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# The canonical Wg and JNK signaling cascades collaborate to promote both dorsal closure and ventral patterning

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## SUMMARY

**Elaboration of the *Drosophila* body plan depends on a series of cell-identity decisions and morphogenetic movements regulated by intercellular signals. For example, Jun N-terminal kinase signaling regulates cell fate decisions and morphogenesis during dorsal closure, while Wingless signaling regulates segmental patterning of the larval cuticle via Armadillo. *wingless* or *armadillo* mutant embryos secrete a lawn of ventral denticles; *armadillo* mutants also exhibit dorsal closure defects. We found that mutations in *puckered*, a phosphatase that antagonizes Jun N-terminal kinase, suppress in a dose-sensitive manner both the dorsal and ventral *armadillo* cuticle defects. Furthermore, we found that activation of the Jun N-terminal kinase signaling pathway suppresses *armadillo*-associated defects. Jun N-terminal kinase signaling promotes dorsal closure, in part, by regulating *decapentaplegic* expression in the dorsal epidermis. We demonstrate that Wingless signaling is also required to activate *decapentaplegic* expression and to coordinate cell shape changes during dorsal closure. Together, these results demonstrate that MAP-Kinase and Wingless signaling cooperate in both the dorsal and ventral epidermis, and suggest that Wingless may activate both the Wingless and the Jun N-terminal kinase signaling cascades.**

Key words: Armadillo, Dorsal closure, Segment polarity, Puckered, Wingless, JNK, Wnt, β-catenin, *Drosophila*

## INTRODUCTION

Proper patterning of multicellular organisms depends on stringent regulation of cell-cell signaling. Members of the Wnt/Wingless (Wg) family of secreted glycoproteins direct cell fates in both insects and vertebrates (reviewed in Wodarz and Nusse, 1998). Genetic studies revealed many of the genes required for Wg signaling in *Drosophila*. Mutations in several of these, including *armadillo* (*arm*), were first identified in a screen for genes whose zygotic expression is required for embryonic viability and proper patterning of the larval cuticle (Nusslein-Volhard and Wieschaus, 1980). *arm* mutant embryos exhibit segment polarity defects characterized by secretion of a ventral lawn of denticles and a concomitant loss of naked cuticle. This phenotype resembles that of *wg* mutants, because Arm functions in the transduction of Wg signal.

Arm is the *Drosophila* ortholog of human β-catenin (β-cat). Both are found associated with cell-cell adherens junctions, in the cytoplasm and in nuclei. Wg/Wnt signaling elicits a cellular response by regulating the free pool of Arm/β-cat. In the absence of Wg/Wnt signal, cytoplasmic Arm/β-cat is rapidly degraded via a proteasome-mediated pathway (reviewed in Polakis, 1999). Cells of the *Drosophila* epidermis that receive Wg signal accumulate Arm in the cytoplasm and nucleus (reviewed in Wodarz and Nusse, 1998). This depends upon Wg binding cell surface receptors of the Frizzled (Fz) family, the activation of Dishevelled (Dsh) and subsequent inactivation of Zeste white-3 (Zw3, the *Drosophila* ortholog of glycogen synthase kinase-3β). From studies of mammals, two additional proteins, APC and Axin, were implicated in regulating β-cat stability (reviewed in Polakis, 1999). Members of the TCF/LEF family of transcription factors are also required for Wnt/Wg signal transduction (reviewed in Wodarz and Nusse, 1998). TCF/LEF transcription factors bind DNA, and recruit Arm/β-cat as a co-activator, thus activating Wg/Wnt-responsive genes. Therefore, Wg/Wnt signaling regulates cell fate choices directly by altering the patterns of gene expression.

In *Drosophila*, upstream components of the Wg pathway are also required during the establishment of planar polarity, the process whereby epithelial cells acquire positional information relative to the body axes of the animal. For example, both Fz and Dsh are required to coordinate the proximal-to-distal orientation of actin-based wing hairs (reviewed in Shulman et al., 1998). Most tests have suggested that planar polarity is independent of Arm function, while genetic and biochemical studies suggest that the Jun N-terminal kinase (JNK) signaling pathway functions downstream of Fz and Dsh to establish planar polarity (reviewed in Boutros and Mlodzik, 1999). It remains unclear whether Fz is activated by Wg, or any Wnt, during the establishment of planar polarity. It has been

suggested that JNK signaling and the canonical Wg pathway function as alternate signal transduction pathways downstream of Fz, with Dsh functioning as a branch point from which different cellular processes are elicited in a tissue-specific manner.

In *Drosophila*, the JNK pathway is best known for its role in dorsal closure (reviewed in Noselli and Agnes, 1999). During stage 13 of embryogenesis, two lateral epidermal sheets migrate toward the dorsal midline where they fuse. Mutations in the *Drosophila* orthologs of JNK kinase (JNKK; Hemipterous; Hep; Glise et al., 1995), JNK (Basket; Bsk; Riesgo-Escovar et al., 1996; Sluss et al., 1996), as well as the transcription factors Fos (Kayak; Kay; Riesgo-Escovar and Hafen, 1997; Zeitlinger et al., 1997) and Djun (Hou et al., 1997; Kockel et al., 1997; Riesgo-Escovar and Hafen, 1997), block dorsal closure. Other regulators of dorsal closure include Misshapen, a Ste20 relative (Paricio et al., 1999; Su et al., 1998), Puckered (Puc), a VH1-like phosphatase (Martin-Blanco et al., 1998), and Decapentaplegic (Dpp), a TGFβ relative (reviewed in Noselli and Agnes, 1999). Both Puc and Dpp are activated in the dorsalmost epidermal cells by JNK signaling (reviewed in Noselli and Agnes, 1999). Dpp then acts over several cell diameters to coordinate cell shape changes throughout the epidermis. Puc, in contrast, antagonizes JNK signaling in cells adjacent to the leading edge. The ligand that initiates JNK signaling in this context remains to be identified.

Mutations in APC and β-cat play roles in both colorectal cancer and melanoma (reviewed in Polakis, 1999), but these mutations fail to account for all cases, suggesting that other Wnt signaling antagonists might be involved in oncogenesis. To identify antagonists that function in embryogenesis and/or oncogenesis, a modifier screen for suppressors of the *arm* zygotic phenotype was performed (Cox et al., 2000). In the course of this screen, an unexpected connection between JNK and Wg signaling was discovered. Previous models suggested that distinct signaling pathways operate in the ventral and dorsal epidermis, with JNK signaling coordinating dorsal closure and Wg signaling acting through Arm to establish segment polarity in the ventral epidermis. Here, we demonstrate that mutations in *puc*, a known negative regulator of JNK signaling, suppress both the dorsal closure and ventral segment polarity defects associated with a decrease in Wg signaling. Furthermore, we present evidence that downstream components of the Wg signaling pathway, like Arm and dTCF, act together with JNK signaling pathways in both ventral patterning and dorsal closure. Our data are consistent with a model whereby Wg activates separate, yet parallel, signaling cascades that are required to promote dorsal closure and establish ventral pattern.

## MATERIALS AND METHODS

### Fly stocks and phenotypic analysis

The wild-type stock was Canton S. Mutants used are described at http://flybase.bio.indiana.edu. Unless otherwise noted, *arm=arm$^{XP33}$/Y*, *puc=puc$^{A251.1}$*, *wg$^{weak}$=wg$^{PE4}$/Df(2)DE* and *wg$^{null}$=wg$^{IG22}$*. *zw3$^{M1-1}$* and *dsh$^{75}$* germline clones were generated as in Peifer et al. (1994). Stocks were obtained as follows: *puc$^{A251.1}$*, *Djun$^{2}$* and *kay$^{1}$*, the Bloomington *Drosophila* stock center; *puc$^{R10}$* and *puc$^{E69}$*, A. Martinez Arias; *UAS-dpp*, *UAS-tkvA*, *UAS-tkv$^{Q253D}$*, *UAS-DTak$^{WT3}$*, *UAS-DTak1* and *UAS-DTak$^{KN(1-1)}$*, M. B. O'Connor; *UAS-Bsk* and *UAS-Hep*, M. Mlodzik; *LE-Gal4*, S. Noselli; *UAS-Wg*, A. Bejsovec; *arm-Gal4>>VP16* and β-*tubulin-flp*, J. P. Vincent and D. St. Johnston. To express Gal4::VP16, *arm-Gal4>>VP16/TM3* females were mated to β-*tubulin-flp* males. Non-*TM3* $F_1$ males were collected and mated en masse to UAS females.

### In situ hybridization and immunofluorescence

Dechorionated embryos were fixed for 5 minutes with 1:1 37% formaldehyde/heptane, devitellinized with 1:1 heptane/methanol, postfixed for 15 minutes in 1.85% formaldehyde/PBT (1× PBS + 0.1% Triton X-100), washed extensively with PBT, and transferred to 1× HYB buffer (50% formamide, 5× SSC, 100 μg/ml salmon sperm DNA, 100 μg/ml *E. coli* tRNA, 50 μg/ml heparin, 0.1% Tween-20, pH 4.5) for 1 hour at 70°C. Embryos were incubated overnight at 70°C with heat-denatured digoxigenin-labeled antisense RNA, washed at 70°C once each with 1× HYB, 2:1 HYB/PBT and 1:2 HYB/PBT, four times with PBT, incubated for 1 hour at 25°C with alkaline phosphatase-conjugated anti-digoxigenin (1:2000; Boehringer Mannheim), washed with PBT, and equilibrated with 1× AP buffer (100 mM Tris (pH 9.5), 100 mM NaCl, 50 mM $MgCl_2$, 0.1% Tween-20). Transcripts were visualized with NBT and BCIP. Immunofluorescence was as in Cox et al. (1996).

## RESULTS

### *puc* mutations suppress loss of Arm function

Wg signaling is required to establish posterior cell fates in each larval epidermal segment. In wild-type embryos, anterior cells of each segment secrete cuticle covered with denticles, while posterior cells secrete naked cuticle (Fig. 1A). *arm* mutants, like other mutations disrupting Wg signaling (Nusslein-Volhard and Wieschaus, 1980), exhibit a lawn of ventral denticles and thus loss of naked cuticle (Fig. 1B). In addition, strong hypomorphic or null *arm* alleles, like *arm$^{XP33}$* and *arm$^{YD35}$*, have a dorsal hole, suggesting problems during dorsal closure. Thus, Arm is required during establishment of segment polarity and during dorsal closure.

During a screen for suppressors of *arm*'s embryonic phenotype (Cox et al., 2000), we found that one suppressor was a P-element-induced allele of *puckered (puc*). *puc* encodes a VH-1-like phosphatase that acts as a negative/feedback regulator of the JNK signaling pathway during dorsal closure (Martin-Blanco et al., 1998). *puc$^{A251.1}$* is a strong *puc* allele, exhibiting the characteristic 'puckering' of the dorsal epidermis (Fig. 1E; unless noted, *puc* henceforth refers to *puc$^{A251.1}$*), and results from the insertion of a P-element enhancer trap in intron 2 (Martin-Blanco et al., 1998).

Heterozygosity for *puc* (*arm;puc$^{A251.1}$/+*) strongly suppresses *arm*'s dorsal closure defects, while ventral patterning defects are moderately suppressed (Fig. 1C). *arm;puc$^{A251.1}$* double mutants have a more pronounced suppression of the segment polarity phenotype, with naked cuticle reappearing (arrowheads in Fig. 1D). In addition, *arm;puc$^{A251.1}$* double mutants have a novel dorsal phenotype, characterized by loss of dorsal cuticle (* in Fig. 1D). Another strong *puc* allele, *puc$^{R10}$* (Martin-Blanco et al., 1998), suppresses *arm* to a similar extent, while weak alleles like *puc$^{E69}$* fail to suppress either the dorsal or ventral defects (data not shown). Thus, a reduction in Puc activity suppresses, in a dose-sensitive manner, both the dorsal and ventral cuticle phenotypes of *arm*.

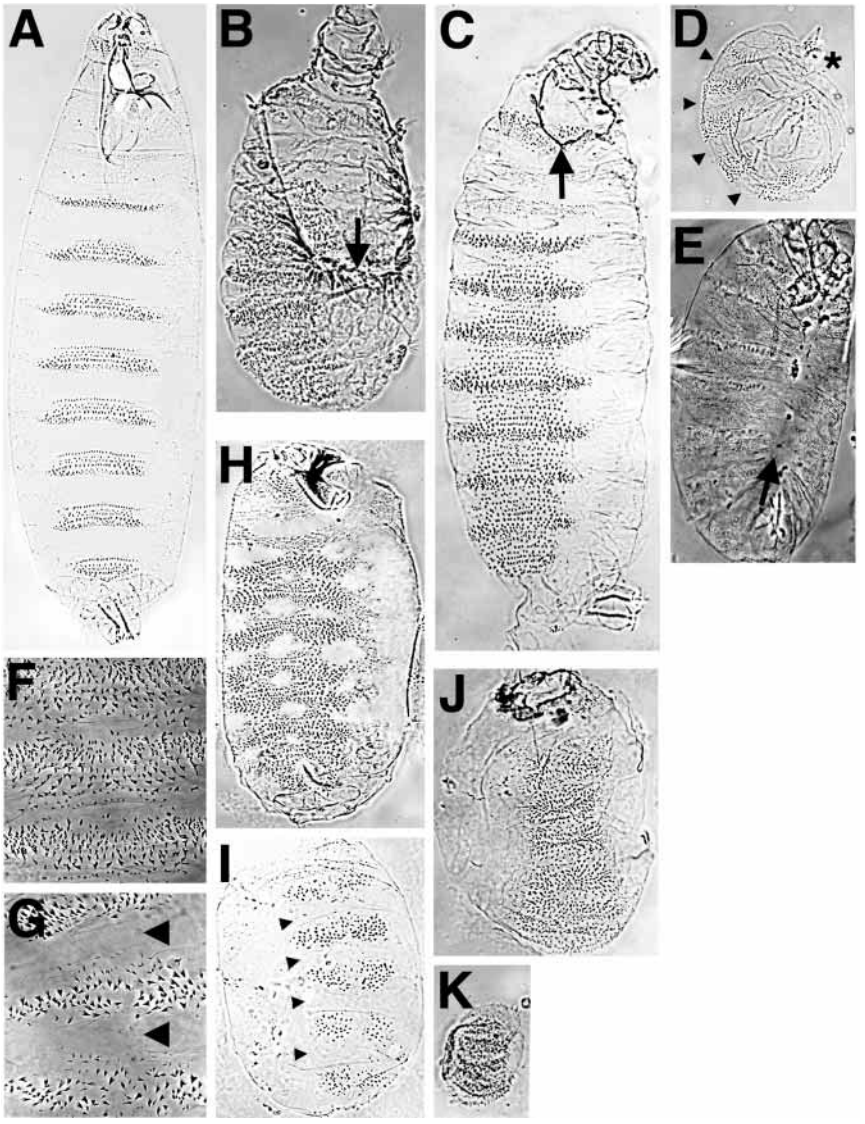


**Fig. 1.** Inactivation of Puc suppresses defects in Wg signaling. Ventral views (except E) of cuticles, with anterior up. **(**A) Wild type; (B) $arm^{XP33}/Y$. Note the hole in the dorsal cuticle (arrow). (C) $arm^{XP33}/Y;puc^{A251.1}/+$. Note increase in cuticle length and lack of a dorsal hole – an anterior hole remains (arrow). (D) $arm^{XP33}/Y;puc^{A251.1}$, * denotes loss of dorsal cuticle. Arrowheads (D,G,I) denote patches of naked cuticle. (E) $puc^{A251.1}$. Note displacement of anterior and posterior ends towards the dorsal surface and loss of patterning at the dorsal midline (arrow). (F) $dTCF^2$. (G) $puc^{A251.1};dTCF^2$. (H) $Df(2)DE/wg^{PE4}$ ($wg^{weak}$). (I) $wg^{weak};puc^{A251.1}$. (J) $wg^{IG22}$. (K) $wg^{IG22};puc^{A251.1}$. No suppression is seen.

### *puc* mutations suppress mutations in Wg signaling

To assess whether this suppression was specific to *arm*, the cuticle phenotypes of *puc*;$dTCF^2$, $wg^{weak}$;*puc* and $wg^{null}$;*puc* double mutants were examined. Loss of Puc function suppressed the ventral segment polarity defects of both $dTCF^2$ (Fig. 1F versus G) and a weak *wg* mutant ($wg^{weak}$; Fig. 1H versus I). In contrast, loss of Puc function did not suppress the segment polarity defects of $wg^{null}$ alleles (Fig. 1J versus K). *puc*;$dTCF^2$, $wg^{weak}$;*puc* and $wg^{null}$;*puc* double mutants all also have a reduction in dorsal epidermis (data not shown) similar to that of *arm*;*puc* double mutants (Fig. 1D). These data suggest that Puc normally antagonizes Wg signaling and suggest that Puc functions either downstream of or in parallel to the Wg pathway.

### Puc antagonizes Wg signaling in ventral patterning, perhaps via the JNK pathway

Puc was previously reported to specifically affect dorsal closure (Martin-Blanco et al., 1998; Ring and Martinez Arias, 1993). To assess whether loss of Puc function also affects cell fate choices in the ventral epidermis, the cuticle pattern of *puc* mutants was examined. In *puc* mutants, the denticle belts are narrowed in the anteroposterior (AP) axis with the strongest narrowing at the ventral midline (compare Fig. 2A to B). This phenotype is similar to that caused by weak activation of the Wg signaling pathway (Pai et al., 1997), further suggesting that Puc normally antagonizes Wg signaling in the ventral epidermis.

The only known biochemical role for Puc is as an antagonist of JNK activity (Martin-Blanco et al., 1998). While the JNK pathway has only been shown to act during dorsal closure, *hemipterous* mRNA is expressed uniformly throughout the epidermis (Glise et al., 1995) and *basket* mRNA is expressed throughout the epidermis but enriched at the leading edge (Riego Escovar et al., 1996; Sluss et al., 1996). To test whether Puc may affect ventral patterning by regulating JNK signaling ventrally, we first examined the expression of the $puc^{A251.1}$ enhancer trap, a JNK target gene, in the ventral epidermis of *puc* mutants. In phenotypically wild-type embryos, expression of β-gal, driven by *puc* enhancer traps, is completely restricted to the dorsalmost epidermal cells (Fig. 2C), where *puc* mRNA is also normally enriched – no expression is seen in the ventral epidermis (Fig. 2C; Glise and Noselli, 1997; Martin-Blanco et al., 1998; Ring and Martinez Arias, 1993).

In contrast in *puc* homozygotes, we found significant activation of this JNK target gene in the ventral epidermis, in addition to its previously reported activation in cells adjacent to the leading edge (Fig. 2D; Glise and Noselli, 1997; Martin-Blanco et al., 1998; Ring and Martinez Arias, 1993). The $puc^{A251.1}$ enhancer trap was activated in intermittent stripes of cells extending from the dorsal epidermis to the ventral midline (Fig. 2E). These stripes are at the anterior margin of the presumptive denticle belts (brackets in Fig. 2F) and co-localize, in part, with Engrailed in the posterior compartment of each segment (data not shown). In the ventral epidermis, substantial activation of the enhancer trap was also observed near the ventral midline with the strongest expression just posterior to and overlapping the prospective denticle belts (Fig. 2F). Ectopic expression of the enhancer trap correlates well with the narrowing of denticle belts in *puc* mutants; the

*puc* enhancer trap is activated in the cells that are converted from a denticle-bearing cell fate to a naked cuticle cell fate (compare Fig. 2B and F). These data are consistent with the idea that Puc normally represses JNK activity in the ventral epidermis.

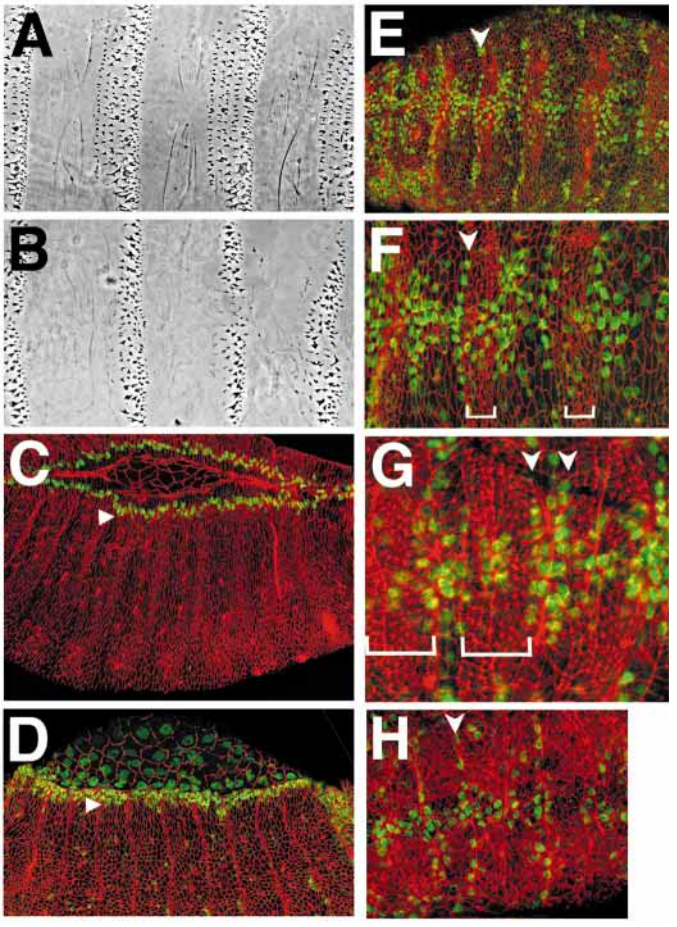


**Fig. 2.** Puc antagonizes Wg and negatively regulates a JNK reporter during ventral patterning. (A) Ventral view of wild-type cuticle; (B) ventral view of $puc^{A251.1}$. Note narrowing of denticle belts and occasional gaps in denticles at the midline. (C-H) Embryos double-labeled with anti-β-gal (green; indicates expression of the *puc* enhancer trap), and anti-PTyr (red; labels adherens junctions). (C) Lateral view of stage 12 wild-type ($puc^{A251.1}$/+) embryo. The enhancer trap is expressed exclusively in cells of the leading edge of the dorsal epidermis (arrowhead) and not expressed at all in the ventral epidermis. (D) Lateral view of stage 12 *puc* mutant ($puc^{A251.1}/puc^{A251.1}$) embryo. Note expansion of enhancer trap expression into several rows of cells adjacent to the leading edge (arrowhead). (E,F) (close-up) Ventral views of stage 14 $puc^{A251.1}$ embryo. β-gal expression is found in the ventral epidermis where it is never observed in a wild-type embryo. Brackets (F) denote prospective denticle belts. Note β-gal expression both near the ventral midline and in a row of cells at the anterior edge of the developing denticle belts (arrowhead); these cells will be converted from denticle to naked cuticle fates. (G) Ventral view of stage 14 $arm^{XP33}/Y;puc^{A251.1}$ embryo. Expanded stripes of enhancer trap expression (arrowheads) correlate with reappearance of naked cuticle (Fig. 1D). Brackets denote prospective denticle belts. (H) Ventral view of stage 14 $wg^{IG22};puc^{A251.1}$ embryo. Segmental expression of the enhancer trap is diminished (arrowhead). Anterior is to the left in all panels.

A correlation between expression of the *puc* enhancer trap and conversion to naked cuticle fate was also observed in *arm;puc* double mutants. Domains of β-gal expression up to three cells wide appear in the ventral epidermis (arrowheads in Fig. 2G). Double labeling for anti-phosphotyrosine (PTyr), to visualize developing denticles, and β-gal demonstrated that cells expressing $puc^{A251.1}$ enhancer trap differentiate into naked cuticle (Fig. 2G). In $wg^{null};puc$ embryos, in contrast, while cells along the midline continue to express β-gal, the segmental stripes of β-gal are diminished (Fig. 2H). This reduction in enhancer trap expression correlates with the failure of $wg^{null};puc$ double mutants to secrete naked cuticle (Fig. 1K). One possible explanation of this observation is that Wg signaling promotes *puc* expression; however, the loss of *puc*-expressing cells might also result from the ectopic apoptosis that occurs in *wg* mutants.

To further test whether *puc* mutations affect ventral patterning via activation of JNK signaling, we directly tested whether activation of the pathway by elevated expression of the kinases mimics the effects of *puc* mutations. We mis-expressed Hep or Bsk, the *Drosophila* JNKK and JNK orthologs (reviewed in Noselli and Agnes, 1999), in an *arm* mutant background using the GAL4-UAS system (Brand and Perrimon, 1993). Ubiquitous expression of either Bsk and Hep (Fig. 3C,D) results in a partial suppression of the *arm* phenotype, to a degree similar to the suppression by *puc* heterozygosity (Fig. 1C). The size of the embryo is expanded and dorsal closure defects are often alleviated, but naked cuticle is not restored. Expression of the *Drosophila* TAK1 ortholog DTak, a putative JNKK kinase (Takatsu et al., 2000), had a more dramatic effect. Mis-expression of DTak in even numbered segments of the developing *arm* epidermis using the *paired-GAL4* driver resulted in the reappearance of naked cuticle in those segments (Fig. 3F). Furthermore, mis-expression of DTak in a wild-type background using the same *paired-GAL4* driver resulted in narrowing of the denticle belts (Fig. 3G), similar to that caused by weak activation of the Wg pathway (Pai et al., 1997) or by loss of Puc function (Fig. 2B). These data are consistent with the idea that Puc's effects on the ventral pattern occur via regulation of the JNK signaling pathway, though other mechanisms, such as a direct role for Puc in the canonical Wg pathway, remain possible.

In both *puc* and *arm;puc* mutants, cells that activate the JNK pathway acquire naked cuticle cell fates. This is consistent with the hypothesis that Puc normally antagonizes production of naked cuticle in the ventral epidermis by suppressing JNK signaling. It should be noted, however, that loss-of-function mutations in JNK pathway components, including *bsk*, *Djun* and *kayak (Dfos)*, do not substantially alter the ventral denticle pattern (data not shown). This suggests that, if the JNK pathway plays a role in ventral patterning, it may function semiredundantly with other MAPK signaling pathways, as it does in planar polarity in the eye (Boutros et al., 1998). This could also explain why DTak has a stronger effect, as it may be upstream of more than one MAPK module.

We also tested a second means by which Puc could influence ventral patterning. The EGF-receptor (EGFR), acting via the ras-ERK pathway, promotes development of denticle cell fates and thus antagonizes Wg signaling, which promotes naked cuticle (O'Keefe et al., 1997; Szuts et al., 1997). If Puc upregulated this pathway, perhaps via effects on ERK, this

might explain Puc's effects on ventral patterning. Therefore, we tested whether heterozygosity or homozygosity for loss-of-function mutations in the EGFR pathway suppressed *arm*. Mutations in the ligand *vein*$^{147-2}$, the receptor *EGFR*$^{C18}$ (heterozygotes only), *rolled* (MAPK/ERK) and *ras85B*$^{e1B}$ did not significantly suppress either the dorsal or ventral *arm* phenotypes (Cox et al., 2000; data not shown). As heterozygosity for these mutations suppresses other phenotypes resulting from activation of MAPK signaling (e.g., Simon et al., 1991), these results suggest that Puc is unlikely to influence ventral pattern via the EGFR pathway, a result consistent with Puc's inability to regulate ERK activity (Martin-Blanco et al., 1998).

### Wg signaling regulates *dpp* expression at the leading edge

Previous studies identified a role for JNK signaling during dorsal closure. The unexpected interaction between Puc and Wg signaling in ventral patterning, as well as *puc's* suppression of *arm*'s dorsal closure defect (Fig. 1C), led us to examine the potential role of Wg signaling during dorsal closure. Cells of the leading edge, which initiate dorsal closure, activate a specific transcriptional program. Thus, we assessed whether Wg signaling plays a role in dorsal closure by regulating expression of *dpp*, a TGF-β family member. Dpp is expressed in cells of the presumptive leading edge before germband retraction and by leading edge cells after germband retraction (Fig. 4A). It is thought to promote dorsal closure by initiating cell elongation in the dorsoventral (DV) axis (reviewed in Noselli and Agnes, 1999). JNK signaling is essential for continued *dpp* expression at the leading edge (reviewed in Noselli and Agnes, 1999); in embryos mutant for the JNK pathway, *dpp* expression in these cells is lost (Fig. 4B,C), resulting in a failure to complete dorsal closure (Fig. 4J).

We thus examined *dpp* expression in embryos where Wg signaling was compromised. In embryos mutant for strong alleles of *wg* (*wg*$^{IG22}$ or *wg*$^{CX4}$), *dpp* expression was initiated at the leading edge but then decayed; by the onset of dorsal closure, *dpp* expression was strongly reduced or absent (Fig. 4D). Similar results were seen in embryos zygotically mutant for strong (*arm*$^{XP33}$; Fig. 4F) or null *arm* alleles (*arm*$^{YD35}$; data not shown). To further reduce Arm function, we made embryos maternally and zygotically mutant for *arm*$^{XM19}$, an allele that preferentially eliminates Arm function in Wg signaling. In these embryos, leading edge expression of *dpp* was reduced substantially, with no detectable expression observed during dorsal closure (Fig. 4G). *dpp* expression was also lost from lateral epidermal cells as well as from the foregut and hindgut, both ectodermal derivatives. In contrast, *dpp* expression remained in several other tissues, including the midgut (white arrows) and clypeolabrum. Similar loss of *dpp* expression both at the leading edge and in the lateral epidermis was seen in embryos maternally and zygotically mutant for a strong *dsh* allele, *dsh*$^{75}$ (Fig. 4E). Together, these results suggest that Wg signaling promotes *dpp* expression in leading edge cells after germband retraction. Furthermore, Dsh and Arm are required for *dpp* expression in other ectodermal tissues.

*dTCF* encodes a transcription factor that co-operates with Arm to activate Wg target genes. *dTCF* is maternally contributed to the embryo; to overcome this maternal pool, a N-terminally truncated form of dTCF, dTCFΔN, which functions as a constitutive repressor of Wg target genes (Cavallo et al., 1998), was overexpressed in the developing epidermis. In such embryos, *dpp* expression in the dorsalmost epidermal cells was attenuated but not eliminated (Fig. 4H); expression of *dpp* in the lateral epidermis was also strongly reduced. This suggests that dTCF may also be required for Wg's activation of *dpp*.

The *puc* enhancer trap is a second JNK target gene activated in leading edge cells (Fig. 5A; reviewed in Noselli and Agnes, 1999). Therefore, we examined its expression in several *wg*-class mutants – zygotic *arm* (Fig. 5B,C), *wg*$^{null}$ (Fig. 5D) and embryos maternally and zygotically mutant for *arm*$^{XM19}$ (Fig. 5E) or *dsh*$^{75}$ (Fig. 5F). In all, enhancer trap expression was reduced or lost in a subset of leading edge cells, although detectable expression of the enhancer trap always remained. To further examine the role Wg signaling plays in regulating the *puc* enhancer trap, we examined *arm*;*puc* and *wg*$^{null}$;*puc* double mutant embryos. In *puc* single mutant embryos, *puc* enhancer trap expression expands during dorsal closure into additional lateral cells (Glise and Noselli, 1997; Ring and Martinez Arias,

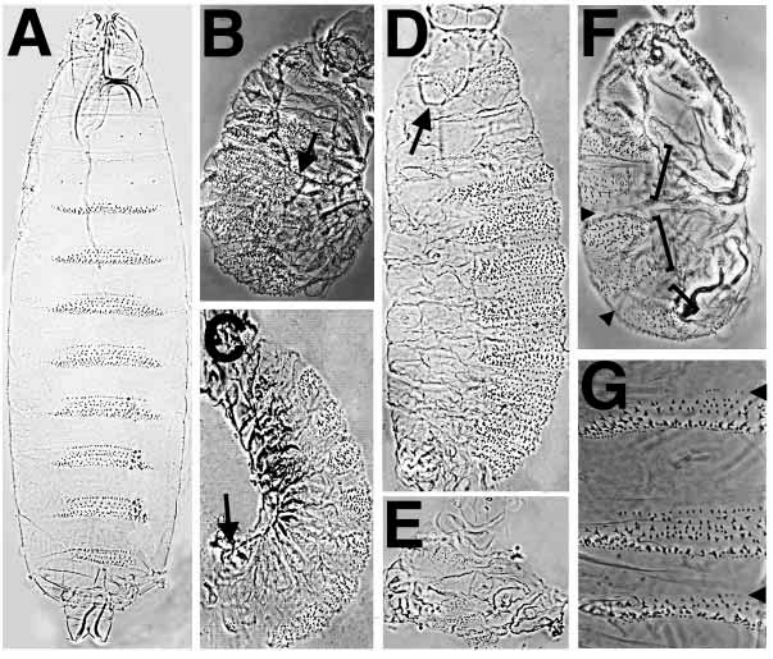


**Fig. 3.** Elevated JNK signaling suppresses *arm*. Cuticle preparations with anterior up. (A) Wild type; (B) *arm*$^{XP33}$/*Y*. Note defects in segment polarity and dorsal closure (arrow). (C) *arm*$^{XP33}$/*Y*;*UAS-Bsk*/*arm-Gal4::VP16*. Note suppression of ventral defects without rescue of dorsal closure (arrow). (D) *arm*$^{XP33}$/*Y*;*UAS-Hep*/*arm-Gal4::VP16*. Note suppression of both dorsal closure (arrow) and ventral defects. (E) *arm*$^{XP33}$/*Y*;*UAS-Hep*/*arm-Gal4::VP16*. A subset of embryos where JNK signaling was elevated, such as this one, secrete cuticles that are fragmented (The frequencies were: *UAS-Hep*=11%, *UAS-Bsk*=20%, *UAS-DTak*$^{WT}$=20%). (F) *arm*$^{XP33}$/*Y*;*UAS-DTak*$^{WT}$;*prd-Gal4*. Note suppression of *arm*'s segment polarity defect, leading to reappearance of naked cuticle (arrowheads) between fused denticle belts (brackets). (G) *UAS-DTak1*;*prd-Gal4*. Anterior rows of denticles are lost in even-numbered segments (arrowheads).

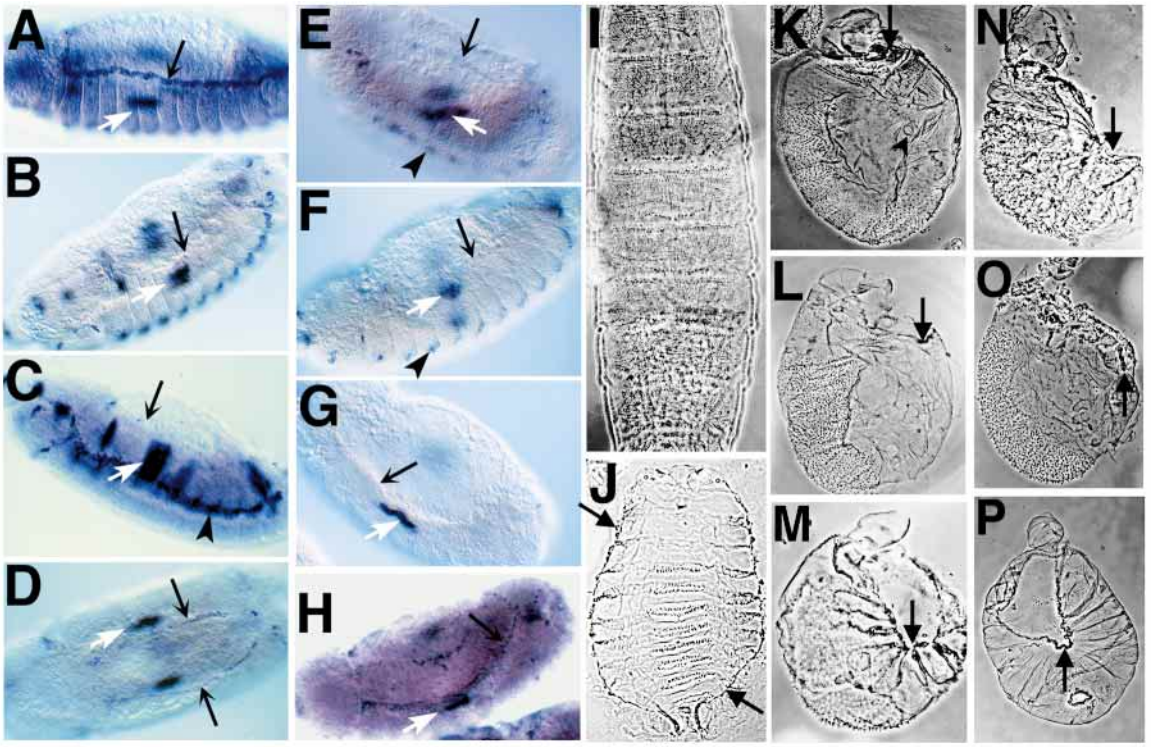


**Fig. 4.** Wg signaling is required for *dpp* expression and dorsal closure. (A-H) Lateral views of stage 13 embryos labeled for *dpp* mRNA, with anterior at left. Note *dpp* at the leading edge (black arrows), in the midgut (white arrows) and in the lateral epidermis (black arrowheads). (A) Wild type; (B) *Djun*$^{1}$; (C) *UAS-puc*/*arm-GAL4::VP16*; (D) *wg*$^{CX4}$; (E) maternal and zygotic *dsh*$^{75}$; (F) *arm*$^{XP33}$/*Y*; (G) maternal and zygotic *arm*$^{XM19}$; (H) *UAS-dTCFΔN*;*arm-GAL4::VP16*. Note loss of *dpp* mRNA at the leading edge (black arrows) while midgut expression (white arrows) remains. (I-P) Cuticles. (I) Dorsal view of wild type. (J) *UAS-puc*/*arm-GAL4::VP16*. The dorsal surface is completely open, with edges of the large dorsal hole indicated by arrows. (K,L) *wg*$^{IG22}$. Many *wg* embryos (K) are dorsally closed but exhibit severe defects in dorsal patterning; note abnormal cuticular structures (arrowhead). (L) 18% are mildly dorsally open. In K-P, the arrow indicates the posterior extent of the dorsal hole. (M) Maternal and zygotic *dsh*$^{75}$ mutant; (N) *arm*$^{XP33}$/*Y* mutant; (O) maternal and zygotic *arm*$^{XM19}$ mutant; (P) *UAS-dTCFΔN; arm-GAL4::VP16* mutant. Note dorsal closure defects.

1993; Fig. 5G), due to hyperactivation of the JNK pathway. In *arm*;*puc* and *wg*$^{null}$;*puc* double mutants, this expansion was attenuated (Fig. 5H,I), consistent with a role for Wg in regulating *puc* expression in dorsal epidermal cells.

### Constitutive activation of Wg signaling drives ectopic *dpp* expression

These data suggest that both the JNK and canonical Wg pathways regulate *dpp* in leading edge cells. When the JNK pathway is ectopically activated, as occurs in *puc* mutants, *dpp* expression expands into a subset of the more lateral epidermal cells (Fig. 6A versus B; reviewed in Noselli and Agnes, 1999). We thus examined whether ectopic activation of the Wg pathway also expands the domain of *dpp* expression. We activated the Wg pathway by removing maternal and zygotic Zw3 (Fig. 6C,D) or by mis-expressing Wg (data not shown) or a constitutively active form of Arm (data not shown) throughout the dorsal epidermis. In all cases, *dpp* expression expanded from the leading edge into more lateral cells.

Ectopic activation of Wg signaling also mimics the effects of *puc* on the dorsal cuticle. *puc* mutants exhibit a characteristic 'puckering' of the anterior and posterior epidermis towards the dorsal surface, as well as defects in dorsal hair diversity and patterning (Fig. 6E versus F; Martin-Blanco et al., 1998; Ring and Martinez Arias, 1993). Activation of Wg signaling throughout the dorsal epidermis, by ubiquitously expressing Wg (Fig. 6G) or Arm$^{S10}$ (Fig. 6I), or in *zw3* mutants (Fig. 6H), results in a very similar disruption of the dorsal cuticle, including 'puckering', dorsal hair patterning and identity defects (4°only), and 'bald' scars along the dorsal midline. Thus, overactivation of either JNK or Wg signaling pathways has a similar effect upon patterning of the dorsal epidermis.

### Activation of JNK or Dpp signaling in the leading edge rescues dorsal closure of *arm* mutants

Misexpression of Hep throughout the epidermis suppresses the phenotype of *arm* (Fig. 3D). To address whether decreases in Puc activity rescue *arm* dorsal closure by upregulating JNK signaling and/or *dpp* expression in leading edge cells, a leading-edge-specific GAL4 driver (*LE-Gal4*) was used to activate either JNK or Dpp signaling in an *arm* mutant. Overexpression of DTak specifically in leading edge cells ameliorated the *arm* dorsal phenotype (Fig. 7B) while ectopic expression of a dominant-negative DTak transgene (*UAS-DTak*$^{KN(1-1)}$) throughout the epidermis augmented *arm*'s dorsal closure defects (Fig. 7C). Furthermore, expression of either *dpp* (Fig. 7D) or activated *thick veins* (*tkv*; Fig. 7E,F), a Dpp receptor, in leading edge cells of *arm* mutants partially suppressed the dorsal closure defects associated with loss of Arm function. Together, these results suggest that elevation in

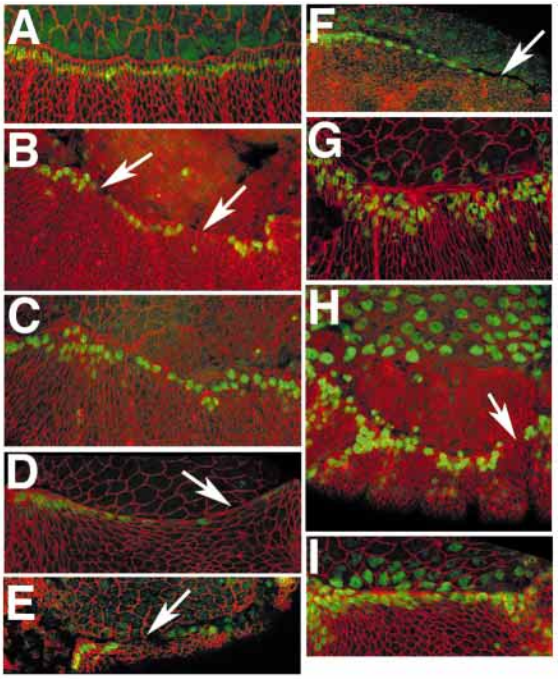


**Fig. 5.** *puc* enhancer trap expression in *wg*-class mutants. Lateral views of stage 14 embryos double-labeled as in Fig. 2. (A) $puc^{A251.1}$/+. Only leading edge cells express the enhancer trap. (B,C) $arm^{XP33}$/Y;$puc^{A251.1}$/+; (D) $wg^{IG22}$;$puc^{A251.1}$/+; (E) Maternal and zygotic $arm^{XM19}$;$puc^{A251.1}$/+; (F) maternal and zygotic $dsh^{75}$;$puc^{A251.1}$/+; (G) $puc^{A251.1}$. Enhancer trap expression expands into 2-4 rows of leading edge cells. (H) $arm^{XP33}$/Y;$puc^{A251.1}$. (I) $wg^{IG22}$;$puc^{A251.1}$. *wg*-class mutants lose enhancer trap expression in a subset of leading edge cells (arrows).

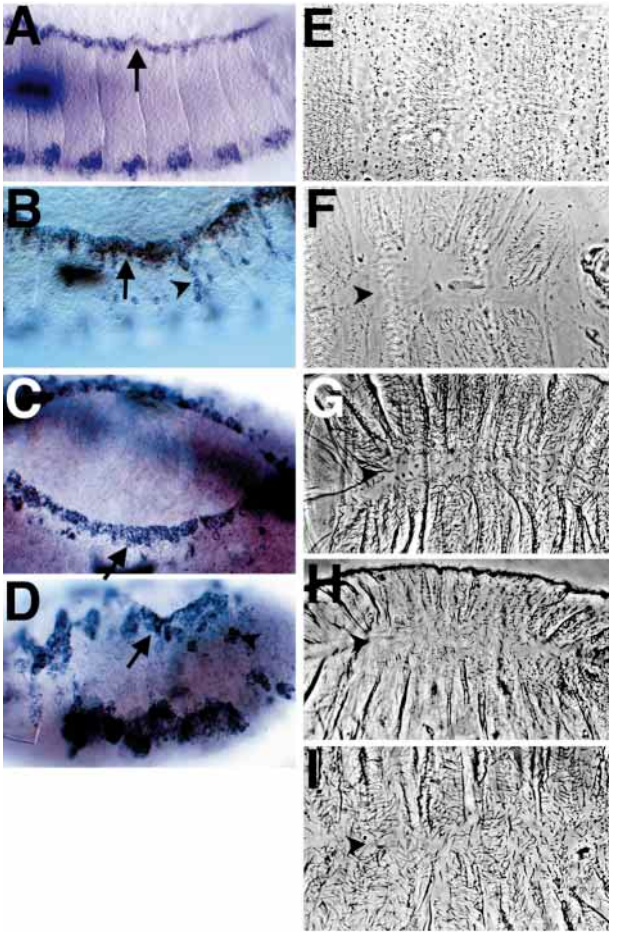


**Fig. 6.** Ectopic activation of Wg signaling mimics loss of Puc function. **(**A-D) Lateral views, stage 14 embryos labeled for *dpp* mRNA. Anterior is at left. (A) Wild type. *dpp* mRNA accumulates at the leading edge (arrow) and in lateral patches. (B) $puc^{A251.1}$; (C,D) Maternally $zw3^{M1-1}$ mutant embryos; (C) presumptive zygotically wild-type embryo; (D) a presumptive maternal and zygotic mutant. Expression of *dpp* expands at the leading edge (arrows) and into the lateral epidermis (arrowheads). (E-I) Dorsal views of cuticles. (E) Wild type. Note the patterning and diversity of dorsal hairs. (F) $puc^{A251.1}$; (G) *UAS-wg*/*arm-GAL4::VP16*; (H) maternal and zygotic $zw3^{M1-1}$; (I) *UAS-*$arm^{S10:}$*arm-GAL4::VP16*. Activation of Wg signaling mimics $puc^{A251.1}$, with loss in dorsal hair diversity, a naked scar at the dorsal midline (arrowheads) and 'puckering' of the cuticle.

JNK signaling, either via inactivation of Puc or over-expression of JNK pathway kinases, can suppress *arm*'s dorsal defects while reductions in JNK signaling in leading edge cells augment *arm*'s dorsal closure defects.

Constitutive activation of the Wg pathway results in the upregulation of *dpp* expression (Fig. 6). To assess whether activation of the Wg pathway is sufficient to suppress defects associated with a loss of JNK signaling, Wg (data not shown) or $Arm^{S10}$ (Fig. 7H) were misexpressed in the $kay^2$ mutant background. Although it was previously demonstrated that activation of Dpp signaling in the dorsalmost epidermal cells rescues JNK pathway mutants (e.g., Hou et al., 1997), constitutive activation of the Wg cascade fails to promote *dpp* expression in leading edge cells (data not shown) or to rescue the dorsal closure defect of *kay* (Fig. 7G versus H). Arm's role during dorsal closure can be bypassed by activating Dpp signaling whereas the requirement for Kayak remains even if the Wg pathway is activated. Therefore, Arm may amplify JNK-dependent expression of *dpp*, JNK may be required for *dpp*-independent events during dorsal closure, or both. Finally, Arm likely contributes to dorsal closure in a Wg-independent way, through its maintenance of cadherin/catenin-based adherens junctions.

## Wg signaling is required to coordinate dorsal closure

The dorsal closure defects of *arm* mutants were previously ascribed to Arm's role in adherens junctions, as $wg^{null}$ mutants (Nusslein-Volhard and Wieschaus, 1980) and *arm* mutants specifically affecting Wg signal transduction are not completely open dorsally. However, given the similar alterations in the transcriptional program of leading edge cells caused by defects in either JNK or Wg signaling, we re-examined dorsal closure in Wg pathway mutants. $wg^{null}$ cuticles have significant defects in dorsal pattern, characterized by loss of dorsal hairs and the presence of abnormal cuticular structures (Fig. 4K,L). While most $wg^{null}$ mutants are closed dorsally, 18% remain open at the dorsal anterior end (Fig. 4L); similar defects are seen in embryos maternally and zygotically mutant for *dsh* (Fig. 4M) or $arm^{XM19}$ (Fig. 4O), or in embryos

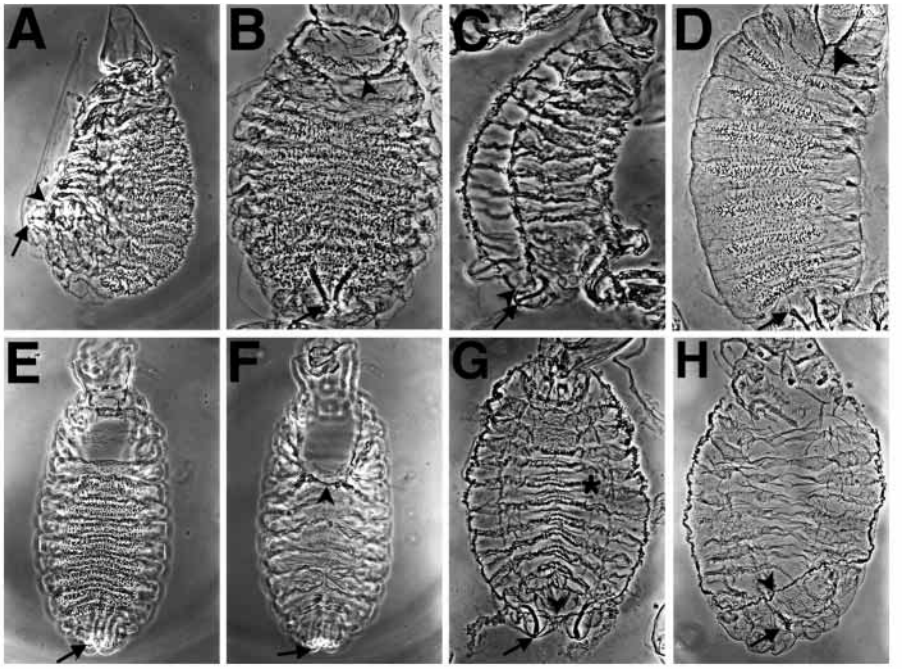


**Fig. 7.** Ectopic Dpp signaling rescues *arm*'s dorsal closure defects while activation of Wg signaling fails to rescue JNK signaling mutants. Arrowheads denote the posteriormost extent of the dorsal hole; arrows denote filzkörper, the posteriormost structure. (A) Lateral view of $arm^{XP33}/Y$; (B) ventral view of $arm^{XP33}/Y$;*LE-Gal4;UAS-DTak*$^{WT3}$. Note rescue of dorsal closure. (C) Lateral view of $arm^{XP33}/Y$;*LE-Gal4/+;UAS-DTak*$^{KN(1-1)}$/+. The dorsal defect is enhanced. (D) Ventral view of $arm^{XP33}/Y$;*LE-Gal4;UAS-dpp*. Note rescue of dorsal closure. (E,F) Ventral and dorsal views of $arm^{XP33}/Y$;*UAS-tkv*$^{Q253D}$/*LE-Gal4*. Note rescue of dorsal closure. (G) Dorsal view of *kay*$^2$ *arm-Gal4::VP16/kay*$^2$. * denotes ventral denticle belts. (H) Dorsal view of *UAS-arm*$^{S10}$/+;*kay*$^2$ *arm-Gal4::VP16/kay*$^2$. Note the loss of denticles.

expressing dTCFΔN throughout the epidermis (Fig. 4P). Thus, the endpoint of dorsal closure, the dorsal cuticle pattern, is altered in *wg*-class mutants.

These analyses of cuticle pattern and *dpp* expression suggest that Wg signaling regulates dorsal closure. To examine this directly, the cell shape changes that accompany dorsal closure were monitored by confocal microscopy, utilizing fluorescent-phalloidin to label filamentous actin and anti-PTyr antibody to label adherens junctions (Fig. 8). In wild-type embryos, cells begin to change shape during germband retraction (Fig. 8A,J). Cells of the leading edge are organized into a single well-defined row, and elongated in the DV axis by the end of germband retraction (Fig. 8B,K). As dorsal closure initiates, an additional 3-4 lateral cell rows also begin to stretch ventrally. Leading edge cells meet first at the posterior (Fig. 8C,L). Next, anterior leading edge cells meet and the embryo completes dorsal closure.

Loss of *wg* completely blocks the well-ordered cell shape changes that normally accompany dorsal closure. Leading edge cells never elongate along the DV axis (Fig. 8D,M); instead they stretch along the AP axis (Fig. 8E,N). This AP stretching may correlate with the 'purse string' tightening of actin filaments thought to help drive dorsal closure (Young et al., 1993). Presumptive leading edge cells of *wg* mutants accumulate actin and PTyr where they contact the amnioserosa, though they do so in an uneven fashion compared to wild type (Fig. 8E,N; data not shown). In addition to these defects, certain lateral cells contract like leading edge cells, stretching along the AP axis (Fig. 8N). Thus lateral cells in *wg* mutants may not acquire proper DV identities.

It has been hypothesized that the concerted cell shape changes characteristic of wild-type embryos are essential for dorsal closure. Thus, we were quite surprised to observe that, despite the severe abnormality in cell shape changes in a *wg* mutant, dorsal closure proceeds, though in a very abnormal fashion (Fig. 8F,O). Due to incomplete germband retraction, cells still cover the dorsal side at the posterior end; this may help bring posterior cells together. Gradually most leading edge cells meet at the midline and resume a cuboidal appearance.

We also examined *wg*$^{null}$*;puc* double mutants (Fig. 9A-D), to determine what happens to cells that normally secrete dorsal and lateral cuticle. *wg;puc* double mutants resemble *wg* single mutants until germband retraction. Several differences then become apparent in *wg;puc* double mutants. First, dorsal closure is never initiated. Second, lateral epidermal cells, which elongate along the AP axis, activate the *puc* enhancer trap, thus further resembling leading edge cells (Fig. 9A). Finally, massive cell degeneration is seen in the lateral and dorsal epidermis of late embryos (Fig. 9B,C), likely explaining the complete absence of dorsal cuticle. The *puc* enhancer trap comes on strongly in the cells destined to degenerate. A small group of ventral epidermal cells survives (Fig. 9D); presumably these are the cells that secrete cuticle.

In *arm* mutants, certain aspects of dorsal closure are more severely affected than in *wg*, while others proceed more normally. This may reflect the fact that *arm* zygotic mutants retain some Wg signaling, albeit greatly reduced, but also have reductions in cadherin-catenin function. *arm* mutants are quite normal through germband retraction. At this point, leading edge cells initiate cell shape changes (Fig. 8G,P), but do not do so in a coordinated fashion, producing a variety of cell shapes (Fig. 8P). A subset of more lateral cells fail to elongate (Fig. 8H,Q). Most *arm* embryos fail to initiate dorsal closure and leading edge cells often curl under their more lateral neighbors. At a stage when dorsal closure would finish in wild-type embryos, the amnioserosa rips away from the leading edge cells (Fig. 8I,R), with detached cells resuming a cuboidal shape. *arm;puc* double mutants resemble *arm* single mutants until the point at which dorsal closure should be complete. At this point, ectopic enhancer trap expression appears in the amnioserosa and at the anterior and posterior ends of the embryos (Fig. 9F,G), as in *puc* single mutants. Enhancer trap expression expands to nearly fill the dorsal and lateral epidermis of the double mutant (Fig. 9I), perhaps leading to eventual cell

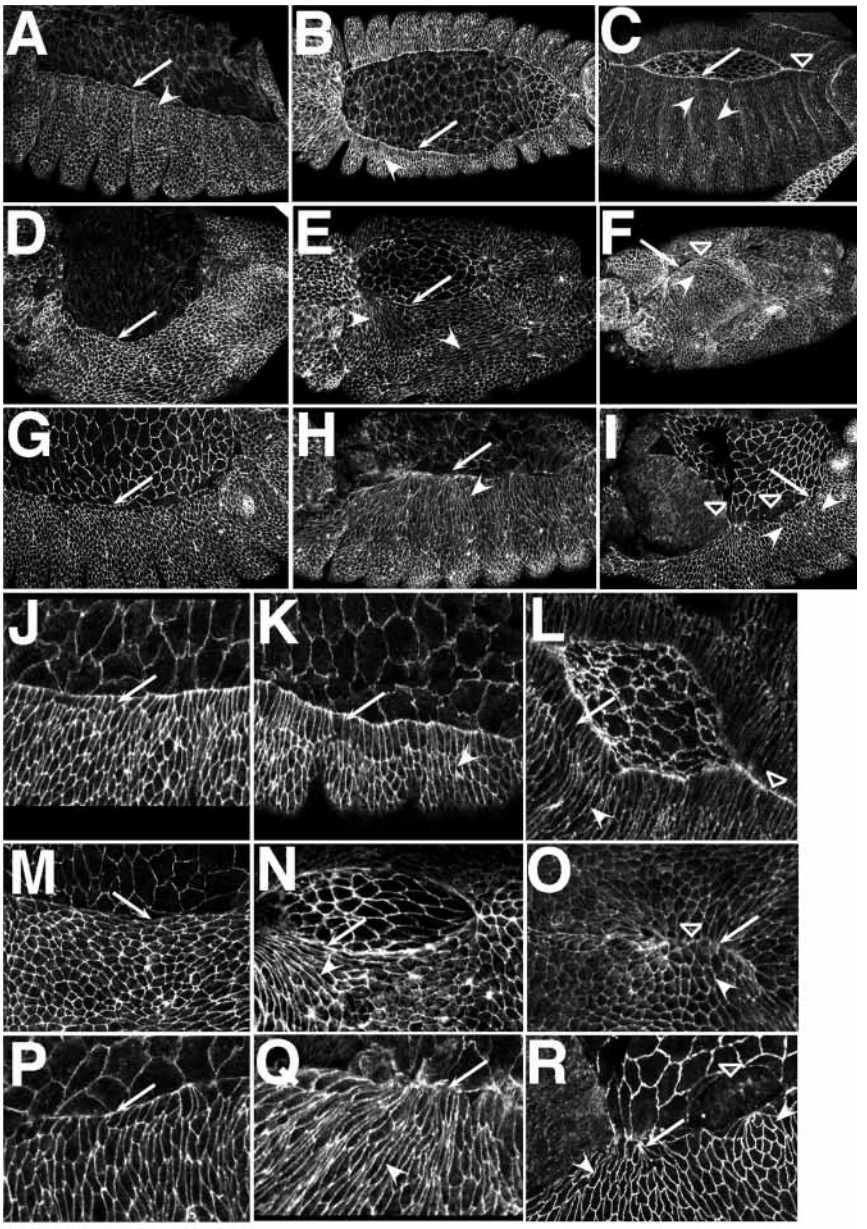


**Fig. 8.** Dorsal closure is aberrant in both *wg* and *arm*. Embryos labeled for PTyr to view cell boundaries. Dorsal closure in successively later wild-type (A-C, J-L), $wg^{IG22}$ (D-F, M-O) and $arm^{XP33}$ (G-I, P-R) embryos. Anterior is to the left. (A,J) Lateral views, late stage 12 wild type. Leading edge cells (arrows) begin change shape and align in a row; lateral neighbors remain cuboidal (arrowhead). (B,K) Dorsal and lateral views of stage 14 wild type. Leading edge cells are elongated (arrows); lateral neighbors are beginning to change shape (arrowheads). (C,L) Late stage 14 wild type. Both leading edge (arrows) and lateral cells (arrowheads) are highly elongated. At the leading edge and where cells join they accumulate PTyr (open arrowheads). (D,M) Lateral views of stage 12-13 $wg^{IG22}$ mutants. Segmentation is not apparent. Germband retraction is not completed. Leading edge cells (arrows) never initiate proper cell shape changes, nor do they form a single row. During stage 13, they elongate in the AP axis, as do certain lateral cells. (E,N) Lateral views of stage 14 $wg^{IG22}$ mutants. Leading edge cells (arrows) and certain lateral neighbors (arrowheads) are highly elongated. Dorsal closure proceeds. (F,O) Lateral/dorsal views of stage 15 $wg^{IG22}$ mutants. Dorsal closure has gone to (O) or nearly to (F) completion. Upon completion of closure, leading edge cells (arrows) resume a cuboidal shape, and their more lateral neighbors remain cuboidal (arrowhead). Cells at the midline are not well-aligned nor do they accumulate high levels of PTyr (open arrowheads). (G,P) Lateral views of stage 13 $arm^{XP33}$ mutants. Leading edge cells initiate elongation, but are much less uniform in shape (arrows). (H,Q) Lateral views of stage 14 $arm^{XP33}$ mutants. Leading edge (arrows) and lateral cells (arrowheads) elongate in the DV axis but do so irregularly. (I,R) Lateral views of stage 14/15 $arm^{XP33}$ mutants. The amnioserosa rips from the leading edge (open arrowheads). Leading edge cells attached to the amnioserosa remain elongated (arrows), but those that detach resume a cuboidal shape (arrowheads).

death of the dorsal and lateral epidermis as in *wg;puc* embryos.

## DISCUSSION

### Wg and JNK signaling co-operate during embryogenesis

The JNK and Wg signaling pathways were thought to function in distinct domains, with JNK regulating dorsal closure and Wg regulating segment polarity. Here we demonstrate that Wg signaling is critical for normal dorsal closure and that a negative regulator of the JNK pathway, Puc, plays an unexpected role in ventral patterning. This connection emerged from the observation that reduction in Puc function suppresses both the dorsal closure and ventral segment polarity phenotypes of non-null mutations in the Wg pathway.

Puc encodes a MAPK phosphatase that antagonizes JNK signaling (Martin-Blanco et al., 1998). Thus the simplest hypothesis to explain our results is that *puc* suppresses *arm* by hyperactivating the JNK pathway. Consistent with this, the *puc* enhancer trap, a JNK target gene, is ectopically activated in certain ventral epidermal cells in *puc* mutants. In addition, activation of JNK signaling suppresses *arm* in a fashion very similar to that resulting from reduction in Puc function, and activation of a JNKKK in a wild-type embryo mimics weak activation of the Wg pathway (Pai et al., 1997).

Zygotic loss-of-function mutations in the JNK pathway fail to appreciably affect ventral segment polarity (data not shown), however. This is reminiscent of the role of JNK signaling in planar polarity (Boutros et al., 1998). Loss-of-function mutations in the JNK pathway suppress dominant activation of Fz or Dsh, but these mutations fail to exhibit planar polarity defects themselves. This may result from functional redundancy and/or cross-talk between different MAPKKs and/or MAPKs (Paricio et al., 1999). Such crosstalk occurs:

both DMKK4 and Hep can activate Bsk, while *Drosophila* p38 orthologs can phosphorylate Djun and ATF2, both known targets of Bsk (Han et al., 1998). Thus, the JNK signaling pathway may function redundantly with other MAPK pathways, both in planar polarity and in segment polarity. As JNK-independent expression of *puc* has also reported (Zecchini et al., 1999), additional studies will be required to assess the ability of Puc to antagonize other MAPK signaling pathways. While these circumstantial arguments are consistent with a role for the JNK pathway in ventral patterning, the caveats raised by the lack of effects of loss-of-function JNK mutations leave open the possibility that Puc has a role in ventral patterning that is independent of its role in regulating JNK activity – for example, it could directly regulate the canonical Wg pathway.

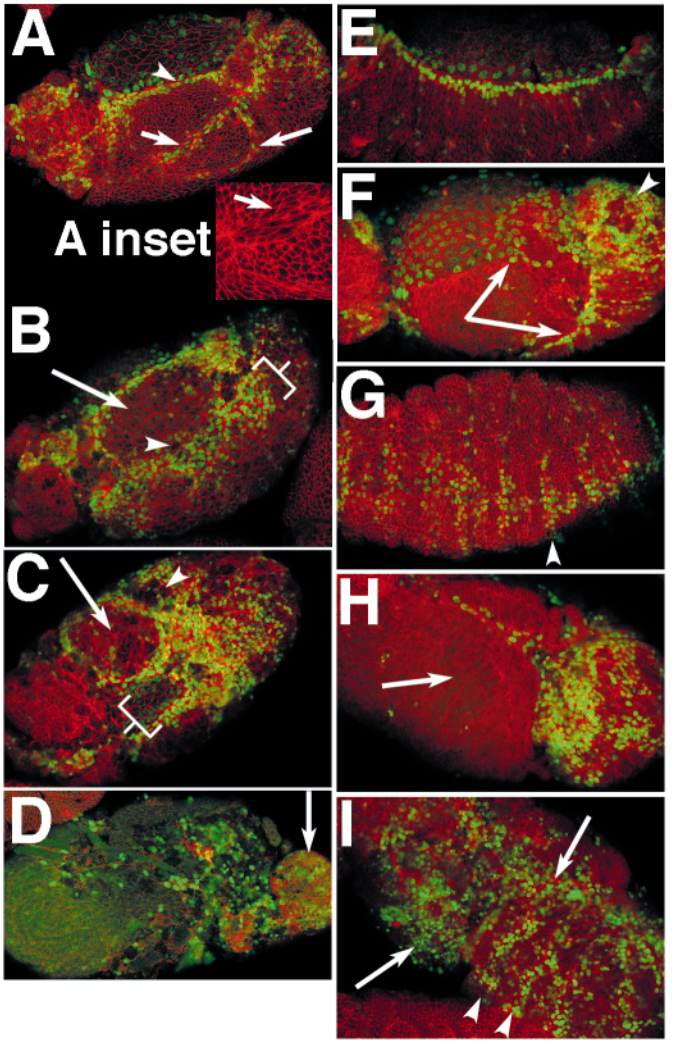


**Fig. 9.** Dorsal closure in *wg;puc* and *arm;puc*. Embryos double-labeled for PTyr revealing cell boundaries (red) and anti-β-gal revealing *puc* enhancer trap expression (green). (A-D) $wg^{IG22}$;$puc^{A251.1}$. (A, A inset) Stage 14; inset shows only PTyr. Enhancer trap expression is seen at the leading edge (arrowhead). Stripes of dorsal and lateral cells also activate the enhancer trap and become highly elongated along the AP axis (arrows), while other cells remain hexagonal. (B,C) Lateral views of successively later embryos. Enhancer trap expression in the lateral epidermis expands (brackets). Dorsal, anterior and posterior cells begin to degenerate (arrowheads). (D) Terminal stage. Only a small ball of ventral epidermal cells remain (arrow). (E-I) $arm^{XP33}$;$puc^{A251.1}$. (E) Stage 14; (F,G) dorsal and ventral views of a slightly later embryo. The amnioserosa has ripped from the epidermis (arrows), and the *puc* enhancer trap is coming on in ventral (arrowhead, G) and posterior (arrowhead, F) cells. (H) Dorsal view of a later embryo. The gut has protruded (arrow). (I) Terminal stage. The epidermis has slipped ventrally. The enhancer trap is on strongly in posterior and lateral epidermal cells (arrows) and in ventral stripes (arrowheads).

### Wg and JNK – one pathway or two?

Our data, combined with previous studies of JNK signaling (reviewed in Noselli and Agnes, 1999), further suggest that Wg and JNK signaling act in parallel during dorsal closure. Both pathways regulate *dpp* expression in dorsal epidermal cells and are required for the proper coordinated cell shape changes to occur. These data are compatible with several different models. It may be that the two pathways both impinge on the same process and the same target gene, but that they do so in response to independent upstream inputs. However, when our data is combined with other recent studies, a potential direct connection between the Wg and JNK pathways is suggested. Using both genetics and in vitro studies, others demonstrated that JNK pathway kinases act downstream of Frizzled and Dsh in planar polarity and that Dsh can activate the JNK signaling cascade directly (Boutros et al., 1998; Li et al., 1999). This suggested that Dsh may function as a binary switch, deciding between the canonical Wg pathway and the JNK pathway during the establishment of segment polarity and planar polarity, respectively. Here, we demonstrate that both the canonical Wg and the JNK pathways are required for proper dorsal closure, and that both pathways affect expression of the same target gene, *dpp*. One plausible model accommodating these data is that Wg, acting via Frizzled receptors and Dsh, activates both the JNK pathway and the canonical Wg pathway simultaneously and in parallel during both dorsal closure and ventral patterning. The possibility that Wg activates both pathways, while exciting in principle, remains quite speculative, and must now be tested by more direct biochemical and cell biological means.

### Wg as a permissive signal

It also is possible that Wg functions as a permissive signal required to allow other effectors to promote *dpp* expression. For example, *dTCF* could repress *dpp* expression in the absence of Wg signaling by recruiting Groucho (Cavallo et al., 1998), a transcriptional repressor, to the *dpp* promotor. Wg signaling might relieve this repression by displacing Groucho with stabilized Arm. Consistent with this hypothesis, constitutive activation of Arm fails to rescue the dorsal closure defects of *kay* mutants. Thus activation of the canonical Wg signaling pathway is necessary but not sufficient to promote *dpp* expression. Wg signaling may thus only amplify JNK-dependent expression of *dpp* in the dorsal epidermis.

One possible intersection between MAPK signaling cascades and TCF-mediated repression has been reported (reviewed in Bowerman and Shelton, 1999). Transcriptional repression of Wnt target genes in *C. elegans* depends upon POP-1, a TCF family member. POP-1 repressor activity is regulated by Mom-4, a Tak1-like kinase, and Lit-1, a Nemo-like MAP kinase relative (Nlk). In mammalian cells, the

transcriptional activity and DNA-binding properties of TCF can be repressed by Tak1/Nlk activation. Therefore, the canonical Wg and MAPK/JNK pathways might converge at dTCF, with MAPK kinase signaling affecting dTCF activity. Additional studies will be required to assess the mechanism by which these pathways interact.

### Cell shape changes and Dpp appear dispensable for dorsal closure

The current model suggests that a sequential series of cellular events drive dorsal closure. Leading edge cells are thought to initiate closure by elongating in the DV axis and upregulating Dpp, thus signaling lateral cells to initiate similar cell shape changes (reviewed in Noselli and Agnes, 1999). We found that the events of dorsal closure apparently do not proceed in lockstep, with each event requiring the successful completion of the previous event. The stereotypical cell shape changes are lost in *wg* mutants; however, the lateral epidermal sheets usually meet at the dorsal midline. In contrast, while cell shape changes are initiated in *arm* mutants, though not in a coordinated fashion, the epidermis does not close. Further, as *dpp* expression in leading edge cells is lost in *wg* mutants, Dpp may not be essential for dorsal closure (a similar model was suggested by Zecchini et al., 1999). Finally, because dorsal closure is more normal in *wg* than in JNK pathway mutants, the JNK pathway likely depends upon activation by signals other than Wg and must affect other processes in addition to Dpp signaling. Further work is required to clarify the semi-redundant mechanisms regulating dorsal closure.

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