A deleted hobo element is involved in the unstable thermosensitive vg al mutation at the vestigial locus in Drosophila melanogaster

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(Received 27 August 1992 and in revised form 3 December 1992)

Summary

We have described a new unstable mutant of the vestigial locus isolated from a natural population. From this mutant, vestigial homo (vg al), wild-type (vg al+), and extreme (vg al+1), alleles arose spontaneously. The molecular analysis of vg al shows that the mutation is due to a 1874 bp hobo element inserted in a vestigial intron. Two distinct kinds of events lead a wild-type phenotype. Three independent vg al+ alleles result from an excision of the hobo element and two other vg al+ alleles have further deletions of hobo sequence. The sequence of one of them shows a 1516 bp hobo insertion at the same place and in the same orientation as the 1874 bp insertion. In the vg al+ alleles, we found a 5' or 3' variably sized deletion of vg sequences. One of them, which has been cloned and sequenced, has a deletion finishing exactly at the left terminal repeat of hobo element. The genetic implications of these different genetic structures are discussed.

1. Introduction

The vestigial locus of Drosophila melanogaster is involved in wing development. In the absence of the vg+ gene, extensive cell death occurs in the third-instar imaginal discs (Fristrom, 1968). The vg locus was cloned by Williams & Bell (1988), and a 19 kb sequence of DNA was shown to be involved in vestigial function. Most of the classical alleles analysed were found to be associated with deletion of vg sequences (vg²⁶, vg²⁶, su(z)²) or insertions (vg²⁴, vg²⁶, vg²⁶, vg²⁶). The two dominant mutants (vg u and vg w) were shown to be due to inversions with one of the breakpoints located in the vestigial locus (Williams & Bell, 1988). A developmentally regulated 38 kb transcript was characterized and shown to be spliced from eight exons (Williams et al. 1990, 1991). The vg²⁶ allele, induced in mutagenesis studies by Alexandrov & Alexandrova (1987), produces an extreme wing phenotype which defines a second complementation unit. This allele is associated with a 4 kb deletion entirely within vg intron two (Williams & Bell, 1988; Williams et al. 1990).

The vg al allele was isolated from a natural French population. This allele is unstable; the genetic instability of the mutant is thermosensitive. At 28 °C vg al extreme (vg al+1) derivatives appear and have a strong wing mutant phenotype, while at 21 °C wild-type revertants (vg al+) are more common. However, the temperature effect is not absolute, as vg al+ could also be isolated at 25 and 21 °C, and vg al+ at 25 °C (Bazin et al. 1991). Further, whilst vg al belongs to the same complementation group as the classical vg al mutant, vg al+ does not complement with either vg al or vg al+.

Southern hybridization analyses of vg al, vg al+, and two independent vg al+ alleles, and the cloning of the vg al mutation, showed that the vg al mutation is due to the insertion of a deleted hobo element. The vg al+ derivative alleles appear to be caused by a deletion of vg sequences, since the hobo element is still present. Two different molecular events can lead to a wild-type revertant phenotype: either the excision of the hobo element as in vg al+1, or a further deletion of hobo sequences as in vg al+2. Hobo elements participate in a third hybrid dysgenesis system (the others being I-R and P-M), which have some similarities with P element (Blackman et al. 1987; Yannopoulos et al. 1987; Louis & Yannopoulos, 1988; Blackman & Gelbart, 1989; Calvi et al. 1991). A complete and functional hobo element is 3 kb long, possesses two terminal inverted repeats of 12 bp and generates an 8 bp duplication at its insertion site.
(Streck et al. 1986; Calvi et al. 1991). Blackman et al. (1989) have shown that the hobo HFL1 element is able to mediate germline transformation and is an autonomous and fully functional element. Mobilization of hobo occurs not only in dysgenic crosses, but also in intrastrain crosses (Blackman et al. 1987; Yannopoulou et al. 1987; Lim 1988), producing molecular rearrangements such as inversions, deletions or new hobo insertions, close to the resident element. Such rearrangements could be a consequence of recombination between two neighbouring hobo elements. In this study we show that the vg" mutation is due to a 1874 bp hobo insertion in the third vestigial intron. The derivative vgext allele is due to a deletion of 2-4 kb of DNA, and other vgext alleles have smaller deletions originating from the same position. The vg" mutation can also revert to wild type. Two different vgext alleles are characterized; one is dominant when heterozygous with a deletion of the vg locus, while the other is only partially dominant. We also observed two different molecular events which can produce wild-type reversions. They are either an excision of the hobo element or a partial deletion of the hobo sequences. For example, the vgext2 wild-type revertant is due to a deletion of 358 bp located in the central part of the 1874 bp hobo element. Herein we discuss the observations that some of the vgext revertants are due to a further deletion of hobo sequences, whereas various deletions of the adjoining vg sequences lead to a vgext phenotype (no wing at all and female sterility).

2. Material and methods

(i) D. melanogaster stocks and culturing

D. melanogaster cultures were grown at 25 or 21 °C and maintained on standard corn, yeast and sugar medium. The wild-type strain used was OregonR and the vestigial mutant strains were vg¹; Df(2R)49D3-4: 50A2-3/CySM5 (Bowling Green Drosophila Center) and vg¹ isolated in a natural population, from France (Bazin et al. 1991). The revertant wild-type strains (vgalt+) were isolated independently from vg¹ cultured at 21 °C: vgalt+ and vgalté or at 25 °C: vgalt+2, vgalt+3, vgalt+4. The derivative vgext strains were isolated from vg¹ cultured at 25 °C. The vgext (vgext1, vgext2, vgext3, vgext5, vgext75 and vgext124) homozygotes display a very pronounced mutant phenotype: no wing, no haltere and the females are sterile. The vgext allele is a recessive lethal mutation. Therefore, the vgext stocks are maintained as heterozygotes with a balancer chromosome.

(ii) DNA manipulation

The culturing and storage of bacteria or lambda phage, preparation of DNA, and plasmid subcloning were performed by standard methods (Maniatis et al. 1982). Genomic D. melanogaster DNA for Southern hybridizations and genomic libraries was prepared by the method of Ish-Horowicz et al. (1979) and repurified by spermine precipitation (Hoopes & McClure, 1981). All gels for Southern hybridization analyses were blotted on to GeneScreen Plus membranes using the capillary blot protocol recommended by the manufacturer (Dupont). Four Southern gels, 5 μg of DNA/lane were used. After hybridization the filters were washed according to GeneScreen Plus specifications. DNA probes were made from restriction fragments resolved on low-melting agarose gels. For the vg¹, vg¹+2 and vg¹+4 libraries, genomic DNA was digested entirely with EcoRI and fragments between 2 and 4 kb, purified within 0.5% agarose gels and electroelution on to dialysis membranes, were cloned in λGT10 and subcloned in bluescribe (Williams & Bell, 1988). All DNA sequencing was performed by double-stranded DNA sequencing of inserts cloned into Bluescribe (Chen & Seeburg, 1985).

3. Results

The vg¹ mutation results from an internally deleted hobo element inserted into the 1-4 kb EcoRI fragment of the vestigial locus (Fig. 1) (Bazin et al. 1991). DNA sequencing of this fragment showed that the insertion is located in the third vg intron, 462 bp 5' to the beginning of the 4th exon. The insertion also generated an 8 bp TACTACAT duplication (Fig. 2). A large number of base changes were found in the vg sequences compared to a wild-type allele (Fig. 2). These are probably due to the fact that vg¹ was isolated from a natural population, and that most intronic sequences are not functionally conserved. The data show that the vg¹ mutation is an insertion of an internally deleted hobo element. The only difference detected between the sequence of hobo vg¹ (hg¹) and the published sequence of a functional complete hobo element called HFL1 (Calvi et al. 1991) is an internal deletion (1086 bp) between positions 995 and 2082, with a ‘G’ inserted at the deletion junction.

(i) Molecular analyses of independent vgalt+ revertant strains

Molecular analysis of five independent vgalt+ revertant strains was undertaken by comparing them to vg¹ and vg (Or¹) strains, utilizing probes covering the whole vestigial locus. In all cases the results indicate that there is a single alteration in the relevant vg¹+14 kb EcoRI fragment. In vg¹ this fragment is 3-4 kb long, due to the hobo insertion. The vgalt+1, vgalt+3 and vgalt+6 strains show the same pattern of hybridization as vg¹ when the 6-5 kb probe is used (see Fig. 1) indicating an excision of the hobo element. However, the relevant EcoRI fragment in vgalt+2 and vgalt+4 is now 3-1 kb (Fig. 3), indicating a partial deletion only of DNA. To localize this deletion, the 3-1 kb vgalt+2 EcoRI fragment was cloned in bluecrire (pvgalt+). A restriction map
A deleted hobo element in Drosophila melanogaster

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Fig. 1. Partial physical map of the vestigial locus. The labelled open boxes designate the known extent of various vg deletions. The hatched boxes below the restriction map denote the exons 3 and 4. The open boxes designate the hobo insertion involved in the vg* mutation. The vg* 3·4 kb and vg*1–2 3·1 kb EcoRI fragments were cloned between the designated sites: R*. The relevant cloned vg*1–2 2·5 kb EcoRI fragment lies between the EcoRI sites designated: R*. The probes used for Southern analyses were the 1·2 kb EcoRI fragment and the 6·5 kb BamHI 1–SstI fragment. The restriction sites on the map are abbreviated as follows: R, EcoRI; P, PstI; X, XhoI; B, BamHI; Pv, PvuII; H, HincII; S, SmaI and N, BglII.

Fig. 2. The limits of the vg* deletion are indicated by asterisks. The deletion extends from 88 bp after the first underlined PstI site of the vg 1-64 EcoRI fragment (see Fig. 1) to the hobo insertion site. The length of the hobo* insertion is also shown: the G TG and CAG indicate the limits of the third vg intron where the hobo* insertion takes place, generating an 8 bp duplication which is underlined, TACTACA CAGAGAACCGC... (in open face lettering) are the hobo terminal repeats, the hobo* sequence is not shown. The polymorphic bases compared to vg* Or sequence, in the neighbouring vg sequence are noted in parentheses ( ).

was made and compared with that of the 3·4 kb EcoRI fragment of vg*. These fragments differ only in the size of the central XhoI fragment, which is 1·5 kb in pgv* and 1·2 kb in pgv*1–2. The sequence of the hobo*1–2 element (hugv*1–2) shows that it is almost identical to, and in the same orientation as, the hugv* sequence. The only difference is a further internal deletion of 358 bp, so that the total internal deletion now extends from positions 938 to 2380.

At the genetic level, we have shown that there are two types of vg* alleles exist. The vg*1–2, vg*1–4 and vg*1–6 alleles display a wild-type phenotype when crossed with vg* (vg* is a complete deletion of the vg locus), whereas the vg*1–2 and vg*1–4 alleles showed a 'notched' phenotype (results not shown). These results do not correlate simply with the molecular alteration observed, since we found a 3·1 kb EcoRI fragment in both vg*1–2 and vg*1–4 (Fig. 3), and yet these alleles were different at the phenotypic level when crossed with vg*. Moreover, vg*1–2 and vg*1–4 gave the same notched phenotype in the heterozygotes with vg*, but differ at the molecular level.

(ii) vg* analyses

A previous analysis of the vg* mutation by Southern hybridization identified that there is a deletion of vestigial sequences within two neighbouring EcoRI fragments (1·4 and 1·64 kb) (Bazin et al. 1991). The relevant EcoRI from vg* fragment (i.e. missing these deleted sequences) was cloned in bluescribe (pgv*) and a restriction map was prepared. Several genomic vg restriction sites are missing: PstI, HincII, BglII, SmaI and EcoRI from the 1·4 kb EcoRI fragment and two PstI sites and BamHI from the 1·64 kb EcoRI fragment (see Fig. 1). The hobo element is still present and had the same characteristics as hugv*. The DNA sequence of the vg* proximal region (Fig. 2) shows that the deletion extends from 88 bp
Fig. 3. Southern blot analysis of OrR, vg^{ext-1}, vg^{ext-2}, vg^{ext-6}, vg^{al+2} and vg^{al} strains. The DNA, digested with EcoRI (lanes 1-7) or PstI (lanes 8-14), was hybridized with the 6-5 kb probe (Fig. 1): OrR lanes 1 and 8, EcoRI (lanes 8-14), was hybridized PstI (lanes 1-7) or hobo insertion site. We analysed five independent vg^{al} strains to see if they all resulted from vestigial deletions. The DNA was digested with XhoI and hybridized with the 1-2 kb EcoRI vestigial fragment to determine the size of the XhoI fragment in the area with the hobo insertion (Fig. 1). This fragment 17 kb long in vg was as against 4-7 kb in vg^{al}, because the hobo insertion contains a XhoI site. In vg^{ext-1} and vg^{ext-2}, the 4-7 kb fragment typical of vg^{al} was observed, showing that there is no detectable change in the vg^{ext-1} and vg^{ext-2} mutations in this region, apart from the hobo insertion (data not shown). Paradoxically, vg^{ext-1} is female sterile and the wing phenotype is dramatically reduced, whilst vg^{ext-2} is a recessive lethal. This result may be explained by a small inversion or deletion in the vestigial sequences, which was not detected in our analyses, or by a point mutation in the exonic sequences. In the vg^{ext-1} mutation we found a 2-5 kb fragment approximately as expected. The equivalent fragment is 3-5 kb in vg^{ext-6} and vg^{ext-7} (data not shown), indicating smaller deletions than in vg^{ext-1}. We also analysed two additional vg^{ext} alleles: vg^{ext-7} and vg^{ext-11-24}. These mutations result from the loss of vg sequences located 5' to the hobo insertion in the case of vg^{ext-7} and 3' to the hobo insertion in the case of vg^{ext-11-24}. The exact end points of the deletions were not located, and we do not know if there are any changes to the 5' or 3' hobo terminal inverted repeats (Fig. 1).

All of these results show that the size of the deletion of genomic vg sequences varies in the vg^{ext} mutations, and that they can be located 5' or 3' to the hobo insertion. At the phenotypic level, all the extreme mutations express a very atrophied wing and the females are sterile. The ovaries are partially developed but no eggs are laid. In addition, some asymmetric thoracic abnormalities are observed. These abnormalities may alter either the scutellum or the thoracic ventral face. In the latter case the legs are modified; in extreme cases there are only five legs. In order to test if these abnormalities are in any way correlated with the extreme wing phenotype, we analysed the thoracic region of several homozygous strains: vg^{al} as a control, vg^{al}, vg^{ext-1} and vg^{ext-2} (Table 1). The results show that the thoracic abnormalities are not correlated with wing size per se, since vg^{al} has no wing (Alexandrov & Alexandrova, 1987; Williams & Bell, 1988) and no significant thoracic abnormality. In the vg^{ext-1} and vg^{ext-11-24} strains we observed opposing thoracic phenotypes (x^2 = 9; 2 df, P < 0-05). The vg^{ext-1} phenotype mainly affects the legs, whilst vg^{ext-11-24} affects the dorsal part of the thorax. Since these two strains differ only by the vg sequence deletions, it would be interesting to test whether these results are correlated. Since the extreme alleles studied herein are derived from the vg^{al} allele, which is caused by an insertion into intron 3, it appears that the wing phenotype and female sterility are correlated with the loss of exon 3 (vg^{ext-1} and vg^{ext-7}) or exon 4 (vg^{ext-11-24}). This is similar to the situation in the vg^{alu} phenotype, which is female sterile and results from a deletion of downstream exons (Lindsley & Zimm, 1992; Williams & Bell, 1988).

Table 1 Thoracic phenotypic analyses of vg^{al}, vg^{ext-7} and vg^{ext-11-24} homozygous flies

| Strain | Wild-type thorax | Thorax abnormalities |
|--------|------------------|---------------------|
| Vg^{al}|                  | Dorsal (%) Legs (%) Total |
| Vg^{ext-7}| 185           | 14 (6) 26 (12) 225 |
| Vg^{ext-11-24} | 399       | 55 (11) 31 (6) 485 |

The number of flies in each category was scored. The parentheses are percentages that the respective group makes up of the total flies scored with that genotype.

4. Discussion

The unstable vg^{al} mutation is due to the insertion of a deleted hobo element into the third intron of the vestigial gene. The hobovg^{al} element has a 1086 bp internal deletion (from bp 996 to bp 2081) as compared to the complete hoboHFL1 (Calvi et al. 1991). We observed one additional base in the hobovg^{al} sequence, namely a guanidine (G) at the position 996 break.
A deleted hobo element in Drosophila melanogaster

point. We did not observe any homology with the 8 bp consensus sequences described by Streck et al. (1986). The vg*1 allele at the site produces different phenotypes according to the length and structure of the hobo element involved. For example, a wing mutant phenotype is associated with vg*1, which has a 1874 bp insertion, whereas vg*1+1 is wild type and has a 1516 bp insertion at the same site. The molecular difference between these two alleles is 358 bp deletion in the centre of the hobo element. The differences leading to the two phenotypes could be due to either hobo or vg transcription, which results in a differing length or quantity of vg mRNA.

Several independent vg*2 mutations arose spontaneously in the vg*1 stock and were analysed. The vg*2 allele was cloned and was shown to have a 2.5 kb deletion of vg sequences extending 5' from the hobo insertion. This deletion ends precisely at the site of the hobo insertion and excises the 8 bp duplication from the mutant, leaving the hobo element intact. The deletion completely removes exon 3 of the vg gene. It partly overlaps with the vg*1 allele deletion, and completely overlaps with the vg*2 allele (Fig. 1). The vg*2 mutations display a strong mutant wing phenotype and female sterility in the homozygous state. This sterility is not observed in the vg*1 allele. The vg*2 mutation completely overlaps with the vg ext allele, whereas the vg ext allele has a lesion of 874 bp insertion, whereas a 8 bp duplication is wild type and has an 8 bp duplication at the same site. The molecular difference between these two alleles is 358 bp deletion in the centre of the hobo element. The differences leading to the two phenotypes could be due to either hobo or vg transcription, which results in a differing length or quantity of vg mRNA.

Two independent molecular events can lead to a wild-type revertant. In the vg*1, vg*1+1 and vg*1+2 revertants, our results suggest a complete excision of the hobo element, whilst in other revertants (like vg*1+2), there is a change in the structure of the hobo element, such as a partial deletion. In the latter case, we cannot exclude the possibility that there was first an excision of the hobo element, and then the insertion of a new deleted hobo element in the same position. However, the vg*1 and the vg*1+2 elements are in the same orientation, tending to argue against an excision and a new insertion. This in turn implies that a partially deleted element is capable of being further deleted, and that there is a part of the hobo element which could be particularly sensitive to deletion, as both vg*1+2 and vg*1+4 seem similar at the molecular level (Fig. 3).

It has been shown that the rearing temperature is an important factor in hybrid dysgenesis systems (P-M and I-R). However, nothing is known regarding the effect of temperature on the occurrence of specific molecular events. We have already shown that breeding temperature can enhance the probability of

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