Barrett, 2004). It has also been proposed that Fog is involved in morphogenesis of the central nervous system during late embryogenesis (Ratnaparkhi and Zinn, 2007). In most of these cases Fog induces apical constriction, although in the CNS the cellular results of Fog's action are not known.

Before cells begin apical constriction proper, they generally have domed apical surfaces which become flat before constriction begins (Fig. 2E; Dawes-Hoang et al., 2007). During apical constriction the myosin in the actin network along the apical membrane of the contracting cells is activated, reducing the size of the network, pulling on apical junctions, and reducing the apical area of the cell (Sweeton et al., 1991). Because of the junctional connections bound to the actin, each cell pulls its neighbors inward during this process. At the same time as their apices are shrinking cells elongate in the apical–basal direction which aids in internalization. After apical constriction is complete, cells shorten apicobasally to become fully internalized (Pouille and Farge, 2008). Apical constriction, along with other concomitant shape changes, in cells of the ventral mesoderm, PMG, and salivary gland eventually results in complete internalization of these cell groups. The cells of imaginal discs only invaginate as far as to form U-shaped folds within the plane of the tissue.

During ventral furrow (VF) formation there are two phases of apical constriction: a stochastic, nonproductive phase, when individual cells contract and relax without any overall reduction in apical area, and a concerted, coordinated phase, when individual cells undergo cyclical ratchet-like rounds of reductions in apical area which are much more stable (Sweeton et al., 1991; Martin et al., 2009). During both phases, actin and myosin periodically coalesce and these concentrations tend to move toward the center of a cell (Martin et al., 2009). *Via* these contractions, the plasma membrane is pulled inward. During random constriction the membrane relaxes to its original position when actomyosin coalescences are disassembled. Once the concerted phase of constriction begins, membrane deformations are stabilized to reduce apical cell

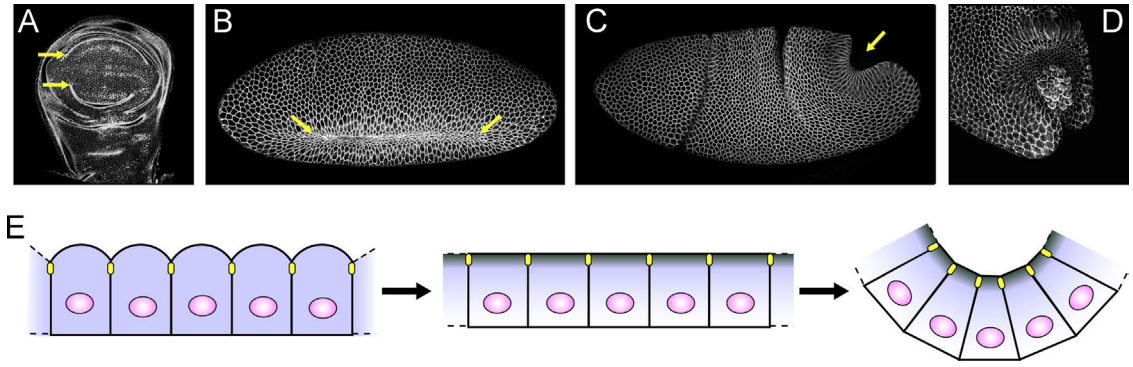

Fig. 2. Morphogenetic changes induced by the Fog pathway: (A) Third instar imaginal wing disc. Actin staining highlights epithelial folds. (B) Ventral furrow invagination. (C) Posterior midgut invagination. (A–C) yellow arrows denote cell groups undergoing Fog pathway induced apical constriction. (D) Closer view of posterior midgut cells undergoing apical constriction. Germ cells are carried in with this invagination. (B–D) embryos are stained for Neurotactin to outline cells. (E) Cartoon of cell shape changes induced by the Fog pathway. When cellularization is complete, adherens junctions (yellow ovals) are sub-apical and apical cell surfaces are rounded. Fog pathway members become apically concentrated (denoted by shading of cells) and apical cell surfaces flatten. When the Fog pathway is activated cell apices constrict and cells elongate apicobasally.