Sternopleural is a Regulatory Mutation of *wingless* With Both Dominant and Recessive Effects on Larval Development of *Drosophila melanogaster*

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ABSTRACT

The Drosophila *wingless* (*wg*) gene encodes a secreted signaling protein that is required for many separate patterning events in both embryonic and larval development. *wg* functions in the development of the adult structures have been studied using the conditional mutant wg^{ts} and also using regulatory mutations of *wg* that reduce larval functions. Here we present evidence that *Sternopleural* (*Sp*) is another regulatory allele of *wg* that affects a subset of larval functions. *Sp* has both a recessive loss-of-function component and a gain-of-function component. The loss-of-function component reflects a reduction of *wg* activity in the notum and in the antenna. The gain-of-function component apparently leads to ectopic *wg* activity in the dorsal first and second leg disc and thereby generates the dominant *Sp* phenotype. *Sp* and other *wg* alleles show a complex pattern of complementation. We present evidence that these genetic properties are due to transvection. These results have implications for the genetic definition of a null allele at loci subject to transvection.

THE Drosophila *wingless* (*wg*) gene is required for many patterning events during embryogenesis and during larval development (reviewed by KLINGENSMITH and NUSSE 1994). Both in the embryo and in the imaginal discs, *wg* is expressed in spatially restricted patterns that undergo dynamic transitions at specific timepoints during development. Many of the different functions of *wg* in embryonic and larval development have been dissected using the temperature-sensitive allele of *wg* (wg^{ts}). For example, studies using this allele have shown that *wg* has several both spatially and temporally distinct roles in the growth and patterning of the wing imaginal disc. Removal of *wg* function during second instar results in loss of distal structures of the wing and their transformation to more proximal ones (BAKER 1988b; COUSO *et al.* 1993). Removal of *wg* later, during third instar, results in the loss of wing margin bristles and of specific bristles in the notum (PHILLIPS and WHITTLE 1993; COUSO *et al.* 1994).

The temporal and spatial complexity in the requirement for *wg* function implies that the regulation of its expression must be equally complex. Indeed, several regulatory mutations of *wg* have been identified that only affect subsets of *wg* function. Such mutants have helped to further dissect these functions. For example, wg^{l} appears to specifically uncover the early function of *wg* in the wing imaginal disc, as its phenotype is identical to that generated by removal of *wg* function in the wing during second instar using the wg^{ts} allele (SHARMA and CHOPRA 1976; MORATA and LAWRENCE 1977; COUSO *et al.* 1993). This mutation has been shown to be due to a 2.5-kb deletion in the 3′ regulatory region of *wg* (BAKER 1987; VAN DEN HEUVEL *et al.* 1993). As wg^{l} does not affect the integrity of the *wg* transcript, it is likely to reduce *wg* transcription in the second instar wing imaginal disc, the pattern of which has been shown to be very different from the *wg* expression pattern in the third instar wing imaginal disc (COUSO *et al.* 1993; WILLIAMS *et al.* 1993). The mutations wg^{CX3} and wg^{P} have also been shown to affect the 3′ regulatory region of *wg* and to reduce some of its larval functions (BAKER 1987, 1988a,b; COUSO *et al.* 1993; VAN DEN HEUVEL *et al.* 1993), while the mutation $spade^{flag}$ (spd^{fg}) specifically reduces *wg* function in the wing hinge during third instar and maps to the 5′ regulatory region of *wg* (C. J. NEUMANN and S. M. COHEN, unpublished observations).

Sternopleural (*Sp*) is a dominant mutation that results in supernumerary sternopleural bristles (LINDSLEY and ZIMM 1992). Homozygous *Sp* animals die at pupal stages before cuticle deposition. *Sp* has been mapped to 2–22.0, and cytologically to the region 27C1–28C1. Within the limits of resolution of these techniques, this is the same chromosomal location as *wg*. Here, we present evidence that *Sp* is a mutation of the 3′ regulatory region of *wg* that affects late larval expression and has both a loss-of-function and a gain-of-function component. In the homozygous state, *Sp* leads to a reduction of *wg* function in the antennal disc and in the notum part of the wing disc, while the dominant phenotype of *Sp* is apparently due to ectopic activity of *wg* in the dorsal first and second leg discs.

TIONG and NASH (1990) have previously suggested that *spd*, *wg* and *Sp* may belong to the same genetic

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unit, but observed a very complicated complementation pattern between *spd, Sp, wg^1, wg^{IG22}*, and two new alleles that they had generated, *l(2L)"H"* and *l(2L)"I."* Notably, none of these alleles failed to complement all of the others, and *Sp* and *l(2L)"I"* fell outside of a deficiency, *Df(2L)DE,* which uncovers the lethality of embryonic lethal *wg* mutants. We now present evidence that this complex complementation behavior is due to transvection at the *wg* locus. This conclusion has implications for the genetic analysis of genes that, like *wg,* have a complex regulation and that are subject to transvection.

MATERIALS AND METHODS

Drosophila stocks: wg^{CX4}, wg^{CX3}, wg^P, and wg^1 are described in BAKER (1987) and VAN DEN HEUVEL *et al.* (1993). Note that wg^P is a chromosomal inversion, and is not associated with a *P*-element insertion. *Sp* is described in (LINDSLEY and ZIMM 1992). The *wg* alleles wg^{r0727}, $Df(2L)spd^{j2}$ and Sp^{revP} are described below under P-element generated alleles. All crosses were performed at 25° under standard conditions.

***P*-element-generated alleles:** wg^{r0727} is an insertion of a *P* element of the PZ class into the *wg* locus kindly provided by U. GAUL. It is embryonic lethal, although the segment polarity phenotype of wg^{r0727} homozygous embryos is weaker than that of wg^{CX4} homozygous embryos (unpublished observation), arguing that it is a strong *wg* hypomorph. By performing plasmid rescue, we mapped the insertion site of wg^{r0727} to ~100 bp 5′ of the *wg* transcription start site (see Figure 4). We used this *P* element to generate both $Df(2L)spd^{j2}$ and Sp^{revP}. $Df(2L)spd^{j2}$ was generated by imprecise excision of the wg^{r0727} *P* element (by scoring for loss of the $rosy^+$ marker). Hybridization of genomic DNA from flies heterozygous for $Df(2L)spd^{j2}$ with a probe spanning the insertion site of wg^{r0727} (data not shown) showed that the left flank is gone, while the right flank is still intact, thus leaving the *wg* transcript untouched. Cytological analysis showed that the imprecise *P*-element excision has generated a large deficiency covering the 27C1–28A1 interval. Sp^{revP} was generated by jumping the *P* element from a genetically marked wg^{r0727}, *b pr cn* chromosome. The genotype of the flies in which the jump occurred is: wg^{r0727}, *b pr cn/Sp;Δ2-3 Sb/+*. These were crossed to *l(2)/CyO* flies. Out of 2000 F_1 progeny, one phenotypic *Sp* revertant was identified on a chromosome lacking the markers *pr* and *cn* (in trans to *CyO, pr cn*). This chromosome was shown to carry a *P*-element insertion into the *wg* 3′ UTR by hybridization with probes from the *wg* genomic region (compared with the *Sp* starting chromosome).

Histochemical methods: Whole mount *in situ* hybridization was performed as described by (TAUTZ and PFEIFLE 1989). *wg* RNA probe was generated according to manufacturers instructions (Boehringer Mannheim), using the full length *wg* cDNA. *Dll* protein was visualized in imaginal discs using antibody raised in mouse, as described in (VACHON *et al.* 1992). X-gal staining was performed on pharate adults carrying a *wg* lac-z insertion, as described by (HAMA *et al.* 1990).

Generation and identification of *wg* expressing clones in the sternum: f^{36a} *hsp70-flp* females were crossed to males homozygous for *Ubx>f^+>wg* on the second chromosome (DIAZ-BENJUMEA and COHEN 1995) and the resulting larvae were subjected to a heat shock of 33° for 25 min. *wg* expressing clones in the area of the sternopleural bristles were identified by the presence of f^{36a} bristles.

RESULTS

***Sp* behaves as a loss-of-function allele of *wg*:** *Sp* was first identified as a dominant mutation that produces an increase in the number of sternopleural bristles (LINDSLEY and ZIMM 1992) (Figure 1, A and B). We observed that an allele of *wg*, wg^P, also produces a weak *Sp*-like dominant phenotype (Figure 1C), suggesting that *Sp* may affect the *wg* locus. Indeed, when *Sp* is crossed to the two pupal lethal *wg* alleles that have been mapped to the *wg* 3′ regulatory region, wg^{CX3} and wg^P (BAKER 1988a,b), the resulting individuals are also pupal lethal (Table 1). Crosses to other *wg* alleles are not lethal, suggesting that *Sp* may be a regulatory allele.

Examination of the few adult escapers of crosses between *Sp* and wg^P and wg^{CX3} reveals that they have two distinct phenotypes, each of which can be attributed to reduced *wg* activity. The escapers often lack one or both anterior dorsocentral bristles and more rarely also the posterior postalar bristle and the presutural bristle (Figure 2 and Table 1). These three bristles are among the six macrobristles in the notum that have been shown to require *wg* function for their development (PHILLIPS and WHITTLE 1993). Removal of *wg* function during late third instar using the wg^{ts} allele leads to loss of these bristles, which lie close to the *wg*-expression domain in the notum. We also noticed that in our allelic combinations, microbristles located inside this *wg*-expression domain may also be lost (Figure 2). The second phenotype is loss of distal antennal segments, and in very rare cases, antennal duplication (Figure 3 and Table 1). This phenotype can also be obtained by shifting animals of the genotype wg^{ts}/wg^{CX3} to the nonpermissive temperature during late third instar (data not shown); also, wg^{CX3} homozygous pharate individuals do not have any antennae at all (BAKER 1988b).

These results suggest that *Sp* is a regulatory mutation of *wg* that compromises *wg* function in the antenna and in the notum. This interpretation is supported by the observation that the *wg* expression in the notum is strongly reduced in the late third instar wing imaginal discs of *Sp* homozygous larvae (Figure 6, E and F). It is noteworthy that there is some *wg* expression left in the posterior notum, which correlates well with the observation that the more posteriorly located *wg*-sensitive bristles (the scutellar bristles and the posterior dorsocentral bristle) are not affected by *Sp*. A corresponding reduction of *wg* expression in the antennal disc could not be found (data not shown), but as the antennal phenotype of *Sp* is relatively weak when crossed to wg^{CX3} (which is known to be a very strong mutant for the *wg* antennal function), it may not be possible to visualize what could be a very subtle reduction in *wg* expression.

Complementation between *Sp* and *wg* alleles may be due to transvection: wg^{CX4} is an RNA null mutant of *wg* (VAN DEN HEUVEL *et al.* 1993). When *Sp* is crossed to this *wg* allele, the resulting individuals are adult viable. However, they do show loss of anterior dorsocentrals and of microbristles located in the *wg*-expression domain of the notum (Figure 2 and Table 1). $Df(2L)spd^{j2}$ is a new allele of *wg* that we have generated by *P*-element

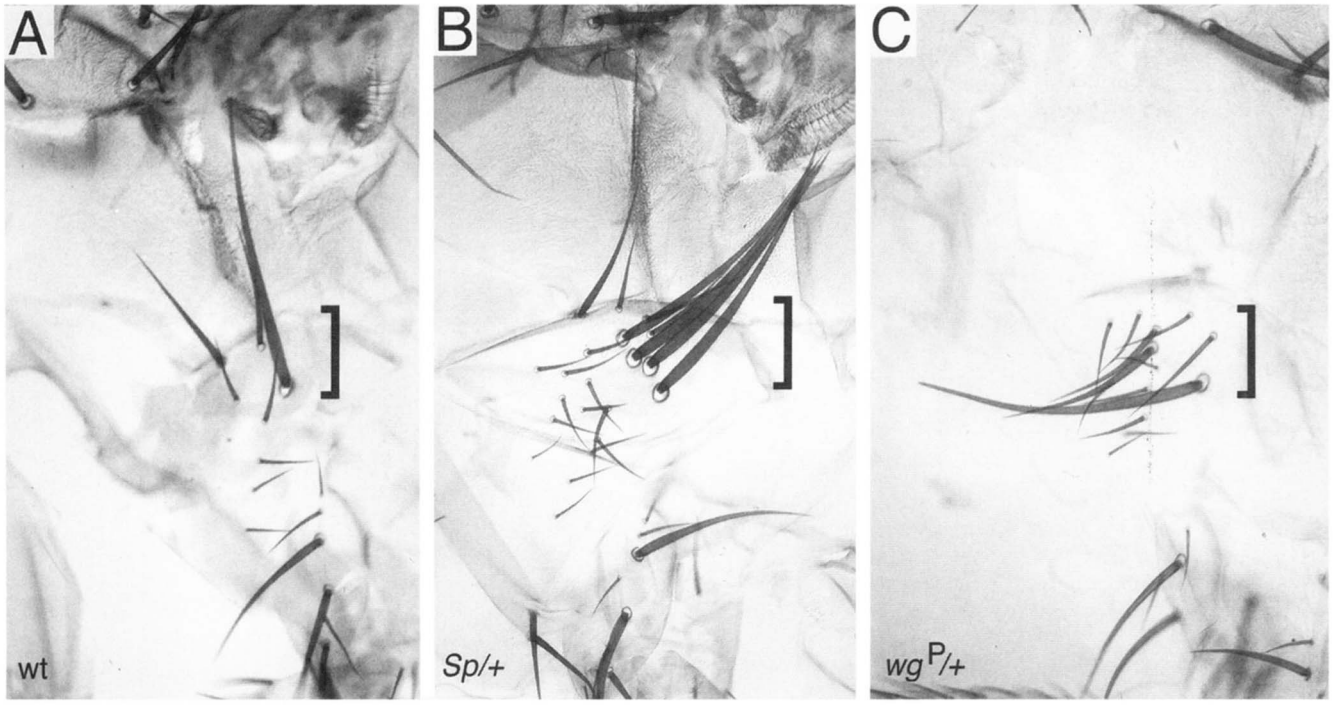

FIGURE 1.—The *Sp* dominant phenotype. (A) A wild-type cluster of sternopleural bristles, indicated by the bracket. Anterior is to the left and dorsal is up. (B) In *Sp*/+ mutants there is an increase in the number of sternopleural bristles, affecting both macrochaetae and microchaetae. (C) In wg^{P}/+ mutants a mild dominant *Sp*-like phenotype can be observed with ~20% penetrance.

mobilization (see MATERIALS AND METHODS and Figure 4). $Df(2L)spd^{j2}$ is a large deficiency that removes all 5′ sequences of *wg*, but leaves the coding region intact. As $Df(2L)spd^{j2}$ breaks ~100 bp upstream of the *wg* transcription start site (Figure 4), it is likely that it does not produce transcript. When crossed to this deficiency, *Sp* is adult viable, showing the same phenotype as over wg^{CX4}, with the addition that distal antennal segments are missing (Table 1).

These two instances of complementation of the lethality of *Sp* by strong *wg* alleles might be explained by transvection: complementation between alleles of a gene that depends on synapsis of the homologous chromosome arms (LEWIS 1954; GELBART 1982; reviewed in PIRROTTA 1990). It has been suggested that transvection phenomena might be explained by enhancer elements on one chromosome directing expression of the homologous transcription unit on the paired sister chromosome. In this example, the relevant enhancers from the 3′ UTR region of the *wg* gene on the wg^{CX4} and $Df(2L)spd^{j2}$ chromatids may be driving the expression of the intact *wg* coding sequences on the *Sp* chromatid, in *trans*. If so, it is expected that *trans* complementation would be pairing-dependent.

Comparison of the complementation behavior of three *wg* alleles that affect the 3′ UTR supports this interpretation. Like *Sp*, two *wg* alleles with relatively minor disruptions of the 3′ UTR, wg^{1} and wg^{CX3} complement $Df(2L)spd^{j2}$ (Table 1). wg^{1} is associated with a small deletion in the 3′ UTR, while wg^{CX3} is associated with a 17-kb insertion 3′ of *wg*. wg^{1} fully complements $Df(2L)spd^{j2}$ and partially complements wg^{CX4}. While wg^{CX3} is homozygous pupal lethal with complete penetrance, individuals of the genotype $wg^{CX3}/Df(2L)spd^{j2}$ are semiviable, dying only after eclosion. These alleles might not be expected to disrupt synapsis. By contrast In(2L)wg^{P}, which is pupal lethal when crossed to wg^{CX3}, is not complemented by $Df(2L)spd^{j2}$; *trans*-heterozygous animals show a more severe phenotype in that they die before pupal stages (Table 1). wg^{P} is associated with an inversion that breaks ~10 kb 3′ of *wg* and so is expected to disrupt synapsis (see Figure 4). Therefore, the fact that wg^{CX3} and *Sp* are partially complemented (and wg^{1} is fully complemented) by $Df(2L)spd^{j2}$ but wg^{P} is not, suggests that this complementation is pairing dependent. A similar argument can be made for wg^{1}, which is extensively complemented by wg^{CX4}, a null allele, but is not complemented by wg^{P}. Taken together, these results suggest that interallelic complementation between *wg* alleles can best be explained by transvection. However, we note that formal proof of this interpretation would require a direct demonstration that complementation between a given pair of alleles is synapsis dependent (*i.e.*, that introducing a chromosomal rearrangement that prevents pairing also disrupts complementation).

The dominant phenotype of *Sp* is due to *wg*: To identify the gene responsible for the *Sp* dominant phenotype, we attempted to revert this phenotype by *P*-element mutagenesis (see MATERIALS AND METHODS). One such revertant was obtained, which we designated Sp^{revP},

TABLE 1

Complementation behavior of several mutations at the *wg* locus

	wg^{1}	wg^{P}	wg^{CX3}	wg^{CX4}	$Df(2L)spd^{j2}$	tSp^{revP}	*Sp*
Sp	Wild type	Semi-lethal; pharates lack distal antenna and adc bristles	Semi-lethal; pharates lack distal antenna and adc bristles	Adult viable; lack adc bristles	Adult viable; loss of distal antenna and adc bristles	Pupal lethal	Pupal lethal
Sp^{revP}	Adult viable wg^{1} phenotype (100)[a]	Pupal lethal	Pupal lethal	Pupal lethal Pharates lack antennae, legs and adc bristles	Pupal lethal	Pupal lethal	
$Df(2L)spd^{j2}$	Wild type	Lethal before pupariation	Semi-lethal; escapers lack antennae and first leg pair	Embryonic lethal	Embryonic lethal		
wg^{CX4}	Adult viable wg^{1} phenotype (15)	Pupal lethal	Pupal lethal	Embryonic lethal			
wg^{CX3}	Adult viable wg^{1} phenotype (35)	Pupal lethal	Pupal lethal				
wg^{P}	Adult viable wg^{1} phenotype (80)	Embryonic lethal[b]					
wg^{1}	Adult viable wg^{1} phenotype (80)[c]						

[a] Values in parentheses are percent penetrance.
[b] Data from VAN DEN HEUVEL *et al.* (1993).
[c] Data from MORATA and LAWRENCE (1977).

in which the dominant *Sp* phenotype is almost completely suppressed. Only 10% of the revertants show a mild *Sp* phenotype. While *Sp* is adult viable over wg^{CX4}, a wg null, individuals of the genotype Sp^{revP}/wg^{CX4} die as pupae (Table 1), suggesting that *wg* activity is further reduced by the mutation reverting the *Sp* dominant phenotype. Molecular analysis revealed that the *P* element in Sp^{revP} had inserted in the 3′ regulatory region of *wg* (Figure 4). Furthermore, Sp^{revP} behaves genetically as a strong 3′ regulatory mutation of *wg*, showing phenotypes similar to wg^{CX3} in combination with different *wg* alleles (Table 1). The *lacZ* reporter gene of this *P* element reflects the pattern of *wg* expression in the wing disc (data not shown). These results indicate that the dominant *Sp* phenotype can be reverted by further reduction of the activity of the *wg* gene on the *Sp* chromosome, suggesting that *wg* is required for the generation of this phenotype.

As the mutation *Sp* genetically maps to a regulatory region of the *wg* gene and as the sternopleural bristles arise from the extreme dorsal part of the second thoracic leg disc (SCHUBIGER 1968), an area where *wg* is not normally expressed, this raises the possibility that the dominant *Sp* phenotype is due to ectopic expression of *wg*. To directly test this hypothesis, we misexpressed *wg* during larval development in random patches of cells using the flip-out technique (STRUHL and BASLER 1993), with a *wg* flip-out construct making use of the *Ubx* promoter (DIAZ-BENJUMEA and COHEN 1995). One adult phenotype obtained from animals treated in this way is a phenocopy of the dominant *Sp* phenotype, and in all cases this was associated with the presence of at least some *wg*-expressing cells in the cluster of sternopleural bristles (Figure 5). This result indicates that the expression of *wg* in the immediate vicinity of the cluster of sensory organ precursor cells giving rise to the sternopleural bristles is sufficient to generate the *Sp* dominant phenotype. Sp phenocopies were obtained in 20% of animals of the genotype in which clones of Wg-expressing cells can be generated, as compared with >50% of animals with wing phenotypes (see DIAZ-BENJUMEA and COHEN 1995). The difference in frequencies probably reflects the smaller number of potential target cells in the region where the Sp bristles are formed (compared with the wing primordium).

To further investigate whether the *Sp* mutation causes

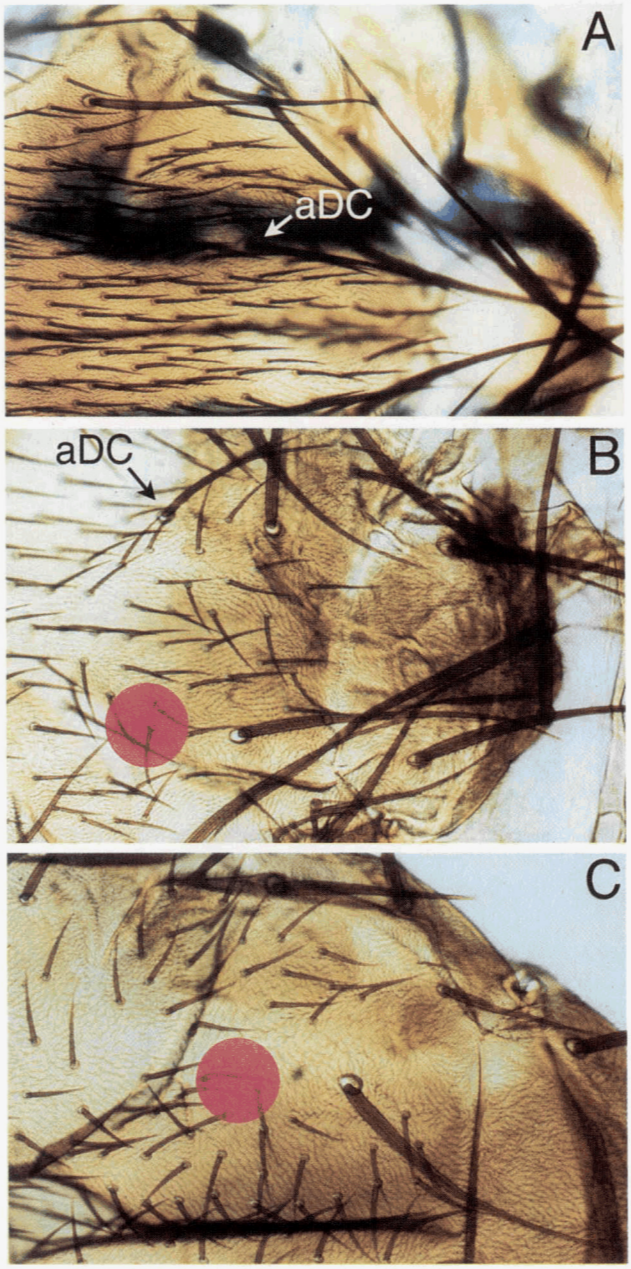

FIGURE 2.—The *Sp* loss-of-function phenotype in the notum. (A) Dorsal view of a part of the notum of a wild-type individual carrying the *wg* lac-z reporter. The anterior dorsocentral bristle (aDC) lies on the edge of the stripe of *wg* expression in the notum (blue label). Anterior is to the left. (B) Individuals of the genotype Sp/wg^{P} frequently lack one or both anterior dorsocentral bristles. The position in which the aDC should be located is marked by the purple circle. (C) Individuals of the genotype Sp/wg^{CX4} also frequently lack the anterior dorsocentral bristles. There also appears to be a reduction in the number of microbristles located in the *wg*-expression domain, suggesting a stronger reduction in *wg* function in this combination.

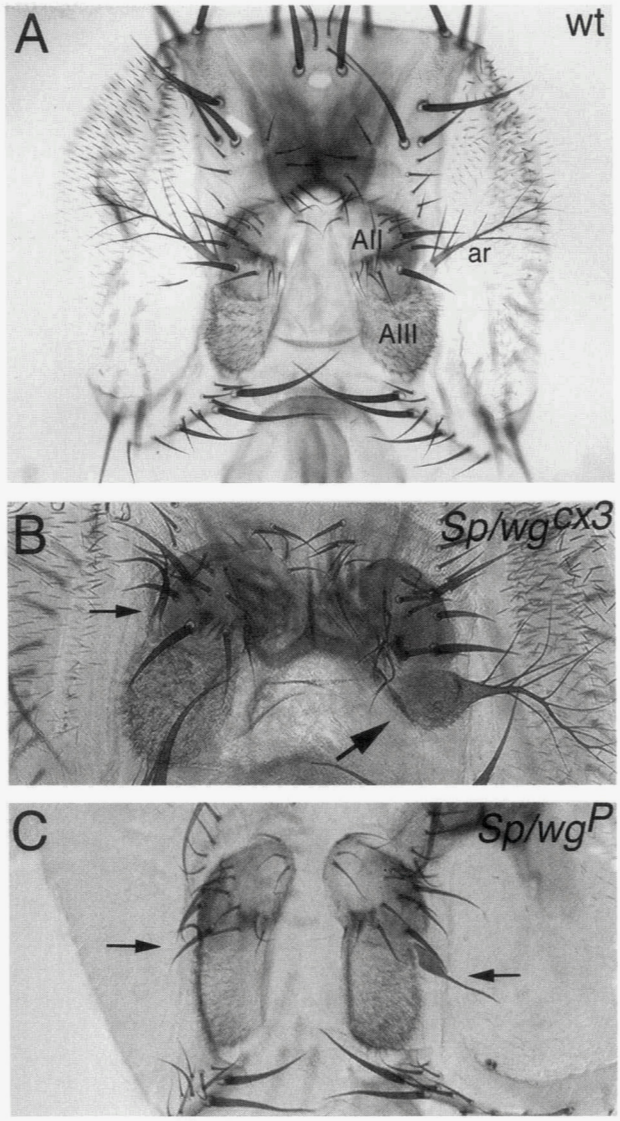

FIGURE 3.—The *Sp* loss-of-function phenotype in the antenna. (A) A wild-type head showing the normal morphology of the antenna. There are three antennal segments. Segments AII and AIII are marked. The arista (ar) is a distal outgrowth of the AIII. (B) Pharate individuals of the genotype Sp/wg^{CX3} show phenotypes ranging from reduction of the arista (small arrow on the left side) to reduction of segment AIII (large arrow on the right). The phenotype is variable and incompletely penetrant. (C) Pharate individuals of the genotype Sp/wg^{P} show a loss or reduction of the arista (arrows).

wg misexpression in the dorsal second leg disc, we stained for *wg* expression by *in situ* hybridization in both *Sp* heterozygotes and homozygotes. In *Sp* heterozygotes all leg discs showed wild-type morphology and no ectopic *wg* expression could be detected (data not shown). In *Sp* homozygotes the third leg disc showed normal morphology and normal *wg* expression, but the first and second leg disc pairs were clearly abnormal on the dorsal side of the disc, although no ectopic *wg* expression could be detected (Figure 6B). In some cases, the dorsal half of the disc was mildly overgrown and resembled a duplication (data not shown). In some cases, this overgrowth was much more extreme (Figure 6B). As it has been shown that expression of *wg* in the dorsal part of the leg disc can cause both bifurcations (STRUHL and BASLER 1993) and overgrowth (WILDER and PERRIMON 1995), these observations support the

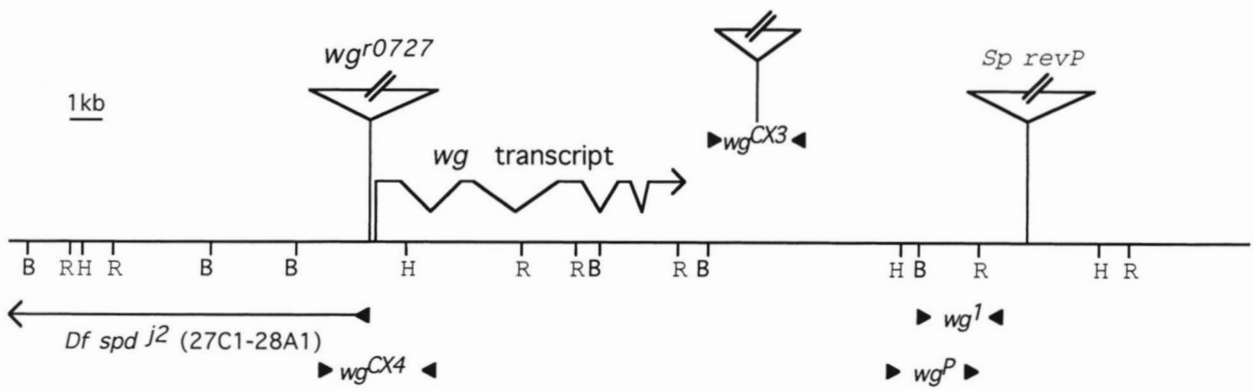

FIGURE 4.—Physical organization of the *wg* gene. The positions of the *wg* transcription unit and of several mutations used in this study are shown on a physical map of the *wg* locus. The locations of insertions associated with wg^{r0727}, wg^{CX3}, and Sp^{revP} are indicated by triangles. The deletions associated with wg^{CX4} and wg^{1} are indicated by: ▶ ◀. The proximal breakpoint of $Df(2L)spd^{j2}$ (◀) is located within 100 bp of the *wg* transcription start site. The distal breakpoint of $In(2L)wg^{P}$ is indicated: ▶ ▶. The positions of molecular alterations in the mutants wg^{CX4}, wg^{CX3}, wg^{1} and wg^{P} have been described in VAN DEN HEUVEL *et al.* (1993). DNA from *Sp*/+ flies was analyzed by Southern blotting with probes from the 3′ region of *wg*, starting at the 3′ end of the transcript and proceeding 34 kb downstream (data not shown). Each fragment of DNA was tested with several different enzymes, including *Xba*I, *Bam*HI, *Hind*III, and *Eco*R1. However, no chromosome rearrangements detectable by this method were identified.

idea that *wg* is misexpressed dorsally in the first two leg discs of *Sp* larvae, albeit at levels that we are unable to detect by *in situ* hybridization.

It has been proposed that *wg* and *decapentaplegic* (*dpp*) cooperate to establish the proximal-distal axis by setting up an organizing center in the middle of the disc, where cells are exposed to both *wg* and *dpp* (CAMPBELL *et al.* 1993; reviewed in CAMPBELL and TOMLINSON 1995), and it has been shown that cells in the leg disc that receive both the *wg* and the *dpp* signal turn on the gene *Distal-less* (*Dll*; DIAZ-BENJUMEA *et al.* 1994), a gene that is required for the formation of the proximal-distal axis (COHEN and JÜRGENS 1989). We thus further characterized the *Sp* leg phenotype by looking at *Dll* expression in *Sp* homozygous first and second leg discs (Figure 6, C and D). In some cases, *Dll* is expressed dorsally in these discs in a manner reminiscent of bifurcations (Figure 6D), although these rarely appeared to be distally complete, consistent with the suggestion that the mutation *Sp* causes ectopic *wg* activity in the dorsal first and second leg discs.

The dorsal leg overgrowth and duplications are a manifestation of a recessive gain-of-function component of *Sp*, which must be distinguished from both its recessive loss-of-function component and its simple gain of function component. The simple gain of func-

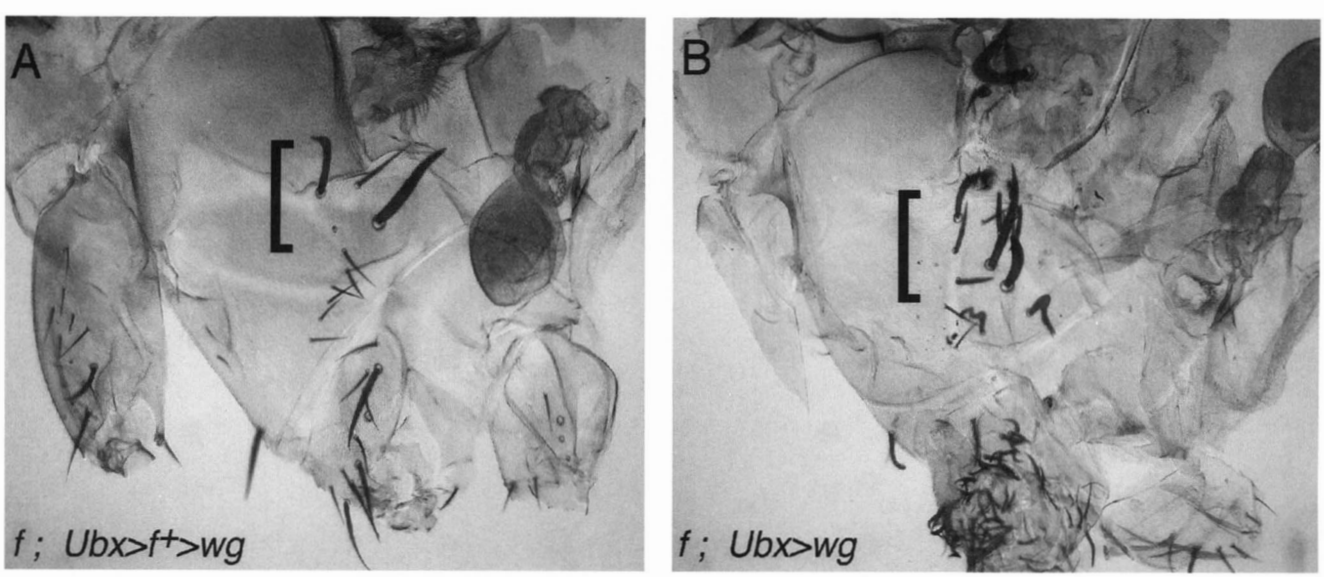

FIGURE 5.—Expression of *wg* in the sternum can phenocopy the *Sp* dominant phenotype. (A) The sternopleural region of an individual of the genotype f^{36a};*Ubx*>f^{+}>*wg*/+. Note that the f^{36a} phenotype in the macrochaetae is not completely rescued by the f^{+} cassette of the *Ubx*>f^{+}>*wg* transgene, but rescue is sufficient to distinguish these bristles from bristles in which the f^{+} cassette is absent. (B) A *wg*-expressing clone occupies part of the cluster of sternopleural bristles, causing an increase in bristle number. Note the strong f^{36a} phenotype in the macrochaetae. Anterior is to the left and dorsal is up.

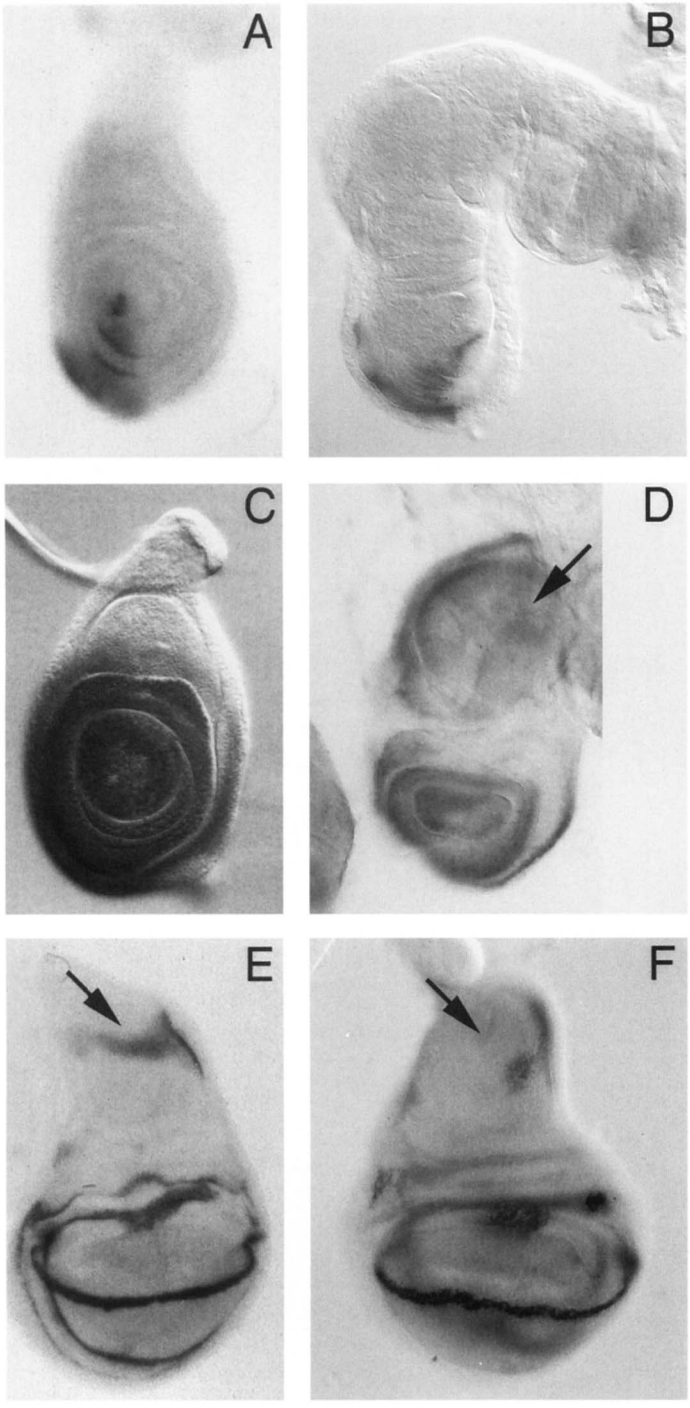

FIGURE 6.—Expression patterns of *wg* and *Dll* in wild-type and *Sp* homozygous leg and wing imaginal discs. (A) A wild-type second leg disc. *wg* RNA visualized by *in situ* hybridization is expressed in a ventral wedge. (B) A *Sp* homozygous second leg disc. Note the severe overgrowth on the dorsal side leading to many additional folds. *wg* RNA expression appears normally restricted to the ventral side. (C) A wild-type second leg disc labeled with anti-Dll. Dll expression marks the distal segments of the leg. The folding pattern of the epithelium reflects the segments of the leg. (D) A *Sp* homozygous second leg disc. Note the ectopic expression of *Dll* in an ectopic fold on the dorsal side (arrow), reminiscent of a *wg*-induced bifurcation (see DIAZ-BENJUMEA *et al.* 1994). (E) A wild-type wing disc labeled for *wg* RNA. The arrow indicates the stripe of *wg* expression in the notum, for comparison with the histochemical stain of the adult notum in Figure 3. (F) A *Sp* homozygous wing disc. Note the loss of *wg* expression in the notum (arrow).

tion component probably leads to ectopic *wg* expression in the dorsal leg disc that is sufficient to cause an increase in sternopleural bristles, while the presence of two doses of this ectopic expression appears to cause extensive repatterning.

DISCUSSION

Our results indicate that *Sp* is a regulatory allele of *wg*. Genetically *Sp* maps to the 3′ regulatory region of *wg*, as it is lethal over two *wg* alleles that affect the 3′ regulatory region of *wg*, wg^{CX3} and wg^{P}, but is viable over $Df(2L)spd^{j2}$, which removes the whole 5′ regulatory region of *wg*. This conclusion is further supported by the observation that wg^{P}, a mutation mapped molecularly to the *wg* 3′ region, also shows a mild *Sp* phenotype, and by the observation that the *P*-element insertion in Sp^{revP} is in the *wg* 3′ region. We have not been able to find any chromosome rearrangements in this region of the *Sp* chromosome, starting at the 3′ end of the *wg* transcript and proceeding ~34 kb in the 3′ direction (see Figure 4). This suggests that *Sp* may be a fairly subtle mutation that cannot be identified at the level of Southern blotting.

The *Sp* loss-of-function phenotype: Our results suggest that the loss-of-function component of the mutation *Sp* includes a reduction of *wg* function in both the antennal disc and in the notum part of the wing disc, as defects in these *wg*-dependent domains are observed when *Sp* is crossed to various *wg* alleles (Table 1). The reduction of *wg* function in the notum (Figure 2) correlates with a reduction of *wg* expression in this part of the wing disc in *Sp* homozygous larvae (Figure 6). A corresponding reduction of *wg* expression in the antennal disc could not be found, but this may be due to the fact that *Sp* only shows a relatively weak antennal phenotype (Figure 3). As *Sp* is pupal lethal over deficiencies that uncover *wg* completely, we conclude that this lethality is due to the *wg* loss-of-function component of *Sp*. However, this loss-of-function component must include more than the reduction of *wg* function in the antenna and in the notum, as these alone are not expected to cause lethality.

The *Sp* gain-of-function phenotype: Our results indicate that the *Sp* dominant phenotype is also due to *wg*, first as this phenotype can be reverted by inserting a *P* element into the 3′ region of *wg* on the *Sp* chromosome (Figure 4), and second because a phenocopy of the *Sp* dominant phenotype can be generated by expressing *wg* in the vicinity of the cells that give rise to the sternopleural bristles (Figure 5). These two observations suggest that ectopic *wg* activity is sufficient to generate the *Sp* dominant phenotype. This conclusion is supported

by the observation that wg^P, a *wg* mutant affecting the 3′ regulatory region of *wg* (BAKER 1987; VAN DEN HEUVEL *et al.* 1993; see also Figure 4), also has a dominant *Sp* phenotype, albeit with a lower penetrance and expressivity than *Sp* (Figure 1). While it is not possible to detect any ectopic *wg* expression in either *Sp* heterozygous or homozygous leg discs (Figure 6), the *Sp* homozygous first and second leg discs show a phenotype that is consistent with *wg* being expressed dorsally, that is, the dorsal part of the disc is highly overgrown and shows ectopic *Dll* expression reminiscent of a *wg*-induced duplication (Figure 6; STRUHL and BASLER 1993; DIAZ-BENJUMEA *et al.* 1994). We conclude that *wg* is expressed dorsally at very low levels in the first and second leg discs due to the *Sp* mutation and that this *wg* expression is sufficient to generate the *Sp* dominant phenotype in the heterozygous state (*i.e.*, the dominant *Sp* gain-of-function component), while it leads to pattern duplication and overproliferation in the homozygous state (*i.e.*, the recessive *Sp* gain-of-function component).

This conclusion is lent plausibility by the fact that all methods that have been used to date to express *wg* ectopically in the leg disc, while having dramatic phenotypic consequences, only supply very low levels of *wg* activity. The *actin 5C>wg* flip-out construct designed by STRUHL and BASLER (1993) gives levels of *wg* expression that are much lower than the endogenous *wg* expression. The UAS-*wg* described by WILDER and PERRIMON (1995) expresses a mutant form wg^{ts} protein, which although producing high levels of Wg antigen, appears to be compromised in its activity as compared to a UAS-Wg construct in which the wild-type protein is expressed (NA and COHEN, unpublished observations). Furthermore, the *Ubx>wg* flip-out construct used in this study, while giving strong expression in the wing (DIAZ-BENJUMEA and COHEN 1995), gives levels of *wg* expression in the leg that are difficult to distinguish from background (data not shown), but that are sufficient to generate both a strong dominant *Sp* phenotype (Figure 5) and to cause leg bifurcations (data not shown).

This leaves open the question of how the presence of *wg* leads to the generation of extra sternopleural bristles, but one possibility is that it interferes with lateral inhibition in these proneural clusters through the inactivation of *shaggy* (SIMPSON *et al.* 1988; SIMPSON and CARTERET 1989; PERRIMON and SMOUSE 1989). It has been shown that *shaggy*$^-$ clones can generate a neurogenic phenotype in the leg, presumably due to interference with lateral inhibition (DIAZ-BENJUMEA and COHEN 1994).

Taken together, our results indicate that a mutation in the 3′ regulatory region of *wg* that apparently does not result from a detectable chromosome rearrangement can affect expression of *wg* in three different imaginal discs and simultaneously lead to loss of expression in some regions and to ectopic expression in others.

The complex genetic behavior of the *wg* locus may be explained by transvection: Our data provide a basis for interpreting the results previously obtained by TIONG and NASH (1990). While performing a genetic analysis of the *adenosine 3* (*Gart*) region, these authors generated several new mutants at the *wg* locus and observed a very complicated pattern of complementation between these and already existing alleles, including *Sp*. Although they suggested that *wg* and *Sp* might belong to the same genetic unit, they did not attempt to explain why *Sp* is complemented by a deficiency, *Df(2L)DE*, that uncovers the lethality of embryonic lethal *wg* mutants. Our results now suggest an explanation for this: as *Df(2L)DE* behaves genetically like a weaker *wg* mutant than our deficiency, $Df(2L)spd^{J2}$, in that it not only fully complements wg^J, but also wg^{CX3} (data not shown), this strongly suggests that its proximal breakpoint is located 5′ of the *wg* transcription start site, and thereby allows transvection.

The mutation *Sp* illustrates how difficult it can be to genetically map different mutations to a locus which, like *wg*, is subject to a complex regulation involving control elements spread out over a large region of DNA, and which may, therefore, be subject to transvection (LEWIS 1954; GELBART 1982; reviewed in PIRROTTA 1990). *Sp* and two other regulatory alleles of *wg*, wg^J and spd^{fg}, are largely complemented by the null allele, wg^{CX4}, and complement each other fully (Table 1; C. J. NEUMANN and S. M. COHEN, unpublished data). As these different regulatory alleles also affect very different subsets of *wg* function, it may be useful, in some contexts, to regard them as separate genetic units (or control modules) that have been acquired during evolution, but that act through one transcription unit. The occurrence of transvection also makes it necessary to reconsider the definition of wg^{CX4} as a null allele, as it does not behave as a true null with respect to the regulatory alleles, but only with respect to alleles that compromise the transcript itself, or in the absence of pairing of the chromatids.

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