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Molecular organization of the vestigial region in *Drosophila melanogaster*

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The vestigial (*vg*) locus of *Drosophila melanogaster* is involved in wing margin development. In the absence of a vg^+ gene, extensive cell death occurs in third instar imaginal discs which results in a complete loss of adult wing margin structures. P-element tagging was used to obtain a molecular clone of the *vg* locus, which led to the molecular characterization of ~46 kb of DNA from the region. Deficiency analysis and molecular mapping identified sequences, spanning ~20 kb of DNA within the larger region, which are necessary for vg function. The molecular map was oriented with respect to a pre-existing genetic fine structure map of the locus. The centromere distal limits of the locus were defined by deficiency analyses while the proximal end has not yet been conclusively established. However, three transcripts, that are apparently unrelated to *vg*, provide circumstantial evidence for the proximal limits of the *vg* locus. The nature of the molecular lesions for several extant recessive or lethal *vg* alleles was determined, and these were placed on the *vg* molecular map. The characterization of the lesions associated with two dominant *vg* alleles and one complex *vg* allele imply interesting regulatory mechanisms for this locus. As well, a revertant of a 412 insertion mutant allele was shown to have resulted from a further insertion of a roo element into the 412 element.
Key words: Drosophila/transposable elements/vestigial/mutations

Introduction

Among the large number of mutations known for *Drosophila melanogaster* the vestigial (vg) mutant phenotype was described relatively early (Bridges and Morgan, 1919), and a considerable number of alleles have been isolated (described in Lindsley and Grell, 1968). Some *vg* mutants reduce the wings to a vestige of their normal size, but considerable phenotypic variation is exhibited by the extant alleles of this locus. The phenotypes range from a cryptic effect (e.g. vg^{ni}/vg^{ni}), to an effect of slight nicking of the wing margins (e.g. vg^{np}/vg^{np}), to phenotypes of virtual wing elimination (e.g. vg^{nw}/vg^{nw}) and homozygous lethality (e.g. vg^{U}/vg^{U}). Classical *vg* alleles may also show a variety of other phenotypic effects including haltere reduction, developmental delay, low viability, female sterility, high penetrance when homozygous, or even homeosis (described in Lindsley and Grell, 1968; Bownes and Roberts, 1981a). Most of the *vg* alleles are recessive under standard laboratory conditions although many act as weak dominants under specific conditions (i.e. low temperature or in a Minute background). In general, any factor (environmental or genetic) that increases the length of the developmental cycle will modulate *vg* expression towards a more severe phenotype (reviewed in Green, 1946). Alleles also exist which always behave as strong dominants, as well as alleles which show a complex complementation pattern with other *vg* alleles.

Several studies have implicated excessive cell death in regions of the imaginal wing disc as the physiological cause of the mutant phenotype (Fristrom, 1969; James and Bryant, 1981; Bownes and Roberts, 1981a,b; O'Brochta and Bryant, 1983), but the reason for this cell death remains uncertain. Bownes and Roberts (1981b) have shown that *vg* wing discs are unable to regenerate margin structures under a variety of experimental conditions, and they postulated that *vg* wing discs have an altered positional information system. Thus, the cell death could be a consequence of an earlier position-specific defect in cell determination. An alternative hypothesis was advanced by Silber and Becker (1981) who speculated that aberrant purine metabolism is the cause of the *vg* mutant phenotype. They showed that *vg* mutants have much higher HGPRTase and 5′ nucleotidase activities than do wild-type flies.

The molecular cloning of the vg^+ gene as well as various mutant alleles was made feasible by the development of P-element tagging, which has been used successfully in cloning many other *Drosophila* structural genes (e.g. Bingham *et al.*, 1981; Searles *et al.*, 1982). We report here the cloning of the vg^+ gene by this means. The cloning of the locus serves as an entry point into the molecular characterization of the wild-type versus the mutant alleles of *vg*. We present data on the partial characterization (at the DNA level) of several extant *vg* alleles of spontaneous or induced origin. Our results are consistent with the pattern emerging that many of the spontaneously derived mutant alleles of *Drosophila* genes are due to gross sequence changes resulting from insertions or deletions of up to several kilobases of DNA (Zachar and Bingham, 1982; Bender *et al.*, 1983a; Coté *et al.*, 1986). We also present data that orient the molecular map of the *vg* locus with respect to the classical genetic fine structure map (Carlson *et al.*, 1980). The characterization of the lesions associated with two dominant *vg* alleles and one complex *vg* allele imply interesting regulatory mechanisms for this locus. The new data accumulated in this study should eventually lead to an understanding of the wild-type function of the locus and hopefully will help to resolve various hypotheses explaining the functional deficiency of *vg* mutants.

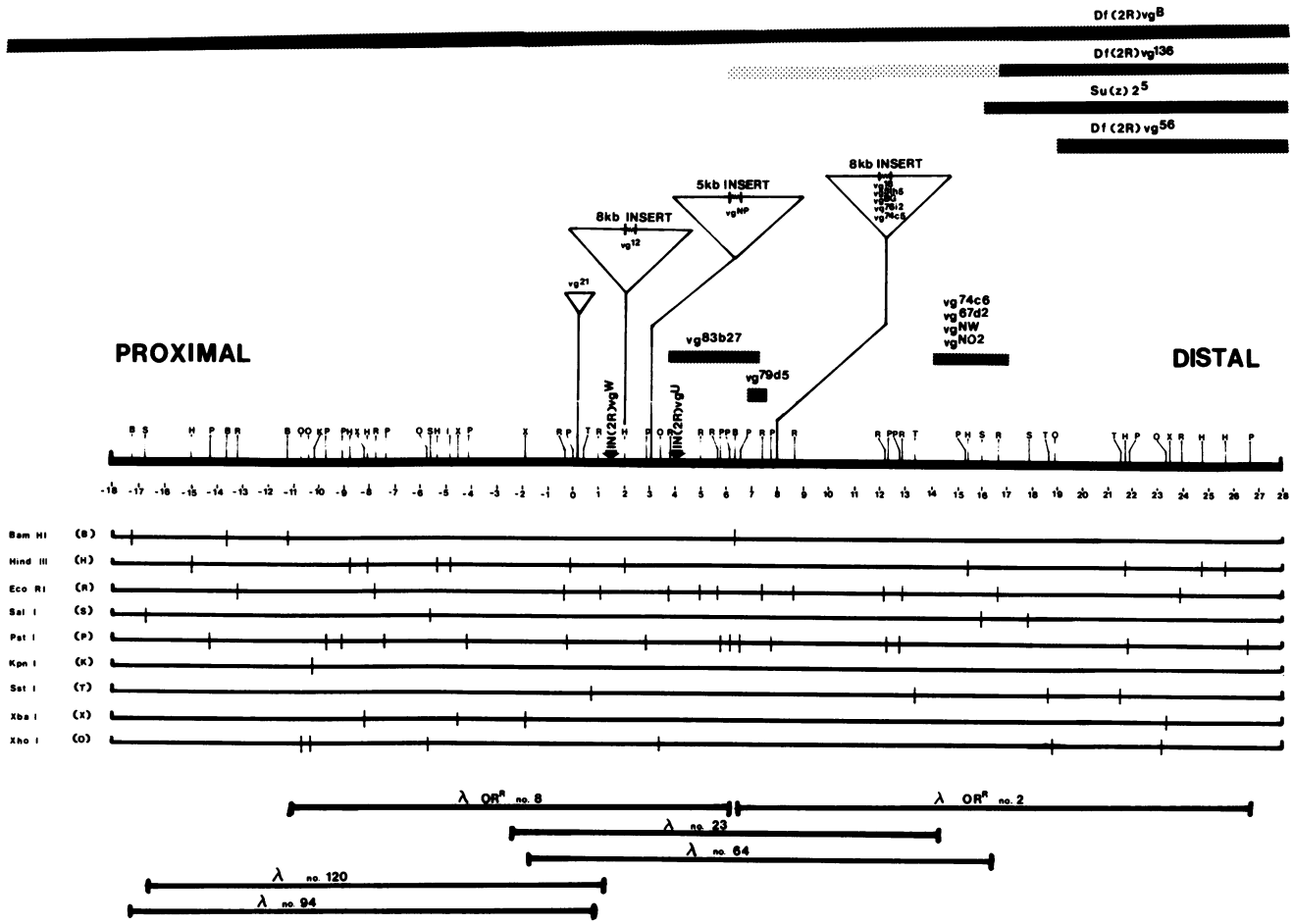

Fig. 1. Molecular map of the *vg* region derived from OR^R with the physical locations of analyzed *vg* alleles added. The EMBL3 λ clones derived from the *vg* region are shown at the bottom with restriction enzyme maps above. Restriction fragments <200 bp are not shown. All of the restriction maps, except *Kpn*I and *Xba*I, were confirmed by genomic Southern hybridizations to OR^R and $Canton^S$ and showed no restriction polymorphisms between the two strains. The positions of various *vg* alleles are shown above the composite restriction map, while the positions of the chromosome deficiencies analyzed are shown at the top as black bars. The lightly shaded portion of $Df(2R)vg^{136}$ designates inverted DNA. The 0 coordinate is arbitrarily assigned to the insert position of vg^{21}, which was used to clone the region. Negative values proceed in the centromere proximal direction while positive values proceed distally.

Results

Cloning of the vg⁺ gene

The *vg* gene has been cytologically mapped to salivary chromosome interval 49D-F by deficiency analysis (Morgan *et al.*, 1938). Due to the relative paucity of rearrangement breakpoints in this region and the lack of any known gene product for the vg^+ locus, transposon tagging appeared to be the method of choice to clone the gene. A dysgenic cross was performed and one *vg* allele was recovered from 2×10^6 flies screened (see Materials and methods). This vg mutant (henceforth called vg^{21}) is a cryptic allele in that it is homozygous wild type, but it exhibits a weak vg phenotype when heterozygous with vg^{BG} (BG denotes Bowling Green, the source of this vg stock). *In situ* hybridizations of radiolabeled P-element sequences to salivary gland chromosomes of vg^{21} larvae revealed several regions that hybridized to P-elements, including 49D-F. The vg^{21} strain was backcrossed to vg^{BG} (M cytotype) for several generations to eliminate most of the extraneous P-elements and a genomic library was constructed (see Materials and methods) and screened with a P-element probe. *In situ* analysis showed that 8 of 150 positive clones hybridized to the *vg* region. Restriction endonuclease analysis yielded a map comprising 34 kb of DNA from the region of the P-element insert. Unique DNA, flanking the P-element sequences, was identified and used to clone vg^+ sequences from an OR^R genomic DNA library. These sequences, in turn, were subjected to restriction-endonuclease analysis and a final map covering ~ 46 kb of DNA from the *vg* region was constructed (Figure 1). A comparison of the OR^R restriction map to that of vg^{21} revealed that the only difference was an ~650 bp insert in vg^{21}, indicated in Figure 1. This insert hybridizes to P-element sequences from the ends of the pΠ25.1 element (O'Hare and Rubin, 1983), so it is an internally-deleted P-element. The insert position is designated as co-ordinate 0 on the restriction map presented. *In situ* hybridization analysis and reverse Southern analysis detected no other repetitive element within the 46 kb DNA interval (results not shown).

Localization of vg gene in cloned region by deficiency analysis

A deficiency map of the vg region is available (P.Lasko, personal communication) which contains four deletions useful for physically locating the *vg* gene within the cloned region. $Df(2R)vg^B$ deletes the *vg* locus, two centromere-

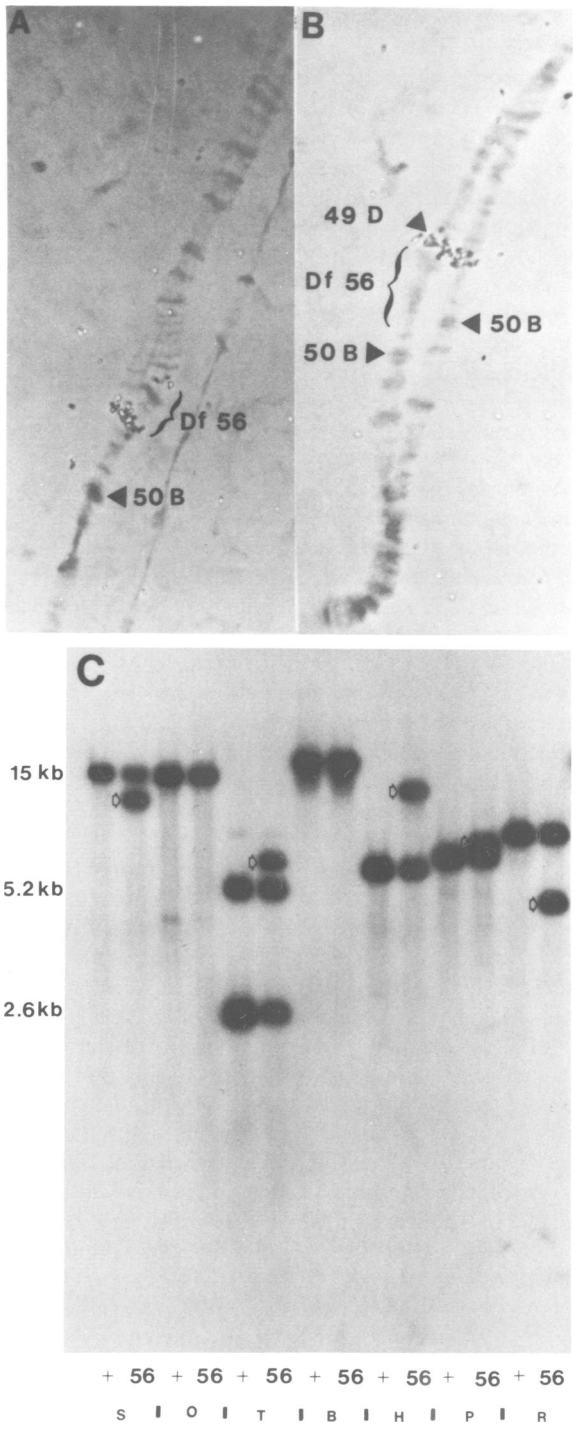

Fig. 2. Demonstration of the position of the Df(2R)vg^{56} proximal breakpoint. Panels (**A**) and (**B**) demonstrate *in situ* hybridizations to Df(2R)vg^{56}/SM5,Cy polytene chromosomes: by a recombinant clone spanning the Df(2R)vg^{56} proximal breakpoint in (A) and by λ OR^{R}2 (Figure 1) in (B). The weak hybridization to the *vg* region in (A) is expected since the breakpoint clone is not entirely composed of non-*vg* region DNA (11 kb/12 kb). Exposure time was 3 days. Panel (**C**) portrays genomic Southern hybridization to restriction digests of Df(2R)vg^{56} DNA (56) or the Canton-S parental strain (+). Restriction enzyme designations are as in Figure 1. The probe used is a *SalI*−*Xho*I DNA fragment from +18 to +19 (Figure 1). Subsequently, the blotted filter was stripped and reprobed with a *Xho*I−*Sst*I DNA fragment from +21 to +23 (Figure 1) and only the DNA bands from the balancer chromosome hybridized (results not shown). The arrows indicate the novel restriction fragments created by the deficiency.

proximal lethal complementation groups, and several polytene chromosome bands distal to *vg*. *In situ* hybridization analyses show that none of the clones from the region hybridized to the deficiency-bearing chromosome of Df(2R)vg^{B}/vg^{+} heterozygotes (results not shown), indicating that the entire cloned region is within the limits of this deficiency. Df(2R)*vg*^{136} deletes *vg* and several distal loci, but it complements the two proximal complementation groups that are missing in Df(2R)vg^{B}. Genomic Southern analyses and recombinant clone analysis of Df(2R)vg^{136} DNA (data not shown) indicate that the deficiency begins at +17 on the restriction map (Figure 1) and in addition there is a small inversion extending from +5 to +17. Df(2R)vg^{56} is a small deletion which removes several lethal complementation groups distal to vg but which almost completely complements *vg*. Since this deficiency gives only a very weak vg phenotype (i.e. only slight nicking when heterozygous with vg^{BG}), it probably does not delete DNA essential to *vg* function. Genomic Southern analyses indicate that the deficiency breakpoint is at +19 on the restriction map (Figure 1). The aberrant *SalI* fragment detected (Figure 2C) was cloned. When this breakpoint clone was radiolabeled and hybridized to salivary gland chromosomes from Df(2R)vg^{56} flies, the results show that the breakpoint clone contains DNA from the distal end of the deficiency (Figure 2A and B). This analysis is important since it places a distal limit on the *vg* locus (at about +19), while the Df(2R)vg^{136} analysis shows that sequences essential to vg function are encoded within the 14 kb of DNA proximal to the Df(2R)vg^{56} breakpoint. Su(z)2^{5} is a small deficiency deleting polytene bands distal to *vg* and gives an intermediate vg phenotype (Ting Wu, personal communication; and this study). This indicates that while it has a greater effect on *vg* function than Df(2R)vg^{56}, it does not act as a null allele and thus retains some *vg* function. Southern analysis of this allele shows that it breaks at ~ +16 (data not shown). This is consistent with the Df(2R)vg^{56} results which indicate that the centromere distal end of the *vg* gene is proximal to +19 on the restriction map (Figure 1).

Localization of vg gene in cloned region by mutant analysis

An intragenic recombination map between three *vg* alleles (vg^{BG}, vg^{np} and vg^{nw}) has been constructed (Carlson *et al.*, 1980), and is shown in Figure 3. We obtained genetic recombinants between vg^{21} and vg^{BG} to place vg^{21} on the genetic fine structure map as well. The vg^{21} allele maps centromere-proximal to vg^{BG}, very close to vg^{np} (Figure 3). Since the number of recombinants recovered between the alleles (Carlson *et al.*, 1980; this study) was low, the genetic distances are only approximate. However, proximal-distal orientation of the alleles is accurate. The molecular mapping of the four *vg* alleles revealed the nature of their respective lesions within the cloned region. The vg^{21} alteration as described above is a small insertion of about 650 bp of P-element sequences into the locus. The vg^{BG} and vg^{np} alleles were characterized by genomic Southern analyses for detection of any alterations. An 8 kb insertion which mapped to +8 on the restriction map was observed in all isolates of vg^{BG} (Figure 3). The genetic recombinants between vg^{21} and vg^{BG} have been shown to have a molecular cross over between the respective inserts. The recombinants recovered

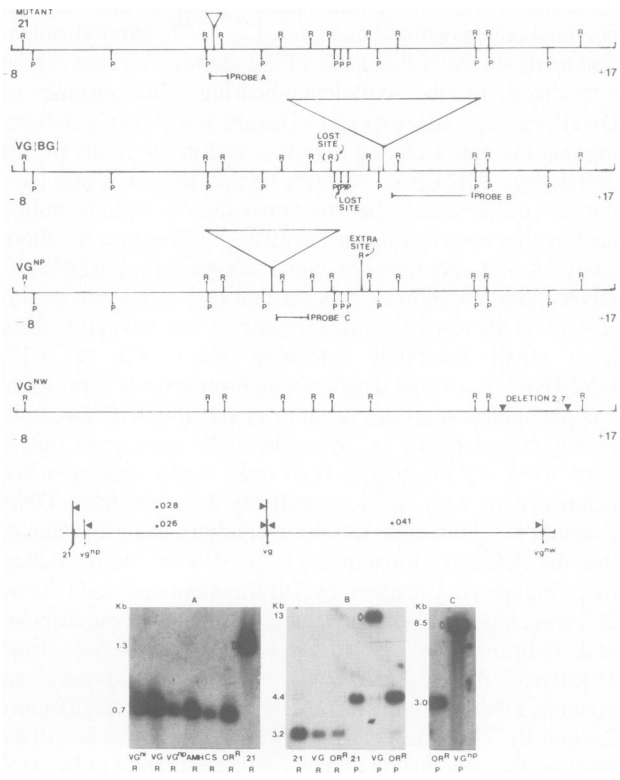

Fig. 3. Concordance of some *vg* alleles from the molecular map with the genetic fine structure map. The molecular lesions associated with each allele, and their relative positions, are indicated in the respective lines for each allele. Southern analyses demonstrating three of these lesions are shown in the panels at the bottom. The probes used for the Southerns are *Eco*RI fragments indicated as **A**, **B**, and **C** on the respective molecular maps above. The arrows indicate novel fragments generated by the respective *vg* alleles. The abbreviations for the various restriction enzymes and alleles are the same as in Figure 1. A genetic map showing recombination distances between the alleles is also shown (Carlson *et al.*, 1980; and this study). The genetic ordering of these alleles and the recombination distances between them is in accordance with molecular locations of the respective lesions.

were vg^+ and lacked both inserts (data not shown). Since this insert was altered in a revertant of vg^{BG} (see below), it seems likely that this 8 kb insertion is the causative lesion for vg^{BG}. The vg^{np} was found to have an insert of 5 kb located at +3 on the restriction map (Figure 3). The only other alteration detected is a restriction site polymorphism which has also been noted with other *vg* alleles, so it appears likely that the insertion is the vg^{np} lesion. Southern analyses of DNA from vg^{nw} flies indicate that a deletion of about 3 kb is present. Subsequent cloning and restriction analyses localized it between +14 and +17 on the restriction map (Figure 3). This was the only detectable genomic change in vg^{nw} flies within the cloned region. This evidence, plus similar deletions in several other lethal *vg* alleles (results below), indicate that the 3 kb deletion is probably the mutant lesion for vg^{nw}.

As is demonstrated in Figure 3, the positioning of the above *vg* alleles on the molecular map of the cloned region is consistent with both genetic data (recombination distances and proximal/distal orientation) and the deficiency analyses, which define the region essential for vg^+ gene function as the same region where the above mutants are distributed. This provides strong evidence that the molecular lesions that are mapped are the lesions responsible for respective mutant effects. The distances between the alleles in kb DNA/recombination unit are similar to those estimated previously (Bender *et al.*, 1983b; Côté *et al.*, 1986). In the present study, both deficiency and mutant recombination analyses served to orient the cloned region (i.e. proximal−distal) and to define a region of ~ 19 kb where *vg* mutants map. The centromere proximal limits of the locus have not yet been rigorously determined, although the identification of *vg* nonspecific transcripts (see below) provide strong circumstantial evidence for placing this limit just proximal to coordinate 0.

After the initial analyses above, which defined the approximate limits within which *vg* mutants map, several other recessive viable and recessive lethal alleles were positioned on the map by genomic Southern analyses to detect the molecular lesions associated with each mutant. The lesions found are summarized in Figure 1. The alterations indicated are the only lesions seen in the entire 46 kb cloned region. The results show that all recessive and lethal *vg* alleles (other than large deficiencies extending into the region) show lesions localized between coordinates 0 and +17. In all cases the alterations detected were insertions or deletions of DNA, except vg^{168} for which no alteration was detected. Small deletions or insertions (<200 bp) would not have been detected in these analyses, so it remains possible that smaller alterations may be associated with vg^{168} or any of the other *vg* alleles analyzed.

Analysis of dominant and complex vg mutants

Two *vg* alleles behave as dominants (i.e. entire wing margin gone when one dose of mutant allele is present) under all known growth regimens and both of these mutants are associated with cytologically detectable inversions. In both cases one end of the inversion is close to the cytological location of *vg*, so it is likely the inversions disrupt normal function and cause the dominant vg phenotype. Figure 4A and B shows *in situ* hybridizations of two different *vg* region EMBL3 λ clones to the salivary gland chromosomes of flies heterozygous for the $In(2R)vg^U$ inversion (henceforth designated as vg^U). Since λ120 hybridizes only to the proximal end of the inversion, while λ64 hybridizes to both ends, the proximal end of the inversion must be within the λ64 clone. Southern analysis of DNA from this region localized the breakpoint to +4 on the restriction map (data not shown). Figure 4C shows *in situ* hybridization of λ64 to both ends of the $In(2R)vg^W$ inversion (henceforth designated as vg^W). *In situ* hybridization of a more distal clone (OR^R2) showed hybridization only to the distal end of the inversion (data not shown) localizing the breakpoint to within λ64. Genomic Southern analysis located the breakpoint at ~ +2 on the restriction map, and the proximal breakpoint was cloned. Figure 4D shows *in situ* hybridization of this breakpoint clone to wild-type salivary bands 48A and 49D providing confirmation that the breakpoint was cloned. Genomic Southern analyses of DNA from flies heterozygous for vg^U and vg^W detected no other alterations within the *vg* region, although as mentioned previously alterations of ≤200 bp probably would not have been detected. The map positions determined for the vg^U and vg^W breakpoints within the *vg* region are recorded in Figure 1.

At least one mutant of *vg* is known which displays a complex complementation pattern (I.Alexandrov, personal com-

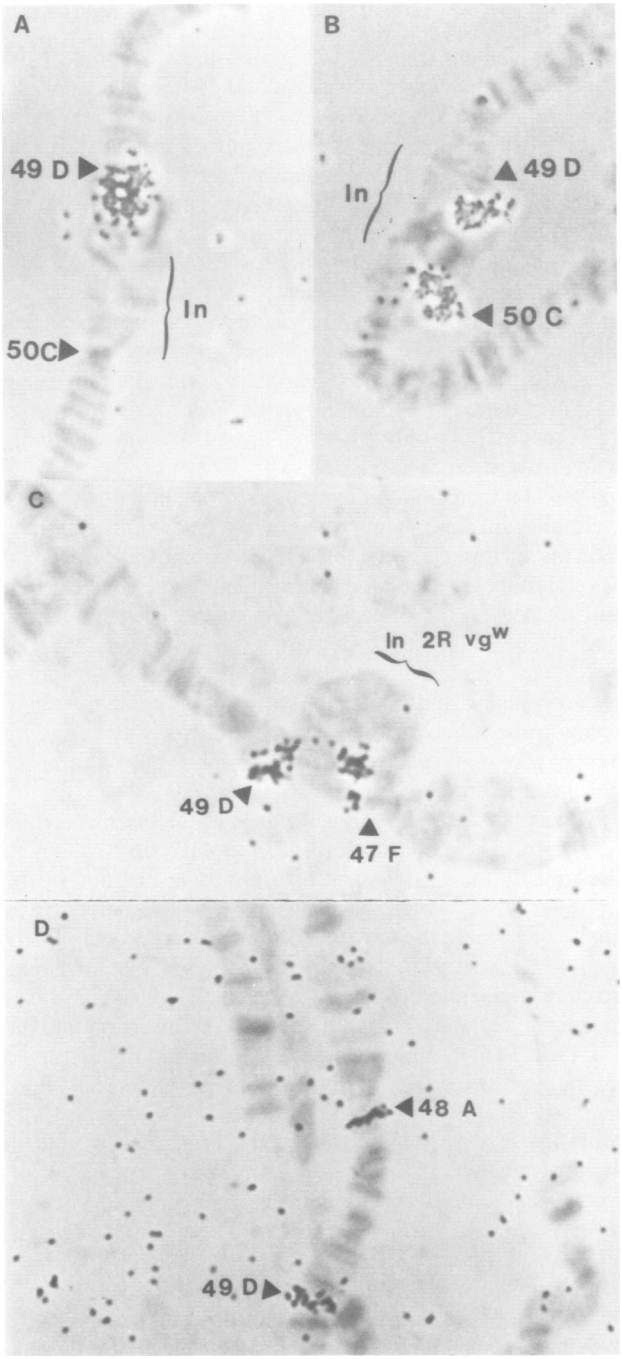

Fig. 4. *In situ* localization of vg^{U} and vg^{W} breakpoints in the *vg* region. Salivary gland chromosomes from *vg*^{U}/Balancer (Table I) larvae were probed with ^{3}H-labeled λ120 (**A**) or λ64 (**B**) (see Figure 1 for origin of two probes used). Panel (**C**) depicts a λ64 probe hybridized to vg^{W}/Balancer (Table I) salivary gland chromosomes. Panel (**D**) shows hybridization of a ^{3}H-labeled λ clone (which spans the vg^{W} proximal breakpoint) to OR^{R}.

munication; our unpublished observations). This mutant, vg^{83b27}, is homozygous viable, but with an extreme vg phenotype. However, when heterozygous with several *vg* alleles, the phenotype is wild-type. This complementation occurs with alleles that map both proximal and distal to vg^{83b27}. Southern hybridization analysis (data not shown) of DNA from the *vg* region of vg^{83b27} flies detected only a 3 kb deletion extending from +5 to +8 on the restriction map (Figure 1).

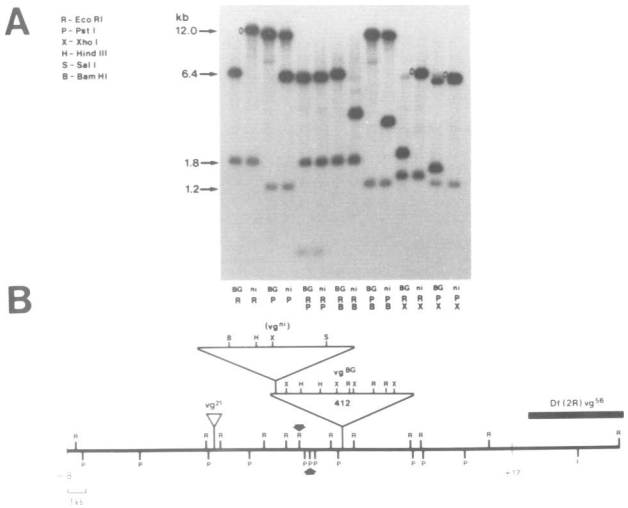

Fig. 5. Molecular analysis of the vg^{ni} revertant of *vg*(BG) **A**. Southern blots of restriction digests of DNA from vg^{BG}(BG) and vg^{ni}(ni) were probed with a 1.2 kb *Eco*RI fragment from +8 (Figure 1). Arrows indicate aberrant restriction fragments seen in the revertant, showing further insertion into this region. **B**. Composite restriction map (omitting *Pst*I sites) from three overlapping λ clones derived from vg^{ni}. The inserts are oriented in the manner indicated from the analysis described in the text. The arrows on the restriction map identify polymorphisms common to vg^{BG} and vg^{ni}.

Analysis of vg^{ni}

The vg^{ni} mutation is a nearly complete phenotypic revertant of vg^{BG} (Lindsley and Grell, 1968). Genomic Southern analyses (Figure 5) indicate that this revertant has a further insertion into the proximal end of the vg^{BG} insert. A genomic recombinant DNA library was made from the vg^{ni} strain, and three overlapping clones were isolated which spanned the *vg* region from coordinates 0 to +15 (Figure 1). This includes the vg^{ni} alteration. Restriction mapping suggested that vg^{ni} is an 8 kb insert into the vg^{BG} insert. The restriction map of the vg^{BG} insert matches that of the 412 mobile element (Shephard and Finnegan, 1984). To confirm this, Southern blots of restriction digests of the vg^{ni} λ clones were probed with a radiolabeled 412 element. This probe hybridized strongly to the vg^{BG} insert which in DNA from vg^{ni} flies was separated into two segments, a strong distal signal and a weak signal proximal to the actual vg^{ni} insert. This confirms that the vg^{BG} insert is indeed a 412 element, and indicates that the vg^{ni} insert is within the 412 element (Figure 5). A more complete restriction map (results not shown) of the vg^{ni} insert revealed an almost perfect match with the roo (Scherer *et al.*, 1982) element. The only difference is a small deletion of roo sequences at the proximal end of the vg^{ni} element. Thus, it appears that vg^{ni} is an insertion of 8 kb of a roo element into the proximal end of the 412 element insert of vg^{BG}.

Centromere proximal limits by identifying non-vg transcripts

Transcription analysis of the cloned region identified several relatively abundant transcripts, centromere proximal to coordinate 0 (Figure 1), which are present throughout ontogeny (Figure 6B). However, none were identified in the expected region from 0 to +19 which our previous mutant analyses identified as essential to *vg*$^{+}$ function. Utilizing embryonic cDNA clone banks (Poole *et al.*, 1985), three cDNAs were

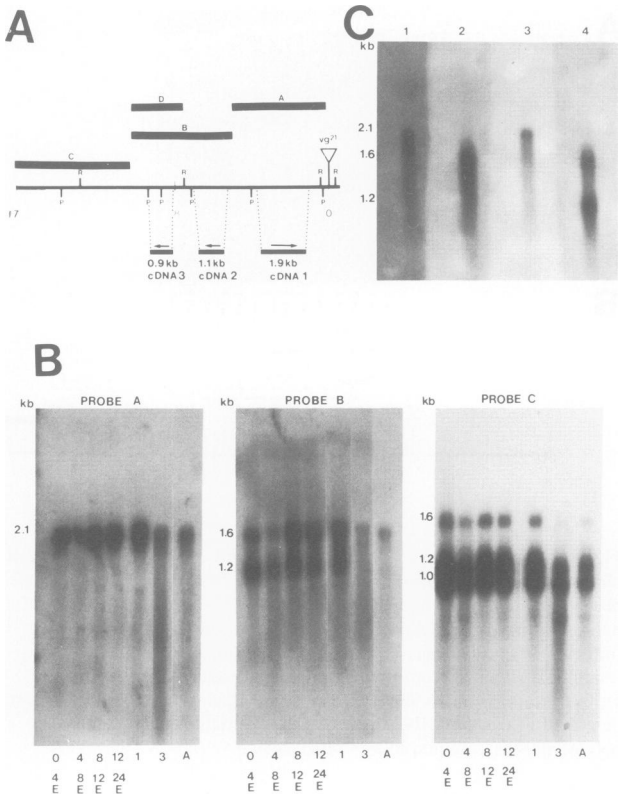

Fig. 6. Analysis of transcription units proximal to *vg*. **A**. The proximal *vg* region map is shown with probes A−D indicated above. The location of three cDNA classes are given below. cDNA1 is probe A specific, cDNA2 is probe B specific and cDNA3 is probe B and D specific. cDNA localization was established by hybridizing the longest cDNA inserts of each cDNA class to Southerns of various restriction digests of EMBL3 clones from the *vg* region. The direction of transcription was determined by probing Northerns with strand-specific RNA probes. **B**. Developmental Northerns were probed with the indicated oligolabeled probes. The RNA size markers are *Drosophila* rRNA and mouse β-globin mRNA. The multiple transcripts recognized by probe C have not been analyzed further. Stages of development used were 0−4 h embryonic (0-4E), 4−8 h embryonic (4-8E), 8−12 h embryonic (8-12E), 12−24 h embryonic (12-24E), first instar larvae (1L), third instar larvae (3L) and adult (A). **C**. Hybridization of labeled cDNAs to Northern blots of first instar mRNA. The oligolabeled probes are cDNA1 (lane 1), cDNA2 (lane 2), probe A (lane 3) and probe B (lane 4).

subsequently identified and mapped in this region (Figure 6A−C). The cDNA1 and cDNA2 (most *vg*-proximal) transcription units were analyzed to see if their expression is altered in *vg* mutants (results not shown). No alteration of expression, in levels or size of transcripts, was seen with any of the *vg* mutants examined in first instar, third instar, or adult stages. Thus, we conclude that these are not likely to be *vg* transcripts. If these transcription units are, indeed, unrelated to vg function they provide strong circumstantial evidence for the centromere-proximal limits of vg^+ function close to coordinate 0 within the cloned region. If this is the case, functional *vg* transcripts within the 0 to +19 interval (Figure 1) must be expressed at very low levels and/or in a small number of cells. A detailed analysis of *vg*-specific transcription is presently underway and will be the subject of a separate report.

Discussion

Using P-element transposon tagging, the *vg* region of the *D.melanogaster* genome was isolated. Molecular analyses of *vg* deficiencies, together with the molecular mapping of several *vg* mutations and cDNA analyses, established that the cloned 46 kb interval contains the vg^+ gene and defined a smaller region, of ~19 kb, required for vg^+ function.

Vestigial mutants cause wing margin loss and haltere reduction due to cell death in the respective imaginal discs. Previous genetic analyses indicate that four general classes of *vg* mutations exist: recessive viable alleles, recessive lethal alleles, completely dominant alleles and complex alleles. Recessive *vg* alleles show variable elimination of wing margin structures when homozygous. Weak recessives (e.g. vg^{np}) may exhibit only slight nicking of the wing margins, while extreme recessives (e.g. vg^{BG}) have the entire wing margin eliminated. Combinations of strong and weak alleles yield intermediate wing phenotypes, indicating that the mutants in this class are probably hypomorphic mutants. Several of these recessive viable *vg* alleles were characterized and located on the molecular map of the *vg* locus in this study. These include: vg^{21}, vg^{np}, vg^{79d5}, vg^{18}, vg^{51h25}, vg^{BG}, vg^{76i2} and vg^{74c5}. Of these, the latter five appear phenotypically and molecularly indistinguishable. There seems to be no correlation between the severity of the vg mutant phenotype and whether the lesion is due to an insertion or a deletion.

Molecular analysis of vg^{ni} indicates that this revertant results from a roo insertion into the 412 insert of vg^{BG}. Presumably the roo element disrupts some function of the vg^{BG} 412 insert which was causing the *vg* phenotype. It is unknown what the disrupted function is, but likely possibilities are transcription, polyadenylation or splicing problems generated by the 412 inserts. Sequence analyses to determine the exact site of roo element insertion into the 412 element may indicate which 412 function is disrupted in the vg^{ni} revertant. This type of revertant has been reported (Collins and Rubin, 1982; Levis *et al.*, 1982; Mizrokhi *et al.*, 1985; Campunzano *et al.*, 1986), but in the previous examples the revertants were either small inserts or caused only slight changes in phenotype. The vg^{ni} revertant demonstrates that parts of the *vg* region are relatively insensitive to insertions *per se*, since the vg^{ni} revertant of vg^{BG} is nearly wild-type for *vg* function yet now has 16 kb of DNA inserted into the locus. An attractive hypothesis is that most recessive viable *vg* alleles are intronic mutations which cause a mutant phenotype by reducing *vg* expression but which do not alter the gene product itself. This would account for the hypomorphic nature of recessive mutants and is consistent with the vg^{ni} analysis.

Several recessive lethal *vg* alleles, that are not associated with cytologically visible deficiencies, were examined in this study. These were: vg^{12}, vg^{168}, vg^{nw}, vg^{no2}, vg^{67d2} and vg^{74c6}. The latter four have molecularly indistinguishable deletion lesions within the *vg* region. The three distinct kinds of molecular lesions (i.e. in vg^{nw}, vg^{168} and vg^{12}) are, respectively, a deletion, a possible point mutant and an insertion. Comparison of these lesions to the types of alterations identified among the recessive viable mutations shows no inherent bias towards the type of mutant (i.e. insertion

Table I. *D.melanogaster* strains

Genotype[a]	Origin	Source
Df(2R)vg^{136}/SM5	γ	P.Lasko
Df(2R)vg^{56}/SM5	γ	P.Lasko
vg^{12}/SM5	γ	P.Lasko
vg^{168}/SM5	γ	P.Lasko
vg^{18}	γ	P.Lasko
vg^{21}	hybrid dysgenesis	This study
tri vg^{NO2}/SM5	spontaneous	Bowling Green Stock Centre
vg^{nw} Hia/SM/	spontaneous	Bowling Green Stock Centre
vg^{ni}	spontaneous derivative of vg	Bowling Green Stock Centre
vg (vg^{BG})	spontaneous	Bowling Green Stock Centre
In(2L)t In(2R) Cy/In(2R) vg^{U}	γ	Bowling Green Stock Centre
Df(2R)vg^{B}/SM5	spontaneous	Bowling Green Stock Centre
vg^{np}	spontaneous	Bowling Green Stock Centre
vg^{51h25}	spontaneous	Amherst College
In(2R) vg^{W}/In(2L)Cy In(2R)Cy	spontaneous	Umea Stock Centre
vg^{83b27}	γ	I.Alexandrov
vg^{79d5}	γ and neutrons	I.Alexandrov
vg^{76i2}	γ	I.Alexandrov
vg^{74c5}	γ	I.Alexandrov
vg^{67d2}/SM5	γ	I.Alexandrov
vg^{74c6}/bPm	γ	I.Alexandrov
sc z w^{15};Su(z)$(2)^{5}$/cyO	induced?	Chao-ting Wu
π2 (P-cytotype)	– – –	W.Engels
Oregon-R (M-cytotype)	– – –	T.Wright
Canton-S (M-cytotype)	– – –	R.Hodgetts
In(2L)Cy In(2R)Cy		
al dp b px cn vg c a px bw mp sp		J.Kennison

[a]Allele designations are as in Lindsley and Grell (1968).

or deletion) or position of the mutant in the locus for producing a lethal or viable allele.

Almost all spontaneous or induced *vg* alleles fall into the recessive viable or recessive lethal classes described above. However, a few behave as completely dominant or complex mutants. The dominant *vg* alleles vg^{U} and vg^{W} are recessive lethals, but also cause a strong vg phenotype when heterozygous with either *vg* or vg^{+} alleles. Since vg^{+} is essentially haplo-sufficient (i.e. heterozygous deficiencies of the region give only slight nicking of the wings), dominant *vg* alleles may be envisioned as somehow blocking the expression of a wild-type allele in heterozygous flies. Alternatively, dominance may be mediated by a neomorphic gene product. When these mutants were analyzed molecularly, both were found to have an inversion breakpoint splitting the locus in two. Since these are the only mutants analyzed which split the locus, it is likely that this disruption generates an aberrant *vg* product which mediates the dominance. This could be by producing an aberrant 5′ gene product or an aberrant 3′ gene product. Alternatively, it is possible that the inversions themselves cause the dominant phenotype, by disrupting normal chromosome synapsis in a transvection-like process (Lewis, 1954). The generation and analysis of phenotypic revertants of the dominant alleles could help resolve this question. Another intriguing question, if transvection can be discounted, is how the aberrant product mediates a dominant phenotype. Is it, perhaps, by a negative complementation-like process or by the action of a neomorphic gene product? Comparisons of the aberrant and normal RNA transcripts of the gene and cDNA analyses should produce some understanding of this process. Another complication of the dominant alleles is found in vg^{W}. This allele, in addition to being a *vg* dominant, is also a homeotic dominant causing transformation of dorsal metathorax to mesothorax and posterior wing duplication (Bownes and Roberts, 1981b). This homeotic effect could be a *vg* specific effect, or could be due to a breakpoint effect caused by the other end of this particular inversion. Since the proximal end of the vg^{W} inversion is cytologically inseparable from engrailed (Bownes and Roberts, 1981b; Figure 4), this is a tantalizing possibility. The cloning of the vg^{W} breakpoints has allowed the isolation of DNA from the engrailed region which is disrupted by this inversion and should facilitate using P-element mediated transformation to attempt to define DNA fragments which give the dominant homeotic effect.

One extant complex *vg* allele, which does not fit any of the three previous classifications, was also analyzed. This mutant, vg^{83b27}, is homozygous viable with an extreme vg phenotype. However, in *trans* with several other *vg*-containing chromosomes, complementation is observed (I.Alexandrov, personal communication, and our unpublished observations). All of the recessive viable *vg* alleles analyzed in this study are completely or almost completely complemented by vg^{83b27}, while the chromosome deletion alleles (i.e. Df(2R)vg^{B} and Df(2R)vg^{136}), the recessive lethal alleles, and the dominant alleles are not complemented.

The molecular analysis of vg^{83b27} revealed a 3 kb deletion of DNA from the central region of the locus. It is unusual for a deletion mutant to complement other alleles, especially since in this case the homozygous deletion is an extreme allele. However, the observation that recessive viable alleles are complemented while recessive lethals are not supports the notion that the viables are hypomorphic mutants and the lethals are structurally aberrant mutants of the locus. It is possible that the deletion removes one or more exons from the gene and produces a product which in some way enhances the expression of the allele on the homologue. In this way, hypomorphic alleles would be complemented, while structurally aberrant alleles (i.e. lethals) would not. This may be envisioned as a reverse process to that seen in the case of the dominant alleles. Clearly, further analysis of these two interesting classes of *vg* alleles will yield valuable insight into how the vg^+ gene is normally regulated.

Materials and methods

Stocks and dysgenic screens

D.melanogaster cultures were grown at 24°C and maintained on a synthetic medium (Nash and Bell, 1968). The genotypes, origin and sources of all strains used in this study are presented in Table I. The dysgenic cross was performed between P-cytotype balancer stock (Π2/SM5,Cy) males and Oregon-R (M cytotype) females. The F_1 OR^R/SM5,*Cy* dysgenic progeny were mated to *vg* flies (M cytotype) and wing-defective F_2 progeny were analyzed. The use of the Π2/SM5,Cy balancer stock allows the selection of transposon insertions onto the OR^R second chromosome by selecting only the Cy^+ F_2 progeny. A total of 100 independent wing-defective flies were isolated from a total of $\sim 2 \times 10^6$ Cy^+ flies analyzed. These mutants were tested for complementation of the vg^{BG} allele and one was found to be allelic. The majority were X-linked dominant and recessive wing defectives and were not analyzed further.

Materials

Restriction enzymes and other DNA modifying enzymes were obtained from BRL or Pharmacia and used according to the manufacturer's instructions. All radioisotopically labeled compounds were purchased from New England Nuclear. Oligolabeled probes were made with [α-^{32}P]dCTP (3000 Ci/mmol), RNA probes were labeled with [α-^{32}P]UTP (3000 Ci/mmol), while nick translations for *in situ* hybridizations utilized [^{3}H]dTTP (50–80 Ci/mmol). The *Drosophila* embryonic cDNA clone banks were gifts from T.Kornberg.

DNA manipulations

Culturing and storage of bacterial or lambda phage, preparation of DNA, restriction analyses and plasmid subcloning were performed according to standard methodology (Maniatis *et al.*, 1982). Genomic *Drosophila* DNA for Southern hybridizations and genomic libraries were prepared according to Ish-Horowitz *et al.* (1979), and repurified by spermine precipitation (Hoopes and McClure, 1981).

Genomic libraries

All genomic libraries were constructed in EMBL-3 according to Frischauf *et al.* (1983) and Maniatis *et al.* (1982). Conditions for partial digestion of genomic DNA with *Sau*3a were established, optimally cut *Drosophila* DNA was purified in 0.4% agarose gels by electroelution onto dialysis membranes and 15–20 kb fragments were retained, ethanol precipitated and resuspended in 5 μl TE buffer. Four μl of vector DNA (1 μg/2 μl) was added, ligated and packaged. Typical results yielded 6×10^5 p.f.u./reaction. Genomic libraries and the cDNA libraries were transferred to biodyne membranes (Pall) and prepared for hybridization by standard methodology. The vg^{nw} and vg^{56} libraries were also constructed as above except that genomic DNA was digested with *Sal*I, rather than *Sau*3a, and ligated into appropriately prepared EMBL3 arms.

Southern and Northern hybridizations

All gels for Southern or Northern hybridization analyses were blotted onto GenescreenPlus membranes using the capillary blot protocol recommended by the manufacturer (DuPont). RNA was extracted, purified by oligo(dT) chromatography, and run on 1.5% formaldehyde agarose gels as in Gietz and Hodgetts (1985). For Northern gels 4 μg RNA/lane were used, while for Southern gels 5 μg DNA/lane were used. Size markers for Southern gels were *Bam*HI digested EMBL3, and size markers obtained from mixing the digestion products of PΠ25.1 treated with a variety of enzymes and calibrated by comparison to the known sequence (O'Hare and Rubin, 1983). Hybridization conditions for all plaque lifts, Southerns and DNA-probed Northerns were as in Klessig and Berry (1983). Southerns and DNA-probed Northerns were washed according to GenescreenPlus specifications. DNA probes were made from restriction fragments resolved on low melt agarose gels and oligolabeled by the method of Feinberg and Vogelstein (1983). RNA probes for Northerns were prepard from restriction fragments cloned into Bluescribe (Vector Cloning Systems) using the transcription protocol of Melton *et al.* (1984), and their methods for hybridization conditions and washes of RNA-probed Northerns were also used. The prehybridization and hybridization temperature was 65°C.

In situ hybridizations

The protocol of Pardue and Gall (1975) as modified by Glew *et al.* (1986) was used for *in situ* hybridizations. Labeled probes were nick translated as in Maniatis *et al.* (1982). At least 5×10^5 c.p.m. of [^{3}H]dTTP labeled probe ($5 \times 10^6 - 10^7$ c.p.m./μg DNA) were used for each slide subjected to *in situ* hybridization.

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