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Molecular Cloning and Genetic Analysis of the suppressor-of-white-apricot Locus from *Drosophila melanogaster*

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We report genetic and molecular analyses of the suppressor-of-white-apricot [*su(w^a)*] locus, one of several retrotransposon insertion allele-specific suppressor loci in *Drosophila melanogaster*. First, we isolated and characterized eight new mutations allelic to the original *su(w^a)^1* mutation. These studies demonstrated that *su(w^a)* mutations allelic to *su(w^a)^1* affected a conventional *D. melanogaster* complementation group. Second, we cloned the chromosomal region containing the *su(w^a)* complementation group by P element transposon tagging. The ca. 14-kilobase region surrounding the *su(w^a)* complementation group contained five distinct transcription units, each with a different developmentally programmed pattern of expression. Third, we used a modified procedure for P-mediated gene transfer to identify the transcription unit corresponding to *su(w^a)* by gene transfer. Fourth, we found that the presumptive *su(w^a)* transcription unit produced a family of transcripts (ranging from ca. 3.5 to ca. 5.2 kilobases) in all developmental stages, tissue fractions, and cell lines we examined, suggesting that the gene is universally expressed.

Several allele-specific suppressor loci are known in *Drosophila melanogaster*. Allelic states of these loci strongly influence the severity of the mutant phenotypes of subsets of mutant alleles at other loci. Most allele-specific suppressor or enhancer loci exert their effects as a result of highly specific interactions with mutations resulting from insertion of specific retrotransposons (10, 17, 23, 30; for a review see reference 19). Furthermore, several allele-specific suppressor loci are known in *Saccharomyces cerevisiae* which specifically interact with the Ty retrotransposon (27).

Retrotransposons are developmentally regulated transcription units. In some cases (and presumably in general) retrotransposons parasitize regulatory information produced by the cellular host (see references 20 and 26 for reviews). While the detailed molecular basis of the highly specific interaction of suppressors with retrotransposon insertion mutations is largely obscure, one attractive interpretation of these various observations is that suppressor and enhancer loci are among the host genes participating in regulating retrotransposon transcription. Equivalently, allele-specific suppressor loci may participate in developmental regulation of fly genes. Thus, allele-specific suppressor loci may present a uniquely favorable opportunity to subject loci producing *trans*-acting transcription factors to genetic and molecular analysis.

Motivated by these considerations, we have undertaken a detailed analysis of one of the allele-specific suppressor mutations in *D. melanogaster*, suppressor-of-white-apricot-one [*su(w^a)^1*)]. The mutation suppressed by *su(w^a)^1* is white-apricot (*w^a*). *w^a* results from an insertion of the copia retrotransposon into the second intron of the white locus primary transcription unit. Substantial evidence exists that the *w^a* copia insertion is necessary for the highly specific action of *su(w^a)* on the *w^a* phenotype (1, 3, 10; see reference 30 for recent, detailed studies).

We report here that the suppressor-of-white-apricot-one affects a conventional complementation group or, equivalently, a conventional genetic locus. We further report the molecular cloning of the unique sequence interval containing this *su(w^a)* genetic locus and experiments establishing the identity of the *su(w^a)* transcription unit and various features of the behavior of this transcription unit.

MATERIALS AND METHODS

Fly strains. Fly strains not described in the text are described in Zachar and Bingham (29) and Zachar et al. (30).

RNA isolation. Unless otherwise specified in the text, flies were reared at temperatures carefully controlled between 24.5 and 25.5°C. RNAs were isolated and oligo(dT)-cellulose fractionated by the methods of Bingham and Zachar (4) and Davison et al. (6).

Northern and Southern gel analysis. Southern gel analysis was performed essentially by the method of Southern (24) as modified by Botchan (5). Sequence probes for Southern gel analysis were double-stranded DNAs ^{32}P labeled by nick translation. Northern gel analysis was performed essentially as described by Maniatis et al. (14). Single-stranded M13 sequence probes for Northern gel analysis were prepared as described by Hu and Messing (9) and used as described by Bingham and Zachar (4). All Northern gel experiments used 5 μg of oligo(dT)-purified RNA per channel.

rp49 is a ribosomal protein gene whose mRNA was used throughout as a control for the amount of polyadenylated RNA in Northern gel channels. This control measurement consisted of running a small, consistent portion of each RNA sample to be analyzed on a second gel (in strict parallel with the experimental gel) and probing a transfer of this second gel with the *Eco*RI-*Hin*dIII fragment containing most of the *rp49* transcription unit (18).

Molecular weight standards for RNA measurements were the following *Drosophila* transcripts (visualized by reprobing Northern filters with the appropriate sequences): copia, white, *Adh*, and *rp49*. We estimate that our size measurements for RNAs of 0.8 to 2.7 kilobases (kb) are accurate to within less than about 5%, while those for RNAs of 2.7 to 5.2 kb could be in error by as much as about 10%. Molecular

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weight standards for Southern gels were various commercially available ladders and restriction digests, and we estimate that our size measurements from these gels are accurate to within less than 5%.

In situ hybridization. In situ hybridization was carried out with nick-translated, tritiated DNA sequence probes as described by Bingham et al. (3).

EMS mutagenesis. Ethyl methanesulfonate (EMS) mutagenesis was carried out by the method of Lewis and Balker (11) except that the EMS concentration was reduced by twofold to allow higher levels of fertility among mutagenized males. Mutagenized $su(w^a)^+w^a$ males were crossed to homozygous $y^2su(w^a)^1w^a$ females, and newly induced $su(w^a)$ mutations were scored by production of females with a suppressed white-apricot eye color phenotype.

X-ray mutagenesis. $su(w^a)^+w^a$ males were mutagenized with ca. 4,000 rads delivered by a cesium-137 source at a rate of ca. 500 rads/min. Mutagenized males were outcrossed to allow scoring for induced mutations as described above for EMS mutagenesis.

P element mutagenesis and transposon tagging. Dysgenic hybrid males were made by crossing homozygous $su(w^a)^+w^a$ females to males from the standard *pi*-2 strain (see reference 2 and references therein). These dysgenic males were outcrossed to allow scoring for induced mutations as described above for EMS mutagenesis.

Recovery of the $su(w^a)^{hd7}$ P element insertion together with contiguous $su(w^a)$ sequences was done as originally described by Bingham et al. (3). Sequences from this initial P element containing phage clone were used to retrieve $su(w^a)$ locus sequences from a library of the Oregon R_{22} wild-type strain. All phage cloning was carried out by conventional procedures with EMBL4 lambda cloning vector (7).

Subcloning. Subclones used as sequence probes were made by using conventional plasmid and M13 cloning vectors (see reference 28 and references therein).

Gene transfer. Germ line gene transfer was carried out essentially by the embryo microinjection procedure of Germeraad (8) as modified by Spradling and Rubin (25) to capitalize on the high germ line-specific transposition rate of the P element (2).

We made several small modifications in this procedure that substantially improved efficiency. First, and most important, we found that control of the ambient relative humidity at which mechanical dechorionation is done is crucial. When the relative humidity is quite low (well below 20%), survival of injected embryos to hatching drops sharply to as low as a few percent in extreme cases. In contrast, when the ambient relative humidity is maintained at very high levels (in excess of 80%), survival is very reproducibly high. We recommend that dechorionation be done in a relatively small room that can be conveniently humidified to high levels with one of the inexpensive, domestic ultrasonic humidifiers widely available. It is also important to carefully control the extent of preinjection desiccation of embryos. The optimal extent of desiccation is slight and is easily determined by a small number of trials. (Conventional chemical dechorionation procedures [50% bleach, 0.7% NaCl, 0.1% Triton X-100; 30 to 90 s of treatment, followed by washing in 0.7% NaCl–0.1% Triton] produce acceptable survival to hatching (ca. 30%) largely independently of the ambient relative humidity. However, in our hands, chemical dechorionation reduced 10- to 100-fold the rates of G_0 rosy expression and of successful gene transfer compared with mechanical dechorionation.) Second, we found that a thin strip (ca. 0.5 mm) of rubber cement (Carter's brand) had reproducibly lower toxicity than other materials widely used for mounting embryos for injection. Strips are conveniently produced by rapidly pulling a microscope slide through a very narrow flow of thinned (commercial thinner) rubber cement streaming from the tip of a pastuer pipette or glass rod. Slides should be cured for a minimum of about 2 h at room temperature before use. Third, mineral oil was significantly less toxic than the more expensive and difficult to obtain halocarbon oils as a covering medium. We used conventional commercial preparations suitable for human ingestion and supplemented coverage (1 to 3 h after beginning incubation to hatching at 18°C) with mineral oil equilibrated at 18°C. This supplementation was necessary to prevent the less viscous mineral oil from settling and uncovering the embryos. Using this modified procedure, we routinely and very reproducibly observed survival to hatching of 35 to 40% (occasionally higher) for our *ry cn* strain. Moreover, with this procedure we commonly observed rates of G_0 ry^+ expression of 40 to 60% and frequencies of fertile G_0 adults producing transformed G_1 progeny of 10 to 20%.

Gene transfer constructions contained sequences from the $su(w^a)^+$ allele from the Oregon R_{22} strain as diagrammed in Fig. 3. In all experiments the gene transfer vector was the ry^+-marked derivative of the original P element containing plasmid p6.1 (2, 21), Carnegie 20 (22). The initial recipient strain was an *ry cn* double mutant outcrossed to the vigorous Oregon R_{22} strain to produce a relatively robust strain.

The *SWAP1* gene transfer construction contained the *Xho*I-*Sal*I $su(w^a)$ segment diagrammed in Fig. 3 inserted into the *Sal*I site in the Carnegie 20 polylinker in the orientation juxtaposing the $su(w^a)$ *Xho*I site to the polylinker *Hpa*I site. The *SWAP17* gene transfer segment contained the segment extending from the *Sal*I site at coordinate 0.0 to an *Nru*I site at coordinate −6.2 kb (Fig. 3) inserted into the Carnegie 20 polylinker so that the $su(w^a)$ *Nru*I site (rendered flush by treatment with the Klenow fragment of DNA polymerase I) was ligated to the polylinker *Hpa*I site and the $su(w^a)$ *Sal*I site to the polylinker *Sal*I site. The *SWAP2* gene transfer construction contained the $su(w^a)$ *Hin*dIII-*Xho*I fragment (Fig. 3) inserted into the Carnegie 20 polylinker so that the $su(w^a)$ *Hin*dIII site (rendered flush as above) was ligated to the polylinker *Hpa*I site and the $su(w^a)$ *Xho*I site was ligated to the polylinker *Sal*I site.

The capacity of a gene transfer segment to confer $su(w^a)^+$ genetic function was assayed as follows. Males from transformed strains in which the gene transfer segment resided on an autosome were crossed to females homozygous for the w^a mutation and the $su(w^a)$ mutant alleles indicated in the text [both white and $su(w^a)$ are X-linked]. Parallel control crosses with nontransformed *ry cn* males produced exclusively male offspring having a suppressed white-apricot eye color phenotype [phenotypically $su(w^a)$], as did crosses in which the male parent carried the *SWAP2* gene transfer segment. In crosses in which the male parent was homozygous for the *SWAP1* or *SWAP17* construction, all of the male offspring had a normal white-apricot eye color phenotype [phenotypically $su(w^a)^+$]. Crosses in which the male parent was heterozygous for the *SWAP1* or *SWAP17* construction produced male offspring that were phenotypically $su(w^a)^+$ and $su(w^a)$ in approximately equal proportions.

RESULTS

Genetic analysis of suppressor-of-white-apricot. The original observation ultimately leading to the studies of allele-

specific suppressors in all organisms was the discovery of dominant, transposon-specific suppressors in maize by McClintock (15, 16). These maize suppressors map to transposon copies. In light of these observations, the possibility must be considered that *D. melanogaster* suppressors have a related origin. It has been pointed out previously that the recessive nature of the *Drosophila* suppressors argues against the hypothesis that suppressor loci are themselves transposons (17). This observation is indeed highly suggestive but does not, of course, unambiguously resolve the issue.

At the outset of our studies a single suppressor-of-white-apricot [$su(w^a)$] mutation was available, $su(w^a)^1$. The hypothesis that this mutation affects an authentic fly gene (rather than a transposon) predicts that it will be possible to isolate at high frequency new mutations that both are allelic to $su(w^a)^1$ and map at the locus of $su(w^a)^l$. We demonstrated this prediction to be fulfilled by isolating and characterizing eight new recessive mutations allelic to $su(w^a)^l$. Four of these were from ca. 40,000 EMS-mutagenized male gametes (superscript allele designations *D5*, *DM17*, *A12*, and *SD10*), two were from ca. 40,000 X-ray-mutagenized male gametes (superscript allele designations *γ44* and *γ107*), and two were from ca. 80,000 PM dysgenic hybrid male gametes (superscript allele designations *hd7* and *hdM8*) (see Materials and Methods for description of mutagenesis). First, each of the mutations arising in PM dysgenic hybrids or after EMS mutagenesis was subjected to meiotic mapping analysis, and each mapped to the region of the distal X containing the $su(w^a)^l$ mutation (0.0 to 1.5 map units). (For each mutation analyzed, two to six $y^l su(w^a)^+ w^a$) crossovers were observed among 300 to 1,000 male progeny of females of genotype $y^l su(w^a)^+ w^{11E4}/y^+ su(w^a)^x w^a$, where $su(w^a)^x$ is any of the new mutations in question. In the same samples of progeny, no $y^+ su(w^a)^+ w^a$ males were observed.) Second, both of the mutations arising in PM dysgenic hybrids contained P element insertions in the same interval of less than 200 bases in the $su(w^a)$ region (see Results). Last, all eight of these mutations resided in the same ca. 6.2-kb interval containing the $su(w^a)^l$ mutation as assessed by duplication mapping with gene transfer segments as described below (Results).

These results demonstrate that all of the various mutations in our study mapped in the same very small interval containing the $su(w^a)^l$ mutation. This observation, in turn, demonstrated that new $su(w^a)$ mutant alleles can be isolated with the genetic properties and at the frequencies predicted by the hypothesis that these mutations affect a conventional *Drosophila* genetic locus.

Molecular cloning of DNA sequences of the suppressor-of-white-apricot locus. One of the two mutations arising in PM dysgenic hybrids described above, $su(w^a)^{hd7}$, was subjected to additional, detailed analysis. This mutation was unstable in PM dysgenic hybrids [reverting in about 1 in 500 gametes from dysgenic $su(w^a)^{hd7}$ females] but was not measurably unstable when crossed into the P cytotype (no revertants detected among ca. 10,000 progeny). As assessed by in situ hybridization, $su(w^a)^{hd7}$ was associated with a P element labeling site on cytological interval 1D,E that was lost when the mutation reverted (results not shown); earlier genetic mapping experiments placed the $su(w^a)^l$ mutation in cytological interval 1D,E (see reference 12 and references therein). Based on the original characterization of P-induced mutations (2, 21), these results indicate that $su(w^a)^{hd7}$ results from insertion of the P element into $su(w^a)$ sequences.

We used the $su(w^a)^{hd7}$ P element insertion allele to retrieve $su(w^a)$ DNA sequences by P element transposon tagging (2, 3) (see Materials and Methods). One phage-cloned segment [$\lambda su(w^a)^{hd7}10$] hybridizing to the $su(w^a)$ region of M chromosomes (such hybridization is due to P-contiguous sequences in the cloned segment) was found among a set of 40 P element-homologous phages taken from a $su(w^a)^{hd7}$ library (results not shown). This phage contained a P element copy approximately 0.9 kb in size, and subcloned segments of P element-contiguous sequences from the phage hybridized in situ to the $su(w^a)$ region (1D,E, Fig. 1) (results not shown). Genomic Southern gel analysis demonstrated that this cloned P element insertion was present in the parental $su(w^a)^{hd7}$ allele and was lost in each of two independently occurring revertants of $su(w^a)^{hd7}$ analyzed (Fig. 2) (results not shown). (Our results demonstrate that these revertants contained P element residues of less than ca. 50 bases and suggest that reversion results from precise excision of the $su(w^a)^{hd7}$ P element insertion.)

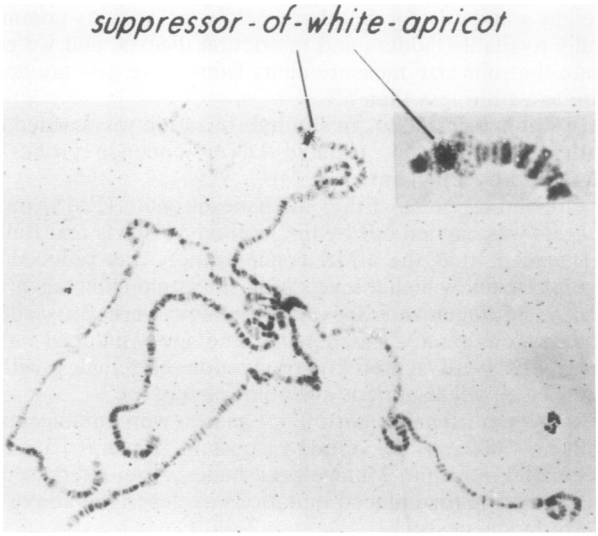

FIG. 1. In situ hybridization of $su(w^a)$ subclone of *D. melanogaster* (Oregon R_{22}) polytene salivary gland chromosomes. The plasmid subclone used as a sequence probe corresponded to the *Xho*I-*Sal*I $su(w^a)$ locus fragment extending from coordinates −7.1 to 0.0 kb (see Fig. 3 for coordinate system). Shown are a low-magnification, bright-field photograph of an entire nucleus and (inset) a high-magnification, phase-contrast photograph of the distal portion of an X chromosome. Sequences homologous to the cloned segment were present at a position indistinguishable from the cytological locus of $su(w^a)$ as assessed by conventional deletion mapping (1D,E; see reference 12 and references therein).

Collectively, these results demonstrate that $\lambda su(w^a)^{hd7}10$ carried the P element insertion responsible for the $su(w^a)^{hd7}$ mutation together with $su(w^a)$ region sequences contiguous to the insertion. We used the P element-contiguous sequences from $\lambda su(w^a)^{hd7}10$ to retrieve a ca. 14-kb interval surrounding the site of the $su(w^a)^{hd7}$ P element insertion from the Oregon R_{22} wild-type strain (Fig. 3). We found this entire interval to consist of unique sequences present only in the 1D,E region (Fig. 1 and 2) (results not shown).

Transcription of the suppressor-of-white-apricot region: The ca. 14-kb $su(w^a)$ interval surrounding the $su(w^a)^{hd7}$ P element insertion contained at least five distinct transcription units (Fig. 3 and 4). Two of these five transcription units (A and D, Fig. 4) appeared to be expressed continuously throughout development, whereas transcription units B, C, and E had more complex developmental patterns (Fig. 4).

The A transcription unit produced a complex array of at

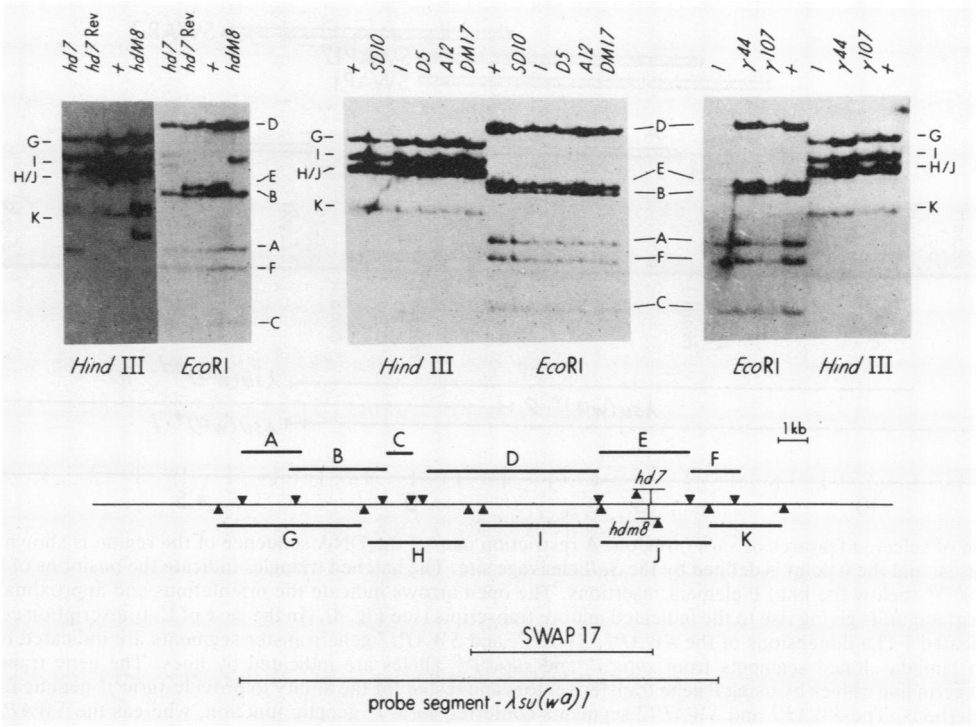

FIG. 2. Southern gel analysis of various $su(w^a)$ alleles. Genotypes of flies from which DNAs were extracted are indicated above each channel by the superscript to $su(w^a)$ that identifies them in the text. DNAs were digested with *Hin*dIII or *Eco*RI as indicated. Shown below the gels is an *Eco*RI (▼) and *Hin*dIII (▲) restriction map of the $su(w^a)$ region surrounding the sites of insertion of the P elements [indicated by labeled lines connected to the $su(w^a)$ map] responsible for the $su(w^a)^{hd7}$ and $su(w^a)^{hdM8}$ mutations. The restriction fragments (A to K) are indicated in the gel panels (note that *Hin*dIII fragments H and J comigrated in these experiments). The dimensions of the $\lambda su(w^a)^1$ cloned segment used as the sequence probe and the *SWAP17* gene transfer segment (see Fig. 3) are indicated. In the middle and rightmost panels, the bottom half of the photograph was exposed about twice as long as the top half to facilitate visualization of small fragments. [The $su(w^a)^{hd7}$ DNA used was isolated from the specific stock yielding the revertant; this particular stock contained an approximately equal mixture of the original $su(w^a)^{hd7}$ allele and a mutant derivative of $su(w^a)^{hd7}$ containing a deletion internal to the P element insertion (our unpublished results). This led to the presence of two bands, each of stoichiometry 0.5, in place of a single band of stoichiometry 1 in the $su(w^a)^{hd7}$ channels. Presumably, either of these two related mutant alleles could give rise to revertants.]

least three partially overlapping mature transcripts, ranging in size from ca. 3.5 to ca. 5.2 kb (Fig. 4 and 5). Each of these mature transcripts was homologous to cloned segments dispersed throughout the A transcription unit (Fig. 5), suggesting that the complexity of the array results from production of substantially overlapping mature transcripts by some combination of differential initiation, termination, and processing of primary transcripts of the region. (The detailed analysis of the structures of this family of transcripts will be the subject of a later report.)

The two P element insertion mutations at $su(w^a)$ described above [$su(w^a)^{hd7}$ and $su(w^a)^{hdM8}$] occurred at a site(s) near the beginning of the A transcription unit and the end of the B transcription unit. We investigated the effects of these two mutations on transcription of the region and found that they exerted discernible effects only on the A transcription unit (Fig. 4). Both of these mutations drastically reduced the levels of the 5.2- and 4.6-kb mature A transcripts and, in some samples, slightly reduced the levels of the minor class of ca. 3.5-kb mature A transcripts. These observations suggest that the A transcription unit is the $su(w^a)$ transcription unit.

The A transcription unit was expressed at all developmental stages examined in our initial analysis (Fig. 5). This observation suggested that this transcription unit might be very widely expressed. We investigated this possibility further by assessing A transcript levels from various sources. We found that the A family of transcripts was produced in all of the following: adult heads, adult thorax abdomens, anterior-half pupae, posterior-half pupae, and the Kc_0 and Schneider line 2 *D. melanogaster* tissue culture cell lines (Fig. 5). Moreover, quantification of levels of the A transcript family (normalizing to the levels of the polyadenylated *rp49* transcript; see Materials and Methods) demonstrated that these transcripts were produced in all stages, tissue fractions, and cell lines we examined at levels differing by about threefold or less (Fig. 5).

High-resolution mapping of $su(w^a)$ gene sequences by gene transfer. The effects of the $su(w^a)^{hd7}$ and $su(w^a)^{hdM8}$ insertions on levels of transcripts from the A transcription unit (see above) strongly suggested that A was the $su(w^a)$ transcription unit. We tested this hypothesis further by duplication mapping with small chromosomal segments introduced by P-mediated gene transfer (see Materials and Methods for description of methodological improvements in gene transfer procedures). We found that two chromosomal segments containing the A transcription unit conferred a $su(w^a)^+$ eye color phenotype on w^a individuals carrying each tested $su(w^a)$ mutant allele (superscript allele designations *1*, *D5*, *SD10*, *DM17*, *A12*, *γ44*, *γ107*, *hd7*, and *hdM8*). One of these two segments (the *SWAP17* segment) extended from coordinates −6.2 to 0 kb and contained only the A transcription unit. The second of these segments (*SWAP1*) extended from coordinates −8.0 to 0 kb and contained both the A transcrip-

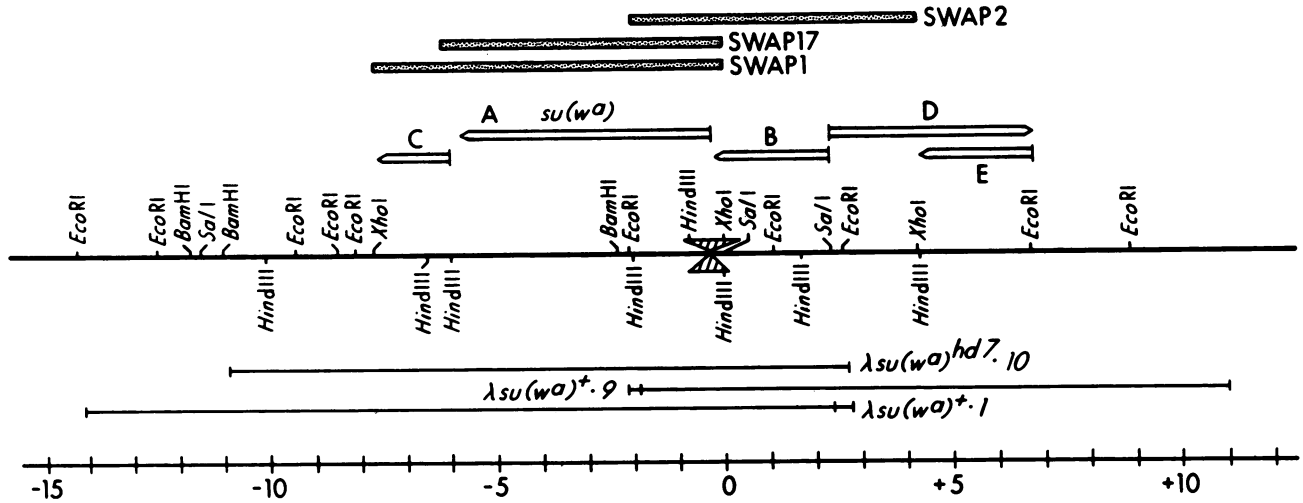

FIG. 3. Diagram of selected features of *su(w^a)* region. A restriction map of the DNA sequence of the region is shown. The scaling grid is calibrated in kilobases, and the 0 point is defined by the *Sal*I cleavage site. The hatched triangles indicate the positions of the $su(w^a)^{hd7}$ (above the line) and $su(w^a)^{hdM8}$ (below the line) P element insertions. The open arrows indicate the orientations and approximate maximal sizes of the primary transcription units giving rise to the indicated mature transcripts (see Fig. 4). [In the case of E, transcription could begin rightward of the interval indicated.] The dimensions of the *SWAP1*, *SWAP2*, and *SWAP17* gene transfer segments are indicated by the stippled bars. The dimensions of lambda cloned segments from $su(w^a)^+$ and $su(w^a)^{hd7}$ alleles are indicated by lines. The gene transfer segments were introduced into the germ line of flies by using P gene transfer vectors and tested for the ability to provide $su(w^a)^+$ genetic function as described in Materials and Methods. The *SWAP1* and *SWAP17* segments conferred $su(w^a)^+$ genetic function, whereas the *SWAP2* segment did not.

tion unit and some or all of the C transcription unit. Furthermore, a segment (*SWAP2*) extending from coordinates −1.8 to +4.5 kb and containing the B transcription unit but only about one-third of the A transcription unit did not restore a $su(w^a)^+$ eye color phenotype in individuals carrying any of the nine *su(w^a)* mutant alleles listed above. These results are summarized in Fig. 3.

These experiments demonstrated that the 6.2-kb chromosomal segment approximately coextensive with the A primary transcription unit(s) and containing none of the other transcription units in the region carried the *su(w^a)* gene. This observation and the observation that the $su(w^a)^{hdM8}$ and $su(w^a)^{hd7}$ P element insertions specifically affected the A transcription unit identified the A transcription unit as the presumptive *su(w^a)* gene.

Evidence that viable *su(w^a)* mutations are leaky mutations. The question arose whether *su(w^a)* was an essential gene. All of the mutations isolated in the genetic studies described here were viable in homozygous females and hemizygous males, in spite of their having been isolated in females heterozygous for the viable $su(w^a)^l$ allele, which should, in the simplest case, support the viability of any recessive lethal *su(w^a)* allele produced by mutagenesis. The viability of all isolated mutations thus suggests that *su(w^a)* is not mutable to a lethal allelic state and is not an essential gene. However, several observations suggest that judgment should be reserved on this issue.

First, the majority class of presumptive *su(w^a)* mutations isolated from among progeny of PM dysgenic hybrids (four of six from among ca. 80,000 gametes) and a minority class of such mutations isolated after EMS mutagenesis (three of seven from among ca. 40,000 gametes) drastically reduced the viability of heterozygous females (life expectancy is a few days and only a few eggs are produced). As a result, these presumptive mutations are, in effect, dominant female sterile mutations under the conditions of isolation and are not recovered. Though this class of mutations could arise from any of a number of sources, it conceivably represents a lethal class of *su(w^a)* mutant alleles.

Second, the two viable P element insertion *su(w^a)* mutant alleles recovered appeared to result from insertion into sequences not incorporated into mature RNAs, based on the observation that all transcript classes produced by $su(w^a)^+$ alleles were produced (although at much reduced levels) by these two mutant alleles (Fig. 4; results not shown). Thus, though a large preponderance of the *su(w^a)* primary transcription unit consisted of presumptive exon sequences, as judged by the relationship between the size of the mature transcripts and the maximal size of the primary transcription unit (see above), the two viable P element insertion alleles apparently did not result from exon insertions. An attractive interpretation of this observation is that P element insertions in *su(w^a)* exon sequences are not recovered by our mutagenesis scheme. (Such exon insertions might, for example, correspond to the class of new presumptive P insertion mutant alleles that are not recoverable, as discussed in the preceding paragraph.)

Lastly, X-rays (frequently) and EMS (occasionally) produce chromosome rearrangement mutations. These include, primarily, small and large deletions and inversions (see, for example, references 6 and 29). In spite of this, all of the X-ray- and EMS-induced *su(w^a)* mutations we isolated, as well as the X-ray-induced $su(w^a)^l$ mutation isolated previously, were not associated with mutational changes detectable by restriction mapping (Fig. 2) (results not shown). These studies would have detected inversions with one breakpoint in *su(w^a)* or with both breakpoints in *su(w^a)* and separated by at least one *Eco*RI or *Hin*dIII cleavage site [see Fig. 3 for a restriction map of *su(w^a)*]. Furthermore, these studies would have detected deletions of greater than ca. 50 bases of *su(w^a)* sequences. These observations are consistent with the hypothesis that rearrangement mutations destroying *su(w^a)* function are not recovered. On this hypothesis, X-ray- and EMS-induced *su(w^a)* mutations recovered

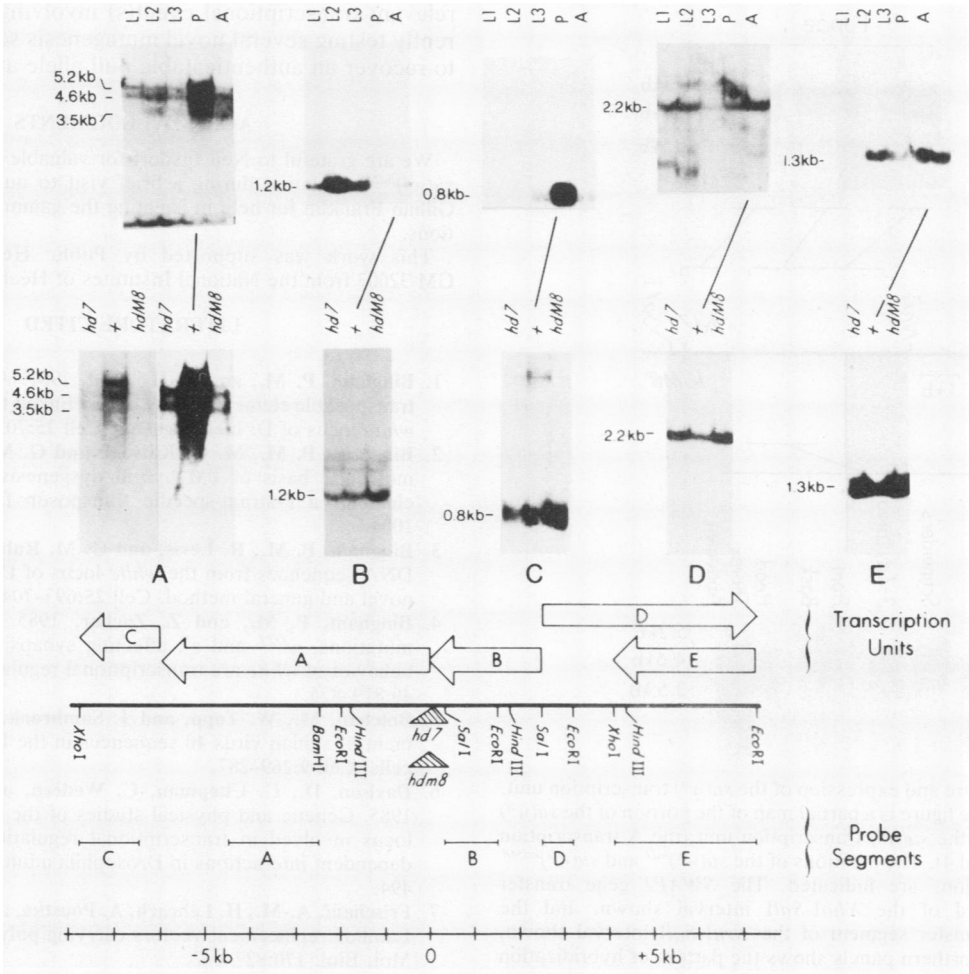

FIG. 4. Northern gel analysis of $su(w^a)$ region transcripts. A restriction map of the central portion of the $su(w^a)$ region and a scaling grid are shown in the bottom third of the figure. The corresponding single-stranded DNA sequence probes, transcription units (direction and approximate maximal dimensions indicated by arrows), and Northern panels are indicated by letters A to E. The positions of the $su(w^a)^{hd7}$ and $su(w^a)^{hdM8}$ P element insertions are indicated by the hatched triangles. In the top row of panels, developmental stages of $su(w^a)^+$ individuals from which RNAs were extracted are indicated above each channel (L1, L2, and L3, 1- to 2-day, 3- to 4-day, and 5- to 6-day old larvae, respectively; P, pupae; A, adults). These filters represent duplicate RNA samples, and the control for the levels of polyadenylated RNA in each channel (described in Materials and Methods) is shown below the leftmost panel. The second row of Northern panels shows the effects of the $su(w^a)^{hd7}$ and $su(w^a)^{hdM8}$ mutations on expression of transcripts from the region. The genotype of individuals donating the RNA is indicated by the appropriate superscript above the channel, and the developmental stage from which the RNAs were isolated is indicated by the line connecting the wild-type channel with the corresponding developmental stage in the top row of Northern panels. [Each of the P insertion mutations also failed to affect L2 levels of the B transcript (results not shown).]

are presumed to consist of small mutational alterations (for example, base substitutions) leading to partial inactivation of the locus.

Collectively, these various observations, while certainly not compelling evidence that $su(w^a)$ is lethal mutable, suggest that judgment should be reserved as to whether $su(w^a)$ is an essential gene until a strategy can be devised allowing the isolation of demonstrably amorphic (null) alleles at the locus.

DISCUSSION

Effective, detailed analysis of *trans*-acting factors participating in the relatively complex regulation and metabolism of transcripts of polymerase II transcription units in metazoans will likely require development of experimental systems in which genes producing such factors are not only identifiable but also accessible to sophisticated genetic manipulation. Allele-specific suppressor loci in *Drosophila* represent one of a very small number of candidates for such experimental systems.

Motivated by these considerations we undertook the analysis of an allele-specific suppressor mutation, $su(w^a)^1$. The results reported here demonstrate that this recessive allele-specific suppressor mutation maps to a conventional, well-behaved complementation group. In turn, we have taken advantage of this observation to clone the DNA sequences corresponding to this $su(w^a)$ complementation group by P element transposon tagging, and our results localized $su(w^a)$ genetic function to a small, unique DNA segment containing a single transcription unit (or an extensively overlapping family of transcription units) producing a complex family of mature transcripts.

An additional, conspicuous feature of our results was the similarity of $su(w^a)$ transcript levels in whole organisms, various tissue fractions, and two tissue culture cell lines.

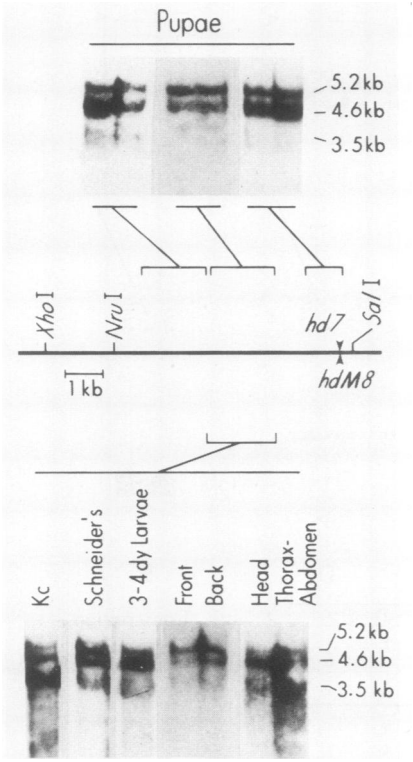

FIG. 5. Structure and expression of the *su(w^a)* transcription unit. At the center of the figure is a partial map of the portion of the *su(w^a)* region containing the *su(w^a)* transcription unit (the A transcription unit; see Fig. 3 and 4). The positions of the $su(w^a)^{hd7}$ and $su(w^a)^{hdM8}$ P element insertions are indicated. The *SWAP1* gene transfer segment consisted of the *Xho*I-*Sal*I interval shown, and the *SWAP17* gene transfer segment of the *Nru*I-*Sal*I interval shown. The top row of Northern panels shows the pattern of hybridization of subsegments of the interval (indicated by the brackets connected to each panel) to the various molecular weight classes of transcripts produced by the region. Each panel of the top row contains a pair of pupal RNA samples. The bottom row of Northern panels shows the expression of the *su(w^a)* family of transcripts in various tissue fractions and cells. The probe segment is indicated by the bracket. Kc and Schneider are two *D. melanogaster* tissue culture cell lines, Front and back refer to the corresponding halves of pupae, and head and thorax-abdomen refer to the corresponding portions of mature adults (Materials and Methods). The various pupal samples in this figure were underexposed by ca. 2.5-fold relative to the other panels.

This observation is most economically interpreted to indicate that the *su(w^a)* transcription unit is expressed at a relatively constant level in all cells in the organism, that is, that *su(w^a)* is a universally expressed gene.

Our studies further suggest that the viable *su(w^a)* mutant alleles currently available might result from leaky mutations. We note that the hypothesis that null alleles of *su(w^a)* are nonrecoverable does not require the supposition that multilocus deletions covering *su(w^a)* be nonrecoverable or that the *su(w^a)* region appear to be haplolethal in segmental aneuploidy studies (see, for example, reference 13). We further note in this regard that the effect of *su(w^a)* mutations on the fate of transcripts of the w^a allele has been shown previously to be relatively small (on the order of twofold [30]). This observation demonstrates that the *su(w^a)* gene product is either incompletely inactivated by the mutations in question or plays only a small, quantitative role in the relevant transcriptional event(s) involving w^a. We are currently testing several novel mutagenesis schemes to attempt to recover an authenticatable null allele at *su(w^a)*.

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LITERATURE CITED

1. **Bingham, P. M., and B. H. Judd.** 1981. A copy of the *copia* transposable element is very tightly linked to the w^a allele at the *white* locus of D. melanogaster. Cell **25**:705–711.
2. **Bingham, P. M., M. G. Kidwell, and G. M. Rubin.** 1982. The molecular basis of PM hybrid dysgenesis: the role of the P element, a P-strain-specific transposon family. Cell **29**:995–1004.
3. **Bingham, P. M., R. Levis, and G. M. Rubin.** 1981. Cloning of DNA sequences from the *white* locus of D. melanogaster by a novel and general method. Cell **25**:693–704.
4. **Bingham, P. M., and Z. Zachar.** 1985. Evidence that two mutations, w^{DZL} and z^1, affecting synapsis-dependent genetic behavior of *white* are transcriptional regulatory mutations. Cell **40**:819–825.
5. **Botchan, M., W. Topp, and J. Sambrook.** 1976. The arrangement of simian virus 40 sequences in the DNA of transformed cells. Cell **9**:269–287.
6. **Davison, D., C. Chapman, C. Wedeen, and P. M. Bingham.** 1985. Genetic and physical studies of the portion of the white locus involved in transcriptional regulation and in synapsis-dependent interactions in Drosophila adults. Genetics **110**:479–494.
7. **Frischauf, A.-M., H. Lehrach, A. Poustka, and N. Murray.** 1983. Lambda replacement vectors carrying polylinker sequences. J. Mol. Biol. **170**:827–842.
8. **Germeraad, S.** 1975. Induction of genetic alterations in Drosophila by injection of DNA into embryos. Genetics **80**: 534–535.
9. **Hu, N., and J. Messing.** 1982. The making of strand-specific M13 probes. Gene **17**:271–277.
10. **Levis, R., K. O'Hare, and G. M. Rubin.** 1984. Effects of transposable element insertions on RNA encoded by the *white* gene of Drosophila. Cell **38**:471–480.
11. **Lewis, E. B., and F. B. Balker.** 1968. Method of feeding ethyl methansulfonate (EMS) to Drosophila males. Drosophila Information Serv. **43**:193.
12. **Lindsley, D. L., and E. H. Grell.** 1968. Genetic variations of *Drosophila melanogaster*. Publication 627. Carnegie Institution, Washington, D.C.
13. **Lindsley, D. L., L. Sandler, B. B. Baker, A. T. C. Carpenter, R. E. Denell, J. C. Hall, P. A Jacobs, G. L. G. Miklos, B. K. Davis, R. C. Gethman, R. W. Hardy, A. Hessler, S. M. Miller, H. Nozawa, D. M. Parry, and M. Gould-Somero.** 1972. Segmental aneuploidy and the genetic gross structure of the Drosophila genome. Genetics **71**:157–184.
14. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual, p. 202–203. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
15. **McClintock, B.** 1954. Mutation in maize and chromosomal aberrations in Neurospora. Carnegie Inst. Washington Year Book **53**:254–260.
16. **McClintock, B.** 1956. Controlling elements and the gene. Cold Spring Harbor Symp. Quant. Biol. **21**:197–216.
17. **Modolell, J., W. Bender, and M. S. Meselson.** 1983. D. melanogaster mutations suppressible by the suppressor-of-Hairy-wing are insertions of a 7.3 kb mobile element. Proc. Natl. Acad. Sci. USA **80**:1678–1682.
18. **O'Connell, P., and M. Rosbash.** 1984. Sequence, structure and

codon preference of the Drosophila ribosomal protein 49 gene. Nucleic Acids Res. **12**:5495–5513.

19. **Parkhurst, S. M., and V. G. Corces.** 1986. Retroviral elements and suppressor genes in Drosophila. BioEssays **5**:52–57.
20. **Roeder, G. S., and G. R. Fink.** 1983. Transposable elements in yeast, p. 299–328. *In* J. Shapiro (ed.), Mobile genetic elements. Academic Press, Inc., New York.
21. **Rubin, G. M., M. G. Kidwell, and P. M. Bingham.** 1982. The molecular basis of PM hybrid dysgenesis: the nature of induced mutations. Cell **29**:987–994.
22. **Rubin, G. M., and A. C. Spradling.** 1983. Vectors for P element-mediated gene transfer in Drosophila. Nucleic Acids Res. **11**:6341–6351.
23. **Searles, L. L., and R. A. Voelker.** 1986. Molecular characterization of the vermilion locus and its suppressible alleles. Proc. Natl. Acad. Sci. USA **83**:404–408.
24. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
25. **Spradling, A. C., and G. M. Rubin.** 1982. Transposition of cloned P elements into Drosophila germ line chromosomes. Science **218**:341–347.
26. **Varmus, H. E.** 1983. Retroviruses, p. 411–505. *In* J. Shapiro (ed.), Mobile genetic elements. Academic Press, Inc., New York.
27. **Winston, F., D. T. Chaleff, B. Valent, and G. R. Fink.** 1984. Mutations affecting Ty-mediated expression of the *HIS4* gene of Saccharomyces cerevisiae. Genetics **107**:179–197.
28. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequence of M13mp18 and pUC19 vectors. Gene **33**:103–119.
29. **Zachar, Z., and P. M. Bingham.** 1982. Regulation of *white* locus expression: the structure of mutant alleles at the *white* locus of Drosophila melanogaster. Cell **30**:529–541.
30. **Zachar, Z., D. Davison, D. Garza, and P. M. Bingham.** 1985. A detailed developmental and structural study of the transcriptional effects of insertion of the *copia* transposon into the *white* locus of Drosophila melanogaster. Genetics **111**:495–515.