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Ectopic expression of *wingless* in imaginal discs interferes with *decapentaplegic* expression and alters cell determination

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SUMMARY

We have expressed the segment polarity gene *wingless* (*wg*) ectopically in imaginal discs to examine its regulation of both ventral patterning and transdetermination. By experimentally manipulating the amount of Wg protein, we show that different thresholds of Wg activity elicit different outcomes, which are mediated by regulation of *decapentaplegic* (*dpp*) expression and result in alterations in the expression of homeotic genes. A high level of Wg activity leads to loss of all dorsal pattern elements and the formation of a complete complement of ventral pattern elements on the dorsal side of legs, and is correlated with repression of *dpp* expression. *wg* expression in dorsal cells of each disc also leads to dose-dependent transdetermination in those cells in homologous discs such as the labial, antennal and leg, but not in cells of dorsally located discs. When *dpp* expression is repressed by high levels of Wg, transdetermination does not occur, confirming that *dpp* participates with *wg* to induce transdetermination. These and other experiments suggest that dorsal expression of *wg* alters disc patterning and disc cell determination by modulating the expression of *dpp*. The dose-dependent effects of *wg* on *dpp* expression, ventralization of dorsal cells and transdetermination support a model in which *wg* functions as a morphogen in imaginal discs.

Key words: *wingless*, *decapentaplegic*, cell determination, transdetermination, blastema, morphogen, *Drosophila*, imaginal disc

INTRODUCTION

Drosophila imaginal discs, the adult limb primordia, acquire a disc-specific determination (legness, wingness, headness) in the embryo. This determination is governed by homeotic or selector genes (Lewis, 1963; Lawrence and Morata, 1994). As discs increase in cell number during larval development, determination is maintained and pattern formation is completed (reviewed in Cohen, 1993). Positional cues within discs are used to specify pattern elements, giving each cell a unique identity (Bryant, 1978). The way each cell receives this positional information could be by one or both of two different mechanisms: through concentration gradients of diffusible factors or via relay of signals from one cell to another. It has been established that the diffusible cell signaling protein encoded by the segment polarity gene *wingless* (*wg*) is important for growth and patterning of discs (Struhl and Basler, 1993; Basler and Struhl, 1994), but whether *wg* initiates a signaling cascade between cells or is a morphogen is not yet clear.

wg is a member of the Wnt family of secreted glycoproteins (Rijsewijk et al., 1987; van den Heuvel et al., 1989) and is required for the formation of imaginal primordia in the embryo (Simcox et al., 1989; Cohen et al., 1993). In all discs, *wg* is expressed in a specific pattern and with *decapentaplegic* (*dpp*) participates in dorsal-ventral and proximal-distal development (Baker, 1988a,b; Couso et al., 1993; Held et al., 1994). In leg discs, *wg* is expressed in ventral cells, and loss of *wg* function leads to loss of ventral fates and duplication of the dorsal pattern (Baker, 1988a,b; Peifer et al., 1991; Held et al., 1994). Ectopic expression of *wg* in cell clones of leg discs induces supernumerary outgrowths on the dorsal side of the adult leg that contain ventral-lateral pattern elements (Struhl and Basler, 1993; Diaz-Benjumea and Cohen, 1994; Maves and Schubiger, 1995). The ventralized pattern elements also contain neighboring cells that do not express *wg*, suggesting that *wg* organizes pattern in surrounding cells (Struhl and Basler, 1993). Ventral, as opposed to ventral-lateral, pattern elements were never observed in these outgrowths, which led to the proposal that higher levels of Wg are needed to produce completely ventralized outgrowths; however, this hypothesis was not supported by subsequent experiments with increased levels of *wg* expression (Wilder and Perrimon, 1995).

Expression of *wg* in dorsal leg disc cells also causes loss of dorsal pattern elements in adult legs (Wilder and Perrimon, 1995; Maves and Schubiger, 1995), and induces transdetermination of dorsal leg cells to wing fates (Maves and Schubiger, 1995). Transdetermination has been previously observed in conjunction with disc patterning after surgical experiments and occurs in all disc types (Schubiger, 1971; Hadorn, 1978). Transdeterminations resemble homeotic transformations phenotypically, and they alter segment identity but not compartmental identity (Hadorn, 1978). A specific region of each disc, which we refer to as the weak point, transdetermines. Weak

points are located in dorsal cells near the anterior/posterior (A/P) compartment boundary of the discs (Hadorn, 1978; Maves and Schubiger, 1995). Transdetermination of leg-to-wing occurs in disc cells that express high levels of *dpp*, which suggests an interaction between *wg* and *dpp* (Maves and Schubiger, 1995). Increasingly, interactions between *wg* and *dpp* that are important for pattern formation have been documented. Segregation of the thoracic imaginal disc primordia in embryos requires an intersection of cells that express *wg* and those that express *dpp* (Cohen et al., 1993). Both *wg* and *dpp* are required for a complete proximal-distal axis in discs (Campbell et al., 1993). Interactions between *wg* and *dpp* also participate in patterning the developing brain (Kaphingst and Kunes, 1994) and midgut (Immerglück et al., 1990; Thüringer and Bienz, 1993).

We have ectopically expressed *wg* in all discs to examine its regulation of both ventral patterning and transdetermination. By experimentally manipulating the level of Wg protein in discs, we show that different outcomes are produced with different thresholds of Wg activity. At moderate levels of Wg activity, some dorsal pattern elements are lost in each disc and, in the ventrally located discs, this is accompanied by transdetermination. At high concentrations, Wg represses expression of *dpp*, leading to legs with a total loss of dorsal pattern, formation of a complete complement of ventral pattern elements on the dorsal side and no transdetermination. These experiments suggest that the dose-dependent effects of *wg* on patterning and transdetermination are mediated by modulation of *dpp* expression, and confirm that *dpp* participates with *wg* to induce transdetermination. These dose-dependent effects of *wg* support a model in which *wg* functions as a morphogen in imaginal discs.

MATERIALS AND METHODS

Fly strains, rearing of flies and ectopic expression of *wg*

Fly strains were kindly provided by M. Hoffmann (*w*;*dpp* lacZ/Cyo;*dpp*40C.6Gal4/TM6B and *w*;*dpp*4a.3Gal4/TM6B), J.-P. Vincent (*w*;UAS-*wg*$^{+}$) and E. Wilder (*w*;*ptc*Gal4 and *yw*;UAS-*wg*^{ts}). The Gal4 system was used to ectopically express *wg* (Brand and Perrimon, 1993). *dpp*Gal4 (Staehling-Hampton et al., 1994) or *patched* (*ptc*)Gal4 (Speicher et al., 1994) were crossed with UAS-*wg*^{ts} or UAS-*wg*$^{+}$ to generate animals containing both UAS*wg*$^{+}$ or UAS*wg*^{ts} and one of the Gal4 drivers. Both 40C.6Gal4 and 4a.3Gal4 showed similar effects under the conditions used here. Flies with the UAS-*wg*^{ts} transgene were maintained at 25°C until 50 hours after egg laying (AEL), early second instar, when they were induced to express *wg* by shifting larvae to 15°C. The animals were then raised at 15°C until fully differentiated. Only control flies (e.g., TM6B/UAS*wg*) eclosed in these experiments; those receiving *wg* expression differentiated into pharate adults. To examine expression of *dpp*lacZ after expression of UAS-*wg*^{ts}, the BS3.0 (*dpp* disc enhancer fused to lacZ; Masucci et al., 1990) chromosome was crossed into flies carrying UAS-*wg*^{ts}. Note that the eye-antennal disc rotates 180°C during development (Struhl, 1981) such that dorsal cells become ventrally located; for convention among discs, we call dorsal cells those that express *dpp*.

Cuticle analysis

Adult flies were boiled in 2 N KOH for 5 minutes, dissected and cuticle structures mounted in Faure medium, and photographed through a Nikon Microphot-FX microscope using Nomarski optics as in Maves and Schubiger (1995).

Immunostaining of discs

Discs were dissected, fixed, stained and mounted essentially as in Maves and Schubiger (1995), except that discs were fixed in 4% paraformaldehyde for 20 minutes. To control for variations in antibody staining, both control and test discs received antibody mixtures made in the same tube. Rabbit anti-Wg was used at a 1:200 dilution, rabbit anti-Proboscipedia at 1:75, and rabbit anti-Deformed, rabbit anti-Labial, mouse anti-Sex combs reduced and rabbit anti-Dpp were used at 1:200. Mouse anti-Engrailed was used at a dilution of 1:50. Mouse anti-BrdU was purchased from Sigma Chemicals and used at a dilution of 1:50. Confocal microscopy was performed on a Biorad 600 Confocal microscope and data processed with Adobe Photoshop.

BrdU labeling of discs

48 to 72 hours after induction of ectopic *wg* expression, discs were dissected in Ringer's solution (Schubiger, 1971) and incubated for 10 to 12 minutes in 100 μg/ml of BrdU in Ringer's at room temperature. After three quick washes in PBS with 0.2% Triton, discs were fixed in formaldehyde as above. Prior to incubation with anti-BrdU antibody, discs were incubated in 2 N HCL for 1 hour, then neutralized three times for 5 minutes each in 0.1 M Borax.

RESULTS

Ectopic expression of *wg* interferes with dorsal patterning and disc-specific cell determination

To test whether *wg* alters disc-specific determination at the weak point of discs other than the leg disc, and to determine how transdetermination correlates with pattern regulation induced by *wg*, we have targeted expression of *wg* to cells along the A/P boundary simultaneously in all major discs using the Gal4 system (Brand and Perrimon, 1993). *dpp*Gal4, containing the *dpp* disc enhancer (Staehling-Hampton et al., 1994), was used to drive expression of an upstream activating sequence-*wg* transgene (*dpp*Gal4/UAS-*wg*$^{+}$) in cells along the A/P boundary of each disc (Masucci et al., 1990). Under these conditions transdeterminations arise in the adult derivatives of the weak points of the antennal and leg discs (Table 1; Fig. 1B,D). Bifurcations and overgrowth of antennae, but not legs, occur in *dpp*Gal4/UAS-*wg*$^{+}$ animals. Substantial loss of dorsal pattern elements occurs in the area of the transdetermined structures in both the head and legs (see below; Table 3). In the head, pseudotrachae are missing or fused in the labial palp, eye tissue is severely reduced and the maxillary palp, a ventrally located adult structure that arises from disc cells that are dorsal prior to the 180° rotation (Struhl, 1981), is frequently missing. Legs from *dpp*Gal4/UAS-*wg*$^{+}$ adults have lost nearly 100% of all dorsal structures, including the most distal dorsal element, the claw (Table 3). *dpp*Gal4/UAS-*wg*$^{+}$ legs fall into

Table 1. Transdeterminations induced in adults after ectopic expression of *wingless* (*dpp*Gal4:UAS-*wg*$^{+}$)

Maxillary palp to antenna	11/50 (22%)
Antenna to maxillary palp	6/50 (12%)
Leg to wing	16/150 (11%)
Leg to haltere	2/50 (4%)

Percentages expressed as number of transdeterminations per total number of appendages examined. Leg to haltere transdeterminations occur in one second and one third leg; transdetermination from leg to wing occurs in all three legs. More than one transdetermination occurs in each animal.

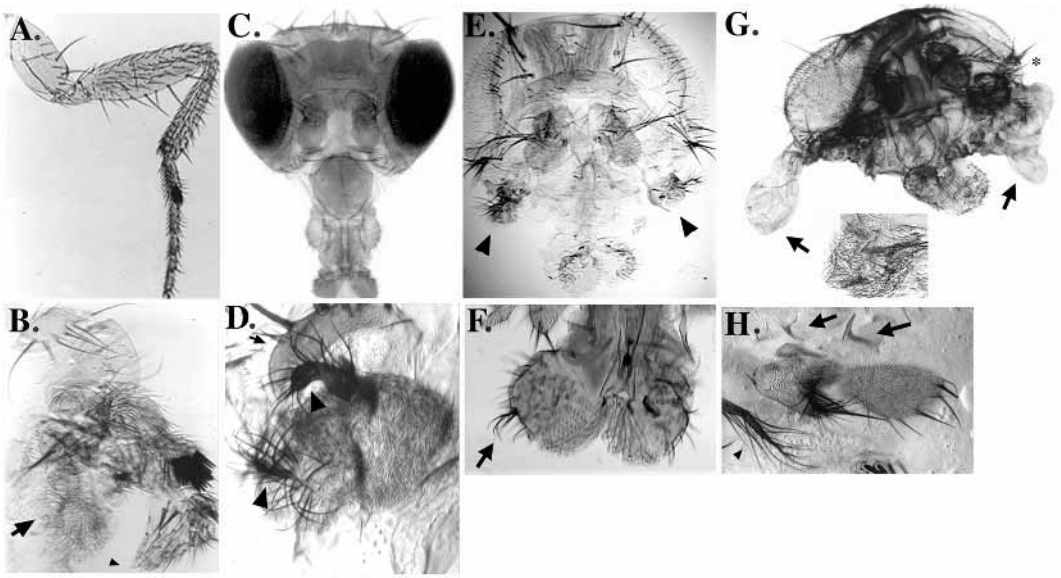

Fig. 1. Ectopic expression of *wingless* exerts dose-dependent effects on disc cell determination in appendages derived from ventral discs. (A) Control leg. (B) Leg from a *dpp*Gal4/UAS-wg^+ animal in which dorsal proximal cells have transdetermined to wing (arrow). The wing cuticle typically includes ventral hinge structures such as yellow club, pleural wing process and other scleral structures, wing hairs, prealar apophysis, axillary pouch, sensilla campaniformia and, more rarely, axillary cord. Note that ventral leg structures are duplicated (e.g., sex comb) and dorsal structures have been lost, causing the leg to curl dorsally, and the claw is missing (arrowhead). No bifurcations or outgrowths arise in these legs. (C) Control head. (D) Example of a bifurcated antenna from a *dpp*Gal4/UAS-wg^+ animal. The first antennal segment (small arrow) is wider than normal and the third segment has bifurcated giving rise to two aristae (arrowheads). (E) Head from *dpp*Gal4/UAS-wg^{ts} animal in which both maxillary palps have transdetermined to antenna (arrowheads). The eyes are smaller than normal due to loss both ventrally and dorsally. (F) *ptc*Gal4;UAS-wg^{ts} labial palp transdetermined to antennal identity, indicated by the presence of an arista (arrow). (G) Head from a *ptc*Gal4;UAS-wg^{ts} animal. The rostral membrane near the maxillary palpus has developed wing cuticle on both sides of this head (arrows). The antennae are normal but maxillary palps have completely transdetermined to antennae (asterisk). The eyes, although approximately normal here, are typically much smaller than wild type. Inset, higher magnification of wing hairs arising from the rostral membrane of the head in G. (H) Transdetermination of a duplicated antennal segment to a maxillary palpus in a *ptc*Gal4;UAS-wg^{ts} animal. The normal antenna (only the arista is shown; arrowhead) is duplicated and includes the brick-like pattern characteristic of the second antennal segment as well as an arista. The duplicated antenna has transdetermined to a maxillary palpus, complete with duplicated lacinia (arrows). Magnification, A, B, C, E and G, 63×. D, 200×. F, inset G and H, 100×.

two categories. In the first, wing structures representing transdetermination are found on the dorsal side, and dorsal structures such as sensilla on the coxa and throchanter are present, but other dorsal pattern elements are lost, including cuticle along the proximal-distal axis that causes the leg to curl dorsally (Figs 1B, 2B). All leg segments are present but fused and truncated. The second category consists of legs that do not transdetermine and completely lack dorsal structures (Fig. 2D; Table 3). Legs with no dorsal structures have a mirror-symmetric duplication of ventral structures, with the axis of symmetry at the dorsal-ventral midline (Fig. 2C,D). The ventral structures induced in dorsal cells by ectopic *wg* contain a complete complement of ventral pattern elements. Thus, ectopic expression of *wg* from *dpp*Gal4/UAS-wg^+ induces dorsal leg cells to take on either true ventral or transdetermined fates, but not both.

In contrast to the legs and antennae, wings from *dpp*Gal4/UAS-wg^+ animals do not transdetermine. However, ectopic expression of *wg* results in pattern abnormalities, including loss of dorsal wing hinge sensory organs and duplication of ventral hinge structures such as the yellow club. In 100% of animals, the wing blade is shortened along the proximal-distal axis, and the distal sensilla campaniformia (sc) normally associated with vein 3 are absent, and replaced with bristles characteristic of both dorsal and ventral wing margin bristles of the double row. These ectopic bristles form a row that is perpendicular to the normal wing margin. In addition, in approximately 80% of animals, a supernumerary wing surrounded by hairs characteristic of the posterior row of the wing margin is observed arising from the hinge region. A row of sc in a region that probably corresponds to vein three is located in the center of these supernumerary wings.

Different thresholds of Wg activity elicit changes in cell determination in a disc-specific manner

Although *wg* is ectopically expressed and results in pattern abnormalities in all *dpp*Gal4/UAS-wg^+ discs, we do not detect transdetermination in the wing, haltere, genitalia or proboscis of these animals. The lack of supernumerary leg bifurcations and substantial loss of dorsal structures in *dpp*Gal4/UAS-wg^+ adults suggests that the level of *wg* expression may be considerably higher than in previous reports (Struhl and Basler, 1993; Maves and Schubiger, 1995). If so, our observations suggest that patterning events are sensitive to levels of Wg activity and thus transdetermination may be also. We therefore asked whether manipulation of the level of *wg* expression might cause transdeterminations in other discs. To test this, we have used a temperature-sensitive UAS-*wg* to control the level of ectopic Wg activity (UAS-wg^{ts}; Wilder and Perrimon, 1995). Secretion of the UAS-wg^{ts} protein occurs at 15°C but not at 25°C, although the protein is expressed at both temperatures (González et al., 1991; Wilder and Perrimon, 1995). When *dpp*Gal4/UAS-wg^{ts} larvae are raised at 25°C, animals differentiate normally and we observe no pattern abnormalities or transdeterminations in the adult. In contrast, raising larvae at 25°C until 50 hours after egg laying (AEL), then shifting to

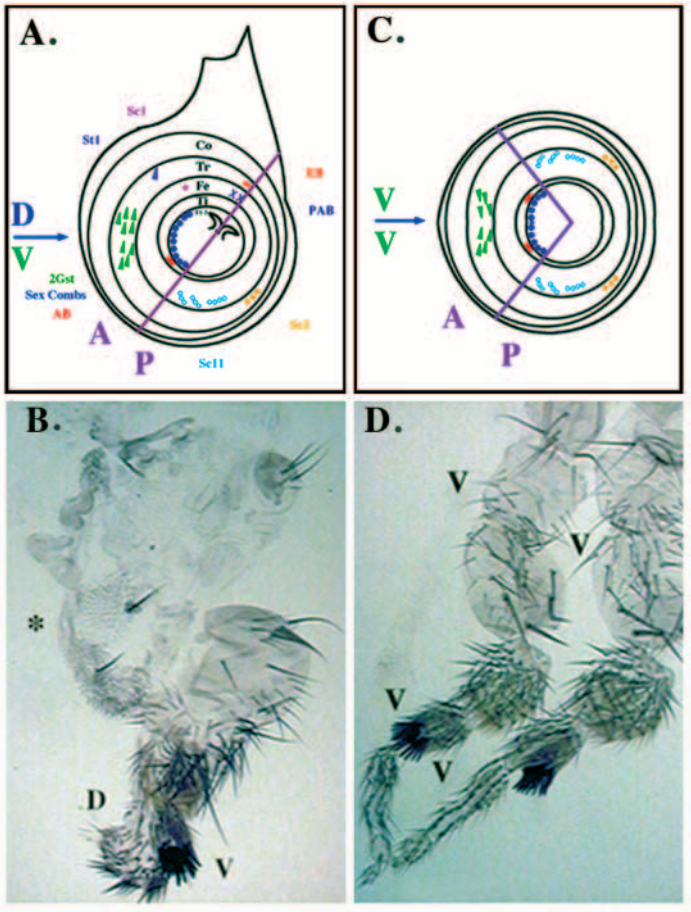

Fig. 2. Loss of dorsal structures, transdetermination and formation of ventral structures on the dorsal side of legs is dependent on the dose of Wingless. (A) Fate map of the leg, modified from Schubiger (1968). The primordia for some adult cuticle structures in the leg are listed, with their respective position on the fate map. D, dorsal; V, ventral; A, anterior; P, posterior. Symbols designating position of structures are color coded with the abbreviation. Co, coxa; Tr, trochanter; Fe, femur; Ti, tibia; St, sensilla trichodea; GSt, groups of sensilla trichodea; AB, apical bristle; PAB, pre-apical bristle; EB, edge bristle; Sc, sensilla campaniformia. This fate map was made for the first leg, but is also appropriate for the second and third legs. (B) Leg from *dpp*Gal4/UAS-wg^{+} animal, with severe dorsal loss (D) and ventral duplications (V), causing the distal part of the leg to curl dorsally. In addition, dorsal cells have transdetermined to wing (asterisk). (C) Fate map of legs illustrated in D with mirror-image ventral and no dorsal structures, showing the line of symmetry (blue arrow). Posterior is to the right, as in A. (D). Pair of legs from *dpp*Gal4/UAS-wg^{+} animal. These legs have lost all dorsal structures, and have duplicated a complete complement of the normal ventral pattern in mirror image symmetry (V/V). Legs such as this one never transdetermine. Magnification of B and D is 63×.

15°C results in two-fold more leg-to-wing transdeterminations than with the UAS-wg^{+} (compare Tables 1 and 2). Although no bifurcations occur in legs from 15°C *dpp*Gal4/UAS-wg^{ts} animals, dorsal leg elements are lost with slightly lower frequencies; for example, only 29% lack a claw (Table 3). The frequency of transdetermination of maxillary palp to antenna and antenna to maxillary palp is reduced, as is the extent of dorsal loss in the head (not shown). Again, transdeterminations

Table 2. Transdeterminations after ectopic expression of UAS-wg^{ts} at different temperatures

	25°C	18°C	15°C
(a) *dpp*Gal4:UAS-wg^{ts}			
Maxillary palp to antenna	0/16		1/32 (3%)
Antenna to maxillary palp	0/16		0/32 (0%)
Leg to wing	0/48		25/96 (26%)
(b) *ptc*Gal4:UAS-wg^{ts}			
Maxillary palp to antenna	0/30	30/64 (46%)	33/34 (99%)
Antenna to maxillary palp	0/30	11/64 (17%)	4/34 (12%)
Labial to antenna	0/30	2/64 (3%)	2/34 (6%)
Head to wing	0/30	0/64 (0%)	8/34 (24%)
Leg to wing	0/30	0/192 (0%)	22/102 (22%)
Genital to maxillary palp	0/30	0/64 (0%)	2/34 (6%)

Percentages expressed as number of transdeterminations per total number of appendages examined.

are limited to appendages derived from the antennal and leg discs (Table 2).

We have also used *patched* (*ptc*) Gal4 to drive expression of the UAS-wg^{ts} transgene, in a pattern that substantially overlaps that of *dpp*Gal4, but at a stronger level in both ventral and dorsal cells (Speicher et al., 1994; Wilder and Perrimon, 1995). Like *dpp*Gal4/UAS-wg^{ts} animals, *ptc*Gal4;UAS-wg^{ts} larvae continuously raised at the restrictive temperature of 25°C do not transdetermine and are phenotypically wild type (Table 2). However, shifting the temperature to 15°C at 50 hours AEL leads to additional transdeterminations: proboscis transdetermines to antenna, genitalia to maxillary palp and head rostral membrane to wing (Fig. 1; Table 2). In addition, transdetermination of maxillary palp to antenna (99%) and antenna to maxillary palp (12%) is enhanced relative to *dpp*Gal4/UAS-wg^{ts} animals. The leg-to-wing transdetermination and loss of dorsal leg elements are similar to *dpp*Gal4/UAS-wg^{ts} animals (Tables 2, 3). As before, no bifurcations occur in the legs. Wings from *ptc*Gal4;UAS-wg^{ts} animals have lost sensory organs in the dorsal hinge and the wing blade is shortened as in *dpp*Gal4/UAS-wg^{+}animals, although less severely. The level of *wg* expression from *ptc*Gal4;UAS-wg^{ts} thus increases the frequency and extends the types of transdeterminations that occur. Extra *wg* expression in ventral cells does not lead to pattern regulation (Struhl and Basler, 1993; Wilder and Perrimon, 1995) or transdetermination (Maves and Schubiger, 1995), thus we conclude that the increase in severity of the phenotype of *ptc*Gal4;UAS-wg^{ts} animals relative to *dpp*Gal4/UAS-wg^{ts} results from the increase in *wg* expression in dorsal cells.

We conclude that experimental manipulation of Wg activity significantly alters transdetermination frequencies in a disc-specific manner, suggesting that different discs respond to distinct threshold of Wg activity. Pattern regulation such as ventralization, loss of dorsal elements and transdetermination thus are linked in ventrally located discs. However, although pattern loss and duplications occur in dorsally located discs such as the eye, wing or haltere (not shown), these discs do not transdetermine.

The severity of adult phenotype correlates with an increase in spread of Wg and En protein in discs

We have correlated the patterning events in adults with gene expression in discs by examining the expression of *wg* and the

Table 3. Percentage of legs with loss of dorsal leg structures after ectopic *wg* expression

		Number animals examined	Proximal → Distal						
			St3/St4	EB	Sc+	St1	Sc1	PAB	Claw
(a) UAS-wg^{ts}									
*ptc*Gal4:UAS-wg^{ts}	15°C	*n*=12	60%	100%	91%	43%	100%	100%	29%
*ptc*Gal4:UAS-wg^{ts}	18°C	*n*=34	60%	90%	83%	17%	40%	100%	0%
*ptc*Gal4:UAS-wg^{ts}	25°C	*n*=10	0%	0%	0%	0%	0%	0%	0%
(b) UAS-wg^{+}									
*dpp*Gal4:UAS-wg^{+}		*n*=15	73%	100%	73%	100%	100%	100%	100%
TM6B:UAS-wg^{+}		*n*=10	0%	0%	0%	0%	0%	0%	0%

Numbers reflect percentage of legs that are missing the particular structure listed. All three legs (prothoracic, mesothoracic, and metathoracic) are included in the assay. St, sensillum trichodea; Sc, sensillum campaniformia; EB, edge bristle; PAB, preapical bristle. All of the lost structures are located near the A/P boundary (see Fig. 2). Loss of dorsal structures in *dpp*Gl4:UAS-wg^{ts} legs is similar to that of *ptc*Gal4:UAS-wg^{ts} (not shown).

posterior-specific gene, *engrailed* (*en*). To examine the activity of Wg^{ts} protein at different temperatures, we have stained *ptc*Gal4;UAS-wg^{ts} discs with antibodies specific for *wg* (van den Heuvel et al., 1989). Granules of Wg protein, corresponding to secretory vesicles containing Wg, have been described in embryos (van den Heuvel et al., 1989; González et al., 1991). In *ptc*Gal4;UAS-wg^{ts} discs at the control temperature of 25°C, the domain of expression of *wg* in cells along the A/P boundary has clearly demarcated posterior and anterior borders, with no granules (Fig. 3B). In contrast, at 15°C, small dots of Wg protein can be seen to extend anteriorly from anterior-most border of the *ptc*Gal4 expression domain (Fig. 3D, arrow). The granular appearance and spread of Wg is much more extensive in *dpp*Gal4/UAS-wg^{+} discs (not shown). The most severe adult phenotype, i.e. complete loss of dorsal elements and replacement with a fully ventralized pattern in *dpp*Gal4/UAS-wg^{+} legs, correlates with the most extensive spread of Wg granules in discs. These data indicate that the highest level of Wg activity can fully ventralize dorsal cells.

en is normally expressed in cells of the posterior compartment of each disc (Kornberg et al., 1985). In 15°C *ptc*Gal4;UAS-wg^{ts} discs, *en* expression has broadened into anterior cells (Fig. 3D). Expansion of *en* into both dorsal and ventral anterior cells occurs in 100% (*n*=25) of antennal discs, whereas in leg discs the *en* expansion is primarily restricted to dorsal cells and occurs in approximately 80% (*n*=60) of leg discs. The anterior *en* expression is dependent upon ectopic Wg (Fig. 3), although we do not know if it is necessary for pattern regulation or transdetermination. However, anterior *en*-expressing cells do not take on posterior fates in the adult. Although the mechanism behind the *en* expansion is not clear, since anterior *en* expression occurs in *ptc*Gal4;UAS-wg^{ts} discs

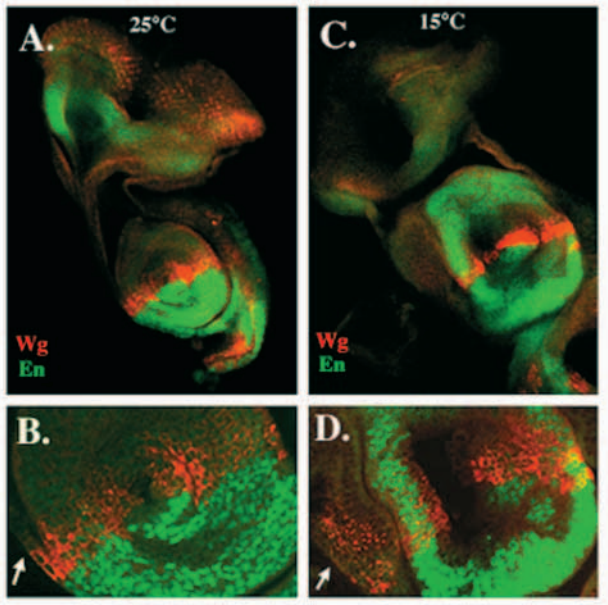

Fig. 3. Increasing Wingless activity correlates with a granular staining pattern and expansion of Engrailed into anterior cells. (A) Control, *ptc*Gal4;UAS-wg^{ts} eye-antenna disc from larva raised continuously at 25°C stained for Wg (red) and En (green). (B) High magnification of A/P boundary in A. Notice that the borders of *wg* expression are relatively sharp, and *en* expression is strictly posterior. (C) *ptc*Gal4;UAS-wg^{ts} eye-antennal disc from larva raised from 50 hours AEL at 15°C. (D) High magnification of disc from C, showing a granular pattern of Wg (red) extending from the anterior-most border of the *ptc*Gal4 expression domain (arrow) (compare with arrow in B.). Also, *en* expression (green) has expanded into the anterior compartment. Magnification: (A,C) 200×; (B,D) 800×.

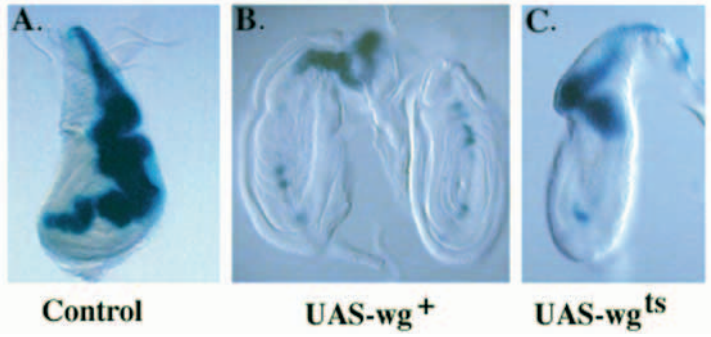

Fig. 4. Wingless down-regulates expression of *dpp* in leg discs. (A) First leg disc from control, *dpp*lacZ;TM6B/UAS-wg^{+} larva, showing the stripe of lacZ staining in the *dpp* expression pattern. (B) Pair first leg discs from *dpp*lacZ;*dpp*Gal4/UAS-wg^{+} larva, showing loss of *dpp*lacZ expression. *dpp*lacZ staining is almost entirely gone in the right disc, but the disc at left has some staining in the dorsal proximal region. (C) Leg disc from 15°C *dpp*lacZ; *dpp*Gal4/UAS-wg^{ts} larva, showing proximal dorsal but not ventral *dpp*lacZ expression. Magnified 200×.

at 15°C, but not at 25°C, it provides an indicator of the increase in Wg activity at 15°C.

Loss of adult dorsal cell fates correlates with down-regulation of *dpp* expression in discs by Wg

Our results indicate that specific dorsal leg structures, centered around the A/P boundary are lost with increasing ectopic Wg activity (Table 3). Furthermore, a comparison of the phenotypes obtained with increasing Wg activity (Tables 1, 2, and 3) indicates that the frequency of transdetermination is inversely proportional to the loss of dorsal pattern elements, suggesting that the weak point has been eliminated from the disc. At the highest level of Wg activity, in *dpp*Gal4/UAS-wg^+ animals, legs have no dorsal structures and the ventral pattern is duplicated (Fig. 2D). These legs resemble legs from severe loss-of-function *dpp* alleles (Held et al., 1994); therefore, we have asked whether *dpp* expression might be affected in the discs. We have examined the expression of *dpp* using a *dpp*-lacZ transgene (BS3.0; Blackman et al., 1991). In discs from *dpp*-lacZ;*dpp*Gal4/UAS-wg^+ larvae, we find two populations of discs, which correspond to the two classes of adult leg phenotypes described above. In the first, corresponding to legs that transdetermine, *dpp*-lacZ is expressed in the proximal-most dorsal cells of the disc at reduced levels, and ventral *dpp*-lacZ expression is absent (Fig. 4C). In the second population of discs, corresponding to legs that do not transdetermine, both dorsal and ventral expression of *dpp*-lacZ is almost completely absent, leaving only a trace of the original expression pattern (compare Fig. 4A and B). In discs from *dpp*-lacZ;*dpp*Gal4/UAS-wg^{ts} larvae that express a moderate level of *wg*, *dpp*-lacZ is expressed in proximal dorsal cells, but is down-regulated in more distal dorsal cells and completely lacking in ventral cells (Fig. 4C). Leg disc cells that transdetermine to wing fates, marked by the presence of Vestigal protein (Maves and Schubiger, 1995), are located precisely in the region of the disc that still retains expression of *dpp*-lacZ (not shown). The lack of supernumerary outgrowths in these legs correlates with down-regulation of *dpp* expression (Campbell et al., 1993; Diaz-Benjumea et al., 1994). Ectopic *wg* expression in the eye-antennal disc also down-regulates *dpp* expression and correlates with less frequent transdetermination (data not shown).

Loss of *dpp* expression prevents *wg*-induced cell proliferation in the weak points

Regeneration and transdetermination are always accompanied by the establishment of a blastema in which cell division is stimulated (Hadorn, 1978). To determine how patterning events induced by *wg* correlate with changes in the pattern of cell division, we have examined cell proliferation in discs. We have labeled disc cells with the thymidine analog, 5-Bromo 2-deoxy-Uridine (BrdU), as a measure of DNA synthesis, in a short pulse (10-12 minutes) and visualized BrdU incorporation with immunostaining. In control late third instar discs, this pulse of BrdU yields few labeled cells of variable intensity, randomly scattered throughout the disc (Fig. 5), reflecting the asynchronous cell division in most discs (Bryant, 1978). However, in 15°C *ptc*Gal4;UAS-wg^{ts} discs, in addition to the random pattern of BrdU labeling, localized patches of uniformly intense BrdU incorporation are located near the A/P boundary of each disc (Fig. 5C-E). In 15°C *ptc*Gal4;UAS-wg^{ts} eye-antennal discs, the BrdU-labeled cell cluster lies in the region of the presumptive maxillary palpus; in the leg, the cluster consists of dorsal proximal cells, corresponding to the weak point of those discs (Fig. 5C-E). When leg and antennal discs are stained for both BrdU and *wg*, the BrdU-labeled cell cluster lies in close proximity to the *wg*-expressing cells; however, cells incorporating BrdU are largely outside of the domain of cells ectopically expressing *wg*. This observation suggests that *wg* may signal to neighboring cells to induce proliferation (Fig. 5C,D; Wilder and Perrimon, 1995).

The loss of *dpp* expression in all but the dorsal-proximal cells of leg discs suggests that *dpp* might be required for stimulation of localized cell division as well as for transdetermination. To test this hypothesis, we have examined both BrdU incorporation and the presence of Dpp protein. We find that the presence of Dpp positively correlates with BrdU incorporation in the weak point of the disc. In the complete absence of Dpp, BrdU incorporation is not clustered, but scattered throughout the disc (Fig. 6). These experiments confirm that Wg down-regulates expression of Dpp protein and strongly suggest that the stimulation of cell division in the weak point of discs requires an ectopic interaction of *wg* and *dpp*.

Homeotic gene expression is altered after ectopic expression of *wg*

The phenotypes of many transdeterminations correspond to that of homeotic mutations (Kauffman et al., 1978). Transdeterminations in the head resemble phenotypes of mutations of homeotic genes within the Antennapedia complex (Ant-C), including *Sex combs reduced* (*Scr*), *proboscipedia* (*pb*), *Deformed* (*Dfd*), *labial* (*lab*) and *Antennapedia* (*Antp*) (reviewed in Jürgens and Hartenstein, 1993). To determine whether changes in expression of the Ant-C homeotic proteins correlates with *wg*-induced transdeterminations, we have immunostained eye-antennal, leg and labial discs. For these experiments, we focus on the *ptc*Gal4;UAS-wg^{ts} larvae raised at 15°C, since they transdetermine at high frequency. The normal pattern of expression of each of these genes is observed in discs from control larvae (Fig. 7). Antennal disc expression of *lab*, *Scr* and *Dfd* is normal in discs dissected from 15°C *ptc*Gal4;UAS-wg^{ts} larvae. Ectopic Dfd protein is observed in ventral anterior cells of first legs (not shown). *pb* expression is severely reduced in 50% (n=44) of labial discs (Fig. 7A). In addition, *pb* is ectopically expressed in 80% (n=44) of presumptive maxillary palpus cells in antennal discs (Fig. 7B) and in leg discs (not shown). In approximately 10% (n=44) of 15°C *ptc*Gal4;UAS-wg^{ts} labial discs, *Scr* expression is reduced, although *Scr* expression in antennal discs is not affected (Fig. 7B). In agreement with the location of transdetermined adult cuticle, the Ant-C alterations in the discs occur in cells located directly in or near the disc weak point.

The leg-to-wing and rostrum-to-wing transdeterminations can be detected in disc cells by staining leg and eye-antennal discs with antibodies to the wing-specific marker, *vg* (Williams et al., 1991; Maves and Schubiger, 1995). *vg* is not normally expressed in leg or antennal disc columnar epithelial cells (Williams et al., 1991). We find Vg protein in the rostral membrane primordia of the antennal discs of 15°C *ptc*Gal4;UAS-wg^{ts} animals at a frequency of 60% (n=28). In addition, *vg* is expressed in approximately 30% (n=30) of *ptc*Gal4;UAS-wg^{ts} leg discs, in a proximal location that corresponds to the transdetermined adult tissue. Vg protein is also

detected in leg discs from both *dpp*Gal4/UAS-wg^{ts} and *dpp*Gal4/UAS-wg^{+} animals with similar frequencies. Staining for both BrdU and markers of transdetermination indicates that transdetermining cells arise from the BrdU-incorporating cluster (Fig. 5E) and demonstrate that the BrdU-incorporating cells in this cluster make up a blastema, in which the cells give rise to more and different structures than expected from the fate map (Bryant, 1978).

DISCUSSION

Dorsal cells are ventralized by Wg in a concentration-dependent manner

A large body of evidence has established that *wg* is necessary for specification of ventral fates in legs and in the head (e.g., Baker, 1988b; Struhl and Basler, 1993). However, whether this is accomplished through a cascade of cell-cell signaling events initiated by *wg*, or through a gradient of Wg protein has not been resolved. Two methods have been used to ectopically express *wg*: the flp-out system to generate cell clones expressing low levels of *wg* (Struhl and Basler, 1993) or the Gal 4 system, in which higher levels of a temperature sensitive *wg* was expressed (Wilder and Perrimon, 1995). If Wg acts as a morphogen then high levels of Wg should induce true ventral, and lower levels induce ventral-lateral pattern elements; however, in both experiments no true ventral structures were found, pointing to a signal cascade as a mechanism for *wg* action. Here we have used both a wild-type Wg as well as the temperature-sensitive Wg in the Gal4 system, and find that the UAS-wg^{ts} functions as a hypomorph when compared to the UAS-wg^{+}. The high level of Wg activity achieved in *dpp*Gal4/UAS-wg^{+} animals causes a loss of all dorsal structures and a complete mirror-symmetric duplication of both anterior and posterior ventral elements in legs. Correlated with this observation is that leg discs from *dpp*Gal4/UAS-wg^{+} animals have lost expression of *dpp*. In contrast, the UAS-wg^{ts} transgene produces a level of Wg activity that is substantially lower than that of the UAS-wg^{+}, by the criteria of both loss of dorsal pattern in adult legs and loss of *dpp* expression in discs.

Wilder and Perrimon (1995) proposed that specification of true ventral fates in dorsal cells of both the anterior and posterior compartments requires not only *wg* but another signal. This was suggested because, in contrast to ectopic *wg*, cell clones lacking the downstream *wg* signaling component *zeste white 3*, (*zw3*), which phenotypically mimic ectopic expression of *wg*, give rise to true ventral structures in dorsal cells of both compartments (Wilder and Perrimon, 1995). Our work suggests that instead of a cooperative signal, an antagonistic signal is transmitted in dorsal cells by *dpp* which prevents complete ventralization by *wg*. Thus, in dorsal cells, Wg presumably via inhibition of *zw3*, specifies ventral fates but only at concentrations high enough to block *dpp* expression. At lower concentrations, only ventral-lateral elements are made. Since loss of *zw3* function produces the same effect as a high concentration of Wg, *zw3* is likely to be downstream of the antagonism between *wg* and *dpp* signaling.

In contrast to requiring the absence of *dpp* to produce ventral fates in dorsal cells, *wg* appears to require the presence of *dpp* to induce cell proliferation there. A moderate level of dorsal Wg is associated with extra cell division in dorsal cells, as previously reported (Wilder and Perrimon, 1995), but we find that, at higher concentrations of Wg, localized cell division in dorsal cells is not stimulated. The direct correlation with inhibition of *dpp* expression suggests that the ability of *wg* to stimulate cell division in dorsal cells requires Dpp and is similar to blastema formation after a confrontation of disparate positional values (Abbott et al., 1981).

Transdeterminations arise in ventral, but not dorsal discs in response to distinct Wg thresholds

Our results extend the previous observation that ectopic expression of *wg* induces leg cells to transdetermine to wing fates (Maves and Schubiger, 1995). Like the specification of ventral cell fates by *wg*, transdetermination is also dose-dependent. For example, leg-to-wing transdeterminations after expression of the UAS-wg^{ts} are more than double the frequency seen after expression from the UAS-wg^{+} transgene. And, although the maxillary-palp-to-antenna transdetermination is induced over a range of Wg concentrations, transdetermination from rostral membrane to wing appears to require a distinct threshold. Thresholds like this one can be achieved by bistable circuits in which feedback loops amplify and stabilize the original effect. The dose-dependent disruption of maintenance of cell determination by Wg in transdetermination suggests that bistable circuits are being switched from one state to another (Kauffman et al., 1978), and is reminiscent of phage lambda (λ), which uses an extracellular signal (ultraviolet light) to decide whether to maintain its lysogenic state or to induce the lytic cycle (reviewed in Ptashne, 1987). The extracellular signal regulates the level of λ repressor protein, which determines whether lysogenic induction takes place (Ptashne, 1987). Similarly, the level of dorsal Wg determines the outcome of transdetermination, possibly by regulating the level of expression of *dpp*. We postulate that, once a circuit such as head to wing is switched, the system is stabilized by the action of selector genes. Wg induces many of the transdeterminations that were previously only observed after disc fragmentation and culture (Fig. 8). Close analysis revealed that transdeterminations occur in sequential order and with frequencies characteristic for each disc (Hadorn, 1978; Kauffman et al., 1978). Our observations confirm that the types of transdeterminations induced by Wg fall into the same sequential order as observed in the fragmentation experiments.

Increasing the Wg activity such that *dpp* expression is eliminated completely prevents transdetermination, supporting the idea that transdetermination requires both *wg* and *dpp* (Maves and Schubiger, 1995). The fact that head rostral membrane, which is derived from cells that express high levels of *dpp* in the antennal disc, transdetermines to wing, but only at a particular threshold of Wg activity, suggests that a distinct balance of *wg* and *dpp* may be required to induce the wing program. If transdetermination results from an interaction between *wg* and *dpp*, then it may not be surprising that *wg* induces transdetermination in ventral discs but not in the wing and haltere discs. Homeotic transformations between the proboscis and leg, and antenna and leg demonstrate a basic homology between these appendages (Postlethwait and Schneiderman, 1971; Kaufman, 1978). Also, the patterns of expression of *wg*, *dpp*, *en* and *hedgehog* (*hh*) in the labial, antennal and leg discs are similar, as are their respective roles (Baker, 1988b; Mohler, 1988; Peifer et al., 1991). In contrast, *wg* and *dpp* are expressed in

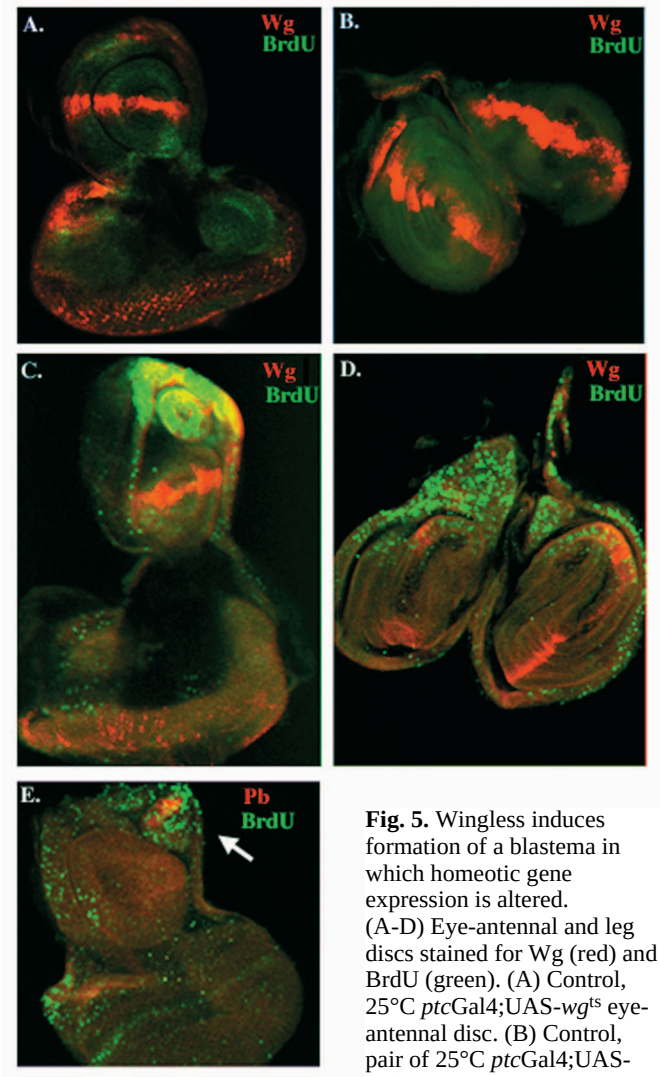

Fig. 5. Wingless induces formation of a blastema in which homeotic gene expression is altered. (A-D) Eye-antennal and leg discs stained for Wg (red) and BrdU (green). (A) Control, 25°C *ptc*Gal4;UAS-wg^{ts} eye-antennal disc. (B) Control, pair of 25°C *ptc*Gal4;UAS-wg^{ts} leg discs. In these discs, Wg protein is expressed in the *ptc*Gal4 domain but is not secreted, and thus is non-functional. (C) 15°C *ptc*Gal4;UAS-wg^{ts} eye-antennal disc, showing intense incorporation of BrdU in the weak point. (D) 15°C *ptc*Gal4;UAS-wg^{ts} leg discs, with incorporation of BrdU in the weak point. (E) Eye-antennal disc, labeled for BrdU (green) and Pb (red), which is ectopically induced in cells that are part of the blastema. Magnified 200×.

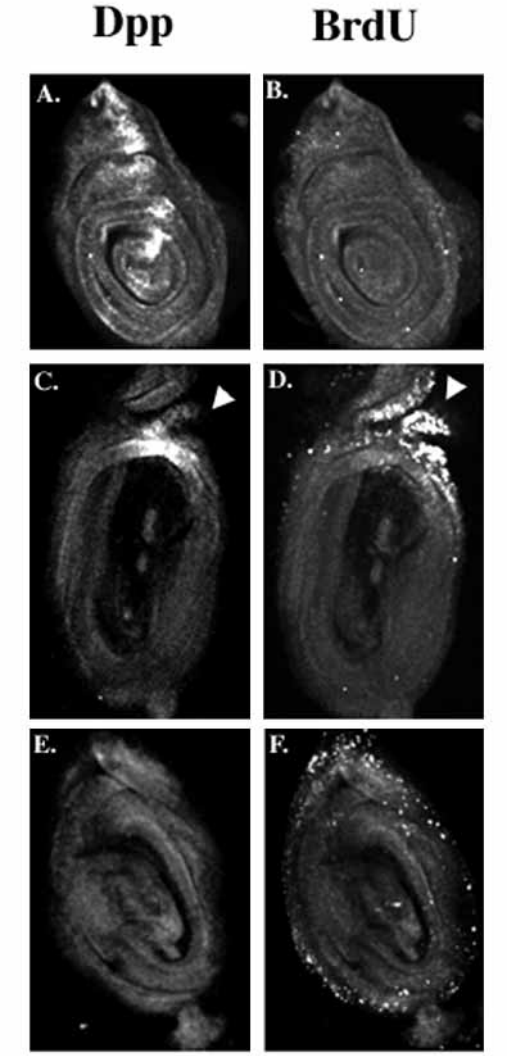

Fig. 6. Stimulation of a blastema by Wingless requires Dpp. Leg discs stained for Dpp protein and BrdU. (A,B) Control, TM6B/UAS-wg^{+} disc showing expression of Dpp, and little or no BrdU incorporation, due to the very short pulse time (see text). (C,D) Leg disc from 15°C *ptc*Gal4;UAS-wg^{ts} larva, showing down-regulation of *dpp* expression except for a small, proximal region of the disc (C), which also has incorporated high levels of BrdU (D). (E,F) *dpp*Gal4/UAS-wg^{+} leg disc, with no detectable *dpp* expression, and only random incorporation of BrdU. The difference in BrdU incorporation between F and B may be due to a low but undetectable amount of Dpp in F. Magnified 200×.

different patterns in dorsally located discs such as the wing and haltere and appear to be utilized in somewhat different ways than in ventral discs (e.g., Basler and Struhl, 1994). Thus, combinations of other patterning genes may induce transdetermination in the dorsally located wing and haltere discs.

The alterations of *pb* expression in labial and antennal discs and the corresponding labial, maxillary palp and antennal transdeterminations correlate well with transformations resulting from mutant alleles of *pb* (Kaufman, 1978; Cribbs et al., 1992). There is also a clear correlation between the expression of *vg* in leg discs and antennal discs and the transdeterminations to wing in each of those adult structures. Recent experiments in which *vg* was expressed in all discs indicate that *vg* is a selector gene that directs a wing developmental program, except in third leg discs, where it directs the formation of haltere structures (Kim et al., 1996). Our results are interesting in view of these results, because all three leg disc types transdetermine to wing, although second and third legs transdetermine to haltere at low frequency. It is possible that the anterior expansion of *en* expression, by repressing *Ubx* (L. Maves, personal communication), shifts transdetermination of third leg discs to wing rather than haltere.

Transdetermination: a mistake during regeneration, or recapitulation of an earlier state?

Many experiments have suggested that proliferation is required for transdetermination (e.g., Wildemuth, 1968), and that trans-

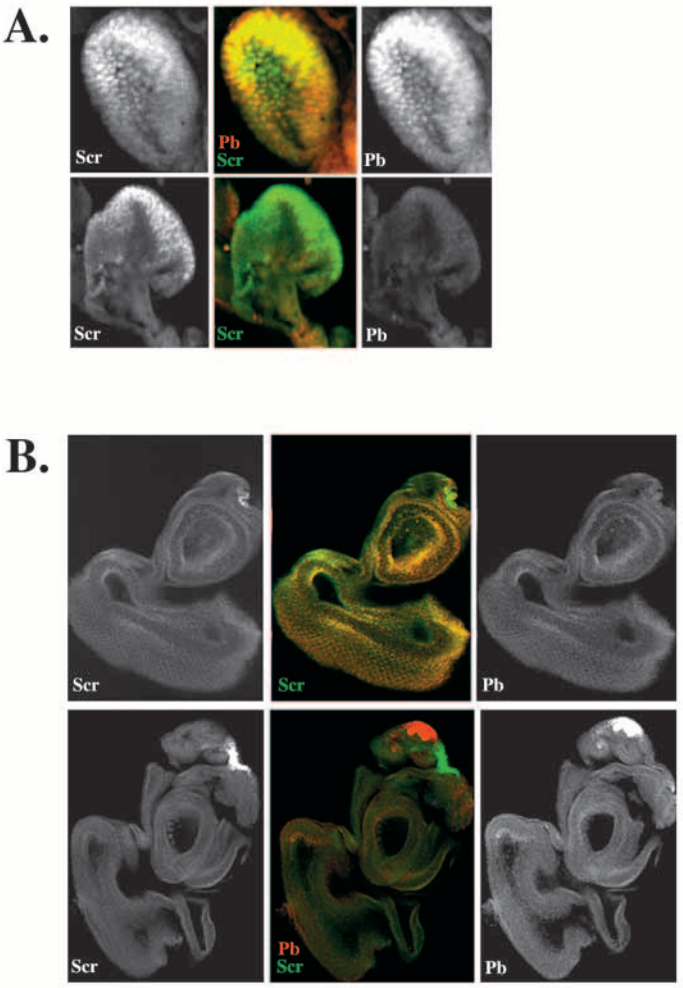

Fig. 7. Ectopic expression of *wingless* represses the expression of *Scr* in labial discs and induces *pb* expression in antennal discs. (A) Top panels, labial discs from control, 25°C *ptc*Gal4;UAS-*wg*^{ts} animals stained with antibodies specific for Scr or Pb. Bottom panels, labial discs from 15°C *ptc*Gal4;UAS-*wg*^{ts} animals. The side panels show the pattern of expression of each protein alone, and the middle panels are merged to show both expression patterns. In control discs, nearly all of the cells of the discs express both Scr (green) and Pb (red) proteins (yellow color in cells expressing both proteins). In discs from *ptc*Gal4;UAS-*wg*^{ts} animals raised at 15°C however, *pb* expression is repressed. (B) Eye-antennal discs from control (top) and 15°C *ptc*Gal4;UAS-*wg*^{ts} (bottom) animals, stained for Scr and Pb proteins. Middle panel of both top and bottom rows shows a merged image of the respective side panels, to show expression of both Scr (green) and Pb (red). In the 15°C *ptc*Gal4;UAS-*wg*^{ts} discs, *pb* is ectopically expressed while the expression of *Scr* is unchanged. Discs are magnified 200×.

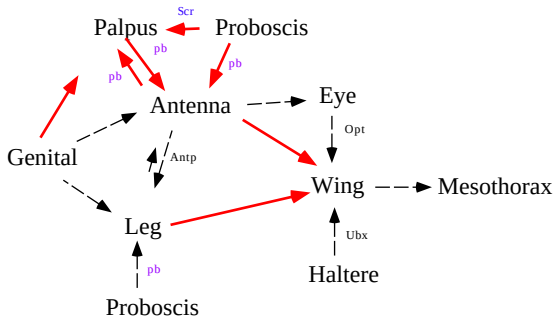

Fig. 8. Transdetermination directions and homeotic counterparts. Transdeterminations between different adult appendages occur with directionality and specific sequence (indicated by arrows; after model by Kauffman et al., 1978). Red arrows are those that are affected by *wg*. Homeotic mutations that correspond to transdetermination events are indicated beside the appropriate arrows. *pb*, *proboscipedia*; *Scr*, *Sex combs reduced*; *Antp*, *Antennapedia*; *Ubx*, *Ultrabithorax*; *Opt*, *Ophthalmoptera*. Colored abbreviations for homeotics designate those affected by *wg*.

determination may result from a 'mistake' during the regeneration process (Hadorn, 1978). Our studies indicating that transdetermination does not occur in the absence of cell proliferation (i.e., when *dpp* expression is inhibited) are consistent with this view. Clearly, in order to make the transdetermined structures (for example, the large areas of wing cuticle in the head and legs), extra cell proliferation is needed. However, 100% of both ventral and dorsal discs show a *dpp*-dependent local stimulation of growth, but transdetermination only occurs in a fraction of ventral discs, and in no dorsal discs. If transdetermination results from loss of information between mother and daughter during cell division, it might be expected that at some frequency this would also occur in dorsal discs.

An alternative explanation is that transdetermination mimics an earlier developmental program. For example, *pb* is expressed in the embryonic maxillary segment, but not in the antennal disc, which is partially derived from the maxillary segment (Jürgens and Hartenstein, 1993). Expression of *wg* in *dpp*-expressing cells that give rise to the maxillary palp causes those cells to induce expression of *pb*, and results in transdetermination of antenna to maxillary palp. Induction of *pb* occurs only in the presumptive maxillary palp of the antennal disc, arguing that this transdetermination may be a recapitulation of an embryonic state.

Wg is a morphogen that specifies ventral fates in the leg and antennal discs

We have shown that both ventralization and transdetermination of dorsal cells in discs occurs with a dose-response to Wg, thus Wg fulfills the definition of a morphogen. Our observations therefore support the hypothesis that, in the ventral discs such as the leg and antenna, Wg functions in a gradient to produce the complete range of ventral and ventral-lateral structures (Struhl and Basler, 1993). We propose that, in normal disc development, the high level of Dpp in dorsal cells antagonizes Wg activity and restricts its function to ventral cells. High concentrations of ventral Dpp represses *wg* expression (Morimura et al., 1996), thus Wg and Dpp appear to regulate each other and plausibly organize the dorsal/ventral boundary in the disc through this cross-regulation. Normally *dpp* is expressed in a subset of ventral leg and antennal disc cells that also express *wg*. Our results are consistent with the idea that the level of ventral *dpp* expression in leg (Held et al., 1994) and antennal (L. Johnston, unpublished observation) discs may be kept low by *wg*. Low levels of *wg* and *dpp* appear to be compatible in ventral cells as opposed to dorsal cells, perhaps via some intrinsic factor. Cross regulatory interactions between Wg and Dpp may also explain why animals lacking *dpp*

function duplicate ventral structures, and those lacking *wg* function duplicate dorsal structures (Held et al., 1994). In the former, we would predict that *wg* expression is induced in dorsal cells and, in the latter, *dpp* expression induced in ventral cells. Consistent with this idea, dorsally located clones of cells that lack *thickvein* (*tkv*), a *dpp* receptor (Nellen et al., 1994; Penton et al., 1994), induce expression of *wg* (Penton and Hoffmann, 1996).

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