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# Wingless signaling and the control of cell shape in *Drosophila* wing imaginal discs

Thomas J. Widmann, Christian Dahmann *

*Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstrasse 108, 01307 Dresden, Germany*

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ABSTRACT

The control of cell morphology is important for shaping animals during development. Here we address the role of the Wnt/Wingless signal transduction pathway and two of its target genes, *vestigial* and *shotgun* (encoding E-cadherin), in controlling the columnar shape of *Drosophila* wing disc cells. We show that clones of cells mutant for *arrow* (encoding an essential component of the Wingless signal transduction pathway), *vestigial* or *shotgun* undergo profound cell shape changes and are extruded towards the basal side of the epithelium. Compartment-wide expression of a dominant-negative form of the Wingless transducer T-cell factor (TCF/Pangolin), or double-stranded RNA targeting *vestigial* or *shotgun*, leads to abnormally short cells throughout this region, indicating that these genes act cell autonomously to maintain normal columnar cell shape. Conversely, overexpression of Wingless, a constitutively-active form of the Wingless transducer β-catenin/Armadillo, or Vestigial, results in precocious cell elongation. Co-expression of Vestigial partially suppresses the abnormal cell shape induced by dominant-negative TCF. We conclude that Wingless signal transduction plays a cell-autonomous role in promoting and maintaining the columnar shape of wing disc cells. Furthermore, our data suggest that Wingless controls cell shape, in part, through maintaining *vestigial* expression.



## Introduction

Animal development requires the orchestration of growth, fate specification, and morphogenesis of cells. It has long been known that growth control and cell fate specification rely to a large extent on the spatiotemporal activities of secreted signaling molecules and their transduction pathways. Recent evidence suggests that the activation of some of these signaling cascades is also tightly linked to the control of cell shape (e.g. Corrigall et al., 2007; Escudero et al., 2007; Gibson and Perrimon, 2005; McClure and Schubiger, 2005; Schlichting and Dahmann, 2008; Shen and Dahmann, 2005). However, how signal transduction pathways control cell shape remains poorly understood.

Signaling molecules of the Wnt family are important for a variety of cellular processes during animal development. Wnts signal through at least three different pathways. Signaling through the noncanonical planar cell polarity (PCP) and the Wnt/$Ca^{2+}$ pathways is involved in cell polarity and cell movements (Kohn and Moon, 2005). Signaling through the canonical β-catenin pathway is important for growth and cell fate specification and mutations in components of this pathway are associated with numerous cancers, including cancer of the intestinal epithelium (Clevers, 2006). Little is known about the involvement of the canonical β-catenin-dependent Wnt pathway in morphogenesis.

The developing *Drosophila* wing is a useful model system for the study of signaling pathways and their influence on growth, fate specification, and morphogenesis (Cohen, 1993). The wing develops from a single-layered epithelium of approximately 50 cells, the wing imaginal disc (wing disc). The wing disc is shaped as a sac-like structure in which the apical sides of cells are facing an internal lumen and the basal sides are facing towards the outside of the tissue (Auerbach, 1936) (Figs. 1A, B). Cells in this epithelium display a cuboidal shape during early larval development. In the late-second instar larval stage, however, cells on one side of the wing disc flatten and become squamous whereas cells on the other side elongate and become columnar (McClure and Schubiger, 2005; Ursprung, 1972). Cells of the wing disc begin to proliferate in the second instar larval stage and give rise to approximately 50,000 cells by the end of larval development. As development proceeds, the wing disc cells are regionalized and specified, giving rise in the adult to the wing blade, the notum (body wall), and the distal and proximal hinge, which join the wing blade to the body wall.

The activity of the β-catenin-dependent Wnt/Wingless signaling pathway is required for the specification, growth, patterning, and morphogenesis of the pouch, the region of the wing disc that during pupal development will be transformed to the adult wing blade (reviewed in Gonsalves and DasGupta (2008)). During the mid-second instar larval development, *wingless* is initially expressed in a ventroanterior wedge of cells within the wing disc (Wu and Cohen, 2002). By the late-second instar larval development, signaling between cells of the dorsal and ventral compartments induces

* Corresponding author. Fax: +49 351 210 1349.
*E-mail address:* dahmann@mpi-cbg.de (C. Dahmann).

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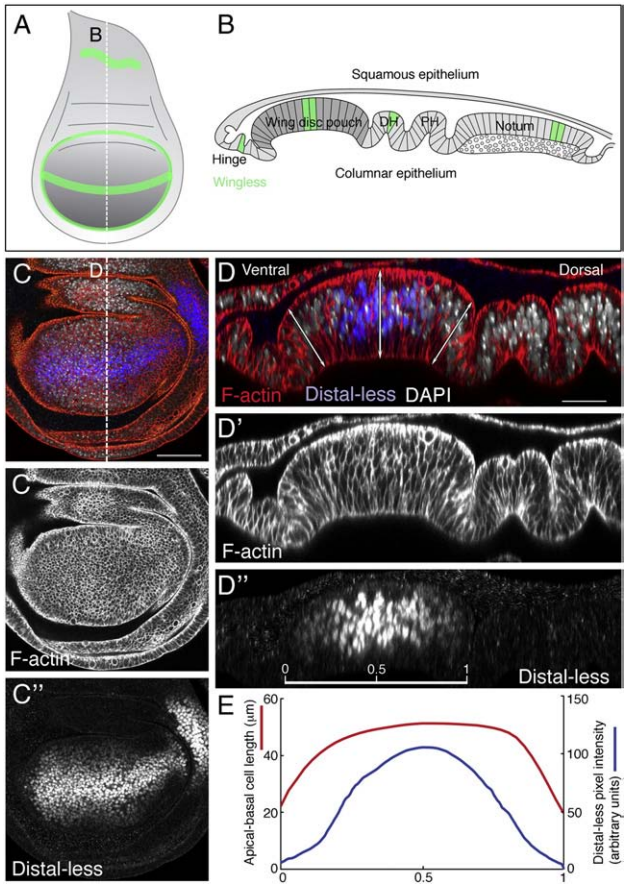


**Fig. 1.** Wingless signal transduction activity correlates with apical–basal cell length in the wing disc pouch. (A, B) Schemes of (A) *xy* and (B) cross-section *xz* views of mid-to-late-third instar wing discs. The wing disc pouch is shaded in grey and the presumptive distal hinge (DH), proximal hinge (PH), and notum regions are indicated. A representation of the Wingless expression domain is in green. (C, D) *xy* (C) and *xz* (D) views of mid-to-late-third instar wing discs stained for F-actin (red), Distal-less (blue), and DAPI (white). In (C) anterior is to the left and dorsal up and in (D) ventral is to the left. (E) Apical–basal cell length and pixel intensity of Distal-less as a function of the position along the dorsoventral axis for the pouch region of the wing disc shown in D, as indicated in D". In these, and all subsequent *xz* sections, apical of the columnar cells is to the top. Dotted lines indicate the position of *xz* or *xy* sections and double-sided arrows indicate apical–basal cell length. Scale bars: 50 µm (C); 25 µm (D).

*wingless* expression in cells adjacent to the dorsoventral compartment boundary (Williams et al., 1993). In the early-third instar larval development, a ring of Wingless expression, the so-called Wingless inner ring, is induced at the distal hinge; a second ring of Wingless expression appears in the proximal hinge region during late-third instar development (Baker, 1988). Wingless protein is secreted from the Wingless-producing cells, forms a protein gradient, and acts at long range to induce target gene expression in surrounding cells (Zecca et al., 1996). Wingless signaling depends on the Frizzled receptors and the co-receptor LDL-receptor-related protein (LRP)/Arrow (reviewed in Stadeli et al. (2006)). Binding of the Wingless ligand to the receptor complex results in the stabilization of β-catenin (Armadillo in *Drosophila*). Armadillo serves a dual role in the cell. By binding to the $Ca^{2+}$-dependent cell–cell adhesion molecule E-cadherin, Armadillo mediates cell adhesion at adherens junctions. Second, Armadillo can enter the nucleus where it binds to the transcription factor T-cell factor (TCF; Pangolin in *Drosophila*) to activate target genes in response to Wingless signaling.

Wingless promotes an increase in wing disc pouch size, at least in part, by feeding an autoregulatory loop of one of its targets, the selector gene *vestigial*, which defines the wing primordium and which is required for its growth (Kim et al., 1996; Zecca and Struhl, 2007). Signaling by Decapentaplegic (Dpp), a member of the Transforming growth factor (TGF)-β superfamily, induces *vestigial* expression also away from the dorsoventral compartment boundary and, thereby, contributes to the fast expansion of the wing primordium (Kim et al., 1997). Clonal analysis indicates that Wingless signaling contributes to wing disc pouch growth mainly by inhibiting apoptosis, and that constitutive activation of Wingless signaling does not speed up cell doubling, but rather slows it down (Johnston and Sanders, 2003). In contrast to the wing disc pouch, Wingless is both necessary and sufficient to drive the proliferation of cells in the wing disc hinge (Neumann and Cohen, 1996b; Zirin and Mann, 2007).

Wingless signaling is also important for specifying cell fates within the wing disc. During early larval development, loss of Wingless signaling results in the loss of wing structures and a transformation to notal structures. Conversely, ectopic expression of Wingless in the notum can result in the formation of wing-like structures (for review see Klein (2001)). During later larval development Wingless induces genes along the dorsoventral compartment boundary, including *senseless*, which is required to specify cell fates at the wing margin (Jafar-Nejad et al., 2006).

Finally, Wingless signaling has been implicated in the control of cell shape and cell adhesion during wing development. The Wingless signaling activity correlates along the dorsoventral axis with, and is required for, a gradient in the size of the apical cell circumference of columnar cells in late-larval wing discs (Jaiswal et al., 2006). Cells transducing the highest level of Wingless signaling display a narrow apical circumference and cells with low Wingless signaling activity are apically wider. Wingless also directs the graded expression of *shotgun* (*shg*), which encodes E-cadherin, indicating that Wingless regulates epithelial cell–cell adhesion (Jaiswal et al., 2006). The mechanisms by which Wingless signaling controls apical cell shape, and whether Wingless signaling also affects apical–basal cell length in the wing disc epithelium, remain unknown.

Here, we have systematically addressed the roles of Wingless signal transduction components, Vestigial, and E-cadherin in influencing the apical–basal length of wing disc pouch cells during larval development. We find that E-cadherin is required to maintain the highly elongated columnar shape of late-larval wing disc pouch cells. Moreover, we provide evidence that canonical Wingless signaling and Vestigial promote cell elongation during early larval development and that they are required to maintain the elongated columnar cell shape during late-larval development. Finally, our experiments indicate that Wingless signaling controls cell shape, in part, by maintaining Vestigial expression.

## Materials and methods

### *Drosophila* stocks

Flies were raised at 25 °C unless indicated otherwise. The following fly stocks were used: $arrow^{2}$ (Wehrli et al., 2000), $arm^{XM19}$ (Cox et al., 1999), $vg^{83b27\text{-}R}$ (Williams et al., 1991), $shg^{R69}$, a partial deletion of the *shg* coding region removing all cadherin repeats (Godt and Tepass, 1998), $shg^{IH}$ (Nüsslein-Volhard et al., 1984), $cpa^{69E}$ (Janody and Treisman, 2006), *Act5C>CD2>GAL4* (Pignoni and Zipursky, 1997), *ap-GAL4* (Calleja et al., 1996), *ubx-GAL4* (de Navas et al., 2006), *nubbin-GAL4* (Baena-Lopez and Garcia-Bellido, 2006), $tubP\text{-}gal80^{ts}$ (McGuire et al., 2003), *UAS-arm* (Pai et al., 1997), $UAS\text{-}arm^{S10}$ (Pai et al., 1997), $UAS\text{-}TCF^{DN}$ (van de Wetering et al., 1997), *UAS-wg* (Simmonds et al., 2001), *UAS-vg* (Kim et al., 1996), *UAS-shg* (Sanson et al., 1996), *UAS-p35* (Hay et al., 1994), and *UAS–CD8–GFP* (Lee and Luo, 1999). $UAS\text{-}vg^{dsRNA}$ and $UAS\text{-}shg^{dsRNA}$ were from the Vienna Drosophila RNAi Center, #16896 and 27081, respectively (Dietzl et al., 2007).

Marked clones were generated by Flp-mediated mitotic recombination (Lee and Luo, 1999; Xu and Rubin, 1993) subjecting larvae to a