wingless refines its own expression domain on the *Drosophila* wing margin

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The imaginal discs of *Drosophila*, which give rise to the adult appendages, are patterned during a period of intense cell proliferation. The specification of differing regions occurs in some cases by subdividing the disc epithelium into lineage compartments[1]. However, in most cases precise boundaries are formed between different cell types without early compartmentalization[2]. One such boundary occurs between the *wingless* (*wg*)-expressing cells of the wing margin and the adjacent proneural cells, which give rise to margin sensory bristles. Here we show that this boundary arises in part by a mechanism of 'self-refinement', by which *wingless* protein (Wg) represses *wg* expression in adjacent cells. Cells unable to receive the Wg signal do not resolve the boundary between *wg*-expressing and proneural cells.

The wing imaginal disc is set aside during embryogenesis as an anlage of 20–40 cells, which by the end of three instars of larval life has proliferated to form a single-layered epithelium of roughly 50,000 cells[1,3]. Midway through the second instar, the disc becomes subdivided into dorsal and ventral lineage compartments; in the prospective wing blade, the cells surrounding the dorsoventral boundary form the wing margin and express a number of margin-specific genes. In early third-instar wing discs (48 h before pupariation), the secreted *Wg* growth factor is expressed at low levels throughout the prospective wing blade. Beginning at mid-third instar (24 h before pupariation), this pattern refines, becoming expressed (Fig. 1*a*) at high levels along the prospective wing margin in a stripe 3–6 cells wide just to either side of the dorsoventral boundary (Fig. 1*b*, *d*). Shortly thereafter, the proneural transcription factors genes *achaete* (*ac*) and *scute* (*sc*) are expressed in a broad stripe 10–12 cells wide along the anterior margin. Proneural expression is reduced within, but is high adjacent to, the *wg*-expressing cells. The sensory mother cells (SMCs) that give rise to the chemosensory margin bristles arise within this proneural region, immediately adjacent to the *wg*-expressing cells (the SMCs are first detected by heightened proneural gene expression; Fig. 1*j*, *k*). Other expression patterns also obey this boundary: the *wg*-expressing cells express *cut*[4], the *vestigial* intron-2–*LacZ* construct[5], and certain *Enhancer of split* complex members[6–8], and are flanked by regions expressing high levels of the Notch ligand genes *Delta* and *Serrate* (not shown) and reduced amounts of *Notch*[9].

Reductions or removal of Wg activity resulted in a failure to resolve this boundary properly. The wg^{IL} allele is temperature sensitive; at the restrictive temperature it acts as a strong hypomorph, but produces an immunologically detectable product[10]. When reared at restrictive temperatures for 12 h, late third instar

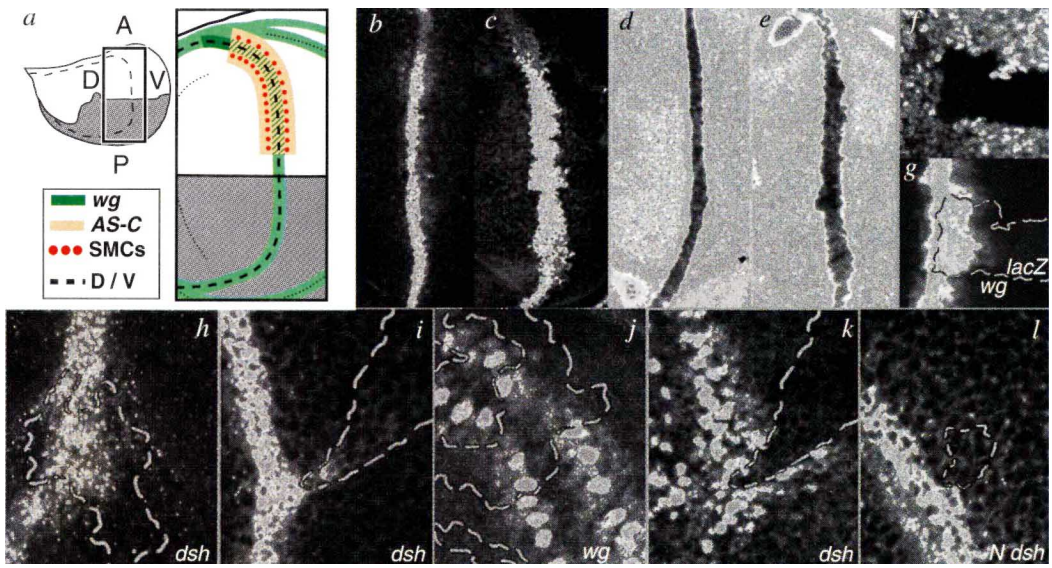

FIG. 1 *a–i*, Margin *wg* expression after loss of *wg* or *dsh* functions. *a*, Diagram of late third instar wing disc. Box outlines margin region shown in the remaining figures, with axes and patterns of gene expression as marked (see text for details). *AS-C*, *acheate–scute complex* expression; D/V, dorsoventral. *b–e*, wg^{ts} (wg^{IL}/wg^{cx4}) margins, stained for Wg protein (*b*, *c*) or messenger RNA (*d*, *e*) expression. *b*, *d*, At permissive temperature, protein and mRNA expression was normal. *c*, *e*, After a 12 h shift to restrictive temperature, protein expression expanded to a region twice the normal width (protein: 55/57 discs; mRNA: 17/19 discs). *f*, *g*, Hypomorphic wg^{LacZ} clone on the margin, shown by the absence of anti-Myc staining (*f*; in *g* and subsequent panels clones are marked by a dotted outline and *). Clones caused expansion of *wg-LacZ* expression (anti-β-gal; 72/97, none showed loss). *h*, *i*, *dsh* clones that intersected (*h*) or sat immediately adjacent to (*i*) the *wg* stripe elevated anti-Wg-staining cell-autonomously (dsh^{75}: 15/17; dsh^{v26}: 12/12). Similar effects were observed in anterior, posterior, dorsal and ventral clones. In some cases, it seemed that the normal *wg* stripe expanded or distorted to meet the ectopic anti-Wg staining within clones away from the margin (see *i*; 7 clones). It is unclear whether ectopic *wg* expression is being induced in wild-type cells between the clone and the margin, or if cells at the margin are distorting or rearranging near the clone. *j*, *k*, Anti-Scute or Achaete staining in wg^- and dsh^- clones. *j*, wg^{cx4} clones that intersected the *wg* stripe showed non-autonomous loss of Sc (18/22 large clones), as did 5/7 wg^{LacZ} clones (not shown). *Sc* was expressed at wild-type levels in *wg* mutant cells that were 1-2 cell diameters from the clone boundary, presumably in response to Wg secreted by wild-type cells. Most small clones, where all cells were close to the boundary, showed normal Sc levels. *k*, dsh^- clones within the margin proneural region showed cell-autonomous loss of sc expression (dsh^{75}: 9/9; dsh^{v26}: 15/15). *dsh* clones that intersected the *wg* stripe, and produced ectopic *wg*, also generated ectopic *sc* expression outside the normal proneural region in *dsh*/+ cells near the clone boundary (arrow; note *wg* expression in same clone in *i*). *l*, Margin *wg* expression after loss of *Notch* (*N*) and *dsh*. $Notch^-$ dsh^- clones that lay adjacent to (*l*) or intersected the *wg* stripe (not shown) showed cell-autonomous loss of anti-Wg staining without expansion of *wg* expression (29/29 clones). $Notch^-$ dsh^- clones in the anterior also lose anti-Sc staining (E.J.R., C.A.M., M. Halevy and S.S.B., manuscript in preparation).

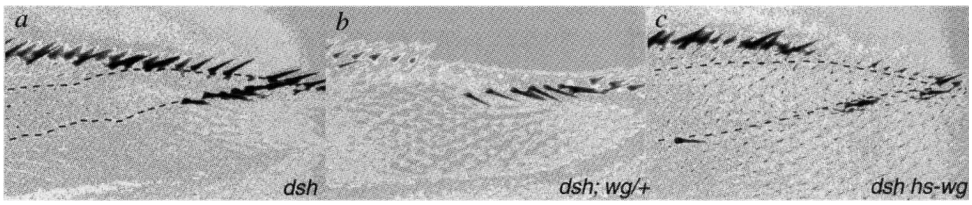

FIG. 2 *dsh*⁻ clones induced ectopic bristles off the wing margin in a *wg*-dosage-dependent fashion. *a*, *dsh*⁻ clone in adult wing, marked with *y* and f^{36a} (dotted outline), extending from the margin into the interior of the wing blade. The average distance from farthest bristle to margin was 4.5 cells (±1.0, range 3–7, $n = 23$). *b*, *dsh* clone induced in a wg^{IG22} heterozygote. The average distance for all clones observed was 2.71 (±0.73, range 2–4, $n = 14$). *c*, *dsh* mutant clone induced in the presence of heat-shock promoter-*wg*. The average distance for all clones observed was 10 cells (1.6, range 8–12, $n = 5$). *hs-wg* produces insufficient activity to generate ectopic bristles in a wild-type background (not shown). The clones in *b* and *c* are not marked with f^{36a}, but are identified by the cell-autonomous tissue polarity defect caused by *dsh*[14] (results not shown). Unmarked *dsh*⁻ and *Notch*⁻ *dsh*⁻ clones were identified in adult wings by the absence of normal margin bristles[6,20]. 22/30 *dsh* clones were accompanied by ectopic bristles distant from the margin, as compared with 5/40 *Notch dsh* clones. The bristles near *Notch* dsh clones were only rarely as contiguously arrayed or as distant from the margin as those near *dsh* clones. Regulation within the margin proneural regions may account for these occasional bristles (data not shown).

wg^{IL}/wg^{null} discs (0–8 h before pupariation) showed widened margin expression of *wg* product (Fig. 1*c*) and mRNA (Fig. 1*e*) in a stripe of roughly twice its normal width. We obtained similar results using mosaic analysis. An enhancer-trap insertion into *wg*, wg^{LacZ}, is a strong hypomorph[11]. Most homozygous wg^{LacZ} clones within the normal *wg*-expressing region induced expansion of *wg–LacZ* expression in flanking cells outside the normal region (Fig. 1*f*, *g*). Thus, cells immediately outside the normal stripe of *wg* expression are capable of expressing *wg* at late third instar, and *wg* expression within the normal *wg* stripe directly or indirectly represses *wg* expression in those adjacent cells.

To test whether this interaction was direct, we used mosaic analysis to render the adjacent cells incapable of receiving the Wg signal. The ubiquitously expressed cytoplasmic protein encoded by *dsh* is required for the reception of Wg signals[12–14]; the evidence suggests that the *dsh* protein, Dsh, is activated by the Wg signal[15,16]. The *wg* gene was ectopically expressed in *dsh*⁻ clones located near the normal *wg* stripe (Fig. 1*h*, *i*), as would be expected if the interaction was direct. Expression of *wg* expanded as many as six cells from the normal *wg* boundary. Thus, reception of Wg signalling is required to repress *wg* expression in a broad region of competence near the normal margin. This novel self-refinement function of Wg cannot on its own account for the normal retention of *wg* expression along the margin, which must result from some preexisting bias (see below). Nonetheless, Wg plays a critical role in localizing the boundary between *wg*-expressing and non-expressing cells.

Self-refinement is one of two identified signalling functions of margin Wg. Previous work has shown that Wg signalling is necessary and sufficient for proneural gene expression and the subsequent formation of margin bristles in cells flanking the *wg*-expressing stripe[11,12,16–21] (Fig. 1*j*, *k*). Thus, removing the ability to respond to the Wg signal by generating *dsh*⁻ clones results in the loss of proneural gene expression (Fig. 1*k*) and bristles in the adult[16,20] (Fig. 2*a*). One might also predict that the ectopic *wg* expressed within the clone might be able to induce ectopic proneural gene expression in wild-type cells surrounding the clone, leading to the formation of margin bristles abnormally distant from the margin. This phenotype has indeed been reported in adult wings[20] (Fig. 2*a*), and we observed clearly ectopic proneural gene expression surrounding *dsh*⁻ clones (Fig. 1*k*). It is likely that the ectopically expressed *wg* plays an important role in this phenotype. The formation of ectopic bristles was sensitive to the expression of *wg*, as ectopic bristles were found a shorter distance from the margin in a $wg^{-}/+$ background (Fig. 2*b*), and further from the margin after *wg* overexpression using heat-shock promoter–*wg* (Fig. 2*c*). Moreover, those manipulations that did not lead to ectopic *wg* expression, such as generating *Notch*⁻ *dsh*⁻ double-mutant clones (see below), only rarely induced the formation of ectopic bristles (Fig. 2 legend).

One possible way that Wg self-refinement might act is by repressing the Notch signalling pathway. The *Notch* gene is required for normal *wg* expression along the margin[3,21], and ectopic expression of Notch ligands or raised Notch activity can induce ectopic *wg* expression and margin-like development[8,21–24]. It has therefore been suggested that heightened Notch activity along the dorsoventral boundary is responsible for the localized expression of boundary-specific genes, including *wg*. Moreover, recent evidence indicates that Dsh-mediated Wg signalling can inhibit Notch activity[16].

Although we cannot rule out other mechanisms, our data are consistent with the hypothesis that Wg inhibits Notch. If Wg signalling inhibited margin Notch activity, any increase in the Wg signal should reduce and narrow the domain of the Notch-mediated *wg* expression along the margin, as does lowering Notch activity[3]. As predicted, the increase in Wg signalling induced by

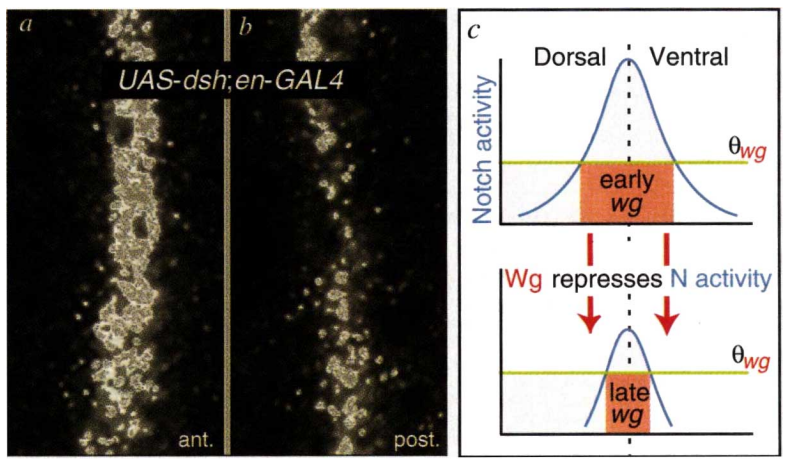

FIG. 3 Interactions between *Notch* and *dsh*. *a*, *b*, *dsh* overexpression in the posterior compartment, using *UAS-dsh* and *en-GAL4*, caused narrowing and occasional loss of margin anti-Wg staining (*b*) when compared with anterior (*a*). Dsh levels, as identified using anti-Dsh, were visibly higher in the posterior compartment (not shown). Both panels are from the same imaginal disc. *c*, Model of Wg self-refinement. Notch signalling activity is highest at the dorsoventral boundary, and levels above the threshold (Θ_{wg}) initially trigger broad *wg* expression (above). Wg represses Notch (N) activity; *wg* expression is maintained only in cells nearest the dorsoventral boundary (below).

the overexpression of Dsh[16] in the posterior compartment had exactly this phenotype (Fig. 3*a*, *b*). A further prediction of this model is that the ectopic expression of *wg* observed in *dsh*$^-$ clones should be reversed by the simultaneous loss of *Notch*; indeed, ectopic *wg* expression was not observed in *Notch*$^-$ *dsh*$^-$ clones (Fig. 1*l*). The mechanism by which Wg inhibits Notch activity is not known, but it has been suggested that this inhibition is mediated by the binding of Dsh to Notch[16]. One prediction of the Dsh–Notch-binding model is that removal of Wg signalling components downstream of Dsh should not affect Notch activity. Our evidence suggests that *Armadillo*, which like *dsh* is required for normal Wg signalling but which acts genetically downstream of *dsh*[25,26], is not required for Wg self-refinement (E.J.R., C.A.M., M. Halevy and S.S.B., manuscript in preparation).

That a narrow region of *wg* expression is normally retained along the margin, even after self-refinement, indicates that these cells are in some manner less sensitive to Wg signalling than cells more distant from the margin. This difference in sensitivity could be explained in two ways. First, some unknown factor specific to the dorsoventral boundary may render boundary cells less sensitive to Wg signalling. Recent evidence suggests that there are as yet uncharacterized signals organized around the dorsoventral boundary[27], and these could be responsible for localized biases in cell behaviour. A simpler hypothesis is that Notch activity at the dorsoventral boundary is initially higher and thus remains above levels required for *wg* expression (Fig. 3*c*). In support of this idea, it should be noted that both *Enhancer of Split* complex members and the *vestigial* second intron enhancer are expressed specifically along the margin, and that this expression depends upon the presence of Su(H) binding sites within their enhancers[6,7,27]; the Su(H) transcription factor is thought to mediate Notch signalling.

The self-refinement function of Wg may have parallels in other situations, including the vertebrate hindbrain. As in the wing margin, the boundary-specific domains of *Wnt-1* expression are initially sloppy, but become refined later in development; moreover, in *Wnt-1*^{sw} mutant mice many of the domains of *Wnt-1* hindbrain expression seem to be expanded when compared with the wild type[28]. □

Methods

wg^{ts} larvae were from *wg*^{IL}/*In(2LR) Gla Bc*^{1} X *wg*^{cx4}/*In(2LR) Gla Bc*^{1}; permissive and restrictive temperatures were 16.5 °C and 30 °C, respectively. Shifted discs and unshifted controls were marked and labelled in the same well. Antibody labelling was as described previously[3], using rabbit or rat (1/1000) anti-Wg (provided by R. Nusse), 1/1000 rabbit anti-Scute or 1/25 mouse anti-Ac (both provided by G. Panganiban), 1/400 rabbit anti-Dsh[15] (provided by R. Nusse), anti-Myc supernatant, and/or anti-β-galactosidase. *In situ* hybridization was as described previously[29] with dig-labelled *wg* complementary DNA (provided by F. M. Hoffmann). Clones were generated using the *FLP/FRT* system as described previously[3] with the following crosses: *wg*^{LacZ} *FRT*^{40A}/*CyO* X *y w FLP1*; *πM*^{21C} *πM*^{36F} *FRT*^{40A} (provided by A. Penton). *svb*^{YP17b} *dsh*^{v26} *FRT*^{101}/*FM7* or *y w dsh*^{75} *FRT*^{101}/*FM7* or *N*^{1081} *svb*^{YP17b} *dsh*^{v26} *FRT*^{101}/*FM7* X *πM*^{5A} *FRT*^{101}; *FLP3, Sb/TM6, Tb. y w dsh*^{v26} *f*^{36a} *FRT*$^{9-2}$/*FM7 X ovo*^{D1} *FRT*$^{9-2}$; *FLP*^{38}/*FLP*^{38}. *y w dsh*^{75} *FRT*^{101}/*FM7; wg*^{lG22}/*Gla X ovo*^{D1} *FRT*^{101}; *FLP*^{38}/*FLP*^{38}. *y w dsh*^{75} *FRT101/+; hs-wg/+ X ovo*^{D1} *FRT*^{101}; *FLP*^{38}/*FLP*^{38}. *dsh*^{v26} and *wg*^{lG22} are protein nulls[15,30], whereas the sole phenotype of *ovo*^{D1} is female sterility. Larvae observed at late third instar (Fig. 1) were heat-shocked during second instar, and those reared to adulthood (Fig. 2) were heat-shocked during third instar. All were reared at 25 °C. Dsh overexpression was induced using the *GAL4/UAS* system as described previously[19], with *UAS-dsh* X *en-GAL4*. These larvae were reared at 22 °C.

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Shared neural control of attentional shifts and eye movements

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We are able to move visual attention away from the direction of gaze, fixating on one object while attending to something else at a different location, within the region of peripheral vision. It has been widely assumed that the attentional neural systems are separate from the motor systems, but some studies challenge this idea[1–5]. It has now been suggested that the attentional system is part of the premotor processing in the brain[6]. This model proposes that attentional processes evolved as part of the motor systems, with isolated attentional shifts representing an artificial separation of a natural linkage. Here we test how attentional shifts might be linked to the preparations for making saccadic eye movements. We studied the superior colliculus in monkeys as they shifted their attention during different tasks, and found that each attentional shift is associated with eye-movement preparation.

Attention can be moved under voluntary or involuntary control[7–9]. A technique developed to study experimentally the dynamics of visual attention involves presentation of a cue, which indicates the target position before onset of that target[8]. This improves accuracy of target detection and decreases the time needed to detect or identify the target. Researchers refer to voluntary control of attention as endogenous and study it with symbolic cues. The involuntary control has been called exogenous (or reflexive), and is studied with cues directly priming a location.

Previous reports have proposed that the superior colliculus participates in shifting attention exogenously[10]. Patients suffering from tectal lesions often have deficits in the ability to shift attention[11]. The superior colliculus is also important in the generation of eye movements[12,13]. We have explored the relationship