

Cloning and Characterization of the *scarlet* Gene of *Drosophila melanogaster*

Richard G. Tearle,*¹ John M. Belote,^{†,2} Michael McKeown,^{†,3} Bruce S. Baker,^{†,4} and Antony J. Howells*

*Department of Biochemistry, Faculty of Science, Australian National University, Canberra, ACT, Australia and [†]Department of Biology B-022, University of California at San Diego, La Jolla, California 92093

Manuscript received November 30, 1988

Accepted for publication March 13, 1989

ABSTRACT

DNA from the *scarlet* (*st*) region of *Drosophila melanogaster* has been cloned by chromosome walking, using the breakpoints of a new X-ray-induced third chromosome inversion (*In(3LR)st-a27*) which breaks in the *scarlet* (73A3.4) and *rosy* (87D13-14) regions. Two spontaneous mutants of *st* (*st*¹ and *st*^{sp}) contain insertions of non-*st* DNA located within 3.0 kb of the site of the inversion breakpoint used to isolate the gene, and a second *scarlet* inversion breaks within 6.5 kb of this site. However no changes detectable by Southern blotting were found in 5 X-ray-induced *st* mutants with cytologically normal third chromosomes. A 2.3-kb transcript arising from the *st* gene region (as defined by mutant analysis and DNA transformation) has been detected. This transcript is present throughout development at low levels, with a peak level during the early to mid-pupal stage. The size and amount of this transcript is altered in *st*¹, and its amount is drastically reduced in *st*^{sp}. Flies carrying the *white*¹ mutation show normal levels of expression of the *st* transcript, suggesting that the *w*⁺ gene does not regulate transcription of the *st*⁺ gene. Nucleotide homology between sequences from the *st* transcription unit and a fragment carrying coding information from the *white* gene has been detected. This suggests that the *st* and *w* proteins are related; they appear to belong to a family of membrane-spanning, ATP-binding proteins involved in the transport of pigment precursors into cells.

THE normal red-brown eye color of *Drosophila melanogaster* is due to the presence of two types of light screening pigments, a brown ommochrome (xanthommatin) and a series of red pteridines (drosop-terins) (PHILLIPS and FORREST 1980; SUMMERS, HOWELLS and PYLIOTIS 1982). The biosynthetic pathways leading to these two pigments are completely different, xanthommatin being derived from tryptophan and the drosop-terins from guanosine triphosphate.

Four eye color genes—*vermillion* (*v*), *cinnabar* (*cn*), *scarlet* (*st*), and *white* (*w*)—encode products that are absolutely required for biosynthesis of xanthommatin. Two of these genes code for pathway enzymes, *v* for tryptophan oxygenase (BAGLIONI 1960; TARTOF 1969; BAILLIE and CHOVNICK 1971) and *cn* for kyn-urenine-3-hydroxylase (GHOSH and FORREST 1967; SULLIVAN, KITOS and SULLIVAN 1973). While the roles of *st* and *w* have not yet been satisfactorily explained, these genes do exhibit similarities in their expression. Mutations in *w* and *st* affect the expression of the pathway in the same organs: the Malpighian tubules of the larva and the compound eyes and ocelli of the

adult (BEADLE and EPHRUSSI 1937), where they perturb the ability of these tissues to take up, transport, or store xanthommatin precursors (SULLIVAN and SULLIVAN 1975; HOWELLS and RYALL 1975). In addition, analysis of temperature-sensitive alleles of the two loci reveal a similar temporal requirement for the two gene products during development, 24–48 hr after pupariation (EPHRUSSI 1942; HOWELLS 1979). *w* and *st* differ in that *w* blocks the formation of both types of pigments whereas *st* affects just xanthommatin accumulation. We are undertaking an analysis of *st* and *w* to establish whether there is a common basis for their similar effects on ommochrome synthesis.

Here we report our initial characterization of the *st* gene. It was isolated by chromosome jumping, using an inversion whose breakpoints lie in salivary chromosome region 73A3.4 (the cytological location of *st*) and 87D13-14 (within the *rosy-Ace* chromosomal walk of BENDER, SPIERER and HOGNESS 1983). We have uncovered gross alterations in the DNA of several *st* mutants which we assume to be associated with the *st* lesions. A developmental profile of the *st* gene expression has also been established and we have shown that lesions in the *w* gene do not affect the level of *st* transcripts. Nucleotide similarity between coding regions of the *st* and *w* genes has been detected, suggesting that the similarity in mutant phenotypes reflects a common biochemical function for the *w* and *st* gene products.

¹ Present address: Department of Biochemistry, University of Adelaide, S.A. 5000, Australia.

² Present address: Department of Biology, Biological Research Laboratories, Syracuse University, Syracuse, New York 13244.

³ Present address: Molecular Biology and Virology Laboratories, The Salk Institute, P.O. Box 85800, San Diego, California 92138.

⁴ Present address: Department of Biological Sciences, Stanford University, Stanford, California 94305.

MATERIALS AND METHODS

Sources of stocks: Many of the *st* mutants used in these experiments were uncovered in the mutant screens described below; these (and others) are listed in Table 1. For the additional stocks used: *st*^{82c3} was obtained from M. M. GREEN; *st*¹ and *st*^{sp} were obtained from the Bowling Green stock center. Of the wild-type strains, Amherst M56i was established by G. LEFEVRE, while Canberra has been maintained in one of our laboratories (A. J. H.) for 16 yr.

Mutagenesis and cytogenetic analysis: Flies were raised at 25° on a cornmeal-molasses-agar-yeast-propionic acid medium seeded with live yeast. For descriptions of the mutants used, see LINDSLEY and GRELL (1968), LINDSLEY and ZIMM (1986, 1987), or the indicated references.

To induce mutations in the *st* region with X-rays, adult males were collected from a *Ki roe p^p* isogenic third chromosome line and aged for 2–7 days prior to irradiation. They were then subjected to an X-ray dose of 4000 r (Torrex 150 X-ray Inspection System, 120 kV, 5 mA, 0.5-mm plexiglass filter). Groups of 25 males were then placed in quarter pint bottles containing fresh media and about 40 *st*^{82c3} virgin females. After 2–3 days, the flies were transferred to fresh bottles for another 2–3 days. The F₁ progeny from both sets of bottles were subsequently examined and those exhibiting the *st* phenotype were singly mated to *Df(3L)st^{81k172}/TM6B, Hu Tb e ca* flies in order to confirm the existence of a new *st* allele or *st* region deficiency, and to make the irradiated chromosome heterozygous over *TM6B*, thus establishing a balanced stock.

For cytological analyses, flies carrying the chromosome of interest were crossed to Canton S flies, and the larvae reared at 18° in uncrowded, well-yeasted vials. Salivary glands from late 3rd instar larvae were dissected in 0.7% saline, fixed for 45 sec in 45% acetic acid, and transferred to a drop of lacto-acetic orcein stain on a coverslip. After 5–8 min a microscope slide was applied and the glands squashed. Chromosome spreads were observed under phase-contrast optics using a Zeiss Universal microscope. The chromosomal rearrangements listed in Table 1 are our determinations; some of these are not in agreement with previously published breakpoints.

Construction of the EMBL3-*In(3LR)st-a27* library and chromosome walk: A genomic library was prepared from flies homozygous for the *In(3LR)st-a27* chromosome. Genomic DNA was partially digested with *Mbo*I, size-fractionated and then ligated into the *Bam*HI sites of the phage cloning vector EMBL3A (FRISCHAUF *et al.* 1983). Preparation of fly and bacteriophage DNAs, DNA fragment isolation, Southern blot analyses, identification of recombinant clones, and enzymatic reactions (*e.g.*, restriction digests, ligations, and nick translations) were carried out using the protocols described in MANIATIS, FRITSCH and SAMBROOK (1982), or the references therein.

In situ hybridization: Recombinant DNA was biotinylated by nick translation using DNA polymerase I and Bio-16-UTP (ENZO Biochemicals). Hybridization was carried out as described in LANGER-SAFER, LEVINE and WARD (1982). The hybridization signal was visualized using streptavidin-biotinylated horseradish peroxidase as described by the supplier (ENZO Biochemicals).

Southern blot analyses of fly DNA: Genomic DNA was isolated from adult flies as described in MIKLOS *et al.* (1984) or by a modification of the method described in COEN, THODAY and DOVER (1982). Restriction digestion and electrophoresis of DNA were carried out as described in WALKER, HOWELLS and TEARLE (1986). Transfer of digested DNA to nylon membrane (Zetaprobe, Biorad) was

carried out as described in REED and MANN (1985). Hybridizations were performed in solutions of 1.5 × SSPE, 1% SDS, 0.5% BLOTTO (Diploma skim milk power), 500 µg/ml salmon sperm DNA and (for genomic Southern analyses) 10% dextran sulfate at 68° for 16–24 hr. Filters were probed with recombinant phage or plasmid DNAs nick translated using [³²P]dCTP (Amersham, 3000 Ci/mmol) to 1–2 × 10⁸ cpm/µg at a final concentration of 2–20 ng/ml, and washed (twice) in 0.1 × SSC, 1% SDS at 42–50° for 30 min.

Transcript analyses: Animals were collected at specific stages of development as described in RYALL and HOWELLS (1974) and stored at –45°. RNA was prepared by the methods of CHIRGWIN *et al.* (1982) or BINGHAM and ZACHAR (1985). Poly(A⁺) RNA was prepared from total RNA using oligo-dT cellulose as described in MANIATIS, FRITSCH and SAMBROOK (1982) or by the use of Messenger Affinity Paper (Hybond-mAP) as described by the supplier (Amersham). Samples of RNA were glyoxylated and run on 0.8% or 1.5% agarose gels with circulating 10 mM Na-phosphate buffer, pH 7.0 (THOMAS 1983). Transfer to nylon membrane was carried out in 10 mM Na phosphate (pH 7.0) and the RNA fixed to the membrane by exposure to UV irradiation. Blots were hybridized in solutions of 50% formamide, 1.5 × SSPE, 1% SDS, 0.5% BLOTTO, 10% dextran sulfate and 500 µg/ml salmon sperm DNA at 60°, and probed with riboprobes synthesized from the T7 promoter of pGEM-1 or pGEM-2 essentially as described in the protocol provided by Promega Biotec., using 20 µCi ³²P-UTP (Amersham, 800 Ci/mmol) per reaction. Filters were washed once in 0.1 × SSC, 0.1% SDS and once in 0.1 × SSC, 1.0% SDS; both washes at 60° for 15 min. Filters were sometimes subsequently treated with ribonuclease (1 µg/ml ribonuclease A in 2 × SSC for 15 min at 20°) in order to reduce the level of nonspecifically bound probe.

DNA sequencing and sequence analysis: Restriction fragments for sequencing were ligated into M13 sequencing vectors (mp8, mp18 or mp19) and single-stranded DNA prepared as described by MESSING (1983). All sequencing was carried out by the dideoxynucleotide chain-termination method developed by SANGER, NICKLEN and COULSEN (1977). Sequence data was analyzed using the DNA Inspector II programs in a Macintosh Plus microcomputer.

RESULTS

Cytological localization of the *scarlet* gene: In order to help identify the location of the *st* gene, we carried out an X-ray mutagenesis experiment designed to induce and recover new *st* mutants (see MATERIALS AND METHODS). Approximately 40,000 F₁ individuals were scored for the bright red eye color phenotype characteristic of *st*. This screen resulted in the isolation of 21 new *st*[–] mutants (Table 1). A subset of these mutants had chromosome rearrangements involving the 73A region of chromosome 3 (2 inversions, 9 deficiencies, and 2 complex rearrangements that involved deletions of the 73A region). In addition to these, twelve chromosome deficiencies obtained from other sources (see Table 1) were examined cytologically and retested for complementation with *st*¹ and/or *st*^{82c3} alleles. The 22 deficiencies that remove the distal half of the chromosome doublet 73A3.4 are *st*[–], whereas *Df(3L)tra*, which removes the proximal

TABLE 1

Mutant alleles and chromosome rearrangements used in the characterization of the *st* locus

I. Mutant alleles not associated with chromosome rearrangements:		
Allele	Source	Reference
<i>st</i> ¹	Spontaneous	1
<i>st</i> ^{1p}	Spontaneous	1
<i>st</i> ^{82k}	Spontaneous	2
<i>st</i> ¹⁴⁷⁵⁴	EMS-induced	3
<i>st</i> ^{82c3}	MR-induced	4
<i>st</i> ²⁴¹	X-ray-induced	5
<i>st</i> ^{a24}	X-ray-induced	5
<i>st</i> ^{a31}	X-ray-induced	5
<i>st</i> ^{b22}	X-ray-induced	5
<i>st</i> ⁸²⁰²	X-ray-induced	5
<i>st</i> ⁸²²	X-ray-induced	5
<i>st</i> ^{k29}	X-ray-induced	5
<i>st</i> ^{L15}	X-ray-induced	5
II. Deficiencies, inversions, duplication:		
Rearrangement	Cytology	Reference
<i>Df(3L)st4</i>	72D7-11;73B7-C11	6, 7
<i>Df(3L)st6</i>	72E1.2;73A3.4	7, 8
<i>Df(3L)st7</i>	73A3.4;74A3	7, 8
<i>Df(3L)st103</i>	73A2-3;74B3-5	7, 9
<i>Df(3L)st106</i>	72E3;73A3.4	7, 9
<i>Df(3L)st100.62</i>	72F3-7;73B3	1
<i>Df(3L)st7P</i>	73A1.2;73A7-9	7, 10
<i>Df(3L)st8P</i>	72E4;73B4	7, 10
<i>Df(3L)st-k10</i>	73A3.4;72D1.2	4, 7
<i>Df(3L)st-k7</i>	73A3.4;74E1.2	4, 7
<i>Df(3L)st</i>	72D7;74A3	11, 12
<i>Df(3L)st-a20</i>	73A3;73A4	5, 7
<i>Df(3L)st-b8</i>	72D5-11;74B2	5, 7
<i>Df(3L)st-b11</i>	72D10.11;73D1.2	5, 7
<i>Df(3L)st-e4</i>	72D5-10;73A5	5, 7
<i>Df(3L)st-e5</i>	72E5-F1 73A2;72F1.2 73C1.2 (deficient for 73A2 to 73C1.2)	5, 7
<i>Df(3L)st-f13</i>	72C1-D1;73A3.4	5, 7
<i>Df(3L)st-g24</i>	72D1.2;73A9-10	5, 7
<i>Df(3L)st-g82</i>	72D2-11;73B1.2 (associated with <i>Tp(3;1)st-g82</i>)	5, 7
<i>Df(3L)st-j7</i>	73A1.2;73B1.2	5, 7
<i>Df(3L)st-j11</i>	72E2;73E1.2	5, 7
<i>Df(3L)st-g18</i>	72E2;74F4-75A1.2	13
<i>Df(3L)tra</i>	73A4;74A1.2	14
<i>In(3LR)st-a27</i>	73A3.4;87D13-14	5
<i>In(3)st-k21</i>	73A3.4;centric heterochromatin	5
<i>Dp(3;3)st⁺-g18</i>	72E1.2 74F4-72E2 74F4- 72E2 75A1 (duplicated for 72E1.2;74F4-75A1)	13
<i>T(Y;3)st</i>	Y 70A1.2-74A4.5 62B3-3L tip	15

The references are: (1) LINDSLEY and GRELL (1968), (2) R. G. TEARLE (unpublished data), (3) HOWELLS (1979), (4) M. M. GREEN (unpublished data), (5) this report, (6) ASHBURNER *et al.* (1981), (7) LINDSLEY and ZIMM (1987), (8) VELISSARIOU and ASHBURNER (1981), (9) ASHBURNER *et al.* (1980), (10) J. J. BONNER (unpublished data), (11) T. C. KAUFMAN, (unpublished data), (12) BAKER and RIDGE (1980), (13) BELOTE and McKEOWN (1985), (14) B. S. BAKER (unpublished data), (15) J. KENNISON (unpublished data).

edge but not the distal two-thirds of this darkly staining band, is *st*⁺. The two *st* inversions, *In(3LR)st-a27*

and *In(3)st-k21*, are broken in the middle of the 73A3.4 doublet. These results are in agreement with those of ASHBURNER *et al.* (1981), who also mapped the *st* locus to 73A3.4.

In addition to these chromosome rearrangements involving the *st* locus, we recovered eight new "point mutants." Here the term "point mutant" is not meant to imply that these are the result of single base changes, but rather it refers to mutants that are cytologically normal, and that complement mutations in the genes flanking *st* (*I(3)73Ab* and *I(3)73Ac-g*). Seven of these *st* alleles have phenotypes that are indistinguishable from the original *st*¹ mutant, while one mutant, *st*²⁴¹ is a hypomorph. The eye color of newly emerged *w^{Bwx};st¹/st²⁴¹* adults is light yellow, in contrast to the pure white eyes of *w^{Bwx};st¹* flies. All of the *st* "point mutants," as well as the two *st* inversions are completely viable over a deficiency for *st*, confirming that the *st*⁺ gene performs no function essential for viability.

Among the chromosome aberrations isolated in this screen is a rearrangement, *Dp(3;3)st⁺-g18*, involving a tandem duplication plus an inversion of region 72E1.2 to 74F4-75A1. This *st*⁺ duplication chromosome was recovered from an F₁ germline mosaic that also carried the complementary deficiency chromosome, *Df(3L)st-g18*, which is deleted for this same region (BELOTE and McKEOWN 1985). Flies homozygous for *Dp(3;3)st⁺-g18* and therefore carrying four doses of *st*⁺ exhibit an eye color that is indistinguishable from flies with a single dose of *st*⁺.

Cloning the *scarlet* region: The *In(3LR)st-a27* inversion chromosome allowed us to "jump" into the 73A3.4 band at, or near, the *st* locus from the previously cloned *rosy* (*ry*) gene region at 87D (BENDER, SPIERER and HOGNESS 1983). The cytological breakpoint of *In(3LR)st-a27* is located in 87D13-14. DNA blotting (Figure 1A) shows that *In(3LR)st-a27* is altered within the 4.1-kb *EcoRI* fragment from the *ry* region recombinant phage R2387 (provided by W. BENDER, Harvard University). No other alterations were detected in the region around this putative breakpoint. The results seen with respect to the *EcoRI* and *BamHI* digests are consistent with the expectation that *In(3LR)st-a27* is the result of a simple inversion event with one of the breakpoints lying within the 3.4-kb *BamHI* fragment of λR2387. The *HindIII* digestion pattern is more complicated and suggests that the inverted chromosome also contains a small deletion that removes DNA containing the *HindIII* site at or near the inversion breakpoint (see below, Figure 1B).

We constructed a recombinant DNA phage library containing DNA isolated from *In(3LR)st-a27* flies (see MATERIALS AND METHODS). We used the 4.1-kb *EcoRI* fragment from λR2387 as a probe to screen this library for phage that contain the *In(3LR)st-a27* re-

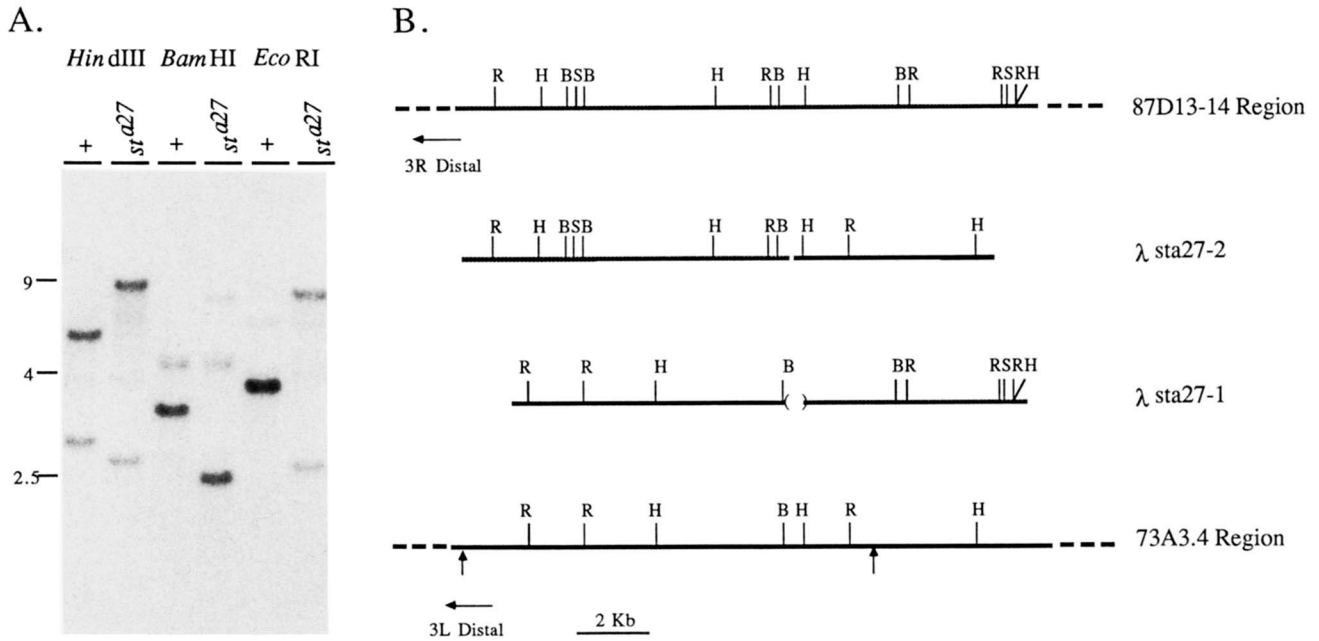


FIGURE 1.—Inversion breakpoints of *In(3LR)st-a27*. (A) Southern blot analysis of genomic DNA isolated from *Ki roe p^f* (lanes labeled +) and *In(3LR)st-a27* (lanes labeled *st^{a27}*), probed with the 4.1-kb *EcoRI* fragment from the *rosy* region clone λ R2387 of BENDER, SPIERER and HOGNESS (1983). (B) Restriction maps of recombinant phage λ sta27-1 and λ sta27-2 with comparisons to the 87D13-14 and 73A3.4 chromosomal regions. B = *BamHI*, H = *HindIII*, R = *EcoRI*, S = *SalI*. The 87D13-14 map is taken from Figure 3 of BENDER, SPIERER and HOGNESS (1983). The region shown corresponds to -168 to -152 on their map. The 73A3.4 map is derived from our chromosome walk in a Canton S strain; the arrows below the map show the endpoints of a recombinant phage λ stR4 which carries DNA from this region.

arrangement region. Two recombinant phage (λ sta27-1 and λ sta27-2) that hybridized to this probe were isolated. Their maps are shown in Figure 1B. Comparison of their restriction maps with the map of the 87D13-14 region, as determined by BENDER, SPIERER and HOGNESS (1983), suggests that these two phage represent each of the inversion breakpoints of the *In(3LR)st-a27* chromosome. λ sta27-1 contains the distal region of 73A3.4 fused to the proximal region of 87D13-14, and λ sta27-2 contains the proximal region of 73A3.4 fused to the distal portion of 87D13-14. The restriction maps of these phage are consistent with our expectations based on the results shown in Figure 1A.

Given the above assumptions, the 4.5-kb *EcoRI-SalI* fragments of λ sta27-2 (the *SalI* site is at the junction of the insert and the vector arm) should represent DNA from the 73A3.4 region. This fragment was used to probe the wild-type Canton S library of MANIATIS *et al.* (1978) for phage containing DNA from 73A3.4 and the endpoints of the DNA of one such phage, λ stR4, are shown on the map in Figure 1B. A comparison of the maps of λ stR4 and λ R2387 to the maps of the breakpoint-containing phage, λ sta27-1 and λ sta27-2, suggests that *In(3LR)st-a27* also contains a deletion of 1 kb from region 87D13-14. This is consistent with the blotting data of Figure 1. *In situ* hybridization confirms that λ stR4 DNA is derived from region 73A3.4 (data not shown).

DNA fragments from λ stR4 were used as probes to isolate overlapping recombinant phage from the Canton S library, and the chromosomal walk was continued in both directions until about 200 kb of contiguous DNA was cloned. An additional 50 kb distal to our walk was provided to us by DEBORAH ANDREW, who had initiated a chromosome walk in salivary gland chromosome region 73A2-3 (D. ANDREW and B. S. BAKER, unpublished data). A detailed description of the genetic and molecular characterization of this region are presented elsewhere (MCKEOWN, BELOTE and BAKER 1987; J. M. BELOTE, F. M. HOFFMAN, M. MCKEOWN and B. S. BAKER, in preparation). Another Canton S genomic DNA library (WALKER, HOWELLS and TEARLE 1986) was also screened with *st* region subclones and a restriction map of *st* region phage from both libraries is shown in Figure 2.

Southern blot analyses of wild-type strains: In order to characterize restriction site and fragment length polymorphisms compatible with wild type *st* function, we examined the DNA of several wild-type strains by DNA blotting. The blots were probed initially with either λ stR4, λ st4 or λ st25, thus covering a region of 29 kb (-11.2 to +17.9), and then with plasmid subclones to map the region more accurately. For convenience, restriction fragments are given codes indicating the restriction sites that define them and their length, *e.g.*, H/R1.4 for the 1.4 kb *HindIII/EcoRI* fragment (co-ordinates 0.0 to 1.4). Figure 3A

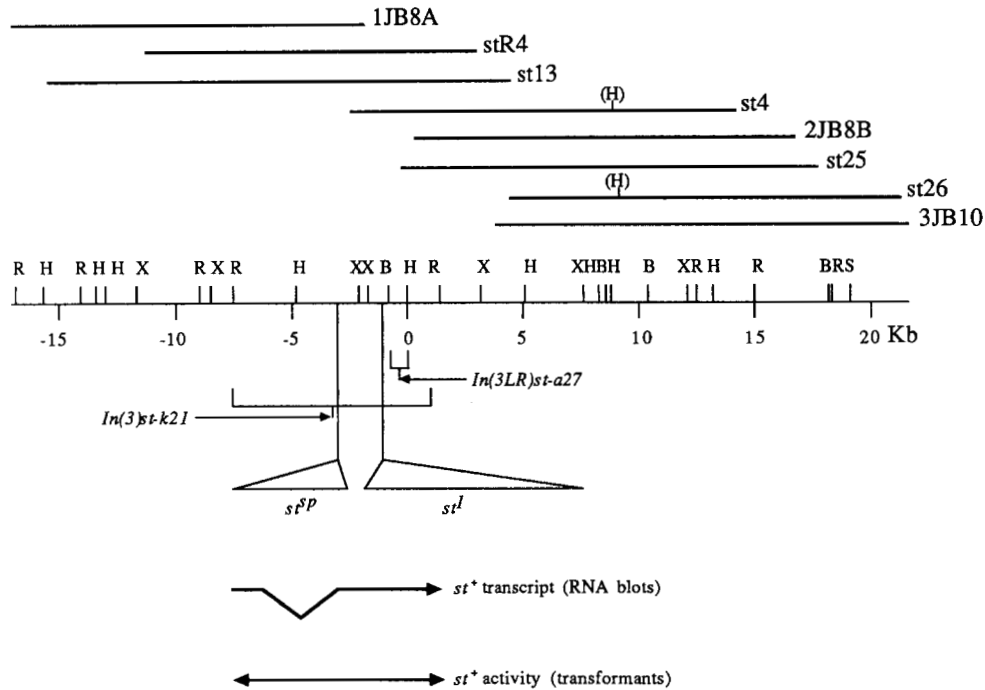


FIGURE 2.—DNA from the *scarlet* region. A restriction map of *st* region DNA from wild-type (Canton S) flies is shown. Restriction enzymes are abbreviated as in Figure 1, plus X = *Xho*I. Position 0 is the *Hind*III site nearest the *In(3LR)st-a27* breakpoint. The positions of recombinant phage from this region are shown above the map and those of various chromosomal rearrangements that disrupt *st*⁺ function are shown below the map. Horizontal lines below the map indicate the restriction fragments that hybridize to *st* RNA and also the fragment that has been tested successfully for *st*⁺ transforming ability.

gives an example of a blot, showing the results of using λ stR4 to probe Canton S and Oregon R DNA.

Our Canton S strain is not isogenic for the 3rd chromosome, and is polymorphic for *Hind*III restriction sites at positions -11.7 and $+8.8$ (shown in parentheses in Figure 2). Thus the presence of a *Hind*III site at -11.7 in some chromosomes results in the appearance of a minor 2.7-kb H/R band as well as the predominant 4.0-kb band (-13.0 to -9.0), while the absence of the *Hind*III site at $+8.8$ in a minority of chromosomes results in contiguous fragments of 0.5 kb and 3.7 kb being replaced by a 4.2-kb H/R band ($+8.3$ + 12.5). The *st* region of our Oregon R strain differs from that of Canton S in two respects: (1) there is an additional *Hind*III site at $+4.0$ and which results in the appearance of a 2.6-kb R/H fragment in place of the 4.0-kb Canton S fragment, and (2) there is a restriction fragment length polymorphism that makes the 4.0-kb H/R fragment seen in Canton S (-13.0 to -9.0) about 4.5 kb in length. We interpret this to be a length polymorphism rather than the loss of one restriction site and the gain of another. Our Canberra strain has a restriction pattern in the *st* region similar to that of Oregon R, while the Amherst M56i strain has a pattern similar to Canton S.

Southern blot analyses of *st* mutant strains: As the first approach toward defining the *st*⁺ functional unit, we examined several *st* mutants for DNA rearrangements associated with the loss of *st*⁺ activity.

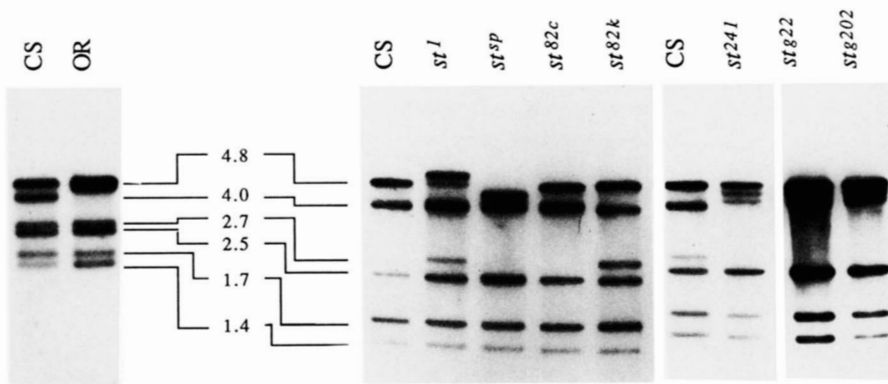
The most useful mutants for this purpose were the X-ray-induced alleles, since they arose in a defined isogenic third chromosome line, *Ki roe p^p*. Most informative among the X-ray-induced mutants were the two *st* inversions. *In(3LR)st-a27*, which was used for the isolation of the *st* locus, has been discussed above. It has a chromosomal breakpoint in the region -0.8 to 0.0 on the molecular map. The *st* region breakpoint of *In(3)st-k21* has not been mapped with the same accuracy, and is known only to lie somewhere between -7.3 and $+1.4$. Five other X-ray-induced *st* mutants (*st*²⁴¹, *st*^{g22}, *st*^{g202}, *st*^{b22}, and *st*^{a24}) that are not associated with cytologically visible rearrangements were examined by Southern blotting and found to be indistinguishable from the parental stock. Patterns of bands for three (*st*²⁴¹, *st*^{g22} and *st*^{g202}) are shown in Figure 3, B and C; although slightly different from Canton S, the patterns are identical to each other and to that obtained with DNA from *Ki roe p^p* (the parental chromosome) (data not shown).

In addition to these newly induced mutants, we examined four other cytologically normal mutant alleles: *st*^l, *st*^{sp}, *st*^{g2k}, and *st*^{g2c3} (Figure 3, B and C). Unfortunately, the parental chromosomes on which these mutations arose are not available for direct comparisons. Three of these mutations, *st*^l, *st*^{sp}, and *st*^{g2c3} are associated with changes within the same region disrupted by the *st* inversions.

The *st*^l mutation is associated with the insertion of

A. *Hin* dIII/*Eco* RI

B. *Hin* dIII/*Eco* RI



C. *Bam* HI/*Xho* I

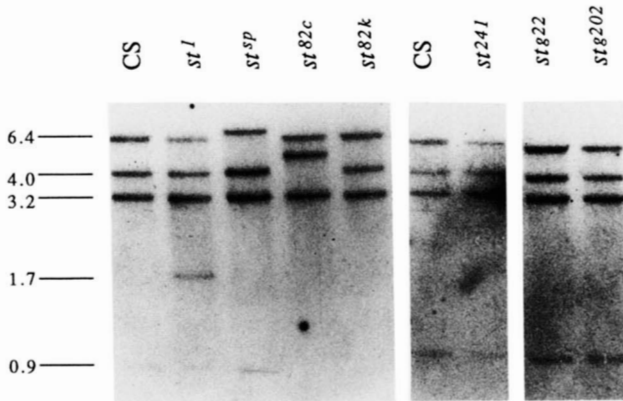


FIGURE 3.—Analysis of wild-type and mutant *scarlet* genes. DNA from each of the strains shown was digested with the enzymes indicated, blotted and probed with radiolabeled λ stR4 DNA. Lengths (kb) of the major bands (shown beside the panels) were determined from the mobilities of marker DNA fragments (*Hind*III-digested λ DNA) run on each gel. CS = Canton S; OR = Oregon R.

approximately 7.6 kb of non-*st* DNA between -1.7 and -0.8 . Thus the 0.9-kb X/B fragment in Canton S is replaced by a 1.7-kb fragment in *st*¹ (Figure 3C; lanes 1 and 2). A second new fragment also appears but is too small to be seen on this blot. The 4.8-kb H/H fragment (-4.8 to 0.0) in Canton S is replaced by bands of 5.5 and 2.7 kb (Figure 3B; lanes 1 and 2). (Residual wild-type 0.9-kb X/B and 4.8-kb H/H bands seen in the *st*¹ lanes were the result of slight contamination of the *st*¹ stock from which this DNA was made; they were not seen on blots involving other preparations of *st*¹ DNA.) A partial map of the *st*¹ insertion (Figure 4), compiled from these and other Southern blots, shows no obvious similarity to published maps of any well-characterized *Drosophila* transposable element.

The *st*^{sp} chromosome contains an insertion of approximately 5.2 kb between -3.0 and -2.1 . This results in the replacement of the 4.8-kb H/H band (-4.8 to 0.0) with fragments of 4.3 and 2.5 kb (Figure 3B; lanes 1 and 3); the 2.5-kb band is obscured by the presence of a 2.5-kb R/H fragment, but comparison with neighboring lanes reveals that this band is a

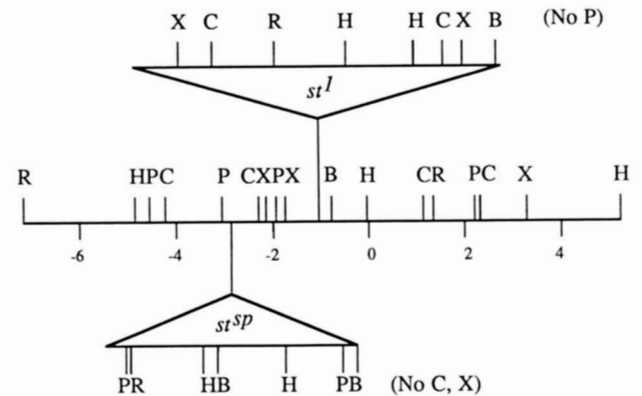


FIGURE 4.—Mutant *st*¹ and *st*^{sp} are associated with insertions. Partial restriction maps of the insertions in *st*¹ and *st*^{sp}. Restriction enzymes are abbreviated as in Figure 2, plus C = *Cl*aI, and P = *P*stI. Sites within the insertions were determined from the sizes of restriction fragments on Southern blots; note that a maximum of two sites can be determined for each enzyme since additional sites lying between them will not be detected. Restriction enzymes that do not cut the insertions are indicated in parentheses. Map coordinates are given as in Figure 2.

doublet. In the *Xho*I/*Bam*HI digest, the 6.4-kb X/X fragment (-8.5 to -2.1) is replaced by bands of approximately 8.0 and 0.9 kb (Figure 3C; lanes 1 and

3). The partial restriction map of this insertion (Figure 4) shows no obvious similarity to published restriction maps of *Drosophila* transposable elements. The spotted phenotype of *st^{sp}* is not particularly obvious in wild-type backgrounds; however, when combined with *w^{Bux}* (to remove the drosopterins) *st^{sp}* shows an eye pigment pattern reminiscent of *w^{sp}* alleles—a pale yellow eye with spots of darker brown.

A putative MR-induced allele, *st^{82c3}* (M. M. GREEN, personal communication), is missing the *Bam*HI site at -0.8 . Thus, the 0.9-kb X/B fragment (-1.7 to -0.8) and the adjacent 4.0-kb B/X fragment (-0.8 to $+3.2$) of Canton S are replaced by a band of 4.9 kb (Figure 3C; lanes 1 and 4). If the loss of the *Bam*HI site is the result of a deletion, it must be rather small, since we can detect no change in the size of the 1.7-kb X/H fragment (-1.7 to 0.0) in the *st^{82c3}* mutant DNA (data not shown).

All of the *st⁻* rearrangements analyzed thus far are located between -7.3 and $+1.4$, suggesting that the *st⁺* functional unit lies at least partly within this region. Recent DNA transformation studies (A. ELIZUR, R. G. TEARLE, R. B. SAINT and A. J. HOWELLS, unpublished data) have confirmed this by showing that a fragment extending from -7.3 to $+1.4$ is capable of supplying *st⁺* function.

Transcription of *scarlet*: In order to characterize the size of the *st* transcription unit we probed blots of poly(A⁺) RNA from wild type third instar larvae with riboprobes made from various restriction fragments from across the region. The probes were strand specific, with transcription being from left to right on the map shown in Figure 2. Figure 5A shows a number of such blots. These blots show that fragments located between the *Pst*I site at -3.0 and the *Eco*RI site at $+1.4$ hybridize to a 2.3 kb transcript. The fragment immediately to the left of -3.0 (1.5-kb P/P) fails to hybridize to any transcript but the next leftward fragment (2.4-kb R/H, -4.5 to -6.9) again hybridizes to a 2.3 kb transcript. Recent sequencing data (Y. HAUPT and A. J. HOWELLS, unpublished data) suggests that part of the *st* coding region is located in the 2.4-kb R/H fragment and that the 1.5-kb P/P fragment is probably part of a relatively long intron. The fragment to the right of 0.0 (1.4-kb H/R, 0.0 to $+1.4$) weakly hybridizes to a 2.3-kb transcript, as well as to transcripts of about 2.6 and 3.6 kb. We interpret the latter two bands to be products of an adjacent proximal transcription unit. The bands seen on these blots are resistant to ribonuclease digestion and are therefore unlikely to be the result of spurious hybridization by the riboprobes. The results are consistent with the size of the gene inferred from our study of *st⁻* chromosomal rearrangements and with the transformation results mentioned above. The putative *st* transcript is present at substantially lower levels than *white* tran-

scripts in the same RNA samples from third instar larvae (data not shown).

The *st^l* and *st^{sp}* mutations involve the insertion of DNA into or near the *st* transcription unit. The mutations might alter the level or structure of the *st* RNA. Figure 5B shows blots of poly(A⁺) RNA from both *st^l* and *st^{sp}* pupae hybridized with region-specific probes. A faint *st* transcript (<5% of wildtype) is detected in the *st^{sp}* sample using a probe covering the region -1.7 to -0.8 ; the higher level of background usually associated with the probe covering the region -0.8 to 0.0 prevents us from determining whether this fragment also detects the transcript in *st^{sp}*. *st^l*, on the other hand, gives rise to an RNA which is slightly smaller and less abundant than the wild-type *st* transcript. This RNA is detected using a probe containing sequences to the left of the *st^l* insertion (-1.7 to -0.8). Although the differences in length and abundance of the *st^l* transcript relative to *st⁺* are small, they are quite reproducible. A probe lying completely to the right of the *st^l* insertion (-0.8 to 0.0) does not detect this RNA, indicating that the *st^l* RNA is truncated near, or within, the *st^l* insertion. The observation that the 2.3-kb RNA is affected by both of these spontaneous *st* mutations provides strong evidence that this is the *st* gene transcript.

Developmental analyses using a temperature-sensitive *st* allele (HOWELLS 1979) indicate that the temperature-sensitive period for eye pigmentation is approximately 24–48 hr after pupariation at 25°. Other studies show that *st⁺* must act in the larval Malpighian tubules. We have used RNA blotting to determine if the different developmental requirements for *st⁺* activity are reflected in changes in expression of the *st* gene at the level of the RNA. Figure 5C shows RNA from different developmental stages hybridized to a probe from region -0.8 to -0.0 . The major *st⁺* transcript is present at all stages. The level of *st⁺* RNA is developmentally regulated such that it is present in about 10-fold higher levels in early to mid pupae than at any other stage. This corresponds well to the time at which the gene would need to be expressed for proper pigmentation of the eye.

Since the *st⁺* and the *w⁺* genes appear to play similar roles in the pathway leading to the deposition of xanthommatin, we wished to determine if *st⁺* expression is regulated by *w⁺*. In other words, is the *w⁺* gene function required for the expression of *st⁺*? We have addressed this question by carrying out an RNA blot analysis of the *w^l* mutation. The blots shown in Figure 5D show that the level of *st⁺* RNA does not change drastically in the presence of *w^l*, suggesting that *w⁺* does not regulate the transcription of the *st⁺* gene.

Homology between *scarlet* region DNA and *white* locus sequences: Because of the similar effects of *st* and *w* mutants on ommochrome deposition, we wished

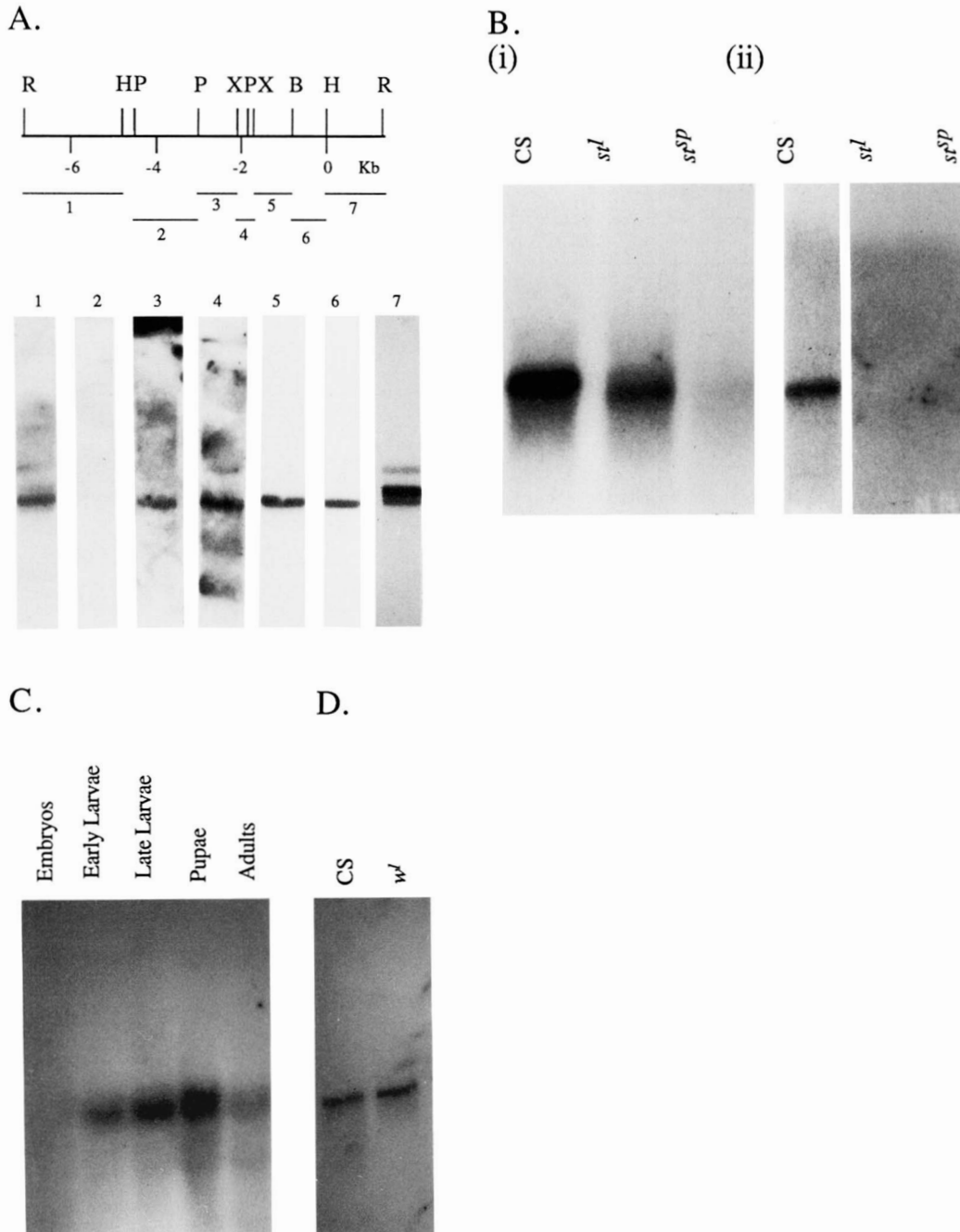


FIGURE 5.—RNA expression of the *scarlet* gene. (A) Location of the *st* transcription unit. An RNA blot (10 μ g poly(A⁺) RNA/lane) was cut into strips and the strips hybridized to riboprobes made from restriction fragments from across the *st* region. After hybridization and washing, the filters were autoradiographed. The autoradiographs were aligned for photography. Positions of the fragments from which the riboprobes were made are shown below the restriction map. (B) Expression of *st* RNA in *st* mutants. Poly(A⁺) RNA (5 μ g/lane) from wild type (Canton S) or *st* mutant early pupae was blotted and probed with riboprobes covering either region -1.7 to -0.8 (left three lanes) or -0.8 to 0.0 (right three lanes) (probes 5 and 6 respectively) (A). The same RNA preparations were used for the blots shown in (i) and (ii) and, for each blot, all three lanes were part of the same gel and were hybridized together. (C) Developmental expression of *st*. Poly(A⁺) RNA (10 μ g/lane) from various developmental stages was separated by electrophoresis, blotted and probed with a riboprobe covering the region -0.8 to 0.0 (probe 6) (A). (D) Expression of *st* RNA in the presence and absence of the *w*⁺ gene. Poly(A⁺) RNA from wild-type (*w*⁺) or mutant (*w*⁻) third instar larvae was blotted and probed as in (C).

to determine if there is any similarity between the coding or regulatory sequences of the two cloned loci. A clone (λ w45A2) carrying the *w*⁺ gene and surrounding DNA (coordinates -5.2 to 9.2 ; LEVIS, BINGHAM and RUBIN 1982) was isolated from the wild-type Canton S library of MANIATIS *et al.* (1978). DNA from

λ w45A2 was digested with various restriction enzymes and probed with subclones from the *st* region (-4.8 to $+3.4$). A subclone carrying the 4.8-kb H/H *st* fragment (-4.8 to 0.0) cross-hybridizes to a 1.1-kb S/Xb fragment of the *w* locus (-0.7 to $+0.4$). A subclone carrying the 2.0-kb S/B fragment (-0.7 to $+1.3$) of *w*

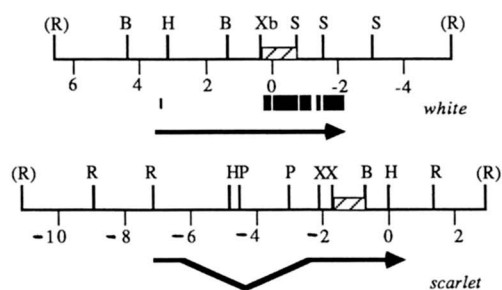


FIGURE 6.—Cross-hybridization between *scarlet* and *white*. The top part of the figure shows restriction maps of phage clones carrying the w^+ ($\lambda w45A2$) and st^+ ($\lambda stR4$) genes. They have been aligned so that transcription of both genes is from left to right. Exons of w are shown as black bars under the w map. The approximate extent of the st transcription unit is shown as a horizontal line under the st map. Restriction enzymes are abbreviated as in Figure 4, plus Xb = *Xba*I. The major cross-hybridizing fragments are indicated by cross hatching. The bottom part of the figure shows DNA from the st or w clones digested with the restriction enzymes indicated. The stained gels (on the left) were blotted and probed, as indicated, with a w specific probe (−0.7 to +1.3) or a st specific probe (−4.8 to 0.0) to give the autoradiographs (on the right). The positions of marker fragments (*Acc*I-digested λ DNA) are indicated. Hybridization conditions were as given for Southern blots (MATERIALS AND METHODS); probes were labeled by nick translation.

cross-hybridizes to the 0.9-kb X/B fragment (−1.7 to −0.8) of st . This hybridization can be detected after hybridization ($1.5 \times$ SSPE at 68°) and washing ($0.1 \times$ SSC at 60°) under stringent conditions. The regions of st and w cross-hybridization are indicated on the restriction maps shown in Figure 6. The region lying downstream of the *Xho*I site (−1.7) of st was sequenced and a 320 bp region of this sequence showing the greatest homology to w is given in Figure 7; this sequence contains a single open reading-frame and the derived amino acid sequence is shown under the nucleotide sequence. Also given in Figure 7 is the

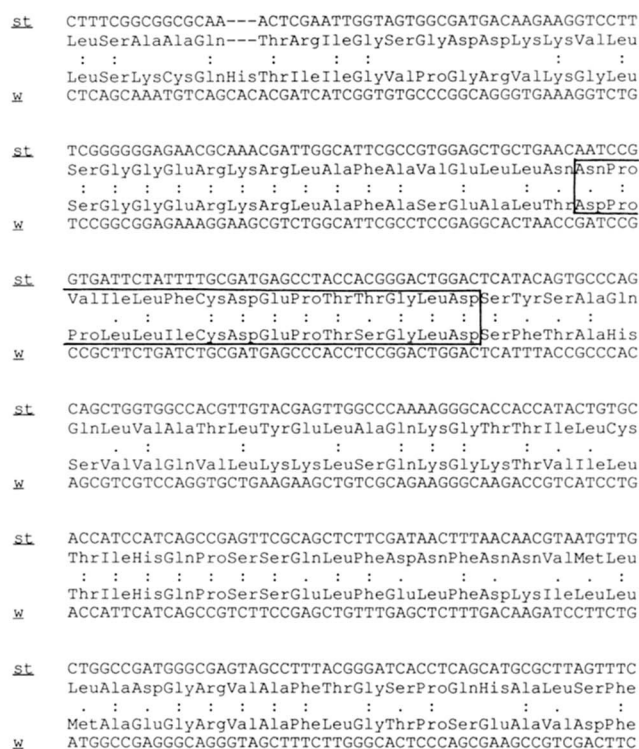


FIGURE 7.—Sequence homology between *scarlet* and *white*. The nucleotide sequence for st starts 281 nucleotides downstream from the *Xho*I site (coordinate −1.7); the w sequence is from O'HARE *et al.* (1984) (position −354 to −678). Colons between the deduced amino acids indicate identical residues; a single dot shows a conservative substitution. The boxed region is strongly homologous to site B of the ATP-binding region of bacterial periplasmic transport proteins (AMES 1986).

sequence of the homologous region from w (O'HARE *et al.* 1984), which shows that the overall homology between the two genes in this region is 59% (identical residues—at both the nucleotide and derived amino acid levels). This sequence includes one of the putative ATP-binding sites of the w protein (MOUNT 1987); the homology of the 100 nucleotides which encodes this site (and its immediate surroundings) is 72%, which probably accounts for the cross-hybridization between the two genes (as shown in Figure 6). Further DNA sequencing (A. ELIZUR, R. G. TEARLE, D. G. BOYLE and A. J. HOWELLS, in preparation) has shown that other smaller patches of homology not seen by hybridization studies are present in other parts of the putative coding regions.

DISCUSSION

The evidence that $\lambda stR4$ contains the st gene is overwhelming. (1) Sequences from the clone correspond to the non-*rosy* region sequences of clones $\lambda sta27-1$ and $\lambda sta27-2$, which span the breakpoints of *In(3LR)st-a27*. This inversion chromosome, which is associated with a st mutant phenotype, has one break in 73A3.4, the cytological position of st ; the correspondence of the st mutant phenotype and the cytological location implies strongly that the breakpoint is

either very close to, or within, the *st* gene. (2) In the DNA of four different mutant stocks showing a *st* phenotype, the restriction map of the region relative to wild type is altered. (3) Successful germline transformation of *st*¹ individuals to *st*⁺ has been achieved following the injection of *st*¹ embryos with a P-element transformation vector carrying an 8.7-kb fragment spanning coordinates -7.3 to +1.4 (A. ELIZUR, R. G. TEARLE, R. B. SAINT and A. J. HOWELLS, unpublished data). (4) A single 2.3-kb transcript is altered in *st*¹ and greatly reduced in abundance in *st*^{sp}. (5) Some of the sequences from within the above transcription unit show significant sequence similarity to the *white* gene, another locus involved in ommochrome metabolism.

The transformation experiments, together with the Northern blot analysis, indicate that the *st* transcription unit is confined to the region from -7.3 to +1.4. When DNA from Canton S is analyzed by Southern blotting using probes covering this region, few restriction site or fragment length polymorphisms are found. In contrast, DNA sequences which lie both distal (-14.1 to -9.0) and proximal (+1.4 to +12.5) to the *st* transcription unit show considerable polymorphism. The distal region is not only polymorphic in our Canton S strain but also shows different restriction patterns in all of the different wild type strains that we have examined. Obviously more detailed data is needed but it does seem that the regions that flank *st* are highly polymorphic. Such variation is not typical of the *D. melanogaster* genome in general, although some highly polymorphic regions, such as those containing the *zeste* locus, have been found (MARIANI, PIRROTTA and MANET 1985).

The developmental pattern of expression of the *st* gene at the RNA level agrees with biochemical and genetic data concerning this gene. Specifically, there are low levels of expression in larvae and adults and an obvious peak of expression in the pupal period. Tissue transplantation experiments (BEADLE, 1937a, b) suggest that the Malpighian tubules normally require expression of both *st* and *w* for storage of ommochrome pigment precursors. The presence of *st*⁺ transcripts throughout larval life (albeit at a low level) probably reflects this expression in the Malpighian tubules. This has been directly demonstrated for RNAs from the *w* gene (FJOSE *et al.* 1984).

Pigment deposition in the eyes commences about 48 hr after pupariation (SUMMERS, HOWELLS and PYLIOTIS 1982). Genetic analysis using a temperature-sensitive *st* allele shows that *st* expression is required just prior to this time (about 24-48 hr post pupariation) for proper pigmentation of the eyes (HOWELLS 1979). This correlates with a tenfold increase in the level of *st* RNA early in the pupal period. Finer-scale analysis of *st* gene expression during pupal life (data

not shown) indicates that the peak level of *st* RNA corresponds to the temperature-sensitive period for the temperature-sensitive mutant (*i.e.*, 24-48 hr after pupariation). As for *w* (LEVIS, O'HARE and RUBIN 1984; PIRROTTA and BROCKL 1984), *st* RNA can be detected in adults. Whether this reflects stability of the mRNA made during pupal life or continued low level transcription has not been determined.

The transcriptional results obtained for *st*¹ are compatible with the position of the non-*st* DNA insertion in this mutant. The use of two probes covering the -1.7 to 0.0 region was particularly informative. The probe covering the region -1.7 to -0.8 (which lies 5' to the *st*¹ insertion) detects a transcript which appears to be slightly smaller than that found in wild type. However a probe covering the region just 3' to the *st*¹ insertion fails to detect a major transcript. Thus, it seems likely that in *st*¹, transcription initiates from the normal *st* promoter (probably located between -7.3 and -4.8) and terminates within the *st*¹ insertion. Since the *st*¹ transcript is polyadenylated, a polyadenylation signal must be located within this insertion. Polyadenylated aberrant transcripts which terminate within inserted transposable elements have been found in several *w* mutants (PIRROTTA and BROCKL 1984; LEVIS, O'HARE and RUBIN 1984; ZACHAR, CHAPMAN and BINGHAM 1985; BINGHAM and CHAPMAN 1986).

The *st*^{sp} mutation is of interest because it gives a spotted pigment pattern reminiscent of the *w*^{sp} alleles. This suggests that the lesion in *st*^{sp}, like those in the *w*^{sp} alleles, may affect regulatory rather than coding sequences. The severely reduced level of the *st* transcript in 6-30 h *st*^{sp} pupae is consistent with this suggestion; however, unlike the situation with the *w*^{sp} alleles, where the lesions lie 5' to the coding region, the insertion in *st*^{sp} appears to be located within an intron. This may mean that some of the regulatory sequences of the *st* gene are located within this intron; however it is also possible that the insertion has a *cis*-acting inhibitory effect on the *st* promoter or that an aberrant *st*^{sp} transcript is unstable and is rapidly degraded.

As outlined in the Introduction, *st* and *w* mutants show marked similarities at the genetic and biochemical levels, raising the question of whether their products perform similar functions, or whether there is a regulatory relationship between them. Our molecular analysis has revealed that there is considerable nucleotide similarity between the two genes. The sequencing data, which has confirmed the hybridization analysis, shows that the *st/w* homology is located in coding regions of these two genes and is just as strikingly apparent in the derived amino acid sequences. Although the functional significance of this similarity between the putative protein products of *st* and *w*

must await further analysis, it would appear that the phenotypic similarity between lesions at the two loci reflects a common functional role for the proteins. MOUNT (1987) has pointed out the sequence similarity between the putative *w* protein and a family of ATP-binding proteins involved in bacterial periplasmic transport systems. As shown in Figure 7, the strongest homology between the derived amino acid sequences of *w* and *st* includes a region thought to be involved in ATP binding. A role for the *w* and *st* proteins in the transport of xanthommatin precursors across the plasma membrane of pigment cells was proposed by SULLIVAN and SULLIVAN (1975). The *w* gene is essential for pteridine as well as ommochrome pigment production. The phenotypic analogue of *st* in the pteridine biosynthesis pathway appears to be the *brown* (*bw*) gene (SULLIVAN *et al.* 1980); recent molecular analysis of the *bw* gene has indicated that there is also strong amino acid homology between the protein products of the *w* and *bw* genes (DRESEN, JOHNSON and HENIKOFF 1988). As discussed by these authors, it seems likely that the *w*, *bw* and *st* gene products are members of a family of membrane-spanning, ATP-binding proteins which associate to provide the permeases involved in the uptake of pigment precursors.

An alternative hypothesis concerning the phenotypic similarities of lesions at *st* and *w*, that one gene regulates the expression of the other, is not supported by the data. The level of the *st* transcript in third instar larvae is not affected by the *w*¹ mutation (our data), and the level of *w* gene expression has previously been shown not to be affected in *st*¹ adults (LEVIS, O'HARE and RUBIN 1984).

Given the similarities in the tissue-specific and temporal expression of the *w* and *st* genes (see Introduction), we consider it possible that conserved regulatory as well as structural sequences might exist between the two genes. Such a possibility is exciting because of the implications it might have for the regulation and function of the ommochrome biosynthetic pathway in particular, and for the coordinate control of functionally related genes in eukaryotes in general. Since the nucleotide sequence of the putative regulatory region upstream of *w* has been established (O'HARE *et al.* 1984) and analyzed in some detail (DAVISON *et al.* 1985; ZACHAR, CHAPMAN and BINGHAM 1985; PIRROTTA, STELLER and BOZZETTI 1985; LEVIS, HAZELRIGG and RUBIN 1986), we are now determining the nucleotide sequences of the upstream and intronic regions of *st* in order to identify any shared regulatory sequences.

We thank K. C. REED and A. ELIZUR for advice about methods and for valuable discussions. This work was supported by grants from the Australian Research Grants Scheme to A.J.H., and by grants from the U.S. National Institutes of Health (NIH) and the National Science Foundation to B.S.B. and J.M.B. R.G.T. was supported by an Australian Commonwealth Postgraduate Award

and M.M. was supported by an NIH postdoctoral training grant, and by a postdoctoral fellowship from the Helen Hay Whitney Foundation.

LITERATURE CITED

- AMES, G. F.-L. 1986 Bacterial periplasmic transport systems: structure, mechanism, and evolution. *Annu. Rev. Biochem.* **55**: 397-425.
- ASHBURNER, M., J. FAITHFULL, T. LITTLEWOOD, G. RICHARDS, S. SMITH, V. VELISSARIOU and R. WOODRUFF, 1980 New mutants. *Drosophila Inform. Serv.* **55**: 193-195.
- ASHBURNER, M., P. ANGEL, C. DETWILER, J. FAITHFULL, D. GUBB, G. HARRINGTON, T. LITTLEWOOD, S. TSUBOTA, V. VELISSARIOU and V. WALKER, 1981 New mutants. *Drosophila Inform. Serv.* **56**: 186-191.
- BAGLIONI, C., 1960 Genetic control of tryptophan pyrrolase in *Drosophila melanogaster* and *Drosophila virilis*. *Heredity* **15**: 87-96.
- BAILLIE, D. L., and A. CHOVIK, 1971 Studies on the genetic control of tryptophan pyrrolase in *Drosophila melanogaster*. *Mol. Gen. Genet.* **112**: 341-353.
- BAKER, B. S., and K. RIDGE, 1980 Sex and the single cell: on the action of major loci affecting sex determination in *Drosophila melanogaster*. *Genetics* **94**: 383-423.
- BEADLE, G. W., 1937a Development of eye colors in *Drosophila*: Fat bodies and Malpighian tubules as sources of diffusible substances. *Genetics* **22**: 146-152.
- BEADLE, G. W., 1937b Development of eye colors in *Drosophila*: Fat bodies and Malpighian tubules in relation to diffusible substances. *Genetics* **22**: 587-611.
- BEADLE, G. W., and B. EPHRUSSI, 1937 Development of eye colors in *Drosophila*: Diffusible substances and their interrelations. *Genetics* **22**: 76-86.
- BELOTE, J. M., and M. MCKEOWN, 1985 Post-replicative repair of an X-ray damaged chromosome following fertilization in *Drosophila melanogaster*. *Drosophila Inform. Serv.* **61**: 33-34.
- BENDER, W., P. SPIERER and D. S. HOGNESS, 1983 Chromosomal walking and jumping to isolate DNA from the *Ace* and *rosy* loci and the *bithorax* complex in *D. melanogaster*. *J. Mol. Biol.* **168**: 17-33.
- BINGHAM, P. M., and C. H. CHAPMAN, 1986 Evidence that *white-blood* is a novel type of temperature-sensitive mutation resulting from temperature dependent effects of a transposon insertion on formation of *white* transcripts. *EMBO J.* **5**: 3343-3351.
- BINGHAM, P. M., and Z. ZACHAR, 1985 Evidence that two mutations, *w*^{Dst} and *z*¹, affecting synapsis dependent genetic behavior of *white* are transcriptional regulatory mutants. *Cell* **40**: 819-825.
- CHIRGWIN, J. M., A. E. PRZYBYLA, R. J. MACDONALD and W. J. RUTTER, 1979 Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* **18**: 5294-5299.
- COEN, E. S., J. M. THODAY and G. DOVER, 1982 Rate of turnover of structural variants in the rDNA family of *Drosophila melanogaster*. *Nature* **295**: 564-568.
- DAVISON, D., C. H. CHAPMAN, C. WEEDEN and P. M. BINGHAM, 1985 Genetic and physical studies of a portion of the *white* locus participating in transcriptional regulation and synapsis-dependent interactions in *Drosophila* adult tissues. *Genetics* **110**: 479-494.
- DRESEN, T. D., D. H. JOHNSON and S. HENIKOFF, 1988 The *brown* protein of *Drosophila melanogaster* is similar to the *white* protein and to components of active transport complexes. *Mol. Cell. Biol.* **8**: 5206-5215.
- EPHRUSSI, B., 1942 Analysis of eye color differentiation in *Drosophila*. *Cold Spring Harbor Symp. Quant. Biol.* **10**: 40-48.
- FJOSE, A., L. C. POLITO, U. WEBER and W. J. GEHRING,

- 1984 Developmental expression of the *white* locus of *Drosophila melanogaster*. *EMBO J.* **3**: 2087–2094.
- FRISCHAUF, A. M., H. LEHRACH, A. POUSTKA and N. MURRAY, 1983 Lambda replacement vectors carrying polylinker sequences. *J. Mol. Biol.* **170**: 827–842.
- GHOSH, D., and H. S. FORREST, 1967 Enzymatic studies on the hydroxylation of kynurenine in *Drosophila melanogaster*. *Genetics* **55**: 423–431.
- HOWELLS, A. J., 1979 Isolation and biochemical analysis of a temperature-sensitive *scarlet* eye colour mutant of *Drosophila melanogaster*. *Biochem. Genet.* **17**: 149–158.
- HOWELLS, A. J., and R. L. RYALL, 1975 A biochemical study of the *scarlet* eye colour of *Drosophila melanogaster*. *Biochem. Genet.* **13**: 273–282.
- LANGER-SAFER, P. R., M. LEVINE and D. WARD, 1982 Immunological method for mapping genes on *Drosophila* polytene chromosomes. *Proc. Natl. Acad. Sci. USA* **79**: 4381–4385.
- LEVIS, R., P. M. BINGHAM and G. M. RUBIN, 1982 Physical map of the *white* locus of *D. melanogaster*. *Proc. Natl. Acad. Sci. USA* **79**: 564–568.
- 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**: 471–481.
- LEVIS, R., T. HAZELRIGG and G. M. RUBIN, 1985 Separable *cis*-acting control elements for expression of the *white* gene of *Drosophila*. *EMBO J.* **4**: 3489–3499.
- LINDSLEY, D. L., and E. H. GRELL, 1968 *Genetic Variations of Drosophila melanogaster*. Carnegie Inst. Wash. Publ. 627.
- LINDSLEY, D. L., and G. ZIMM, 1986 The genome of *Drosophila melanogaster*. Part 2: Lethals; maps. *Drosophila Inform. Serv.* **64**: 72–73.
- LINDSLEY, D. L., and G. ZIMM, 1987 The genome of *Drosophila melanogaster*. Part 3: Rearrangements. *Drosophila Inform. Serv.* **65**: 48.
- MANIATIS, T., E. F. FRITSCH and J. SAMBROOK, 1982 *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- MANIATIS, T., R. C. HARDISON, E. LACY, J. LAUER, C. O'CONNEL, D. QUON, G. K. SIM and A. EFSTRADIATIS, 1978 The isolation of structural genes from libraries of eukaryotic DNA. *Cell* **15**: 687–701.
- MARIANI, C., V. PIRROTTA and E. MANET, 1985 Isolation and characterization of the *zeste* locus of *Drosophila*. *EMBO J.* **4**: 2045–2052.
- MCKEOWN, M., J. M. BELOTE and B. S. BAKER, 1987 A molecular analysis of *transformer*, a gene in *Drosophila melanogaster* that controls female sexual differentiation. *Cell* **48**: 489–499.
- MESSING, J., 1983 New M13 vectors for cloning. *Methods Enzymol.* **101**: 20–78.
- MIKLOS, G. L. G., M. J. HEALY, P. PAIN, A. J. HOWELLS and R. J. RUSSELL, 1984 Molecular and genetic studies on the euchromatin-heterochromatin transition region of the X chromosome of *Drosophila melanogaster*. I. A cloned entry point near to the *uncoordinated* locus. *Chromosoma* **89**: 218–227.
- MOUNT, S. M., 1987 Sequence similarity. *Nature* **325**: 487.
- O'HARE, K., C. MURPHY, R. LEVIS and G. M. RUBIN, 1984 DNA sequence of the *white* locus of *Drosophila melanogaster*. *J. Mol. Biol.* **180**: 437–455.
- PHILLIPS, J. P., and H. S. FORREST, 1980 Ommochromes and pteridines, pp. 542–623 in *The Genetics and Biology of Drosophila*, Vol. 2d, edited by M. ASHBURNER and T. R. F. WRIGHT. Academic Press, New York.
- PIRROTTA, V., and C. BROCKL, 1984 Transcription of the *Drosophila white* locus and some of its mutants. *EMBO J.* **3**: 563–568.
- PIRROTTA, V., H. STELLER and M. P. BOZZETTI, 1985 Multiple upstream regulatory elements control the expression of the *Drosophila white* gene. *EMBO J.* **4**: 3501–3508.
- REED, K. C., and D. MANN, 1985 Rapid transfer of DNA from agarose gels to nylon membranes. *Nucleic Acids Res.* **13**: 7207–7221.
- RYALL, R. L., and A. J. HOWELLS, 1974 Ommochrome biosynthetic pathway of *Drosophila melanogaster*: Variations in the levels of enzyme activities and intermediates during adult development. *Insect Biochem.* **6**: 135–142.
- SANGER, F., S. NICKLEN and A. R. COULSEN, 1977 DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74**: 5463–5467.
- SULLIVAN, D. T., R. J. KITOS and M. C. SULLIVAN, 1973 Developmental and genetic studies on kynurenine hydroxylase from *Drosophila melanogaster*. *Genetics* **75**: 651–661.
- SULLIVAN, D. T., and M. C. SULLIVAN, 1975 Transport defects as the physiological basis for eye color mutants of *Drosophila melanogaster*. *Biochem. Genet.* **13**: 603–613.
- SULLIVAN, D. T., L. A. BELL, D. R. PATON and M. C. SULLIVAN, 1980 Genetic and functional analysis of tryptophan transport in Malpighian tubules of *Drosophila*. *Biochem. Genet.* **18**: 1109–1130.
- SUMMERS, K. M., A. J. HOWELLS and N. A. PYLIOTIS, 1982 Biology of eye pigmentation in insects. *Adv. Insect Physiol.* **16**: 119–166.
- TARTOF, K. D., 1969 Interacting gene systems: I. The regulation of tryptophan pyrrolase by the *vermilion* suppressor of *vermilion* system in *Drosophila*. *Genetics* **62**: 781–795.
- THOMAS, P. S., 1983 Hybridization of denatured RNA transferred or dotted to nitrocellulose paper. *Methods Enzymol.* **100**: 255–266.
- VELISSARIOU, V., and M. ASHBURNER, 1981 Cytogenetic and genetic mapping of a salivary gland secretion protein in *Drosophila melanogaster*. *Chromosoma* **84**: 173–185.
- WALKER, A. R., A. J. HOWELLS and R. G. TEARLE, 1986 Cloning and characterization of the *vermilion* gene of *D. melanogaster*. *Mol. Gen. Genet.* **202**: 102–107.
- ZACHAR, Z., C. H. CHAPMAN and P. M. BINGHAM, 1985 On the molecular basis of transvection effects and the regulation of transcription. *Cold Spring Harbor Symp. Quant. Biol.* **50**: 337–346.

Communicating editor: V. G. FINNERTY