Heritable Somatic Excision of a *Drosophila* Transposon

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A mutation in the *white* gene of *Drosophila mauritiana* that results from insertion of the transposable element *mariner* is genetically unstable in both germ cells and somatic cells. Somatic instability is indicated by the occurrence of animals having mosaic eyes with patches of pigmented cells on a peach-colored background. Normally uncommon, the frequency of mosaicism is so greatly enhanced in a particular mutant strain that virtually every animal in the strain is an eye-color mosaic. The molecular basis of the mosaicism is the excision of the *mariner* element from its location in the DNA of the *white* gene in somatic cells. The phenomenon results from a single dominant genetic factor located in chromosome 3. Genetic control over the excision of transposable elements may play a role in determining the persistence of transposable elements in the genome.

A NOVEL TRANSPOSABLE GENETIC element, designated *mariner*, has recently been characterized in *Drosophila mauritiana*, a sibling species of *D. melanogaster* (*1*). The element was identified during the molecular analysis of a spontaneous mutation in the X-linked gene *white*. Wild-type function of the *white* gene is necessary for the normal brick-red eye color of adult flies (*2*). The mutant allele, *white-peach* (w^{pch}), results in peach-colored eyes and is associated with an insertion of the 1.3-kb *mariner* element slightly upstream from the first exon (*1*). The *white-peach* allele exhibits genetic instability in the germline and reverts to wild type at a frequency of approximately 10^{-3} per gene per generation (*3*). Mosaic individuals with patches of pigmented cells in the eyes are also found at a frequency of approximately 10^{-3} (*3*). However, we have recently discovered a strain in which patches of pigmented cells occur in virtually every fly and in which the somatic mosaicism is highly heritable. We now report that the heritable somatic mosaicism results from a dominant autosomal factor in chromosome 3. The molecular mechanism of the phenomenon is the excision of the *mariner* element from the *white-peach* allele during development.

Representative mosaics from the strain with heritable mosaicism are shown in Fig. 1. The mosaic strain, designated E25H, arose from a cross of *white-peach* females with males carrying a *white* mutation obtained from the Bowling Green *Drosophila* Stock Center. Male and female mosaic offspring from the cross were selected and inbred in subsequent generations to eliminate the *white* mutation from the strain. At the time of this analysis, E25H had been maintained for approximately 45 generations.

Superimposed on the background peach eye color typical of the *white-peach* allele, sectors of pigmented tissue occur in the eyes of E25H flies (Fig. 1). The pigmented sectors vary widely in number, size, and shape. Within the E25H mosaic strain, it is clear that the variegation is strongly heritable because more than 90% of flies exhibit some degree of mosaicism. The E25H strain also exhibits reverse mutation in the germline at a higher frequency than that of the w^{pch} strain from which it was derived. The frequency of reverse mutations in the germline of E25H is at least 10^{-2} per gene per generation, and a significantly higher rate of germline reversion occurs in males than in females (*4*). The revertants are phenotypically wild type and, among more than 10,000 progeny, have not been observed to mutate. We have also isolated *white* mutant derivatives of w^{pch} from E25H. To explain these observations, we hypothesized that the E25H strain contains a genetic factor promoting both somatic and germinal excision of the *mariner* element inserted in the *white* gene.

Genetic studies were carried out to localize the postulated mosaicism factor in the genome of E25H. The occurrence of nonmosaic flies within the E25H strain implied either that the mosaicism factor was not expressed in all flies containing the factor, or that the factor was not completely homozygous in the strain. Lack of homozygosity was suggested by the observation that crosses of individual flies from the E25H strain with *white-peach* flies resulted in progenies in which the frequency of mosaics was either 100% or approximately 50%. The same result was observed in reciprocal crosses, and mosaic progeny were observed in equal proportions in both sexes.

When mosaic males from E25H were mated individually with *white-peach* females, 47 males produced exclusively mosaic progeny and 72 males produced approximately 50% mosaic progeny. Nonmosaic progeny from the latter crosses were tested for the absence of the mosaicism factor by backcrossing to *white-peach* flies. Among 28 nonmosaic progeny from 14 crosses that were tested, none produced any mosaic offspring (1446 progeny in total). Four progenies of matings yielding exclusively mosaic flies were sib-mated and subcultured three times, and all gave the 3:1 ratio expected of dominant autosomal inheritance. The mean numbers of progeny from the four crosses were 1502 mosaics versus 506 nonmosaics. These results suggest that the allele frequency of the mosaicism factor was approximately 70% when these tests were carried out. Strains now exist that are completely homozygous for the mosaicism factor.

The genetic results are consistent with the hypothesis that the E25H strain contains an autosomal dominant genetic factor, not completely homozygous, the presence of which results in eye-color mosaicism in all *white-peach* flies that contain the factor. Moreover, occurrence of the mosaicism is not specific to the particular *white-peach* allele present in the E25H strain because mosaicism occurs in male progeny from the cross of *white-peach* females with E25H males, even though the male progeny receive their *white-peach*–bearing X chromosome from their nonmosaic mothers.

Because few genetic markers have been isolated in *D. mauritiana*, further genetic localization of the mosaicism factor was carried out in a strain of the sibling species *D. simulans* into which both *white-peach* and the mosaicism factor had been introduced by repeated backcrossing. Females from this strain were mated with males of a *D. simulans* strain carrying the mutations *f, net pm,* and *st e* (*forked* bristles, *net* wing veins with *plum* eyes, and *scarlet* eyes with *ebony* body) on the X, 2, and 3 chromosomes, respectively. Mosaic sons were backcrossed with the *f, net pm, st e* strain, and homozygous *net pm* and homozygous *st e* female progeny were selected. Single females, either *net pm* (and heterozygous for *st e*, $n = 53$) or *st e* (and heterozygous for *net pm*, $n = 56$), were crossed with w^{pch} males. The *net pm* females were found to genetically transmit the mosaicism, but the *st e* ones did not. Therefore, the dominant factor for mosaicism is localized to chromosome 3.

A simple explanation for the mosaic phenotype in the E25H strain is that it results from excision of the *mariner* element from the *white-peach* allele in somatic cells. If this were the case, then mosaic flies should contain two classes of DNA molecules homolo-

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gous to the *white* gene—a larger one still containing the *mariner* insert and a smaller one from which the insert had been excised (Fig. 2A). To test this prediction, genomic DNA from mosaic flies of both sexes from the E25H strain was digested with restriction enzymes, separated by agarose gel electrophoresis, and transferred to nitrocellulose (*5–7*). We then hybridized the filter with a 3.0-kb Bam HI fragment from the wild-type *white* gene (Fig. 2B) (*1, 8*). The nonmosaic *white-peach* strain contains an insertion of approximately 1.3 kb (the *mariner* element) in the *white* gene (Fig. 2B, lane b). As expected, w^+/w^{pch} heterozygous females show both the normal 3.0-kb and the larger 4.3-kb restriction fragments (Fig. 2B, lane c). DNA from E25H mosaic flies has the same restriction fragment pattern as heterozygous w^+/w^{pch}, but the smaller molecular weight band (of the size predicted from somatic excision of the 1.3-kb *mariner* element from the 4.3-kb Bam HI fragment) is less intense (Fig. 2B, lane d).

Phenotypically wild-type revertants from E25H are indistinguishable from normal wild-type flies at this resolution (Fig. 2B, lane e) and nonmosaic flies of the E25H strain do not show any detectable excision in other tissues (Fig. 2B, lane f). This latter observation is consistent with the genetic evidence indicating that the putative excision factor is highly penetrant and that its presence in the genome is strongly correlated with the mosaic eye phenotype.

Somatic excision occurs in other parts of the body, not only in the eyes (Fig. 2B, lanes g, h, and i). The 3.0-kb DNA band resulting from somatic excision occurs in extracts of heads (lane g), thoraxes (lane h), and abdomens (lane i). Although the excision band is clearly more intense in the extracts of heads, this may reflect the fact that these animals were selected because of their visible eye-color mosaicism. The genome of *D. mauritiana* contains approximately 20 copies of the *mariner* sequence (*1*), and it is not known whether somatic excision also occurs with elements other than the one inserted in *white-peach*.

Our data demonstrate somatic excision of the *mariner* transposable DNA sequence from the *white* gene in a *D. mauritiana white-peach* strain; the excision is highly heritable and segregates as a single autosomal-dominant factor. Although the mosaicism factor has not yet been identified, it may be an overexpressed copy of the *mariner* element itself, with increased expression due to the site of insertion or perhaps an alteration in regulatory sequences within the element. On the basis of this hypothesis, one might expect the mosaicism factor itself to be genetically unstable or to undergo transposition, but to this point we have no evidence of such phenomena. Alternatively, the mosaicism factor could be a mutant form of a normal *Drosophila* gene that promotes excision of the *mariner* insert from *white-peach*.

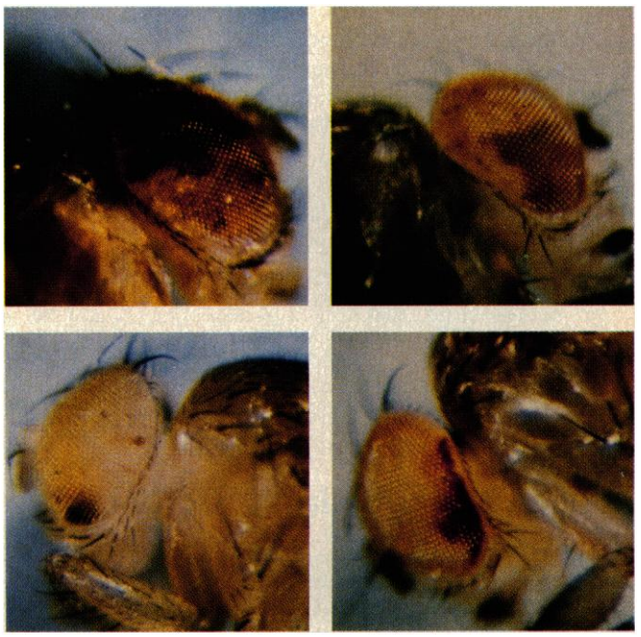

Fig. 1. A representative sample of flies from the E25H mosaic line of *Drosophila mauritiana*.

Frequent instability in somatic cells distinguishes the *mariner* element from other transposable elements described in *Drosophila*. In the case of the transposable element P in *D. melanogaster*, for example, transposition is normally restricted to the germline, apparently by differential RNA splicing, and somatic instability is observed only with an artificial construct that lacks one key intron (*9*). The characteristics of the *mariner* element are similar to those observed for certain transposable elements in maize, in which variegated phenotypes resulting from somatically unstable mutations in genes that affect kernel morphology and pigmentation are well documented (*10*). Certain strains of the nematode *Caenorhabditis elegans* undergo somatic excision of the transposable element *Tc1*, with excision in cells of the germline being a much rarer event (*11*).

Further direct and comparative studies of transposable elements should reveal why some are active in both somatic and germ cells while others are active only in the germline. The existence of high rates of both somatic and germline excision of the *mariner* element in *D. mauritiana* suggests a possible mechanism for genomes to lose entire families of transposable elements when the rate of germline excision exceeds the rate of transposition. If excision process-

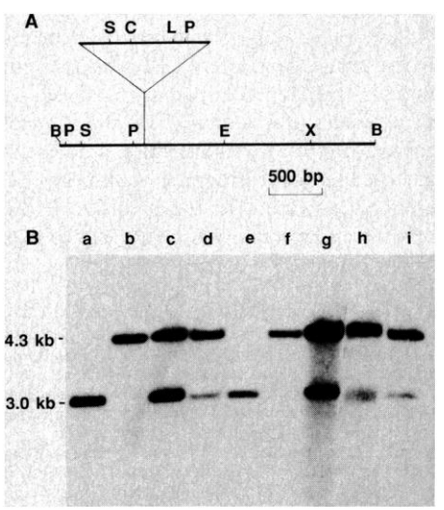

Fig. 2. (**A**) Restriction maps of the 3.0-kb Bam HI fragment from the *white* region in wild-type *D. mauritiana* (plasmid pJJ3) and the 1.3-kb *mariner* insert in the *white-peach* mutation (plasmid pJJ1) (*1*). Abbreviations for restriction endonucleases are as follows: B, Bam HI; P, Pvu II; S, Sph I; E, Eco RV; X, Xho I; C, Sac I; L, Sal I. (**B**) Autoradiograph of Southern transfer hybridization of genomic DNA from mosaic and nonmosaic flies. Genomic DNA was digested with Bam HI, separated on a 0.7% agarose gel, and transferred to nitrocellulose. The filter was then hybridized with the nick-translated 3.0-kb Bam HI fragment from plasmid pJJ3, which contains only wild-type *white* sequences. DNA was obtained from the following flies: a, w^+; b, w^{pch}; c, w^+/w^{pch} heterozygous females; d, E25H mosaics; e, wild-type revertants from E25H; f, phenotypically w^{pch} nonmosaic flies from E25H; g, E25H heads; h, E25H thoraxes; and i, E25H abdomens. Each lane contains approximately 3 μg of DNA except for lane g, which contains >> 3 μg. See (*1, 5–8*) for procedures for isolation of genomic DNA, Southern transfer, nick translation, and filter hybridization.

es analogous to the one described here occur with other transposable elements, then the proposed excision-loss mechanism could explain unusual patterns in the distribution and abundance of transposable elements among related species (*12*).

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Human Lipoprotein Lipase Complementary DNA Sequence

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Lipoprotein lipase is a key enzyme of lipid metabolism that acts to hydrolyze triglycerides, providing free fatty acids for cells and affecting the maturation of circulating lipoproteins. It has been proposed that the enzyme plays a role in the development of obesity and atherosclerosis. The human enzyme has been difficult to purify and its protein sequence was heretofore undetermined. A complementary DNA for human lipoprotein lipase that codes for a mature protein of 448 amino acids has now been cloned and sequenced. Analysis of the sequence indicates that human lipoprotein lipase, hepatic lipase, and pancreatic lipase are members of a gene family. Two distinct species of lipoprotein lipase messenger RNA that arise from alternative sites of 3′-terminal polyadenylation were detected in several different tissues.

LIPOPROTEIN LIPASE (LPL) PLAYS A major role in the regulation of lipid metabolism. Its primary function is the hydrolysis of the core triglycerides of circulating chylomicrons and very low density lipoproteins (VLDL). In a broader sense, LPL affects the maturation of several classes of lipoprotein particles and may facilitate the transfer of cholesteryl esters to endothelial cells. Thus, the fate of plasma cholesterol, as well as triglycerides, depends on the proper function of LPL. Lipoprotein lipase is synthesized in parenchymal cells of many tissues, but functions on the luminal surface of vascular endothelium, where it is anchored by a membrane-bound glycosaminoglycan chain. In the presence of the cofactor apoliprotein C-II, LPL hydrolyzes dietary or endogenous triglycerides to monoglycerides and free fatty acids, which are taken up by cells for oxidation (muscles) or storage (adipose). The enzymatic activity is regulated by nutrients and hormones to allow tissues to respond to energy requirements and storage needs (*1*). To obtain new insight into structural, regulatory, and genetic features of LPL, we have cloned and sequenced LPL complementary DNA (cDNA) derived from human adipose tissue.

LPL clones from a mouse macrophage cDNA library were identified by screening with antibodies to bovine milk LPL (*2*). We used a 500–base pair (bp) mouse partial cDNA clone to identify clones from a human adipose tissue cDNA library (*3*). Sequence analysis of overlapping cDNA clones yielded the DNA and predicted protein sequence of the 448–amino acid LPL protein, which is preceded by a 27-residue prepeptide (Fig. 1).

The human LPL cDNA clones that were initially sequenced (designated LPL35 and LPL37) contain 174 bp of 5′ and 1556 bp of 3′ untranslated sequence and contain 3155 bp overall. The poly(A) tail is preceded by the common polyadenylation signal AATAAA. However, when cloned LPL cDNA was radiolabeled and hybridized to poly(A)$^+$ RNA derived from several human tissues, two bands of roughly equal intensity were seen in each positive tissue (Fig. 2). The two RNA species are approximately 3350 and 3750 nucleotides in length. The LPL messenger RNA (mRNA) is seen most abundantly in adipose and adrenal tissues, in moderate intensity in kidney and intestine, and in barely detectable amounts in pancreas and placenta. We did not detect LPL mRNA in liver, HepG2 (hepatocyte) cells, or white blood cells.

The two different sized RNA species might be due to alternative sites of polyadenylation, splicing, or transcription initiation of the RNA. Several experiments were undertaken to address these possibilities. Oligonucleotide-primed cDNA synthesis of adipose mRNA suggested that only one 5′ end of the message occurs at about 175 nucleotides upstream of the translation initiation codon. This is about 39 nucleotides farther 5′ than the beginning of the cDNA sequence shown in Fig. 1. To explore the possibility of alternative splicing, which could affect the protein coding portions of the mRNA, we subjected nine independent LPL cDNA clones to restriction enzyme analysis. The only differences seen could be attributed to the clones being of various lengths. The issue was resolved by the eventual isolation of clones that extended farther in the 3′ direction. The original cDNA library was rescreened with a 210-bp restriction fragment derived from the 3′ terminus of clone LPL37. This fragment was used as a probe because eight of the nine originally characterized LPL clones were found to terminate within or near a stretch of ten adenosine residues contained within the 3′ untranslated region. This oligo(A) stretch is not a true poly(A) tail; no polyadenylation signal sequence precedes it and clone LPL37 extends through this region and terminates farther downstream in a poly(A) tail. This

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