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REVIEW

The roles and regulation of multicellular rosette structures during morphogenesis

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ABSTRACT

Multicellular rosettes have recently been appreciated as important cellular intermediates that are observed during the formation of diverse organ systems. These rosettes are polarized, transient epithelial structures that sometimes recapitulate the form of the adult organ. Rosette formation has been studied in various developmental contexts, such as in the zebrafish lateral line primordium, the vertebrate pancreas, the *Drosophila* epithelium and retina, as well as in the adult neural stem cell niche. These studies have revealed that the cytoskeletal rearrangements responsible for rosette formation appear to be conserved. By contrast, the extracellular cues that trigger these rearrangements *in vivo* are less well understood and are more diverse. Here, we review recent studies of the genetic regulation and cellular transitions involved in rosette formation. We discuss and compare specific models for rosette formation and highlight outstanding questions in the field.

KEY WORDS: Myosin II, *Drosophila* epithelium, Morphogenesis, Rosette, Zebrafish lateral line

Introduction

The proper formation of adult organs relies on cellular rearrangements that occur during development. How these morphogenetic movements are regulated at a molecular and cellular level remains a central question in developmental biology. Recently, it has become apparent that polarized epithelial rosettes are common intermediates that are observed during the organogenesis of multiple organs in diverse species. These rosettes are multicellular structures in which five or more cells interface at a central point and their remodeling contributes to the formation of a functional organ. Such rosettes are observed in a variety of developmental and adult contexts (Blankenship et al., 2006; Gompel et al., 2001; Lienkamp et al., 2012; Villasenor et al., 2010). In addition, rosettes have also been observed in stem cell populations, both *in vitro* and *in vivo* (Chen et al., 2012; Elkabetz et al., 2008; Elkabetz and Studer, 2008; Mirzadeh et al., 2008; Zhang et al., 2001). Whereas the intracellular mechanisms that participate in rosette formation are relatively well conserved between species and organ systems, the extracellular cues that regulate rosette formation are diverse. Despite these differences, the many contexts in which rosettes are formed suggest that they constitute a broadly utilized mechanism during morphogenesis. Thus, an understanding of the signals and cellular events that drive rosette formation will be important for understanding tissue formation and maintenance.

In this Review, we first summarize the processes mediating the cellular rearrangements that lead to rosette formation. Next, we discuss the molecular mechanisms responsible for rosette formation in different contexts. Finally, we compare and contrast the mechanisms driving rosette formation to highlight similarities and differences across organ systems.

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Mechanisms of rosette formation

During development, there appear to be at least two structurally distinct classes of rosettes (Fig. 1): those that form through apical constriction (see Glossary, Box 1) and those that from through planar polarized constriction (see Glossary, Box 1). Both rosette types are transient, although their persistence varies. In some cases, the cellular mechanism driving rosette formation is not fully elucidated but appears to involve features of both apical constriction and planar polarized constriction; it is not yet clear whether these cases represent a distinct mechanism of rosette formation. Generally, rosettes that are formed through the planar polarized mechanism resolve relatively quickly and typically contribute to processes involving tissue elongation. By contrast, rosettes that are formed through apical constriction can persist for extended periods of time, may or may not resolve, and often remodel to form a functional structure or organ. In this Review, we focus on the mechanisms that induce rosette formation, rather than those involved in rosette resolution. However, it is important to note that the functional significance of rosettes is tightly linked to both their formation and resolution. For example, the resolution of rosettes that are formed during convergent extension (see Glossary, Box 1) drives tissue elongation.

Making rosettes via apical constriction

During embryonic development, apical constriction promotes cell shape changes that are important for diverse developmental processes, including gastrulation and neural tube closure (Sawyer et al., 2010; Martin and Goldstein, 2014). This cellular behavior is characterized by the narrowing of the apical domain of a columnar cell. Apical constriction of multiple neighboring cells can result in changes in tissue architecture, including the bending of sheets of cells. Similarly, the coordinated apical constriction of neighboring cells can also result in the formation of a multicellular rosette containing cells that have their apical domains pointed towards a common center. Because apical constriction is reviewed thoroughly elsewhere (Sawyer et al., 2010; Martin and Goldstein, 2014), here we only summarize the general principles that underlie narrowing of the apical domain.

The apical constriction that underlies rosette formation is typically dependent on contraction of an acto-myosin network (see Glossary, Box 1) (Ernst et al., 2012; Harding and Nechiporuk, 2012; Nishimura and Takeichi, 2008) and, in many contexts, the assembly of an apical acto-myosin meshwork is a prerequisite for apical constriction (Sawyer et al., 2010; Martin and Goldstein, 2014). Proper establishment of the apical domain appears to specify the location of acto-myosin assembly, and the localization of acto-myosin cytoskeletal components is often dependent on the polarity regulators Par-6 and Par-3 [Bazooka (Baz) in *Drosophila*] and atypical protein kinase C (aPKC) (Putzke and Rothman, 2003;

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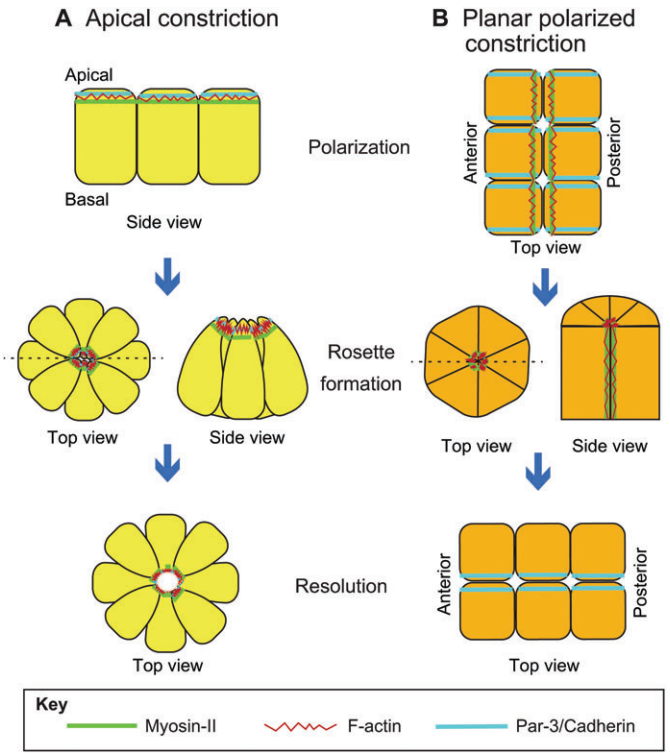

Fig. 1. Mechanisms of rosette formation. (A) Rosette formation via apical constriction. Prior to apical constriction, cytoskeletal molecules, including F-actin and myosin-II, Par-3 and N-cadherin, are in the apical domains of columnar cells. Apical constriction leads to the formation of a rosette around an acto-myosin-rich center. Note that only apical, but not basal, domains of rosette cells are constricted. Following rosette formation, rosette centers may open to form a central lumen. (B) During rosette formation via planar polarized constriction, cytoskeletal molecules are distributed in a planar polarized fashion throughout the developing tissue, prior to rosette formation. Actin and myosin-II are localized to the AP axis, and Par-3 (Baz) and cadherin are localized to perpendicular membranes. This distribution allows the formation of a rosette with a myosin-II-rich center. Note that cells constrict along their lateral edges. Rosettes formed in this manner often resolve along the axis perpendicular to that of the initial cellular arrangement. Dashed lines indicate the plane of the cross-sections shown.

Sawyer et al., 2010). The apical localization of cell junction proteins, including adherens junction (AJ) proteins (cadherins) and tight junction proteins (ZO-1; also known as Tjp1) is also crucial for driving the apical assembly of F-actin and non-muscle myosin-II (referred to hereafter as myosin-II), the central components of the acto-myosin cytoskeleton. Activation of the apical meshwork is mediated by phosphorylation of the myosin-II subunit myosin regulatory light chain (MRLC).

Overall, the coordinated apical constriction of a community of cells can lead to the formation of a rosette (Fig. 1A). Within these rosettes, apically constricted cells take on a teardrop shape, with the rosette resembling a garlic bulb. As discussed later, the development of several tissue types relies on rosettes formed in this manner. Interestingly, despite using a similar intracellular mechanism, the extracellular cues that trigger the formation of apical constriction-dependent rosettes are diverse and are discussed in more detail below.

Making rosettes via planar polarized constriction

A second, distinct cellular mechanism that leads to the formation of cellular rosettes depends on planar polarized myosin activity (Fig. 1B). Planar polarity (see Glossary, Box 1) is generated within the plane of a tissue, orthogonal to apicobasal polarity. This type of polarity coordinates the asymmetric distribution of molecules within individual cells along epithelial sheets (Gibson and Perrimon, 2003). It is crucial for the processes of gastrulation, neurulation and organogenesis, all of which require multicellular rearrangements such as tissue elongation. In certain contexts, the planar polarized distribution of molecules involved in acto-myosin constriction and cell adhesion can give rise to rosette formation (Blankenship et al., 2006; Lienkamp et al., 2012; Simões et al., 2010; Tamada et al., 2012). In a subset of these cases, molecules involved in the planar cell polarity (PCP) pathway, including non-canonical Wnt, are clearly required for rosette formation (Lienkamp et al., 2012). In other examples, the distribution of cytoskeletal molecules, including myosin-II and F-actin, resembles the distribution of PCP proteins, but this localization is independent of PCP-based signaling (Zallen and Wieschaus, 2004). The localization of Par proteins in these examples is distinct from its apical localization in examples of apical constriction-based rosette formation (Fig. 1).

Although the same cytoskeletal molecules and polarity cues are involved in planar polarized-based and apical constriction-based rosette formation (myosin-II and Par-3), in the former these molecules are localized to complementary domains along the anterior-posterior (AP) or dorsal-ventral (DV) axes, instead of colocalizing in the apical domain (Blankenship et al., 2006; Zallen and Wieschaus, 2004). As such, these rosettes are structurally distinct from those formed by apical constriction, as the constricted region spans the lateral edge of cells within the rosette (Fig. 1). As

Box 1. Glossary

Acto-myosin network. A dynamic meshwork of the cytoskeletal molecules myosin-II and F-actin that can drive changes in cellular architecture.

Anterior visceral endoderm (AVE). A group of extraembryonic cells that specifies anterior patterning in the mouse embryo and is responsible for orienting the anterior-posterior axis.

Apical constriction. The narrowing of the apical domain of an apicobasal polarized cell, resulting in the formation of a teardrop-shaped cell.

Convergent extension (CE). The process by which a tissue elongates and narrows in one direction and lengthens in the perpendicular direction.

Dorsal forerunner cells (DFCs). A group of cells that migrate at the leading edge of the developing zebrafish organizer during gastrulation but do not involute. At the end of gastrulation, forerunner cells migrate deep into the embryo and organize to form the mature Kupffer's vesicle.

Epiboly. A coordinated cell movement that occurs in the embryo during gastrulation and results in the spreading of cells into sheets.

Pair-rule genes. Patterning genes found in insect embryos that are expressed in narrow stripes and act in combination to assign each cell a distinct fate along the anterior-posterior axis.

Planar polarity. The coordination of asymmetries within cells, orthogonal to the plane of apicobasal polarity. In multicellular tissues, planar polarity results in alignment of these asymmetries.

Planar polarized constriction. Constriction of the lateral domain of cells that occurs perpendicular to the plane of apicobasal polarity.

Primitive streak. Defines the axis of bilateral symmetry and is the site of gastrulation in avian, reptilian and mammalian embryos.

Ventricular-subventricular zone (V-SVZ). Paired structures in the adult brain that are composed of the lateral ventricles, which contain the cerebral-spinal fluid, and the subventricular zone, which is composed of multiple cell types and acts as a niche for adult neural stem cells that give rise to neurons and glial cells.

Visceral endoderm (VE). An extraembryonic layer of cells that covers the early mouse embryo and is important for primitive streak formation and anterior-posterior patterning.

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discussed below, the extracellular cues that drive the planar polarized distribution of cytoskeletal molecules are diverse, and include Nodal, epidermal growth factor (EGF) and Wnt.

Rosette formation during development

Rosettes were first observed during *Drosophila* eye development. Since then, rosettes have been appreciated in a variety of tissues in both vertebrates and invertebrates. In many cases, either apical constriction or planar polarity is the primary mode of rosette formation, whereas in other cases the primary mode is either unknown or shows features of both apical constriction and planar polarized constriction (Table 1). Below, we first discuss the better understood examples of rosette formation, including zebrafish lateral line formation, *Drosophila* germ band extension and *Drosophila* eye morphogenesis. This is followed by a discussion of more recently appreciated but less well-studied cases in which rosette formation occurs, including kidney tubule elongation, pancreatic branching and neural development.

Zebrafish lateral line development

One of the better-studied examples of apical constriction-based rosette formation occurs during the development of the zebrafish lateral line. This mechanosensory system allows detection of water movement, promoting behaviors such as predator avoidance, prey detection and schooling in aquatic vertebrates. The lateral line is composed of mechanosensory organs called neuromasts (NMs), arranged along the body of the animal (Dijkgraaf, 1963; Ma and Raible, 2009; Montgomery et al., 2000). Recently, the zebrafish posterior lateral line (pLL; a collection of trunk NMs) has emerged as an attractive model for studying organ patterning and morphogenesis due to its experimental accessibility. The primary pLL is derived from a thickening of the ectoderm called the pLL placode; the posterior portion of this placode begins migrating caudally at 22 h post fertilization (hpf) as a cohesive group of ~100 cells called the pLL primordium (pLLp) (Ghysen and Dambly-Chaudière, 2004). As it migrates, the pLLp deposits clusters of cells (proto-NMs) from its rostral (trailing) region; by 48 hpf, seven or eight clusters are deposited along the trunk as the pLLp reaches the tail (Ghysen and Dambly-Chaudière, 2007). Each of these proto-NMs will differentiate into a functional NM. The migrating pLLp is organized into two or three rosettes (Fig. 2A,B). Each rosette corresponds to a proto-NM composed of ~20 cells (Gompel et al., 2001). As a proto-NM is deposited from the trailing region of the pLLp, a new rosette is assembled in the leading region (Chitnis et al., 2012; Nechiporuk and Raible, 2008). Newly deposited rosettes/proto-NMs subsequently open apically to form a lumen. Recent studies have revealed that the molecular and cellular cues coordinating rosette assembly in the pLLp appear to be generally conserved, compared with other models of rosette formation.

Within rosettes, cells are apically constricted and nuclei are basally displaced, as demonstrated by three-dimensional reconstructions of individual cells (Fig. 2C-E) (Harding and Nechiporuk, 2012). The formation of these garlic bulb-shaped rosettes depends on coordinated apical constriction, whereby a subset of polarized cells in the leading region of the pLLp collectively constricts apically (Fig. 2C-E) (Harding and Nechiporuk, 2012). This constriction requires activation of myosin-II, which becomes localized to the apical domains of cells just prior to rosette formation (Ernst et al., 2012; Harding and Nechiporuk, 2012). Myosin-II is activated by phosphorylation, which is dependent on both Rho kinase 2a (Rock2a) and the scaffolding molecule Shroom3; loss of Rock or Shroom3 results in failure of rosette formation (Ernst et al., 2012; Harding and Nechiporuk, 2012). As Shroom3 has previously been shown to anchor Rock in the apical domains of cells (Nishimura and Takeichi, 2008), it is likely that Shroom3-mediated apical accumulation of Rock2a may activate rosette formation in the pLLp.

One identified extracellular cue that drives pLLp rosette formation is the fibroblast growth factor (FGF) signaling pathway. Fgf3 and

Table 1. Summary of the tissues in which rosette formation has been observed

Developing structure	Adult tissue	Method	Species	Number of cells	Extracellular signaling	Other signals	Resolve?
Anterior visceral endoderm	Vertebrate axis	?	Mouse	>5	PCP/non-canonical Wnt (Trichas et al., 2012)	?	Resolve
Epithelial elongation	Epithelium	PPC	*Drosophila*	5-11	?	Myo-II/Baz/Abl/β-catenin/F-actin/Rho/Shroom	Resolve
Kidney tubule formation	Kidney	PPC	Mouse/*Xenopus*	5-6	Wnt9b (Lienkamp et al., 2012)	dishevelled 2/Myo-II	Open to form lumen
Kupffer's vesicle	Zebrafish axis	?	Zebrafish	?	Wnt (Oteíza et al., 2010)	Prickle1a	Open to form lumen
Lateral line primordium	Lateral line	AC	Zebrafish	18-24	FGF (Nechiporuk and Raible, 2008; Lecaudey et al., 2008)	Shroom3/Rock/Myo-II	Open to form lumen
Neural tube closure	Neural tube	AC	Chick	>5	?	Shroom3/Rock/Myo-II	?
Ommatidia formation	Eye	PPC	*Drosophila*	5	EGF (Brown et al., 2006)	Atonal/E-cadherin/Baz/Myo-II/Drak/Rok	Resolve
Pancreatic branching	Pancreas	AC	Mouse	?	EphB (Villasenor et al., 2010)	Myo-II/Stard13	Resolve
Primitive streak	Vertebrate axis	?	Chick	>6	Nodal (Yanagawa et al., 2011)	?	Resolve

The method utilized for rosette formation is indicated as either apical constriction (AC) or planar polarized constriction (PPC).

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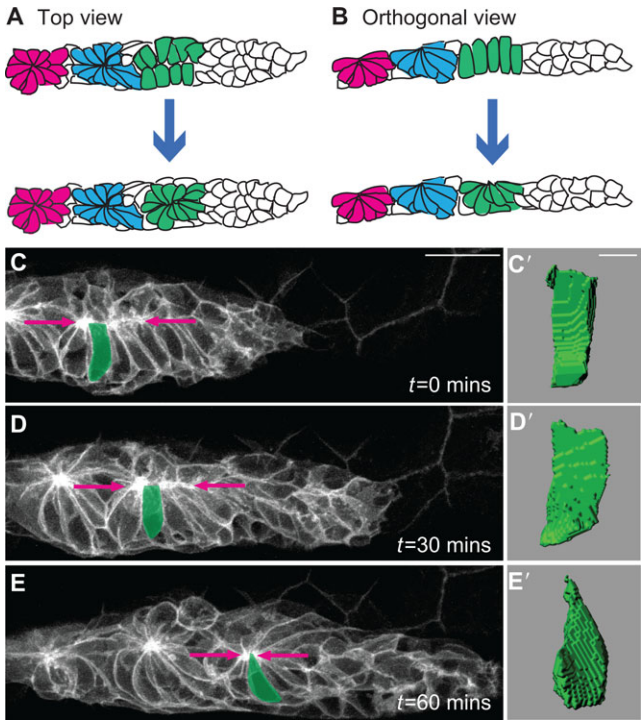

Fig. 2. Rosette formation in the zebrafish posterior lateral line. (A,B) In the zebrafish posterior lateral line (pLL), rosettes form in the leading (caudal) part of the pLL primordium, where columnar cells constrict apically to form a new rosette. Note that the apical ends of teardrop-shaped cells are oriented towards the rosette center. (C-E) Live imaging of rosette formation in a *Tg(claudinB:lynEGFP)* zebrafish embryo (Haas and Gilmour, 2006; modified with permission from Harding and Nechiporuk, 2012). Over the course of 1 hour, cells apically constrict (arrows) to form a rosette. (C′-E′) Three-dimensional reconstructions of the pseudocolored cell in C-E demonstrate progressive apical constriction of rosette-forming cells over time. Scale bars: 20 μm in C-E; 4 μm in C′-E′.

Fgf10a are expressed in the rosette-forming, leading region of the pLLp. Although the requirement for FGF ligands during apical constriction has not been explicitly tested, FGF receptor (FGFR) activation is required for the formation of new rosettes (Harding and Nechiporuk, 2012; Lecaudey et al., 2008; Nechiporuk and Raible, 2008). Following conditional inhibition of FGFR signaling, cells in the leading portion of the pLLp fail to apically constrict (Harding and Nechiporuk, 2012), presumably underlying the loss of rosette formation, while previously assembled rosettes remain intact. Recent work showed that the Ras/MAP kinase (MAPK) pathway is activated downstream of FGFR in the pLLp (Harding and Nechiporuk, 2012); an absence of Ras/MAPK signaling phenocopies the loss of FGFR activity. The absence of FGFR/Ras/MAPK signaling results in the loss of myosin-II activation and apically localized Rock2a, suggesting that this pathway acts to control apical constriction by mediating Rock localization (Harding and Nechiporuk, 2012). Additionally, *shroom3* is a transcriptional target of FGF signaling in the pLLp (Ernst et al., 2012). Collectively, these data suggest that FGFR/Ras/MAPK signaling activates the acto-myosin cytoskeleton by inducing *shroom3* transcription. Subsequently, newly activated Shroom3 drives apical localization of Rock2a, resulting in activation of myosin-II.

Interestingly, the loss of FGFR, Ras, MAPK or Shroom3 does not affect the establishment of apical polarity in cells prior to rosette formation; when these molecules are inhibited, cells are polarized with cytoskeletal components properly assembled in apical domains of columnar cells that are poised to apically constrict and form rosettes (Ernst et al., 2012; Harding and Nechiporuk, 2012). This evidence suggests that, in addition to the FGFR/Ras/MAPK pathway, unidentified signal(s) promote the establishment of polarity and cytoskeletal recruitment.

Drosophila epithelial morphogenesis

During *Drosophila* embryogenesis, a series of cellular rearrangements promote the doubling of body length and the formation of an elongated body axis (Bertet et al., 2004; Blankenship et al., 2006; Irvine and Wieschaus, 1994), and live-imaging studies have shown that these rearrangements include the formation and resolution of rosettes (Blankenship et al., 2006). Similar to rosette formation during zebrafish NM development, this process is myosin-II dependent. The rosettes formed during *Drosophila* epithelial morphogenesis also display enrichment of myosin-II and F-actin at the rosette center, although they are composed of fewer (5-11) cells than pLLp rosettes. In contrast to pLLp rosettes, *Drosophila* epithelial rosettes meet at a vertex that spans the lateral height of cells (Fig. 3). Furthermore, the majority of cells within the developing tissue appear to participate in rosette formation (Blankenship et al., 2006), suggesting a crucial role for rosette formation and resolution in *Drosophila* epithelial morphogenesis and a mechanism by which local cell rearrangements drive global changes in tissue shape.

In contrast to rosette formation in the zebrafish pLLp, rosette formation during *Drosophila* embryonic epithelial elongation relies on planar polarized constriction and is driven by the differential recruitment of cytoskeletal molecules to cell-cell junctions (Blankenship et al., 2006). Intercalating cells in the epithelium initially establish planar polarity, which is first identifiable by the asymmetric enrichment of F-actin at the AP borders between cells (Blankenship et al., 2006). Myosin-II and its activator Rho kinase (Rok, a homolog of Rock) also accumulate at AP borders and promote localized junctional contraction (Bertet et al., 2004; Zallen and Wieschaus, 2004; Simões et al., 2010) and, recently, the scaffolding protein Shroom was shown to be required for Rok and myosin-II junctional activation, downstream of Rok signaling (Simões et al., 2014). Subsequently, AJs, as assayed by staining for β-catenin (Armadillo) and E-cadherin (Shotgun), become enriched

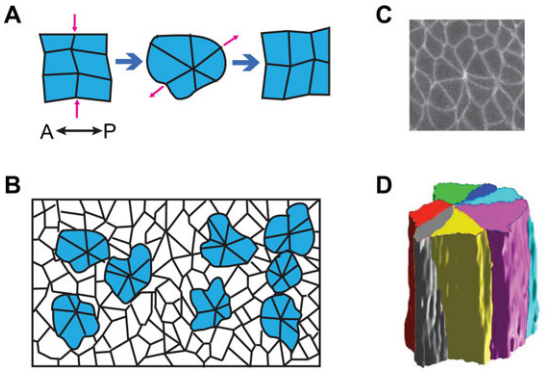

Fig. 3. Rosette formation during *Drosophila* epithelial elongation. (A) Rosettes form and resolve along perpendicular axes to drive cellular rearrangement and epithelial elongation. A, anterior; P, posterior. (B) Schematized apical view of the epithelium. Note that many cells in the epithelium participate in rosette formation (see Blankenship et al., 2006). (C) A rosette in a wild-type *Drosophila* embryo expressing Resille:GFP, a membrane marker. (D) Three-dimensional reconstruction of an epithelial rosette. Note that in this rosette type, cells are joined along their lateral lengths, in contrast to apically constricted rosettes.

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at DV cell interfaces (Blankenship et al., 2006). Developing rosettes are thus identifiable by F-actin and myosin-II accumulation at AP interfaces and the accumulation of Par-3 and E-cadherin at new cell membrane interfaces (Blankenship et al., 2006). The cues that regulate this positioning of planar polarized molecules along the AP axis are independent of the Frizzled-PCP pathway (Zallen and Wieschaus, 2004). Instead, they require striped expression of pair-rule genes (see Glossary, Box 1) such as *even-skipped* (*eve*) and *runt* (Zallen and Wieschaus, 2004). In the absence of Eve and Runt, the expression of planar polarized components during epithelial elongation is lost. By contrast, when Eve and Runt are ectopically expressed, planar polarized components are reoriented. Further work is necessary to provide a link between pair-rule gene expression and planar polarity.

After the assembly of polarized cytoskeletal molecules, cells along the DV axis form a transient rosette (Figs 1 and 3). The rosette subsequently resolves into an array along the AP axis. The planar polarized distribution of cytoskeletal molecules allows shrinkage of AP cell interfaces by two related mechanisms: (1) planar polarized junctional contraction (Fernandez-Gonzalez et al., 2009; Rauzi et al., 2010); and (2) alternating myosin-II flow directed by polarized fluctuations in E-cadherin levels (Levayer and Lecuit, 2013; Rauzi et al., 2010). Notably, another process, the contraction of apically localized myosin-II (also called medial myosin-II), contributes to convergent extension during *Drosophila* epithelial elongation. Recent work has demonstrated that this separate, apically localized pool of pulsating myosin-II is connected to AJs. Whereas this apical myosin-II clearly contributes to body elongation, it seems dispensable for rosette formation; in mutants in which apical myosin-II is specifically disrupted, rosettes still form, although apical myosin-II forms rings around vertices instead of its usual tight localization around rosette centers (Sawyer et al., 2011). Thus, myosin-II-dependent rosette formation and resolution is just one of at least two myosin-dependent mechanisms that lead to epithelial tissue elongation.

Rosette-based epithelial elongation is dependent on cytoskeletal activity. Elongation is reduced in embryos deficient for myosin-II heavy chain, myosin regulatory light chain (MRLC), Rok, which activates MRLC, or Shroom (Bertet et al., 2004; Simões et al., 2010, 2014). Furthermore, disruption of Par-3, Par-6 or aPKC also impairs elongation (Blankenship et al., 2006; Simões et al., 2010; Zallen and Wieschaus, 2004), potentially due to roles for these proteins in rosette formation. In addition, microtubules are important for the asymmetric localization of a mobile E-cadherin pool and Par-3 complex (Bulgakova et al., 2013).

Finally, it has been shown that the tyrosine kinase Abl is required for rosette formation via phosphorylation of β-catenin at the interface of cells that will shrink to form the rosette center (Tamada et al., 2012). This phosphorylation is required for the planar polarized distribution of β-catenin prior to rosette formation. The upstream activator of Abl has not been identified in the context of *Drosophila* axis elongation, although Abl is regulated by EGF (Hopkins et al., 2012), platelet-derived growth factor (PDGF) (Brasher and Van Etten, 2000), EphA (Harbott and Nobes, 2005) and Robo (Rhee et al., 2002) in other contexts. Thus, these extracellular cues are prime candidates for activators of Abl, and hence rosette formation, during epithelial morphogenesis in *Drosophila*. Interestingly, other cellular rearrangements called neighbor exchanges, which involve four cells that meet at a central vertex, also occur during epithelial elongation (Bertet et al., 2004; Rauzi et al., 2010); however, although these rearrangements promote epithelial elongation, they are not dependent on Abl (Tamada et al., 2012).

Drosophila eye development

Morphogenesis of the *Drosophila* compound eye also relies on the formation of multicellular rosettes. The compound eye, which is composed of 800 photosensory units called ommatidia, is derived from the eye imaginal disc. The imaginal disc is a sheet of epithelial cells that proliferate during larval life, differentiating during the third instar stage (Wolff and Ready, 1993). During this stage, the morphogenetic furrow, an indentation in the eye disc, sweeps across the eye disc from posterior to anterior. Following passage of the furrow, cells at the posterior margin of the furrow rearrange into organized multicellular structures that progress to form 'arcs' and then 'rosettes' (Fig. 4). As rosettes form, photoreceptors begin to differentiate, with rosettes finally giving rise to 'preclusters' composed of photoreceptors (Ready et al., 1976).

The rosettes that form during compound eye development evolve from nine- to ten-cell-wide lines of cells (Fig. 4) (Brown et al., 2006; Escudero et al., 2007; Robertson et al., 2012). These lines evolve into arcs, which are composed of six to seven cells surrounding the 'core' cells that are eventually excluded from the rosette. Each arc then 'zips up' into a rosette conformation, which initially contains up to seven cells joined at a central vertex (Fig. 4). During this period, markers of neuronal maturation are detectable in presumptive photoreceptors (Brown et al., 2006). Over time, core cells are excluded, progressively giving rise to a six-cell intermediate and then a five-cell rosette (Robertson et al., 2012).

In the absence of three-dimensional reconstruction studies, it is difficult to determine whether rosette formation in the *Drosophila* eye is based on apical or planar polarized constriction, although the localization of Par-3 and myosin-II to reciprocal domains prior to rosette formation implies a planar polarity-based mechanism (Robertson et al., 2012). Similar to rosette formation in other contexts, rosette formation in the *Drosophila* eye disc is myosin-II dependent; rosettes and their precursors (arcs) are completely lost

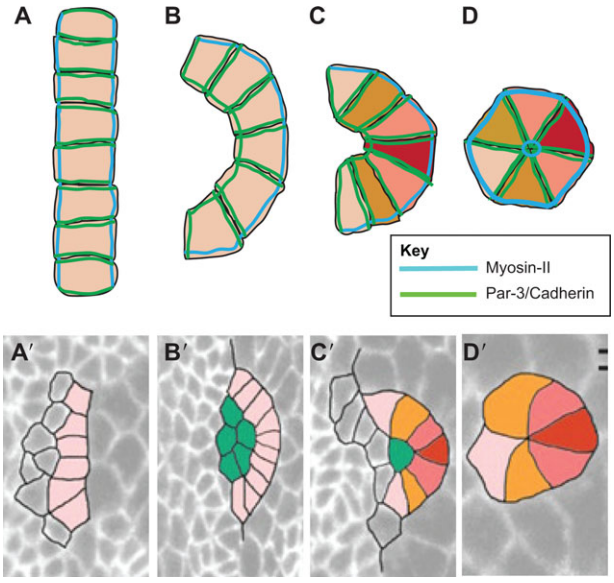

Fig. 4. Rosette formation during *Drosophila* ommatidial development. (A) Lines of up to ten cells form prior to rosette formation, with complementary localization of myosin-II and Par-3/cadherin. (B-D) Cells transition into arcs (B,C), which subsequently form the five-cell rosette (D), which contains a myosin-II-rich rosette center. During rosette formation, ommatidial cell fate also begins to be specified; the different colors represent distinct cell fates. (A′-D′) Formation of lines (A′), arcs (B′,C′) and rosettes (D′) in the eye disc (modified with permission from Robertson et al., 2012).

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following overexpression of dominant-negative myosin-II heavy chain (Escudero et al., 2007; Robertson et al., 2012). Activated myosin-II is first enriched at the posterior AJs of aligned cells, prior to arc/rosette formation. As rosettes form, myosin-II remains localized at the outer rosette membranes, but is also present in the rosette center (Escudero et al., 2007; Robertson et al., 2012). By contrast, E-cadherin and Par-3 are enriched in the mediolateral AJs, perpendicular to posterior AJs. This mediolateral enrichment of E-cadherin and Par-3 is dependent on myosin-II, as disruption of myosin-II causes uniform distribution of E-cadherin and Par-3 (Robertson et al., 2012). Interestingly, Par-3 seems to reciprocally limit myosin-II localization. The kinases Drak and Rok, which are important for the phosphorylation of Sqh (a MRLC homolog), are also redundantly required for myosin-II-dependent rosette formation, although neither kinase is required for E-cadherin or Par-3 localization (Robertson et al., 2012). Overall, the arrangement of myosin-II and Par-3 is similar to the distribution of cytoskeletal components observed during *Drosophila* embryonic epithelial elongation, in which Par-3 is required to restrict myosin to specific planar edges (Simões et al., 2010).

The factors that drive rosette formation in the eye disc include EGF receptor (EGFR), the activity of which is required for the formation of both rosettes and arcs (Brown et al., 2006). The basic helix-loop-helix transcription factor Atonal (Ato) is required upstream of EGFR (Baonza et al., 2001); *ato*-expressing cells upregulate the expression of *rhomboid*, and these cells presumably release ligand to activate EGFR. *ato* is initially expressed in all cells before they enter the morphogenetic furrow, but becomes restricted to clusters of cells in the furrow and finally to single cells within rosettes (Brown et al., 2006). Although both Atonal and EGFR are required for cluster formation at the posterior margin of the morphogenetic furrow, they play distinct roles in the cell shape changes that drive rosette formation (Brown et al., 2006). Independent of EGFR activation, Ato is required for the upregulation of cadherins (Brown et al., 2006), for directing the localization of Par-3 and myosin-II to their appropriate membranes, and for photoreceptor differentiation (Robertson et al., 2012). EGFR activation also plays a distinct role in rosette formation, as EGFR is required for the formation of arcs and rosettes and its overactivity leads to the over-recruitment of cells into rosettes (Brown et al., 2006) and increased levels of Sqh (Escudero et al., 2007). By contrast, loss of EGFR signaling causes loss of the myosin-II rings that surround rosettes (Escudero et al., 2007). The molecular mediators of EGFR signaling in this context have been elucidated; overexpression of activated Ras leads to a similar over-recruitment phenotype, apparently upstream of the ETS transcription factor Pointed (Brown et al., 2006). Interestingly, Ato-dependent upregulation of cadherin occurs in the absence of EGFR, suggesting that E-cadherin levels are regulated independently of EGFR signaling. These data suggest a two-step model of rosette formation in the *Drosophila* eye in which Ato activates E-cadherin, and EGFR-Ras-MAPK-Pointed signaling subsequently promotes rosette formation by an as yet unknown mechanism. Interestingly, EGFR signaling is apparently dispensable for the formation of the cellular lines that are prerequisite for arc and rosette formation (Robertson et al., 2012), suggesting that it acts during the later stages of rosette formation.

Kidney tubule elongation

Recently, rosettes have also been identified in the developing vertebrate kidney. Lienkamp et al. discovered rosettes in the mouse kidney tubule collecting duct epithelium (Lienkamp et al., 2012). Live imaging of the single giant nephron of the *Xenopus laevis* kidney revealed similar rosettes that form along the mediolateral axis and resolve in perpendicular orientation (along the proximodistal axis) (Lienkamp et al., 2012). As in other tissues, myosin-II activity is required for rosette formation in this context, and for nephron morphogenesis (Lienkamp et al., 2012). This process is strikingly similar to that occurring during *Drosophila* epithelial morphogenesis; myosin-II is enriched along mediolateral cell-cell boundaries, perpendicular to the axis of kidney epithelium elongation (Lienkamp et al., 2012). Notably, a four-cell neighbor exchange is observed in the context of kidney tubule elongation, similar to that observed during *Drosophila* epithelial elongation. In this case, four cells meet at a vertex; however, these four-cell transitions are not arranged in a polarized manner and are therefore likely to be under different molecular control than higher order (5+ cell) rosettes (Lienkamp et al., 2012). Overall, this similarity between kidney tubule elongation and *Drosophila* epithelial elongation suggests that rosette formation and resolution are deeply conserved mechanisms driving tissue morphogenesis.

Non-canonical Wnt signaling mediated by the cytoplasmic PCP component Dishevelled has been implicated in kidney tubule elongation. Consistent with this, the expression of dominant-negative dishevelled 2 or the conditional deletion of *Wnt9b* in mice results in a reduction in rosette number and causes a failure of rosettes to resolve in the proper orientation; both manipulations disrupt tubule elongation (Karner et al., 2009; Lienkamp et al., 2012). By contrast, disruption of PCP signaling molecules, including Dishevelled, has no impact on rosette formation during *Drosophila* embryonic epithelial morphogenesis (Zallen and Wieschaus, 2004). These findings suggest that similar rosette-based cellular rearrangements are required for kidney tubule elongation and *Drosophila* epithelial elongation but that the upstream signaling is evolutionarily divergent. This role for PCP signaling in driving rosette formation is not an isolated case, as PCP-dependent rosette formation mediates proper development of the mouse visceral endoderm (see below; Trichas et al., 2012).

Pancreatic branching morphogenesis

The adult pancreas is a highly branched tubular network consisting of exocrine glands required for digestion and endocrine glands required for glucose homeostasis. How endocrine pancreatic branches arise is largely unknown, but recently it has been shown that apically constricted rosettes form transiently during pancreas morphogenesis (Villasenor et al., 2010). Pancreatic progenitor cells undergo numerous shape changes and rearrangements during lumen formation, but it was shown that single cells undergo asynchronous apical constriction, with the progressive emergence of apically constricted cells corresponding to the formation of rosettes (Fig. 5A), reminiscent of those seen in other systems. These pancreatic rosettes are transient: as development progresses, rosettes open at the center to form microlumens (Villasenor et al., 2010). These microlumens then connect to one another to form a continuous lumen.

The formation of pancreatic rosettes involves the activation of Eph receptors; the concomitant inhibition of EphB2 and EphB3 in mice causes gross morphological defects in the pancreas, including disorganized rosettes (Villasenor et al., 2010). Loss of EphB signaling also correlates with a decrease in membrane-associated β-catenin and E-cadherin, suggesting a loss of junctional integrity (Villasenor et al., 2010). This indicates that Eph signaling regulates the levels of β-catenin, thereby controlling rosette formation. In addition, the RhoGAP Stard13 is required for rosette formation during pancreas development (Petzold et al., 2013), as pancreatic cells lacking Stard13 show loss of apical F-actin and phospho-myosin-II, and reduced rosette formation (Petzold et al., 2013). These

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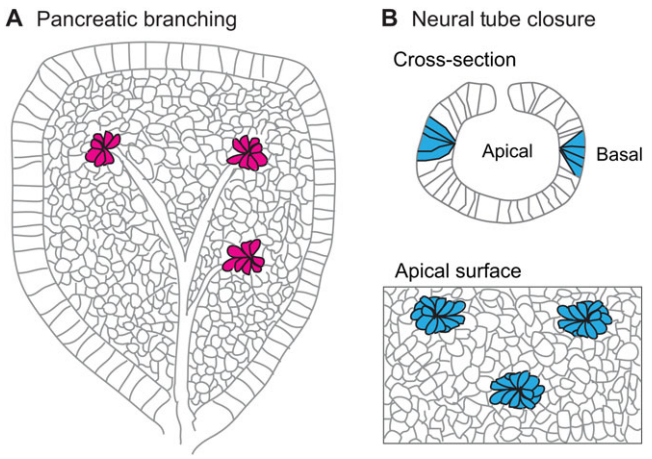

Fig. 5. Rosette formation during pancreatic branching morphogenesis and neural tube closure. (A) Rosettes in the developing pancreas. The rosettes formed subsequently open up to form microlumens. Modified with permission from Villasenor et al. (2010). (B) Rosettes in the developing neural tube form via apical constriction. Multiple rosettes are visible simultaneously in the apical surface of the neural tube.

data suggest that, although the extracellular signals that direct rosette formation are divergent, the cytoskeletal molecules involved in this process, including the Rho GTPases, are conserved across species.

Vertebrate axis formation

The primitive streak (see Glossary, Box 1) defines the axis of bilateral symmetry and the site of gastrulation. During primitive streak formation, epiblast cells ingress to form new germ layers, and cell intercalation is one of the mechanisms underlying the cellular rearrangements required for this event (Chuai and Weijer, 2008). Recently, rosettes have been described during this process. In the epiblast of the chick embryo, rosettes with actin-rich centers are visible within the developing primitive streak (Wagstaff et al., 2008), and rosette number increases as development progresses (Chuai and Weijer, 2008; Wagstaff et al., 2008; Yanagawa et al., 2011). These rosettes are dependent on Nodal signaling, as inhibition of Nodal pathway members results in reduced rosette numbers (Yanagawa et al., 2011). However, other molecules involved in the formation of these rosettes have not yet been elucidated, and it is also unclear whether this process is dependent on apical constriction or planar polarized constriction.

Rosettes have also been observed in an extraembryonic structure, termed the visceral endoderm (VE, see Glossary, Box 1), that is important for formation of the vertebrate axis in amniotes. The VE forms the outer layer of the egg-cylinder stage embryo. A specialized set of VE cells, the anterior visceral endoderm (AVE, see Glossary, Box 1), migrates within the VE and is responsible for correct orientation of the AP axis. The AVE must migrate through an intact epithelium, which is achieved though intercalation (Trichas et al., 2012). Recently, the migration of the AVE within the VE has been linked to rosette formation. The formation of these rosettes, and the orderliness of cells, is dependent on PCP signaling (Trichas et al., 2012); however, a more detailed description of these rosettes is necessary to determine whether apical or planar polarized constriction underlies their formation.

Zebrafish Kupffer's vesicle development

Rosettes have also been identified during the early stages of zebrafish development. The first organ formed in zebrafish embryos is Kupffer's vesicle (KV), a ciliated organ homologous to the mouse ventral node. KV is important for establishing left-right asymmetry (D'Amico and Cooper, 1997; Essner et al., 2005) and is derived from progenitor cells known as dorsal forerunner cells (DFCs, see Glossary, Box 1) (D'Amico and Cooper, 1997). During epiboly (see Glossary, Box 1), the DFCs become increasingly compact, eventually detach from the enveloping layer from which they derived, and form multiple rosette structures (Oteíza et al., 2008). These rosettes subsequently merge into a single rosette, which opens to form a lumen at the apical rosette center (Oteíza et al., 2008).

The molecular mechanisms that regulate rosette formation in the context of KV development remain elusive. However, PCP/non-canonical Wnt signaling molecules are important for rosette opening to form the KV lumen. Knockdown of the core PCP/non-canonical Wnt signaling component *prickle1a* results in the formation of multiple rosettes that fail to coalesce, possibly by affecting cellular adhesion properties (Oteíza et al., 2010). Although PCP signaling molecules are clearly important for the formation of these rosettes, it is not clear whether this process requires a planar polarized distribution of molecules or, alternatively, whether a non-canonical Wnt pathway triggers apical constriction, driving rosette formation. The requirement for myosin-II during rosette formation in the context of KV development is also currently unclear; however, myosin-II activity is required during later stages of KV development (Wang et al., 2012). Future experiments will reveal if KV rosettes form by a myosin-II-based mechanism, as would be predicted by the high conservation of myosin-II dependence in other systems.

Embryonic neurogenesis

Additionally, rosette structures have also been observed during formation of the vertebrate nervous system, including the inner surface of the closing neural tube of chicken embryos (Nishimura and Takeichi, 2008) and the neuroepithelium (Afonso and Henrique, 2006). Neurulation depends on a series of morphogenetic cellular movements that lead to neural tube closure (Copp and Greene, 2010). The importance of apical constriction in this process has been recognized and highly studied, and is dependent on myosin-II (Rolo et al., 2009). The scaffolding molecule Shroom3 is also important for neurulation in both mice and *Xenopus* (Haigo et al., 2003; Hildebrand and Soriano, 1999), as it is required in the apical localization of myosin-II (Hildebrand, 2005).

The neuroepithelial rosettes that arise during neural tube closure contain five or more cells joined at a vertex (Fig. 5B). The vertices are enriched for ZO-1 and phospho-MRLC. Similar to the situation seen in other contexts, Rock activity is required for rosette formation (Nishimura and Takeichi, 2008), and proper Rock localization is dependent on its molecular interaction with Shroom3 (Nishimura and Takeichi, 2008). Recently, a role for the FERM protein Lulu has also been described in the regulation of Shroom3-dependent neural tube formation in *Xenopus* (Chu et al., 2013), suggesting that a Lulu-Shroom3-Rock-MRLC pathway could regulate rosette formation. The extracellular, upstream signaling molecules that induce this pathway have not yet been elucidated. BMP has been implicated in the establishment of apicobasal polarity during neural tube closure (Eom et al., 2011), suggesting a potential role in rosette formation.

Rosettes have also been observed in the neuroepithelium of the closed chick neural tube following ectopic expression of Par3. These ectopic rosettes accumulate Par3 in rosette centers, as well as N-cadherin and β-catenin. These cells undergo cell cycle progression and neurogenesis, suggesting that Par3 is important for regulating the apical domain during neurogenesis; this also underscores the importance of Par3 in rosette formation (Afonso

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and Henrique, 2006). Although similar rosettes may not form under normal conditions of embryonic neurogenesis, this observation is intriguing because rosettes are found during neurogenesis in the adult brain (see below).

Rosette formation in adults and *in vitro*

In addition to the multiple modes of rosette formation observed during embryonic development, rosettes have been described in non-developmental contexts. For example, rosette-like structures termed 'pinwheels' have been found in the adult mammalian brain. Multiple types of tumors and cultured neural stem cells also form multicellular rosettes (Chen et al., 2012; Elkabetz and Studer, 2008; Elkabetz et al., 2008; Koch et al., 2009). These models highlight the importance of rosettes during both the development and the homeostasis of neural tissue.

Rosette structures in the adult neural stem cell niche

Recent work has identified rosette-like structures, originally termed pinwheels, in the adult mammalian brain (Mirzadeh et al., 2008). These rosettes are found in the ventral subventricular zone (V-SVZ, see Glossary, Box 1) and act as a niche for the neural stem cells (NSCs) that give rise to specific subtypes of olfactory neurons and glial cells (Merkle et al., 2007; Giachino et al., 2014). The rosette structure of the V-SVZ is unique to the lateral ventricular wall of the brain and is only found in areas of high neurogenic activity, suggesting that this cellular arrangement plays a crucial role in the adult NSC niche (reviewed by Ihrie and Alvarez-Buylla, 2011). Unlike the transient embryonic rosettes seen in many other systems, V-SVZ rosettes appear to be maintained throughout adulthood, although there is a decrease in rosette number with age (Shook et al., 2012).

The rosettes of the V-SVZ are composed of ependymal cells that contact both the cerebral-spinal fluid (CSF) of the ventricle and astrocyte-like NSCs, called type-B1 cells. On the lateral ventricle surface, the apical domains of ependymal cells surround the small apical ends of one or more type-B1 cells to form the rosette (Fig. 6) (Mirzadeh et al., 2008; Shen et al., 2008). The rosettes form the outer region of the V-SVZ NSC niche; internal to the rosette, the cell bodies of type-B1 cells are associated with type-C cells (which are the immediate progeny of B1 cells and act as intermediate progenitor cells), type-B2 cells (astrocyte-like cells that do not contact the ventricle), and type-A cells (more differentiated neuroblasts). The basal processes of type-B1 cells also contact blood vessels from within the rosette (reviewed by Ihrie and Alvarez-Buylla, 2011; Fuentealba et al., 2012). This arrangement of type-B1 cells allows them to sample the environment of the ventricle through their apical processes and to interact with the internal brain and vascular environment with their basal processes, thus promoting their quiescence or cell division appropriately (Fuentealba et al., 2012). In fact, recent work demonstrated that the loss of this rosette structure results in the premature differentiation of type-B1 NSCs and disruption of the stem cell niche (Kokovay et al., 2012). These data suggest that the rosette arrangement of the V-SVZ stem cell niche might allow type-B1 cells to respond to changes in the molecular makeup of the CSF.

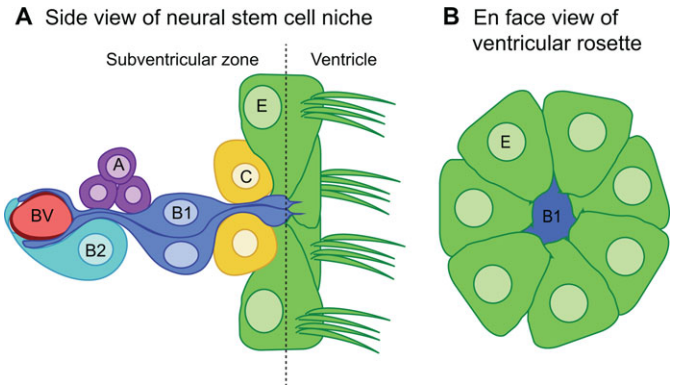

Fig. 6. Rosette structures in the adult neural stem cell niche. (A) The ventricular-subventricular (V-SVZ) adult neural stem cell niche. The niche is composed of ependymal (E) cells on the ventricular wall and astrocyte-like stem cells (B1 and B2) that, in the case of type-B1 cells, extend from the ventricular space to the subventricular space and make contact with blood vessels (BV) of the central nervous system. Type-B1 and -B2 cells give rise to rapidly proliferating progenitor cells (C and A) that will form new neurons and glial cells. (B) En face view of the lateral ventricular wall showing the rosette arrangement of ependymal cells surrounding the apical tips of type-B1 cells. See Fuentealbe et al. (2012).

Human embryonic stem cell-derived neural rosettes

During neuronal differentiation, human embryonic stem cells (hESCs) undergo morphogenic changes to form multicellular, radial structures that are similar to the rosettes described in *in vivo* systems (Chen et al., 2012; Elkabetz et al., 2008; Elkabetz and Studer, 2008; Zhang et al., 2001). The formation of these rosettes is dependent on the presence of FGF2 (Zhang et al., 2001), indicating that FGF may act as an extracellular activator of rosette formation in multiple contexts (hESCs and the zebrafish lateral line). These rosettes show apical accumulation of ZO-1 and N-cadherin (Elkabetz et al., 2008), both of which then correspond to the rosette center. Interestingly, these rosettes have the capacity to differentiate into broad neuronal subtypes (Elkabetz et al., 2008). Although the significance of rosette formation during this process is currently unknown, cells within rosettes have multipotent potential and are precursors for multiple cell types. Thus, it is tempting to speculate that rosettes are essential structural intermediates, creating a niche for NSCs, similar to the niche observed in the V-SVZ *in vivo*.

Common themes and principles

The appearance of cellular rosettes in several developmental contexts raises the question of the biological importance of rosettes in tissue morphogenesis. After analyzing the examples presented in this Review, we argue that there are at least three distinct cellular behaviors mediated through rosette formation: tissue elongation, lumen formation and maintenance of stemness.

Convergent extension (CE) is a dramatic morphogenetic event that culminates in tissue elongation and often requires cell intercalation. This intercalation results in tissue shrinkage in one direction and expansion in the perpendicular direction through relatively small movements by individual cells. The rosette-based mechanism of intercalation appears to be broadly utilized during CE, as many tissues undergoing CE form rosette intermediates, including the *Drosophila* epithelium, the kidney, the vertebrate primitive streak, and the neural tube. In these cases, rosettes are transient, often contain a relatively small number of cells (5-11), and resolve relatively quickly. At least in some instances, rosette resolution has been shown to be required for tissue elongation, implying that rosette formation might be a generalized mechanism contributing to tissue elongation. For example, during *Drosophila* epithelial elongation, interfering with rosettes through the knockdown of Abl reduces elongation (Tamada et al., 2012), arguing that rosette formation is essential for CE in some cases.

By contrast, rosettes in the contexts of the zebrafish pLLp, KV development and pancreatic branching, although also transient,

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undergo apical 'opening' to form a lumen, and, in the case of the pLLp, can include 20 or more cells. This implies that, in these cases, rosettes form intermediates that ultimately promote or are required for the formation of a lumen. The molecular mechanisms that lead to the transition from a rosette to an open lumen are still poorly understood and undoubtedly will be a focus of future studies.

Finally, and in at least two of the cases described above (adult neurogenesis in the SVZ and ESCs), rosettes appear to maintain the stemness of the tissue. It is thus likely that rosettes generate a specific microenvironment or niche that is required for a subset of cells associated with the rosettes to maintain their pluripotent state. This idea is supported by experiments in the adult V-SVZ that disrupt rosettes. When rosettes are lost, a subset of cells within the rosette prematurely differentiates. It is tempting to speculate that a very similar mechanism might be at play during the neuronal differentiation of hESCs; additional experiments are required to demonstrate whether this is indeed the case.

Our comparison of multiple examples of rosette-based tissue development argues that there are many extracellular signals that can trigger rosette formation, whether via apical constriction or planar polarity. Perhaps, as the extracellular cues that drive rosette formation in more contexts begin to be appreciated, some relationship between the extracellular cues that promote rosette formation and the resultant tissue might be discovered. Despite these differences, the core cytoskeletal molecules involved in rosette formation are highly conserved and broadly utilized. Given this high degree of conservation, it is not surprising that cytoskeletal molecules are essential for rosette formation. In addition, this finding underscores the importance of cytoskeletal molecules in cellular rearrangements. The recent appreciation of rosettes as a common cellular rearrangement during tissue formation suggests that rosettes are an essential feature of development and adult tissue maintenance in the mammalian brain.

Conclusions

As discussed here, multicellular rosette formation is a conserved feature of embryonic development and also occurs in adult and *in vitro* models. In the past 20 years, rosettes have been described as important intermediates in a wide variety of species and tissues. Although our understanding of the extracellular and intracellular changes that drive rosette formation is incomplete, recent studies have revealed both diversity and similarity among molecular regulators of rosette formation (see Table 1). Additionally, the many recently discovered developmental models of rosette formation imply that additional examples are likely to exist and might be uncovered in the near future. These yet-to-be described models might shed further light on the molecular mechanisms that promote rosette formation and the biological function of these unique structures.

Our relatively recent appreciation that rosette formation is a conserved developmental process in multiple tissues and species suggests that it must be a particularly biologically advantageous method of organ formation or tissue remodeling. Additionally, the majority of cytoskeletal molecules involved in rosette formation are evolutionarily conserved, suggesting that many biological organisms have the capacity to form such rosettes. Perhaps this is the reason for the relatively widespread use of rosettes during development across many species.

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Competing interests

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References

Afonso, C. and Henrique, D. (2006). PAR3 acts as a molecular organizer to define the apical domain of chick neuroepithelial cells. *J. Cell Sci.* **119**, 4293-4304.

Baonza, A., Casci, T. and Freeman, M. (2001). A primary role for the epidermal growth factor receptor in ommatidial spacing in the Drosophila eye. *Curr. Biol.* **11**, 396-404.

Bertet, C., Sulak, L. and Lecuit, T. (2004). Myosin-dependent junction remodelling controls planar cell intercalation and axis elongation. *Nature* **429**, 667-671.

Blankenship, J. T., Backovic, S. T., Sanny, J. S. P., Weitz, O. and Zallen, J. A. (2006). Multicellular rosette formation links planar cell polarity to tissue morphogenesis. *Dev. Cell* **11**, 459-470.

Brasher, B. B. and Van Etten, R. A. (2000). c-Abl has high intrinsic tyrosine kinase activity that is stimulated by mutation of the Src homology 3 domain and by autophosphorylation at two distinct regulatory tyrosines. *J. Biol. Chem.* **275**, 35631-35637.

Brown, K. E., Baonza, A. and Freeman, M. (2006). Epithelial cell adhesion in the developing Drosophila retina is regulated by Atonal and the EGF receptor pathway. *Dev. Biol.* **300**, 710-721.

Bulgakova, N. A., Grigoriev, I., Yap, A. S., Akhmanova, A. and Brown, N. H. (2013). Dynamic microtubules produce an asymmetric E-cadherin-Bazooka complex to maintain segment boundaries. *J. Cell Biol.* **201**, 887-901.

Chen, Y., Wang, Z., Xie, Y., Guo, X., Tang, X., Wang, S., Yang, S., Chen, K., Niu, Y. and Ji, W. (2012). Folic acid deficiency inhibits neural rosette formation and neuronal differentiation from rhesus monkey embryonic stem cells. *J. Neurosci. Res.* **90**, 1382-1391.

Chitnis, A. B., Nogare, D. D. and Matsuda, M. (2012). Building the posterior lateral line system in zebrafish. *Dev. Neurobiol.* **72**, 234-255.

Chu, C.-W., Gerstenzang, E., Ossipova, O. and Sokol, S. Y. (2013). Lulu regulates shroom-induced apical constriction during neural tube closure. *PLoS ONE* **8**, e81854.

Chuai, M. and Weijer, C. J. (2008). The mechanisms underlying primitive streak formation in the chick embryo. *Curr. Top. Dev. Biol.* **81**, 135-156.

Copp, A. J. and Greene, N. D. E. (2010). Defining a PARticular pathway of neural tube closure. *Dev. Cell* **18**, 1-2.

Dijkgraaf, S. (1963). The functioning and significance of the lateral-line organs. *Biol. Rev.* **38**, 51-105.

D'Amico, L. A. and Cooper, M. S. (1997). Spatially distinct domains of cell behavior in the zebrafish organizer region. *Biochem. Cell Biol.* **75**, 563-577.

Elkabetz, Y. and Studer, L. (2008). Human ESC-derived neural rosettes and neural stem cell progression. *Cold Spring Harb. Symp. Quant. Biol.* **73**, 377-387.

Elkabetz, Y., Panagiotakos, G., Al Shamy, G., Socci, N. D., Tabar, V. and Studer, L. (2008). Human ES cell-derived neural rosettes reveal a functionally distinct early neural stem cell stage. *Genes Dev.* **22**, 152-165.

Eom, D. S., Amarnath, S., Fogel, J. L. and Agarwala, S. (2011). Bone morphogenetic proteins regulate neural tube closure by interacting with the apicobasal polarity pathway. *Development* **138**, 3179-3188.

Ernst, S., Liu, K., Agarwala, S., Moratscheck, N., Avci, M. E., Nogare, D. D., Chitnis, A. B., Ronneberger, O. and Lecaudey, V. (2012). Shroom3 is required downstream of FGF signalling to mediate proneuromast assembly in zebrafish. *Development* **139**, 4571-4581.

Escudero, L. M., Bischoff, M. and Freeman, M. (2007). Myosin II regulates complex cellular arrangement and epithelial architecture in Drosophila. *Dev. Cell* **13**, 717-729.

Essner, J. J., Amack, J. D., Nyholm, M. K., Harris, E. B. and Yost, H. J. (2005). Kupffer's vesicle is a ciliated organ of asymmetry in the zebrafish embryo that initiates left-right development of the brain, heart and gut. *Development* **132**, 1247-1260.

Fernandez-Gonzalez, R., Simões, S. d. M., Röper, J.-C., Eaton, S. and Zallen, J. A. (2009). Myosin II dynamics are regulated by tension in intercalating cells. *Dev. Cell* **17**, 736-743.

Fuentealba, L. C., Obernier, K. and Alvarez-Buylla, A. (2012). Adult neural stem cells bridge their niche. *Cell Stem Cell* **10**, 698-708.

Ghysen, A. and Dambly-Chaudière, C. (2004). Development of the zebrafish lateral line. *Curr. Opin. Neurobiol.* **14**, 67-73.

Ghysen, A. and Dambly-Chaudière, C. (2007). The lateral line microcosmos. *Genes Dev.* **21**, 2118-2130.

Giachino, C., Basak, O., Lugert, S., Knuckles, P., Obernier, K., Fiorelli, R., Frank, S., Raineteau, O., Alvarez-Buylla, A. and Taylor, V. (2014). Molecular diversity subdivides the adult forebrain neural stem cell population. *Stem Cells* **32**, 70-84.

Gibson, M. C. and Perrimon, N. (2003). Apicobasal polarization: epithelial form and function. *Curr. Opin. Cell Biol.* **15**, 747-752.
Gompel, N., Cubedo, N., Thisse, C., Thisse, B., Dambly-Chaudière, C. and Ghysen, A. (2001). Pattern formation in the lateral line of zebrafish. *Mech. Dev.* **105**, 69-77.
Haas, P. and Gilmour, D. (2006). Chemokine signaling mediates self-organizing tissue migration in the zebrafish lateral line. *Dev. Cell* **10**, 673-680.
Haigo, S. L., Hildebrand, J. D., Harland, R. M. and Wallingford, J. B. (2003). Shroom induces apical constriction and is required for hingepoint formation during neural tube closure. *Curr. Biol.* **13**, 2125-2137.
Harbott, L. K. and Nobes, C. D. (2005). A key role for Abl family kinases in EphA receptor-mediated growth cone collapse. *Mol. Cell. Neurosci.* **30**, 1-11.
Harding, M. J. and Nechiporuk, A. V. (2012). Fgfr-Ras-MAPK signaling is required for apical constriction via apical positioning of Rho-associated kinase during mechanosensory organ formation. *Development* **139**, 3130-3135.
Hildebrand, J. D. (2005). Shroom regulates epithelial cell shape via the apical positioning of an actomyosin network. *J. Cell Sci.* **118**, 5191-5203.
Hildebrand, J. D. and Soriano, P. (1999). Shroom, a PDZ domain-containing actin-binding protein, is required for neural tube morphogenesis in mice. *Cell* **99**, 485-497.
Hopkins, S., Linderoth, E., Hantschel, O., Suarez-Henriques, P., Pilia, G., Kendrick, H., Smalley, M. J., Superti-Furga, G. and Ferby, I. (2012). Mig6 is a sensor of EGF receptor inactivation that directly activates c-Abl to induce apoptosis during epithelial homeostasis. *Dev. Cell* **23**, 547-559.
Ihrie, R. A. and Alvarez-Buylla, A. (2011). Lake-front property: a unique germinal niche by the lateral ventricles of the adult brain. *Neuron* **70**, 674-686.
Irvine, K. D. and Wieschaus, E. (1994). Cell intercalation during Drosophila germband extension and its regulation by pair-rule segmentation genes. *Development* **120**, 827-841.
Karner, C. M., Chirumamilla, R., Aoki, S., Igarashi, P., Wallingford, J. B. and Carroll, T. J. (2009). Wnt9b signaling regulates planar cell polarity and kidney tubule morphogenesis. *Nat. Genet.* **41**, 793-799.
Koch, P., Opitz, T., Steinbeck, J. A., Ladewig, J. and Brüstle, O. (2009). A rosette-type, self-renewing human ES cell-derived neural stem cell with potential for in vitro instruction and synaptic integration. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 3225-3230.
Kokovay, E., Wang, Y., Kusek, G., Wurster, R., Lederman, P., Lowry, N., Shen, Q. and Temple, S. (2012). VCAM1 is essential to maintain the structure of the SVZ niche and acts as an environmental sensor to regulate SVZ lineage progression. *Cell Stem Cell* **11**, 220-230.
Lecaudey, V., Cakan-Akdogan, G., Norton, W. H. J. and Gilmour, D. (2008). Dynamic Fgf signaling couples morphogenesis and migration in the zebrafish lateral line primordium. *Development* **135**, 2695-2705.
Levayer, R. and Lecuit, T. (2013). Oscillation and polarity of E-cadherin asymmetries control actomyosin flow patterns during morphogenesis. *Dev. Cell* **26**, 162-175.
Lienkamp, S. S., Liu, K., Karner, C. M., Carroll, T. J., Ronneberger, O., Wallingford, J. B. and Walz, G. (2012). Vertebrate kidney tubules elongate using a planar cell polarity-dependent, rosette-based mechanism of convergent extension. *Nat. Genet.* **44**, 1382-1387.
Ma, E. Y. and Raible, D. W. (2009). Signaling pathways regulating zebrafish lateral line development. *Curr. Biol.* **19**, R381-R386.
Martin, A. C. and Goldstein, B. (2014). Apical constriction: themes and variations on a cellular mechanism driving morphogenesis. *Development* **141, 1987-1998**.
Merkle, F. T., Mirzadeh, Z. and Alvarez-Buylla, A. (2007). Mosaic organization of neural stem cells in the adult brain. *Science* **317**, 381-384.
Mirzadeh, Z., Merkle, F. T., Soriano-Navarro, M., Garcia-Verdugo, J. M. and Alvarez-Buylla, A. (2008). Neural stem cells confer unique pinwheel architecture to the ventricular surface in neurogenic regions of the adult brain. *Cell Stem Cell* **3**, 265-278.
Montgomery, J., Carton, G., Voigt, R., Baker, C. and Diebel, C. (2000). Sensory processing of water currents by fishes. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **355**, 1325-1327.
Nechiporuk, A. and Raible, D. W. (2008). FGF-dependent mechanosensory organ patterning in zebrafish. *Science* **320**, 1774-1777.
Nishimura, T. and Takeichi, M. (2008). Shroom3-mediated recruitment of Rho kinases to the apical cell junctions regulates epithelial and neuroepithelial planar remodeling. *Development* **135**, 1493-1502.
Oteíza, P., Köppen, M., Concha, M. L. and Heisenberg, C.-P. (2008). Origin and shaping of the laterality organ in zebrafish. *Development* **135**, 2807-2813.
Oteíza, P., Köppen, M., Krieg, M., Pulgar, E., Farias, C., Melo, C., Preibisch, S., Müller, D., Tada, M., Hartel, S. et al. (2010). Planar cell polarity signalling regulates cell adhesion properties in progenitors of the zebrafish laterality organ. *Development* **137**, 3459-3468.
Petzold, K. M., Naumann, H. and Spagnoli, F. M. (2013). Rho signalling restriction by the RhoGAP Stard13 integrates growth and morphogenesis in the pancreas. *Development* **140**, 126-135.
Putzke, A. P. and Rothman, J. H. (2003). Gastrulation: PARtaking of the bottle. *Curr. Biol.* **13**, R223-R225.
Rauzi, M., Lenne, P.-F. and Lecuit, T. (2010). Planar polarized actomyosin contractile flows control epithelial junction remodelling. *Nature* **468**, 1110-1114.
Ready, D. F., Hanson, T. E. and Benzer, S. (1976). Development of the Drosophila retina, a neurocrystalline lattice. *Dev. Biol.* **53**, 217-240.
Rhee, J., Mahfooz, N. S., Arregui, C., Lilien, J., Balsamo, J. and VanBerkum, M. F. A. (2002). Activation of the repulsive receptor Roundabout inhibits N-cadherin-mediated cell adhesion. *Nat. Cell Biol.* **4**, 798-805.
Robertson, F., Pinal, N., Fichelson, P. and Pichaud, F. (2012). Atonal and EGFR signalling orchestrate rok- and Drak-dependent adherens junction remodelling during ommatidia morphogenesis. *Development* **139**, 3432-3441.
Rolo, A., Skoglund, P. and Keller, R. (2009). Morphogenetic movements driving neural tube closure in Xenopus require myosin IIB. *Dev. Biol.* **327**, 327-338.
Sawyer, J. M., Harrell, J. R., Shemer, G., Sullivan-Brown, J., Roh-Johnson, M. and Goldstein, B. (2010). Apical constriction: a cell shape change that can drive morphogenesis. *Dev. Biol.* **341**, 5-19.
Sawyer, J. K., Choi, W., Jung, K.-C., He, L., Harris, N. J. and Peifer, M. (2011). A contractile actomyosin network linked to adherens junctions by Canoe/afadin helps drive convergent extension. *Mol. Biol. Cell* **22**, 2491-2508.
Shen, Q., Wang, Y., Kokovay, E., Lin, G., Chuang, S.-M., Goderie, S. K., Roysam, B. and Temple, S. (2008). Adult SVZ stem cells lie in a vascular niche: a quantitative analysis of niche cell-cell interactions. *Cell Stem Cell* **3**, 289-300.
Shook, B. A., Manz, D. H., Peters, J. J., Kang, S. and Conover, J. C. (2012). Spatiotemporal changes to the subventricular zone stem cell pool through aging. *J. Neurosci.* **32**, 6947-6956.
Simões, S. d. M., Blankenship, J. T., Weitz, O., Farrell, D. L., Tamada, M., Fernandez-Gonzalez, R. and Zallen, J. A. (2010). Rho-kinase directs Bazooka/Par-3 planar polarity during Drosophila axis elongation. *Dev. Cell* **19**, 377-388.
Simões, S. d. M., Mainieri, A. and Zallen, J. A. (2014). Rho GTPase and Shroom direct planar polarized actomyosin contractility during convergent extension. *J. Cell Biol.* **204**, 575-589.
Tamada, M., Farrell, D. L. and Zallen, J. A. (2012). Abl regulates planar polarized junctional dynamics through β-catenin tyrosine phosphorylation. *Dev. Cell* **22**, 309-319.
Trichas, G., Smith, A. M., White, N., Wilkins, V., Watanabe, T., Moore, A., Joyce, B., Sugnaseelan, J., Rodriguez, T. A., Kay, D. et al. (2012). Multi-cellular rosettes in the mouse visceral endoderm facilitate the ordered migration of anterior visceral endoderm cells. *PLoS Biol.* **10**, e1001256.
Villasenor, A., Chong, D. C., Henkemeyer, M. and Cleaver, O. (2010). Epithelial dynamics of pancreatic branching morphogenesis. *Development* **137**, 4295-4305.
Wagstaff, L. J., Bellett, G., Mogensen, M. M. and Münsterberg, A. (2008). Multicellular rosette formation during cell ingression in the avian primitive streak. *Dev. Dyn.* **237**, 91-96.
Wang, G., Manning, M. L. and Amack, J. D. (2012). Regional cell shape changes control form and function of Kupffer's vesicle in the zebrafish embryo. *Dev. Biol.* **370**, 52-62.
Wolff, T. and Ready, D. F. (1993). Pattern formation in the Drosophila retina. In *The Development of Drosophila Melanogaster*, pp. 1277-1326. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
Yanagawa, N., Sakabe, M., Sakata, H., Yamagishi, T. and Nakajima, Y. (2011). Nodal signal is required for morphogenetic movements of epiblast layer in the pre-streak chick blastoderm. *Dev. Growth Differ.* **53**, 366-377.
Zallen, J. A. and Wieschaus, E. (2004). Patterned gene expression directs bipolar planar polarity in Drosophila. *Dev. Cell* **6**, 343-355.
Zhang, S.-C., Wernig, M., Duncan, I. D., Brüstle, O. and Thomson, J. A. (2001). In vitro differentiation of transplantable neural precursors from human embryonic stem cells. *Nat. Biotechnol.* **19**, 1129-1133.