Review

Transcriptional regulation of tissue organization and cell morphogenesis: The fly retina as a case study

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A B S T R A C T

Understanding how a functional organ can be produced from a small group of cells remains an outstanding question in cell and developmental biology. The developing compound eye of *Drosophila* has long been a model of choice for addressing this question by dissecting the cellular, genetic and molecular pathways that govern cell specification, differentiation, and multicellular patterning during organogenesis. In this review, the author focussed on cell and tissue morphogenesis during fly retinal development, including the regulated changes in cell shape and cell packing that ultimately determine the shape and architecture of the compound eye. In particular, the author reviewed recent studies that highlight the prominent roles of transcriptional and hormonal controls that orchestrate the cell shape changes, cell–cell junction remodeling and polarized membrane growth that underlie photoreceptor morphogenesis and retinal patterning.

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Introduction

During animal development, specific genetic and molecular programs orchestrate the differentiation of the various cell types that assemble into organs. Cell differentiation can include the acquisition of specific morphological features that in turn enables the cell to perform specialized functions. Epithelial cells and neurons are two striking examples of cell types whose differentiation is based on the acquisition of a highly specialized polarized morphology. During development, epithelial cells acquire an apico-basal axis of polarity and discrete cell–cell contacts required for organogenesis, whereas neurons have to elaborate dendrites and an axon to participate in neural circuit formation. In both cases, the final shape and function of the cell emerges over developmental time from a combination of genetic and molecular programs.

Epithelial cells have the ability to adhere to each other to form coherent cellular sheets. This feature is crucial to generate organs such as the gut, kidney and lung, in which epithelia separate an internal luminal space from the outside world. The production of such epithelial structures in a reproducible manner requires stringent regulatory mechanisms to control the shape, packing, and positioning of cells within epithelia. Regulated epithelial cell shape changes and positioning depend on regulated remodeling of cell–cell contacts.

Invertebrate epithelial cells have two main junctional domains in their plasma membrane: the apical *zonula adherens* and the lateral paranodal-like septate junction (Fig. 1A). In the past few years, there has been especially intense study of apical junction remodeling during epithelial patterning. The *zonula adherens* not only mediates cell–cell adhesion; it also generates intracellular signals in response to mechanical tension within the epithelium (reviewed in (Guillot and Lecuit, 2013)). This is in part due to the ability of E-cadherin located in the junction to associate with the F-actin cytoskeleton and the motor protein non-muscle Myosin-II (Fig. 1B). Myosin-II is able to promote the formation of actin-Myosin-II foci and meshworks, which are associated with the *zonula adherens* or discrete *adherens junction* domains in developing epithelia (Blankenship et al., 2006a; Levayer and Lecuit, 2013; Martin et al., 2009; Rauzi et al., 2010; Robertson et al., 2012) (Fig. 1B). Myosin-II can generate contractile force to increase tension in the cell cortex (Fernandez-Gonzalez et al., 2009; Rauzi et al., 2008) and can also promote the endocytosis of *adherens junction* proteins, including E-cadherin (Levayer et al., 2011) (Fig. 1C). In developing epithelia, the actin-Myosin-II cortex can promote the suppression of *adherens junctions* between cells but also the creation of new *adherens junctions* (Bardet et al., 2013; Bertet et al., 2004; Blankenship et al., 2006a) (Fig. 1C). Thus, Myosin-II is a major regulator of cell shape, adhesion and packing within developing epithelia and is therefore at the core of much of organogenesis.

Organogenesis requires a high degree of coordination between epithelial cells to produce the folds or tubular structures within an organ tissue. It also requires temporal coordination of cell
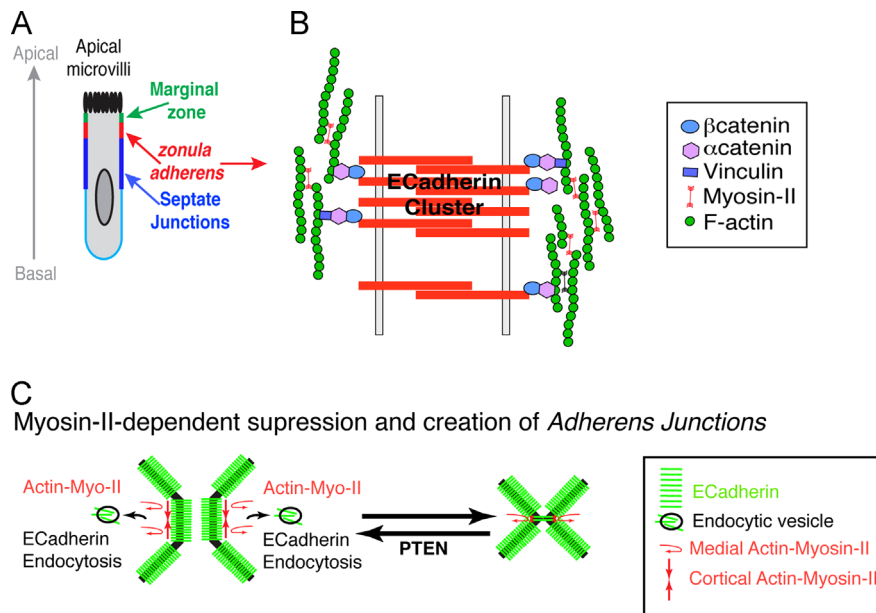
Fig. 1. *adherens junction* remodeling during organogenesis. (A) Depiction of an invertebrate epithelial cell. (B) Simplified schematic of the epithelial cell *zonula adherens*. (C) Depiction of the activity of Myosin-II (Medial meshwork and cortical; Red arrows) during *adherens junction* suppression and elongation. The lipid phosphatase PTEN is required for Myosin-II-dependent *adherens junction* elongation (Bardet et al., 2013).

differentiation. This coordination is governed by both long- and short-range signaling, which often operate in a tissue specific manner. To gain a fully integrated view of organogenesis remains a challenging task. The developing compound eye of *Drosophila melanogaster* is a particularly well-suited *in vivo* model system for attacking the problem. It consists of approximately 750 basic units called ommatidia (Fig. 2). Each ommatidium consists of eight photoreceptor neurons, four cone cells, two primary pigment cells, six shared secondary pigment cells, three shared tertiary pigment cells, and three shared mechanosensory bristles (Tomlinson, 1985a; Waddington and Perry, 1960) (Figs. 2A D). The compound eye originates from an unpatterned, pseudo-stratified, columnar epithelium, where all of the different cell types are induced from a common pool of equipotent epithelial precursors (Lawrence and Green, 1979; Ready et al., 1976).

Photoreceptor differentiation is initiated by the expression of the pro-neuronal basic helix-loop-helix transcription factor Atonal (Greenwood and Struhl, 1999; Jarman et al., 1994). The intracellular Ras/Raf/MAPK signaling pathway, acting reiteratively through the ETS transcription factor Pointed (Freeman, 1996; Xu and Rubin, 1993), is a major inducer of photoreceptor neurogenesis. This pathway is also subsequently required to generate the full complement of accessory cells (i.e., cone and pigment cells) that complete the ommatidium (Freeman, 1996). These steps of cell-fate commitment and early cell differentiation begin during the imaginal disc stage of eye development (reviewed in (Treisman, 2012)) and are completed during the first 10% of the pupal stage of fly development (where 0% is a newly formed white pupa, and 100% corresponds to the hatching of the adult fly from the pupal case) (Fig. 3).

Following this early phase of retinal cell differentiation, pupal eye development proceeds by an orderly sequence of morphogenetic events. As the ommatidial lattice is established, two consecutive transformations occur in the photoreceptors. First, at ∼30% after puparium formation, the cells begin to establish their precise pattern of axon projections to the neuropil of the fly optic lobe (Fig. 3B). This process is genetically hard-wired and is referred to as *neural super-position* (Clandinin and Zipursky, 2000; Meinertzhagen and Hanson, 1993). Second, photoreceptors begin to undergo a striking remodeling of their plasma membrane to form a new *zonula adherens* domain, as well as the subapical stalk membrane (Fig. 3C E). This transformation

begins at ∼37% after puparium formation and leads to a 90 degree rotation of the cell's apico-basal axis (Fig. 3C–E). This step of polarity remodeling is required to align the future light-gathering organelle, the rhabdomere, with respect to the lens-to-brain axis of the retina. Finally, after the ommatidial lattice has been established, there is a further transformation at ∼78% after puparium formation, when the photoreceptors express the visual pigment Rhodopsin (Earl and Britt, 2006; Kumar and Ready, 1995). This event culminates in the terminal differentiation of the rhabdomere (Fig. 3F), which includes the elaboration of a meshwork of F-actin called the rhabdomere terminal web that is required to support rhabdomere morphogenesis and maintenance (Chang and Ready, 2000; Kumar and Ready, 1995; Pinal and Pichaud, 2011).

Apical constriction initiates retinal patterning

Retinal differentiation begins in the fly larva at the morphogenetic furrow (Fig. 3A), a morphogenetic wave that sweeps across the retina from the posterior to the anterior margin of the epithelium (Ready et al., 1976) (Fig. 4A–B). Cells in the morphogenetic furrow undergo apical constriction, and, in its wake, one column of ommatidia is generated about every 2 h. Once the epithelium is fully patterned, a total of approximately 32 columns will have been laid down with exquisite reproducibility (Cagan and Ready, 1989; Campos-Ortega and Hofbauer, 1977).

Epithelial cell apical constriction occurs commonly when a developing epithelium invaginates to form a fold. The morphogenetic furrow is an example of transient apical constriction that does not lead to a permanent fold of the epithelium but instead promotes the onset of tissue patterning. Among the effector proteins that promote epithelial cell apical constriction are the small GTPase RhoA, its effector kinase ROCK, as well as Myosin-II and F-actin (for a recent review, see (Sawyer et al., 2010)) (Fig. 4C). Other factors that are also critical for promoting apical constriction in the morphogenetic furrow are the kinase DRak (Robertson et al., 2012), the F-actin polymerization factor Diaphanous and an increase in apical microtubule concentration (Corrigall et al., 2007) (Fig. 4C). This role for microtubule accumulation during apical constriction resembles that in neural tube closure in *Xenopus*, where parallel microtubule arrays accumulate at