The white, brown, and scarlet genes of Drosophila melanogaster encode three proteins that belong to the Traffic ATPase superfamily of transmembrane permeases and are involved in the transport of guanine and tryptophan (precursors of the red and brown eye pigments). We have determined the nucleotide sequences of two mutant white alleles (w<sup>red</sup> and w<sup>bur</sup>) that cause reduced red pigmentation but have no effect on brown pigmentation. In w<sup>red</sup> the effect is only observed when interacting with the bw<sup>6</sup> allele or a newly isolated allele (bw<sup>T6′</sup>). These alleles of the brown gene were cloned and sequenced. In w<sup>red</sup> the codon for glycine 588 is changed to encode serine; in w<sup>bur</sup> the triplet ATC encoding threonine in bw<sup>6</sup>, and glycine 578 is changed to aspartate in bw<sup>T6′</sup>. No other relevant changes to the gene structures were detected. P-element-mediated germline transduction was used to construct a fly strain containing a white gene with a mutation of the nucleotide binding domain. Such flies had white eyes, indicating that the mutated white gene was unable to support either guanine or tryptophan transport. The implications of these mutations are discussed in terms of a model of the Drosophila pigment precursor transport system.

The red-brown eye color of wild-type Drosophila melanogaster is due to the biosynthesis and deposition, in the pigment cells of the eyes, of two pigment types; drosopherins, which are red colored and are synthesized from GTP, and ommochromes, which are brown and are synthesized from tryptophan (Ames et al., 1982). According to a current model (discussed below), the precursors for these pathways, guanine and tryptophan, are transported into the pigment cells by separate membrane permeases that are members of the Traffic ATPase superfamily of transmembrane transporters (terminology of Ames et al. (1990)) or ABC (terminology of Hyde et al. (1990)) family of membrane transporters.

Early work on the physiological characterization of Drosophila eye color mutant strains by Sullivan’s group (Sullivan et al., 1979, 1980; Sullivan and Sullivan, 1975) identified three genes, white (w<sup>+</sup>), scarlet (st<sup>+</sup>), and brown (bw<sup>+</sup>), as being involved in the uptake of the pigment precursors by cells in developing eyes. Explanted tissues from mutants with null alleles at either scarlet (st<sup>6</sup>) or white (w<sup>6</sup>) had reduced capability to take up tryptophan, and tissue from mutants with null alleles at brown (bw<sup>6</sup>) or white (w<sup>6</sup>) had reduced capability to transport guanine. When subsequent cloning and sequencing of the cDNAs revealed that the w<sup>+</sup>, bw<sup>+</sup>, and st<sup>+</sup> genes encode three related proteins belonging to the Traffic ATPase family with hydrophobic characteristics of membrane proteins, it was proposed that the guanine transporter contains heterodimers of subunits encoded by the white and brown genes, and the tryptophan transporter contains heterodimers of subunits encoded by the white and scarlet genes (O’Hare et al., 1984; Pepling and Mount, 1990; Dreesen et al., 1988; Tearle et al., 1989).

Traffic ATPase transporters in general are involved in a wide variety of membrane transport processes in nature and include periplasmic permeases of bacteria (Ames, 1986; Cox et al., 1988; Dassa and Hofnung, 1985); the yeast STE 6 protein, which transports the a-factor mating pheromone (Berkower and Michealis, 1991); as well as human proteins of medical significance; the cystic fibrosis transmembrane conductance regulator (CFTR) (Collins, 1992); the P-glycoprotein responsible for development of multidrug resistance in tumor cells (Higgins and Gottesman, 1992; Chen et al., 1986; Gros et al., 1986); and the proteins involved in delivering antigenic oligopeptides to the major histocompatibility complexes in the endoplasmic reticulum (Pows et al., 1992). The guanine and tryptophan transporters of Drosophila are unusual among the eukaryotic Traffic ATPases in that they transport their substrates into the cell rather than pumping molecules out of the cell (see Higgins, 1992).

Comparison among Traffic ATPase proteins reveals that, although the conservation of amino acid sequence may be low between any two members of the family, the overall predicted structural arrangement is highly conserved (Higgins, 1992). Consequently, the general model of these transporters indicates that they contain four domains; two nucleotide binding folds located in a hydrophilic portion of the complex and two membrane spanning regions where the polypeptide chain is predicted to form five or six membrane spanning a-helices (Hyde et al., 1990). An additional domain on a separate subunit, called the periplasmic substrate binding protein, is present in the bacterial periplasmic permease complexes, but involvement of such a subunit has not been established for any eukaryotic members of the family. The nucleotide binding folds are conserved in many proteins that bind ATP or GTP and they are
characterized by a stretch of around 200 amino acids containing two highly conserved smaller sequence elements corre- sponding to the Walker A and B motifs (Walker et al., 1982). The role of the nucleotide binding folds in the Traffic ATPase transporters is presumably to provide the energy, by hydrolysis of ATP, to drive conformational changes in the proteins that are necessary for the transport mechanism. The requirement for ATP hydrolysis has been convincingly demonstrated for the histidine permease of S. typhimurium (Ames and Joshi, 1990) and nonhydrolyzable analogues of ATP have been shown to be incapable of supporting chloride ion transport by CFTR (Anderson et al., 1991a; Anderson and Welsh, 1992).

Although the four domains are always present, they can be located on one from one to four polypeptides, depending on the individual transporter. In the case of the Drosophila tryptophan and guanine transporters, each of the putative subunits encoded by the white, brown, and scarlet genes contains one nucleotide binding fold and one transmembrane domain. The apparent requirement for at least two copies of each domain in the native Traffic ATPase transporters is consistent with the proposed heterodimeric composition of the Drosophila transporters discussed above (see also Dreesen et al., 1988).

In this paper we describe the identification of the nucleotide changes in two alleles of each of white and brown that affect function of the Drosophila guanine transporter, but not the tryptophan transporter. The corresponding changes to the amino acid sequences are all located in the C-terminal hydrophobic portion of the two proteins. In addition, we report site-directed alterations of highly conserved amino acids in the nucleotide binding domain of the white-encoded subunit that cause loss of function of both the guanine and tryptophan transporters.

EXPERIMENTAL PROCEDURES

Fly Strains—The w10 u,bw6 fly strain was obtained from Dr. James Farmer (Brigham Young University, Provo, UT). The strain containing wbw6 was obtained from the Drosophila Stock Center, California Institute of Technology, Pasadena, CA. The strain T50, which contained the novel bw6 allele, came from the wild-type stock collection of Prof. J. Gibson (The Australian National University, Canberra, Australia).

Amplification of white and brown Gene Fragments from Genomic DNA—Genomic DNA was isolated from approximately 100 flies according to the method of Linthorst as modified by Bender et al. (1983) and was quantitated by measurement of the Oligonucleotides to be used as primers for PCR and sequencing experiments (see Table I) were designed by reference to the white gene sequence of O'Hare et al. (1984) or the brown cDNA sequence of Dreesen et al. (1988). PCR amplifications were performed on a Perkin-Elmer Cetus DNA thermocycler in 50-pl reaction volumes containing template genomic DNA (200 ng), primers (20 pmol each), dNTPs (200 μM each), and thermophile DNA polymerase from Pyrococcus furiosis (native Pfu polymerase; 1.25 units; from Stratagene); in the recommended buffer supplied with the enzyme.

The temperature profiles programmed to the thermocycler for amplification experiments were as follows: for white gene fragments, 30 cycles of 94 °C for 45 s, 55 °C for 1 min, 72 °C for 3 min; for brown gene fragments, 35 cycles of 94 °C for 45 s, 63 °C for 1 min, 72 °C for 3 min.

Restriction Analysis of DNA Using Escherichia coli—The w10, wbw6, and bw6 alleles were amplified from agarose gels purified and used as template DNA in the PCR reaction. The PCR products were digested with the restriction enzymes indicated in Table I and were separated by electrophoresis in 0.8% agarose gels. The DNA was stained with ethidium bromide, and the gels were then photographed with a transilluminator to identify the expected bands.

Site-directed Mutagenesis of the white Gene and P-element-mediated Transformation of Drosophila—The E. coli plasmid pRK3 was obtained from Dr. P. Schedl (Princeton University, Princeton, NJ). It harbors a P-element vector containing the white minigene and regulatory sequences flanked by the boundary domains sex and sex' that insulate the white gene against chromosomal position effects (Kellem and Scholl, 1991). To construct a P-element transformation vector with a selectable marker independent of the white gene, the bacterial neomycin resistance gene under control of the Drosophila hsp70 promoter was isolated from the plasmid pUCHneo (Steller and Proctor, 1985) as a 1.6-kb AseI/HindIII fragment and ligated into the unique XhoI site of pRK3. Between the white regulatory sequences and the hsp70 promoter, a 3.2-kb P-element vector containing the neomycin resistance gene was constructed (Klenow) by creating blunt ends and inserting the linearized hsp70 vector into the blunt-ended ligation products.

Barber (1982) described a technique for generating the D. melanogaster selection transformant of D. melanogaster that was performed essentially as described by Spradling (1986). Freshly laid eggs were obtained from a w; Fρy('A2--3') (99B) strain and injected with either pRK3hanoi (Pw; neo') or pRK3hano1.mut1 (Pw'; neo'); surviving G0 flies were crossed to a w1118 strain, and germline transformants were selected on the basis of eye color and/or G418 (0.2 mg/ml) resistance, as described in the kit's instruction manual, with the exception that Pw1 was used for the nicking step prior to T5 exonuclease treatment. An XbaI/SacI fragment containing the desired mutation was then subcloned back into pRK3hano1, replacing the corresponding wild-type sequence and producing the plasmid pRK3hano1.mut1.

Materials—All enzymes and chemicals used were of the highest quality available. Oligonucleotides were synthesized by the Biomolecular Resource Facility, The Australian National University, Canberra, Australia. [α-32P]dATP was obtained from Amerham (Australia) Pty. Ltd. All standard molecular biological methods not otherwise described were performed essentially as described in Sambrook et al. (1989).

RESULTS

Amino Acid Substitutions in the Interacting Alleles w10 and bw6—The specific linkage method between the eye pigmentation alleles w10 and bw6 was first described by Farmer and Fairbanks (Farmer, 1977; Farmer and Fairbanks, 1986). Characterization of the mutations in these alleles is of particular interest because both are phenotypically silent except in w10 bw6.
double mutant flies that contain no other functional alleles of white or brown. In that case, flies with brown eyes are observed due to the almost complete absence of the red pteridine pigments. Flies homozygous for hu or hnts white (white is an X-linked gene) for \( w^{ord} \) have apparently wild-type eyes in the presence of just one copy of \( w^{+} \), and similarly, flies homozygous for \( w^{tr} \) appear wild-type if \( w^{+} \) is present. In terms of the heterodimer model of the D. melanogaster eye pigment precursor permeases (O'Hare et al., 1984; Pepling and Mount, 1990; Dreesen et al., Tearle et al., 1989), these observations clearly indicate that both the \( w^{ord} \) and \( w^{tr} \) alleles must produce proteins that are capable of folding and assembling into the membrane in a near wild-type conformation. Apparently, a guanine transporter containing one of the other mutant \( w^{ord} \) and \( w^{tr} \) encoded subunits is functional. Only when both subunits of the transporter are mutant is activity severely compromised. Furthermore, the observation that ommochrome levels are close to wild-type in \( w^{ord}, \) \( w^{tr} \) homozygous flies is evidence that the \( w^{ord} \) encoded protein is able to dimerize normally with the scarlet-encoded subunit to form an active transporter for tryptophan.

The strategy for determining the nucleotide sequence of the protein coding regions of the \( w^{ord} \) allele is depicted in Fig. 1. The \( w^{ord} \) gene was amplified by PCR from genomic DNA as two fragments: one fragment of 220 bp containing exon 1 flanked by short stretches of \( 5' \)-untranslated and intron 1 DNA and another fragment of 2624 bp containing exons 2–6 and corresponding untranslated DNA. The PCR products were cloned for sequencing, and the pair 92-57/92-44 for the 5' end of the gene, and the other four observed differences are silent with respect to the amino acid sequence and probably represent polymorphisms due to the different backgrounds of the strains being compared.

Comparison with the published wild-type \( w^{ord} \) cDNA sequence revealed five single base differences in the coding region of the \( w^{ord} \) allele (Table II). One of these results in the substitution of a threonine for asparagine at position 638 of the putative amino acid sequence. The other four observed differences are silent with respect to the amino acid sequence and probably represent polymorphisms due to the different background strains being compared.

Isolation and Characterization of a Second \( w^{ord} \)-interacting Brown Allele—From a survey of wild-type and laboratory D. melanogaster stocks in crosses to the \( w^{ord} \) strain, Farmer (1977) observed surprisingly high incidence of strains carrying an allele that behaved like \( w^{tr} \) (failing to suppress \( w^{ord} \)). Accordingly, in an attempt to identify other \( w^{tr} \)-like alleles, we screened our laboratory strains with apparently normal eye
**Drosophila Eye Pigmentation Mutants**

**TABLE I**

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<th>Primer</th>
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<td>Exon 1 PCR</td>
<td>-</td>
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<td>91–136</td>
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<td>3132</td>
<td>Exon 2–6 PCR</td>
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<td>TTAGGAACGCCAGATAGATCTAG</td>
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* Recognition sequences for restriction by EcoRI, HindIII and PstI endonucleases are underlined.

‡ Binding sites are numbered with +1 as the A of the translation start codon in the published white genomic (O'Hare et al., 1984) and brown cDNA (Dreesen et al., 1988) sequences.

† + indicates the coding strand; − indicates the complementary strand.

**TABLE II**

<table>
<thead>
<tr>
<th>Allele</th>
<th>Phenotype</th>
<th>Mutation in DNA*</th>
<th>Effect on amino acid sequence</th>
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<td>w190</td>
<td>Interaction with w6 and wTfs reduces red pigments</td>
<td>G to A (5311)</td>
<td>Gly588 to Ser</td>
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<tr>
<td>w6</td>
<td>Interaction with w190 reduces red pigments</td>
<td>A to C (1913)</td>
<td>Asn638 to Thr</td>
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<tr>
<td>wTfs</td>
<td></td>
<td>A to T (822)</td>
<td>Silent</td>
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<tr>
<td></td>
<td></td>
<td>A to C (843)</td>
<td>Silent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A to G (1335)</td>
<td>Silent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A to C (1365)</td>
<td>Silent</td>
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<tr>
<td>wbox</td>
<td>Interaction with w190 reduces red pigments</td>
<td>G to A (1733)</td>
<td>Gly578 to Asp</td>
</tr>
<tr>
<td>wTfs</td>
<td>Brown eye color due to reduced red pigments</td>
<td>Δ(5290)ATC</td>
<td>Ile851 deleted</td>
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</table>

* The nucleotide positions in parentheses are relative to the first base of the start codon in the published white genomic (O'Hare et al., 1984) and brown cDNA (Dreesen et al., 1988) sequences.

color, as follows. Male flies from our various strains (bw/bw'; where * indicates an unidentified allele) were mated to female w190; wTfs flies and eye color of the male progeny (w190 w; bw9/bw) was assessed. Males with reduced red pigments were carriers of a potentially novel w190-interacting allele. The vermilion (v) marker was incorporated in the screen to block omochrom synthesis and thereby enable small changes in the level of pteridine pigments to be more easily observed.

From a total of the 36 strains that were screened in this way, one of the crosses produced male flies with yellow eyes among the progeny. These flies contained a bw9-like allele, which we have named bwTfs (after the strain T50 from which it was

Fig. 2. Strategy for amplification, cloning, and sequencing of brown gene fragments from genomic DNA. The relative locations of binding in the cDNA are shown for the two pairs of primers used for amplification (92–57 and 92–64 for the 5’ end and 92–63 and 92–68 for the 3’ end) of bw6 and bwTfs allele sequences. The EcoRI (E) or HindIII (H) restriction enzyme recognition sequences incorporated in their 5’ ends are also indicated. In the diagram of cDNA structure, the thick line indicates the protein coding sequences. The mutant PCR products, after digestion with EcoRI and HindIII, are shown in the inset gel photograph: lane 1, the 3’-terminal fragment; lane 2, size markers; lane 3, the 5’-terminal fragment (an additional site for HindII in the ~1150 bp product was seen). The three fragments were cloned to pBluescript SK+ and sequenced as discussed in the text. The region sequenced from the primer 92–67 is indicated as starting with the asterisk. All other sequence data was obtained using either the PCR primers or the universal or reverse sequencing primers that have binding sites in the pBluescript vector. The deduced arrangement of exons and introns in the brown gene is illustrated in the lower portion of the diagram.
isolated. To rule out the possibility that this new allele was a brown null, flies from the T50 strain were also crossed to bw0 (w0 v') flies. All of the resulting progeny had wild-type eye color, consistent with the bw76 allele requiring a specific interaction with w0 to produce a detectable reduction in pteridine pigmentation. If bw76 had been a null allele, that proportion of the progeny that were bw0/bw76 should have had brown eyes.

For characterization of the mutation in the bw76 allele, DNA was isolated from four of the male flies with yellow eyes produced in the initial cross to w0 bw0 females. The brown gene fragments were amplified and cloned for sequencing as described for bw0 in the previous section (results not shown). Sequencing revealed that the bw76 allele was not identical to bw0. The new allele contains a single missense mutation with a G to A transition corresponding to position 1733 of the cDNA sequence. This results in the substitution of glycine 578 by aspartate.

Identification of a Single Codon Deletion in the wbus Allele: Another Mutation in White That Affects the Guanine Transporter but Not the Tryptophan Transporter—Although the double-Gly-Lys subunits are involved in transport of both guanine and tryptophan, in wbus there is a preferential reduction in the pteridine level, whereas the ommochrome level is virtually unaffected (Zachar and Bingham, 1982; Lindsay and Grelle, 1968). As for w0, this indicates that the wbus allele produces a protein that is capable of inserting in the membrane in such a way that it interacts with the scarlet-encoded subunit, to form an active tryptophan transporter in Drosophila eyes. However, clearly the mutant form of the wbus protein is unable to support normal function of the guanine transporter.

To identify any mutations in the wbus-encoded subunit, we amplified and sequenced the allele from genomic DNA as described for the w0 allele (results not shown). The sequence data showed that the triplet ATC, encoding Ile5', has been deleted from the wco2 allele (results not shown). The sequence data also showed that the triplet ATC, encoding Ile5', has been deleted from the wbus allele (Table II). Otherwise, the sequence of the entire coding region and all splice donor and acceptor sites was identical to the published white sequences.

A Site-directed Mutation of Residues in the Nucleotide Binding Domain of the White-encoded Subunit That Results in Loss of Function of Both Transporters—the invariant amino acid residues in the Walker motif A have been proposed to be directly involved in binding of ATP in the nucleotide binding fold (Parsonage et al., 1987), and mutations to these residues in various members of the Traffic ATPase family are known to eliminate or severely reduce function (Berkower and Michaelis, 1991; Azzaria et al., 1989; Cox et al., 1989). To establish the significance of the nucleotide binding fold in the white-encoded subunit of Drosophila guanine and tryptophan transporters, we used oligonucleotide-directed mutagenesis to alter the codons for the Gly269-Lys286/287 pair in the putative nucleotide binding fold to encode Leu-Gln. The mutated white minigene replaced the wild-type gene in pRK3hsneo (P[w', neo']) (Fig. 3), to create the plasmid pRK3hsneo.mut1 (P[wmut, neo']). Plasmid constructs containing either the wild-type or mutated white genes were subsequently used in P-element-mediated germline transformation of D. melanogaster. For characterization of the mutation in the bw76 allele, DNA was isolated from four of the male flies with yellow eyes produced in the initial cross to w0 bw0 females. The brown gene fragments were amplified and cloned for sequencing as described for bw0 in the previous section (results not shown). Sequencing revealed that the bw76 allele was not identical to bw0. The new allele contains a single missense mutation with a G to A transition corresponding to position 1733 of the cDNA sequence. This results in the substitution of glycine 578 by aspartate.

Fig. 3. The plasmid pRK3hsneo used in P-element-mediated transformation of D. melanogaster. The arrangement and approximate relative sizes is shown for genes and DNA motifs referred to in the text that are flanked by the P-element inverted repeat sequences. The unique Xbal and SacI restriction endonuclease sites, used for cloning the equivalent fragment of the white minigene containing the site-directed mutation in pRK3hsneo.mut1 (see "Experimental Procedures"), are also shown. The site-directed mutation derived from pUC8 and the 3-bacteriophage gene (confering ampicillin resistance) are located in the segment external to the P-element termini.

To confirm that the P[wmut, neo'] G2 survivors of G418 selection were authentic transformants, PCR was used to amplify the 5' end of the white minigene from genomic DNA isolated from individual G418 survivors. One primer was designed to bind to the 5'-nontranslated region, and the other was designed to bind in exon 3, downstream of the mutation site. The production of a 1.2-kb fragment after PCR confirmed that the white minigene (containing a shortened intron 1) was present in the genome of the flies, and digestion of the PCR product by PstI into two fragments of approximately 940 and 300 base pairs was proof that the gene contained the site-directed mutation (see Fig. 4).

Therefore, we conclude that a white gene with a Gly269-Lys286/287 Leu-Gln mutation in the putative nucleotide binding fold is unable to complement eye color in recipient flies with a defective white gene. This result also demonstrates that a functional nucleotide binding fold on the white-encoded subunit is necessary for activity of both the guanine and tryptophan transporters of D. melanogaster. The nucleotide binding folds of neither the scarlet nor brown gene products are able to support transport on their own.

DISCUSSION

The mutations detected in the two white (w0, wbus) and two brown (bw6, bw76) alleles described in the present work result in changes to amino acids located in the hydrophobic C-terminal regions of the two encoded proteins. The hydropathy plots of the amino acid sequences of the two proteins (Dreesen et al., 1988), together with a comparison with other members of the ABC transporter family, suggest that there are six membrane-spanning regions for each of the subunits. Models of the orientation of these putative a-helices and the amino acids predicted to be buried in the membrane are depicted in Fig. 5, and the residues altered in the w0, wbus, bw6, and bw76 alleles are indicated.

In the model of the white-encoded subunit the G688S mutation in wbus is located in the C-terminal end of the fifth putative transmembrane helix. Since this mutation does not affect eye color in bw+ flies, it can be concluded that the change does not
P[w+, neo+] or P[wmut1, neo−]
Injection into w−, Δ2-3 host
↓
G0 fly x w1118 flies
↓
G1 flies (assess eye colour)
G418 (0.2 mg/ml) selection
↓
G2 neo− transformants
PCR test on genomic DNA from single flies to confirm mutant white mini-gene is present

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Fig. 4. The strategy for construction, selection, and PCR screening of P[wmut1; neo−] transformant flies. For the PCR test, genomic DNA was isolated from single flies and approximately 100 ng was used in PCR reactions with oligonucleotides 91-134 and 91-70 to amplify the 5' portion of the white minigene (across the shortened intron 1). A product of 1.2 kb indicates that the flies contained the P-element-borne white gene. The PCR products were separated from unreacted primers and "primer dimers" by the Geneclean method and were then tested for the presence of an internal PstI site that would have been introduced by successful mutagenesis with oligonucleotide 91-122. Lanes 1 and 7, DNA markers with sizes (bp) indicated on the far right-hand side. Lanes 2-6, purified PCR products, before (+) and after (−) digestion with PstI, amplified from the following flies: lane 2, a noninjected w, Δ2-3 fly; lane 3, a G2 fly (with wild-type eye color) derived from an egg injected with pRK3hsneo. Lanes 4-6, three G2 flies (white-eyed) derived from eggs injected with pRK3hsneo.mut1.

appreciably affect the ability of the wmut−-encoded subunit to function in active guanine and tryptophan transporters. Therefore, the global structure of the mutant subunit must be nearly identical to wild-type, and any structural alterations due to the introduction of a hydroxyl moiety or the increased size of the serine residue compared with glycine must be relatively localized and insignificant with respect to function of the heterodimers. Similarly, it can be argued that the amino acid substitutions in either bu6 (N638T) or bu70G (G578D) do not cause disruption of subunit structure or function in heterodimers with wmut−-encoded subunits. As for wmut2, the mutant residues in the bu6 and bu70G-encoded subunits are predicted to be near the external surface of the membrane. In bu70G the G578D mutation is in a similar position in transmembrane helix 5 as the change in wmut2, and in bu6 the N638T change is located near the membrane surface in transmembrane helix 6. Notably, the three mutations all give rise to residues capable of H-bonding, and it would seem likely that H-bonding between serine at position 588 in the white-encoded subunit and either aspartate 578 or threonine 638 in the brown-encoded subunit is responsible for the observed phenotype although other explanations are possible. These interactions would imply that helix 5 of the white protein is close to helices 5 and 6 of the brown protein (see Fig. 5). As such interactions cause loss of function, yet individually have no effect, it is possible that some dynamic aspect of transporter function has been affected. Furthermore, with reference to the folding models, the predicted location of all three mutations near the external surface of the membrane makes it tempting to speculate that function associated with the mouth of the pore through which the substrate passes may be affected.

The wmut6 allele, containing a mutation that results in the deletion of Ile516, causes almost no pteridine pigment to be synthesized while leaving the ommochrome levels unaffected (Zachar and Bingham, 1982; Lindsay and Grell, 1968). This is an identical phenotype to that produced by the wmut bu6 interaction, and again it indicates that the mutant white-encoded subunit is capable of interacting with the scarlet-encoded subunit to form an active tryptophan transporter and is therefore assembled and is sufficiently structurally similar to the wild-type subunit to form this functional interaction. We propose, however, that the deletion of Ile516 from transmembrane helix 5 in the wmut6-encoded subunit causes loss of a functional interaction with the brown protein. Deletion of an amino acid from an α-helix changes the faces of the helix due to the rotation in position, by 1 amino acid residue, of the helical surface on the C-terminal side of the deletion relative to the surface on the N-terminal side. Obviously, this could have significant effects on the interactions of that helix with the other transmembrane helices of the complex. Furthermore, if a face of the helix lined the channel through which the substrate is transported, then the deletion may alter the chemical properties of the channel surface by changing the positions of residue side chains which might be critical to the transport mechanism. Therefore, we propose that transmembrane helix 5 of the white-encoded subunit is involved in specific interactions with the brown-encoded subunit in formation of the guanine transporter. This conclusion is consistent with the interaction between helices 5 and 6 of these subunits proposed on the basis of the changes observed in the wmut, bu6, and bu70G alleles, and it may be of relevance that the amino acid deleted is just two turns of an α-helix away from the interacting residue affected in wmut. A corollary to this deduction is that a similar interaction between transmembrane helix 5 of the white-encoded subunit and the scarlet-encoded subunit does not occur in the tryptophan transporter, since ommochrome levels are wild-type in both wmut and wmut,bu6 flies.

Although the existence of additional mutations in the non-coding DNA of the wmut6 allele has not been ruled out in this work, it seems unlikely that the phenotype could be caused by effects on the regulation of gene expression. It should be noted that previous molecular investigation of the wmut6 allele using Southern blotting has shown it to have a gross gene structure identical to the wild-type gene with the exception of a 150-bp deletion between positions −18 and −19.5 on the gene map of white (Zachar and Bingham, 1982). However, a causative effect of this deletion on the expression of the gene is excluded by the results of Hazelrigg et al. (1984) who showed that flies transformed with a white gene construct which does not contain DNA that far downstream of the coding region regulate expression of white normally.

Another important property of the wmut allele which indi-
Fig. 5. a, model of the white-encoded subunit. b, model of the brown-encoded subunit. The amino acids predicted to be in the transmembrane helices in the white- and brown-encoded subunits of the guanine transporter are as indicated. The numbers inside the intra-helical loops indicate the number of amino acids in the loop. The relative distribution of charged amino acids is indicated by + or −. Amino acid residues that were found to be altered in the mutant alleles sequenced are in boldface type and have their residue position superscripted (see Table II). The putative nucleotide binding fold (NBF) is discussed in the text.
cates that the mutation in the protein is responsible for the phenotype is that it has a semidominant effect on pteridine production (Lindsay and Grell, 1968): \( w^{hyw} \) heterozygotes have an eye color that is noticeably less red than wild-type. This is in contrast to the majority of white alleles in which the eye color phenotype is wild-type in heterozygotes. This suggests the interesting possibility that the native permease complex might be tetrameric (or multimeric), possessing two or more copies each of the white-encoded and brown-encoded subunits.

Then it is conceivable that the presence of just one copy of the \( w^{hyw} \)-encoded subunit in the oligomeric complex may be sufficient to disrupt the function of the transporter and that partial pigmentation results from the activity of the small proportion of complexes that form containing only the wild-type white-encoded subunit.

Function-altering mutations within the transmembrane helices have been identified in the MDR (Devine et al., 1992; Gros et al., 1991; Safa et al., 1990; and Parsonage et al., 1991) proteins. In the case of the CFTR protein, 53 missense doublets and 3 single amino acid deletions have been reported that probably cause cystic fibrosis (Tsui, 1992). Although the majority (32 mutants) of these are associated with either of the two nucleotide binding domains, other apparent hotspots for mutations are a predicted cystoplastic loop in the C-terminal hydrophobic domain (nine mutants) and transmembrane helices 5 and 6 (six mutants). Interestingly, it has been suggested previously that these helices may have roles in substrate association and the mechanism of transport in Traffic ATPases (Devine et al., 1992; Gros et al., 1991; Webb et al., 1992).

The effects of the site-directed mutation resulting in Gly-Lys being replaced by Leu-Gln in the Walker motif A of the white protein is consistent with the effects of similar mutations on other ABC transporters and on the F,F,-ATPase (Berkower and Michaeili, 1991; Parsonage et al., 1987; Azzaria et al., 1989; Cox et al., 1989). Structural analysis of adenylate kinase has shown that these amino acids are directly involved in binding of the phosphoryl groups of the nucleotide (Hyde et al., 1990). Similar changes in the \( \beta \)-subunit of the F,F,-ATPase affect the binding and hydrolysis of ATP (Parsonage et al., 1987). The results reported here are therefore consistent with the proposition that ABC transporters are energized by ATP hydrolysis. With respect to the models depicted in Fig. 5, the location of the nucleotide binding domains to the \( \beta \)-terminal side of the transmembrane helices in the \( Drosophila \) transporters is unusual among the eukaryotic ABC permeases. Similarly, the \( Drosophila \) transporters are also unusual among eukaryotic ABC permeases so far described, in that they transport their substrates into the cell instead of pumping molecules out of the cell (Higgins, 1992). As more and more examples of ABC permeases are characterized it will be interesting to see whether a correlation persists between the order of the hydrophilic and hydrophobic domains on the polypeptides and the direction of pumping.

REFERENCES