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Recruitment of cells into the *Drosophila* wing primordium by a feed-forward circuit of *vestigial* autoregulation

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The *Drosophila* wing primordium is defined by expression of the selector gene *vestigial* (*vg*) in a discrete subpopulation of cells within the wing imaginal disc. Following the early segregation of the disc into dorsal (D) and ventral (V) compartments, *vg* expression is governed by signals generated along the boundary between the two compartments. Short-range DSL (Delta/Serrate/LAG-2)-Notch signaling between D and V cells drives *vg* expression in 'border' cells that flank the boundary. It also induces these same cells to secrete the long-range morphogen Wingless (Wg), which drives *vg* expression in surrounding cells up to 25-30 cell diameters away. Here, we show that Wg signaling is not sufficient to activate *vg* expression away from the D-V boundary. Instead, Wg must act in combination with a short-range signal produced by cells that already express *vg*. We present evidence that this *vg*-dependent, *vg*-inducing signal feeds forward from one cell to the next to entrain surrounding cells to join the growing wing primordium in response to Wg. We propose that Wg promotes the expansion of the wing primordium following the D-V segregation by fueling this non-autonomous autoregulatory mechanism.

KEY WORDS: *Drosophila* wing, Morphogen, Organ growth, Selector gene, Vestigial, Wingless signaling

INTRODUCTION

All animals are composed of distinct body parts and organs, many of which are specified by particular combinations of 'selector' genes that control cell and tissue behavior by regulating downstream 'realisator' genes (Garcia-Bellido, 1975). A central problem in animal development has been to determine how selector genes come to be active in the appropriate primordia, and how their activities govern the growth, size and pattern of the body parts they specify.

The first selector genes identified were those that specify developmental compartments in *Drosophila* (Garcia-Bellido, 1975; Blair, 1995). These include the Hox genes of the Bithorax complex, which control segment type, as well as the *engrailed* (*en*) and *apterous* (*ap*) genes, which govern the anterior-posterior (A-P) and dorsal-ventral (D-V) compartments within segments (Garcia-Bellido et al., 1973; Morata and Lawrence, 1975; Diaz-Benjumea and Cohen, 1993; Blair et al., 1994). All of these selector genes are initially activated in small, discrete groups of founder cells, and their states of expression, whether 'on' or 'off', are then stably inherited in descendent cells that constitute the compartment.

Despite the importance of compartment-specific selector genes, it is now apparent that they constitute a special class, and that most other selector genes are expressed in discrete 'organ' domains that are defined by active signaling rather than by epigenetic inheritance (Mann and Morata, 2000). For example, much of the fuselage of the adult *Drosophila* thorax as well as the wing derives from a single 'wing' imaginal disc. During development, this disc is subdivided into several sub-domains of organ-specific selector gene expression, each controlling a different portion of the adult structure: *vestigial* (*vg*) specifies the wing, *homothorax* (*hth*) specifies the hinge, and *teashirt* (*tsh*), *pannier* (*pnr*) and genes of the Iroquois (Iro-C) complex specify the body wall (notum), as well as distinct portions therein (Williams et al., 1991; Williams et al., 1993; Kim et al., 1996; Diez del Corral et al., 1999; Azpiazu and Morata, 2000; Calleja et al., 2000; Casares and Mann, 2000; Cavodeassi et al., 2000). For all these selector genes, the growth, size and shape of the organs they specify depend on the signaling mechanisms that control the expansion of the cell populations in which they are expressed. Here, we focus on how the morphogen Wingless (Wg) promotes the expansion of the population of *vg*-expressing cells that comprise the developing wing.

The *vg* gene is first activated in a small cluster of embryonic cells, defining the nascent wing imaginal disc, and is then expressed in a central portion of the disc during early larval life until the disc is segregated into dorsal (D) and ventral (V) compartments by the heritable activation of *ap* in D compartment cells (Williams et al., 1993; Wu and Cohen, 2002). Following the D-V segregation, cells in both compartments are programmed to send short-range Delta/Serrate/LAG-2 (DSL) signals across the compartment boundary (reviewed in Blair, 1995; Irvine and Rauskolb, 2001), activating the receptor Notch and inducing *vg* expression in a thin stripe of 'border' cells flanking the boundary (Williams et al., 1994; Couso et al., 1995; Kim et al., 1995; Neumann and Cohen, 1996; Kim et al., 1997). DSL-Notch signaling also induces border cells to secrete Wg (Diaz-Benjumea and Cohen, 1995; Rulifson and Blair, 1995; de Celis et al., 1996), which acts at long range to drive *vg* expression in surrounding cells (Zecca et al., 1996; Neumann and Cohen, 1997). Decapentaplegic (Dpp), secreted by A compartment cells along the A-P compartment boundary, also upregulates *vg* away from the D-V compartment boundary (Kim et al., 1996; Kim et al., 1997; Guss et al., 2001), centering a rapidly expanding population of *vg*-expressing cells on the intersection between the D-V and A-P boundaries.

Although generally accepted, the well-defined roles of DSL-Notch, Wg and Dpp signaling in wing development present a paradox: Wg and Dpp signaling coincide in many different contexts during *Drosophila* development, yet they only induce cells to express *vg* in the wing disc. Hence, it appears that Wg and Dpp can only recruit cells to express *vg* if they are already defined as prospective wing, a state that should itself depend on pre-existing

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Vg activity (Halder et al., 1998; Klein and Martinez-Arias, 1998; Guss et al., 2001; Curtiss et al., 2002). This paradoxical requirement suggests a crucial, but as yet unresolved, role for *vg* in controlling its own expression in response to Wg and Dpp.

Here, and in the accompanying paper (Zecca and Struhl, 2007), we provide evidence that Wg promotes the rapid expansion of the wing primordium following the D-V segregation by fueling a non-autonomous circuit of *vg* autoregulation. In this first paper, we show that *vg*-expressing cells send a short-range feed-forward signal that is required to entrain neighboring cells to upregulate *vg* in response to Wg. We also show that this process can reiterate from one cell to the next, propagating the recruitment of surrounding cells into the wing primordium. In the second paper, we show that the quadrant enhancer (*QE*) of the *vg* gene mediates this autoregulatory response and that activity of this enhancer is required for normal wing growth.

MATERIALS AND METHODS

Mutant alleles

ap^{56f}, *wg*^{CX4}, *vg*^{83b27R} and *vg*^{83b27} (see http://flybase.bio.indiana.edu/).

Previously described transgenes

Tubα1-Gal4, *UAS-GFPnls* (Struhl and Greenwald, 2001); *Tubα1-Gal80* (Lee and Luo, 2001); *C765-Gal4*, *UAS>CD2,y*$^{+}$*>Nrt-flu-wg*, *UAS>CD2,y*$^{+}$*>flu-Δarm*, *UAS-flu-wg* (Zecca et al., 1996); *UAS>CD2,y+>N*^{intra} (Struhl and Adachi, 1998), *UAS-N*^{ECN} (Struhl and Greenwald, 2001); *Tubα1>flu-GFP,y*$^{+}$*>Gal4*, *Tubα1>Gal80,y*$^{+}$*>Gal4* (Zecca and Struhl, 2002a; Zecca and Struhl, 2002b), *UAS-wg* (Struhl and Basler, 1993); *UAS-vg*, *1XQE-lacZ* (Kim et al., 1996), *BE-lacZ* (Williams et al., 1994), *rn-lacZ* (*rn*^{89}) (Couso and Bishop, 1998), *wg-lacZ*; *dpp-Gal4* (Wilder and Perrimon, 1995), *Dll-lacZ* (*Dll*^{01092}) (Spradling et al., 1995).

New transgenes

The *UAS>CD2,y*$^{+}$*>vg*, *Tubα1>CD2,y*$^{+}$*>Nrt-flu-wg*, *Tubα1>DsRed,y2>vg*, *Tubα1>flu-GFP,y*$^{+}$*>vg*, *BE-vg*^{GFP}, *5XQE-DsRed* and *Tubα1-DsRed* transgenes were assembled using the following DNAs: *UAS* promoter (Brand and Perrimon, 1993); *Tubα1* promoter, *Tubα1* 3′ UTR (Greenwood and Struhl, 1997; Casali and Struhl, 2004); *vg QE* enhancer/promoter (Kim et al., 1996), *vg BE* enhancer/promoter (Williams et al., 1994), *DsRed* coding sequence (Bevis and Glick, 2002), *vg* coding sequence (Kim et al., 1996); *Nrt-flu-wg* coding sequence (Zecca et al., 1996) and *flu-GFP* coding sequence (Zecca and Struhl, 2002b). The *>flu-GFP,y+>* and *>DsRed,y2>* Flp-out cassettes are derivatives of the *>CD2,y+>* cassette (Zecca et al., 1996) containing either the *flu-GFP* or *DsRed* coding sequences instead of CD2, respectively, and, in the case of the *>DsRed,y2>* cassette, a truncated form of the *y+* genomic fragment yielding a '*y2*' phenotype in *y* flies.

To generate the *BE-vg*^{GFP} transgene, the 750 bp *Eco*RI fragment defining the 'minimal' *vg BE* (Williams et al., 1994) was inserted in (–750 to –1) orientation upstream of the minimal *Hsp70*-promotor (Lis et al., 1983) and the coding sequence for *vg*^{GFP}. The Vg^{GFP} chimera consists of the first 20 amino acids of Vg joined via one copy of the flu-tag to GFP, followed by two flu-tags, joined to the rest of Vg at amino acid 25. The N- and C-terminal joints to Vg are, respectively, <u>PYLYGR</u>/GSYPYDVPDYA and YAGPYDVPDYags/<u>RSFYQYE</u> (Vg sequences are underlined and slashes highlight the joints).

To generate the *5XQE* promoter, the main portion of the 750 bp *BE* (–750 to –138) within the *BE-vg*^{GFP} transgene was replaced with five copies of the 806 bp *QE* fragment of the *vg* gene (Kim et al., 1996), arrayed each in (–806 to –1) orientation, while the *vg*^{GFP} coding sequence was replaced with that of *DsRed*. The remaining 137 bp fragment of the original *BE* sequence includes a Su(H)-binding site that is essential within the context of the intact 750 bp *BE* for its activity in D-V border cells. However, the presence of this binding site is not sufficient, within the context of the *5XQE-DsRed* transgene [and related *5XQE* transgenes reported in the accompanying study (Zecca and Struhl, 2007)], to drive detectable marker gene expression within boundary cells, whether within the wing pouch, or in the presumptive hinge or notum primordia (e.g. Fig. 1B,C). In addition, *5XQE* reporter derivatives in which this Su(H)-binding site is inactivated as in Kim et al. (Kim et al., 1996) or deleted, generate a pattern of expression that is indistinguishable from *5XQE* reporter transgenes in which the site remains intact (as in Fig. 1B,C).

Generation and analysis of clones

Flp-out (Struhl and Basler, 1993; Zecca et al., 1996), Gal4/UAS (Brand and Perrimon, 1993) and MARCM (Lee and Luo, 2001) techniques were used to manipulate gene activity in marked clones of cells or entire discs. To obtain clones ectopically expressing two coding sequences, we used the *Tubα1>flu-*GFP,*y*$^{+}$*>Gal4* transgene to activate gene expression of two *UAS-* transgenes within the same cells (e.g. *UAS-vg* and *UAS-wg*; as in Fig. 7A,B) or, alternatively, a Flp-out transgene generating clones of cells expressing one gene under *Tubα1*-control in discs that express the other gene under *C765-Gal4/UAS* control (e.g. *Tubα1>vg* clones in *C765-Gal4/UAS-Nrt-wg* discs; as in Fig. 4B). To obtain discs containing clones ectopically expressing one coding sequence adjacent to clones or tissue ectopically expressing a second, we employed various permutations of the Flp-out and *Gal4/UAS* methods (e.g. *Tubα1>vg* Flp-out clones next to *UAS>Nrt-wg* Flp-out clones in *C765-Gal4* discs; as in Fig. 5). Finally, the MARCM technique was used to generate *wg*^{cx4} mutant clones ectopically expressing one or more coding sequences (e.g. *UAS-vg* plus *UAS-Nrt-wg* in Fig. 3E).

Clones were generated by heat shock-induced Flp recombinase as described previously (e.g. Zecca and Struhl, 2002b). Unless otherwise stated, clones were induced during the first larval instar (24-48 hours after egg laying), prior to when the D-V compartmental segregation normally occurs (mid- to late second instar), and mature wing discs dissected, fixed and analyzed at the end of the third larval instar using standard protocols (e.g. Zecca and Struhl, 2002b). Antisera employed: anti-Wg (Brook and Cohen, 1996), anti-Vg (Williams et al., 1991), anti-Flu (Roche), anti-β-gal (Cappel) and anti-CD2 (OX34, Serotec).

Genotypes

Genotypes are listed below by figure panel; except where stated otherwise, the X chromosome was *y w Hsp70-flp*.

1E: *1XQE-lacZ ap*^{56f}*/1XQE-lacZ ap*^{56f}.

1F: *y w Hsp70-flp UAS-GFPnls/y w Hsp70-flp; 5XQE-DsRed ap*^{56f} *vg*^{83b27R}*/ap*^{56f} *UAS-N*^{ECN}*; Tubα1>Gal80,y*$^{+}$*>Gal4/rn-lacZ* (no heat shock treatment).

1G: *y w 5XQE-DsRed/y w Hsp70-flp; UAS-Nrt-flu-wg ap*^{56f}*/ap*^{56f}; *C765-Gal4/+*.

1H: *UAS-Nrt-flu-wg ap*^{56f}*/1XQE-lacZ ap*^{56f}*; C765-Gal4/BE-vg*^{GFP}.

2A: *1XQE-lacZ ap*^{56f}*/ap*^{56f}; *Tubα1>Gal80,y*$^{+}$*>Gal4/UAS>CD2,y*$^{+}$*>N*^{intra}.

2B: *y w Hsp70-flp UAS-GFPnls/y w Hsp70-flp; 5XQE-DsRed ap*^{56f} *vg*^{83b27R}*/ap*^{56f} *UAS-N*^{ECN}*; Tubα1>Gal80,y*$^{+}$*>Gal4/rn-lacZ.*

2C,D: *ap*^{56f} *UAS-N*^{ECN}*/1XQE-lacZ ap*^{56f}*; Tubα1>Gal80,y*$^{+}$*>Gal4/BE-vg*^{GFP}.

2E: *5XQE-DsRed ap*^{56f} *vg*^{83b27R}*/ap*^{56f} *UAS-N*^{ECN}*; Tubα1>flu-GFP,y*$^{+}$*> Gal4/BE-lacZ.*

3A: *y w Hsp70-flp Tubα1-Gal4 UAS-GFPnls/y w Hsp70-flp; Tubα1-Gal80 FRT39 ap*^{56f}*/FRT39 ap*^{56f}*; UAS-vg/1XQE-lacZ.*

3B: *wg-lacZ ap*^{56f}*/ap*^{56f} *UAS-vg; Tubα1>flu-GFP,y*$^{+}$*>Gal4/+.*

3C: *y w Hsp70-flp Tubα1-Gal4 UAS-GFPnls/y w Hsp70-flp; Tubα1-Gal80 FRT39 ap*^{56f}*/wg*^{cx4} *FRT39 ap*^{56f}*; UAS-vg/1XQE-lacZ.*

3D: *y w Hsp70-flp Tubα1-Gal4 UAS-GFPnls/y w Hsp70-flp; Tubα1-Gal80 FRT39 ap*^{56f}*/wg*^{cx4} *FRT39 ap*^{56f}*; UAS-wg UAS-vg/1XQE-lacZ.*

3E: *y w Hsp70-flp Tubα1-Gal4 UAS-GFPnls/y w Hsp70-flp; Tubα1-Gal80 FRT39 ap*^{56f}*/wg*^{cx4} *FRT39 ap*^{56f}*; UAS-Nrt-flu-wg UAS-vg/1XQE-lacZ.*

4A: *1XQE-lacZ ap*^{56f} *vg*^{83b27}*/1XQE-lacZ ap*^{56f}*; Tubα1>flu-GFP,y*$^{+}$*>vg UAS-Nrt-flu-wg/+.*

4B: *1XQE-lacZ ap*^{56f} *vg*^{83b27}*/ap*^{56f}*; Tubα1>flu-GFP,y*$^{+}$*>vg UAS-Nrt-flu-wg/C765-Gal4.*

5: *UAS>CD2,y*$^{+}$*>Nrt-flu-wg ap*^{56f}*/1XQE-lacZ ap*^{56f}*; Tubα1>DsRed,y*^{2}*> vg/C765-Gal4.*

6: *1XQE-lacZ ap*^{56f}*/1XQE-lacZ ap*^{56f}*; Tubα1>DsRed,y*^{2}*>vg C765-Gal4/UAS>CD2,y*$^{+}$*>flu-Δarm.*

7A: *UAS-vg/+; Tubα1>flu-GFP,y*$^{+}$*>Gal4 UAS-wg/+.*

7B: *y w 5XQE-DsRed/y w Hsp70-flp; UAS-vg/vg*^{83b27R}*; Tubα1>flu-GFP,y*$^{+}$*>Gal4 UAS-wg/rn-lacZ.*

7D-F: *Tubα1>CD2,y⁺>Nrt-flu-wg/1XQE-lacZ; dpp-Gal4/UAS-vg.*
7G: *UAS>CD2,y⁺>Nrt-flu-wg/+; UAS>CD2,y⁺>vg/C765-Gal4.*
7H: *UAS>CD2,y⁺>flu-Δarm/+; UAS>CD2,y⁺>vg/C765-Gal4.*

RESULTS

Control of *vg* expression by the boundary and quadrant enhancers

Following segregation of the wing disc into D and V compartments, *vg* expression is driven by DSL-Notch signals received by border cells flanking the D-V boundary, and by Wg and Dpp received by neighboring cells in the wing pouch, the central portion of the disc that normally gives rise to the adult wing (reviewed by Blair, 1995; Irvine and Rauskolb, 2001). The 'border' and 'pouch' expression domains are mediated by distinct boundary and quadrant enhancers (*BE* and *QE*) (Williams et al., 1994; Kim et al., 1996). Here, and in the accompanying paper (Zecca and Struhl, 2007), we monitor the activities of these enhancers by assaying transgenes that express reporter proteins such as β-gal, rat CD2 and DsRed, or a functional Vg-GFP chimeric protein, under their control (Fig. 1A-D). In some experiments, we assay *QE* activity using transgenes containing a tandem array of five quadrant enhancers; these *5XQE* reporters appear to provide a more sensitive and faithful indicator of Wg- and Vg-dependent pouch expression than the more standard *1XQE* reporters (Fig. 1B,C) (Zecca and Struhl, 2007).

Wg signaling is necessary but not sufficient to activate the quadrant enhancer

In the generally accepted model of wing development, Wg emanating from D-V border cells drives *vg* expression in the surrounding pouch cells via activation of the *QE*. Accordingly, if the D-V segregation is blocked, as in *ap* mutant (*ap⁰*) discs, border cells are not specified and the early expression of *vg* that normally precedes the D-V segregation and defines the nascent wing pouch rapidly dissipates (Williams et al., 1993) (Fig. 1E; data not shown). In mature, third instar discs, the wing pouch is normally surrounded by the inner of two rings of Wg expression (e.g. Fig. 1A,B) in the prospective wing hinge; in *ap⁰* discs, the pouch is absent and the inner ring reduced to a cluster of cells (e.g. Fig. 1E,F).

In agreement with the model, clones of cells that express Notch^{intra} (N^{intra}) or Notch^{ECN} (N^{ECN}) – constitutively active forms of Notch that are under *Gal4/UAS* control (Struhl and Greenwald, 2001) (henceforth *UAS-N** clones) – in *ap⁰* discs behaved as ectopic border cells and rescued wing growth even when generated up to 48 hours after the time at which D-V segregation would normally occur (Fig. 2). This rescuing activity was associated with the autonomous activation of *wg*, *vg* and *BE* reporter gene expression within the clone and, more strikingly, with the long-range non-autonomous induction of *vg* and *QE* reporter gene expression in surrounding cells (Fig. 2). As in wild-type discs (Fig. 1B), the restored *vg* and *QE* expression did not extend all the way to the inner ring of Wg expression, but instead was separated from it by a zone of cells distinguished by expression of the transcription factor gene *rotund* (*rn*), without detectable *vg* (the '*rn*-only' domain; Fig. 1B, Fig. 2B).

In conflict with the model, supplying ectopic Wg to *ap⁰* discs, whether by making *UAS-wg* clones or by ubiquitously expressing *UAS-wg* throughout, failed to rescue the expression of *vg*, *QE* reporter genes, or other 'pouch genes' such as *Distal-less* (Fig. 1G,H; data not shown) (see also Klein and Martinez-Arias, 1998; Klein and Martinez-Arias, 1999). Hence, Wg is not sufficient to activate *QE*-dependent *vg* expression or to rescue wing development in the absence of D-V border cells.

Non-autonomous activation of the quadrant enhancer in response to ectopic Vg-expressing cells and Wg

The contrasting results obtained with *UAS-N** and *UAS-wg* clones suggests that, in *ap⁰* discs, cells might need to express *vg* to be able to induce neighboring cells to upregulate *vg* expression. In support, we found that *UAS-vg* clones mimic the effects of *UAS-N** clones: when generated in *ap⁰* discs, they were able to induce

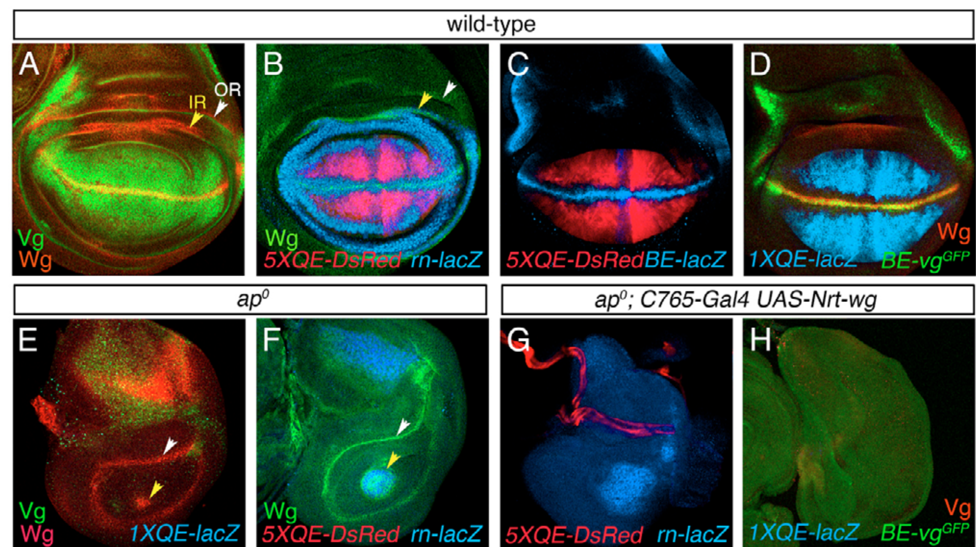

Fig. 1. Wg is not sufficient to activate *vg* in *ap⁰* wing discs. (**A-D**) Wild-type *Drosophila* discs. Vg protein (green in A) is expressed in D-V 'border' cells (yellow in A owing to co-expression with Wg, red) as are *BE* reporter genes (*BE-lacZ*, blue in C; *BE-vg^{GFP}*, green in D). Vg is also expressed in surrounding cells of the 'pouch' and wing primordium, as are *QE* reporter genes (*5XQE-DsRed*, red in B,C; *1XQE-lacZ*, blue in D). Wg (red in A,D, green in B) is expressed in border cells within the pouch, and in an inner and outer ring (IR, yellow arrowhead; OR, white arrowhead) in the surrounding hinge primordium. *rn-lacZ* (blue in B) is expressed throughout the pouch and in the surrounding '*rn*-only' territory extending up to the inner ring of Wg. (**E-H**) *ap⁰* discs. Neither Vg (green in E, red in H), nor *BE-vg^{GFP}* (green in H), nor *QE* reporters (blue in E,H, red in F,G) are expressed, and the pouch is either absent or present only as a small cluster of *rn*-only cells (blue in F) surrounded by the inner ring of Wg (red in E, green in F). Uniform ectopic expression of *UAS-Nrt-wg* under *C765-Gal4* control does not rescue expression of either *QE* reporter gene, endogenous *vg* expression or wing pouch development (G,H; red stain in G is non-specific tracheal staining). Here, and in the remaining figures, all discs are from mature third instar larvae, anterior is left, dorsal is up, protein or reporter gene stains are indicated by color and relevant genotypes are indicated above each image.

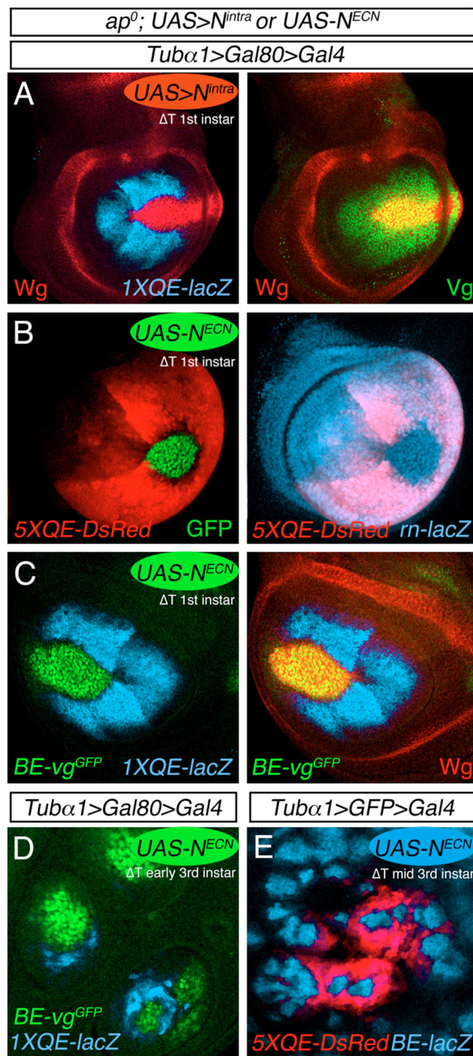

Fig. 2. Rescue of *Drosophila* wing development and *vg* reporter expression in ap^0 discs by *UAS-N*^{intra} and *UAS-N*^{ECN} clones. *Tubα1-Gal4/UAS-N*^{intra} (**A**) and *Tubα1-Gal4/UAS-N*^{ECN} (**B-E**) clones monitored by autonomous expression of Wg (red in A,C), *UAS-GFP* (green in B), *BE-vg*^{GFP} (green in C,D), or *BE-lacZ* (blue in E), induce expression of both *QE* reporter genes (*1XQE-lacZ*, blue in A,C; *5XQE-DsRed*, red in B,E), as well as *rn-lacZ* (blue in B) in surrounding cells (note that *rn-lacZ* expression extends beyond that of *5XQE-DsRed*). Restored growth of the wing pouch and surrounding *rn*-only territory is indicated by the greatly expanded inner ring of Wg expression (A,C; compare with Fig. 1E,F). Clones induced in early (D) or mid- (E) third instar also induce non-autonomous *QE* reporter expression, although the range of the response is greater for clones induced during the first instar (A-C). Here, and in the remaining figures, clones were induced during the first larval instar (except for D,E), and the clone genotypes are indicated by colored ovals (representing presence of marker expression within the respective clones), as indicated in each experiment.

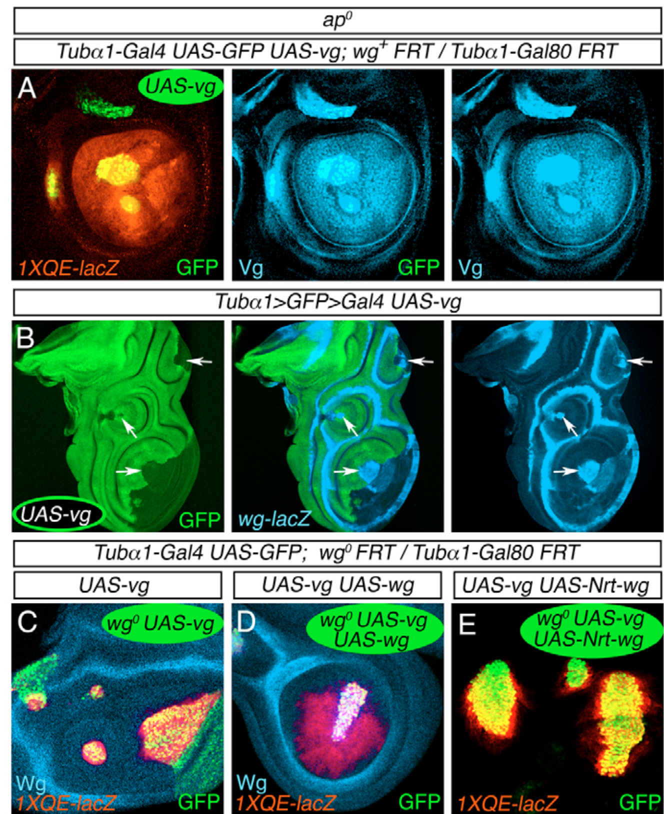

Fig. 3. Rescue of *Drosophila* wing development and *QE* reporter expression by *UAS-vg* clones. (**A-E**) *Tubα1-Gal4/UAS-vg* clones [marked by *UAS-GFP* (green in A,C-E), or the absence of GFP (black in B); clone genotype indicated by colored outline with black filling representing absence of marker expression within the clone] induce Vg (blue in A) and *1XQE-lacZ* (red in A,C-E) in surrounding cells in ap^0 discs. *Tubα1-Gal4/UAS-vg* clones ectopically express *wg* (*wg-lacZ*, blue in B, white arrows) and their ability to induce *1XQE-lacZ* in surrounding cells depends on the Wg they secrete. *Tub-Gal4/UAS-vg* clones that are also wg^0 fail to do so (C), unless they co-express exogenous Wg (D), or membrane-tethered Wg (Nrt-Wg, E), in which case they do so at long range, or only in adjacent cells, respectively. Coincident *GFP* and *1XQE-lacZ* expression appear yellow in C,E, and white when co-stained for Wg (blue) in D.

surrounding cells up to several cell diameters away to express endogenous *vg* as well as *QE* reporters and to develop as wing cells (Fig. 3A).

ap^0 discs lack border cells and hence the normal source of Wg, posing the question of how *UAS-vg* clones induce surrounding cells within such discs to activate the *QE*. *UAS-vg* clones express levels of exogenous Vg that greatly exceed the peak levels normally expressed in the developing wing (Fig. 3A; data not shown): such physiologically abnormal levels might artifactually activate *wg* expression, providing an ectopic source of Wg required for the non-autonomous activation of the *QE*.

To test this, we first asked whether *UAS-vg* clones in ap^0 discs ectopically express *wg*, as monitored by the expression of a *wg-lacZ* reporter gene. As shown in Fig. 3B, this was indeed found to be the case.

We next asked whether the long-range rescuing activity of such *UAS-vg* clones depends on their ability to ectopically express *wg*, using the MARCM technique (Lee and Luo, 2001) to make *UAS-vg* clones that are also wg^0. In contrast to *UAS-vg* clones that were otherwise wild-type (Fig. 3A), wg^0 *UAS-vg* clones in ap^0 discs failed to induce surrounding cells to express *vg* or the *1XQE-lacZ* reporter gene (Fig. 3C).

Finally, we asked whether the long-range rescuing activity of *UAS-vg* clones depends on the long-range action of the ectopic Wg they secrete. To do this, we generated wg^0 *UAS-vg* clones that co-

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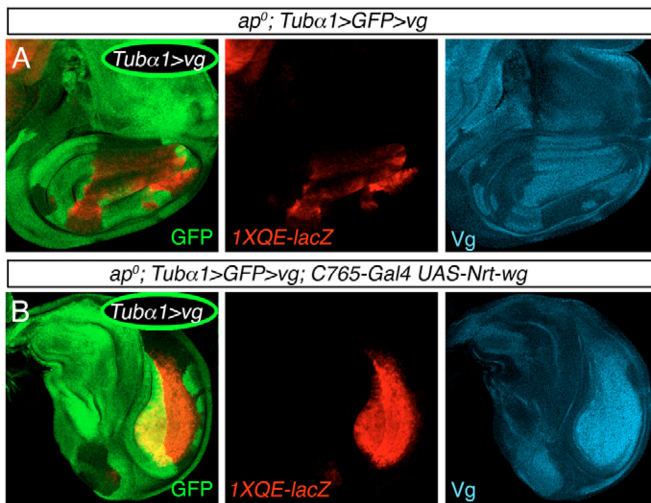

Fig. 4. Rescue of wing development and *QE* reporter expression by *Tubα1>vg* clones depends on Wg input. (**A**) *Tubα1>vg* clones (black by absence of GFP, green) in ap^0 discs express moderate levels of Vg (dull blue) and autonomously rescue *Drosophila* wing growth when located in the prospective wing pouch; *Tubα1>vg* cells within the rescued pouch also express low levels of the *1XQE reporter* (dull red). (**B**) *Tubα1>vg* clones in ap^0; *C765-Gal4/UAS-Nrt-wg* discs (which overexpress Nrt-Wg throughout the disc) autonomously express normal peak levels of Vg (bright blue) as well as *1XQE-lacZ* expression (bright red) within the prospective wing pouch and induce surrounding cells up to several cell diameters away to do the same (coincident *GFP* and *1XQE-lacZ* reporter expression in non-autonomously rescued cells appears yellow).

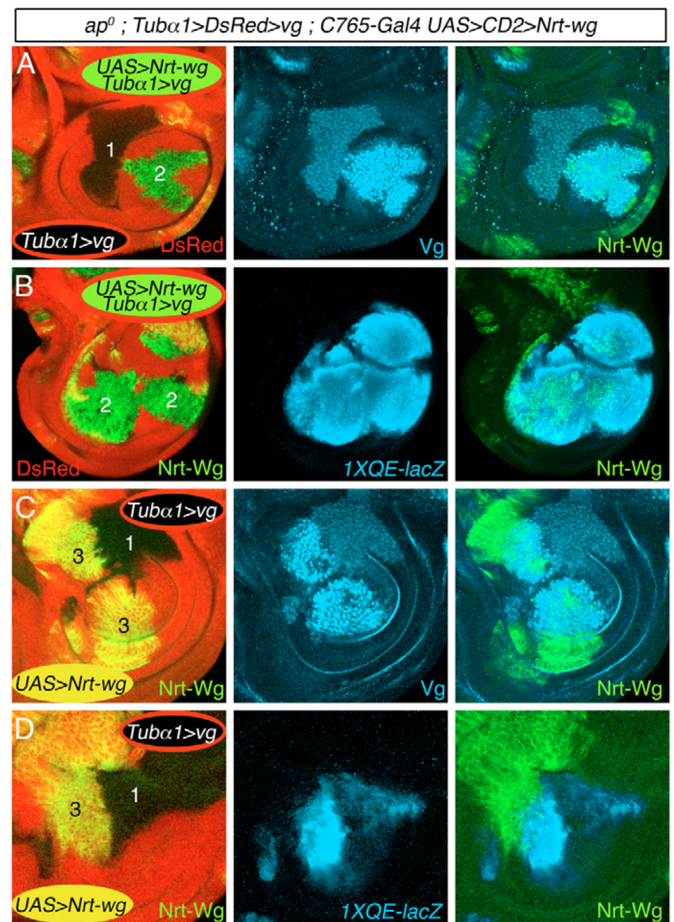

Fig. 5. *Tubα1>vg* clones act at short range to induce *vg* and *QE* reporter expression in neighboring *UAS-Nrt-wg* clones. (**A-D**) ap^0 discs containing *Tubα1>vg* clones (black by absence of DsRed, left column), *C765-Gal4/UAS-Nrt-wg* clones (yellow by coincident expression of Flu-tagged Nrt-Wg, green, and DsRed), and 'double' *Tubα1>vg C765-Gal4/UAS-Nrt-wg* clones (green by expression of Nrt-Wg in the absence of DsRed) are shown stained for Vg (A,C) or *1XQE-lacZ* (B,D) expression (blue). (A,B) Double clones (green) within the prospective *Drosophila* wing pouch (A,B, 2) show peak expression of Vg (bright blue) and *1XQE-lacZ* and induce peak expression in adjacent cells outside of the clone (note that the green territories are nested inside the larger bright blue territories, right column). *Tubα1>vg* clones (A, black, 1) show only moderate Vg expression (derived only from *Tubα1>vg*, dull blue). (C,D) *Tubα1>vg* clones (1, black) that abut *C765-Gal4/UAS-Nrt-wg* clones (3, yellow) induce cells in the latter to express peak levels of Vg and *1XQE-lacZ*. The induction is not limited to those *C765-Gal4/UAS-Nrt-wg* cells that abut the *Tubα1>vg* clone, but propagates over many cell diameters into the *C765-Gal4/UAS-Nrt-wg* clone. Vg and *1XQE-lacZ* expression are also upregulated in the *Tubα1>vg* cells that abut the *C765-Gal4/UAS-Nrt-wg* clone.

overexpress either a *UAS-wg* or *UAS-Neurotactin-wg* (*Nrt-wg*) transgene, the latter encoding a membrane-tethered immobile form of Wg (Zecca et al., 1996). Co-overexpression of *UAS-wg* restored the ability to induce *vg* and *QE* reporter gene expression in surrounding cells up to several cell diameters away (Fig. 3D); co-overexpression of *UAS-Nrt-wg* did so as well, but only at short range – in cells near, or next to the clone (Fig. 3E).

We conclude that Vg-overexpressing cells have the capacity to induce neighboring cells to activate *QE*-dependent *vg* expression, but only if the responding cells also receive Wg.

Moderate levels of ectopic Vg can act together with Wg to activate the quadrant enhancer in neighboring cells

The non-autonomous *vg*-inducing activity of *UAS-vg* clones might reflect either a normal role for *vg* in controlling its own expression in neighboring cells, or an artifactual outcome resulting from Vg overexpression within the clone. To distinguish between these possibilities, we made clones that express moderate levels of Vg in ap^0 discs, and asked whether they could induce neighboring cells to express *vg* in a Wg-dependent fashion.

Clones of cells that express *vg* under the direct control of the *Tubα1* (*αTub84B* – Flybase) promoter (henceforth *Tubα1>vg* clones) generated moderate levels of Vg that were 2- to 3-fold below the normal peak of endogenous Vg expression (Fig. 4, Fig. 5A,C, Fig. 6A), but still sufficient to rescue wing development within the clone in vg^0 wing discs (Zecca and Struhl, 2007).

Tubα1>vg clones also autonomously rescued wing development in ap^0 discs (Fig. 4A). However, they appeared to have little or no capacity to rescue expression of the endogenous *vg* gene (or that of *BE* or *QE* reporter genes), either inside or outside of the clones, except for a weak, cell-autonomous activation of *QE* reporter genes (Fig. 4A; data not shown). The failure of these clones to induce *vg* expression in surrounding cells correlates with the absence of *wg*-expressing border cells (normally specified by DSL-Notch signaling across the D-V compartment boundary, but absent from ap^0 discs).

By contrast, when we repeated this experiment in ap^0 discs that express *UAS-Nrt-wg* uniformly throughout, we found that *Tubα1>vg* clones that were located within the prospective wing

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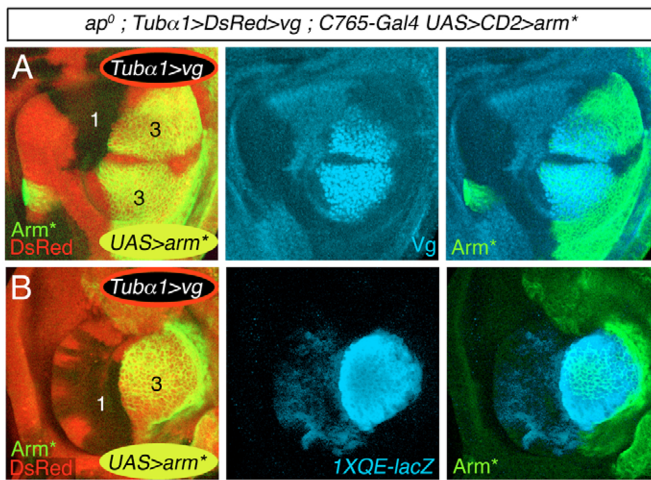

Fig. 6. *Tubα1>vg* clones act at short range to induce *vg* and *QE* reporter expression in neighboring *UAS-arm clones.** *ap⁰* discs containing *Tubα1>vg* clones (#1, black by absence of DsRed) that abut *C765-Gal4/UAS-arm** clones (#3, yellow by coincident expression of Flu-tagged Arm* and DsRed) are shown stained for Vg (**A**) or *1XQE-lacZ* (**B**) expression (blue). Peak levels of both responses propagate throughout the *C765-Gal4/UAS-arm** tissue located within the prospective *Drosophila* wing pouch, but not into neighboring cells outside of the clones (compare with the local non-autonomous induction of both responses by *C765-Gal4/UAS-Nrt-wg* clones in Fig. 5).

pouch were associated with robust activation of *QE* reporters as well as of the endogenous *vg* gene to normal levels (Fig. 4B). Significantly, this upregulation occurred not only within the clone, but also in surrounding cells up to many cell diameters away, providing a general rescue of wing development. By contrast, when we generated *Tubα1>vg* clones that co-express *UAS-Nrt-wg* in *ap⁰* discs, the upregulation of *QE*-dependent *vg* expression was restricted to cells within the clone and their immediate wild-type neighbors, as expected given the restricted range of membrane-tethered Nrt-Wg (Fig. 5A,B).

Thus, cells that express moderate levels of exogenous Vg, well within the normal physiological range, can drive *QE*-dependent *vg* expression in neighboring cells, provided that the responding cells also receive Wg signal.

Distinct Wg- and Vg-dependent signals act combinatorially to recruit prospective wing disc cells to the wing fate

The preceding experiments indicate that Wg is not sufficient to induce wing disc cells to express high levels of *vg* and develop as wing cells. Instead, Wg can only do so in the presence of cells that already express Vg. One explanation for this unexpected requirement is that Vg activity programs cells to make a second intercellular signal that is required in combination with Wg.

To test this, we analyzed *ap⁰* wing discs that contained neighboring *Tubα1>vg* and *UAS-Nrt-wg* clones. As described above, *ap⁰* discs that contain only one or the other type of clone do not express either *vg* or the *1XQE-lacZ* reporter (except for weak cell-autonomous expression of the reporter detected within *Tubα1>vg* clones). However, in discs that contain both types of clones, *Tubα1>vg* clones were able to induce cells within neighboring *UAS-Nrt-wg* clones to express peak levels of both *vg* and the *1XQE* reporter, provided that the two types of clones abutted and that the *UAS-Nrt-wg* cells were located in the prospective wing pouch (Fig. 5C,D). Importantly, neither output was limited to those *UAS-Nrt-wg* cells in direct contact with *Tubα1>vg* cells. Instead, *vg* and *QE* reporter gene expression typically propagated many cell diameters into the *UAS-Nrt-wg* clone and could extend to abutting wild-type neighbors on the opposite side of the clone (Fig. 5C,D). Similar results were obtained when we replaced clones expressing Nrt-Wg with clones expressing Arm*, a truncated form of the cytosolic protein Armadillo that constitutively activates the Wg transduction pathway (Zecca et al., 1996). *Tubα1>vg* clones were able to induce cells in neighboring *UAS-arm** clones to express *vg* and *QE* reporter genes, much as they do when they abut *UAS-Nrt-wg* clones, except that expression did not propagate further to adjacent wild-type cells (as expected, because these *UAS-arm** cells should not provide Wg signal, in contrast to *UAS-Nrt-wg* cells).

Thus, *Tubα1>vg* clones can induce neighboring *UAS-Nrt-wg* and *UAS-arm** cells to activate *QE*-driven expression of *vg*, and the effect can then propagate from one such cell to the next, presumably via reiterative cycles of short-range induction of endogenous *vg* by cells already expressing Vg. In both cases, we only observed induction when the two types of clones abutted, and the effect could only propagate in cells in which the Wg transduction pathway was active, whether in response to Wg signal or to constitutive activation of Arm. We conclude (1) that Vg-expressing cells send an independent signal that acts combinatorially with Wg to induce *QE*-dependent *vg* expression in neighboring cells; (2) that this *vg*-dependent *vg*-inducing signal has a range of only one, or very few, cell diameters, possibly because it is contact-mediated; and (3) that this signal is integrated with Wg downstream of the cytosolic activation of Arm.

Recruitment of notal cells to the wing fate by the combined action of Wg- and Vg-dependent signals

In the experiments described above, we have been able to substitute for the absence of border cells within the prospective wing pouch of *ap⁰* discs by generating clones of ectopic Vg- and Wg-expressing cells: such clones restore *QE*-dependent *vg* expression and rescue wing development. Here, we ask whether ectopic Vg- and Wg-expressing clones can activate the *QE* and induce the formation of ectopic wing primordia in more proximal portions of the wing disc that would normally give rise to the notum.

UAS-wg, *UAS-Nrt-wg* and *UAS-arm** clones in otherwise wild-type discs upregulate *vg* and *QE* reporter gene expression within the prospective wing pouch, but do not induce ectopic *vg* expression in the proximal hinge and notum primordia (data not shown) (Zecca et al., 1996; Klein and Martinez-Arias, 1998; Baena-Lopez and Garcia-Bellido, 2003). By contrast, most *UAS-vg* clones activated *QE* reporter gene expression in the hinge and notum primordia, but unlike *UAS-vg* clones in the prospective wing pouch (Fig. 2), they did not express *wg* ectopically and did not induce *QE*-dependent *vg* expression in surrounding cells (data not shown). Strikingly, *UAS-wg UAS-vg* clones that co-overexpressed Wg and Vg in these primordia were able to induce *QE*-dependent *vg* expression non-autonomously, seeding the formation of ectopic wing primordia (Fig. 7A,B) (see also Baena-Lopez and Garcia-Bellido, 2003). To assess whether this reflects a spatially inappropriate, but otherwise normal, response of wing disc cells to the combination of Wg- and Vg-dependent signals, we performed the following two sets of experiments.

First, we used *dpp-Gal4* to drive *UAS-vg* expression in a stripe of A compartment cells abutting the A-P boundary in the notum and asked whether these cells could induce *QE*-driven *vg* expression in neighboring P clones of *Tubα1>Nrt-wg* cells across the A-P

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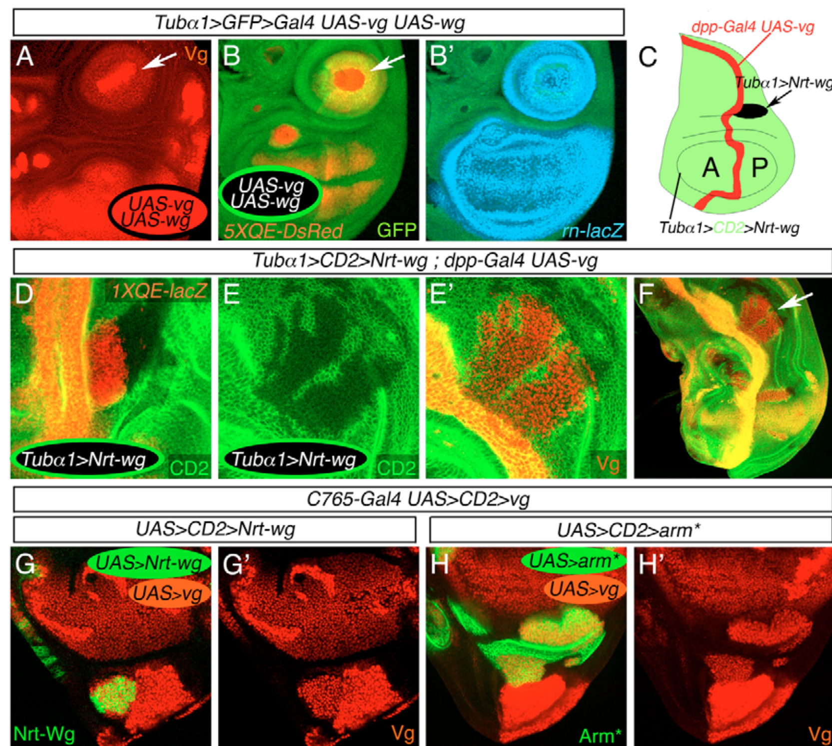

Fig. 7. Recruitment of presumptive notal cells to the wing fate by ectopic Wg- and Vg-expressing cells. (**A-B′**) Wild-type *Drosophila* discs containing *Tubα1>Gal4/UAS-vg UAS-wg* clones located within the presumptive notum, probed for Vg (A, red), *5XQE-DsRed* (B, red) and *rn-lacZ* (B′, blue) expression and marked by Vg overexpression (A, bright red) or loss of GFP (B). Arrows indicate clones that have induced 'ectopic' wing pouches; note that *rn-lacZ* expression (B′) extends beyond that of *5XQE-DsRed* (B), as in the normal pouch. (**C-F**) Discs that express *UAS-vg* in A compartment border cells under *dpp-Gal4* control [red by ectopic *1XQE-lacZ* (D) and Vg (E,F) expression] and contain P compartment clones of *Tubα1>Nrt-wg* cells (indicated by the arrow in C; black by the absence of CD2, green. The arrowed clone in F is shown at higher magnification in E,E′. Cells within the clones that are located within 10-20 cell diameters of the A-P boundary ectopically express normal peak levels of *1XQE-lacZ* (D) and endogenous Vg (E′) and induce immediately adjacent cells across the clone border to do the same (appear yellow in overlap with CD2). (**G-H′**) *UAS>Nrt-wg* (G,G′) or *UAS>arm** (H,H′) clones (marked green by Flu epitope staining of Nrt-Wg and Arm*) located within the presumptive notum ectopically express normal peak levels of endogenous Vg (dull red) when they abut *UAS>vg* clones (bright red by Vg overexpression) and are located within 10-20 cell diameters of the A-P boundary. The *UAS>Nrt-wg* clones, but not the *UAS>arm** clones, also induce their immediate neighbors to do the same.

boundary (Fig. 7C). Although such *Tubα1>Nrt-wg* clones expressed only moderate levels of Nrt-Wg and were normally unable to induce either *vg* or *QE* reporter gene expression in this portion of the disc, we found that they could do so if they were located next to the *UAS-vg*-expressing cells in the A compartment (Fig. 7D-F). Furthermore, *vg* and *QE* reporter gene expression could spread many cell diameters into the *Tubα1>Nrt-wg* clone, and extend outside of the clone to the adjacent wild-type cells (Fig. 7D-F). Propagation did not, however, extend more than ~20-25 cell diameters from the A-P boundary (Fig. 7D,E), possibly reflecting a requirement for Dpp secreted by A cells along the compartment boundary.

In the second set of experiments, we expressed *UAS-vg* in clones, rather than under *dpp-Gal4* control, and examined the response of *UAS-arm** as well as of *UAS-Nrt-wg* clones. We observed that *UAS-vg*-expressing clones can induce *UAS-Nrt-wg* as well as *UAS-arm** clones within the prospective notum to express *vg*, provided that they abut (Fig. 7G,H). Moreover, *vg* expression was able to spread through the responding clone, and even extended to neighboring cells outside in the case of *UAS-Nrt-wg* clones (but not *UAS-arm** clones).

Thus, Vg-overexpressing cells in the prospective notum can induce neighboring cells to express *vg*, provided that the latter also receive Wg input, and once initiated, *vg* expression can propagate to cells further away. Importantly, the levels of Vg expression and Wg signaling within such *Tubα1>Nrt-wg* clones fall within the physiological range, arguing that propagation is not an artifact of either Vg or Wg overexpression, but rather a normal signaling process operating in a spatially inappropriate context.

DISCUSSION

Evidence for a Wg-dependent feed-forward circuit of *vg* autoregulation

Following the D-V segregation, local DSL-Notch signaling across the compartment boundary induces the differentiation of specialized border cells that express *vg*, secrete Wg, and organize a dramatic ~200-fold expansion of the wing primordium. In ap^0 wing discs, D-V segregation fails to occur, border cells are not specified, and the early expression of *vg* that initially defined the wing primordium fades away. We have used this mutant condition to explore how *vg* and *wg* activity in border cells controls wing growth by asking what happens when we replace the missing border cells with cells that ectopically express Wg, Vg or both.

Our main finding is that Wg is not sufficient to sustain or induce *vg* expression in ap^0 discs, even when the morphogen is overexpressed, continuously, in all cells. Instead, Wg can only drive *vg* expression in these discs when the responding cells are near or

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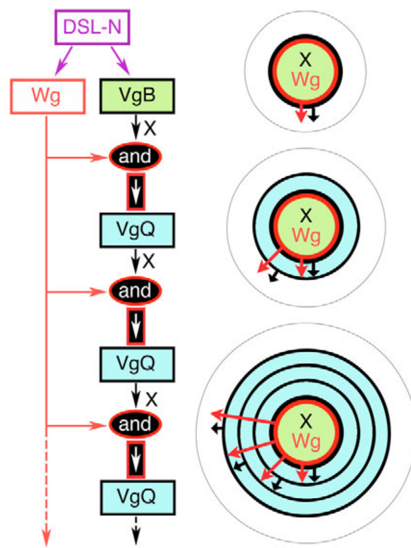

Fig. 8. The feed-forward circuit of Wg-dependent *vg* autoregulation in *Drosophila*. DSL-Notch signaling (purple) induces *wg* (red) and *BE*-dependent Vg (VgB, green) expression in border cells, causing them to send Wg (red arrows), as well as the short-range feed-forward signal, 'X' (black arrows). X entrains neighboring cells to activate *QE*-dependent Vg expression (VgQ, blue) in response to Wg, and these cells become a new source for X so that the process reiterates, propagating recruitment of surrounding cells into the growing wing primordium (as illustrated on the right). Continuous exposure to X (short black arrows on right), together with Wg, may also be required to sustain *QE*-dependent *vg* expression in prospective wing cells once they are recruited. This circuitry is integrated with other signaling processes, including the stimulation of growth of the surrounding cell population (white), from which *vg*-expressing cells are recruited, to control the expansion in wing size as discussed in the text and the accompanying paper (Zecca and Struhl, 2007).

next to cells that express exogenous Vg. The clearest demonstration of this is the experiment in which we generated two types of clones in the same ap^0 disc: clones that express Nrt-Wg, a membrane tethered immobile form of Wg, and clones that express moderate levels of exogenous Vg (Fig. 5C,D). Neither type of clone, alone, can restore normal expression of the endogenous *vg* gene. However, ectopic Vg-expressing clones can induce neighboring Nrt-Wg-expressing clones to express *vg*, provided that they abut. Moreover, this *vg* expression can spread through the Nrt-Wg-expressing clone and extend to adjacent cells outside the clone.

These results indicate that *vg*-expressing cells send a short-range, possibly contact-dependent signal that is required to entrain neighboring cells to express *vg* in response to Wg. Furthermore, they indicate that once the responding cells express *vg*, they can in turn entrain their neighbors in the same way, propagating the recruitment of additional cells into the wing primordium. These findings establish the existence of a Wg-dependent feed-forward circuit of *vg* autoregulation and suggest that D-V border cells normally organize wing growth by providing Wg, as well as the initial Vg-dependent entraining signal that triggers reiteration of this autoregulatory circuit from one cell to the next (Fig. 8) [see also Fig. 7 in the accompanying paper (Zecca and Struhl, 2007)]. Thus, feed-forward regulation in this context has a spatial component, mediating the expansion (in mass and cell number) of a developing primordium by a process of recruitment.

We note that our results are concordant with previous reports that Wg signaling cannot drive *vg* expression in the wing imaginal disc in the absence of border cells (Klein and Martinez-Arias, 1998; Klein and Martinez-Arias, 1999), and that co-overexpression of Wg and Vg can synergize to drive *vg* expression in surrounding cells (Klein and Martinez-Arias, 1999; Baena-Lopez and Garcia-Bellido, 2003). However, our findings advance these results in three significant ways. First, we show that *vg*-expressing cells provide a discrete second signal, required together with Wg, to induce vg expression in surrounding cells. Second, we demonstrate that production of this signal can propagate from one cell to the next, establishing a feed-forward autoregulatory mechanism fueled by morphogen. Third, we show that physiologically normal levels of *wg* and *vg* activity are sufficient to initiate and propagate this feed-forward mechanism, establishing that it is a natural process and not an overexpression artifact.

Identity of the feed-forward signal

Although we do not know the identity of the Vg-dependent feed-forward signal, our results argue that it should have a range of only one or very few cell diameters and that mutations that block its production or reception should prevent *QE*-dependent *vg* expression following D-V segregation. DSL ligands appear unlikely candidates, as high-level DSL-Notch signaling represses the *QE* (Go et al., 1998; Klein and Martinez-Arias, 1999; Nagaraj et al., 1999) (but see Baonza and Garcia-Bellido, 2000). Another possibility is an antagonist of the *Drosophila* Epidermal growth factor receptor (DER)/Ras pathway. DER/Ras and Wg pathways have generally opposing activities during wing disc development, with the DER/Ras pathway promoting notum and hinge development and the Wg pathway promoting wing blade development (Wang et al., 2000; Zecca and Struhl, 2002a; Zecca and Struhl, 2002b). Hence, *vg*-expressing cells might induce surrounding cells to upregulate *vg* by damping down activity of the DER/Ras pathway, which would otherwise block *vg* expression in response to Wg. A final, intriguing possibility would be a regulator of the Dachsous/Fat (Ds/Ft) pathway, which has been implicated in the control of proximal wing growth by prospective distal wing tissue (Cho and Irvine, 2004; Rodriguez, 2004; Jaiswal et al., 2006). Because loss of either Ds or Ft causes extra wing growth, the feed-forward signal would presumably promote expansion of the wing primordium by acting as an antagonist of the pathway.

Wg signaling, *vg* autoregulation and wing growth

The capacity of Wg to drive recruitment of new cells into the wing primordium by fueling *vg* feed-forward autoregulation provides one mechanism for promoting wing growth. However, it appears to operate within the context of other mechanisms for promoting wing growth, as well as for limiting where and when such growth occurs.

We can distinguish at least three additional mechanisms for promoting wing growth, all dependent on Wg. First, in addition to recruiting new cells into the wing primordium, Wg acts continuously to retain cells that were previously recruited: wing cells in which Wg transduction is abrogated rapidly lose *vg* expression and either die, or sort out (Zecca et al., 1996; Neumann and Cohen, 1997). We suggest that retention, like recruitment, depends on the same Wg-dependent *vg* autoregulatory circuit. Specifically, we posit that the feed-forward signal is required both to induce *vg* expression in cells about to enter the primordiium, as well as to maintain *vg* expression in cells after they enter (Fig. 8).

Second, independent of its role in fueling *vg* autoregulation, Wg also appears necessary for the survival and proliferation of *vg*-expressing wing cells. As we describe in the accompanying paper,

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it is possible to bypass the requirement for Wg-dependent *vg* autoregulation by using a *Tubα1>vg* transgene to express exogenous Vg: nevertheless, such 'rescued' *Tubα1>vg* wing cells still require Wg input to survive, grow and proliferate (Zecca and Struhl, 2007).

Third, cells are normally recruited into the *vg*-expressing population from a surrounding population defined by detectable expression of *rn* but not *vg*. Accordingly, the '*rn*-only' population must proliferate in conjunction with the growth of the wing primordium; otherwise, it would be depleted, limiting further recruitment and compromising the development of more proximal structures. In support, we find that the rescue of the wing primordium by Wg-dependent *vg* autoregulation is associated with the rescue and expansion of the surrounding population of *rn*-only cells (e.g. Fig. 2) (see also Liu et al., 2000; del Alamo Rodriguez et al., 2002; Kölzer et al., 2003; Cho and Irvine, 2004). Hence, once cells are recruited into the wing primordium in response to Wg, they may send an additional signal to sustain the source population of *rn*-only cells from which additional wing cells will be recruited (Fig. 8) [see also Fig. 7 in the accompanying paper (Zecca and Struhl, 2007)].

Conversely, we can distinguish at least three mechanisms that appear to constrain operation of the feed-forward circuit, limiting expansion of the wing primordium in space and time. First, is the early segregation of the wing imaginal disc into distinct distal (pre-blade) and proximal (pre-hinge/notum) compartments, only one of which, the pre-blade, is competent to engage the feed-forward autoregulatory circuit. This event, which occurs before D-V compartmental segregation, appears to be governed by an early burst of Wg signaling that selectively and heritably represses *tsh* expression in the founder cells of the putative pre-blade (tsh^{OFF}) compartment (Wu and Cohen, 2002; Zirin and Mann, 2004) [see Fig. 7 in the accompanying paper (Zecca and Struhl, 2007)]. Although Wg-dependent *vg* autoregulation normally appears to operate only within the resulting pre-blade (tsh^{OFF}) compartment (which includes the *rn*-only domain, as well as the presumptive wing pouch), this limit can be exceeded if cells are exposed to ectopic Wg signal before they would otherwise segregate into the pre-hinge/notum (tsh^{ON}) compartment (Ng et al., 1996; Wang et al., 2000; Baena-Lopez and Garcia-Bellido, 2003) (Fig. 6). We suggest that this ectopic Wg activity inappropriately blocks *tsh* activity in the prospective pre-hinge/notum, creating an ectopic pre-blade compartment in which feed-forward regulation can occur.

Second, is the availability of Dpp secreted by A compartment cells along the A-P compartment boundary. Dpp, like Wg, is essential for *vg* expression and wing growth (Posakony et al., 1990; Zecca et al., 1995; Burke and Basler, 1996; Kim et al., 1997; Guss et al., 2001). Hence, operation of the feed-forward mechanism might depend on the combined inputs of Wg and Dpp, centering the expanding domain of Wg-dependent *vg* expression on the intersection between the D-V and A-P compartment boundaries. In agreement, we only observe evidence for Wg-dependent feed-forward propagation in cells located within ~25 cell diameters of the A-P boundary, the expected range of Dpp emanating from A cells along the boundary.

Third, operation of the *vg* feed-forward circuit might be temporally constrained. We find it striking that *vg* is initially expressed in *ap*-null discs up until the time the D-V compartmental segregation would normally occur; yet, flooding such discs with exogenous Wg signal (e.g. as in Fig. 1G,H) is not sufficient to sustain and propagate this early *vg* expression. By contrast, clones of *Tubα1-vg* cells generated in these same discs are effective in triggering the propagation of *vg* expression in surrounding cells (Fig. 4B), suggesting that cells within the 'pre-blade' only become competent to operate the feed-forward autoregulatory circuit after the time at which the D-V segregation normally occurs, concomitant with the differentiation of *wg*- and *vg*-expressing border cells.

Thus, we propose that following the D-V segregation, Wg drives wing growth by at least four distinct outputs: first, by recruiting new cells into the wing primordium; second, by maintaining the recruited cells and their descendents within the primordium; third, by sustaining the survival and proliferative growth of cells defined as 'wing' by the selector activity of Vg; and finally, by acting through the agency of newly recruited wing cells to induce the expansion of the surrounding population of *rn*-only cells from which additional wing cells will be recruited. Counterbalancing these effects would be a requirement for heritable repression of *tsh*, availability of Dpp, and transition to a discrete phase of wing disc development during which the feed-forward circuit can operate. Within these constraints, the size of the wing primordium at any point following the D-V segregation would reflect the increasing range of Wg emanating from the D-V border cells via its capacity to propagate and sustain the *vg* autoregulatory circuit and, separately, its capacity to promote the proliferative growth of *vg*- and *rn*-only-expressing cells.

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