Non-equivalent roles of *Drosophila* Frizzled and Dfrizzled2 in embryonic Wingless signal transduction

Melissa M. Moline*†, Herman A. Dierick*†, Cozumel Southern* and Amy Bejsovec*‡

The highly conserved Wnt family of growth factors is essential for generating embryonic pattern in many animal species [1]. In the fruit fly *Drosophila*, most Wnt-mediated patterning is performed by a single family member, Wingless (Wg), acting through its receptors Frizzled (Fz) and DFrizzled2 (Dfz2). In the ventral embryonic epidermis, Wg signaling generates two different cell-fate decisions: the production of diverse denticle types and the specification of naked cuticle separating the denticle belts. Mutant alleles of *wg* disrupt these cellular decisions separately [2], suggesting that some aspect of ligand–receptor affinity influences cell-fate decisions, or that different receptor complexes mediate the distinct cellular responses. Here, we report that overexpression of Dfz2, but not Fz, rescues the mutant phenotype of *wg^PE2*, an allele that produces denticle diversity but no naked cuticle. Fz was able to substitute for Dfz2 only under conditions where the Wg ligand was present in excess. The *wg^PE2* mutant phenotype was also sensitive to the dosage of glycosaminoglycans, suggesting that the mutant ligand is excluded from the receptor complex when proteoglycans are present. We conclude that wild-type Wg signaling requires efficient interaction between ligand and the Dfz2–proteoglycan receptor complex to promote the naked cuticle cell fate.

Addresses: *Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, 2153 Sheridan Road, Evanston, Illinois 60208-3500, USA.

‡Present address: Department of Biology, Duke University, B336 LSRC, Research Drive, Durham, North Carolina 27708, USA.

Correspondence: Amy Bejsovec
E-mail: bejsovec@northwestern.edu

†M.M.M. and H.A.D. contributed equally to this work.

Results and discussion

The *wg^PE2* allele contains a single amino-acid substitution in the carboxyl terminus of the molecule, changing Val453 to Glu [2]. Unlike *wg^CX4* loss-of-function mutants, which

Figure 1
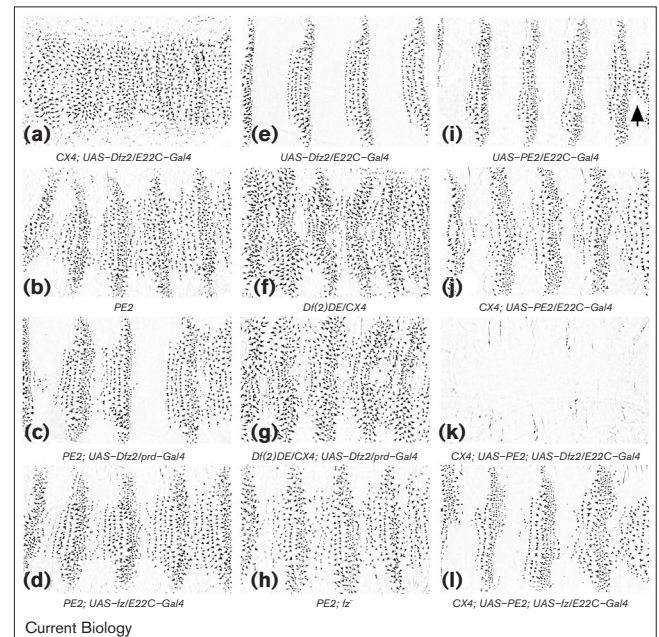
Overexpression of *Dfz2*, but not *fz*, restores naked-cuticle specification in *wg^PE2* mutant embryos. **(a)** The *wg* null mutant (*wg^CX4*) phenotype was not altered by overexpressing *UAS–Dfz2* with the high level, ubiquitously expressed *E22C–Gal4* driver. **(b)** The *wg^PE2* mutant phenotype and **(c)** its rescue by overexpression of *UAS–Dfz2* with *prd–Gal4*, which drives high-level expression in odd-numbered segments. No rescue was observed when *UAS–fz* was overexpressed with either **(d)** *E22C–Gal4* or *prd–Gal4* (data not shown). **(e)** Overexpression of *UAS–Dfz2* did not alter the wild-type pattern. **(f)** The hypomorphic *wg* pattern was not altered by overexpressing **(g)** *UAS–Dfz2*. **(h)** Maternal and zygotic loss of *fz* function (*fz⁻*) did not alter the *wg^PE2* mutant phenotype. **(i,j)** Overexpression of *UAS–wg^PE2* slightly disrupted denticle pattern and naked cuticle formation (arrow) in (i) wild-type embryos, and rescued denticle diversity in (j) *wg* null mutants. **(k)** Co-expression of *UAS–wg^PE2* with *UAS–Dfz2* produced uniform naked cuticle, as does overexpression of wild-type Wg alone in a *wg* null mutant [3]. **(l)** Co-expressing *UAS–wg^PE2* with *UAS–fz* produced a slight rescue of naked cuticle.

produce a cuticle pattern lacking both naked cuticle and denticle diversification, *wg^PE2* mutants lack only naked cuticle and secrete an essentially wild-type array of denticle types in each segment [2,3] (Figure 1a,b). This pattern also differs from that of reduced *wg* expression levels. *Df(2)DE* disrupts the *wg* promoter and results in low-level expression of wild-type *wg* RNA [4]. These hypomorphic mutants produce small patches of naked cuticle in addition to a diverse array of denticles (Figure 1f). As this pattern is distinct from that of the *wg^PE2* mutants, the

Figure 2
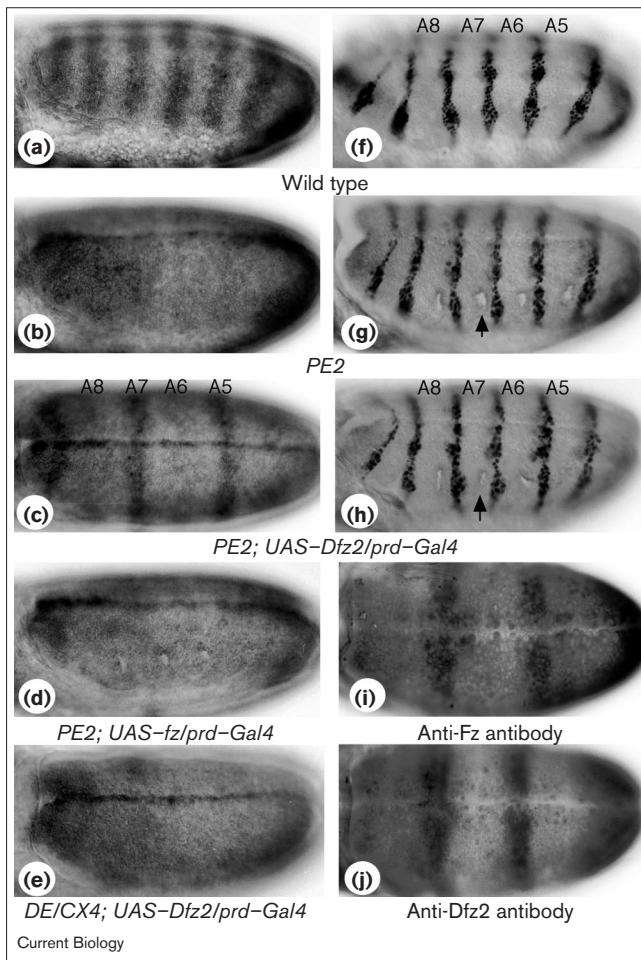
Overexpression of *Dfz2* restores molecular response to *wg*^{PE2} signaling.
(a) Arm is stabilized by wild-type Wg signaling. Stabilization was not observed in **(b)** *wg*^{PE2} mutants, but was restored in odd-numbered segments when **(c)** *prd–Gal4* was used to drive *UAS–Dfz2* expression. Arm stabilization was not observed when **(d)** *UAS–fz* was overexpressed in *wg*^{PE2} mutants, nor when **(e)** *UAS–Dfz2* was overexpressed in hypomorphic *wg* mutants. **(f)** Wild-type epidermal cells express En protein in a 2–3-cell-wide stripe in each segment. **(g)** These stripes were variably narrowed in *wg*^{PE2} mutants, and tracheal pit morphology was disrupted (arrow). **(h)** Both *wg*^{PE2} defects were rescued in odd-numbered segments when *prd–Gal4* was used to drive *UAS–Dfz2* expression. **(i)** Fz and **(j)** Dfz2 proteins were detected at roughly equivalent levels when transgenes were overexpressed with *prd–Gal4*.

wg^{PE2} pattern defect appears to represent a qualitative rather than a quantitative change in Wg activity levels.

The receptors Fz and Dfz2 are thought to function redundantly in embryonic Wg signaling because neither mutation alone produces a pattern defect, but double mutant embryos phenocopy *wg* loss of function [5,6]. Nevertheless, we found that they do not function equivalently, with respect to the *wg*^{PE2} mutant phenotype. Overexpression of wild-type *Dfz2*, but not *fz*, rescued naked cuticle specification in *wg*^{PE2}

mutant embryos. Expression of a *UAS–Dfz2* transgene under the control of a *prd–Gal4* driver promoted proper naked cuticle secretion in odd-numbered segments, where the transgene is expressed, whereas unaffected even-numbered segments remained mutant (Figure 1b,c). These effects were not an indiscriminate consequence of raising the activity level of Wg. Driving ubiquitous *Dfz2* overexpression with *E22C–Gal4* or *arm–VP16–Gal4* (data not shown) had no effect on epidermal patterning in *wg* null mutant embryos (Figure 1a) nor in wild-type embryos (Figure 1e). Furthermore, the pattern produced by the hypomorphic allele *Df(2)DE* was not rescued by overexpression of *Dfz2* (Figure 1f,g). Overexpression of Fz did not rescue the *wg*^{PE2} phenotype (Figure 1d), even though roughly equivalent levels of protein product were produced by both transgenes (Figure 2i,j). This suggests that the *wg*^{PE2} mutant phenotype reflects a specific problem in activation of the endogenous Dfz2 receptor. Furthermore, Fz function could not account for the denticle diversity that is present in *wg*^{PE2} mutants. We saw no effect on denticle diversity when we removed maternal and zygotic *fz* gene product from *wg*^{PE2} mutants (Figure 1h).

Wg signaling results in stabilization of Armadillo (Arm) protein, which activates Wg target genes, such as *engrailed* (*en*). Wild-type embryos show broad stripes of intense Arm staining centered over the *wg*-expressing cells [7] (Figure 2a). No striped increase in Arm staining was detected in *wg*^{PE2} mutant embryos (Figure 2b); only membrane-associated Arm was detected in these embryos, as in *wg* null mutants [7]. Nevertheless, *wg*^{PE2} mutants retained almost wild-type levels of *en* expression throughout development (Figure 2f,g), whereas *wg* null mutants lost all epidermal *en* expression by stage 10. Thus, the *wg*^{PE2}-encoded ligand is able to maintain *en* expression and promote denticle patterning, but it does so without stabilizing detectable amounts of Arm. This suggests either that amounts of Arm below the level of detection suffice for some Wg functions, or that Arm is not directly required for those functions.

Restoration of naked cuticle in *wg*^{PE2} mutant embryos, by *prd–Gal4*-driven expression of *Dfz2*, correlated with stabilization of Arm in odd-numbered segments (Figure 2c). No Arm elevation was observed when *fz* was overexpressed (Figure 2d), nor when *Dfz2* was overexpressed in *Df(2)DE* mutant embryos (Figure 2e), consistent with the lack of naked cuticle specification in such embryos. Furthermore, *prd–Gal4*-driven *Dfz2* expression restored a normal width to *en* expression domains and corrected defective tracheal pit morphogenesis in odd-numbered segments of *wg*^{PE2} mutant embryos (Figure 2g,h), suggesting that all aspects of the *wg*^{PE2} mutant phenotype were rescued by *Dfz2* overexpression.

When ubiquitously expressed in a wild-type embryo, *wg*^{PE2} subtly changes the denticle pattern [3] and shows a

slight dominant-negative effect on naked cuticle formation (Figure 1i). This contrasts with ubiquitous expression of wild-type *wg*, which produces uniform naked cuticle [3,8]. Ubiquitous expression of *wg^PE2* in a *wg* null mutant embryo rescues denticle diversity, but does not significantly rescue naked-cuticle formation [3] (Figure 1j). Coexpression of *Dfz2* and *wg^PE2* in *wg* null mutants produced uniform naked cuticle (Figure 1k), however, as does ubiquitous expression of wild-type *wg* alone [3]. Thus, the ability of *wg^PE2* to generate the naked-cuticle cell fate depends on overexpression of *Dfz2*. We also detected a slight interaction with Fz under conditions of high-level coexpression (Figure 1l), suggesting that amounts of Wg in excess of physiological concentrations permit interaction with Fz receptor.

Indeed, this observation offers an explanation for the apparent genetic redundancy of Fz and Dfz2 in embryonic Wg signaling. In the absence of zygotic Dfz2 receptor, Wg protein may accumulate to a level sufficient to activate Fz receptor, which then promotes normal epidermal patterning. We detected an increased accumulation of Wg protein in embryos zygotically deficient for *Dfz2* (Figure 3c), compared either with wild-type embryos (Figure 3a) or embryos maternally and zygotically deficient for *fz* (Figure 3b). This suggests that Wg ligand is not internalized and degraded as efficiently when Dfz2 is absent from the cell surface, thereby permitting interactions with Fz that are not relevant under wild-type conditions. We also observed abnormal accumulation of Wg protein in *wg^PE2* mutant embryos, which similarly showed a broader and less punctate pattern of Wg antibody staining (Figure 3d). This staining pattern was restored to a more wild-type appearance by overexpressing Dfz2 in *wg^PE2* mutant embryos (Figure 3e), further supporting the idea that the *wg^PE2* lesion compromises interaction with the Dfz2 receptor.

It is curious that ectopic Dfz2 restored the interaction with *wg^PE2* ligand, whereas ectopic *wg^PE2* alone did not (Figure 1c,j). This may indicate either that endogenous levels of Dfz2 are limiting for naked-cuticle specification or that overproduction of Dfz2 saturates a modification system that regulates its interaction with Wg, and with which the *wg^PE2* mutant molecule has a defective interaction. For example, glycosaminoglycans have been shown to be required for efficient Wg signal transduction [9,10], and the *Drosophila* glypican encoded by *dally* appears to act as a co-receptor in the Fz receptor complex [11,12]. Therefore, we looked for possible involvement of proteoglycans in the *wg^PE2*–Dfz2 interaction.

In *wg^PE2* mutant embryos that were zygotically mutant for *dally* or *sugarless*, which encodes an enzyme involved in polysaccharide synthesis, a substantial expanse of naked cuticle was produced (Figure 4a–c). Both mutations are hypomorphic, semi-lethal *P*-element insertions that do

Current Biology
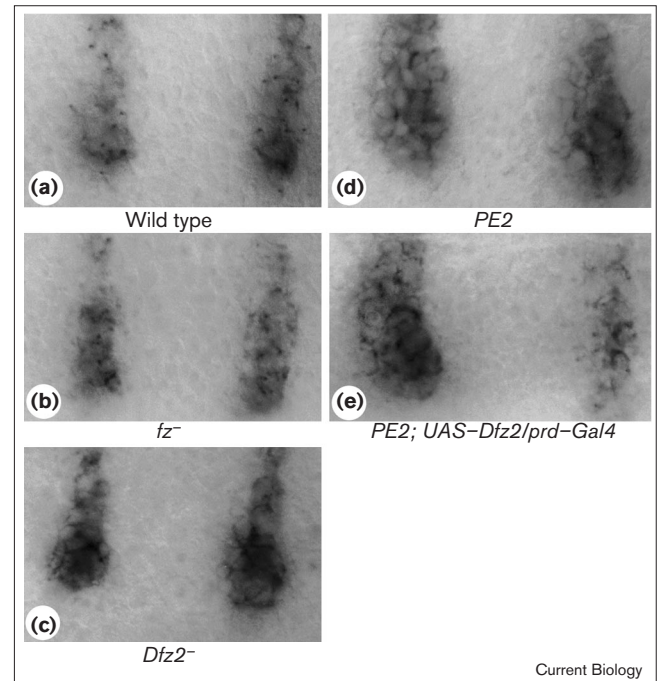
Dfz2 and *wg^PE2* mutants show abnormal Wg protein distribution. **(a)** Wild-type embryos show a punctate pattern of Wg protein staining over several cell diameters surrounding the *wg*-expressing row of cells in each segment. Abdominal segments 6 and 7 of stage 10 embryos are shown with anterior to the left. **(b)** Embryos maternally and zygotically deficient for *fz* (*fz⁻*) showed a wild-type Wg protein distribution, but **(c)** embryos zygotically deficient for *Dfz2* (*Dfz2⁻*) showed less punctate staining and high accumulation of Wg surrounding the cells. **(d)** The *wg^PE2* mutant protein showed a similar distribution, which **(e)** was rescued to a more wild-type appearance in odd-numbered segments when *Dfz2* was overexpressed with the *prd–Gal4* driver.

not affect embryonic patterning in the context of wild-type Wg. Therefore, mild reductions in sugar modification suffice to restore functionality to the *wg^PE2* mutant ligand. Moreover, ectopic expression of *dally*, using a *hs–dally* transgene, worsened the *wg^PE2* mutant phenotype (Figure 4c,d). These effects were specific for the *wg^PE2* phenotype; the hypomorphic *Df(2)DE* phenotype was not affected by zygotic loss of *sugarless* or *dally* and was partially suppressed, rather than enhanced, by providing ectopic *dally* (Figure 4e,f). Thus, excess Dally improves signaling efficiency for low levels of wild-type Wg, as has been demonstrated for other hypomorphic *wg* phenotypes [12], but has the opposite effect on the partial signaling activity of *wg^PE2*.

Finally, overexpression of *dally* reversed the rescuing effect of overexpressing *Dfz2* in *wg^PE2* mutants (Figure 4g,h). This suggests that ectopic *Dfz2* expression allows interaction with the mutant ligand because it shifts the ratio of Dfz2 to Dally molecules at the cell surface, presumably

Figure 4
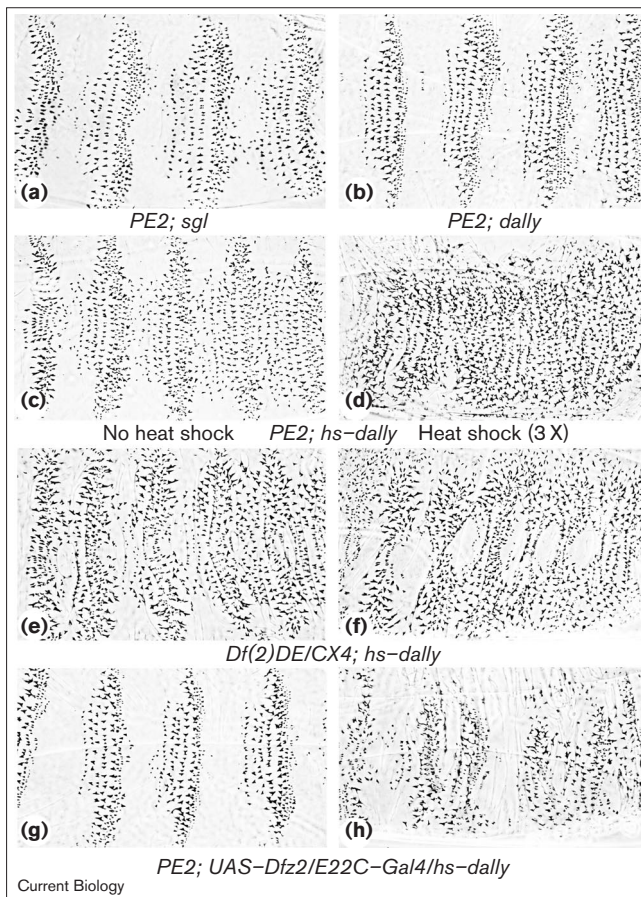
Altering the level of sugar modification modifies the wg^{PE2} mutant phenotype. Embryos homozygous for both wg^{PE2} and **(a)** a *sugarless* allele, sgl^{05007}, or **(b)** a *dally* allele, $dally^{06464}$, showed increased naked cuticle compared with wg^{PE2} single mutants. **(c,e)** Phenotype of (c) wg^{PE2} and (e) *Df(2)DE* mutants. **(d,f)** Overexpression of *dally* by inducing expression of the *hs−dally* transgene (d) worsened the wg^{PE2} phenotype, but (f) increased naked-cuticle specification in *Df(2)DE* mutants. (e) No heat shock; (f) heat shock. **(g)** Overexpressing *UAS−Dfz2* with the ubiquitous *E22C−Gal4* driver substantially rescued naked cuticle in wg^{PE2} mutants, but **(h)** this effect was reversed by co-expressing *dally*. (g) No heat shock; (h) heat shock.

increasing the number of Dfz2 receptor complexes that lack Dally co-receptor and can bind mutant ligand. As the wg^{PE2} genetic lesion changes an uncharged valine to a negatively charged glutamic acid, it is conceivable that introduction of a negative charge in the carboxyl terminus prevents proper binding between Wg ligand and negatively charged sulfated sugar groups.

In conclusion, we propose that interactions between Wg and proteoglycans are required for promoting naked-cuticle specification, but not denticle diversification, and that wg^{PE2} cannot promote this high-level response because of abnormal interactions with proteoglycans. We further conclude that the Fz receptor is able to substitute for Dfz2 under conditions of excess Wg ligand, but under normal circumstances does not appear to have a major role in transducing the naked-cuticle cell fate.

References
1. Dierick H, Bejsovec A: **Cellular mechanisms of Wingless/Wnt signaling activity.** In *Current Topics in Developmental Biology*. Edited by Pederson R, Schatten G. New York: Academic Press; 1999: **43:**153-190.
2. Bejsovec A, Wieschaus E: **Signaling activities of the *Drosophila wingless* gene are separately mutable and appear to be transduced at the cell surface.** *Genetics* 1995, **139:**309-320.
3. Hays R, Gibori GB, Bejsovec A: **Wingless signaling generates epidermal pattern through two distinct mechanisms.** *Development* 1997, **124:**3727-3736.
4. Dierick HA, Bejsovec A: **Functional analysis of Wingless reveals a link between intercellular ligand transport and dorsal-cell-specific signaling.** *Development* 1998, **125:**4729-4738.
5. Bhanot P, Fish M, Jemison J, Nusse R, Nathans J, Cadigan KM: **Frizzled and DFrizzled-2 function as redundant receptors for Wingless during *Drosophila* embryonic development.** *Development* 1999, **126:**4175-4186.
6. Chen C-M, Struhl G: **Wingless transduction by the Frizzled and Frizzled2 proteins of *Drosophila*.** *Development* 1999, **126:**5441-5452.
7. Peifer M, Sweeton D, Casey M, Wieschaus E: *wingless* signal and Zeste-white 3 kinase trigger opposing changes in the intracellular distribution of Armadillo. *Development* 1994, **120:**369-380.
8. Noordermeer J, Johnston P, Rijsewijk F, Nusse R, Lawrence PA: **The consequences of ubiquitous expression of the *wingless* gene in the *Drosophila* embryo.** *Development* 1992, **116:**711-719.
9. Reichsman F, Cumberledge S: **Glycosaminoglycans can modulate extracellular localization of the wingless protein and promote signal transduction.** *J Cell Biol* 1996, **135:**819-827.
10. Haecker U, Lin X, Perrimon N: **The *Drosophila sugarless* gene modulates Wingless signaling and encodes an enzyme involved in polysaccharide biosynthesis.** *Development* 1997, **124:**3565-3573.
11. Lin X, Perrimon N: **Dally cooperates with *Drosophila* Frizzled 2 to transduce Wingless signalling.** *Nature* 1999, **400:**281-284.
12. Tsuda M, Kamimura K, Nakato H, Archer M, Staatz W, Fox B, *et al.*: **The cell surface proteoglycan Dally regulates Wingless signalling in *Drosophila*.** *Nature* 1999, **400:**276-280.