

# Tissue patterning and cellular mechanics

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In development, cells organize into biological tissues through cell growth, migration, and differentiation. Globally, this process is dictated by a genetically encoded program in which secreted morphogens and cell–cell interactions prompt the adoption of unique cell fates. Yet, at its lowest level, development is achieved through the modification of cell–cell adhesion and actomyosin-based contractility, which set the level of tension within cells and dictate how they pack together into tissues. The regulation of tension within individual cells and across large groups of cells is a major driving force of tissue organization and the basis of all cell shape change and cell movement in development.

The complex networks of gene expression and changes in epigenetic state that accompany the adoption of cell fates in development ultimately serve to modify the physical properties and behaviors of cells. Operating at the largest scale are the systems that organize cells into patterns. These self-propagating systems of secreted morphogens and cell–cell interactions generate tissue domains at regular intervals and produce gradients of chemical and mechanical signals that evolve as an organism develops. This confers unique identities to cells as a function of distance from the source of the signal. These mechanisms of tissue patterning achieve their effects by altering the mechanical properties of large groups of cells, enabling them to segregate from their peers on the basis of differential adhesion and cortical tension.

Further down, acting within and between cells, are highly conserved mechanisms of spatially regulating actin dynamics, myosin II–dependent contractility, and membrane trafficking. These events enable cells to refine large-scale tissue patterns by polarizing intracellular components with respect to tissue axes and coordinating this polarity over large distances. Finally, at the smallest scale are molecules associated with cell–cell and cell–matrix junctions that sense and respond to the forces experienced by the cell, which modulate the strength of adhesion and cortical contractility, the activity of mechanosensitive signaling pathways, and feed back into large-scale patterning mechanisms.

Advances in our understanding of cell and developmental biology over the last 50 years and the powerful technologies that have supported them (Abercrombie and Heaysman, 1953; Petran et al., 1986; Denk et al., 1995; Keller et al., 2008;

Lippincott-Schwartz, 2011; Chen et al., 2014) have allowed us to uncover fundamental mechanical principles underlying tissue organization and patterning. These principles all involve the spatial regulation of cell–cell adhesion, actin dynamics, and actomyosin-based contractility.

## Mechanisms of tissue patterning

Ordered patterns are found throughout nature, but their frequency and diversity are perhaps best appreciated in biology in the spots and stripes of mammals and fish (Kondo and Asai, 1995; Yamaguchi et al., 2007; Kondo and Miura, 2010), the pigmentation patterns of bird feathers (Richardson et al., 1990; Prum and Williamson, 2002), and the spiral growth of plant leaves (Holloway, 2010) and mollusk shells (Meinhardt, 2003). In his legendary book, *On Growth and Form*, D'Arcy Thompson (1917) ingeniously hypothesized a connection between the principles of self-organization that drive the emergence of patterns in inorganic matter and those underlying biological order, delineating simple mathematical principles that could explain patterns of tissue growth in nature and suggesting simple relationships between the anatomies of related species.

## Turing reaction–diffusion systems

Inspired by this work, Alan Turing devised a theoretical framework that bridged the inorganic world of chemical reactions and physical law and the world of biological pattern formation. In *The Chemical Basis of Morphogenesis*, Turing (1952) formulated the conditions in which cells, essentially as autonomous machines processing and secreting diffusible morphogens according to certain rules, could give rise to the repeating patterns we observe in nature. In a nutshell, if a cell produces two morphogens with different rates of diffusion, one an activator and the other an inhibitor, and the first morphogen stimulates both its own production as well as that of its inhibitor, the two could give rise to a stable equilibrium with well-defined regions of activation and inhibition. The activator, because it diffuses slowly, concentrates and acts locally, whereas the inhibitor diffuses quickly but can only act over a limited distance, leading to a standing wave pattern of activation and the generation of a long-range, periodic tissue pattern (Fig. 1 A). A key feature of a Turing reaction–diffusion system is that very small, transient differences in morphogen concentration within a homogeneous population of cells can be rapidly amplified and propagated over large distances. By tuning the parameters of these systems, virtually all of the breathtaking array of patterns observed in nature, from the spots of leopards and stripes of fish

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Abbreviations used in this paper: AP, anterior–posterior; BMP, bone morphogenetic protein; FGF, fibroblast growth factor; PCP, planar cell polarity.

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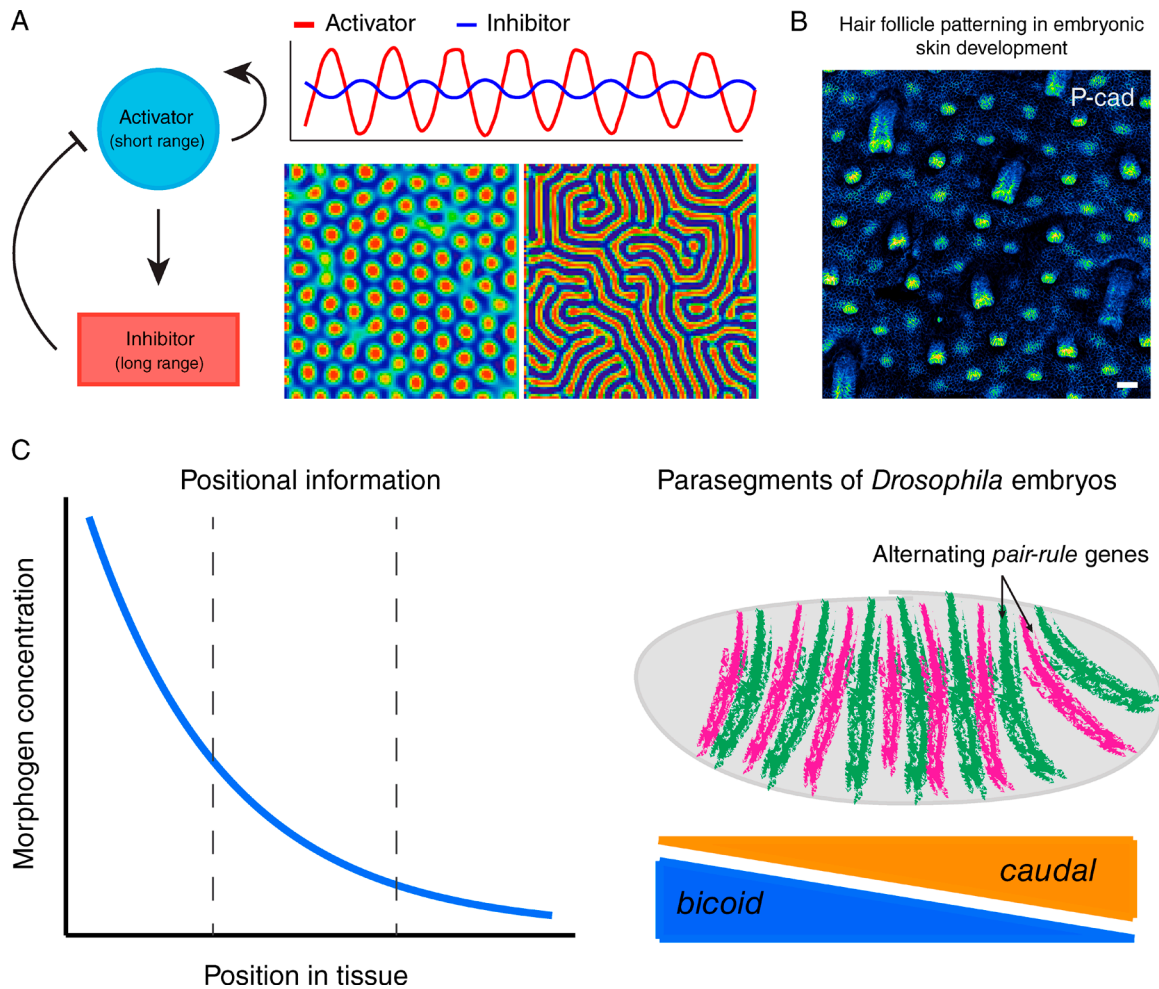


Figure 1. **Mechanisms of tissue patterning.** (A) Turing reaction–diffusion systems, in which cells produce a system of two morphogens with different rates of diffusion, one an activator and the other an inhibitor of a tissue pattern. The activator stimulates both its own production as well as that of its inhibitor. Because the activator diffuses slowly, it concentrates and acts locally, whereas the quickly diffusing inhibitor can only act over a limited distance before it is degraded. The two acting together yield a standing wave pattern of activation, which can give rise to periodic patterns in nature. A simulated reaction–diffusion system is illustrated on the lower right, illustrating the nature of the activator and inhibitor’s diffusion through a tissue and two types of patterns that can emerge. (B) An immunofluorescence image of P-cadherin (P-cad) in embryonic day 16.5 mouse epidermis, revealing the regular pattern and AP angling of hair follicles. The positions of hair follicles are believed to be determined by a reaction–diffusion system comprised of Wnt and Dkk4. Bar, 10  $\mu$ m. (C) Positional information as an alternative patterning mechanism, in which cells interpret their position within a concentration gradient (left). Different threshold levels of a morphogen can specify multiple cell fates. An example of such a system is the patterning of *Drosophila* body segments (right), in which *pair-rule* genes are expressed in alternating stripes, giving each segment a unique identity. The pattern of *pair-rule* gene expression, in turn, is specified by expression of *gap* genes that interact with maternal gradients of *bicoid* and *caudal* expression. Simulations in A were produced using code from Leppänen (2015).

to the pigmentation patterns of sea shells can be accounted for (Kondo and Miura, 2010).

Reaction–diffusion systems are feasible and attractive models for how repeating spatial patterns emerge from an initially homogeneous group of cells. Indeed, theoretical work has long suggested that such systems underlie the patterning of plant vasculature (Dimitrov and Zucker, 2006), the segmentation of *Drosophila* embryos (Kauffman et al., 1978; Bieler et al., 2011), the spacing and morphologies of mammalian hair follicles (Nagorcka and Mooney, 1982, 1985), and limb patterning in tetrapods (Fig. 1, A and B; Newman and Frisch, 1979; Sheth et al., 2012; Raspopovic et al., 2014). However, the challenge has been to identify the morphogens involved, as such efforts have frequently uncovered gene regulatory networks that are too complex to be understood only in terms of a small number of diffusible molecules (Akam, 1989).

Only very recently have advances in genetics and molecular biology, particularly in vertebrate systems, enabled us to identify the morphogens relevant to tissue patterning and to revisit the underlying mechanisms. For example, recent work on the patterning of avian feathers (Jung et al., 1998; Jiang et al., 1999) and mouse hair follicles (Sick et al., 2006) that combine computer simulation with genetic and experimental manipulation of the relevant morphogens has provided direct evidence that reaction–diffusion systems are used as a strategy for tissue patterning in development. Many of the tissue patterns initially thought to be generated by a reaction–diffusion system indeed involve such a mechanism. That said, it should be noted that they frequently operate in the context of geometric constraints and signaling from adjacent tissues and are thus more complex than a two-component system of activator and inhibitor. In some cases, such as pigmentation patterns of zebrafish, Turing-like

patterns are generated not by secreted molecules but by short- and long-range cell–cell interactions that induce cell migration in pigment cells (Watanabe and Kondo, 2015). In others, such as in the *Drosophila* germband, a hierarchy of gene expression rather than a Turing mechanism is responsible for patterning (Zallen and Wieschaus, 2004; Paré et al., 2014).

In the case of the avian feathers, patterning appears to be controlled by secretion of sonic hedgehog (SHH) downstream of fibroblast growth factor (FGF)-4, which promotes placode formation and controls the expression of bone morphogenetic proteins (BMPs) 1 and 4. These BMPs, in turn, act as inhibitors and specify interfollicular fate (Jung et al., 1998). In the mouse epidermis, the WNT pathway, essential for hair placode formation (DasGupta and Fuchs, 1999; Huelsken et al., 2001), has been shown to mediate expression of the WNT inhibitor DKK4 (Sick et al., 2006), similarly defining a reaction–diffusion system whose genetic manipulation affects the density and distribution of hair follicles according to the predictions of the Turing model. Because FGF and BMP expression are frequently downstream of WNT signaling, the patterning of epidermal appendages by a WNT/DKK-based reaction–diffusion system may represent a widely exploited mechanism in which other pathways, including FGF and BMP signaling, serve to modulate and refine the patterns initially established by WNT signals. Indeed, similar mechanisms have been implicated in the positioning of stem cells in intestinal crypts (Zhang et al., 2012) and the positioning of pigment cells into stripes in zebrafish skin (Nakamasu et al., 2009).

#### Alternative mechanisms of pattern formation: morphogen gradients and mechanical self-organization

Reaction–diffusion models perhaps make the most sense in patterning tissues whose physiology is defined by a 2D array of repeated functional units. Although this arises frequently in many organ systems, including the skin, intestine, and inner ear, there are many aspects of our anatomy that are clearly patterned, such as the digits of our hands and feet and the branched tubules of our vascular system, lungs, kidneys, and pancreas (Iber and Menshykau, 2013), that nevertheless appear too intricate to be patterned by such a simple mechanism.

Noting this disparity, Lewis Wolpert championed the notion of “positional information” as the major driver of tissue patterning (Wolpert, 1969, 1989). In some ways, the simplest and most logical explanation for how tissue patterns arise, it simply involves cells differentiating (adopting a certain fate or exhibiting a phenotype) according to their position within a chemical or mechanical gradient (Fig. 1 C). In this model, unlike reaction–diffusion systems, the resulting tissue pattern has no requirement of mirroring the underlying morphogen gradient: cells, known to be sensitive to very small differences in concentration of morphogens or chemotactic agents (Tostevin et al., 2007), can “interpret” their environment and adopt cell fates according to intricate gene regulatory networks.

Morphogen gradients that instruct the acquisition of cell fates in well-defined positions are ubiquitous in development. This is fundamental to the determination of anterior–posterior (AP), dorsal–ventral, and left–right embryonic axes with respect to which the tissues in our bodies are laid out. For example, the apparently simple, segmented body plan of *Drosophila*, in which stripes of *pair-rule* gene expression define the identity

of each segment (Nüsslein-Volhard and Wieschaus, 1980; Ridehough and Ish-Horowicz, 1991; Small et al., 1991), is established by an AP gradient of *bicoid* gene expression (Fig. 1 C; Driever and Nüsslein-Volhard, 1988). In gastrulation, a process common to all animals that specifies the three germ layers, the endoderm, mesoderm, and ectoderm, from which all adult tissues are derived, signals emanating from discrete locations in the embryo ensure both the correct timing of induction and positioning of these layers relative to each other, a process that involves both WNT signaling and members of the TGF- $\beta$  superfamily (for review, see Solnica-Krezel and Sepich, 2012). Similarly, patterning of the vertebrate axial skeleton involves the assignment of unique identities to somites, paired blocks of mesoderm on either side of the notochord that give rise to the skeleton, skeletal muscles, and parts of the dermis (Baker et al., 2008), through combinatorial expression of *Hox* genes (Zákány et al., 2001; Deschamps and van Nes, 2005).

Of course, the mechanisms by which tissues are patterned need not fit neatly into one model or another. It stands to reason that systems of activators and inhibitors should be constrained by both the geometry of developing embryos and the chemical and mechanical gradients that develop. Recent work has borne this out: the patterning of vertebrate digits, a subject of historical controversy between Wolpert’s “positional information” camp and Stuart Newman’s reaction–diffusion camp, appears to be a combination of the two. Within the limb bud, a reaction–diffusion system consisting of WNT, BMP, and SOX9 appears to be responsible for the self-organization of mesenchymal cells into digit precursors, whereas an AP gradient of SHH and downstream *Hox* gene expression module the wavelength of the Turing pattern to fine-tune digit morphology (Sheth et al., 2012; Raspopovic et al., 2014). Studies of Nodal and Lefty signaling in early patterning of zebrafish embryos have similarly revealed that the dynamics of these morphogens, in spite of their origin in a discrete signaling center, follow a reaction–diffusion model (Müller et al., 2012). Models of tissue patterning are thus most useful as a general framework, reaction–diffusion systems describing how diffusible systems of activators and inhibitors interact as they spread through a tissue and morphogen gradients and geometric constraints dictating the final outcome of these systems and nuanced morphology of a tissue.

This perspective is particularly useful when considering that, in a loose interpretation of the Turing model, mechanical rather than chemical instabilities may underlie tissue patterns. Although not widely considered in the realm of patterning, it is well known that within a certain concentration range, fibroblasts can remodel collagen gels to form geometric arrangements of fibroblast clusters, with aligned tracts of collagen fibers connecting the clusters and directing the movement of fibroblasts between them (Harris et al., 1984). It was proposed more than 30 years ago that this type of mechanical self-organization could underlie the formation of dermal condensates in feather and hair follicle formation, but to date, how this might operate within the context of the recently identified reaction–diffusion systems in the epidermis remains unexplored. As highlighted in a recent review of the subject (Green and Sharpe, 2015), technological advances in genetics, molecular biology, and systems biology approaches have the capacity to shed light on the full complexity of patterning mechanisms and how they work together to lay out our elaborate body plans.

### Regulation of actomyosin-based tension by tissue patterning mechanisms

Although considerable theoretical and experimental work has gone into understanding how tissue patterns are generated, much less attention has been paid to how the patterned structures themselves are formed. For instance, in the formation of feathers and hair follicle placodes, what characterizes the transformation of a set of epidermal progenitor cells into a polarized structure comprising multiple differentiated cell types? As these structures are formed, what prevents follicular cells from reintegrating into the epidermal sheet? Given the nature of the pathways found to mediate tissue patterning, including WNTs, SHH, and BMPs, we favor the hypothesis eloquently put forward by Gerald Edelman: that the primary effectors of tissue patterning are genes that modify the physical properties of cells (Edelman, 1992).

The intimate relation between the physical properties of cells and the signaling pathways that regulate tissue patterning and cell fate decisions can be appreciated in the mass cell movements that lay out the body plan during gastrulation. Both WNTs and TGF- $\beta$  family members play a central role by establishing key signaling centers with respect to which drastic changes in cell adhesion and motility take place (Keller, 2005; Solnica-Krezel and Sepich, 2012). Cell movements orchestrated by these signaling centers are both a manifestation of a change in cell fate and an important determinant of tissue identity by dictating the final arrangement of tissues with respect to each other, and thus reciprocal signaling interactions.

For example, WNT signals in the early gastrula play a role in establishing the first, dorsaling signaling center, the Nieuwkoop center in amphibians and the posterior marginal zone in birds and mammals (Kelly et al., 2000; Vonica and Gumbiner, 2007). This signaling center is important in the induction of the embryonic organizer (Spemann-Mangold center in amphibians), which, through members of the TGF- $\beta$  superfamily, Nodal and Vg1, drives the internalization of the surface epithelium by an epithelial-to-mesenchymal transition. The process of internalization, in turn, is coupled to specification of the mesoderm and endoderm. Other TGF- $\beta$  family members are similarly essential in cardiac, lung, skeletal, and neural development (Karttinen et al., 1995; Sanford et al., 1997; Nomura and Li, 1998), and WNT signaling plays as many roles in gastrulation and organ development (Christian et al., 1991; Habas et al., 2001; Lickert et al., 2005; ten Berge et al., 2008), highlighting a universal role of these pathways in tissue morphogenesis and patterning.

Recent studies have begun to uncover a complex interplay at the molecular level between cellular mechanics and pathways that control cell fate. Perhaps the most important implication of this work is that, through changes in adhesion and tension at cell–cell and cell–matrix contacts, mechanosensitive signaling complexes often feed back into the same pathways that mediate global tissue patterning. For example, the TGF- $\beta$  signaling network interacts with multiple pathways that regulate cytoskeletal dynamics and cell motility, including PAK family members, the PI3K–AKT–mTORC1 pathway, and Rho GTPases (Barrios-Rodiles et al., 2005; Lamouille et al., 2014). It is also directly involved in the dissolution of tight junctions through the interaction of TGF- $\beta$ RI, occludin, and Par6, which can down-regulate RhoA activity at tight junctions and lead to their disassembly (Ozdamar et al., 2005). Conversely, tension exerted by cells on the extracellular matrix is known to be important in the release of latent TGF- $\beta$ , and thus cellular

mechanics are likely to be equally important upstream of developmental signaling pathways (Buscemi et al., 2011).

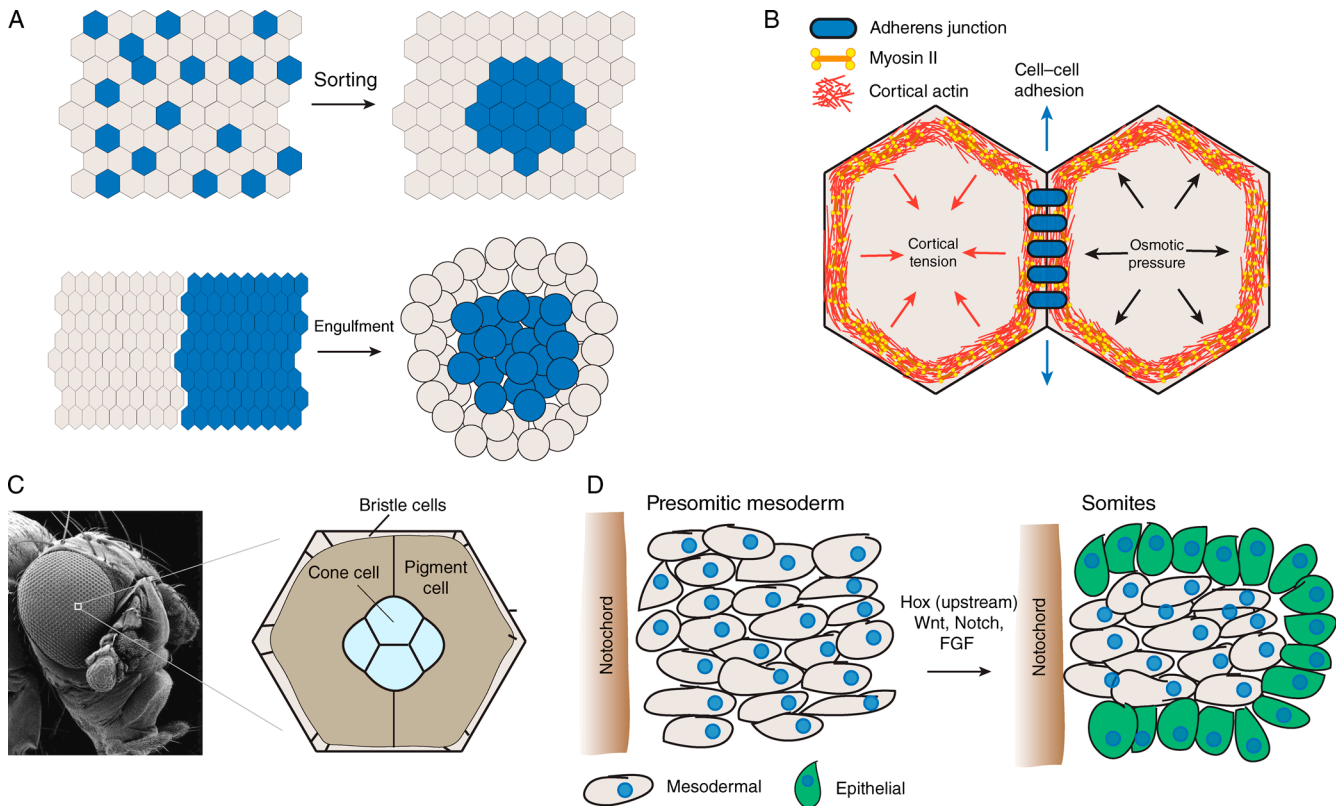
WNT signaling is known to reduce E-cadherin–based cell adhesion through transcriptional repression, leading to a concomitant activation of integrin and Rho signaling to promote cell motility (Jamora et al., 2003; Nelson and Nusse, 2004; Heuberger and Birchmeier, 2010; Livshits et al., 2012). However, modulation of WNT signaling also occurs downstream of tension in association with the Hippo/YAP pathway. In quiescent epithelial cells subjected to strain, YAP has been shown to promote cell cycle reentry and  $\beta$ -catenin to facilitate progression through G1 into S phase (Benham-Pyle et al., 2015). The response of YAP to changes in tissue tension may be mediated downstream of Rho signaling, as suggested from studies of human embryonic stem cells (Ohgushi et al., 2015); however, YAP/TAZ has also recently been shown to directly control tissue tension by activating Rho through ARHGAP18 (Porazinski et al., 2015). Although the relationship between WNT and Hippo signaling is not fully understood, given that YAP/TAZ is known to participate in the  $\beta$ -catenin destruction complex and to facilitate transcription of  $\beta$ -catenin targets downstream of WNTs (Azzolin et al., 2014) in addition to responding directly to tension (Dupont et al., 2011), the cooperation of these two pathways is likely to play an important role in the generation of epithelial appendages through its regulation of both proliferation and tension.

For further discussion on the WNT, TGF- $\beta$ , and SHH pathways and their various interrelationships and context-dependent roles, we refer the reader to several excellent reviews on the subject (Logan and Nusse, 2004; Nusse, 2005; Clevers, 2006; Wu and Hill, 2009; Massagué, 2012). We will focus instead on how their downstream effects on the physical properties of cells govern tissue morphogenesis.

### Cortical tension and cell sorting in tissue patterning

The idea that differences in the adhesive and mechanical properties of cells can direct their sorting and assembly into distinct tissues dates back at least 100 years to the work of H. V. Wilson, who studied the regeneration of freshwater sponges, which undergo a natural degeneration in the winter (Wilson, 1907a,b). Wilson observed that when experimentally degenerated sponges were allowed to recover, undifferentiated amoeboid cells coalesced into masses, recruited other cell types, and eventually differentiated into the full range of tissues that comprised the mature organism. In experiments in which the dissociated cells of different sponge species were mixed, he also discovered that cells only interacted with cells from the same species (Wilson, 1907a). His work thus laid the groundwork for two of the most exciting and active fields of biological research today: how the adhesive and mechanical properties of cells dictate the organization of cells into tissues and the notion that stem cell populations with unique regenerative potential exist within most, if not all, tissues.

Extending and formalizing Wilson's work in what are now considered classic experiments of developmental biology, Townes and Holtfreter (1955) studied the ability of cells dissociated from amphibian embryos to self-organize. They found that, starting from a random mixture of different cell types, cells reproducibly sorted out and adopted positions relative to each other that mirrored their arrangement *in vivo* (Fig. 2 A). This phenomenon was not limited to dissociated cells; in ex-



**Figure 2. Cell sorting on the basis of cortical tension in development.** (A) Illustrations of cell sorting on the basis of surface tension. Differences in cell–cell adhesion between cell types or cortical tension drive the spontaneous segregation of cell types, with those exhibiting the higher surface tension sorting to the center of the tissue. When two cell types with different surface tensions are juxtaposed, one of the tissues engulfs the other, with cells exhibiting higher surface tension sorting internally. (B) The determinants of cortical tension. A cell’s shape and mechanical properties come from its actomyosin cortex, a thin, dense meshwork of cross-linked actin filaments, myosin motors, and actin-binding proteins immediately beneath and tethered to the plasma membrane. This meshwork resists external mechanical deformation and withstands intracellular osmotic pressure, similar to the function of the cell wall in bacteria and plants, but is a much more dynamic structure, turning over in its entirety within 1 min. Cadherin binding in cell–cell adhesions acts to expand the interfaces between cells by reducing the surface tension at sites of contact, directly regulating actomyosin dynamics in adhesions, and mechanically couples cells, stabilizing adhesions against the pulling forces of the cytoskeleton. (C and D) Examples in development where cell sorting on the basis of surface tension function to refine large-scale tissue patterns. (C) In the *Drosophila* compound eye, signals from the morphogenetic furrow recruit cells into ommatidial precursors. Within each of these facets of the eye, however, differential adhesion and contractility dictate the arrangement of cone cells, whose configurations mimic groups of soap bubbles. (D) In the formation of the somites, paired blocks of mesoderm on either side of the notochord that give rise to the skeleton, skeletal muscles, and parts of the dermis, differences in surface tension are believed to be important in maintaining distinct boundaries. Photograph in C courtesy of Walter Gehring, Biozentrum, University of Basel.

periments in which sheets of cells from the neuroectoderm and endoderm were juxtaposed, the neuroectoderm was found to be completely engulfed by the endoderm. These observations led Holtfreter to propose that differences in “tissue affinity” drive the self-organization of cells within tissues. With the advent of DNA recombinant technology, it was soon discovered that this concept encompasses differential adhesion and motility driven by cell surface proteins, the paradigm of which are the cadherins (Nose et al., 1988).

That cell sortings on the basis of differences in adhesion and contractility are likely to be key effectors of tissue patterning systems is perhaps best illustrated by two recent studies of feather placode morphogenesis. Using an *in vitro* reconstitution assay, in which dissociated placode mesenchymal cells were cultured in the presence of an intact epithelium, Jiang et al. (1999) demonstrated that placodes can self-assemble on the basis of a reaction–diffusion system with components in both dermis and epidermis. An important aspect of this self-assembly is an increase in NCAM expression as placodes develop, suggesting that changes in adhesion are a key feature of follicle patterning. Along a similar vein, in the elongation of the feather

bud, the local enrichment of myosin IIB downstream of WNT signaling was shown to be essential in driving cell rearrangement (Li et al., 2013). A more detailed analysis of tension and cellular dynamics in this system will provide key insights into the connections between reaction–diffusion systems and the regulation of adhesion and cortical tension.

### Theories of cell sorting

Cell sorting involves the segregation of a mixture of cells with different fates and mechanical properties into distinct domains, and the maintenance of this segregated state. Although tissues rarely begin as truly random mixtures of different cell types *in vivo*, cell sorting has important functions in forming and maintaining tissue boundaries in embryonic, adult, and diseased tissues. The modern view of cell sorting explains it in terms of tissue surface tension, a function of the strength of adhesion between cells and the contractility of their actomyosin cortex (for an historical perspective, see Krens and Heisenberg, 2011). Initially proposed on the basis of cell adhesion alone, the cells of an aggregate essentially behave as the molecules of immiscible liquids, where the molecules with stronger intermolecular

attraction (higher surface tension) coalesce and separate from the bulk to minimize their surface free energy (Steinberg, 1962; Foty et al., 1996).

There is a subtle interplay between cell adhesion and contractility in determining the surface tension of a tissue. Tension within the cell cortex is regulated in three main ways: the tethering of cortical actin to the plasma membrane through adaptor proteins, actin dynamics within the cortex, including actin cross-linking and bundling and the activity of myosin and microtubule motors, and the coupling of the cortex to sites of cell–cell and cell–ECM adhesion (Salbreux et al., 2012b).

All of these components are mutually interdependent. The formation of both cell–cell and cell–matrix adhesions, for example, depend on mechanical force exerted on components of their respective adhesion complexes, which typically respond by reinforcing adhesion through increased tethering to the cytoskeleton (Schwartz and DeSimone, 2008). In the case of cell–cell adhesion, local actin polymerization and contractility result in the coalescence of nascent, punctate adhesions into a linear structure and their eventual remodeling into a cortical actin belt associated with mature adherens junctions (Vasioukhin et al., 2000; Vaezi et al., 2002). A similar process of maturation occurs in the formation of focal adhesions and their linkage to the cytoskeleton through the recruitment of scaffolding proteins (Chrzanowska-Wodnicka and Burrige, 1996). Developmental regulation of adhesion, in turn, is an essential determinant of the amount of tension a cell can exert on its surroundings. As a result of their interdependence and combined effect on tissue tension, the context-dependent regulation of adhesion and contractility gives rise to a multiplicity of motile and sorting behaviors in tissue morphogenesis.

The final volume occupied by a cell within a tissue is the product of multiple opposing forces. Osmotic pressure seeks to expand the cell, whereas contractile forces within the cortex seek to shrink it. Contractility is also opposed by cell–cell adhesion, where energy released by the ligation of cadherins acts to expand the interface (Maître and Heisenberg, 2011, 2013). The balance of these forces throughout a tissue dictates the final morphology of cells as well as their sorting behavior (Fig. 2 B). Taking these opposing forces into account, researchers have modeled epithelial tissues as a network of cell–cell interfaces, each subject to expansive adhesive forces and cortical contractility. This has identified various regimes of contractility, adhesion, and elasticity that describe the organization and behaviors of cells in development (Farhadifar et al., 2007). Such a framework has been used to accurately describe packing geometries in the development of the *Drosophila* wing imaginal disc and to predict cell rearrangements that occur during tissue growth. Likewise, simulation of cell shapes in the *Drosophila* eye using a model that accounts for cell–cell adhesion and cortical contractility was able to recapitulate the arrangement of pigment and cone cells in both wild-type and mutant conditions (Fig. 2 C; Hayashi and Carthew, 2004; Hilgenfeldt et al., 2008).

It has been a matter of historical debate whether adhesion or contractility is the major determinant of tissue surface tension. In some cases, the organization of cells within tissues can be explained on the basis of differences in cell adhesion alone, such as the precise arrangement of cone cells within *Drosophila* ommatidia (Hayashi and Carthew, 2004). However, the notion that cell adhesion might play a secondary role to contractile forces within the cell dates from the early studies of Holtfreter, who noticed that the means by which sheets of neuroectoder-

mal tissue were engulfed by endodermal cells bore a certain resemblance to the process of invagination that occurs during formation of the neural tube (Townes and Holtfreter, 1955), and Steinberg, whose studies of cell sorting in the presence of cytochalasin B led him to propose that internally generated forces might be important (Steinberg and Wiseman, 1972).

Recent pioneering studies that experimentally measured both adhesion strength and contractility by using micropipette aspiration and atomic force microscopy have borne this out. In zebrafish embryos, dissociated cells from the three germ layers sort on the basis of their contractility rather than the strength of their adhesions, with the ectoderm sorting internally (indicating high surface tension) despite exhibiting the weakest adhesion (Krieg et al., 2008). Subsequent work has suggested that the main role of adhesion is to mechanically couple cells, allowing cortical contractility to control the level of tension, predominantly acting at the cell–medium interface, within the tissue (Maître et al., 2012). It should be noted, however, that adhesion between adjacent tissues and between cells and the extracellular matrix, as well as active cell movement, are likely able to override these general principles. One obvious example is the positioning of the ectoderm during gastrulation. Despite exhibiting the highest tension and sorting internal to the mesoderm and endoderm in vitro, the ectoderm universally remains on the outer surface of the embryo either through interactions of the germ layers with the enveloping layer and yolk sac (Krieg et al., 2008) or because of the mass cell movements that drive the internalization of mesendodermal precursors.

### Cell sorting in development

Regardless of the mechanisms involved, aspects of cell sorting play a major role in development (Krens and Heisenberg, 2011). In the early stages of mouse blastocyst formation, pluripotent epiblast cells and prospective endoderm cells are initially specified at random positions and sorted into distinct domains (Plusa et al., 2008). This is thought to have an important role in fate specification later in development, as it establishes the positions of cell populations within the long-range signaling gradients that evolve. As cells adopt different fates, differences in surface tension are thought to play a role in keeping them separate. After the internalization of mesoderm and endoderm in gastrulation, this is at least part of what keeps the germ layers from intermixing, a role that appears to also apply to different regions of the mesoderm (Ninomiya et al., 2004) and to neural crest and epidermal cells (Davis et al., 1997).

The process of epiboly, the thinning and spreading of the epiblast that occurs concurrently with gastrulation, involves the movement of cells from deep to superficial layers in a process of radial intercalation (Keller, 1980). In zebrafish, this is known to require E-cadherin and regulators of E-cadherin endocytosis such as the EGF pathway, implicating a role for cell sorting in either driving or maintaining the positions of cells after radial intercalation (Kane et al., 2005). Finally, cell sorting can play an active role in the generation of polarized embryonic structures. Studies of chick development have revealed that the limb bud forms by a process of cell segregation from the somatopleural mesoderm, where FGF signaling triggers both changes in cell surface tension as well as proliferation (Damon et al., 2008). The *Drosophila* wing disc, a polarized structure that is compartmentalized along the AP as well as dorsal–ventral axes, likewise requires cell sorting downstream of Decapentaplegic (Dpp, a homologue of BMP) and Notch signaling to form and maintain

tissue boundaries. Proper expression of E-cadherin and other adhesion molecules as well as regulation of cell cortex tension plays a role (Landsberg et al., 2009). A similar process of boundary formation is believed to be at play in vertebrate development in the formation of the somites, which are paired blocks of mesoderm on either side of the notochord that give rise to the skeleton, skeletal muscles, and parts of the dermis (Fig. 2 D; Baker et al., 2008).

Other processes are likely to contribute to cell sorting in vivo, such as chemotaxis, differences in cell migration rates, and cell attraction and repulsion (such as through Eph-Ephrin signaling). Overall, cell sorting on the basis of adhesion and surface tension alone represents an important class of collective cell movements in tissue morphogenesis.

### Refining tissue patterns

Although few studies have explicitly explored a connection between the two, it is easy to imagine that changes in adhesion and cortical tension are important downstream effectors of tissue patterning mechanisms in driving the formation of the patterned structures and in refining or elaborating an initial pattern. In the vertebrate neural tube, for example, progenitors form sharply bordered domains along the dorsal–ventral axis in response to a gradient of SHH secreted by the underlying notochord (Stamatiki et al., 2005; Chamberlain et al., 2008). In toto imaging in zebrafish embryos revealed that cell sorting is required downstream of SHH to segregate cells of different fates, effectively refining a noisy positional signal (Xiong et al., 2013). Similar processes are likely at play in the formation of hair follicles, melanin stripes of fish, and within the *Drosophila* retina.

The *Drosophila* retina consists of a hexagonally packed array of ommatidia, each comprised of 20 cells in a precise arrangement. The ommatidia themselves are specified by a complex patterning mechanism involving signals from the morphogenetic furrow, an epithelial invagination that sweeps in an anterior direction across the eye imaginal disc, recruiting cells into ommatidial precursors as it does so (Ready et al., 1976). Within each facet, however, differential adhesion between cell types dictates their final arrangement, fine-tuning the initial, large-scale pattern (Hayashi and Carthew, 2004).

Similarly, recent studies of pigmentation patterns in zebrafish have suggested that differential cell movement in the two types of pigment cells, initiated by the membrane depolarization of melanophores upon contacting xanthophores, contributes to a cell-sorting process in a Turing-like mechanism (Inaba et al., 2012; Watanabe and Kondo, 2015). In the case of hair follicles, early specification is marked by a reduction of E-cadherin expression and upregulation of P-cadherin (Hirai et al., 1989). Although overexpression of E-cadherin in the skin prevents hair follicle formation (Jamora et al., 2003), it remains to be seen whether this switch in cadherin expression underlies cellular behaviors that are important in forming the follicles. It will be interesting to see what diversity of cellular behaviors are at play beneath reaction–diffusion systems, morphogen gradients, and other long-range patterning mechanisms.

### Polarization of patterned tissue structures

Patterning involves more than the segregation of cell types and their positioning within a tissue. The cells that constitute a patterned structure are often polarized with respect to each other and with respect to the body axes. This is an example of what is known as planar polarity, the uniform polarization

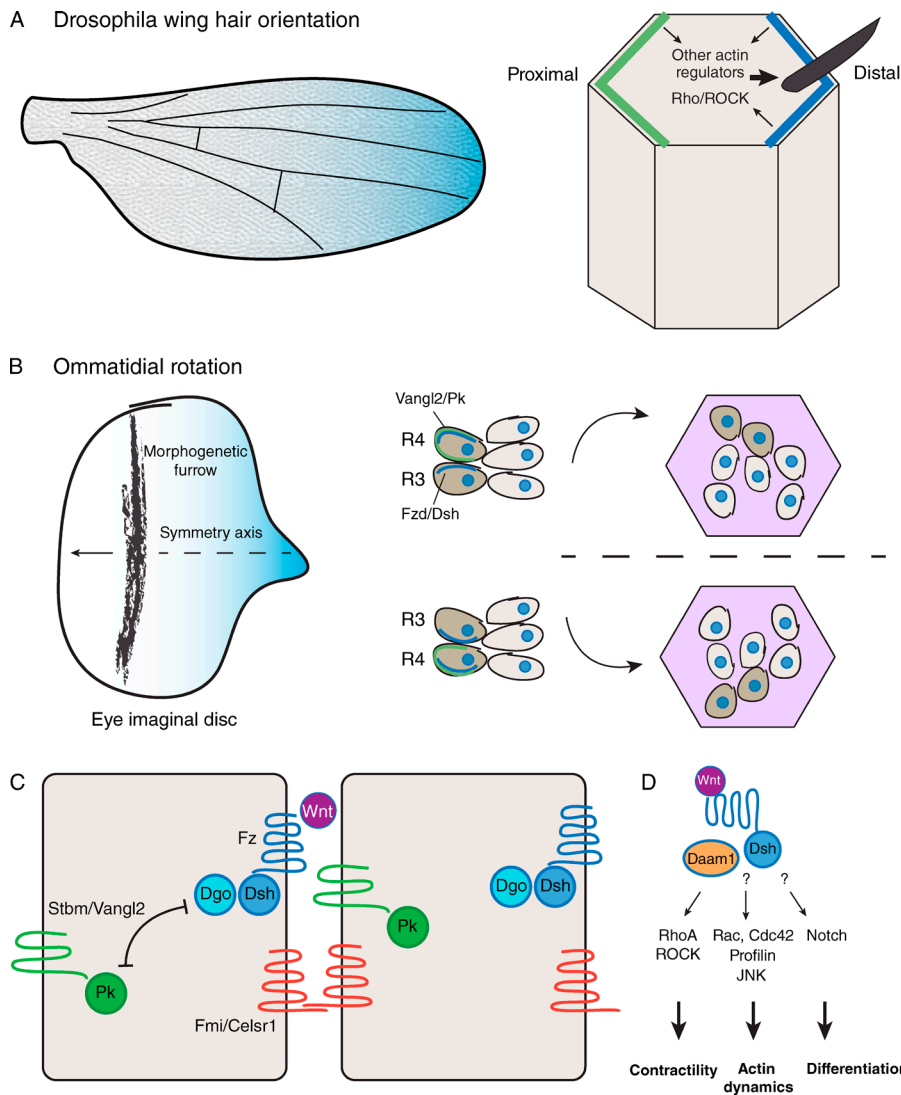
of cells in a tissue across a 2D plane. Examples of this widespread phenomenon include the precise distal orientation of fly wing hairs and the orientation of photoreceptor clusters in the fly eye (Fig. 3, A and B; Zallen, 2007; Seifert and Mlodzik, 2007; Devenport, 2014) and the AP orientation of body hair and the orientation of stereocilia bundles in the inner ear of mammals (Wang et al., 2006).

A key feature of mechanisms that establish planar polarity is that they allow cells to propagate global directional information, such as that encoded by morphogen or mechanical gradients, through local interactions. In some tissues, such as the *Drosophila* germband, the molecules whose local interactions confer polarity, the Toll-like receptors, are targets of the transcription factors that establish the identities of body segments according to a “positional information” model (Paré et al., 2014). Tissues patterned by a Turing or alternative mechanism, on the other hand, frequently use a means of propagating cell polarity whose relationship to an upstream patterning system is less clear and which may operate in parallel. Known as the planar cell polarity (PCP) pathway, its molecular determinants are conserved from flies to humans, and it represents an important way in which tissue patterns are refined at the level of individual cells. Fundamentally, mechanisms of establishing planar polarity accomplish this through the spatial regulation of actin dynamics, myosin II–dependent contractility, and adhesion within cells.

### Planar polarity and the spatial regulation of contractility and adhesion

The means by which tissue patterns are refined at the level of individual cells depends on context. A common theme that has emerged from studies of both *Drosophila* and vertebrates is the utilization of a conserved PCP pathway that spatially regulates local actin remodeling, myosin II–dependent contractility, and adhesion through a host of tissue-specific effectors, many of which have not been fully characterized (Wallingford, 2012). The components and effectors of the PCP pathway have been reviewed extensively elsewhere (Seifert and Mlodzik, 2007; Wallingford, 2012; Devenport, 2014). In a nutshell, PCP proteins are first localized to the apical surface of a cell, where they segregate into mutually antagonistic subcomplexes likely via directional transport along polarized microtubules. In the *Drosophila* wing, the subcomplexes consist of Fz, Dsh, and Dgo on the distal surface, antagonizing Pk activity, and Pk and Stbm on the proximal surface, antagonizing Dsh. In mammals, PCP proteins similarly segregate into mutually antagonistic complexes but do not distribute in exactly the same way (Fig. 3 C; Wang et al., 2006). Recruitment of downstream effectors, many of them binding partners of Dsh, including regulators of myosin II activity, such as RhoA and ROCK (Strutt et al., 1997; Habas et al., 2001; Winter et al., 2001; Nishimura et al., 2012), actin polymerization, such as profilin, Rac, and Cdc42 (Eaton et al., 1996; Habas et al., 2003; Sato et al., 2006), and cell surface regulation of components of juxtacrine signaling pathways, such as the Notch receptor (Das et al., 2002; Strutt et al., 2002; Capilla et al., 2012), are then ultimately responsible for the polarization of cellular behaviors and actin-based structures within cells (Fig. 3 D).

Although it is tempting to speculate that the asymmetric localization of core PCP proteins in these and other systems translates into asymmetries in regulators of actomyosin, it should be noted that this has not been directly observed outside of the



**Figure 3. PCP and the refinement of tissue patterns.** (A and B) Examples in development in which PCP spatially regulates actomyosin to refine the positioning of an epithelial structure. (A) The orientation and number of *Drosophila* wing hairs are controlled by the PCP pathway. Loss of core PCP proteins leads to the misorientation of wing hairs, whereas ROCK activity downstream of Dsh regulates the number of hairs by restricting actin bundling activity to a single site. PCP similarly controls the global AP angling of mammalian hair follicles, which may involve apical constriction of cells along one side of a follicle. (B) In the *Drosophila* eye, the PCP pathways controls the rotation of ommatidia, which establishes an axis of mirror symmetry along the dorsal-ventral axis in the eye imaginal disc. Here, PCP likely regulates the remodeling of specific cell-cell junctions in ommatidial precursors to control the degree of cluster rotation. (C) The core PCP pathway. After their apical localization, PCP components distribute into mutually antagonistic complexes, consisting of Frizzled, Dishevelled, and Diego at the distal end, and Strabismus (Vangl2) and Prickle at the proximal end in *Drosophila* wing cells. PCP proteins are recruited into the region of the adherens junction by the atypical cadherin Flamingo (Celsr1). (D) PCP spatially controls myosin II activity, local actin remodeling, and adhesion through tissue-specific effectors. In vertebrate systems such as the chick neural tube and *Xenopus* mesoderm, this involves activation of ROCK downstream of Dsh and DAAM1. Other systems use different effectors that may either be binding partners of Dsh or may function independently. The PCP pathway is also known to control differentiation in the *Drosophila* eye and leg through Notch signaling, both by transcriptional expression of Delta and regulation of Notch receptor endocytosis, which has the potential to transcriptionally regulate the cytoskeleton. Illustration in C based on Seifert and Mlodzik (2007).

*Drosophila* germband (Simões et al., 2010, 2014), which itself does not use the PCP pathway (Zallen and Wieschaus, 2004), and precisely how effectors of PCP lead to polarized myosin II activity or local actin remodeling remains an area of intense interest.

Through its various effectors, the PCP pathway has multiple roles in the morphogenesis and patterning of both epithelial and mesenchymal tissues. Although the molecular details often differ significantly, the spatial control of actomyosin activity and adhesion has emerged as a universal theme. To highlight the ways in which PCP regulates the physical properties of cells in the elaboration of tissue patterns, we draw on the many examples of patterned epithelial structures discussed earlier. During the formation of the *Drosophila* retina, ommatidial precursors undergo a 90-degree rotation to establish mirror symmetry along the dorsal-ventral axis (i.e., clusters above the equator rotate clockwise and clusters below counterclockwise; see Fig. 3 B). The process involves a precise remodeling of cell-cell contacts and depends both on proper cadherin expression (Mirkovic and Mlodzik, 2006) and myosin II activity (Fiehler and Wolff, 2007). Defects in ommatidial rotation are a hallmark of PCP mutants, in which ommatidia are randomly oriented, highlighting an important role of PCP in fine-tuning both adhesion and contractility downstream of a larger-scale patterning mechanism.

How the PCP pathway regulates contractility, adhesion, and local actin dynamics to achieve different ends in tissue patterning remains a fascinating but unresolved question. As a parallel to the process of germband extension in *Drosophila*, which exhibits planar polarization of ROCK and myosin II independently of PCP, cell intercalation in the chick neuroectoderm during neural tube closure requires PCP for a similar process of polarized, myosin II-dependent apical junction remodeling (Nishimura et al., 2012). In this system, DAAM1, an effector of Dsh, is responsible for the recruitment and activation of ROCK through PDZ-RhoGEF, leading to the polarized activation of myosin II. Convergent extension in mesodermal tissues, which involves a process of mediolateral intercalation thought to depend more on oriented protrusive activity than cell junction remodeling (Keller et al., 2000; recently challenged in Shindo and Wallingford, 2014), is also regulated by the PCP pathway. Similar to the neuroectoderm, intercalation in the *Xenopus* mesoderm also requires Rho activation downstream of DAAM1 (Habas et al., 2001) and may additionally use profilin (Sato et al., 2006). Although PCP plays a role in orienting cell protrusive activity (Wallingford et al., 2000; Kinoshita et al., 2003), the regulation of cadherins (Ulrich et al., 2005; Kraft et al., 2012), and the polarized deposition of extracellular matrix



components (Goto et al., 2005), which effectors of PCP are responsible for these behaviors has not been fully clarified.

In the case of *Drosophila* wing hairs, actin-based organs acquire a precise AP orientation thought to properly direct airflow (Wootton, 1992). The PCP pathway specifies both the number and location of hairs within cells, likely through multiple effectors. Whereas loss of core PCP proteins leads to the misorientation of wing hairs (Gubb and García-Bellido, 1982), ROCK activity downstream of Dsh regulates the number of hairs but not their orientation, with down-regulation leading to the aberrant formation of multiple hairs and overexpression leading to a reduction in hairs (Winter et al., 2001). Thus, whereas one set of effectors appears to spatially regulate local actin remodeling in the selection of a site for hair formation, Dsh and ROCK are required to restrict this activity to a single site. PCP also plays a role in the orientation of stereocilia bundles in the mammalian inner ear by independently regulating convergent extension movements through differential cadherin expression (Chacon-Heszele et al., 2012) and directing the migration of the primary cilium (kinocilium) to a specific site within hair cells (Rida and Chen, 2009). Whether there are additional parallels to *Drosophila* wing hair formation in terms of downstream effectors such as ROCK, however, remains to be explored.

Finally, the orientation of mammalian body hair, in which entire hair follicles (rather than the actin-based hairs in *Drosophila*) are globally angled along the AP axis, similarly depends on the activity of PCP proteins (Devenport and Fuchs, 2008). Although the downstream mechanism for how PCP controls hair follicle angling remains to be determined, it may involve apical constriction in a subset of basal epidermal cells on the posterior side of the follicle (Devenport and Fuchs, 2008), a possibility made more plausible by recently uncovered links between PCP and effectors of apical constriction (Ossipova et al., 2014).

However, unlike other PCP-dependent processes in vertebrates, no polarization in myosin II activity or its upstream regulators has yet been observed in the epidermis, and loss of myosin II in the skin leads to a general reduction in the number of hair follicles rather than an obvious effect on orientation (Schramek et al., 2014). Other well-known effectors of PCP appear to regulate the differentiation and cycling of hair follicles rather than their orientation. Fuzzy, an effector that likely plays a role downstream of Dsh in the *Drosophila* wing (Collier and Gubb, 1997), for example, regulates hair follicle differentiation through formation of the primary cilia and SHH signaling (Zilber et al., 2013). Regulators of the cytoskeleton downstream of PCP, such as Rac1 and Cdc42, are required for hair follicle integrity (Chrostek et al., 2006) and differentiation of epidermal cells along a hair follicle lineage (Wu et al., 2006), respectively, but have not been directly linked to PCP. With multiple roles for PCP at different stages of its development, the hair follicle may therefore prove an ideal model for studying the interplay between PCP and regulation of the cytoskeleton in the progressive development of a tissue pattern.

### Tension upstream and downstream of planar polarity

Although we have implied that PCP acts downstream of longer-range patterning mechanisms, it is important to note that effectors of PCP both dictate and respond to tension within epithelia (Salbreux et al., 2012a). In a seminal study, Eaton and colleagues demonstrated that, in the *Drosophila* wing, external tension arising from contraction of the wing hinge both elon-

gates cells along the proximal–distal axis and dictates the orientation of planar polarity (Aigouy et al., 2010). This raised the intriguing possibility that cell mechanics in general, including regulation of the actomyosin cortex and changes in cell elongation and packing in development, might cooperate with components of the PCP pathway to determine the final polarity of a tissue or the orientation of an epidermal appendage.

Loss of cortical actin-remodeling proteins such as cofilin have been shown to exacerbate PCP defects in mice by perturbing the trafficking of PCP proteins (Mahaffey et al., 2013), and in *Drosophila*, mutations in *flare*, which encodes the cofilin-interacting protein Aip1/Wdr1, exhibit a complex phenotype that includes disruption of planar polarity (Ren et al., 2007). A recent study of Wdr1 and cofilin/destrin mutants in the mouse epidermis further demonstrated a requirement for cofilin-mediated actin severing in maintaining cortical tension, which is required upstream of PCP establishment (Luxenburg et al., 2015). This study further documents Wdr1- and tension-dependent cell shape changes that occur around the time PCP is established, including the AP elongation of cells before PCP establishment and the rounding of cells afterward, a process that is perturbed when tension is inhibited by depleting cells of Wdr1 or pharmacologically inhibiting myosin II in the epidermis. Thus, in addition to a potential role for PCP components in spatially controlling and coordinating tension and actin dynamics within cells, both external tension from the growth or morphogenetic movements of surrounding tissues and internally generated, cortical tension are important contributors to tissue polarity. A fascinating area of future study will be understanding this interrelationship in molecular detail.

### Concluding remarks

Although a great achievement of modern cell biology was elucidating in molecular detail how individual cells migrate in vitro, during development, and in vivo, many cells must move within the confines of cell–cell adhesion (Heller et al., 2014; Shindo and Wallingford, 2014; Williams et al., 2014) and in the context of morphogen gradients and reaction–diffusion systems (Watanabe and Kondo, 2015). This raises a fundamental question for future study: precisely how do cells move within their natural environments in vivo? Likely to involve the regulation of cellular mechanics at multiple scales, systems approaches and advances in in vivo imaging techniques will no doubt shed light on the complexities of cell movement as they relate to the formation and patterning of tissues in development.

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