A DETAILED DEVELOPMENTAL AND STRUCTURAL STUDY OF THE TRANSCRIPTIONAL EFFECTS OF INSERTION OF THE *COPIA* TRANSPOSON INTO THE WHITE LOCUS OF *DROSOPHILA MELANOGASTER*

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Manuscript received May 7, 1985
Revised copy accepted July 17, 1985

ABSTRACT

The *copia* insertion responsible for the w^a mutation is 3′ to the white promotor and in the same transcriptional orientation as white. First, we have analyzed the effects of the w^a *copia* insertion on levels of polyadenylated white transcripts and find large, developmentally programmed effects. Second, we have isolated and sequenced an LTR-excision event involving the *copia* insertion at w^a. This represents the first documented case of an LTR-excision event in Drosophila. This single *copia* LTR has developmentally programmed effects on white transcript levels qualitatively similar to the intact *copia* element. Third, we have characterized the structures of white transcripts from w^a. We find polyadenylated white transcripts apparently having 3′ termini in or near the 3′ LTR of the w^a *copia* insertion, as has been reported in limited studies of w^a transcription in adults by others. These earlier studies also revealed w^a transcripts apparently corresponding to polyadenylated terminus formation in the 5′ LTR of the *copia* transposon; however, our more detailed studies reveal that these transcripts probably have other origins and that little, if any, polyadenylated terminus formation for white transcripts occurs in the 5′ LTR of the w^a *copia* insertion. Moreover, we find no polyadenylated terminus formation for *white* transcripts occurring in the single LTR of the w^a LTR-excision product. Fourth, we find that each of three mutant alleles at $su(w^a)$ produces elevated levels of several classes of RNAs apparently corresponding to transcriptional readthrough of the w^a *copia* transposon. Elevated levels of one presumptive readthrough transcript were observed previously in one $su(w^a)^1$ mutant strain. Fifth, we have confirmed the existence of a transcript initiated in the 3′ LTR of the w^a *copia* insertion and find the levels of this transcript to be strongly influenced by developmental stage and genetic background. Lastly, we have analyzed white transcripts produced by the $w^{hd81b11}$ allele, which carries an insertion of *copia* in the opposite transcriptional orientation and in a different position than the w^a *copia* insertion. In contrast to the w^a *copia* insertion allele, the $w^{hd81b11}$ allele produces polyadenylated white transcript levels very similar to the w^+ case at the stages examined. Moreover, the $w^{hd81b11}$ *copia* element apparently produced polyadenylated terminus formation in white transcripts and we observe no effect of the allelic state of $su(w^a)$ on apparent readthrough of this stop site. We discuss some possible implications of our results for the properties of retrotransposons as developmentally complex insertional mutagens and for the functional organization of the *copia* transcription unit.

SEVERAL retroviruses and retrotransposons in Drosophila, vertebrates and yeast behave as developmentally regulated transcription units (SCHWARTZ, LOCKETT and YOUNG 1982; SCHERER *et al.* 1982; VARMUS 1983; ERREDE *et al.* 1980). These elements appear to directly parasitize regulatory information produced by the cellular host in several well-characterized cases and, probably, in general (ERREDE *et al.* 1980; VARMUS 1983; ROEDER and FINK 1983; WINSTON *et al.* 1984). Thus, retrotransposons represent a useful opportunity to study developmental regulation of transcription in their hosts.

As transposons, these elements are capable of inserting into genetically active regions of the host genome. A large fraction of spontaneous mutations in several organisms result from such insertion events (ERREDE *et al.* 1980; ZACHAR and BINGHAM 1982; COPELAND, JENKINS and LEE 1983; BENDER *et al.* 1983; SCOTT *et al.* 1983; GOLDBERG *et al.* 1983; O'HARE, LEWIS and RUBIN 1983; KIDD, LOCKETT and YOUNG 1983; ROEDER and FINK 1983), and such mutant alleles represent an opportunity to study the local interaction of two developmentally programmed transcription units.

In yeast and in Drosophila, suppressor loci exist that have allelic states that strongly influence the phenotypes produced by mutant alleles resulting from insertion of retrotransposons (GREEN 1959; BINGHAM, LEVIS and RUBIN 1981; BENDER *et al.* 1983; MODOLLEL, BENDER and MESELSON 1983; WINSTON *et al.* 1984). In view of the properties of retrotransposon, suppressor loci are candidates for host genes that have products that participate in regulation, production and metabolism of transcriptional products. Thus, these loci may provide valuable opportunities to isolate and study such genes.

Motivated by the rich potential of retrotransposons for analysis of mechanisms of transcriptional regulation, we have investigated the effects of insertion of one such element into the white locus of Drosophila. The w^a and $w^{hd81b11}$ alleles at white result from the insertion of the *copia* retrotransposon (BINGHAM and JUDD 1981; BINGHAM, LEVIS and RUBIN 1981; RUBIN, KIDWELL and BINGHAM 1982). While this work was in progress, two independent reports were published demonstrating that the w^a *copia* insertion is in an intervening sequence of the white transcription unit and that white transcripts probably terminate in this insertion with high frequency in adult tissues (PIRROTTA and BROCKL 1984; LEVIS, O'HARE and RUBIN 1984). We have substantially extended these earlier structural analyses, and our results have several unexpected implications for the behavior of *copia* LTRs as terminators. Further, we have characterized the developmental pattern of accumulation of w^a transcripts, and our results suggest that *copia* influences expression of a nearby promotor. In addition, we report the results of analysis of transcripts from an LTR-excision derivative of w^a and from the w^a allele in the presence of several mutant alleles at the suppressor-of-white-apricot locus. Lastly, we report results of analysis of white transcripts produced by the $w^{hd81b11}$ *copia* insertion allele.

MATERIALS AND METHODS

Flies: Description of fly strains used, other than the w^a-1A strain, can be found in LINDSLEY and GRELL (1968); BINGHAM and JUDD (1981); BINGHAM, LEVIS and RUBIN (1981); ZACHAR and

BINGHAM (1982); and LEVIS, O'HARE and RUBIN (1984). Eye color phenotypes were scored in flies which were cultured at 25°. The w^a-1A strain is a w^a strain derived from a P-M dysgenic hybrid, for which the female parent was a w^a *M* strain and the male parent was the *pi*-2 *P* strain; the strain was carried as a bottle stock for about 20 generations before the experiments described here were carried out.

Cloned DNA sequences: White locus DNA sequences were originally cloned by BINGHAM, LEVIS and RUBIN (1981). All sequence probes used herein were derived from these cloned segments and from cloned segments of various white alleles retrieved by using these cloned segments (LEVIS, BINGHAM and RUBIN 1982; ZACHAR and BINGHAM 1982). The DNA sequences corresponding to the w^{aLTR1} allele were retrieved by using a bacteriophage lambda vector (FRISCHAUF *et al.* 1983) and conventional procedures (*e.g.*, see ZACHAR and BINGHAM 1982); the cloned segment retrieved and characterized extended from *ca.* 1.5 kb rightward (toward the centromere) of the *copia* insertion at w^a to *ca.* 10 kb leftward.

DNA sequence determination: DNA sequences were determined essentially as described by SANGER, NICKLEN and COULSON (1977); SANGER and COULSON (1978); and BARNES, BEVAN and SON (1983). For the wild-type sequence, all portions of the sequence were determined in both orientations.

DNA sequence probes: M13 clones used to generate single-stranded white sequence probes were of the appropriate polarity to detect transcripts proceeding from right to left in Figure 2 and have the following dimensions (cleavage sites shown in Figure 2): First exon—from the *Hin*dIII cleavage rightward for *ca.* 800 bases; first intron—from the *Xba*I site to the *Bam*HI site; first intron plus second and third exons—from the *Hin*dIII site leftward to the middle *Sal*I site; second and third exons—from the *Bam*HI site leftward to the first *Sal*I site; third exon—the smaller (rightmost) *Sal*I fragment; last exon—the larger (leftmost) *Sal*I fragment.

The ribosomal protein (rp49) gene probe used is the *Hin*dIII to *Eco*RI fragment containing the 5′ portion of the rp49 transcription unit (O'CONNELL and ROSBASH 1984).

RNA isolation: All flies were cultured at temperatures carefully controlled between 24.5° and 25.5°, except for those in Figure 6. Adults were harvested by etherization, pupae by scraping from sides of glass bottles and larvae by aqueous elution from food. Staging of larvae was achieved by seeding bottles for a 16- or 24-hour period, using large numbers of healthy, young (2–5 days old) adults, followed by removal of adults and culture of the bottles for the appropriate additional time period. Pupal samples represent an approximately continuous mixture of all pupal stages. RNAs were isolated as described (BINGHAM and ZACHAR 1985).

Northern and Southern gel analysis: Nucleic acids were fractionated on 1 or 1.25% formaldehyde-agarose gels and were transferred to nitrocellulose, essentially as described previously (MANIATIS, FRITSCH and SAMBROOK 1982). Single-stranded sequence probes were made essentially as described by HU and MESSING (1982). Hybridization was carried out as described by BINGHAM and ZACHAR (1985). Molecular weight standards for RNAs consisted of previously sized Drosophila RNAs: the *copia*, *Adh* and rp49 transcripts. Molecular weight standards for the DNA fragments in Figure 3B consisted of *Hpa*II, *Sal*I + *Pst*I and *Ava*II + *Bam*HI digests of pBR322.

S_1 protection: S_1 protection was carried out as described in BINGHAM and ZACHAR (1985). In addition, each hybridization reaction (*ca.* 30 μg of polyadenylated RNA) contained *ca.* 50 ng of the *Eco*RI + *Hin*dIII fragment of the rp49 gene. One-twentieth of each completed S_1 reaction was run on a separate gel and was probed with rp49 sequences to assess the accuracy of matching of amounts of RNA. [Reducing or increasing by twofold the RNA inputs into S_1 protection reactions produced corresponding reductions or increases of, approximately, twofold in the intensities of the rp49 protection product. This control experiment has been carried out using pupal and adult polyadenylated RNAs, and it demonstrates that the rp49 protector is present in effective excess (results not shown).] Although experimental RNA inputs were sometimes mismatched by as much as twofold based on the rp49 internal control, the samples in the developmental series in the rightmost panel of Figure 3B were well matched. The result of the parallel matching experiment in this case is superimposed on the results with the white protector.

RESULTS

A partial revertant of w^a *is an LTR-excision product*: We isolated a partial revertant of w^a as a y^+ $su(w^a)^+$ $sp1^+/Y$ son of a P-M dysgenic female of genotype

A.
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TGTTGGAATA TACTATTCAA CCTACAAAAA TAACGTTAAA CAACACTACT
TTATATTTGA TATGAATGGC CACACCTTTT ATGCCATAAA ACATATTGTA
AGAGAATACC ACTCTTTTTA TTCCTTCTTT CCTTCTTGTA CGTTTTTTGC
TGTGAGTAGG TCGTGGTGCT GGTGTTGCAG TTGAAATAAC TTAAAATATA
AATCATAAAA CTCAAACATA AACTTGACTA TTTATTTATT TATTAAGAAA
GGAAATATAA ATTATAAATT ACAACA
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B.

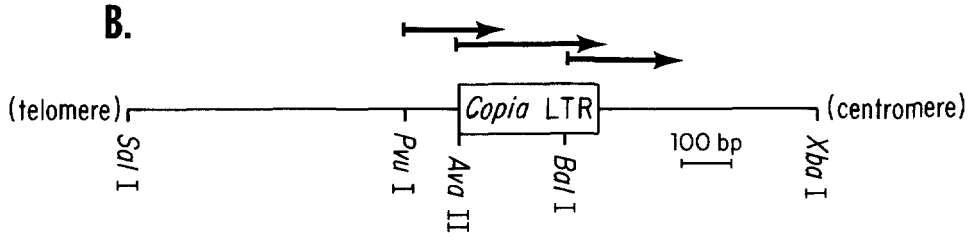


FIGURE 1.—DNA sequence analysis of the w^{aLTR1} allele. Panel A shows the sequence of the *copia* residue in the w^{aLTR1} derivative of w^a (see text). The sequence of this solo LTR is indistinguishable from that of one of the previously sequenced *copia* LTRs (LEVIS, DUNSMUIR and RUBIN 1980). This solo LTR is present as a simple insertion into white sequences and retains the target site duplication from the original copia insertion at w^a (results not shown; O'HARE *et al.* 1984; Figure 2). The two individual bases, in italics, are bases that have been found previously to vary between different copies of the *copia* element (LEVIS, DUNSMUIR and RUBIN 1980). Panel B shows the design of the sequencing strategy. The cleavage sites shown can be related to the white locus map by referring to Figure 2. Sequencing runs began at the *Pvu*I, *Ava*II and *Bal*I site shown and proceeded rightward (toward the centromere). Each run was repeated at least twice, and each overlaps more than 50 bases with the appropriate flanking runs.

w^a/y^2 *su*$(w^a)^1$ w^a *sp1*. We have characterized this derivative by detailed restriction mapping (results not shown) and DNA sequence analysis (Figure 1). We find this allele to contain a single copy of *copia* LTR inserted in white sequences at the insertion point of the w^a *copia* element; the w^a *copia* target site duplication is preserved. We refer to this allele as w^{aLTR1} (or w^{aLTR}). Analogous LTR-excision events are known or thought to occur in the cases of several other retrotransposons [see VARMUS (1983) and ROEDER and FINK (1983) for reviews], and events that may be LTR excisions have been implicated in the reversion of a retrotransposon insertion mutation at the bithorax locus in Drosophila (BENDER *et al.* 1983). By analogy to terminology used to described LTR-excision products of Ty elements, we refer to the single, isolated *copia* LTR in w^{aLTR} as a solo LTR.

We isolated w^{aLTR} during casual inspection of several thousand progeny of dysgenic females. We found no such derivatives among 60,000 progeny of dysgenic w^a/Y males.

Evidence that the copia *insertion at* w^a *is in an intervening sequence*: We have sequenced the portion of the white locus immediately surrounding the *copia* insertion at w^a (from 275 bases to the left to 510 bases to the right of the *copia* insertion; results not shown). The sequence of the region demonstrates that this *copia* insertion does not occur in uninterrupted peptide coding sequences (results not shown; see Figure 2 for a diagram). The insertion is, however, immediately flanked on each side by extended segments of coding sequence, as also was recently reported by O'HARE *et al.* (1984); our sequence

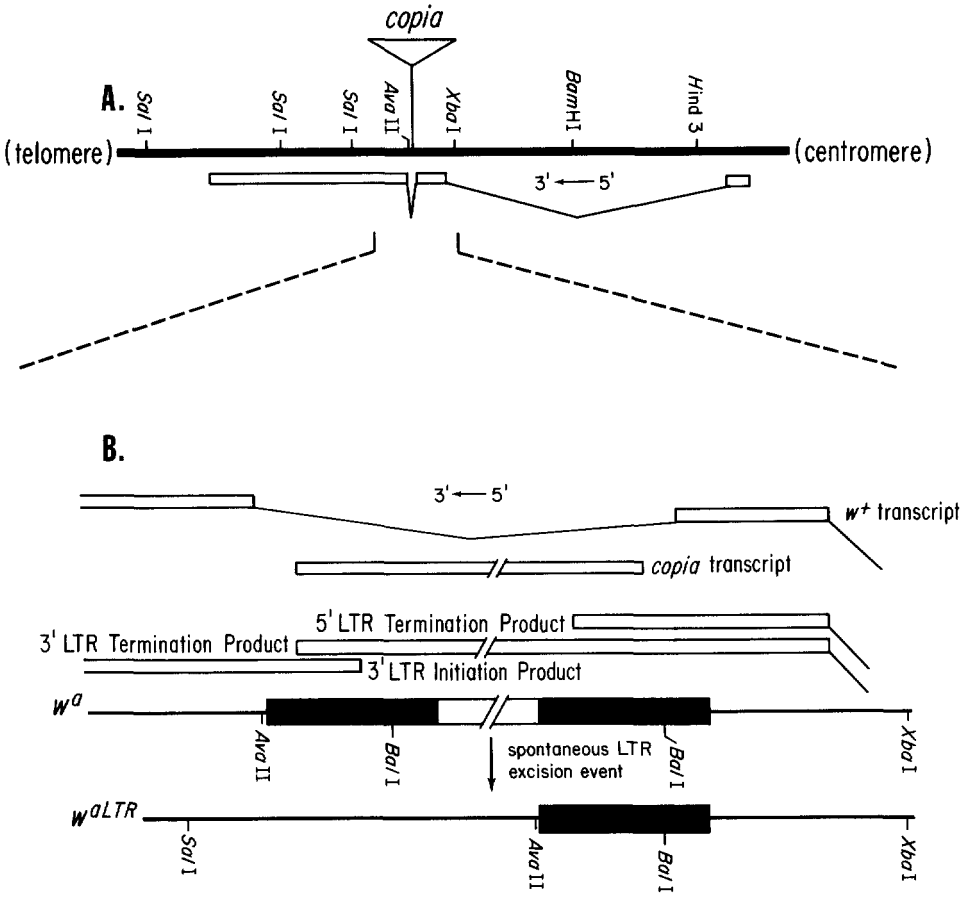


FIGURE 2.—Panel A shows a map of the major w^+ transcript and the surrounding white locus region (PIRROTTA and BROCKL 1984; O'HARE *et al.* 1984; RESULTS). The direction of transcription of both white and *copia* is right to left, and only two of white intervening sequences are shown. (PIRROTTA and BROCKL 1984; O'HARE *et al.* 1984; RESULTS). Also shown is the position of the *copia* insertion in the second white intervening sequence. The direction of transcription of the w^a *copia* transcription unit is likewise right to left; the start site for this transcript has been relatively accurately mapped while the 3′ end of the transcript is not precisely known (BINGHAM, LEVIS and RUBIN 1981; FLAVELL *et al.* 1981; SCHWARTZ, LOCKETT and YOUNG 1982; STELLER and PIRROTTA 1984; PIRROTTA and BROCKL 1984). The distance from the *copia* insertion to the *Hin*dIII site shown is *ca.* 3.1 kb, and from the *copia* insertion to the *Xba*I site is 430 bp. Panel B shows the structures of the w^a and w^{aLTR} alleles and of the appropriate portions of the classes of polyadenylated transcripts that w^a is expected to produce. The boxed portions of the lines representing transcripts correspond to the portion of the primary transcripts expected to be present in mature RNAs; the thin, diagonal portions of these lines represent intervening sequences. The 3′ LTR termination product shown presumably corresponds to the 3′ termination product described in the text.

of this interval agrees precisely with theirs (results not shown). These results and those of S_1 protection analysis (PIRROTTA and BROCKL 1984; Figures 3A and B) demonstrate that the *copia* insertion at w^a is in a small intervening sequence.

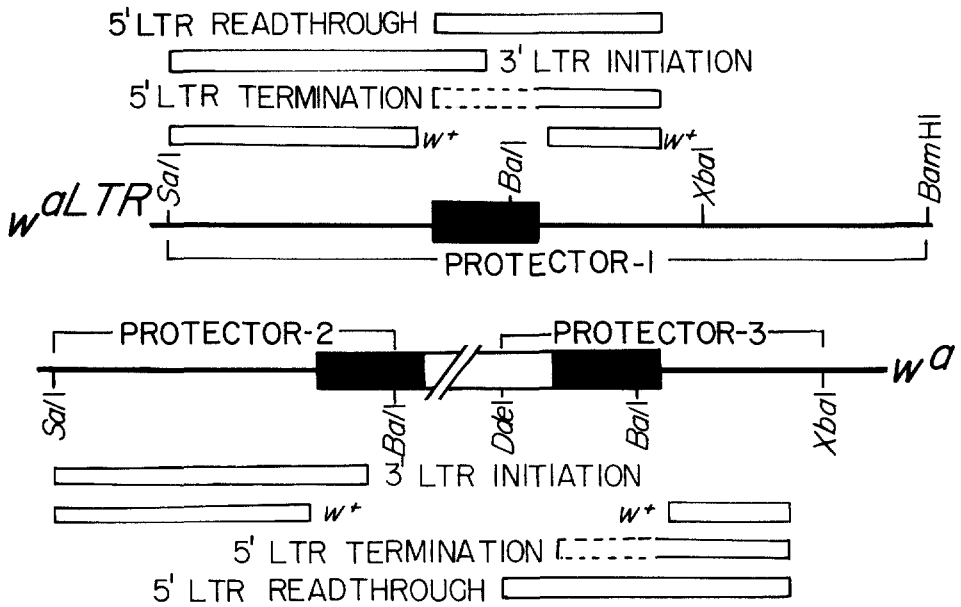


FIGURE 3A.—Structures of S_1 protectors and protection products (see also Figures 1 and 2). Protector-1 contains the *Bam*HI + *Sal*I fragment containing the LTR-excision product of the w^a *copia* insertion. Protector-2 extends from the *Bal*I site in the 3′ LTR of the w^a *copia* insertion to the first white *Sal*I site leftward of the w^a *copia* insertion. Protector-3 extends from the white *Xba*I site leftward to the first *Dde*I site in the w^a *copia* insertion. The dimensions of these protectors are indicated, and each of these M13-cloned fragments is in the appropriate orientation to be homologous to transcripts proceeding from right to left. The expected S_1 resistant products produced by each protector and various w^a transcripts are indicated by the labeled open bars. The site of hypothetical polyadenylated terminus formation for the white transcript in the 5′ LTR is not precisely known, and the maximal uncertainty in the expected size of the corresponding protection product is indicated by the dashed portion of the bar. (Notice that this range of sizes does not overlap with the protection product produced by the intact second white exon.) The 5′ LTR readthrough protection product is produced by all white transcripts reading through the 5′ LTR for protector-1 and by the 5′ LTR plus the first 137 bases of the nonrepeated portion of *copia* for protector-3. Our results suggest that most, and possibly all, of this protection product results from the w^a transcript referred to as the 3′ termination product (see RESULTS). The 276-bp LTR is shown as a solid bar and serves as the scaling object.

These sequence data further strongly suggest that white locus protein coding sequences include the *ca.* 300-base exon immediately 5′ to the w^a *copia* insertion. Thus, the w^a transcript initiating in the 3′ LTR of the w^a *copia* insertion (see RESULTS below) is unlikely to allow the production of a fully functional white polypeptide and is unlikely to contribute to the partial restoration of white function presumably responsible for suppression of the w^a mutant eye-color phenotype (see RESULTS).

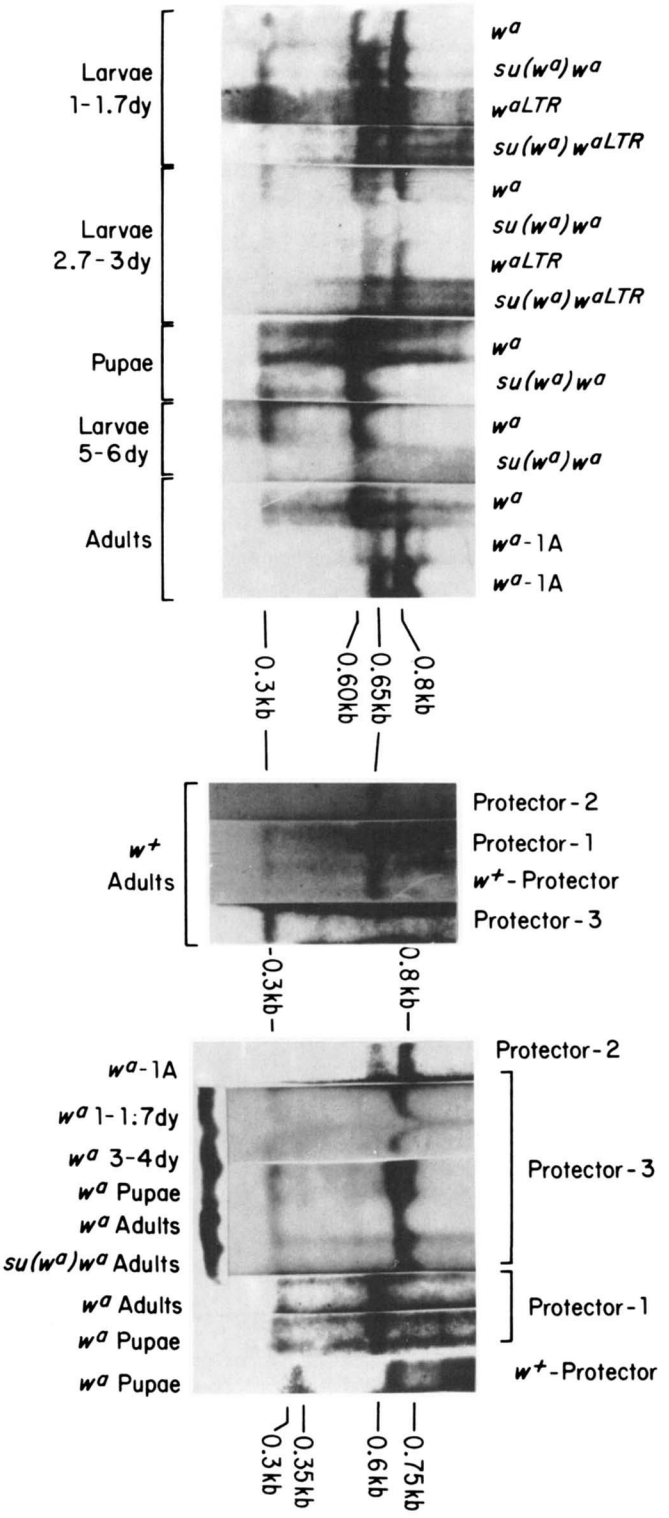
The w^a copia *insertion results in production of new classes of white transcripts terminating or initiating in the* copia *element*: The direction of transcription of the *copia* insertion at w^a is the same as that of the surrounding white transcription unit (BINGHAM, LEVIS and RUBIN 1981; SCHWARTZ, LOCKETT and YOUNG 1982; O'HARE, LEVIS and RUBIN 1983; PIRROTTA and BROCKL 1984). Two

previously published reports include limited analyses of white transcripts produced by w^a adults and also the S_1 protection products generated when these RNAs were used to protect wild-type white DNA sequences (PIRROTTA and BROCKL 1984; LEVIS, O'HARE and RUBIN 1984). In addition to suggesting the existence of low levels of normal white transcripts, these results suggested that white transcripts (initiating 5′ to the *copia* transposon) frequently terminate in both LTRs of the w^a *copia* insertion in adult tissues. (See Figure 2 for a diagram of the various classes of transcripts of the w^a allele expected on this hypothesis.) We have substantially extended the analysis of structures of w^a transcripts.

We have studied the S_1-resistant products obtained by protection of fragments consisting of various portions of the *copia* insertion at w^a together with contiguous white sequences (Figure 3A). Each class of transcript expected to be produced by w^a will produce diagnostic S_1 resistant products with these protectors. Moreover, we have found that some classes of w^a transcripts show developmental or genotypic variation in levels (Figures 4 and 5; see RESULTS), allowing us to look for corresponding diagnostic changes in levels of S_1-resistant products. The results of these studies are as follows:

First, young w^a larvae and old w^a-1A adults produce relatively high levels of a transcript, the molecular weight (*ca.* 2.1 kb) and white homology (Figures 4 and 5; PIRROTTA and BROCKL 1984; LEVIS, O'HARE and RUBIN 1984) of which suggest that it results from transcription initiating in the 3′ LTR of the w^a *copia* insertion and proceeding leftward to produce a mature transcript terminating at the white polyadenylation site. We refer to this transcript as the 3′ LTR initiation product. Other stages and w^a strains examined produce lower levels of this transcript. Our interpretation of this transcript predicts an identical *ca.* 800-base S_1-resistant product, with both protectors 1 and 2 corresponding to *ca.* 130 bases of the 3′ *copia* LTR, *ca.* 30 bases of the second intron and *ca.* 640 bases of the third exon extending from the second intron leftward to the *Sal*I site nearest the w^a *copia* insertion (Figure 3A). A product of the predicted molecular weight and developmental and genotypic dependence is observed (Figure 3B).

Second, a w^a RNA species is observed that has a molecular weight and white homology that suggest it corresponds to a white transcript extending through most of the *copia* element and having a polyadenylated terminus near the 3′ end of the element. Specifically, this transcript is homologous to probes corresponding to the first and second white exons, but not to white probes 3′ to the w^a *copia* insertion, and has a molecular weight (*ca.* 5.8 kb) in good agreement with that expected of a transcript consisting of the first two white exons and the *ca.* 5.2-kb *copia* element (Figures 6 and 7; SCHWARTZ, LOCKETT and YOUNG 1982; PIRROTTA and BROCKL 1984; LEVIS, O'HARE and RUBIN 1984). We observe this species at relatively low levels in larvae and adults and at high levels in pupae. The hypothesis that this transcript extends into *copia* and ends in its 3′ portion predicts a diagnostic S_1-resistant product with protector-1 consisting of the *ca.* 300-base second white exon, *ca.* 50 bases of the second intron and the entire *copia* LTR and totaling *ca.* 630 bases (Figure 3A). In


Larvae 1-1.7dy
Larvae 2.7-3dy
Pupae
Larvae 5-6dy
Adults
w^a
$su(w^a)w^a$
w^{aLTR}
$su(w^a)w^{aLTR}$
w^a
$su(w^a)w^a$
w^{aLTR}
$su(w^a)w^{aLTR}$
w^a
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w^a
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w^a
w^a-1A
w^a-1A
0.3kb
0.60kb
0.65kb
0.8kb
w^+ Adults
Protector-2
Protector-1
w^+-Protector
Protector-3
-0.3kb-
0.8kb-
w^a-1A
w^a 1-1:7dy
w^a 3-4dy
w^a Pupae
w^a Adults
$su(w^a)w^a$ Adults
w^a Adults
w^a Pupae
w^a Pupae
Protector-2
Protector-3
Protector-1
w^+-Protector
0.3kb
0.35kb
0.6kb
0.75kb

addition, protector-3 should produce a fragment corresponding to the resistant product obtained with protector-1 plus the first 137 bases of the nonrepeated portion of the *copia* element; this product should be *ca.* 770 bases in size. We observe products of the appropriate size and developmental stage dependence with protectors 1 and 3 (Figure 3B). The 3′ termination product presumably has its polyadenylated terminus in the 3′ LTR of the *copia* element; however, it is very difficult to design an experiment excluding the possibility that the transcript terminates near, but not in, the 3′ LTR.

Third, several polyadenylated w^a RNA species are observed that have molecular weights (0.8–1.3 kg) that are consistent with their being white transcripts having polyadenylated termini in the 5′ LTR of the w^a *copia* insertion (Figure 4; PIRROTTA and BROCKL, 1984; LEVIS, O'HARE and RUBIN 1984) or in the solo LTR of w^{aLTR} (Figure 4). However, transcripts comigrating with some or all of these are present in non-w^a genotypes (Figure 4; see RESULTS). Moreover, although the levels of the 3′ termination product are quite reproducible, the levels of these putative 5′ LTR termination products are apparently quite sensitive to culture conditions (compare Figures 4 and 6; see RESULTS).

The hypothetical 5′ LTR (or solo LTR) termination product should produce an identical, diagnostic S_1-resistant product with both protectors 1 and 3 (Figure 3A). This resistant product would consist of the *ca.* 300-base second white exon, *ca.* 50 bases of the second intron and some portion of the 5′ LTR and would be between *ca.* 350 and 630 bases in size. [Note that the smallest allowable size for this product is measurably larger than the size of the *ca.* 300-base protected fragment produced by the second white exon alone (Figures 3A and B).] No fragment diagnostic of the 5′ LTR (or solo LTR) termination product is observed at significant intensity in our experiments with w^a or w^{aLTR} RNAs (Figure 3B). These RNAs were prepared under culture conditions producing relatively high levels of transcripts migrating as presumptive 5′ or solo LTR termination products (MATERIALS AND METHODS; Figure 4). [Some minor S_1-resistant products (intensity *ca.* 0.1 of the major product) in the appropriate size range to correspond to a 5′ LTR termination product can be seen on long exposure of experiments with protectors 1 and 3 (results

FIGURE 3B.—S_1 protection analysis of white transcripts. The structures of M13-cloned fragments used as DNA protectors is shown in Figure 3A. Protector-1 was used for all samples in the leftmost panel except for the rightmost channel (the second w^a-1A Adult channel), in which protector-2 was used. S_1 protection reactions were carried out as described in MATERIALS AND METHODS, and each reaction was analyzed on a 1.25% formaldehyde-agarose gel. All transfers were probed with the white *Bam*HI + *Sal*I fragment covering the w^a site, and in the appropriate orientation to detect the DNA protection products (Figure 2). The developmental stages and genotypes of individuals donating the polyadenylated RNAs used are indicated. Experiments with w^a RNA were exposed approximately twice as long as those with w^+ RNA. The RNA inputs to these reactions were generally matched to within about twofold (see MATERIALS AND METHODS). The results of the matching experiment (see MATERIALS AND METHODS) for the set of samples using protector-3 in the right-hand panel are superimposed at the bottom of this portion of the panel; notice that this quantitative protection experiment corroborates the developmental variation in polyadenylated white transcripts seen in the Northern gel experiments in Figure 4.

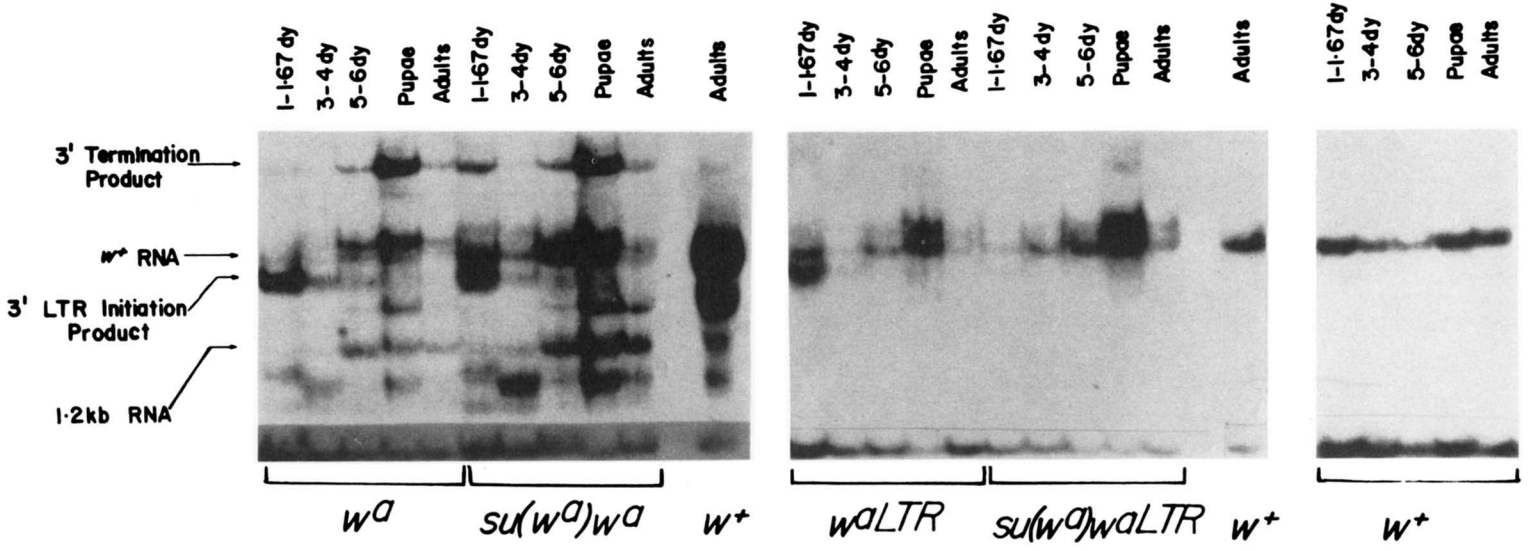

FIGURE 4.—Northern gel analysis of the developmental profiles of polyadenylated transcripts produced by the w^a, w^{aLTR} and w^+ alleles. The genotype and developmental stage of the individuals donating the RNAs is indicated. Stages indicated according to days of age are larval. The gels are 1% formaldehyde-agarose gels, and each channel contains *ca.* 10 μg of polyadenylated RNA (see MATERIALS AND METHODS). Probes were synthesized and hybridization was carried out as described in MATERIALS AND METHODS. The large top portion of each panel shows the white region transcription pattern. The white probe used is the second and third exon probe (see MATERIALS AND METHODS); this probe labels transcripts proceeding into the *copia* insertion from the right, or out of the *copia* insertion toward the left. RNAs corresponding to the product of termination of white transcription in the 3′ portion of the *copia* transcription unit, to the major w^+ transcript, to the product of initiation in the 3′ LTR of the w^a *copia* insertion are indicated, as is the 1.2-kb species discussed in the text (see also Figure 2). The relatively low levels of the 3′ termination product shown for 3–4-day larval material is characteristic of larval material from 3–5 days of age (midlarval stages: results not shown). The leftmost panel was exposed approximately threefold longer than the other panels to show transcripts present at lower levels. We note that $su(w^a)w^{aLTR}$ 1–1.67-day larvae usually show the same high level of the 3′ LTR initiation product as do the other three genotypes; however, the transcript is not present at high levels in the particular experiment shown. The small bottom portion of each panel shows the result of probing duplicate samples (prepared simultaneously and in strict parallel with the samples in the top portion) with rp49 sequences. These panels constitute a control for the amount of polyadenylated RNA in each channel.

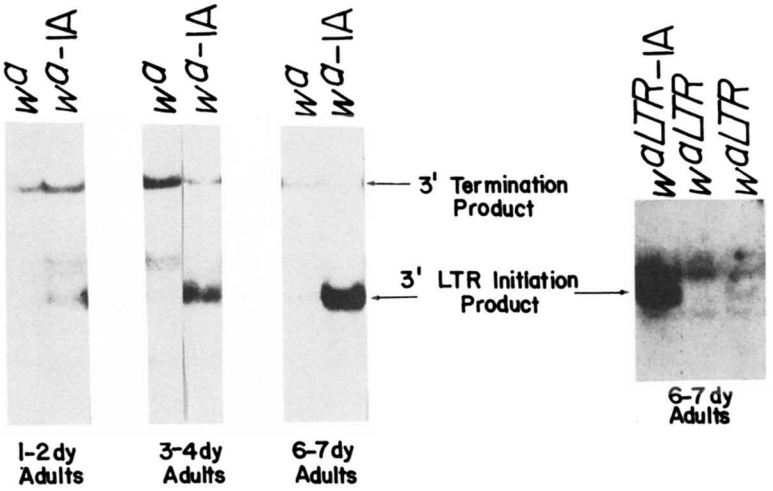

FIGURE 5.—Northern gel analysis of genetic background-dependent accumulation of 3′ LTR initiation product in aging adults. Polyadenylated RNAs were analyzed as described in Figure 4. The developmental stage and genotype of individuals donating the RNAs in the left-hand panel is indicated. Individuals donating RNAs in the righthand panel were all 5- to 6-day-old adults. The w^a-1A strain is described in MATERIALS AND METHODS. The w^{aLTR}-1A strain results from four generations of outcross of the w^{aLTR} strain to the w^a-1A strain, followed by segregation of w^{aLTR} to homozygosity. Notice that the w^a-1A genetic background causes a dramatic accumulation of the 3′ LTR initiation product in aging adults carrying either w^a or its LTR excision derivative.

not shown). If any of these minor products correspond to the 5′ LTR termination product, this product is much more rare than the 3′ termination product and the 3′ LTR initiation product and probably has not been visualized by Northern gel analysis.]

Fourth, in addition to the expected S_1-resistant products described above, we consistently see an S_1-resistant product with w^a RNA and protectors 1 and 3 (but not protector-2) comigrating on our gels with the 300-base w^+ product corresponding to the second white exon (Figure 3A). [A similar product was observed by PIRROTTA and BROCKL (1984) to result from protecting w^+ sequences with w^a RNA.] Our results strongly suggest that this product corresponds to the second white exon. However, the levels of this product are substantially higher than expected from the levels of w^+ transcripts seen on Northern gels (Figure 4). Moreover, the levels of this product covary approximately with the levels of the 3′ termination product, but do not covary with the levels of the 650-base protection product corresponding to the third white exon. (For example, compare 2.7- to 3-day and 5- to 6-day w^a larval protection patterns in the left-hand panel of Figure 3B.) If the 300-base w^a protection product results exclusively from the presence of structurally w^+ transcripts, the levels of the 300- and 650-base w^a protection products should covary. One simple interpretation of these results is that only some of the 300-base w^a protection product results from the presence of structurally w^+ transcripts, and the remainder results from some other class of transcripts. We discuss the probable source of these other transcripts in more detail below.

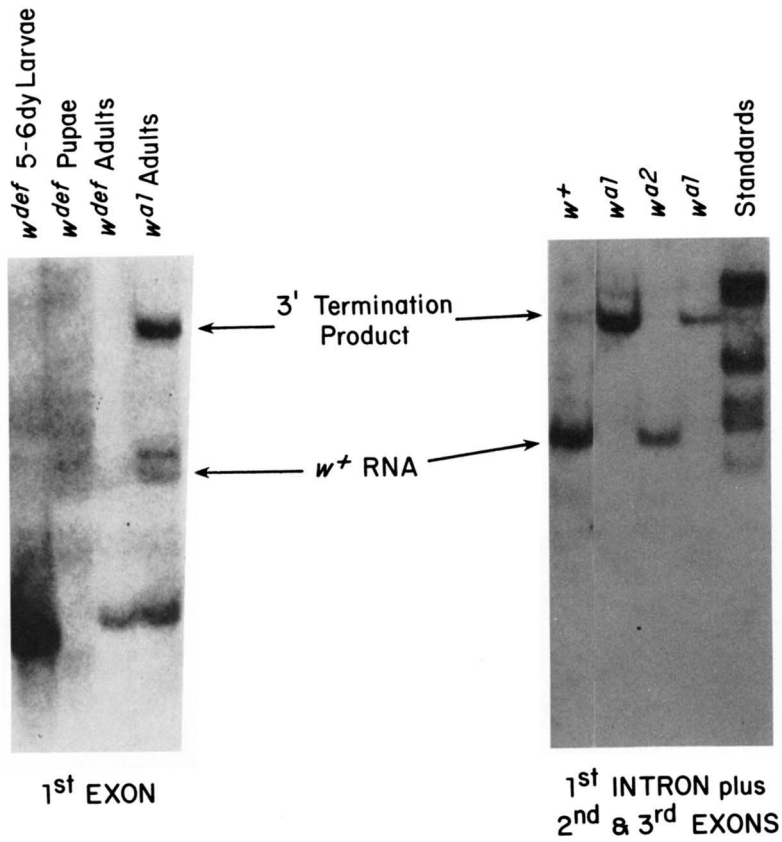

FIGURE 6.—Northern gel analysis of polyadenylated white transcripts. Each channel contains 10 μg of polyadenylated RNA extracted from organisms of the genotype and developmental stage indicated. In contrast to all other experiments described here, organisms donating RNAs were reared at room temperature (varying daily between 20° and 25°). DNA sequence probes are described in MATERIALS AND METHODS. w^{def} is a white deficiency allele in which sequences from at least 2 kb to the left of the w^a *copia* insertion to *ca.* 10 kb to the right of this insertion are deleted. Thus, transcripts seen in the w^{def} do not originate at white (see text for discussion). The mobilities of the major w^+ and 3′ termination product are indicated.

The w^a copia *insertion produces developmentally programmed effects on levels of polyadenylated white transcripts*: white is apparently transcribed during all developmental stages of the fly and produces a mature transcript of about 2.6 kb (Figure 4; O'HARE, LEVIS and RUBIN 1983; PIRROTTA and BROCKL 1984; BINGHAM and ZACHAR 1985). The steady-state levels of this transcript remain nearly constant throughout larval, pupal and adult stages (see MATERIALS AND METHODS for details of staging) in w^+ individuals (Figure 4; PIRROTTA and BROCKL 1984).

Examination of the developmental pattern of accumulation of polyadenylated transcripts of the w^a allele (Figure 4) shows that this allele behaves quite differently from w^+.

First, the 3′ termination product varies in amount by at least fivefold as a function of developmental stage. Quantitative S_1 protection results (Figure 3B)

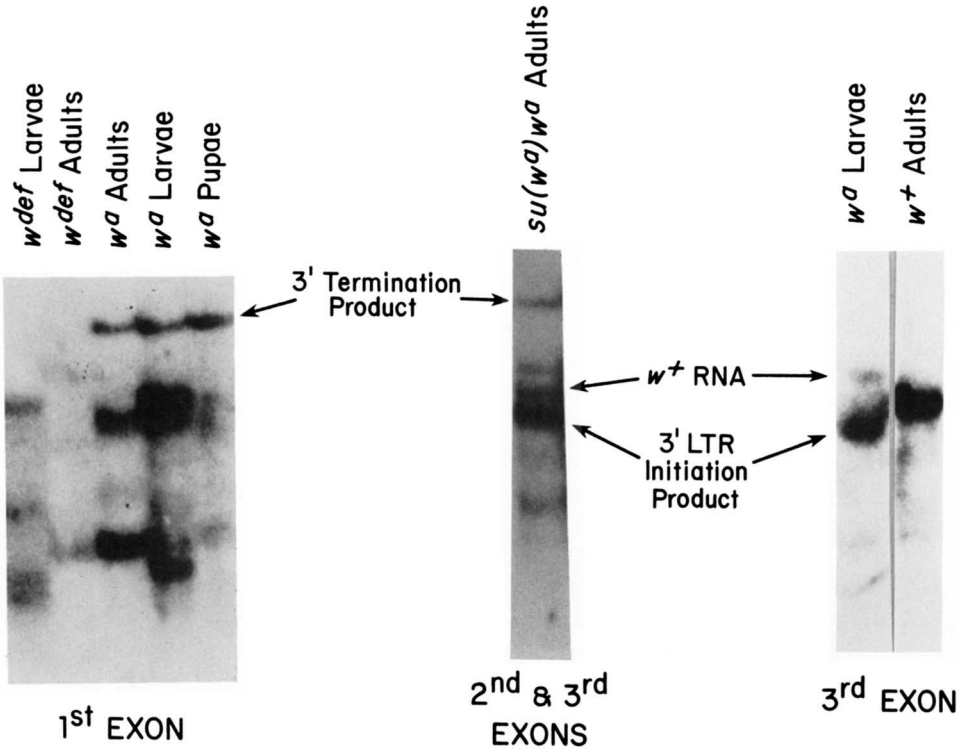

FIGURE 7.—Pattern of hybridization of polyadenylated transcripts from various genotypes to subsegments of white. The larval samples were extracted from 1- to 1.67-day larvae, and these channels contain about twice as much RNA as the other w^a samples. DNA sequence probes are described in MATERIALS AND METHODS. The w^{def} strain is the white deletion described in Figure 6. The middle panel shows all the major classes of transcripts proceeding right to left into or out of the *copia* insertion (see also Figure 4). The mobilities of the major w^+ transcripts, the 3′ LTR initiation product and the 3′ termination product are indicated. The first exon probe is expected to label transcripts from the 5′ side of the w^a *copia* insertion, and the third exon probe transcripts from the 3′ side. (See text for discussion of these results).

suggest that this product represents a large and relatively constant fraction of the polyadenylated transcripts initiated at the white promotor. Thus, the w^a *copia* insertion causes substantial developmental variation in the levels of polyadenylated transcripts initiated at the white promotor. White transcript levels are reduced during larval stages. During pupal stages the levels of the 3′ termination product increase to nearly the wild-type level. In adults the levels of the 3′ termination product are again lower than the wild-type white transcript level (Figure 4).

We observe developmentally programmed variation in w^+ transcript levels produced by the w^{aLTR1} allele that are qualitatively similar to the variation observed in w^a transcripts (Figure 4).

Second, levels of the transcript initiated in the 3′ LTR of the w^a *copia* insertion show developmental variation (Figure 4). Transcripts initiated from the solo *copia* LTR in w^{aLTR} show a similar developmental pattern (Figure 4).

Mutational inactivation of the suppressor-of-white-apricot locus elevates levels of w^a *transcripts corresponding to transcriptional readthrough of the copia transposon*: Suppressor-of-white-apricot is the locus of recessive suppressor mutations specific for the w^a mutation at white (GREEN 1959). We have investigated the effects of mutation at $su(w^a)$ on the transcriptional events involving w^a and w^{aLTR}.

Our $su(w^a)^1w^a$ strain shows consistent elevation in levels of several transcripts, including one indistinguishable from the major w^+ transcript in size, transcriptional polarity and pattern of hybridization to white sequences (Figure 4 and results not shown). Elevated levels of w^+ transcripts were reported previously in adults of one strain carrying the $su(w^a)^1$ allele (LEVIS, O'HARE and RUBIN, 1984). We have tested the hypothesis that mutational alteration of $su(w^a)$ is responsible for this elevated production of w^+ transcripts in two ways: First, our $su(w^a)^1$ and $su(w^a)^+$ strains (Figure 4) have been extensively outcrossed (six outcross generations) to a standard Oregon-R strain (selecting for retention of the yellow-white interval). This experiment demonstrates that the transcriptional phenotype of the $su(w^a)^1$ strain results from genetic elements mapping in or near the yellow-white interval (including $su(w^a)$). Second, we have analyzed w^a transcripts in the presence of two newly isolated mutant $su(w^a)$ alleles (also crossed into the same genetic background as the $su(w^a)^1$ and w^a strains immediately above). Both of these alleles were isolated after EMS mutagenesis and are very similar in eye-color phenotype to $su(w^a)^1$ (D. GARZA, Z. ZACHAR and P. BINGHAM, unpublished observations). Each produces the same elevated levels of w^+ transcripts in midlarval stages, as does the $su(w^a)^1$ mutant allele (Figure 8). w^+ transcripts are the expected product when transcription proceeds entirely through the w^a *copia* insertion and the insertion is spliced out with the second white intron (Figure 2; PIRROTTA and BROCKL 1984; LEVIS, O'HARE and RUBIN 1984).

In addition to elevated w^+ transcript levels in $su(w^a)^1w^a$ individuals, we find (in late larvae, pupae and adults) elevated levels of several transcripts between *ca.* 0.8 and 1.8 kb in size (Figure 4). Transcripts comigrating with these are found in w^+ individuals under the conditions of our experiment (Figure 4). A detailed description of these transcripts will be presented elsewhere (C. CHAPMAN and P. BINGHAM, unpublished observations); however, the high levels of the presumptive second white exon S_1 protection product described above suggest that these transcripts might result from aberrant splicing events involving the splice donor immediately preceding the w^a *copia* insertion and various splice acceptors 3′ to this splice donor. Thus, some or all of these smaller transcripts could be products of readthrough of the w^a *copia* insertion.

Further consideration of transcription termination in the 5′ LTR of the w^a copia *insertion*: The question arises as to whether termination might occur in the 5′ LTR of the *copia* transposon, with the terminus not being polyadenylated. Such transcripts would escape detection in the experiments so far described. The results shown in Figure 9 strongly suggest that such termination events occur rarely, if at all. Specifically, a large fraction of larval white transcription occurs in Malpighian tubule tissues (FJOSE *et al.* 1984; Z. ZACHAR, D. DAVISON,

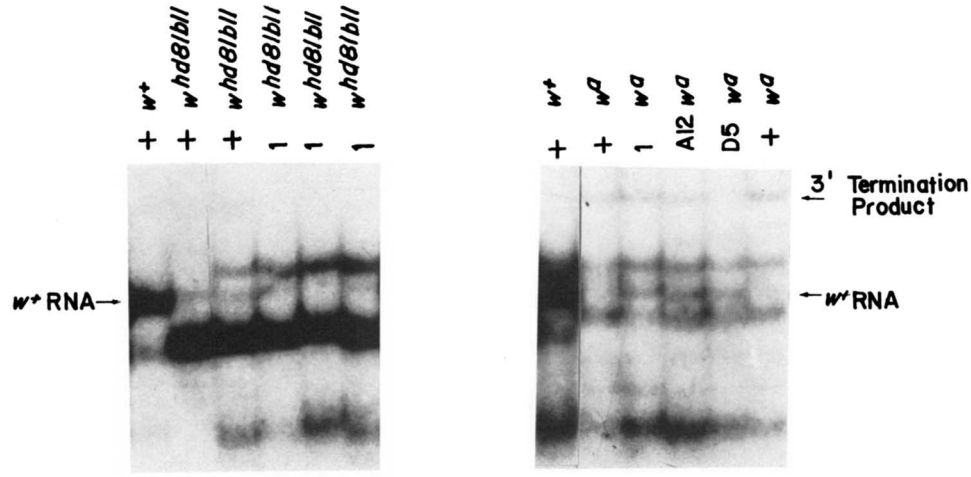

FIGURE 8.—Northern gel analysis of transcripts from the w^a and $w^{hd81b11}$ alleles in the presence of various alleles at $su(w^a)$. The polyadenylated 3- to 4-day larval RNAs were analyzed as in Figure 4. The genotypes donating the RNAs are indicated. The genotype at $su(w^a)$ is indicated by the allele designation: + is the wild-type allele, *1* is the $su(w^a)^1$ mutant allele, *A12* is the $su(w^a)^{EMSA12}$ mutant allele and *D5* is the $su(w^a)^{EMSD5}$ mutant allele (see text). The positions of the major w^+ transcript and the 3′ termination product are indicated. In the left-hand panel, notice no reproducible effects of $su(w^a)^1$ on $w^{hd81b11}$ transcripts (see Figure 10 and text for description of $w^{hd81b11}$ transcripts). In the right-hand panel, notice that relatively high levels of the major w^+ transcript in w^a strains is seen only when the suppressor-of-white-apricot locus is mutationally inactivated.

D. GARZA and P. M. BINGHAM, unpublished results). This allows us to enrich for white transcripts adequately to detect them on Northern gels by extracting RNA from purified larval Malpighian tubules, rather than by fractionating RNA on oligo-dT cellulose. Thus, if a class of w^a transcripts terminate in the 5′ LTR of the w^a *copia* insertion in this tissue, this class should be detected whether or not its members are polyadenylated. Figure 9 shows that, if such a 5′ LTR termination product exists, it must be very much less abundant than the 3′ termination product. [The rare Malpighian transcript at 1.2 kb (Figure 9) comigrates with the 1.2-kb polyadenylated transcript and is presumably identical to it.]

Interaction of the w^{aLTR} *allele with suppressor-of-white-apricot*: In some experiments we observe a very small elevation in w^+ transcript levels produced by w^{aLTR} in the presence of the $su(w^a)^1$ mutant allele (Figure 4), whereas in other experiments this small effect is not detected.

In addition, the following set of experiments, collectively, demonstrate that w^{aLTR} does not detectably respond to the allelic state of suppressor-of-white apricot as assessed by eye color phenotype: First, the following observations demonstrate that the effect of $su(w^a)^1$ on w^a eye pigmentation is of the order of twofold. The phenotype of $su(w^a)^1w^a/su(w^a)^1w^a$ females is substantially darker than that of w^a/w^a females. The eye color phenotype of $su(w^a)^1w^a/su(w^a)^1w^x$ females is essentially identical to that of w^a/w^a females (where w^x is either a white deficiency (w^{pD79k}) or a point mutant allele (w^{65a25}) behaving phenotypically as a null). Second, w^{aLTR}/w^x females have substantially less pig-

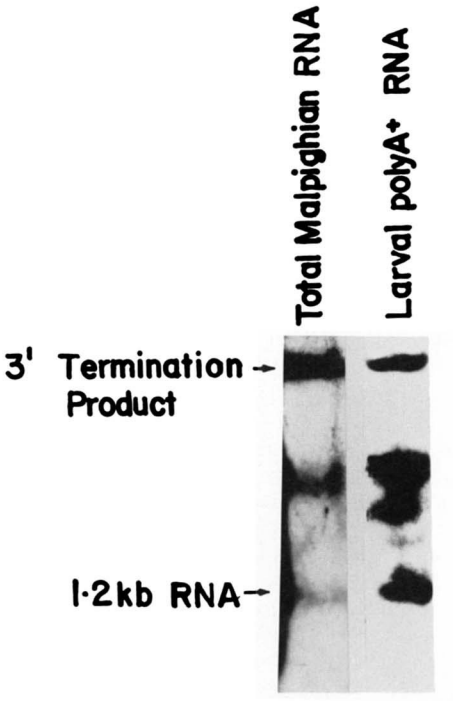

FIGURE 9.—Northern gel analysis of white transcripts in larval w^a tissues. In the left-hand panel is the RNA from about 50 Malpighian tubules dissected from 5- to 6-day larvae; this RNA was not fractionated on oligo-dT cellulose. In the right-hand panel is the polyadenylated RNA from about 25 whole larvae. The white probe was the second and third exon probe. The positions of the 3′ termination product and the 1.2-kb species described in the text are indicated.

mented eyes than do w^{aLTR}/w^{aLTR} females. Thus, a twofold increase in the level of w^{aLTR} eye pigmentation would be detected in w^{aLTR}/w^x females if it occurred. No such increase is seen; $su(w^a)^1w^{aLTR}/su(w^a)^1w^x$ females are indistinguishable in eye color phenotype from w^{aLTR}/w^x females.

Collectively, these experiments suggest that $su(w^a)$ exerts either a less extreme effect on w^{aLTR} than on w^a or no effect at all.

Levels of transcripts initiated in the w^a *solo* copia *LTR and in the 3′ LTR of the intact* w^a copia *are strongly affected by genetic background*: In one strain that we have examined (designated w^a-1A; MATERIALS AND METHODS), we observe a very large genetic background effect on the levels of the 3′ LTR initiation product. Specifically, as adults of this strain age, the 3′ LTR product accumulates dramatically. By 6–7 days of age, adults of this strain have a level of this transcript of the order of tenfold higher than do w^a adults from the other strains that we have examined (Figure 5), a transcript level comparable to wild-type white transcript levels at this same stage. A similar effect is seen when the w^a LTR excision derivative is crossed into this genetic background (Figure 5). This last observation argues that the sequences necessary to respond to this genetic background effect are contained within the LTR. We are currently characterizing the genetic basis of this effect in more detail. However, we note

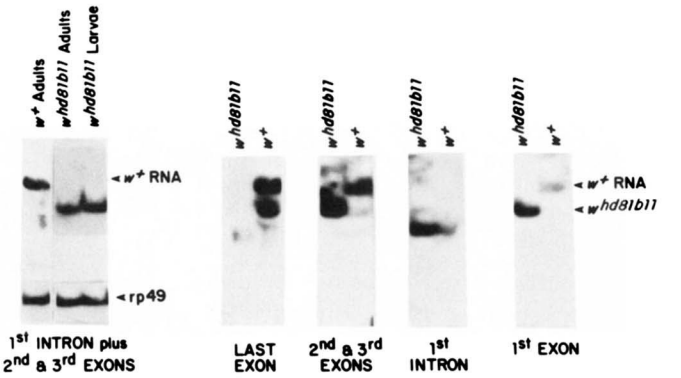

FIGURE 10.—Northern gel analysis of polyadenylated transcripts produced by the $w^{hd81b11}$ *copia* insertion mutant allele at white. The $w^{hd81b11}$ *copia* insertion occurs approximately in the middle of the smaller (rightmost) *Sal*I fragment in Figure 2. Polyadenylated RNAs were analyzed as in Figure 4. Genotypes and developmental stages of individuals donating RNAs in the leftmost panel are indicated. The RNAs in the remaining panels were isolated from adults. DNA sequence probes are described in MATERIALS AND METHODS. The right-hand four panels show the pattern of hybridization of the $w^{hd81b11}$ transcript to subsegments of white. The RNA species labeled by the first intron probe and migrating below the major w^+ and $w^{hd81b11}$ transcripts is observed in some other white genotypes as well and is of uncharacterized origin, as is the species migrating below the w^+ transcript in the last exon panel.

that this strain does not carry an overtly mutant allele either suppressing or enhancing the w^a eye color phenotype.

The $w^{hd81b11\ copia}$ *insertion causes the production of a new class of white transcripts apparently terminating in the* copia *element*: The $w^{hd81b11}$ allele arose in a P-M dysgenic hybrid and results from the insertion of *copia ca.* 1300-bp leftward (Figure 2) from and in the opposite orientation to the *copia* insertion at w^a (BINGHAM, KIDWELL and RUBIN 1982; RUBIN, KIDWELL and BINGHAM 1982; O'HARE *et al.* 1984). In both larvae and adults of $w^{hd81b11}$ we observe a new transcript *ca.* 600 bases smaller than the major w^+ transcript. This new transcript has homology to probes containing white exon sequences 5′ to the point of $w^{hd81b11}$ *copia* insertion, but lacks homology to a probe containing the sequences of the large exon 3′ to the point of *copia* insertion (Figure 10). These results strongly suggest that white transcripts acquire a polyadenylated terminus in or very near the $w^{hd81b11}$ *copia* insertion.

The $w^{hd81b11}$ insertion occurs 8 bp into the penultimate white exon, leaving *ca.* 850 bases of white exon sequences between the insertion point and the polyadenylation signal (O'HARE *et al.* 1984). If the white splicing event immediately 5′ to the *copia* insertion occurs properly (removing about a 220-base intron) our results place the presumptive polyadenylated terminus of the $w^{hd81b11}$ transcript *ca.* 250 bp into the *copia* insertion. However, if this white splice is not made, the polyadenylated terminus of the $w^{hd81b11}$ transcript presumably occurs within *ca.* 30 bases of the *copia* insertion point. Further analysis will be necessary to resolve this issue.

We have examined the effect of the $su(w^a)^1$ mutant allele on white transcripts in $w^{hd81b11}$ 3- to 4-day larvae (Figure 8). We observe neither new size classes

of $w^{hd81b11}$ transcripts nor systematic changes in the stoichiometries of size classes previously observed. This result suggests that the white polyadenylated terminus formation event in the 3′ portion of the $w^{hd81b11}$ *copia* insertion is not influenced by the *su*(w^a) locus product.

DISCUSSION

Copia *insertion can produce developmentally variable effects on accumulation of white transcripts*: The presence of the *copia* transposon in the w^a allele has complex effects on levels of polyadenylated white transcripts. The w^a *copia* insertion causes a large reduction in levels of polyadenylated white transcripts during larval and adult stages and a much smaller reduction in pupal stages. These large developmental effects on polyadenylated white transcript levels from w^a are influenced little, if any, by tested mutant alleles of *su*(w^a) (see RESULTS).

White transcript levels are reduced in larvae, the larval period being one of heavy accumulation of *copia* transcripts (SCHWARTZ, LOCKETT and YOUNG 1982; Z. ZACHAR, D. DAVISON, D. GARZA and P. M. BINGHAM, unpublished results). Moreover, *copia* transcripts actively accumulate in the larval tissue (Malpighian tubules; Z. ZACHAR, D. DAVISON, D. GARZA and P. M. BINGHAM, unpublished results), in which much, and possibly most, of larval white transcription occurs (Figure 9; FJOSE *et al.* 1984). One attractive interpretation of these results is that expression of the w^a *copia* element represses expression of the white promotor *ca.* 3.6 kb 5′ to it. Possibly related expression-associated, negative, long-range *cis* effects have been observed previously (BINGHAM and ZACHAR 1985; EMERMAN and TEMIN 1984). It is also noteworthy that expression-associated repression of contiguous genes by retrotransposons (the transcription of which is developmentally programmed) could account for some of the developmentally complex effects of such elements on genes into which they are inserted (*e.g.*, BENDER *et al.* 1983; KIDD, LOCKETT and YOUNG, 1983; SCOTT *et al.* 1983).

Levels of polyadenylated w^+ transcripts from the w^{aLTR1} allele show developmental variation qualitatively similar to that of white transcripts from w^a (Figure 4). This observation suggests that some or all of the *copia*-internal elements responsible for the effects on white transcript levels map in the LTR.

The behavior of copia *as a transcription terminator and the influence of suppressor-of-white-apricot on* w^a *transcription*: The structure of the transcription units of retrotransposons requires them to solve a basic problem: transcription must proceed through the point in the 5′ LTR at which polyadenylated terminus formation will occur in the 3′ LTR [see VARMUS (1983) for a recent review]. Although this problem has probably been solved differently by different elements, the conventional picture of retrotransposon organization suggests that a single LTR will serve as a terminator or polyadenylation signal when placed downstream from a transcription start site (so that transcription is directed through the entire LTR, rather than beginning within it).

In contrast to this expectation, our studies detect no discreet, stable white transcript resulting from a termination event in the 5′ LTR of the w^a *copia* insertion or in the solo *copia* LTR in w^{aLTR1}, in spite of the existence of a very

major transcript apparently corresponding to polyadenylated terminus formation in the 3′ portion (presumably the 3′ LTR) of the w^a *copia* insertion. Collectively, our results suggest that termination in the 3′ region of the *copia* transposon requires the participation of sequences internal to the nonrepeated portion of the transposon as well, presumably, as sequences in the 3′ LTR itself. VARMUS, QUINTRELL and ORTIZ (1981) observed transcription patterns in Moloney-murine-leukemia-virus provirus insertion alleles, suggesting that the LTRs of this retrovirus behave similarly. It will be of interest to know how general this solution is.

In light of our results, the effects of $su(w^a)$ on w^a transcription suggest that the $su(w^a)$ locus product might be required for efficient functioning of the complex *copia* terminator and/or polyadenylation signal. It is also noteworthy that genetic evidence exists suggesting that the $su(w^a)$ mutant alleles used in this study do not entirely inactivate the $su(w^a)$ locus. Specifically, a very extreme allele of $su(w^a)$ tightly linked to (and possibly synonymous with) a lethal allele produces a nearly wild-type eye color phenotype in the very rare w^a individuals carrying the allele and surviving to late pupal stages (D. GARZA, Z. ZACHAR and P. M. BINGHAM, unpublished observations). This is in contrast to the $su(w^a)$ mutant alleles used here, which darken the w^a eye color phenotype to a level still considerably less-pigmented than wild type. Thus, studies to date may substantially underestimate the dependence of the transcriptional behavior of the w^a allele on the $su(w^a)$ gene product.

Properties of the promotors in copia *and its LTR excision derivative as activators of contiguous genes*: The w^a allele is unusually favorable for examination of transcription promoted from the 3′ LTR of the *copia* element. We have identified this class of transcripts and characterized its developmental pattern of accumulation (Figures 4 and 5). We find that the 3′ LTR promoted transcripts accumulate to high levels early in larval development and that these levels gradually decline severalfold during larval stages and remain relatively low through all subsequent stages examined. The pattern of accumulation of transcripts promoted from the LTR-excision derivative of w^a is essentially indistinguishable from that of 3′ LTR of the intact transposon (Figure 4). This last observation suggests that the information necessary to determine the developmental pattern of expression of the 3′ *copia* LTR is contained entirely within the LTR. It is also noteworthy that this pattern of expression differs markedly from that of the 5′ LTR of the intact *copia* transposon (SCHWARTZ, LOCKETT and YOUNG 1982).

This work was supported by a United States Public Health Service grant to P.M.B.

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Communicating editor: W. M. GELBART