Isolation and Characterization of *Drosophila* Multidrug Resistance Gene Homologs

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Mammalian multidrug-resistant cell lines, selected for resistance to a single cytotoxic agent, display cross-resistance to a broad spectrum of structurally and functionally unrelated compounds. These cell lines overproduce a membrane protein, the P-glycoprotein, which is encoded by a member(s) of a multigene family, termed *mdr* or *pgp*. The amino acid sequence of the P-glycoprotein predicts an energy-dependent transport protein with homology to a large superfamily of proteins which transport a wide variety of substances. This report describes the isolation and characterization of two *Drosophila* homologs of the mammalian *mdr* gene. These homologs, located in chromosomal sections 49EF and 65A, encode proteins that share over 40% amino acid identity to the human and murine *mdr* P-glycoproteins. Fly strains bearing disruptions in the homolog in section 49EF have been constructed and implicate this gene in conferring colchicine resistance to the organism. This work sets the foundation for the molecular and genetic analysis of *mdr* homologs in *Drosophila melanogaster*.

The analysis of multidrug resistance has led to the identification of a multigene family, designated *mdr* or *pgp*, which encodes a family of membrane glycoproteins, termed P-glycoproteins (9, 15, 21). The *mdr* gene is amplified and overexpressed in multidrug-resistant cell lines which are cross-resistant to a broad spectrum of structurally and functionally unrelated compounds, many of which are used in cancer chemotherapy. The P-glycoprotein appears to function as an energy-dependent transport pump related to a large class of evolutionarily and functionally diverse transport proteins (2, 26, 28). The deduced amino acid sequence of the P-glycoprotein predicts a polypeptide composed of two highly conserved halves, each containing six membrane-spanning domains followed by a cytoplasmic domain containing the consensus sequence for a nucleotide binding fold (5, 20, 23). The region surrounding the nucleotide binding fold is homologous to a component of a class of bacterial permeases (2), while more extensive similarity extends into the transmembrane domains of the bacterial transporters HlyB, LtkB, ChvA, CyaB, and NdvA (28). This homology across large evolutionary distances suggests that a highly conserved functional unit involved in membrane transport is present in the P-glycoprotein.

The mammalian *mdr* multigene family is differentially expressed in normal tissues (10, 19). The highest levels of expression are found in a variety of secretory epithelial surfaces, suggesting that specific transport functions are associated with each of the genes (3, 16, 46). The human *mdr* multigene family consists of two members, *mdr1* and *mdr3*, of which only *mdr1* is capable of conferring drug resistance (47, 49). Of the three members in rodents, the two that are most similar to human *mdr1*, rodent *mdr1* and *mdr3*, are also capable of conferring multidrug resistance (8, 12, 22), while rodent *mdr2*, more similar to human *mdr3*, does not appear to confer drug resistance (24).

Preliminary observations have indicated that *mdr* ho-

mologs are present as multigene families in a wide range of species (28). One *Saccharomyces cerevisiae* homolog is *STE6*, which may be responsible for transporting the mating pheromone α -factor (29, 33, 46a). Two genes are present in *Plasmodium falciparum* (50), at least one of which has been proposed to confer resistance to antimalarial agents (18). Partial sequences of four *mdr*-like genes have been cloned from *Entamoeba histolytica* (42). Three homologs have been reported for *Leishmania tarentolae*, one of which exists on an extrachromosomal element whose amplification is correlated with methotrexate resistance (38).

This report extends the analysis of the *mdr* multigene family to *Drosophila melanogaster* from which two members have been isolated and characterized. The facility of genetic manipulations in *Drosophila* spp. has permitted the construction and analysis of lines bearing mutations in one of the *mdr* homologs.

MATERIALS AND METHODS

Isolation and analysis of cDNA clones. The mouse *mdr1* probe, pcDR1.3 (22), was used to screen a *Drosophila* adult head cDNA library (27). Hybridization was performed in 50% formamide–1 M NaCl–1% sodium dodecyl sulfate–10% dextran sulfate–100 μ g of denatured salmon sperm DNA per ml at 42°C, and the filters were washed to 30 mM NaCl–3 mM sodium citrate–0.5% sodium dodecyl sulfate at 55°C. The cDNA inserts were subcloned into pUC19. Representative clones were sequenced on both strands, crossing all restriction sites used in subcloning, with Sequenase (U.S. Biochemicals) primed with pUC forward and reverse primers (Promega).

Nucleic acid identities were compared with the University of Wisconsin Genetics Computer Group programs Compare and Dotplot, using a window of 21 and a stringency of 14. The nucleic acid and predicted amino acid sequences of the *Drosophila*, human, and murine *mdr* homologs were aligned by using the University of Wisconsin Genetics Computer Group program Gap to identify identities. Final alignment of

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the sequences in Fig. 1 was done manually. Hydropathy and acid/base analysis was plotted by DNA Strider (version 1.1). Human and murine *mdr* sequences were from GenBank.

***Drosophila* strains.** Control strains include two wild-type strains, Oregon R and Canton S, and *dpp^{db}/CyO* (gift of W. Gelbart). The *dpp^{db}* mutation is far from *Mdr49* (44). *CyO* and *SM5* (see below) are balancer chromosomes which are used to maintain mutations in stock (31) and are designated in the figures and text as +. Four chromosomes bearing deletions in the region of *Mdr49*, *b8* [abbreviation for *Su(z)2^{1.b8}*], *vg¹³³*, *vg^C*, and *vg¹³⁵* (see text) (the latter three the gift of P. Lasko), are maintained in stocks as *b8/CyO*, *vg¹³³/SM5*, *vg^C/SM5*, and *vg¹³⁵/CyO*.

The three double-deletion lines *vg¹³³/b8*, *vg^C/b8*, and *vg¹³⁵/b8* were made by crossing the *vg¹³³/SM5*, *vg^C/SM5*, and *vg¹³⁵/CyO* flies to *b8/CyO* flies and selecting balancer-free progeny. The *CyO* and *SM5* balancer chromosomes each carry a dominant mutation which makes the wings curl upward, so balancer-free progeny were easily identified by their straight wings. Two independent constructions were made for each double-deletion line, and the progeny of each construction were split into sibling lines which were then maintained separately. In the final stocks, random segregation is permitted for the X, Y, third, and fourth chromosomes, and random recombination is allowed throughout the chromosomes except for the small region on the second chromosome where *Mdr49* is located.

Nucleic acid hybridization. DNA from one-third of an adult fly (41) was digested with *EcoRI* and separated on a 1% agarose gel containing 0.04 M Tris acetate (pH 8) and 0.002 M disodium EDTA. Southern blots (GeneScreen Plus; New England Nuclear) were hybridized with 10⁶ cpm of probe (8) in 50% formamide–1 M NaCl–1% sodium dodecyl sulfate–10% dextran sulfate–100 µg of denatured salmon sperm DNA per ml at 42°C and washed to 30 mM NaCl–3 mM sodium citrate–0.5% sodium dodecyl sulfate at 55°C. Adult fly RNA (20 µg) was fractionated on a formaldehyde gel (8), transferred to Hybond-N (Amersham), and hybridized as described above.

Cytological localization. *Drosophila* polytene chromosomes were hybridized with nick-translated biotinylated probes (Bioprobe; Enzo Diagnostics, Inc.) generated from the *Mdr49* (probe 1210) and *Mdr65* (probe 103) cDNAs in 50% formamide–300 mM NaCl–30 mM sodium citrate–10% dextran sulfate at 37°C. Slides were washed to 300 mM NaCl–30 mM sodium citrate at 37°C and visualized with horseradish peroxidase (DETEK I-hrp; Enzo Diagnostics, Inc.).

Functional analysis of *Drosophila* lines. Assays for colchicine resistance were performed according to a protocol developed by C. Cheney (5a). Flies were allowed to lay eggs on drug-free medium. The eggs were washed with water, counted, and placed onto food containing 0, 2, 5, 10, 20, or 50 µM colchicine. Colchicine was added to liquified *Drosophila* media with food coloring as a marker to ensure even mixing. Viability was calculated as the number of adults emerging divided by the number of starting eggs. Scores were normalized to those obtained at 0 µM colchicine and expressed as percentages.

Nucleotide sequence accession numbers. The sequences reported have been assigned GenBank accession numbers M59076 (*Mdr49*) and M59077 (*Mdr65*).

RESULTS

Isolation and characterization of the *Drosophila mdr* homologs. A cDNA library prepared from adult head RNA was screened with probe pcDR1.3 from the mouse *mdr1* cDNA (22). This fragment encodes a nucleotide binding site, the region with the highest nucleic acid identity to *mdr*-related genes across a wide range of species (20, 23). Sequence analysis classified the 13 clones obtained into two independent sets of cDNAs with homology to the mammalian *mdr* multigene family. The library was rescreened with probes from the proximal ends of each set to identify cDNA clones with complete open reading frames. A total of 39 clones were identified from 350,000 recombinant plaques.

Sequence analysis indicated that cDNAs with complete open reading frames for two *Drosophila mdr* homologs had been isolated. The longest clone of each homolog includes a 5' untranslated region followed by a continuous open reading frame of 1,302 amino acids (Fig. 1) and terminates with a 3' untranslated region which contains a signal for polyadenylation. The chromosomal locations of the *Drosophila mdr* homologs were identified by in situ hybridization to polytene chromosomes. One of the cDNAs localized to region 49EF on chromosome 2 (see below), and the other localized to region 65A on chromosome 3 (Fig. 2). Each of the *Drosophila mdr* homologs will be designated relative to its chromosomal location, i.e., *Mdr49* and *Mdr65*.

Preliminary sequence analysis suggested several nucleic acid polymorphisms among the *Mdr49* cDNAs. Differences were found at 26 sites among the longest *Mdr49* cDNAs, *dsp12*, *dsp20*, and *dsp44*, which were entirely sequenced. These differences were not evenly distributed throughout the cDNAs (see Fig. 5B). One polymorphism accounts for an *EcoRI* restriction site polymorphism (see Fig. 5B, asterisk) which is found in several *Drosophila* stock lines (see below). The polymorphisms at each site are shared by two of the three cDNAs; *dsp12* differs from *dsp20* at 16 sites and from *dsp44* at 22 sites, while *dsp20* differs from *dsp44* at 14 sites. Three of the polymorphisms result in amino acid substitutions (Fig. 1, asterisks). The predicted amino acid at position 697 in *dsp12* and *dsp44* is serine, while in *dsp20* it is asparagine. The predicted amino acid at position 712 in *dsp12* and *dsp44* is aspartic acid, while in *dsp20* it is asparagine. The predicted amino acid at position 957 in *dsp12* is valine, while in *dsp20* and *dsp44* it is isoleucine.

DNA sequence alignments indicate that both *Mdr49* and *Mdr65* have 50 to 52% nucleic acid identity to the entire coding region of both human *mdr* cDNAs and 68% identity in the 200-bp region surrounding the nucleotide binding sites (Fig. 3A). The two *Drosophila mdr* homologs also have 53% nucleic acid identity with each other (Fig. 3B). This is contrasted, however, by the human *mdr* multigene family members, which share 76% nucleic acid identity (Fig. 3C).

The predicted amino acid sequences of the two *Drosophila mdr* homologs indicate that a striking similarity exists with the mammalian P-glycoproteins. Both of the *Drosophila* homologs are identical to the human and murine polypeptides at 42 to 45% of the amino acid positions along the entire length of the polypeptide. The alignment of the two *Drosophila mdr* homologs and human *mdr1* and *mdr3* indicates that the amino acids are identical at approximately 50% of the positions in three of the four sequences (Fig. 1).

The Kyte-Doolittle hydropathy values of the predicted amino acid sequences of *Mdr49* and *Mdr65* reinforce these sequence similarities. They predict polypeptides with two halves, each half composed of six transmembrane domains

FIG. 1. Amino acid alignment of *Drosophila* *Mdr49* and *Mdr65* and human *mdr1* and *mdr3*. Shaded boxes represent identical amino acids in three of the four sequences. Predicted transmembrane domains are underlined, and nucleotide binding folds are double underlined. Asterisks indicate positions of polymorphisms in *Mdr49* as described in the text. Numbers represent residue positions for this figure and reflect gaps.

followed by an intracytoplasmic domain (Fig. 4A) containing the consensus sequence for a nucleotide binding site (26) (Fig. 1). The hydrophobicity plots of the *Drosophila* homologs are virtually identical to the hydrophobicity plots of the mammalian homologs except for an extremely hydrophilic domain in *Mdr49* which is located between residues 650 and 715. This domain includes a cluster of acidic amino acids followed by a cluster of basic amino acids (Fig. 4B) and is in a position analogous to the hydrophilic domain found in the *P. falciparum* *mdr* homolog (18) and to the R group in the cystic fibrosis gene product, which contains clusters of charged amino acids (40).

A comparison of the predicted amino acid sequences of *Mdr49* and *Mdr65* with each other indicates that they are identical at 46% of the positions. The similarity is highest surrounding the nucleotide binding sites and decreases as the sequences extend proximally (Fig. 1). This is in contrast to the human *mdr* gene family members, which share 77% identical amino acid residues (49). In addition, the proximal and distal halves of each *Drosophila* *mdr* homolog have

FIG. 2. Cytological localization of *Mdr65*. In situ hybridization of polytene chromosomes from *Drosophila* larvae with a probe from the *Mdr65* cDNA places the gene in region 65A on the left arm of chromosome 3. The arrow points to the hybridization signal; parallel lines denote limits of region 65A.

FIG. 3. Dotplot analysis of nucleic acid identity between *mdr* homologs. (A) *Drosophila Mdr49* (horizontal) versus human *mdr1* (vertical); (B) *Drosophila Mdr49* (horizontal) versus *Drosophila Mdr65* (vertical); (C) human *mdr1* (horizontal) versus human *mdr3* (vertical).

FIG. 4. Hydropathy and acid/base plots of *Drosophila Mdr49* and *Mdr65*. (A) Kyte-Doolittle hydropathy scores plotted with a window of 11. Horizontal, amino acids; vertical, hydropathy score. (B) Acid/base composition of amino acids. A, acidic residues; B, basic residues. Note the hydrophilic domain (amino acids 650 to 715) in *Mdr49*, which includes a cluster of acidic followed by a cluster of basic amino acids.

diverged further from each other than have the proximal and distal halves of each mammalian homolog. The two halves of each *Drosophila mdr* homolog share 34 to 35% identical amino acid residues, while the proximal and distal halves of human *mdr1* and *mdr3* share 41 to 42% identical residues.

There are four and eight consensus sequences for N-linked glycosylation (NXT/S) (23) in *Mdr49* and *Mdr65*, respectively. Only one site, surrounding residue 102, is shared by both polypeptides. This is also the only N-linked glycosylation site predicted to be external to the cell, and it is included in the first predicted external domain. A potential N-linked glycosylation site is found in an analogous position in the mammalian *mdr* homologs (15).

The consensus sequence for phosphorylation by protein kinase A is present at positions in *Mdr49* comparable to the locations where it is found in mammalian *mdr* homologs (25). *Drosophila Mdr49* and each of the mammalian *mdr* homologs which are capable of conveying multidrug resistance—human *mdr1* and mouse *mdr1* and *mdr3*—have protein kinase A phosphorylation sites at the beginning of the second half of the polypeptide. This potential phosphorylation site is not present in *Drosophila Mdr65*, human *mdr3*, or mouse *mdr2*. A potential protein kinase A phosphorylation site is also present in *Drosophila Mdr49* at the amino-terminal end in a location similar to one found in both human *mdr1* and *mdr3*. Additional potential protein kinase A phosphorylation sites are present in both *Mdr49* and *Mdr65* which are not conserved in the mammalian homologs.

Molecular and genetic characterization of *Mdr49*. *Mdr49* is a newly identified *Drosophila* gene in a region that has been the subject of previous genetic analyses. Figure 5A shows

A.

B.

FIG. 5. (A) Genetic map of the *Mdr49* region. The top line shows the positions of seven genes in the neighborhood of *Mdr49* relative to the centromere (circle). Five chromosomal deletions in the region are depicted below. Solid lines represent DNA that is present; broken lines indicate ambiguities in the endpoints. *Mdr49* lies between *Psc* and *l(2)C* in the zone defined by the vertical dashed lines. (B) Molecular map of the *Mdr49* transcription unit. The heavy portions of the top line represent the coding region. *EcoRI* sites (circles) and the probes used in Fig. 6 are identified above the cDNA. The asterisk denotes the polymorphic *EcoRI* site, and the vertical markings indicate the locations of the 26 nucleic acid polymorphisms found among the three cDNAs, *dsp20* and *dsp44*, with complete open reading frames, and *dsp12* starting at amino acid 192, as described in the text. The molecular endpoints for *vg*¹³⁵, *vg*^C, and *vg*¹³³ are depicted as determined in Fig. 6. Heavy portions of the line represent the coding sequences that remain. Broken lines indicate ambiguity in the endpoint.

the genetic map of the region in which *Mdr49* lies and the endpoints of five chromosomal deletions (1, 4, 30, 51, 51a, 52). Southern analyses using probe 1208 (Fig. 5B) showed that *Mdr49* DNA had been lost from each deletion (data not shown). Thus, at least a portion of *Mdr49* must lie in the overlap of the *vg*^C and *b8* deletions. Because *b8* does not delete the *l(2)C* gene and *vg*^C does not delete the *Psc* gene, yet both deletions remove at least a portion of *Mdr49*, *Mdr49* cannot be either of these two genes but must be a newly identified gene lying, at least in part, between them.

The precise molecular localization of *Mdr49* was further characterized by Southern blot analyses which include two additional deletions, *vg*¹³⁵ and *vg*¹³³, that have breakpoints genetically similar to that of *vg*^C in region 49EF (30). The first six lanes of Fig. 6 represent *EcoRI*-restricted genomic DNA from two control fly lines, Oregon R and *dpp*^{48/+}, and the heterozygous deletion lines *b8/+*, *vg*^{133/+}, *vg*^{C/+}, and *vg*^{135/+}. Figure 6 illustrates the results of hybridization with probes representing the 5' (Fig. 6A), central (Fig. 6B and C), and 3' (Fig. 6D) regions of *Mdr49*. The hybridization band of the *b8/+* lane in each panel is one half the intensity of those in the control lanes indicating that the entire *Mdr49* gene is removed by the *b8* deletion (Fig. 6A to D). The higher-molecular-weight bands in the Oregon R lanes when probed

FIG. 6. Southern blot analyses of fly DNA probed with the *Mdr49* cDNA. The probes from *Mdr49* (see Fig. 5) are designated at the left of each panel, and the genotypes of flies are above the lanes. The presence of wild-type *Mdr49* is indicated by +. The two control lines are Oregon R and *dpp*^{48/+}. Wild-type hybridization bands (filled arrowheads) and breakpoint bands (open arrowheads) are indicated at the right (sizes given in kilobases). Asterisks indicate lanes without hybridization bands. Arrows indicate the polymorphic *EcoRI* bands in panels B and C. Panels E, F, and G correspond to panels A, D, and B and C, respectively, reprobed with the control probe *rp49* (36).

with 1210 and 1201 (Fig. 6B and C, arrows) reflect the polymorphism which removes the *EcoRI* site described above (Fig. 5B, asterisk).

The *vg*¹³⁵, *vg*^C, and *vg*¹³³ deletions were examined for breakpoints within the *Mdr49* gene. In each case, a hybridization band of altered size is present with one of the *Mdr49* probes in addition to the wild-type band. As expected, each of these bands is half the intensity of the control bands. Chromosomal breakpoints occurred within the DNA which hybridized to the 5' probe 44B in *vg*^{135/+} flies (Fig. 6A), to the central probe 1210 in *vg*^{C/+} flies (Fig. 6B), and to the 3' probe 1208 in *vg*^{133/+} flies (Fig. 6D). These results predict a deletion map and identify the direction of transcription as indicated in Fig. 5B.

Generation of *Drosophila* lines deficient for *Mdr49*. Three fly lines have been generated which are deficient for genomic DNA in the *Mdr49* region by placing *vg*¹³⁵, *vg*^C, and *vg*¹³³ in

FIG. 7. Northern blot of fly RNA probed with 44B. Genotypes are indicated above the lanes as for Fig. 6. Canton S is a wild-type strain. Positions of the 4.5-kb transcript (filled arrowhead) and 18S rRNA (open arrowhead) are indicated.

trans to *b8*. This is possible because *b8* does not remove any of the vital genes deleted by the other three deletions. The deficiencies in these three lines have been confirmed by Southern analyses (Fig. 6) and reinforce the map shown in Fig. 5B. Hybridization of DNA from the *vg*¹³⁵/*b8* line with probe 44B indicated that only the breakpoint band from the *vg*¹³⁵ chromosome was present without any contribution from the *b8* chromosome (Fig. 6A), while hybridization with probes 1210, 1201, and 1208 gave no signal, indicating the absence of the corresponding genomic regions from both the *vg*¹³⁵ and *b8* chromosomes (Fig. 6B to D). Hybridization of *vg*^C/*b8* DNA with probe 44B revealed a wild-type band at half the intensity of the controls (Fig. 6A), while probe 1210 revealed only the breakpoint band from the *vg*^C chromosome (Fig. 6B). Hybridization with probes 1201 and 1208 indicated loss of these regions from *vg*^C (Fig. 6C and D). Hybridization of *vg*¹³³/*b8* DNA with probes 44B, 1210, and 1201 revealed a wild-type band at half the intensity of the controls (Fig. 6A to C) and the presence of only the breakpoint band with probe 1208, indicating the location of the *vg*¹³³ breakpoint within the terminal *Eco*RI fragment (Fig. 6D). These hybridization patterns confirm the complete loss of *Mdr49* from *b8* and the breakpoints of *vg*¹³⁵, *vg*^C, and *vg*¹³³ within *Mdr49* as depicted in Fig. 5B.

Analysis of RNA expression in the mutant lines further confirms the deletions of *Mdr49*. Figure 7 is a Northern (RNA) blot hybridized with probe 44B. Wild-type flies express a major 4.5-kb *Mdr49* transcript and less prominent higher-molecular-weight RNA transcripts. In contrast, both *vg*¹³⁵/*b8* and *vg*^C/*b8* flies express a series of truncated RNA species consistent with loss of distal genetic material. The large size of the chromosomal deletions in *vg*¹³⁵ and *vg*^C suggests that the higher-molecular-weight transcripts are due to genetic material upstream of the identified coding region. There are no transcripts detected in *vg*¹³⁵/*b8* flies when probed with 1210 nor in *vg*^C/*b8* flies when probed with 1208 (data not shown). Normal-size transcripts are present in *vg*¹³³/*b8* flies, suggesting that the *vg*¹³³ breakpoint lies distal to or at the very end of the *Mdr49* coding sequence.

Functional analysis of mutant *Mdr49* *Drosophila* lines. The three mutant lines are viable and fertile. An analysis was undertaken to determine whether disruption of *Mdr49* would result in increased sensitivity during development to colchicine, a cytotoxic agent included in the resistance pattern of the mammalian multidrug-resistant phenotype (9, 15, 21). Figure 8A shows that *vg*^C/*b8* is approximately twofold more sensitive to colchicine during development than are the controls. The range of control viabilities was determined by the response of 19 randomly chosen stocks. The vertical lines accompanying the survival curve of these controls represent 1 standard deviation from the mean and demonstrate that a wide range of responses is seen and that the viabilities of the parental lines, *b8*/+ and *vg*^C/+, are encompassed by them (Fig. 8B). Nevertheless, none of the control or parental lines show the degree of lethality at 10 μ M colchicine that is seen for *vg*^C/*b8* (Fig. 8C). In contrast to *vg*^C/*b8*, neither of the other deficiency lines, *vg*¹³⁵/*b8* (Fig. 8B and C) or *vg*¹³³/*b8* (data not shown), is associated with an increased sensitivity to colchicine.

DISCUSSION

The *Drosophila mdr* homologs *Mdr49* and *Mdr65* bear striking sequence similarities to the mammalian *mdr* homologs along the entire polypeptide, sharing 42 to 45% identical amino acid residues. The *Drosophila* and human homologs are much more similar to each other than either is to *S. cerevisiae* (22 and 28%, respectively), *P. falciparum* (22 and 33%), or *Leishmania* (23 and 22%) homologs (data not shown). The putative transmembrane and nucleotide binding domains and several of the N-linked glycosylation and protein kinase A phosphorylation sites are found in analogous positions in the *mdr* homologs. These sequence and structural similarities are highlighted by the observation that when the two *Drosophila* and two human *mdr* homologs are aligned, three of the four amino acids are identical in 50% of the positions (Fig. 1).

The *Drosophila* white and brown genes are structurally related to the *mdr* gene and consist of a nucleotide binding fold followed by a series of transmembrane domains (13, 35, 37). A partial sequence of the scarlet gene also suggests the presence of a nucleotide binding fold (45). The products of these genes have been proposed to transport pigment precursors, possibly as heterodimers which function in a manner similar to the *mdr* homologs (13). A comparison of the coding regions of the *Drosophila mdr* homologs with those of white and brown reveals stretches of amino acids that are approximately 25% identical and stretches of nucleic acids that are 47 to 55% identical (data not shown). These homologies complicate the interpretation of low-stringency hybridization studies with *Mdr49* and *Mdr65* probes which indicate that there may be two additional *mdr* homologs (data not shown).

The two *Drosophila mdr* homologs are located on different chromosomes in contrast to the mammalian *mdr* multigene families, which form a cluster on a single chromosome in humans, mice, and hamsters (6, 39, 48). This may account in part for the divergence between the two *Drosophila* homologs, which are evolutionarily as distant from each other as each is from the mammalian genes. There appears to be a great deal of mutational drift even among three different isolates of *Mdr49* from a single cDNA library. Polymorphisms were observed at 26 nucleotide positions, 3 of which resulted in amino acid substitutions. One of the nucleotide changes is found as an *Eco*RI polymorphism in a variety of

FIG. 8. Viability of fly lines in response to colchicine. (A) Viability of *vg^C/b8* flies and random control flies; (B) viability of *vg¹³⁵/b8*, parental, and random control flies; (C) viability at 10 μ M colchicine. Controls consist of 19 randomly chosen stocks that included 4 wild-type strains and 15 mutant lines representing homozygous recessive or heterozygous dominant lesions at 24 loci. Random controls are shown ± 1 standard deviation of the mean in A, B, and C (*), and standard errors are given for the other lines in panel C. The Tukey-Kramer, GT2, and T' statistical tests indicated that at $P = 0.01$, the viability of *vg^C/b8* flies is significantly different from the viability of all other lines at 10 μ M colchicine. No other significant differences were detected. These three tests are complementary analyses of variance powerful for multiple samples and sample sizes (43).

Drosophila lines. Further analysis is required to ensure that the other polymorphisms do not represent cloning artifacts. A mutation in the human *mdr1* gene appears to increase colchicine resistance (7), and the analysis of a *P. falciparum* *mdr* homolog suggests that specific mutations may be associated with resistance to antimicrobial agents (17). These observations indicate that mutations within the *Drosophila* *mdr* homologs may be a means for generating functional diversification.

Drosophila Mdr49 falls in a chromosomal region for which a detailed genetic map and chromosomal deletions are available. *Mdr49* is a newly identified gene lying between *Psc* and *l(2)C*, in the overlap of the *b8* deletion with *vg¹³⁵*, *vg^C*, and possibly *vg¹³³* (Fig. 5). Three lines of flies that are missing sequences from the *Mdr49* region have been made by placing *b8* in *trans* to *vg¹³⁵*, *vg^C*, and *vg¹³³*. As two of these lines, *vg¹³⁵/b8* and *vg^C/b8*, definitely lack a wild-type *Mdr49* gene (Fig. 5B), *Mdr49* is clearly not essential for viability. This conclusion is consistent with the results of independently conducted genetic studies which have not yet uncovered any vital genes between *Psc* and *l(2)C* (30, 51a). Whether *Mdr49* is nonvital because it encodes nonessential or redundant functions or because one or more of the other members of the *Drosophila* *mdr* multigene family are able to compensate for its loss is not known. Similarity of function among related genes has been noted for a number of *Drosophila* loci, including metallothionein (32, 34), polyhomeotic (14), the achaete-scute complex (11), the Enhancer of split complex (53), and the Suppressor 2 of zeste complex (4a, 42a). Analogously, viability with a disrupted *STE6* gene has been noted in *S. cerevisiae* in which mutant cells have been shown to exhibit increased sensitivity to the antibiotic valinomycin (29).

The three mutant *Drosophila* lines provide a means of examining whether the loss of an *mdr* gene in a multicellular organism results in increased drug sensitivity. Mammalian studies have been able to address only the opposite issue in tissue cultured cells, that drug resistance is associated with the increased expression of *mdr* genes (9, 15, 21). One of the three mutant lines, *vg^C/b8*, does show heightened sensitivity to colchicine. On the other hand, *vg¹³⁵/b8* and *vg¹³³/b8* do not display an increased sensitivity when allowed to mature on colchicine. The lack of colchicine sensitivity in *vg¹³³/b8* is not surprising because the *vg¹³³* breakpoint occurs distal to or at the very end of the *Mdr49* coding sequence (data not shown), as indicated by the presence of normal-size *Mdr49* transcripts. The *vg¹³⁵/b8* line, however, deletes nearly all of the gene, as indicated by a very short RNA transcript. Apparently, the loss of a wild-type *mdr* homolog in and of itself does not cause the heightened colchicine sensitivity during development.

It is necessary to determine whether the colchicine sensitivity observed with the *vg^C/b8* flies is due to abnormal *Mdr49* gene. It is unlikely that the phenotype is due to a mutation in a gene other than *Mdr49* for two reasons. First, the viability trials of the *vg^C/b8* line were done on four stocks derived from two independent constructions, all of which behave indistinguishably. Second, the *vg^C/b8* stocks are maintained such that recombination occurs freely throughout the genome except for the small region containing the deletions (<1% of the genome). Therefore, if a hypothetical second mutation does exist, it must lie near the *vg^C* and *b8* deletions or have become homozygous in all four stocks such that recombination would not be able to eliminate it. Given these arguments, it is most likely that the increased sensitivity to colchicine is due to the abnormal *Mdr49* gene.

If loss of wild-type *Mdr49* does not cause increased sensitivity to colchicine, as in *vg¹³⁵/b8*, what is the basis for the phenotype of *vg^C/b8* flies? One possibility is that the remaining portion of *Mdr49*, representing approximately one-half of the coding region, gives rise to a truncated or fusion protein or RNA species which is deleterious. The abnormal protein might interact with normal products of *mdr* homologs or accessory proteins and interfere with transport. Alternatively, the existence of an altered protein or mRNA could signal that a full complement of functional *mdr* homologues is present. Thus, in contrast to the *vg¹³⁵/b8* flies, in which the cells might sense and compensate for the loss of *Mdr49*, cells in *vg^C/b8* flies would not activate an alternative pathway, resulting in increased sensitivity to colchicine. Preliminary observations indicate that the *vg¹³⁵/b8* flies which developed on 10 μ M colchicine did not appear to express increased levels of *Mdr65* as a compensatory mechanism at the level of detection by Northern blots (data not shown).

It is possible to test the premise that the abnormal *Mdr49* in *vg^C/b8* flies is responsible for increased colchicine sensitivity. Mutagenesis of the *vg^C/b8* line with selection on 10 μ M colchicine, at which concentration *vg^C/b8* is nonviable, should give rise to survivors which have new lesions in *Mdr49* that eliminate the functional capabilities of the truncated gene. This type of selection would also determine whether it is possible to restore resistance through lesions in genes other than *Mdr49*. Mutations in other *mdr* homologs, or perhaps more excitingly in previously unrecognized genes involved in the *mdr* transport function, might be identified. Clearly, the ability to rapidly and precisely manipulate the *Drosophila* genome provides a powerful genetic system for determining the normal actions and interactions of the *mdr* genes and the consequences of inhibiting their functions.

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