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

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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

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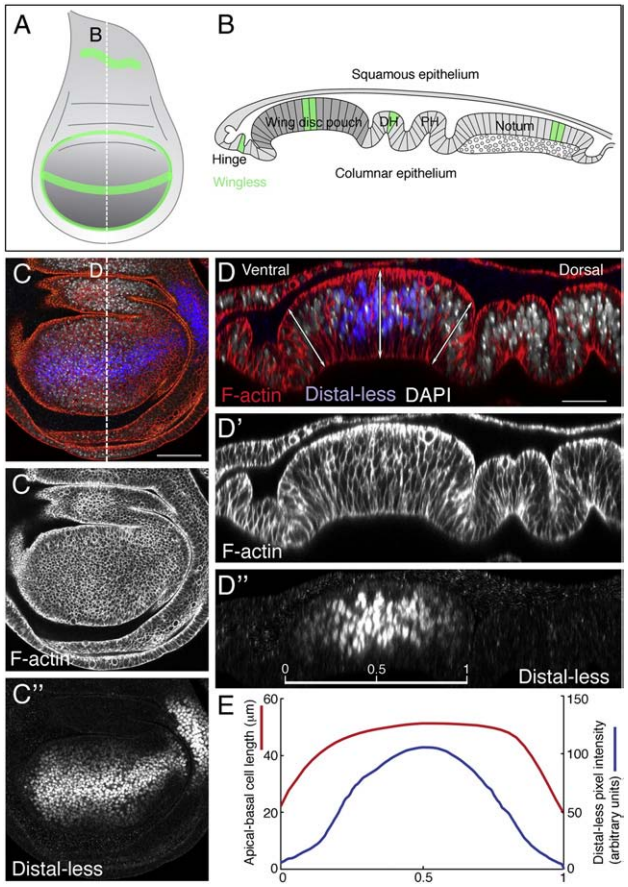

Fig. 1. Wingless signal transduction activity correlates with apical–basal cell length in the wing disc pouch. (A, B) Schemes of (A) *xy* and (B) cross-section *xz* views of mid-to-late-third instar wing discs. The wing disc pouch is shaded in grey and the presumptive distal hinge (DH), proximal hinge (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

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35.5 °C–38.5 °C heat-shock for 30 min. Transgenes were expressed using the GAL4-UAS system (Brand and Perrimon, 1993). To increase the level of transgene expression, larvae used in Figs. 4F–H and 9C were shifted 24 h before dissection to 29 °C. Crosses using *nubbin-GAL4* were set up at 18 °C and shifted (Figs. 4D, E) 37 h or (Figs. 6B, C) 52 h before dissection to 29 °C. To reduce the expression level of E-cadherin in clones of cells (Figs. S6A, B), larvae were raised at 18 °C. To control the timing of transgene expression using TARGET (McGuire et al., 2003), larvae raised at 18 °C were shifted for the indicated time to 29 °C before analysis.

Immunohistochemistry

Unless otherwise stated, wing discs from late-third instar larvae are shown. Discs were fixed and stained according to standard protocols (Klein, 2008). Wing discs, except in Figs. 4F–H, 6D, and 9C, were mounted using double-sided tape (Tesa 05338, Beiersdorf, Hamburg) with the apical side of the columnar epithelium facing the cover slip, except in Figs. 7, 8, and S8 where the basal side faced the cover slip to allow better visualization of basal cell structures. Primary antibodies used were mouse anti-GFP, 1:100 (Santa Cruz), rabbit anti-GFP, 1:2000 (Clontech), rat anti-Distal-less, 1:200 (Vachon et al., 1992), rabbit anti-Vestigial, 1:50 (Kim et al., 1996), rat anti-DE-cadherin (DCAD2), 1:50 (Developmental Studies Hybridoma Bank, DSHB), mouse anti-Wingless, 1:100 (DSHB), mouse anti-Nubbin, 1:10 (Averof and Cohen, 1997), and rabbit anti-p35, 1:500 (Biocarta). Secondary antibodies (Molecular Probes), all diluted 1:200, were anti-mouse Alexa 488, anti-rabbit Alexa 488, anti-mouse Alexa 555, anti-mouse CY5, anti-rat CY5, and anti-rabbit CY5. Rhodamine–phalloidin and DAPI (both Molecular Probes) were diluted 1:200 and 1:500, respectively. Images were recorded on a LSM510 Zeiss confocal microscope.

Measurements

The plot shown in Fig. 1E was generated by first discretizing into 37 bins the basal surface of the wing disc as displayed in Fig. 1D for the region indicated in Fig. 1D". For each bin, the orthogonal distance from the basal surface to the apical surface of the columnar epithelium was determined. The mean pixel intensity of the Distal-less staining was measured using Image J in a 35.0 μm-wide stripe centering on the nuclei. For determining the relative apical–basal length of cells, the distances between the apical and basal surfaces of the columnar epithelium were measured in confocal images showing *xz* cross-section views of wing discs (see for example Fig. 4A) using Image J. Distances were measured approximately 15 μm to the left and 15 μm to the right of the dorsoventral compartment boundary. In Fig. 3I, the apical–basal length of peripodial membrane cells was measured in *xz* sections of late-third instar wing discs in approximately the region of the peripodial membrane that overlaid the center of the pouch. Statistical analysis was performed using a Welch's *t*-test.

Results

Wingless signaling correlates with apical–basal cell length in the wing disc pouch

To test whether Wingless signal transduction activity correlates with the apical–basal length of cells, we stained mid-to-late-third instar wing discs for the two transcription factors Distal-less and Vestigial, whose gene expressions are regulated by the Wingless signaling activity (Couso et al., 1995; Kim et al., 1996; Neumann and Cohen, 1996a; Zecca et al., 1996), and F-actin to visualize cell shape. The wing disc pouch is a single-layered epithelium in which the apical–basal length of individual cells corresponds to the width of the epithelium (Figs. S1A, B). To determine the apical–basal length of cells we therefore measured the width of the wing disc pouch epithelium in optical cross-sections (*xz*). Apical–basal cell length correlated along the dorsoventral axis of the wing disc, in a graded manner, with the level of Distal-less and Vestigial expression (Figs. 1C–E; Figs. S1C, D and S2). Cells in the center of the wing disc pouch, which expressed high levels of Distal-less and Vestigial, were most elongated. Cells were increasingly shorter with decreasing levels of Distal-less and Vestigial. We conclude that the Wingless signaling activity gradient correlates along the dorsoventral axis with the apical–basal length of cells within the wing disc pouch.

arrow mutant clones invaginate and extrude from the wing disc pouch epithelium

To test whether Wingless signaling is required to maintain the highly elongated shape of cells in the center of the wing disc, we decreased Wingless signal transduction in mutant clones of cells and analyzed the epithelial morphology. Clones of cells lacking Wingless signal transduction grow poorly in the wing disc pouch. Clone growth is partly restored by the expression of baculovirus protein p35, an inhibitor of caspases (Hay et al., 1994), indicating that the reduced clone growth is partly due to apoptosis (Giraldez and Cohen, 2003; Johnston and Sanders, 2003). Clones of cells expressing p35 and mutant for $arrow^{2}$, a null allele of *arrow*, which encodes an essential co-receptor for Wingless (Wehrli et al., 2000), were more frequently observed in the hinge region compared to the pouch region of late-third instar larval wing discs (Fig. 2A). In the wing disc hinge, cells within most clones displayed a normal columnar morphology. In a few hinge clones, however, cells invaginated (Fig. 2A, and data not shown). In the wing disc pouch, cells within $arrow^{2}$ mutant clones expressing p35 were frequently invaginated from the apical side of the epithelium and formed a funnel-like lumen with their apical sides facing each other (Figs. 2A–C). Mutant cells at the base of these lumina displayed a strongly reduced length along their apical–basal axis compared to neighboring control cells in the wing disc pouch. In some clones, a fraction of cells was extruded from the epithelium and was located beneath the basal side of the epithelium (Figs. 2D, E, G, H). Mutant cells extended F-actin rich protrusions. We rarely recovered clones that were extruded in their entirety from the wing disc and formed patches of cells beneath the basal surface of the epithelium (Figs. 2F, I). To test whether the morphogenetic defects of $arrow^{2}$ mutant clones were due to reduced Wingless signal transduction in these cells, we restored Wingless signaling by expression of Arm^{S10}, a constitutively-active form of Armadillo (Pai et al., 1997). Expression of Arm^{S10} reverted cell shape in $arrow^{2}$ mutant clones, that were located within the wing disc pouch, to normal (Fig. 2J), demonstrating that the morphogenetic defects of $arrow^{2}$ mutant clones were due to reduced Wingless signal transduction in these cells. Similar to $arrow^{2}$ mutant clones, arm^{XM19} mutant clones, carrying a signaling-defective allele of *armadillo* that retains activity in cell–cell adhesion (Cox et al., 1999), or clones co-expressing a dominant-negative form of TCF, TCF^{DN}, and p35, formed epithelial invaginations (Fig. S3). Taken together, these results show that clones of cells lacking canonical Wingless signal transduction are in part removed from the wing disc pouch epithelium by basal cell extrusion. The decreased apical–basal length of cells in these mutant clones provides a first indication that Wingless signal transduction is required to maintain the highly elongated shape of wing disc pouch cells during late-larval development.

Clones of cells expressing Arm^{S10} in the wing disc hinge or the squamous epithelium invaginate

We have shown above that an inappropriate reduction in Wingless signal transduction activity in clones of cells resulted in the formation of epithelial invaginations and cell extrusion in the wing disc pouch.

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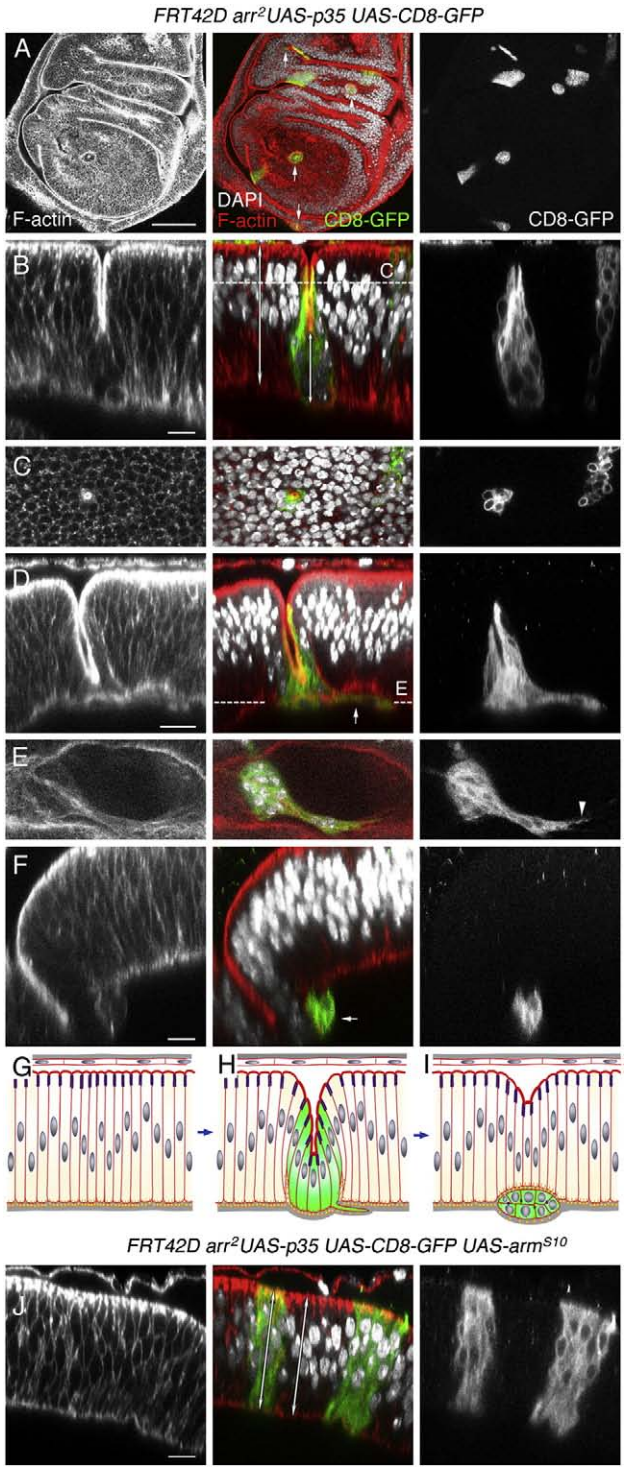

Fig. 2. *arrow*^{2} mutant clones invaginate and extrude from the wing disc pouch. (A–F) Clones of *arrow*^{2} (*arr*^{2}) mutant cells expressing p35 and marked by the presence of CD8–GFP (green) were induced and stained 72 h later for F-actin (red) and DNA (white). (A) *xy* view. Arrows point to invaginating clones. (B) *xz* view. *arrow*^{2} cells are part of a deep epithelial invagination and, at the base of the invagination, are much shorter along the apical–basal axis compared to control cells (double-sided arrows). (C) *xy* view. (D) *xz* and (E) basal *xy* views. Some *arrow*^{2} cells within a clone extruded from the epithelium (arrow) and form protrusions (arrowhead). (F) *xz* view of a clone of extruded *arrow*^{2} cells (arrow). (G–I) Schematic representation of loss of Wingless signaling in *arrow*^{2} clones (green) on epithelial morphology and cell shape. The positions of adherens junctions (blue lines) and focal adhesions (yellow) in the clone cells are speculative. (J) *xz* view of clones of *arrow*^{2} mutant cells co-expressing p35 and Arm^{S10} marked and stained as in (A–F). Cells in the clones have an apical–basal length comparable to neighboring control cells (double-sided arrows) and do not invaginate. Scale bars: 50 μm (A); 10 μm (B, D, F, J).

We next tested whether an inappropriate increase in Wingless signal transduction activity results in similar morphological alterations. Expression of Arm^{S10} in wild-type embryos has been shown to mimic phenotypes that are observed when Wingless is overexpressed (Pai et al., 1997), indicating that expression of Arm^{S10} activates Wingless signal transduction. When located in the wing disc pouch, the region in which Wingless signaling is high, cells in clones expressing Arm^{S10} had a normal appearance (Figs. 3A, B, G). Clones expressing Arm^{S10} in the presumptive hinge region of the wing disc, however, were larger than normal and these clones often bulged out from the epithelium and formed outpockets (Fig. 3A). Clonal cells invaginated and formed lumina that, based on enriched F-actin, were limited by their apical sides (Figs. 3C, D, G). The apical–basal length of cells in these Arm^{S10}-expressing clones in the wing disc hinge was difficult to compare with the length of wild-type wing disc hinge cells due to the heterogeneity in size of the wild-type cells. In the squamous epithelium of the peripodial membrane, where Wingless signaling normally is absent (Baena-Lopez et al., 2003), clones expressing Arm^{S10} also bulged out from the epithelium and formed outpockets (Figs. 3E–G). Arm^{S10}-expressing clones invaginated from the apical side of the peripodial membrane. Cells within these clones were longer along their apical–basal axis compared to neighboring control squamous cells (Baena-Lopez et al., 2003) (Figs. 3E–G). Expression of Arm^{S10} under control of the *ubx-GAL4* driver throughout the peripodial membrane resulted in cells that were greatly elongated along their apical–basal axis, however, in this case the peripodial membrane remained flat and did not invaginate (Figs. 3H, I). Apical–basal lengthening of peripodial membrane cells was also reported as a consequence of ectopic expression of Wingless in this epithelium (Baena-Lopez et al., 2003). This finding corroborates the conclusion that the cell shape changes we have observed upon Arm^{S10} expression are due to an increased level of Wingless signaling activity in Arm^{S10} cells. Taken together, these results show that both the clonal inactivation of Wingless signaling in regions of high Wingless signal transduction and clonal overactivation of Wingless signaling in regions of low Wingless signal transduction, result in epithelial invaginations and abnormal cell shape.

Wingless signaling is required cell autonomously to maintain apical–basal cell length in the wing disc pouch

We have shown that clones of cells in which Wingless signal transduction is severely reduced invaginate and extrude when located next to wild-type cells that transduce the Wingless signal. To test whether Wingless signaling acts cell autonomously to maintain the highly elongated cell shape of wing disc pouch cells during late-larval development, we expressed TCF^{DN} in a large area of the wing disc, the dorsal compartment. Cell morphology was analyzed and compared within the same wing disc to the cell morphology in the ventral compartment, which served as an internal control. Co-expression of TCF^{DN} and p35 in a time-controlled fashion within the dorsal compartment using the GAL4/UAS system (TARGET) (McGuire et al., 2003) resulted in a strong decrease in Distal-less and E-cadherin expression in that region (Fig. 4A and Fig. S4), suggesting that under these conditions Wingless signal transduction was severely compromised. Expression of the Pdm1 protein Nubbin, a marker for the pouch and distal hinge region of the wing disc (Cifuentes and Garcia-Bellido, 1997), was sustained in dorsal cells co-expressing TCF^{DN} and p35 (Figs. 4B, C), indicating that these cells, consistent with a previous report (Johnston and Sanders, 2003), maintained their identity. Dorsal cells close to the dorsoventral compartment boundary invaginated and the dorsal pouch region was smaller and appeared to be bent compared to the control ventral compartment (Figs. 4A–C). Notably, cells throughout the dorsal compartment of the wing disc pouch were shorter along their apical–basal axis compared to the control ventral cells (Figs. 4A–C, I). Expression of TCF^{DN} under the control of *nubbin-*

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GAL4 throughout the wing disc pouch and distal hinge resulted in a similar reduction of apical–basal cell length throughout this region (Figs. 4D, E). In this case, the wing disc pouch epithelium was also reduced in size, but remained flat, indicating that the invagination of cells co-expressing TCF^{DN} and p35 only in the dorsal compartment was a result of the sharp apposition of Wingless transducing cells and non-Wingless transducing cells at the dorsoventral compartment boundary. The extent of the distal hinge fold, which partly coincides with the inner ring of Wingless expression, was greatly reduced in wing discs expressing TCF^{DN} under the control of *nubbin-GAL4* (Figs. 4D, E). Expression of p35 alone had no effect on cell shape (data not shown). We conclude that Wingless signaling, in a cell-autonomous manner, is required to maintain the highly elongated cell shape of wing disc pouch cells during late-larval development. The requirement for TCF in the maintenance of normal apical–basal cell length suggests that Wingless controls cellular morphology through the canonical Wingless signal transduction pathway leading to a change in gene expression.

Wingless signaling is sufficient to promote cell elongation in the wing disc pouch

Cells of the wing disc pouch start to elongate along their apical–basal axis during late-second instar larval development and undergo a cuboidal-to-columnar cell shape transition (McClure and Schubiger, 2005). To test whether Wingless is sufficient to promote cell elongation during early wing disc development, we expressed Wingless from a transgene in dorsal cells from mid-second instar onwards and analyzed cell morphology in early-third instar wing discs. Ventral and dorsal pouch cells of control early-third instar wing discs had a similar apical–basal length (Figs. 4F, I). In contrast, dorsal pouch cells over-expressing Wingless were longer along their apical–basal axis compared to control ventral cells located away from the dorsoventral compartment boundary (Fig. 4G). Ventral cells close to the dorsoventral compartment boundary were also elongated. We attribute this non-cell autonomous effect to the ability of the Wingless signaling molecule to spread within the tissue. To induce Wingless signaling cell autonomously, we expressed the constitutively-active form of Armadillo, Arm^{S10}, in dorsal cells. Dorsal pouch cells expressing Arm^{S10} from mid-second instar onwards were in early-third instar wing discs longer along their apical–basal axis compared to control ventral cells (Figs. 4H, I). Distal-less expression was increased in dorsal cells compared to control ventral cells, indicating that Wingless signal transduction was elevated in Arm^{S10}-expressing cells (Fig. 4H). We conclude that the elevation of Wingless signaling is sufficient to promote cell autonomously the elongation of cells within the wing disc pouch during early larval development.

shg^{R69} mutant clones invaginate and extrude from the wing disc epithelium

Wingless signaling controls cell–cell adhesion by transcriptionally regulating the activity of E-cadherin/*shg* (Jaiswal et al., 2006). To test whether E-cadherin is required to maintain the shape of wing disc cells, we analyzed the morphology of cells in clones mutant for two different alleles of *shg*. shg^{IH} is a strong hypomorphic allele carrying two missense mutations in conserved amino acids of E-cadherin that

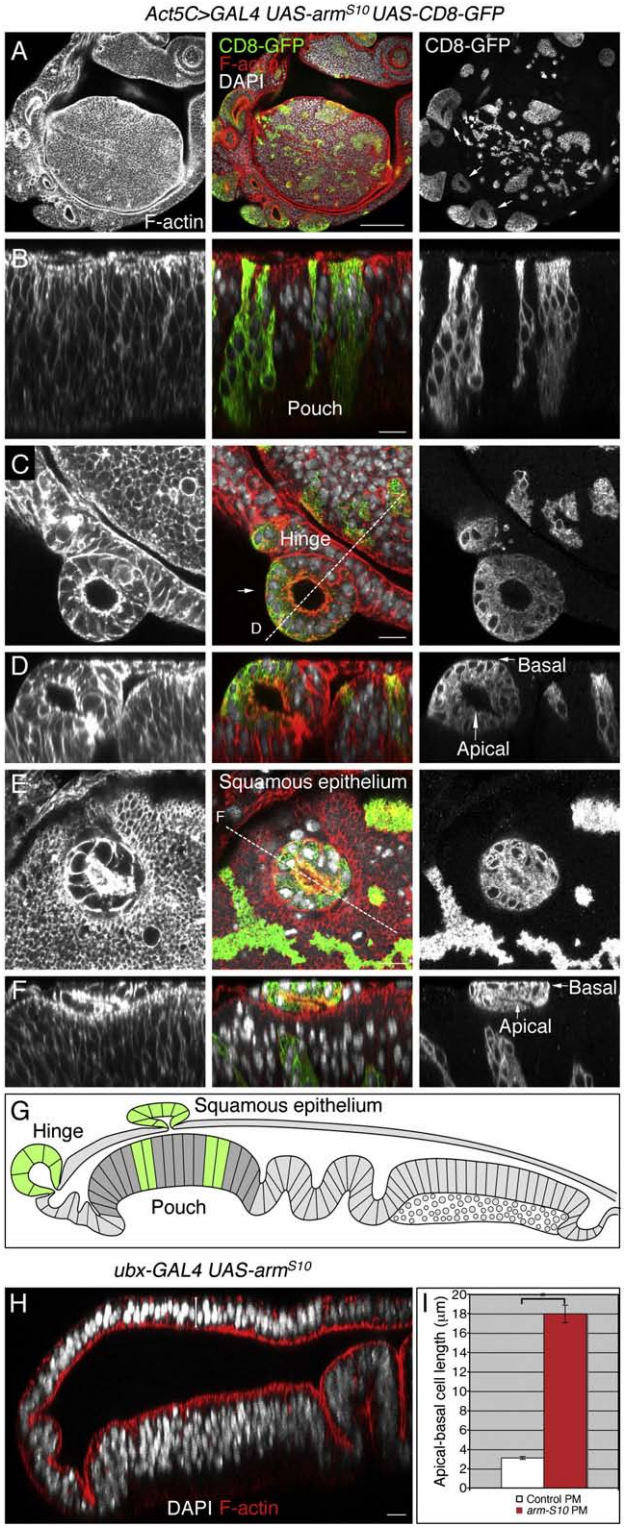

Fig. 3. Clones of cells expressing Arm^{S10} in the wing disc hinge or squamous epithelium invaginate and form outpockets. (A–F) Clones of cells expressing Arm^{S10} and marked by the presence of CD8–GFP (green) were induced and stained 48 h later for F-actin (red) and DNA (white). (A) *xy* view. Clones that express Arm^{S10} and form outpockets are indicated by arrows. (B) *xz* view of clones in the wing disc pouch. Cell morphology appears normal. *xy* (C) and *xz* (D) views of clones in the wing disc hinge. Clones form outpockets (arrow in C) and cells invaginate. *xy* (E) and *xz* (F) views of Arm^{S10}-expressing clones in the squamous epithelium of the peripodial membrane. Clones form outpockets and cells invaginate. The apical and basal sides of cells in Arm^{S10}-expressing clones are indicated in (D) and (F). (G) Schematic representation of the approximate position and morphology of the Arm^{S10}-expressing clones shown in (B), (D), and (F). (H) *xz* view of a wing disc from a late-third instar larva expressing Arm^{S10} under control of the peripodial membrane specific driver *ubx-GAL4*. Cells of the peripodial membrane are elongated along the apical–basal axis compared to controls (e.g. Fig. 1D; double-sided arrow). Cell density is increased in the peripodial membrane. (I) Apical–basal length of peripodial membrane (PM) cells of control wing discs or wing discs expressing Arm^{S10} in the peripodial membrane under control of *ubx-GAL4*. Means ± s.e.m. are indicated ($n = 9$ wing discs (control); $n = 7$ (arm^{S10}); $^*P < 0.001$). Scale bars: 50 µm (A); 10 µm (B–F, H).

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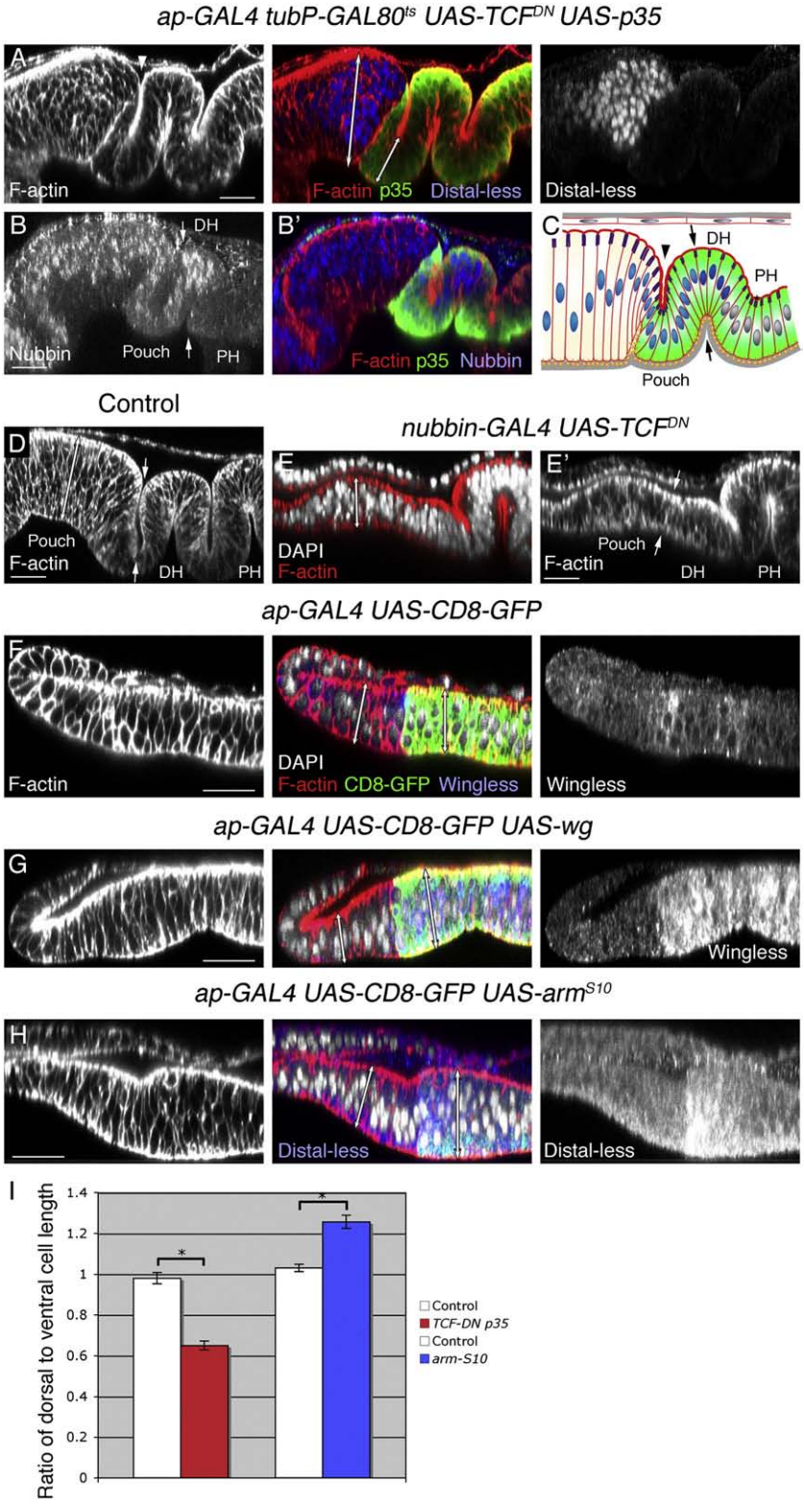
leads to a disruption of embryonic epithelia to varying degrees (Fox et al., 2005). In the wing disc, however, *shg*^{IH} clones, even though displaying highly reduced levels of E-cadherin, did not disrupt the integrity of the epithelium and cells within these clones had a normal apical–basal length (Fig. S5). In contrast, clones of cells mutant for a null allele of *shg*, *shg*^{R69}, displayed severe morphological defects. Clones were part of invaginations of the apical surface of the epithelium (Figs. 5A, B). *shg*^{R69} mutant cells at the base of the invagination were shorter along their apical–basal axis compared to neighboring control cells (Fig. 5A). *shg*^{R69} mutant clones were often extruded from the basal side of the wing disc epithelium (Fig. 5C). Mutant cells displayed long actin-filled protrusions not seen in control cells (Fig. 5D). To test whether the morphological alterations in *shg*^{R69} mutant clones were due to the mutation in *shg*, and not to an unrelated mutation that might have been present on this chromosome, we attempted to rescue clones by expression of a wild-type *shg*



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transgene using the GAL4–UAS system. Overexpression of E-cadherin/*shg* using the GAL4/UAS system in otherwise wild-type clones, however, often resulted in the formation of epithelial invaginations (Figs. S6A, B). Cells over-expressing E-cadherin had decreased levels of Distal-less, indicating that Wingless signaling was compromised (Fig. S6C), and were shorter along their apical–basal axis (Figs. S6C, D). Co-expression of Armadillo in clones of cells expressing E-cadherin restored normal clone and cell shape (Figs. S6E, F). This finding suggests that overexpression of E-cadherin results in morphological defects, similar to those observed when Wingless signaling is reduced, by sequestering Armadillo to the zonula adherens. Defective cell shape in shg^{R69} mutant clones was reversed to normal by co-expression of E-cadherin and Armadillo using the GAL4/UAS system (Figs. 5E, F), demonstrating that the extrusion of shg^{R69} clones was not due to an unrelated mutation on that chromosome, but specific to the mutation in *shg*.

E-cadherin is required to maintain apical–basal cell length

To determine whether E-cadherin plays a cell-autonomous function in maintaining cell shape within the wing disc epithelium, we down-regulated E-cadherin expression by RNA interference. Co-expression throughout the dorsal compartment of a double-stranded RNA targeting E-cadherin, and p35 to suppress apoptosis, resulted in highly reduced levels of E-cadherin in these cells (Fig. 6A). The epithelium of the dorsal compartment was within the pouch region bent towards its basal side. Dorsal cells expressing double-stranded RNA targeting E-cadherin and p35 were shorter along their apical–basal axis compared to cells of the control ventral compartment (Figs. 6A, E). To corroborate these findings, we used the *nubbin-GAL4* driver to co-express double-stranded RNA targeting E-cadherin and p35 throughout the wing disc pouch. The wing disc pouch was also bent towards the basal side and cells were shorter along their apical–basal axis compared to controls (Figs. 6B, C). The epithelium appeared to remain intact under these conditions, since nuclei displayed a normal position within cells and were not located at the basal side of the epithelium as is frequently observed when cells die. Compartment-wide co-overexpression of E-cadherin and Armadillo during early larval development, however, did not result in an apical–basal cell elongation (Figs. 6D, E). We conclude that E-cadherin is required to maintain the highly elongated shape of wing disc pouch cells during late-larval development.

vestigial mutant clones in the wing disc pouch invaginate

vestigial (*vg*) is a target gene of Wingless required for the specification and growth of the wing disc pouch (Couso et al., 1995; Kim et al., 1996; Zecca and Struhl, 2007). To test whether Vestigial also plays a role in the shaping of wing disc cells, we generated and analyzed *vestigial* mutant clones of cells. vg^{83b27R} mutant clones are poorly recovered and often only comprise two to three cells when induced in the wing disc pouch (Kim et al., 1996). To overcome this limitation, we expressed p35 within vg^{83b27R} mutant clones. Large clones (>20 cells) mutant for vg^{83b27R} expressing p35 were recovered within the wing disc pouch, albeit at a low frequency (Fig. 7A). Mutant cells invaginated from the apical side of the epithelium and formed a funnel-like lumen (Figs. 7A–C). At the base of these lumina, mutant cells displayed a strongly reduced length along their apical–basal axis compared to neighboring control cells in the wing disc pouch (Baena-Lopez and Garcia-Bellido, 2006) (Fig. 7B), similar to clones of cells compromised for Wingless signal transduction.

Clones of cells expressing Vestigial in the wing disc hinge invaginate

We have shown above that an inappropriate reduction in Vestigial activity in clones of cells results in the formation of epithelial invaginations in the wing disc pouch. To test whether an inappropriate increase in Vestigial protein results in similar morphological alterations, we overexpressed Vestigial in clones of cells. Cells in clones over-expressing Vestigial, even though they proliferated poorly, had a normal appearance when located in the wing disc pouch (Figs. 8A, B). When located in the hinge, however, these clones bulged out of the epithelium and formed outpockets (Fig. 8A). Clonal cells in these outpockets invaginated from the apical side of the epithelium (Fig. 8C). Taken together, these results show that both the clonal inactivation of Vestigial within the Vestigial expression domain, and clonal overexpression of Vestigial in regions where Vestigial is not expressed, results in epithelial invagination and abnormal cell shape.

Vestigial is required to maintain apical–basal cell length in the wing disc pouch

To test whether Vestigial is required in a cell-autonomous manner to maintain the highly elongated shape of wing disc pouch cells during late-larval development, we reduced the activity of *vestigial* by RNA interference. Expression of a double-stranded RNA targeting *vestigial* (vg^{dsRNA}) throughout the dorsal compartment of wing discs led to a strong reduction in Vestigial expression in these cells and to a reduced size of the dorsal wing disc pouch (Figs. 9A, B). Sections through the center of the wing disc pouch, where Vestigial levels are normally high, showed that cells expressing vg^{dsRNA} were moderately, but reproducibly, shorter along their apical–basal axis compared to the ventral control cells within the same wing disc (Fig. 9A). At more lateral sides in the wing disc pouch, where Vestigial levels normally are low, the difference in length between vg^{dsRNA}-expressing cells and ventral control cells was more pronounced (Figs. 9B, D). We conclude

Fig. 4. A cell-autonomous role for Wingless signal transduction in promoting a highly elongated cell shape in the center of the wing disc pouch. (A) *xz* view of a late-third instar wing disc co-expressing TCF^{DN} and p35 (green) in the dorsal compartment 39 h after temperature shift to inducing conditions and stained for F-actin (red) and Distal-less (blue). TCF^{DN} and p35-co-expressing cells have highly reduced levels of Distal-less, invaginate close to the dorsoventral compartment boundary (arrowhead), and are shorter along the apical–basal axis compared to control ventral cells (double-sided arrows). (B) *xz* view of late-third instar wing disc co-expressing TCF^{DN} and p35 (green) in the dorsal compartment 39 h after temperature shift to inducing conditions and stained for F-actin (red) and Nubbin (blue). Nubbin is expressed at low levels in the pouch and at elevated levels in the distal hinge (DH), and its expression remains in cells co-expressing TCF^{DN} and p35. Nubbin is not detectable in proximal hinge cells (PH). Arrows point to the approximate border between pouch and distal hinge, as revealed by Nubbin staining. (C) Schematic representation of (B). (D, E) *xz* views of a late-third instar (D) control wing disc or (E) a wing disc expressing TCF^{DN} under the control of the *nubbin-GAL4* driver stained for F-actin (red) (and DNA in (E)). TCF^{DN}-expressing cells are shorter along the apical–basal axis compared to control cells (double-sided arrows). Arrows point to the approximate border between pouch and distal hinge, as inferred from the folding of the epithelium. (F) *xz* view of an early-third instar control wing disc expressing CD8–GFP (green) under the control of *ap-GAL4* in the dorsal compartment and stained for F-actin (red), DNA (white), and Wingless (blue). Dorsal and ventral cells have a similar apical–basal cell length (double-sided arrows). (G) *xz* view of an early-third instar wing disc co-expressing Wingless (Wg) and CD8–GFP (green) under the control of *ap-GAL4* in the dorsal compartment and stained as in (F). Dorsal cells, and ventral cells in the vicinity of the dorsoventral compartment boundary, are elongated along the apical–basal axis compared to cells elsewhere (double-sided arrows). (H) *xz* view of an early-third instar wing disc co-expressing Arm^{S10} and CD8–GFP (green) under the control of *ap-GAL4* in the dorsal compartment and stained for F-actin (red), DNA (white), and Distal-less (blue). Arm^{S10}-expressing cells display elevated levels of Distal-less and are elongated along the apical–basal axis compared to the ventral control cells (double-sided arrows). (I) Ratio of apical–basal cell length between dorsal and ventral cells in late-third instar wing discs expressing p35 (control) or TCF^{DN} and p35 (TCF^{DN} *p35*), or in early-third instar wing discs expressing CD8–GFP (control), or Arm^{S10} and CD8–GFP (arm^{S10}) in the dorsal compartment. Means ± s.e.m. are indicated ($n = 5$ wing discs (p35, control); $n = 13$ (TCF^{DN} *p35*); $n = 11$ (CD8–GFP, control); $n = 5$ (arm^{S10}); *$P < 0.001$). *xz* sections in A, B, D–H, and in Figs. 6, 9, 10, S4, and S6C were taken approximately through the center of the wing disc perpendicular to the dorsoventral compartment boundary, except otherwise stated; dorsal is shown to the right. Scale bars: 20 μm.

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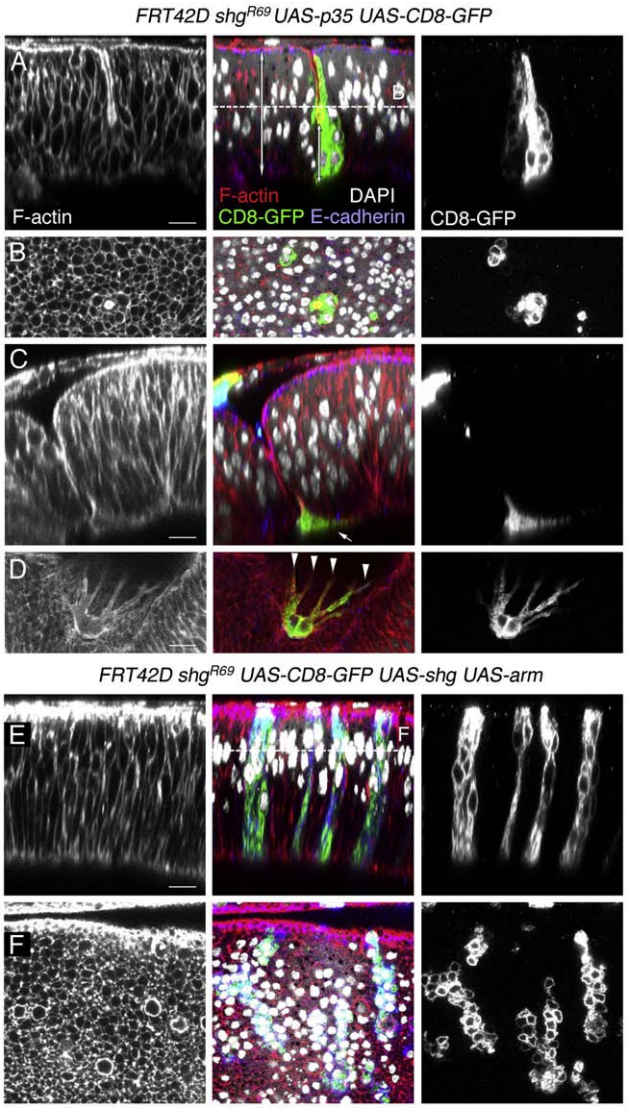

Fig. 5. *shg*^{R69} mutant clones expressing p35 invaginate and extrude from the wing disc epithelium. (A–D) Clones of *shg*^{R69} mutant cells expressing p35 and marked by the presence of CD8–GFP (green) were induced and stained 48 h later for F-actin (red), DNA (white), and E-cadherin (blue). (A) *xz* view. *shg*^{R69} cells are part of a deep epithelial invagination and, at the base of the invagination, are much shorter along the apical–basal axis compared to control cells (double-sided arrows). (B) *xy* view. *xz* (C) and basal *xy* (D) views of an extruded *shg*^{R69} clone (arrow). Mutant cells form long protrusions (arrowheads). (E, F) Clones of *shg*^{R69} mutant cells co-expressing E-cadherin and Armadillo (Arm) marked by the presence of CD8–GFP (green) were induced and stained as in (A–D). *xz* (E) and *xy* (F) views are shown. Clonal cells have a normal morphology and do not invaginate or extrude. Scale bars: 10 μm.

that Vestigial is required to maintain, in a cell-autonomous manner, the normal elongated shape of wing disc pouch cells during late-larval development.

Vestigial is sufficient to promote cell elongation in the wing disc pouch

To test whether Vestigial is sufficient to promote cell elongation during early larval development, we expressed Vestigial from a transgene in the dorsal compartment of wing discs. When analyzed in early-third instar larval stage, dorsal cells over-expressing Vestigial from second instar onwards were elongated along their apical–basal axis compared to the neighboring ventral control cells (Figs. 9C, D). These data indicate that Vestigial is sufficient to promote apical–basal cell elongation during early larval development.

Vestigial mediates a morphogenetic response to Wingless signaling

To test whether Wingless signaling controls wing disc pouch cell shape by maintaining expression of Vestigial, we generated *arrow*^{2} mutant clones of cells expressing Vestigial in second instar larval stage and analyzed the frequency of clones and the shape of cells in these clones in late-third instar larval development. Control *arrow*^{2} mutant clones expressing GFP were rarely recovered (Fig. 10A; 1.1 ± 0.92 (mean $\pm$ s.d.) clones per wing disc pouch, $n = 9$). *arrow*^{2} mutant clones expressing Vestigial were recovered at a significantly higher frequency (Fig. 10B, 3.6 ± 1.8 (mean $\pm$ s.d.) clones per wing disc pouch, $n = 25$; $P < 0.0001$). However, as shown in Fig. S7, such clones were still part of epithelial invaginations, indicating that expression of Vestigial might have slowed down extrusion of *arrow*^{2} mutant clones, but did not efficiently suppress their morphogenetic defects. The juxtaposition of wild-type cells and *arrow*^{2} mutant cells at clone borders might, in part, lead to exaggerated morphogenetic defects inside the clone. Therefore we tested whether the expression of Vestigial can suppress the shortening of cells that is a consequence of time-controlled induction of compartment-wide TCF^{DN} expression. At a time after induction when TCF^{DN}-expressing cells showed a severely reduced apical–basal cell length, cells co-expressing Vestigial and TCF^{DN} still had a nearly normal apical–basal length (Figs. 10C–E). Distal-less remained undetectable in these cells (Fig. 10D), indicating that Wingless signaling remained repressed. These results show that the cell shape defects caused by reducing Wingless signal transduction can be partially suppressed by Vestigial. Vestigial, therefore, is an important mediator of the morphogenetic response to Wingless signaling.

Discussion

The developmental control of cell shape is important for animal morphogenesis. Here, we have addressed the role of the Wnt/Wingless signaling pathway and two of its target genes, *vestigial* and *E-cadherin*/*shg*, in shaping *Drosophila* wing disc pouch cells during development. Clones of mutant cells lacking Wingless signal transduction, Vestigial, or E-cadherin invaginate and are lost from the epithelium by basal extrusion. Compartment-wide expression of TCF^{DN}, or double-stranded RNA targeting *vestigial* or *shg*, led to short cells throughout this region, indicating that these genes act cell autonomously to maintain normal cell shape. Conversely, overexpression during early development of Wingless, Arm^{S10}, or Vestigial resulted in precocious cell elongation. The abnormal cell shape caused by TCF^{DN} expression was partially suppressed by co-expression of Vestigial. Our results show an important role for the canonical Wingless signaling pathway, E-cadherin, and Vestigial in shaping wing disc pouch cells. Furthermore, they suggest that Vestigial is an important mediator of the morphogenetic response to Wingless.

A cell-autonomous role for Wingless signaling in shaping wing disc cells

Clones of cells defective for Wingless signaling are only poorly recovered within the wing disc pouch and are small compared to control clones. Inhibition of apoptosis partially suppresses the slow clone growth (Giraldez and Cohen, 2003; Johnston and Sanders, 2003). Our observation that these clones extrude from the wing disc pouch epithelium suggests that cell extrusion might also account for the poor recovery of these clones. Cell extrusion has been previously reported, for example, for clones defective in Dpp signal transduction, actin capping proteins, or C-terminal Src kinase activity (Corrigall et al., 2007; Gibson and Perrimon, 2005; Janody and Treisman, 2006; Shen and Dahmann, 2005; Vidal et al., 2006; Widmann and Dahmann, 2009), indicating that it is a common mechanism to remove inappropriate cells from wing disc epithelia. Previous reports showed that cell extrusion appears to be a consequence of the local

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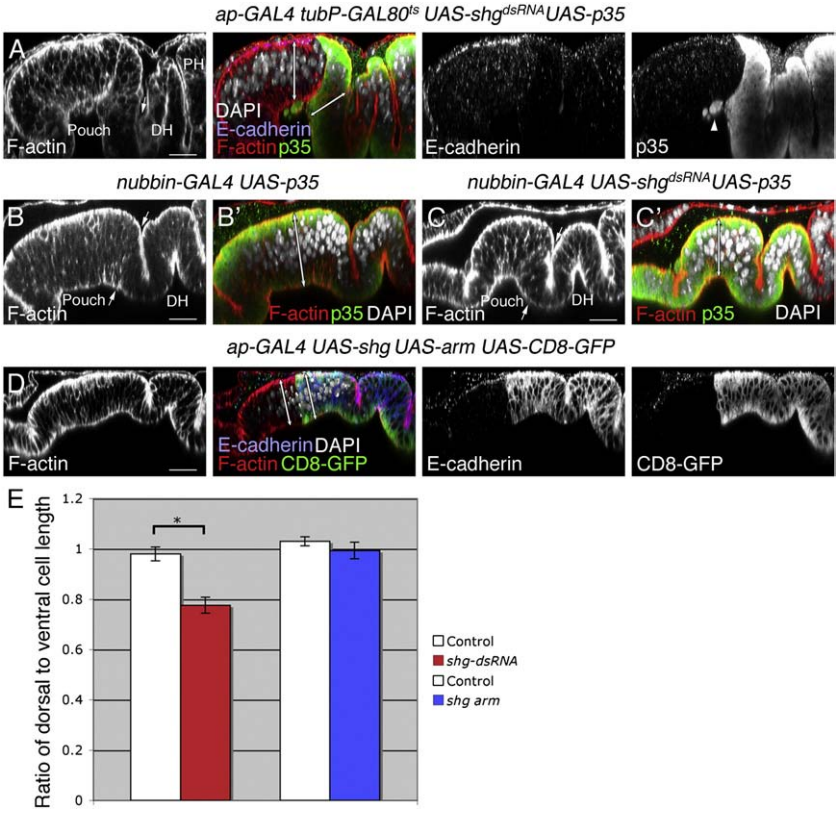

Fig. 6. E-cadherin is required to maintain apical–basal cell length. (A) *xz* view of a late-third instar wing disc co-expressing double-stranded RNA targeting *shg* (shg^{dsRNA}) and p35 (green) in the dorsal compartment at 52 h after temperature shift to inducing conditions and stained for F-actin (red), DNA (white), and E-cadherin (blue). shg^{dsRNA} and p35-co-expressing cells in the wing disc pouch have highly reduced levels of E-cadherin and are shorter along the apical–basal axis compared to control ventral cells (double-sided arrows). Arrow points to the approximate border between pouch and distal hinge, as inferred from the folding of the epithelium. A few single shg^{dsRNA} and p35-co-expressing cells extrude from the epithelium (arrowhead). (B, C) *xz* sections of late-third instar wing discs expressing (B) p35 or (C) p35 and shg^{dsRNA} under the control of *nubbin-GAL4* in the wing disc pouch and stained as in (A). shg^{dsRNA} and p35-co-expressing cells are shorter along the apical–basal axis compared to control p35-expressing cells (double-sided arrows). Arrows point to the approximate border between pouch and distal hinge. (D) *xz* section of an early-third instar wing disc co-expressing E-cadherin, Armadillo, and CD8–GFP (green) under the control of *ap-GAL4* in the dorsal compartment and stained for F-actin (red), DNA (white), and E-cadherin (blue). Dorsal cells and ventral cells display a similar apical–basal length (double-sided arrows). (E) Ratio of apical–basal cell length between dorsal and ventral cells in late-third instar wing discs expressing p35 (control) or shg^{dsRNA} and p35 (shg^{dsRNA}), or in early-third instar wing discs expressing CD8–GFP (control), or E-cadherin, Armadillo and CD8–GFP (*shg arm*) in the dorsal compartment. Means ± s.e.m. are indicated ($n = 5$ wing discs (p35, control); $n = 6$ (shg^{dsRNA}); $n = 11$ (CD8–GFP, control); $n = 5$ (*shg arm*); $^*P < 0.001$). Scale bars: 20 μm.

environment of the mutant cells, i.e. the apposition of mutant and wild-type cells (Vidal et al., 2006; Widmann and Dahmann, 2009). Similarly, we observed invagination and extrusion of mutant cells only when Wingless signaling was compromised in clones, but not when it was compromised throughout the pouch region of the wing disc. The invagination and extrusion of clones of cells with defective Wingless signal transduction, therefore, might be the consequence of the apposition of wild-type cells and cells with inappropriately reduced Wingless signal transduction at the clone border. The initial mechanisms that result in the extrusion of clones of cells that are compromised for Wingless signal transduction activity remain to be analyzed. Our finding that Wingless signal transduction is required to maintain proper epithelial cell shape suggests that the disparate cell shapes at the mutant clone border might contribute to the initiation of cell extrusion.

Blocking Wingless signaling and apoptosis within the dorsal compartment or throughout the pouch region of the wing disc by co-expression of TCF^{DN} and p35 resulted in reduced apical–basal cell length, indicating that Wingless signal transduction is required in a cell-autonomous manner to maintain the normal elongated cell shape within the pouch region of late-larval stage wing discs. Cells forced to survive after Wingless deprivation retain markers of wing fates (Johnston and Sanders, 2003) (Fig. 4B), indicating that the observed alterations in cell shape are not caused by changes in cell identity, but rather reflect a direct role of Wingless signaling in maintaining normal cell shape. Our observation that expression of Wingless or a constitutively-active form of the Wingless transducer Armadillo/β-catenin (Arm^{S10}) during early larval development resulted in precocious cell elongation, suggests furthermore that Wingless signal transduction plays an instructive role during the normal cuboidal-to-columnar shape transition that takes place in late-second instar wing discs.

Expression of constitutively-active Armadillo in clones of cells located in the wing disc hinge or peripodial membrane resulted in increased clone size, epithelial invagination, and outpocketing during late-larval development (Figs. 3C–F; data not shown). In contrast, when located in the wing disc pouch, such clones have fewer cells compared to control clones (Johnston and Sanders, 2003) (Fig. 3A) and maintain their normal columnar shape (Fig. 3B), indicating regional differences in the response of wing disc cells to elevated levels of Wingless signaling. Expression of Wingless stimulates growth and division of wing disc hinge cells (Giraldez and Cohen, 2003) and increases cell number in the peripodial membrane (Baena-Lopez et al., 2003). These results suggest that increased cell proliferation, in addition to alterations in the cytoskeleton and cell–cell adhesion, might contribute to the invagination and outpocketing of Arm^{S10}-expressing clones located in the wing disc hinge and peripodial membrane. The slow rate of cell division, yet normal columnar shape, of cells expressing constitutively-active Armadillo in the wing disc pouch indicates that slowing down cell division in clones does not inevitably affect cellular morphology.

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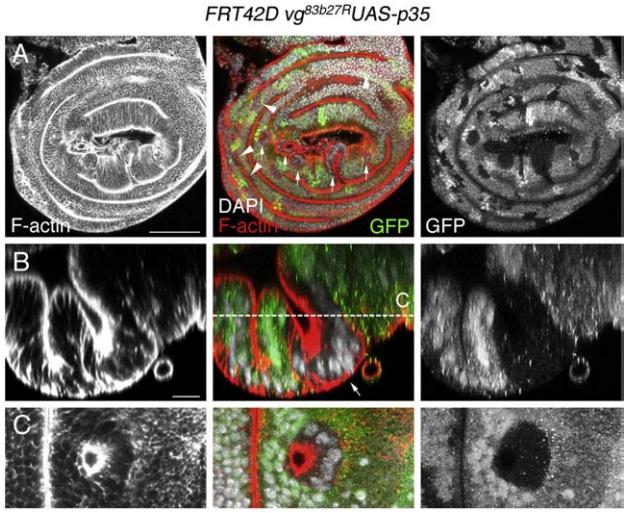

Fig. 7. vg^{83b27R} mutant clones in the wing disc pouch invaginate. (A–C) Clones of vg^{83b27R} mutant cells marked by the absence of GFP (green) were induced and stained 60 h later for F-actin (red), and DNA (white). (A) *xy* view. vg^{83b27R} clones have a normal appearance in the hinge (arrowheads) but invaginate in the pouch region of the wing disc (arrows). (B) *xz* view. vg^{83b27R} mutant cells are part of a deep epithelial invagination (arrow) and, at the base of the invagination, are much shorter along the apical–basal axis compared to control cells. The fold to the left in the image is part of the hinge. (C) *xy* view. Scale bars: 50 μm (A); 10 μm (B).

E-cadherin and columnar cell shape

E-cadherin is a component of adherens junctions and is required for cell–cell adhesion. In the *Drosophila* epidermis, for example, mutations in E-cadherin result in the disintegration of morphogenetically active epithelia (Tepass et al., 1996). We found that, in the wing disc, shg^{R69} mutant clones lose their normal columnar cell shape and extrude from the basal side of the epithelium. Extruded cells displayed long actin-rich protrusions, consistent with the notion that these cells underwent an epithelial-to-mesenchymal transition, as has been observed in other epithelia for the loss of E-cadherin (Thiery and Sleeman, 2006). In the wing disc, E-cadherin appears to have a cell-autonomous function in maintaining columnar cell shape, because compartment-wide down-regulation of E-cadherin through RNA interference initially resulted in a decreased apical–basal length of these cells without detectable epithelial disintegration. It is difficult, however, to demonstrate a direct role for E-cadherin in controlling columnar cell shape because of its predominant role in maintaining cell adhesion. Nevertheless, these data indicate that E-cadherin mediated cell–cell adhesion is important for maintaining a highly columnar cell shape.

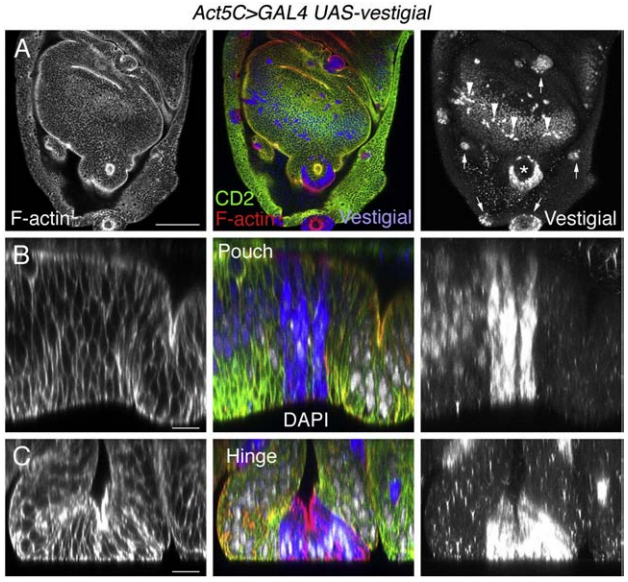

Fig. 8. Clones of cells expressing Vestigial in the wing disc hinge invaginate and form outpockets. (A–C) Clones of cells expressing Vestigial and marked by the absence of CD2 (green) were induced and stained 60 h later for Vestigial (blue), F-actin (red) and DNA (white). (A) *xy* view. (B, C) *xz* views of clones in the (B) pouch and (C) hinge region of the wing disc. Vestigial-expressing clones in the pouch are unusually small but display a normal morphology (arrowheads in (A)); in the hinge, these clones lead to outpocketing and invagination (arrows in (A)). The invaginating group of cells marked by an asterisk is composed of both clonal and wild-type cells. Scale bars: 50 μm (A); 10 μm (B, C).

Vestigial mediates a cell shape response to Wingless signaling

Several observations indicate that Vestigial mediates, at least in part, a morphogenetic response to Wingless signaling. First, similar to cells in clones lacking Wingless signal transduction, cells in *vestigial* mutant clones change shape and form epithelial invaginations. Second, similar to expression of TCF^{DN}, compartment-wide reduction of Vestigial function resulted in shorter cells, albeit to a lesser degree compared to the expression of TCF^{DN}. This difference might indicate that, in addition to Vestigial, other target genes of TCF might mediate

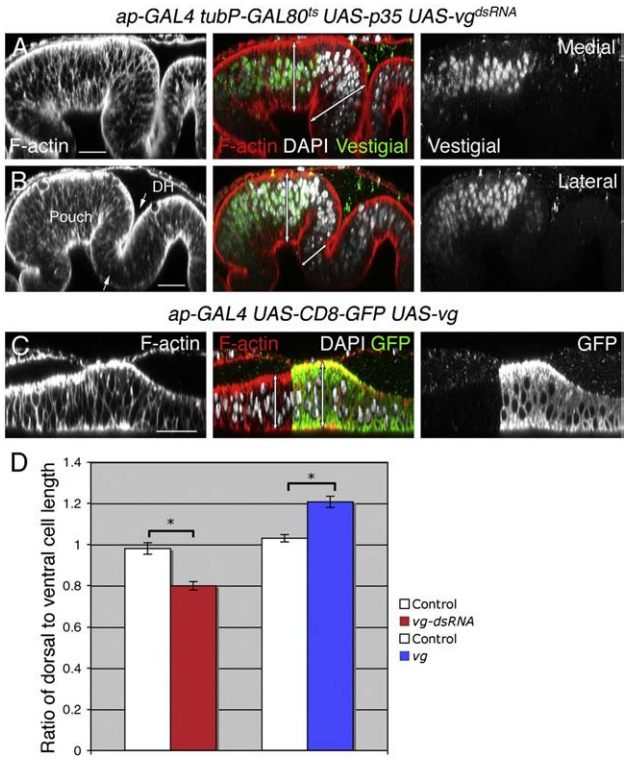

Fig. 9. A cell-autonomous role for Vestigial in promoting a highly elongated cell shape in the wing disc pouch. (A, B) *xz* views of a (A) medial or (B) lateral section of a late-third instar wing disc pouch co-expressing $vestigial^{dsRNA}$ and p35 in the dorsal compartment at 74 h after temperature shift to inducing conditions and stained for F-actin (red), Vestigial (green), and DNA (white). $vestigial^{dsRNA}$ and p35-co-expressing cells have highly reduced levels of Vestigial and are shorter along the apical–basal axis compared to control ventral cells (double-sided arrows). Arrows indicate the approximate border between pouch and distal hinge, as inferred from the folding of the epithelium. (C) *xz* section of an early-third instar wing disc co-expressing Vestigial and CD8–GFP (green) under the control of *ap-GAL4* in the dorsal compartment and stained for F-actin (red), and DNA (white). Vestigial-expressing cells are elongated along the apical–basal axis compared to the ventral control cells (double-sided arrows). (D) Ratio of apical–basal cell length between dorsal and ventral cells in late-third instar wing discs expressing p35 (control) or vg^{dsRNA} and p35 (vg^{dsRNA}), or in early-third instar wing discs expressing CD8–GFP (control), or Vestigial and CD8–GFP (*vg*) in the dorsal compartment. Means ± s.e.m. are indicated ($n = 5$ wing discs (p35, control); $n = 11$ (vg^{dsRNA}); $n = 11$ (CD8–GFP, control); $n = 4$ (*vg*); $^*P < 0.002$). Scale bars: 20 μm.

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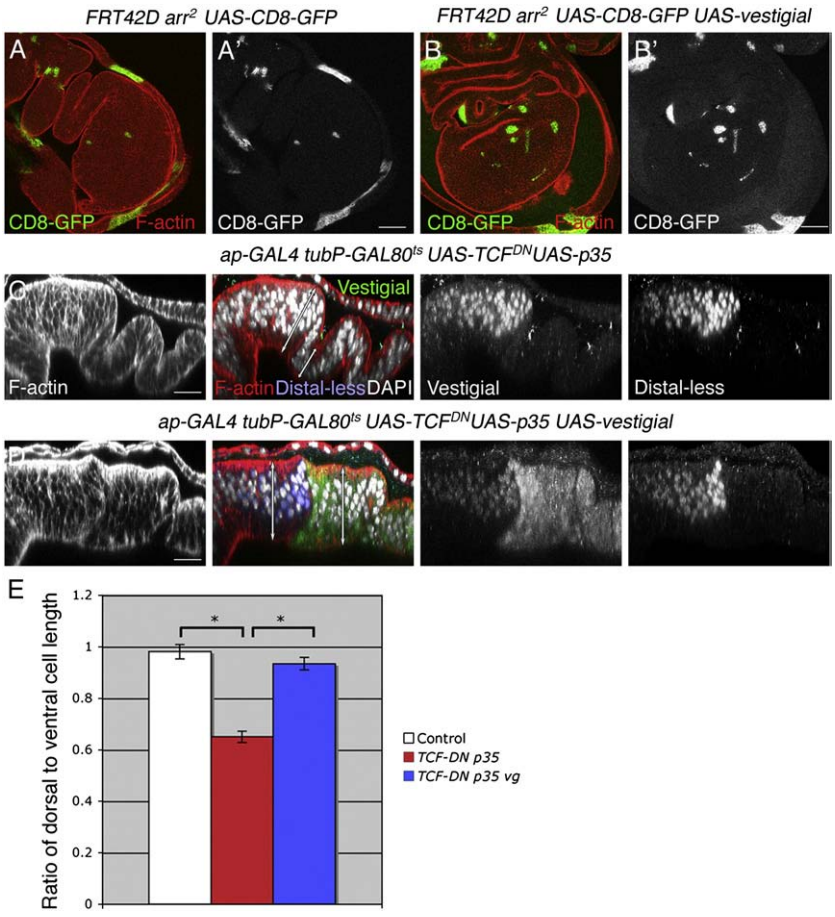

Fig. 10. Vestigial suppresses shape defects of TCF^{DN}-expressing cells in the wing disc pouch. (A, B) Clones of *arrow*^{2} mutant cells expressing (A) CD8–GFP or (B) CD8–GFP and Vestigial were induced and stained 75 h later for F-actin (red) and CD8–GFP (green). *xy* views are shown. Expression of Vestigial increases the number of observable *arrow*^{2} mutant clones. (C) *xz* view of a wing disc co-expressing TCF^{DN} and p35 in the dorsal compartment 37 h after temperature shift to inducing conditions and stained for F-actin (red), Vestigial (green), and Distal-less (blue). TCF^{DN} and p35-co-expressing cells are shorter along the apical–basal axis compared to control ventral cells (double-sided arrows). (D) *xz* view of a wing disc co-expressing TCF^{DN}, p35, and Vestigial as described in (C) and stained for F-actin (red), Vestigial (green), and Distal-less (blue). TCF^{DN}, p35, and Vestigial-co-expressing cells have an apical–basal length comparable to control ventral cells (double-sided arrows). (E) Ratio of apical–basal cell length between dorsal and ventral cells in late-third instar wing discs expressing p35 (control), or TCF^{DN} and p35 (*TCF^{DN} p35*), or TCF^{DN}, p35, and Vestigial (*TCF^{DN} p35 vg*) in the dorsal compartment. Means ± s.e.m. are indicated ($n = 5$ wing discs (control); $n = 11$ (*TCF^{DN} p35*); $n = 6$ (*TCF^{DN} p35 vg*); $^*P < 0.001$). Scale bars: 20 μm.

the morphogenetic response to Wingless. Distal-less is required for the specification of the wing margin, but apparently dispensable for other aspects of wing development (Campbell and Tomlinson, 1998; Gorfinkiel et al., 1997). Moreover, RNAi-mediated down-regulation of *distal-less* had no observable effect on wing disc cell shape (data not shown), indicating that Distal-less might not be involved in shaping wing disc cells in response to Wingless signaling. Third, clones of cells over-expressing Vestigial in late-larval development display, similar to clones expressing the constitutively-active Wingless transducer Arm^{S10}, a normal morphology when located in the wing disc pouch, however, invaginate and form outpockets when present in the wing disc hinge. Fourth, similar to the activation of Wingless signal transduction, overexpression of Vestigial during early larval development results in precocious cell elongation. The fifth, and most compelling piece of evidence that Vestigial mediates the morphogenetic response to Wingless is that expression of Vestigial is sufficient to partly suppress the cell shape defects caused by reducing Wingless signal transduction through expression of TCF^{DN}.

How does Vestigial mediate a morphogenetic response to Wingless signaling? The actin cytoskeleton plays a pivotal role in shaping cells within tissues (for a recent review, see Lecuit and Lenne (2007)). Recent results show that Vestigial induces the expression of *actin capping protein α* (*cpa*) in the wing disc pouch (Janody and Treisman, 2006). Capping proteins inhibit the extension of barbed ends of actin filaments and thereby restrict actin polymerization (Schafer et al., 1995). Mutations in either *cpa* or *cpb*, the gene encoding the capping protein β, result in the invagination and extrusion of Vestigial-expressing cells (Janody and Treisman, 2006) (Fig. S8), similar to the observations for mutations in Wingless signal transduction components (Figs. 2, S3) or *vestigial* (Baena-Lopez and Garcia-Bellido, 2006) (Fig. 7). These results are consistent with the notion that Wingless signal transduction, at least in part, controls cell shape by maintaining *vestigial* expression, which in turn induces *cpa* expression to modify the actin cytoskeleton and thereby cell shape. We have recently provided evidence that Dpp signaling controls cell shape in the wing disc pouch by regulating Rho1 and non-muscle myosin II (Myosin II) (Widmann and Dahmann, 2009). Thus, it will be interesting to investigate whether Wingless signaling might also act on Rho1 and Myosin II to control cell shape.

Dpp signaling (Gibson and Perrimon, 2005; Shen and Dahmann, 2005; Widmann and Dahmann, 2009) and Wingless signaling (Jaiswal et al., 2006) (this study) control the shape of cells in the wing disc pouch by regulating gene expression. These findings are consistent with the view that the positional information provided by the graded expression of specific Dpp and Wingless target genes is used to direct cell shape and epithelial morphology within wing imaginal discs.

The outpocketing of clones of cells expressing the constitutively-active Wingless transducer Arm^{S10} in the wing disc hinge presents an interesting parallel to the outpocketing of the vertebrate intestinal

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epithelium during the formation of adenomas. In addition to the superficial similarity in cellular morphology, adenomas are frequently associated with mutations in genes that activate Wnt signaling. For example, mutations in the gene *adenomatous polyposis coli* (*APC*), which lead to elevated levels of β-catenin, are responsible for familial adenomatous polyposis and sporadic cancers of the digestive tract (reviewed in de Lau et al. (2007)). A role for β-catenin-dependent Wnt/Wingless signaling in epithelial morphogenesis might thus be conserved in vertebrates.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ydbio.2009.07.013.

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