A molecular basis for transdetermination in *Drosophila* imaginal discs: interactions between *wingless* and *decapentaplegic* signaling

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Accepted 4 November 1997; published on WWW 8 December 1997

SUMMARY

We are investigating how *Drosophila* imaginal disc cells establish and maintain their appendage-specific determined states. We have previously shown that ectopic expression of *wingless* (*wg*) induces leg disc cells to activate expression of the wing marker *Vestigial* (*Vg*) and transdetermine to wing cells. Here we show that ectopic *wg* expression non-cell-autonomously induces *Vg* expression in leg discs and that activated *Armadillo*, a cytosolic transducer of the *Wg* signal, cell-autonomously induces *Vg* expression in leg discs, indicating that this *Vg* expression is directly activated by *Wg* signaling. We find that ubiquitous expression of *wg* in leg discs can induce only dorsal leg disc cells to express *Vg* and transdetermine to wing. Dorsal leg disc cells normally express high levels of *decapentaplegic* (*dpp*) and its downstream target, *optomotor-blind* (*omb*). We find that high levels of *dpp* expression, which are both necessary and sufficient for dorsal leg development, are required for *wg*-induced transdetermination. We show that dorsalization of ventral leg disc cells, through targeted expression of either *dpp* or *omb*, is sufficient to allow *wg* to induce *Vg* expression and wing fate. Thus, *dpp* and *omb* promote both dorsal leg cell fate as well as transdetermination-competent leg disc cells. Taken together, our results show that the *Wg* and *Dpp* signaling pathways cooperate to induce *Vg* expression and leg-to-wing transdetermination. We also show that a specific *vg* regulatory element, the *vg* boundary enhancer, is required for transdetermination. We propose that an interaction between *Wg* and *Dpp* signaling can explain why leg disc cells transdetermine to wing and that our results have implications for normal leg and wing development.

Key words: *wingless*, *vestigial*, *decapentaplegic*, *optomotor-blind*, Imaginal disc, Transdetermination, *Drosophila*, Signaling

INTRODUCTION

A significant problem in developmental biology is understanding how cells maintain a particular fate. To address this problem, we are studying how *Drosophila* imaginal disc cells maintain their determined states. Cell transplantation experiments as well as molecular and genetic analyses have revealed that imaginal cells acquire a disc-specific determined state as early as stage 11-12 of embryogenesis (Simcox and Sang, 1983; Meise and Janning, 1993; Cohen et al., 1993; Fuse et al., 1996). The imaginal discs grow and maintain their determined states through the larval stages until metamorphosis, when they differentiate to give rise to the appendages of the adult fly. Imaginal disc cells, however, can undergo transdetermination, or a switch in their determined state to that of another disc type (reviewed in Hadorn, 1978). When imaginal disc fragments undergo extra cell proliferation during in vivo culture, a few cells of a fragment might transdetermine and, upon differentiation, produce cuticular structures of a different appendage. Fragments from all imaginal discs can transdetermine. Many transdetermination events resemble the effects of homeotic mutations. For example, transdetermination from antenna to leg mimics the phenotype of an *Antennapedia* mutation. Imaginal disc transdetermination can be induced in situ by direct misexpression of homeodomain proteins (Schneuwly et al., 1987; Halder et al., 1995) and the nuclear protein *Vestigial* (Kim et al., 1996). Such observations indicate that the proper localized expression of these nuclear regulatory factors is critical for maintaining proper cell fate determination. However, it is not known what mechanisms might cause such factors to become misregulated during transdetermination and cause imaginal disc cells to change their determined states. Because transdetermination is a switching of cell fate, an understanding of transdetermination at a molecular level will provide significant insight into how proper cell fate is normally maintained.

We have previously shown that ectopic expression of *wingless* (*wg*), a member of the *Wnt* signaling family (reviewed in Nusse and Varmus, 1992), can induce transdetermination events in all ventral appendages of *Drosophila* (Maves and Schubiger, 1995; Johnston and Schubiger, 1996). *wg*-induced transdeterminations and disc fragmentation-induced transdeterminations both occur in the same specific regions within particular discs and only switch in particular directions to produce specific structures of other discs. For example, in both *wg*-induced and fragmentation-induced transdetermination, dorsal leg disc cells transdetermine.
to produce ventral wing hinge structures (Schubiger, 1971; Maves and Schubiger, 1995). Localized regions, such as dorsal leg disc cells, that can transdetermine have been termed 'weak points' (Hadorn, 1978; Schneuwly et al., 1987; Johnston and Schubiger, 1996). We want to know what gene activities define such weak points as well as why transdeterminations proceed in specific directions.

We have focused on understanding leg-to-wing transdetermination because the fate maps for both appendages are known in detail (Schubiger, 1968; Bryant, 1975) and because the expression patterns and functions of genes involved in leg and wing development are well understood (reviewed in Blair, 1995, and in Held, 1995). In both leg and wing discs, engrailed (en) is expressed in posterior cells (Kornberg et al., 1985; Fig. 1A-D). en activates expression of hedgehog (hh; Lee et al., 1992; Tabata et al., 1992). In the leg disc, Hh signaling activates expression of wg in an anterior-ventral quadrant of cells (Baker, 1988; Basler and Struhl, 1994; Fig. 1A) and decapentaplegic (dpp), which encodes a secreted signaling molecule of the TGF-β family (Gelbart, 1989), in a stripe of anterior cells along the anterior-posterior (AP) compartment boundary, at high levels dorsally and lower levels ventrally (Masucci et al., 1990; Basler and Struhl, 1994; Fig. 1B). optomotor-blind (omb), which encodes a T-box transcription factor (Pflugfelder et al., 1992), is a downstream target of Dpp signaling and is expressed specifically in anterior-dorsal leg disc cells (Brook and Cohen, 1996; Fig. 1C). wg and dpp are believed to interact synergistically to promote distal outgrowth in the center of the leg disc (Campbell et al., 1993; Diaz-Benjumea et al., 1994). wg and dpp also interact antagonistically to control dorsoventral leg patterning (Penon and Hoffmann, 1996; Morimura et al., 1996; Jiang and Struhl, 1996; Brook and Cohen, 1996; Theisen et al., 1996). In the wing disc, Hh signaling activates expression of dpp in a stripe of anterior wing disc cells (Basler and Struhl, 1994; Fig. 1E). Dpp signaling organizes growth and patterning along the A/P axis of the wing disc (Posakony et al., 1990; Zecca et al., 1996) by activation of target genes such as omb (Grimm and Pflugfelder, 1996; Nellen et al., 1996; Lecuit et al., 1996; Fig. 1E). Dorsal wing identity is specified by apterous (Diaz-Benjumea and Cohen, 1993; Fig. 1F). wg may play a role in ventral wing patterning during mid-larval development (Couso et al., 1993; Williams et al., 1993). In late third instar, wg is expressed in stripes in the presumptive notum, hinge and blade regions (Couso et al., 1993; Fig. 1D). vestigial (vg) encodes a nuclear protein that is both necessary and sufficient for wing identity (Williams et al., 1991; Kim et al., 1996; Fig. 1F). vg expression is regulated by Dpp as well as by signals from the dorsal-ventral (D/V) boundary (Kim et al., 1996).

Here we show that ectopic wg expression non-cell-autonomously induces Vg expression in leg discs and that activated Armadillo, a cytosolic transducer of Wg signaling, cell-autonomously induces Vg expression in leg discs, indicating that this Vg expression is directly activated by Wg signaling. We find that ubiquitous expression of wg in leg discs can induce only dorsal leg disc cells to express Vg and transdetermine to wing. We show that high levels of dpp expression are required for wg-induced transdetermination. We also show that both dpp and omb specify dorsal leg cell fate and allow wg to induce Vg expression and wing fate. We discuss these results in terms of how cell-signaling interactions can both initiate transdetermination as well as control the direction of transdetermination.

**MATERIALS AND METHODS**

*Drosophila* stocks, the flp-out technique and Gal4/UAS crosses

Flies were raised at 25°C on standard *Drosophila* medium supplemented with yeast unless otherwise mentioned.

We used the flp-out technique (Struhl and Basler, 1993) to ectopically express Wg (or activated Armadillo) in imaginal disc cell clones. Act5C>wg clones were induced as described in Maves and Schubiger (1995). UAS>flu-wg or UAS>flu-omb clones were induced by crossing hs-flp; UAS>CD2, y>flu-wg (or UAS>CD2, y>flu-omb; transgenes described in Zecca et al., 1996) females to *C765-Gal4* males and heat shocking the progeny. Heat shocks were either at 34°C for 30 minutes (which induces about 3-4 clones per leg; L. M., data not shown), at 37°C for 60 minutes (which induces a low level of ubiquitous expression of wg, Struhl and Basler, 1993; L. M. and G. S., data not shown), or, for UAS>flu-wg and UAS>flu-omb clones, at 37°C for 20 minutes (which induces multiple clones per leg; L. M., data not shown).

We used the Gal4/UAS system (Brand and Perrimon, 1993) to target expression of wg, dpp or omb. The following Gal4 drivers were used: *C765-Gal4* (drives ubiquitous expression in imaginal discs; Nellen et al., 1996); *dpp-Gal4* (lines 4A.3 and 40C.6, Morimura et al., 1996). The following UAS lines were used: *UAS-wg* (Lawrence et al., 1995), *UAS-dpp* (lines 42C.1, Morimura et al., 1996 and 94B.1, provided by M. Hoffmann) and *UAS-omb* (lines 2-17 and 4-15, Grimm and Pflugfelder, 1996). Gal4/UAS crosses were typically raised at 25°C, however, the *C765-Gal4/UAS-wg* cross was raised at 18°C to generate a lower level of ubiquitous wg expression (the Gal4/UAS system is temperature-sensitive; see Morimura et al., 1996).

We also used the following reporter genes: wg-lacZ (Struhl and Basler, 1993), dpp-lacZ BS3.0 (Masucci et al., 1991), omb-lacZ (Grimm and Pflugfelder, 1996), apterous-lacZ (Diaz-Benjumea and Cohen, 1993), vg

**Fig. 1.** Patterning gene expression in wild-type *Drosophila* leg (A-C) and wing (D-F) discs. In this and in subsequent figures, discs are oriented dorsal side up, anterior to the left. (A-C) Anti-En expression is shown in green. *LacZ* reporter gene expression is detected with anti-β-gal (red). (A) wg-lacZ (red); (B) dpp-lacZ (red); (C) omb-lacZ (red); (D) wg-lacZ (red) and En (green); (E) dpp-lacZ (red) and Omb (green); (F) apterous-lacZ (green) and Vg (red). Overlap of expression patterns appears yellow. Scale bar, 100 μm (A-C); 100 μm (D-F).
boundary enhancer-lacZ (Williams et al., 1993) and vg quadrant enhancer-lacZ (Kim et al., 1996), as well as the dpp hypomorphic alleles dpp16 and dpp18 (Spencer et al., 1982) and the vg allele vg<sup>vg83b27</sup> (Williams et al., 1991). Standard genetic crosses were used to bring Gal4/UAS transgenes, lacZ reporter transgenes, and dpp and vg mutations into the wg flip-out fly stocks. When necessary, transgenes and mutations were balanced using TM6B or T(2;3) chromosomes marked with Tubby, allowing Tubby progeny to be used as controls.

We observe a developmental delay in animals with ectopic wg expression. The delay is variable from animal to animal but can be up to 2-3 days delay to begin wandering; also, larvae can remain in the wandering stage for up to about 2 days. We reproducibly observe lower frequencies of Vg expression in leg discs dissected from larvae that are early in the wandering stage. We therefore dissect larvae that are late in the wandering stage (i.e. not crawling vigorously but not yet pupariated). Reproducibly, wg-expressing leg discs from such larvae will show a consistent, high frequency of Vg expression (as reported in Tables 1 and 2).

**Immunocytochemistry and X-gal staining**

Discs were prepared for fixation and staining by inverting the anterior halves of late wandering stage larvae in Ringer’s solution (Ephrussi and Beadle, 1936). Gut and fat body were removed and the carcasses with the attached discs were accumulated in Ringer’s. Discs were fixed and stained as described in Maves and Schubiger (1995), except that fixation was for 20 minutes at room temperature. All incubations and washes were performed at room temperature, except that primary antibody incubations were performed at 4°C. Primary antibodies were used at the following dilutions: rabbit anti-Vestigial 1:200, mouse anti-Engrailed monoclonal 4D9 1:500, mouse anti-Optomotor-blind 1:100, mouse anti-β-galactosidase (Boehringer Mannheim) 1:1000, rabbit anti-β-galactosidase (Cappel) 1:1000, mouse anti-CD2 (Serotec) 1:500. The following secondary antibodies were used at 1:200 dilution: goat anti-rabbit Texas Red (Molecular Probes), goat anti-mouse BODIPY FL (Molecular Probes) and goat anti-mouse BODIPY Texas Red (Molecular Probes). Images were collected 1:200 dilution: goat anti-rabbit Texas Red (Molecular Probes), goat anti-mouse BODIPY FL (Molecular Probes), and goat anti-mouse BODIPY Texas Red (Molecular Probes). Images were collected using Adobe Photoshop 3.0. The orientation of leg discs is assessed using the nerve (posterior-ventral), the stalk (dorsal) and molecular markers such as Engrailed.

X-gal staining of discs was performed as in Morimura et al. (1996).

**Disc transplantations and cuticle analysis**

For disc transplantations, leg discs were isolated from late wandering stage larvae in Ringer’s solution (Ephrussi and Beadle, 1936). Gut and fat body were removed and the carcasses with the attached discs were accumulated in Ringer’s. Discs were fixed and stained as described in Maves and Schubiger (1995), except that fixation was for 20 minutes at room temperature. All incubations and washes were performed at room temperature, except that primary antibody incubations were performed at 4°C. Primary antibodies were used at the following dilutions: rabbit anti-Vestigial 1:200, mouse anti-Engrailed monoclonal 4D9 1:500, mouse anti-Optomotor-blind 1:100, mouse anti-β-galactosidase (Boehringer Mannheim) 1:1000, rabbit anti-β-galactosidase (Cappel) 1:1000, mouse anti-CD2 (Serotec) 1:500. The following secondary antibodies were used at 1:200 dilution: goat anti-rabbit Texas Red (Molecular Probes), goat anti-mouse BODIPY FL (Molecular Probes). Images were collected with a Bio-Rad MRC 600 confocal microscope system and assembled into figures using Adobe Photoshop 3.0. The orientation of leg discs is assessed using the nerve (posterior-ventral), the stalk (dorsal) and molecular markers such as Engrailed.

Cuticle was prepared and analyzed according to Maves and Schubiger, 1995). To improve tissue clearing, we boiled flies in 5N KOH for 2 minutes, then rinsed the cuticle with water before dissection. Implants were not boiled in KOH.

**RESULTS**

**Wingless signaling directly induces Vestigial expression in leg discs**

To determine whether Wg signaling directly induces transdetermination in leg discs, we wanted to know whether components of the Wg signaling pathway could induce transdetermination and also what is the relationship between ectopic Wg signaling and the transdetermined cells. We therefore analyzed the ability of either ectopic wg-expressing cell clones (UAS>flu-wg clones) or clones of cells expressing a constitutively active form of Armadillo (UAS>flu-Δarm clones), which functions cell-autonomously to transduce the Wg signal (Peifer et al., 1991; Zecca et al., 1996), to induce Vg expression, a marker for transdetermined cells (Maves and Schubiger, 1995), in leg discs. UAS>flu-wg-expressing clones can induce Vg expression in a non-autonomous manner, in cells either within or outside of the clones (Fig. 2A), although Vg-expressing cells are not found more than a few cell diameters away from UAS>flu-wg cells (Fig. 2A). However, autonomous activation of the Wg signaling pathway, using UAS>flu-Δarm-expressing clones, only induces Vg expression in cells within the clones (Fig. 2B). We conclude that ectopic Wg signaling in leg discs directly activates Vg expression and, therefore, directly induces transdetermination.

**Ubiquitous expression of wingless in leg discs induces leg-to-wing transdetermination only in dorsal leg cells**

To determine which leg disc cells are competent to activate Vg expression and transdetermine in response to ectopic wg expression, we ubiquitously expressed wg in leg discs, using either the flip-out technique or the Gal4/UAS system. Ubiquitous expression of wg results in a high frequency of both ectopic Vg expression in leg discs and wing cuticle in adult legs (Table 1). To map which leg disc cells activate Vg expression, we labeled wg-expressing leg discs for Vg expression and for regional markers of the leg disc (see Fig. 1). Ubiquitous expression of wg induces ectopic Vg expression in dorsal leg disc cells adjacent to, but never overlapping with, cells expressing high levels of dpp (Fig. 3A). We find that cells in the leg disc with ectopic Vg expression are coincident with or adjacent to cells expressing high levels of omb (Fig. 3B). The ectopic Vg may be expressed in anterior or posterior cells (Fig. 3C) but is always situated dorsally. Ectopic wing structures are induced only in the dorsal region of the leg (Fig. 3E). The wing structures induced, ventral wing hinge structures and wing blade hairs, are the same as those induced by random Act5C>wg clones (Maves and Schubiger, 1995; Fig. 3F). The ectopic Vg-expressing leg disc cells do not express Apterous (28 C765-Gal4/UAS-wg leg discs with Vg expression showed no Vg/Apterous colocalization), further confirming that leg cells transdetermine to ventral, not dorsal, wing cells. Thus, only dorsal leg disc cells are competent to activate Vg expression and transdetermine to ventral wing cells in response to wg.

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<tr>
<th>Genotype</th>
<th>Frequency of leg discs with Vg expression</th>
<th>Frequency of legs with wing tissue</th>
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<tr>
<td>Act5C&gt;wg</td>
<td>69% (20/29 discs)</td>
<td>44% (11/25 legs*)</td>
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<tr>
<td>C765-Gal4/UAS-wg</td>
<td>59% (13/22 discs)</td>
<td>91% (40/44 legs)</td>
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Data shown are from foreleg discs and adult forelegs. Similar results were observed for all three leg pairs, except that third legs can transdetermine to haltere or wing. C765-Gal4/UAS-wg flies were raised at 18°C.

*Ubiquitous Act5C>wg expression causes pupal lethality. To obtain adult cuticle, discs were transplanted into wild-type host larvae (see Materials and Methods). Both forelegs and second legs were analyzed.
Wingless signaling directly activates Vg expression in leg disc cells. (A) Vg expression (red) induced in a foreleg disc with UAS>flu-wg clones, labeled by the absence of the CD2 (green) marker. Vg is expressed in both UAS>flu-wg-expressing cells and non-UAS>flu-wg-expressing cells (arrow). 42/271 leg discs with UAS>flu-wg clones showed ectopic Vg expression. In 31/42 discs, Vg was expressed within a clone; in 6/42 discs, Vg was expressed outside of a clone; in 5/42 discs, Vg expression straddled a clone boundary, as shown in (A). (B) Vg expression (red) induced in a second leg disc with UAS>flu-arm clones, labeled by the absence of the CD2 (green) marker. Vg is expressed within the UAS>flu-arm-expressing cells. 38/418 leg discs with UAS>flu-arm clones showed ectopic Vg expression. In all 38 discs, Vg was expressed within a clone. For both UAS>flu-wg and UAS>flu-arm clones, Vg expression was induced only on the dorsal side of leg discs. Scale bar: 10 μm (A-B).

Fig. 2. Wingless signaling directly activates Vg expression in leg disc cells. (A) Vg expression (red) induced in a foreleg disc with UAS>flu-wg clones, labeled by the absence of the CD2 (green) marker. Vg is expressed in both UAS>flu-wg-expressing cells and non-UAS>flu-wg-expressing cells (arrow). 42/271 leg discs with UAS>flu-wg clones showed ectopic Vg expression. In 31/42 discs, Vg was expressed within a clone; in 6/42 discs, Vg was expressed outside of a clone; in 5/42 discs, Vg expression straddled a clone boundary, as shown in (A). (B) Vg expression (red) induced in a second leg disc with UAS>flu-arm clones, labeled by the absence of the CD2 (green) marker. Vg is expressed within the UAS>flu-arm-expressing cells. 38/418 leg discs with UAS>flu-arm clones showed ectopic Vg expression. In all 38 discs, Vg was expressed within a clone. For both UAS>flu-wg and UAS>flu-arm clones, Vg expression was induced only on the dorsal side of leg discs. Scale bar: 10 μm (A-B).

**dpp is required for wg-induced transdetermination**

Because wg-induced Vg expression occurs in dorsal leg disc cells near high levels of dpp expression, we asked whether the high endogenous level of dorsal dpp expression in leg discs is required for wg-induced transdetermination. To reduce the level of dpp expression in leg discs, we used transheterozygote mutants for two dpp reduction-of-function alleles: dpp<sup>d6</sup> and dpp<sup>d8</sup>. These alleles disrupt the 3' regulatory region of dpp (St Johnston et al., 1990), and the transheterozygotes have reduced levels of Dpp expression in imaginal discs, as observed by immunostaining (data not shown). Like other dpp ‘disc’ mutants (Spencer et al., 1982), dpp<sup>d6/dpp</sup><sup>d8</sup> transheterozygotes have defects in both wing and leg development. Wing blades are severely reduced in size and the wing hinge shows loss of some pattern elements, although these dpp mutants can produce the ventral wing hinge structures that are found in transdetermined legs. dpp<sup>d6/dpp</sup><sup>d8</sup> legs lack claws (60/60 legs) and have variable loss of other dorsal leg pattern elements, consistent with a requirement for dpp in dorsal leg development (Held et al., 1994). When ubiquitous Act5C>wg expression is induced in dpp<sup>d6/dpp</sup><sup>d8</sup> larvae, the frequency of transdetermination is significantly reduced (Table 2). These results show that a high level of dpp expression in the leg disc is required to interact with ectopic wg to induce transdetermination.

**High levels of dpp expression promote transdetermination-competent cells**

We next asked whether a high level of dpp expression is sufficient to interact with wg to induce transdetermination. Neither ubiquitous expression of dpp, using C765-Gal4/UAS-dpp, nor overexpression of dpp, using dpp-Gal4/UAS-dpp, induces leg-to-wing transdetermination (302 forelegs scored from C765-Gal4; 417 forelegs scored from dpp-Gal4, Morimura et al., 1996). However, ectopic or overexpression of...
Initiation of transdetermination by *wg* and *dpp* causes loss of endogenous *wg* expression, induces ectopic expression of *omb* in ventral leg disc cells, and can dorsalize ventral leg cells (Morimura et al., 1996; Jiang and Struhl, 1996; Brook and Cohen, 1996; Theisen et al., 1996). Because Wg signaling directly activates Vg expression in transdetermination (see above), we postulate that transdetermination can not occur simply by overexpressing *dpp* because *wg* expression is lost. We therefore asked whether the dorsalization of ventral leg disc cells by *dpp* is sufficient to induce leg disc cells that are competent to transdetermine in

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<th>Frequency of legs with wing tissue</th>
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<tr>
<td>Act5C&gt;wg</td>
<td>64% (23/36 discs)</td>
<td>16% (14/90 legs)</td>
</tr>
<tr>
<td>Act5C&gt;wg; dpp^60/dpp^68</td>
<td>4% (2/52 discs)</td>
<td>2% (2/88 legs)</td>
</tr>
<tr>
<td>Act5C&gt;wg; dpp-Gal4/UAS-dpp</td>
<td>97% (28/29 discs)</td>
<td>45% (17/38 legs)</td>
</tr>
<tr>
<td>Act5C&gt;wg; vg^63027/vg^63027</td>
<td>1% (3/270 discs†)</td>
<td>2% (3/176 legs)</td>
</tr>
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</table>

Data shown are from foreleg discs and adult forelegs (*data from second leg discs; †data from foreleg and second leg discs). Similar results were observed for all three leg pairs, except that third legs can transdetermine to haltere or wing. Multiple sites of Vg expression in a single disc (or of wing tissue in a single leg) are scored as one transdetermination event. Vg frequencies are generated from 37˚C heat shocks to induce ubiquitous *wg* expression; wing cuticle frequencies are generated from 34˚C heat shocks, which induces random ectopic *wg* clones and a lower transdetermination frequency (see Materials and Methods).

### Table 2. *dpp* and *vg* expression levels affect the frequency of *wg*-induced transdetermination

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**Fig. 4.** *dpp* overexpression promotes transdetermination-competent leg cells. (A) Act5C>wg; dpp-Gal4/UAS-dpp third leg disc showing Vg expression (red, arrows) relative to Engrailed expression (green). 26/29 Act5C>wg; dpp-Gal4/UAS-dpp leg discs have Vg expression at both dorsal and ventral sites, 2/29 have dorsal Vg expression, and 1/29 discs show no Vg expression. (B) Act5C>wg; dpp-Gal4/UAS-dpp second leg disc showing Vg expression (red, arrows) relative to omb-lacZ (green) expression. 19/19 Act5C>wg; dpp-Gal4/UAS-dpp leg discs have both dorsal and ventral Vg-expressing cells that are coincident with or near cells with high levels of omb-lacZ expression. Low levels of ectopic omb-lacZ expression are induced in anterior lateral cells. The spotted omb-lacZ expression in the center of the disc is in adepithelial cells. (C) Proximal leg (and wing) tissue from a transplanted Act5C>wg; dpp-Gal4/UAS-dpp second leg disc. The bracket marks the reduced coxa, trochanter and femur segments. Ventral wing hinge structures, such as the yellow club (arrows) and wing hinge hairs (star) are observed at opposite regions (dorsal and ventral) within the mesothoracic leg tissue. We observe similar phenotypes in cuticle derived from non-transplanted Act5C>wg; dpp-Gal4/UAS-dpp leg discs. Scale bars: 100 μm (A,B); 100 μm (C).

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**Fig. 5.** Ectopic *omb* expression induces dorsal leg cell fate. (A) A dpp-Gal4/UAS-omb foreleg with dorsal-dorsal symmetry. 33/50 forelegs show dorsal-dorsal symmetry, which is most evident in proximal segments (arrows point to mirror-image dorsal edge bristles in the trochanter segment). Phenotypes in the other 17/50 legs include loss of ventral leg structures, fusion of proximal foreleg segments and ventral leg bifurcations; this range of phenotypes is similar to that observed with *dpp* overexpression (Morimura et al., 1996). Similar results were obtained with two independent UAS-omb lines raised at 25˚C. (B) dpp-Gal4/UAS-omb foreleg disc with loss of wg-lacZ expression (arrow; no X-gal staining). 90/90 leg discs from 15 larvae showed no wg-lacZ expression; all wing discs from those larvae stained positively for wg-lacZ. (C) dpp-Gal4/UAS-omb foreleg disc with mirror-image dpp-lacZ expression (red), shown relative to Engrailed expression (green); dpp-lacZ expression is strong on both the dorsal and ventral (arrow) sides of the disc. 24/24 leg discs show enhanced ventral dpp-lacZ expression. Scale bars: 50 μm (A,B); 100 μm (C).
response to ectopic \( wg \) expression. When ubiquitous \( Act5C\rangle w g \) expression is induced in \( dpp-Gal4/UAS-dpp \) larvae, we find ectopic \( Vg \) expression in endogenous dorsal leg disc cells as well as in cells in the ventral region of the leg disc (Fig. 4A,B). In \( Act5C\rangle w g \); \( dpp-Gal4/UAS-dpp \) leg discs, ectopic \( Vg \) expression is localized in or near cells expressing high levels of \( omb \) (Fig. 4B) in proximal cells at the edges of these leg discs (Fig. 4A,B). The presence of \( omb \) expression on the ventral side of these leg discs suggests that the ventral leg disc cells are dorsalized. However, ectopic \( wg \) expression has prevented Dpp signaling from inducing high levels of \( omb \) in proximal cells at the edges of these leg discs (Fig. 4A,B). We also note the tremendous overgrowth of these discs; \( Act5C\rangle w g \); \( dpp-Gal4/UAS-wg \) leg discs are three to five times the size of wild-type leg discs (compare Fig. 4A,B with Fig. 1). We find ventral wing hinge structures in the endogenous dorsal leg region as well as on the opposite side of \( Act5C\rangle w g \); \( dpp-Gal4/UAS-dpp \) legs (Fig. 4C). Small areas of wing tissue occur in proximal segments of these legs (Fig. 4C). In addition to generating transdetermination-competent cells in the ventral region of the leg, the overexpression of \( dpp \) also promotes a higher frequency of \( wg \)-induced transdetermination in the dorsal region of the leg (Table 2; Fig. 4 legend), an effect reciprocal to the effect of reduced \( dpp \) expression on \( wg \)-induced transdetermination. Therefore, overexpression of \( dpp \) enhances the ability of \( wg \) to induce transdetermination. These results show that high levels of \( dpp \) expression, although not sufficient to induce transdetermination, play a critical role in dorsalizing leg cells to promote transdetermination-competent leg cells.

**optomotor-blind** can dorsalize ventral leg cells and promotes transdetermination-competent leg cells

The results presented above suggest that transdetermination-
competence is an inherent property of dorsal leg cells. omb is a downstream target of Dpp signaling (Grimm and Pflugfelder, 1996; Nellen et al., 1996; Lecuit et al., 1996; Brook and Cohen, 1996) and omb expression correlates with both dorsal leg cell fate and with transdetermination-competent leg disc cells. omb expression is not activated in leg discs until mid-third instar (data not shown), after dpp expression is present (Masucci et al., 1990), further supporting omb as a downstream target of dpp. Therefore, we asked whether omb is sufficient to dorsalize leg cells. We find that ectopic expression of omb, using dpp-Gal4/UAS-omb, dorsalizes ventral leg cells but is not sufficient to induce transdetermination. dpp-Gal4/UAS-omb legs lack ventral leg structures and have a mirror-image dorsal-dorsal symmetry (Fig. 5A). The dorsalization of ventral leg cells by omb is accompanied by both the loss of wg expression (Fig. 5B), consistent with the loss of ventral leg structures, as well as the ectopic expression of dpp (Fig. 5C). These same effects are also observed upon overexpression of dpp (Morimura et al., 1996; Brook and Cohen, 1996; Theisen et al., 1996). Therefore, omb is sufficient to specify dorsal leg cell fate.

To determine whether leg cells dorsalized by omb expression are also competent to transdetermine, we induced ubiquitous Act5C>wg expression in dpp-Gal4/UAS-omb leg discs. 100% of Act5C>wg; dpp-Gal4/UAS-omb leg discs have Vg expression in both dorsal and ventral regions (30/30 leg discs; Fig. 6A). The Vg expression often (19/50 discs) appears in patches resembling a stripe along the dorsal-ventral axis of the leg disc (Fig. 6A), approximately where omb is driven by dpp-Gal4. Ectopic wg expression in wild-type leg discs or in leg discs overexpressing dpp induces Vg expression only in proximal leg disc cells where high levels of omb expression are present (see above). The ability of wg to induce Vg expression in the central, distal region of dpp-Gal4/UAS-omb leg discs suggests that omb makes leg disc cells competent to express Vg more directly than does dpp. However, we observe a non-autonomous effect of omb on wg-induced Vg expression: Vg expression can arise in cells where omb is not expressed (En-expressing cells in Fig. 6A; also see Figs 3B, 4B). This suggests that omb, a nuclear protein, induces the expression of another signal that acts with wg to induce Vg expression. To observe the effect of Act5C>wg; dpp-Gal4/UAS-omb expression on the leg pattern, we transplanted leg discs into wild-type host larvae because Act5C>wg; dpp-Gal4/UAS-omb pupae die before differentiating adult cuticle. We find an extensive area of wing tissue grown from Act5C>wg; dpp-Gal4/UAS-omb legs (Fig. 6B,C). The overgrowth of wing tissue is due to duplications of ventral wing hinge and wing blade structures; we do not observe any additional wing structures that are not induced in legs by wg. We note that proximal leg cells transdetermine to produce proximal wing hinge structures whereas distal leg cells transdetermine to produce distal wing blade tissue (although we note that we do not observe wing margin tissue; Figs 4C, 6B-C). Because wg induces more extensive Vg expression and more extensive area of wing structures in dpp-Gal4/UAS-omb leg discs than in dpp-Gal4/UAS-dpp leg discs, we conclude that omb promotes transdetermination-competent leg disc cells more directly than does dpp. Taken together, our results indicate that the Wg and Dpp signaling pathways are cooperating to induce Vg expression and leg-to-wing transdetermination.

The vg boundary enhancer is used in transdetermination

During normal wing disc development, Vg expression is up-regulated in the mid-second instar stage (Williams et al., 1993) through the vg boundary enhancer (Fig. 7A; Williams et al., 1994). The activation of the boundary enhancer is controlled by signaling interactions at the D/V boundary of the wing disc, specifically by Notch signaling acting through Suppressor of Hairless, a transcription factor that binds to the boundary enhancer (Kim et al., 1996). A second stage of Vg up-regulation occurs during the early-third instar stage, when the Vg quadrant enhancer is activated by Dpp signaling coupled with a signal from the D/V boundary (Fig. 7B; Kim et al., 1996). To determine how Wg and Dpp signaling induce Vg expression during transdetermination, we asked whether the Vg boundary or quadrant enhancers are activated in transdetermined leg discs. We find that the quadrant enhancer is not expressed in leg discs with ubiquitous Act5C>wg expression (0/156 discs show quadrant enhancer-lacZ expression; 100/156 discs show Vg expression). The boundary enhancer, however, is activated in 100% of transdetermined leg discs (Fig. 7C). Activation of the boundary enhancer in leg disc cells does not occur until late third instar, later than it is normally activated in wing discs (Williams et al., 1993), even when wg is ectopically expressed in the second instar stage (data not shown). Boundary enhancer-lacZ expression and Vg expression generally colocalize in leg disc cells (Fig. 7C), suggesting that the boundary enhancer is the Vg regulatory region activated by Wg and Dpp signaling in transdetermination. To test whether activity of the boundary enhancer is required for transdetermination, we induced Act5C>wg expression in vg°83b27 mutants, which have a deletion of the Vg intron that contains the boundary enhancer (Williams et al., 1991, 1994). Transdetermination is virtually absent in vg°83b27 homozygotes (Table 2), demonstrating that the Vg boundary enhancer plays a critical role in incorporating Wg and Dpp signals to induce leg-to-wing transdetermination.

DISCUSSION

We show that Wg signaling directly induces Vg expression, and thus transdetermination, in leg discs. A direct, long-range effect of Wg signaling on Vg expression has also been observed in wing discs (Zecca et al., 1996; Neumann and Cohen, 1997). However, neither Wg, nor activated Armadillo, can induce Vg expression in every leg disc cell. This led us to address what other factors participate with wg in transdetermination.

Dorsalizing factors Dpp and Omb as competence factors in transdetermination

We show that dorsalization of leg disc cells by Dpp signaling is both necessary and sufficient to make leg disc cells competent to transdetermine in response to wg. High levels of dpp expression are necessary and sufficient to promote dorsal leg cell fate (Held et al., 1994; Morimura et al., 1996; Jiang and Struhl, 1996; Brook and Cohen, 1996; Theisen et al., 1996). The reason why dpp is not sufficient to induce transdetermination can be understood because of the dose-
dependent interactions between dpp and wg (Morimura et al., 1996; Johnston and Schubiger, 1996). If a low level of dpp is driven in ventral, wg-expressing leg disc cells, those cells will not activate omb and will not be dorsalized (Morimura et al., 1996; L. M., unpublished observations). If a high level of dpp is driven such that omb is activated and ventral leg cells are dorsalized, then endogenous wg expression is repressed (Morimura et al., 1996; Brook and Cohen, 1996; Theisen et al., 1996). wg is critical for activating Vg in transdetermination, therefore no transdetermination can occur simply by overexpressing dpp. If a high level of wg is driven in dorsal leg disc cells, dpp expression is repressed, dorsal leg structures are lost and transdetermination does not occur (Johnston and Schubiger, 1996). In our experiments, we drive wg at a lower level that does not repress dpp expression (see Fig. 3) and still allows transdetermination. The fact that we can drive exogenous wg in addition to dpp overexpression and thus get dorsal and dorsalized ‘ventral’ leg disc cells to transdetermine (see Fig. 4) supports our proposal that dorsalized leg disc cells are the cells that are competent to transdetermine.

We show that omb is sufficient to promote dorsal leg cell fate. We find, however, that Wg can block the ability of Gal4-targeted Dpp to activate omb in, and thus dorsalize, distal leg disc cells. Dpp can prevent Wg from activating a target gene in leg disc cells (Brook and Cohen, 1996). Thus, in addition to having antagonistic effects on each other’s expression (Penton and Hoffmann, 1996; Morimura et al., 1996; Jiang and Struhl, 1996; Brook and Cohen, 1996; Johnston and Schubiger, 1996; Theisen et al., 1996), Wg and Dpp have antagonistic effects on each other’s signaling in leg disc cells. These antagonistic interactions are likely significant not only for maintaining dorsoventral leg patterning but also for maintaining leg identity.

Targeted omb expression allows wg to induce more extensive transdetermination than does targeted dpp expression, supporting the conclusion that omb makes leg disc cells transdetermination-competent more directly than does dpp. However, the non-autonomous effect of omb on wg-induced transdetermination suggests that omb activates the expression of a secreted signal that acts with Wg signaling to induce transdetermination (Fig. 8). This signal could be Dpp, which we show can be activated by omb, but Dpp would have to promote transdetermination in leg disc cells dorsalized by omb. Our observation that the vg boundary enhancer, which does not appear to be directly regulated by Dpp (Kim et al., 1996, 1997), is required for transdetermination supports a role for signals normally used to establish D/V wing patterning to also be used in transdetermination.

The use of the vg boundary enhancer in transdetermination has two implications for normal leg and wing patterning. First, it suggests that, in leg discs, Dpp and Omb may activate a Notch ligand, such as Serrate or Delta, which could act with Wg in transdetermination to activate the vg boundary enhancer. The role of Notch signaling in leg patterning is not known; however, Serrate can enhance the ability of Wg to activate vg expression (Couso et al., 1995). Second, it suggests that Wg and Dpp signaling play an early role in D/V wing patterning, as we further discuss below.

Why do dorsal leg cells switch specifically to ventral wing cells?

The elucidation of interactions between Wg and Dpp signaling in leg-to-wing transdetermination allows us to formulate hypotheses as to why dorsal leg cells transdetermine specifically to ventral wing cells. One hypothesis is that an interaction between Wg and Dpp in transdetermination mimics an interaction between Wg and Dpp normally acting in ventral wing disc cells. During mid-larval wing disc development, wg, dpp and omb expression overlap specifically in ventral wing disc cells (Couso et al., 1993; Burke and Basler, 1996; Grimm and Pflugfelder, 1996). wg, dpp and omb expression overlap later in wing disc development in regions outside of the presumptive ventral wing (Grimm and Pflugfelder, 1996). The only other imaginal disc tissue that exhibits an overlap between wg, dpp and omb expression is in the eye disc (Zecca et al., 1996; L. M., unpublished observations). [The haltere disc has similar wg, dpp and omb expression patterns as the wing disc. Metathoracic legs can transdetermine to either wing or haltere, depending on whether Ubx expression is present (L. M., unpublished observations.)] In leg discs, antagonistic interactions between Wg and Dpp normally prevent wg expression from overlapping with high levels of dpp expression and with omb expression. Our results show that when wg is ectopically expressed in leg discs, Wg signaling interacts with high levels of dorsal Dpp signaling to induce Vg expression. We propose that forcing this synergistic interaction in leg discs may mimic a Wg/Dpp synergistic interaction normally acting in ventral wing disc cells to promote Vg expression. wg function is required to promote ventral wing development (Couso et al., 1993; Williams et al., 1993). Our results predict that Wg signaling directly activates the vg boundary enhancer during wing disc development, presumably in conjunction with Notch signaling through Suppressor of Hairless (Kim et al., 1996). Dpp signaling may promote the competence of wing disc cells to respond to Wg signaling. We note that even though the vg boundary enhancer, which is expressed along the presumptive wing margin (Williams et al., 1994), is used in transdetermination, we do not observe wing margin structures in transdetermined legs. This could be for several reasons, including that the levels of Vg expression in transdetermined leg disc cells are not high enough to promote wing margin fates (L. M., unpublished observations).

A second hypothesis is that an interaction between Wg and Dpp in transdetermination mimics the interaction between Wg and Dpp normally used to establish the wing disc primordium.
In *Drosophila* embryos, leg and wing imaginal disc cells arise from a common primordium (Wieschaus and Gehring, 1976; Cohen et al., 1993). The segregation of Vg-expressing wing primordium cells from dorsal leg primordium cells requires wg and a high level of Dpp signaling (Goto and Hayashi, 1996). Because the interactions between wg and dpp in leg-to-wing transdetermination and in the establishment of the wing primordium are similar, transdetermination may be a reactivation of the embryonic establishment of wing determination. Dorsal leg disc cells may remain competent during larval development to re-establish wing fate, possibly because they maintain high levels of Dpp signaling.

**A cell-signaling basis for transdetermination and for determination**

Our results provide a mechanistic basis for the phenomenon of transdetermination. An interaction between Wg and Dpp signaling may also initiate transdetermination in fragmented leg discs. Leg disc fragments that have the ability to transdetermine are those with cuts through dorsal as well as through ventral leg disc cells (Schubiger, 1971). During wound healing in culture, the fragmented disc cut edges heal together, thereby effectively juxtaposing wg-expressing (ventral) leg cells with dpp-expressing (dorsal) leg cells. Intercellular signaling interactions can thus explain how nuclear regulatory factors that play critical roles in the specification of segmental identity and imaginal disc cell fate, such as Vg, could become activated following a wounding event. Gehring (1967) showed that transdetermination occurs in polyclonal groups of cells, supporting the significance of cell signaling in inducing transdetermination.

The original discovery of imaginal disc transdetermination prompted the proposal that an understanding of transdetermination would provide insight into how determination is normally controlled (Hadorn, 1967). We point out that there are two components to determination: establishment and maintenance. There is evidence that Wg and Dpp signaling are used to establish disc-specific determined states (Cohen et al., 1993; Goto and Hayashi, 1996). Are Wg and Dpp signaling also used to maintain disc-specific determined states? Transdetermination results when wg and dpp interact out of their proper context. Antagonistic interactions between wg and dpp during leg disc development normally prevent Wg from interacting with high levels of Dpp signaling (Penton and Hoffmann, 1996; Morimura et al., 1996; Jiang and Struhl, 1996; Brook and Cohen, 1996; Theisen et al., 1996). Because the interactions between wg and dpp in leg-to-wing transdetermination and in the establishment of the wing primordium are similar, transdetermination may be a reactivation of the embryonic establishment of wing determination. Dorsal leg disc cells may remain competent during larval development to re-establish wing fate, possibly because they maintain high levels of Dpp signaling.

We thank K. Basler, S. Carroll, M. Hoffmann, G. Pflugfelder, G. Struhl, J.-P. Vincent and B. Wakimoto for fly stocks, and S. Carroll, T. Kornberg and G. Pflugfelder for antibodies. We also wish to thank L. Johnston and S. Carroll for discussions and comments on the manuscript. This work is supported by NIH grant GM33656 and NSF grant IBN9600662 to G. S. L. M. is supported by an NIH predoctoral training grant in Developmental Biology.

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