Using the *P{wHy}* Hybrid Transposable Element to Disrupt Genes in Region 54D-55B in *Drosophila melanogaster*

Stephanie E. Mohr and William M. Gelbart[1]

Department of Molecular and Cellular Biology, Harvard University, Cambridge, Massachusetts 02138

Manuscript received December 3, 2001
Accepted for publication June 6, 2002

ABSTRACT

Understanding the function of each gene in the genome of a model organism such as *Drosophila melanogaster* is an important goal. The development of improved methods for uncovering the mutant phenotypes of specific genes can accelerate achievement of this goal. The *P{wHy}* hybrid transposable element can be used to generate nested sets of precisely mapped deletions in a given region of the Drosophila genome. Here we use the *P{wHy}* method to generate overlapping, molecularly defined deletions from a set of three *P{wHy}* insertions in the 54E-F region of chromosome 2. Deletions that span a total of 0.5 Mb were identified and molecularly mapped precisely. Using overlapping deletions, the mutant phenotypes of nine previously uncharacterized genes in a 101-kb region were determined, including identification of new loci required for viability and female fertility. In addition, the deletions were used to molecularly map previously isolated lethal mutations. Thus, the *P{wHy}* method provides an efficient method for systematically determining the phenotypes of genes in a given region of the fly genome.

THE availability of the genome sequence of *Drosophila melanogaster* has given researchers a glimpse of the complete set of Drosophila genes. The annotation of these genes continues to improve with computational and experimental methods, making the sequence of the ~13,600 Drosophila genes available from any web terminal on the globe (Adams *et al.* 2000). Even so, understanding the function of these genes, alone and in concert with one another, is a daunting task.

Classic genetic approaches have yielded invaluable insight into gene function at the levels of biochemical function, function in a particular pathway, and function in a particular cell type, tissue, or organ. Indeed, the Berkeley Drosophila Genome Project (BDGP) has set a goal of obtaining a mutation in every gene in the fly genome to help elucidate gene function (Spradling *et al.* 1999). The BDGP gene disruption project and others have focused on genes that mutate to lethality, sterility, or other readily definable phenotypes (Cooley *et al.* 1988; Spradling *et al.* 1999). A similar, smaller-scale effort was made to obtain *P*-element insertions in the 2.9-Mb Adh region. In this case, all insertions (not just those with definable phenotypes) were mapped, yielding insertions in a larger proportion of genes (Ashburner *et al.* 1999).

A major advantage of *P*-element disruption over chemical or high-energy radiation mutagenesis is that *P* elements provide a molecular foothold from which researchers can identify the site of insertion by molecular methods such as inverse PCR (iPCR; Ochman *et al.* 1988; Triglia *et al.* 1988) or the recently reported universal fast-walking technique (UFW; Myrick and Gelbart 2002). Another advantage is that transposon insertions in or near genes can be used to generate new mutant alleles and local deletions by mobilization of *P*-element insertions (Robertson *et al.* 1988) or by *P*-induced male recombination (Preston *et al.* 1996). On the other hand, a disadvantage of this approach is that *P*-element insertions are not random. Thus, as efforts to obtain *P*-element insertions into genes increase in scale, the majority of insertions will fall into regions in which a *P* element has already been identified and/or into intergenic regions.

Clearly, new methods could accelerate the pace of understanding gene function by facilitating the study of mutant phenotypes. Although both RNA-mediated interference and homologous recombination approaches have been reported (Kennerdell and Carthew 1998; Rong and Golic 2000, 2001), it remains to be determined if these techniques can be used for a large-scale survey of mutant phenotypes of all genes in Drosophila.

Another approach is the *P{wHy}* hybrid transposable-element-based method for creating genomic deletions (Huet *et al.* 2002). With this method, the *P{wHy}* element is used to make nested sets of deletions in a particular region of the fly genome. The *P{wHy}* element (Figure 1A) consists of a transposase-deficient *hobo* element flanked by the *white* and *yellow* marker genes and by *P*-element ends that enable the entire *P{wHy}* hybrid element to be mobilized by a source of *P*-element transposase (note that *P* and *hobo* transposases do not cross-react; Eggleston *et al.* 1988; Calvi 1993). Subsequently, mobilization of *hobo* can be used to create nested sets

[1]*Corresponding author:* 16 Divinity Ave., Cambridge, MA 02138. E-mail: gelbart@morgan.harvard.edu

of deletions that extend in one direction or the other from a given starting insertion of the *P{wHy}* element (Figure 1, B and C; Huet *et al.* 2002). Deletion of flanking sequence is thought to be caused by insertion of the *hobo* element into a nearby site followed by recombination between the resulting adjacent *hobo* insertions, thereby excising the intervening sequence (Blackman *et al.* 1987; Sheen *et al.* 1993; Lim and Simmons 1994). Because the *hobo* element remains intact in the deletion strains, iPCR or other techniques can be used to molecularly identify the deletion endpoints, resulting in a deletion mapped with to-the-base-pair accuracy (Ochman *et al.* 1988; Triglia *et al.* 1988; Huet *et al.* 2002; Myrick and Gelbart 2002).

Here we use the *P{wHy}* method to disrupt and characterize genes by generating deletions from adjacent starting insertion sites and then placing overlapping deletions *in trans* to one another. The deletions cover an ~0.5-Mb region in cytological region 54D-55B of chromosome 2 between the cytogenetically defined loci *abero* (54E; Kania *et al.* 1995) and *staufen* (*stau*; 55B; St. Johnston *et al.* 1991). Although the region contains many genes based on cDNA evidence and computational prediction (Adams *et al.* 2000; Rubin *et al.* 2000), few mutations were known to disrupt genes in the region. We show that the method proves effective for the 54D-55B region: complementation analyses with overlapping deletions uncover the mutant phenotypes of several loci, including loci required for viability and female fertility, and the deletions were also used to molecularly map anonymous, EMS-induced mutations in the region.

MATERIALS AND METHODS

Fly stocks and culturing: Fly strains were maintained at 25° using standard protocols for media preparation and culturing. Strains carrying the following mutations were obtained from the National Drosophila Stock Center (Bloomington, IL): *grh*^{s2140}, *grh*^{IM}, *grh*^{06850}, *hal*^{DB48}, *stau*^{1}, *stau*^{D9}, *thr*^{1}, *thr*^{k07805b}, *Df(2R)Pcl7B*, *l(2)k11505*, *l(2)k11311*, *l(2)k09924*, and *l(2)PC4-A*^{139}, *l(2)PC4-B*^{110}, *l(2)PC4-D*^{202}, *l(2)PC4-E*^{119}, *l(2)PC4-F*^{198}, *l(2)PC4-G*^{223}, and *l(2)PC4-M*^{420} (Duncan 1982; Dynlacht *et al.* 1989; Schüpbach and Wieschaus 1991; St. Johnston *et al.* 1991; D'Andrea *et al.* 1993; Flybase 1999; Spradling *et al.* 1999). The *EP(2)616* insertion (Rørth *et al.* 1998) was provided by Exelixis (San Francisco). The *adrift*^{1} (Englund *et al.* 1999) mutation and the *Df(2R)30W* deletion were provided by C. Samakovlis. The *P{wHy}14F06*, *14H10*, and *02B10* insertion strains have unique insertions of the *P{wHy}* element in a *y*^{1} *Df(1)67c* background, hereafter referred to as *y*$^{-}$ *w*$^{-}$. All crosses were performed at 25° and stocks were maintained at 22° or 25°.

***P{wHy}* insertion site identification:** Autosomal insertions of *P{wHy}* were molecularly mapped on the 3′ end using iPCR (Ochman *et al.* 1988; Triglia *et al.* 1988). Genomic DNA was digested with *Sau*3AI or *Hin*PI. PCR was performed using the following primers: for 5′ end mapping, Plac1, cacccaaggctctgctcccacaat and Plac4, gactgtgcgttaggtcctgttca; for 3′ end mapping, P4-2, caatcatatcgctgtctccactcagact and Pye1, gttgcgatttcggagctacaatcgg. The iPCR amplicons were sequenced using the Plac1 or P4-2 oligonucleotide primers.

The *P{wHy}14F06*, *14H10*, and *02B10* insertion sites were confirmed by using genomic DNA-specific primers 5′ or 3′ of the insert site (below) with Pendout2 (cgacgggaccaccttatgtt) in PCR assays, and PCR products were sequenced with Pendout2 to confirm the insertion sites. The genomic DNA-specific primers were as follows: for 14F06, 5′-atctcctcctcgctggactcggact and 5′-cttttgcacgcaagcgcagc; for 14H10, 5′-agtttaaataggatctcgcc and 5′-gatgtagtagtgttgaaaggtga; for 02B10, 5′-gatgtggaaattgtatgagagtag and 5′-tagtagtctttttcgaagctctg.

Generation and identification of genomic disruptions: To mobilize *hobo* in *P{wHy}14F06*, *14H10*, and *02B10*, homozygous or heterozygous males carrying an insertion were crossed to virgin females carrying a source of *hobo* transposase (*y*$^{-}$ *w*$^{-}$*;Gla/CyO-P{hsH\T-2}*). The resulting larvae were heat-shocked for 30 min at 37° on the second, fourth, and sixth days after crossing the adults. In the F_1 cross, individual *y*$^{-}$ *w*$^{-}$*;P{wHy}/CyO-P{hsH\T-2}* males were crossed to *y*$^{-}$ *w*$^{-}$*; Gla/SM6a* virgin females. Phenotypically w^{+} y^{-} and w^{-} y^{+} lines were recovered from the F_2 and crossed to *y*$^{-}$ *w*$^{-}$*; Gla/SM6a* virgin females to establish balanced stocks. The 5′ and 3′ genomic DNA-specific primers that flank the insertion site described above were used with the Pendout2 primer in a one-tube PCR assay to test for the presence of 5′ and 3′ ends. Lines in which both the 5′ and 3′ products were present were discarded (these represent deletions of the marker genes without associated disruption of flanking genomic DNA). Lines in which only the predicted *P* end was detected were retained (see Figure 1, B and C).

Mapping of *hobo* insertion sites: The 5′ *hobo* insertion sites (w^{+} y^{-} lines) and 3′ *hobo* insertion sites (w^{-} y^{+}) were mapped by iPCR (Ochman *et al.* 1988; Triglia *et al.* 1988) or UFW (Myrick and Gelbart 2002). For iPCR, genomic DNA was digested with *Eco*RI, *Alu*I, or *Mse*I. PCR was performed with the following primers: for 5′ *hobo* flanking DNA, Ph5-1, acg caaaacaccgtattattcgg and Ph5-2, cgtaggtagtcgagtcaaatggc; for 3′ *hobo* flanking DNA, Ph-EA1, gggcataatctatttcgcttttct and Ph3-2, cgagtattttgtgtgccgcaagt. PCR amplicons were sequenced with Ph5-1 or Ph3-2.

For UFW 5′ *hobo* mapping, the following primers were used (listed in the order in which they were used): h5-1, 5′-actacct acgagaccactcg; h5-2, 5′-tttaggcactgtgtgagcgg(n_{10}); h5-3, 5′-taa cggtatacccacaagtg; h5-4, 5′-acgcaaacacctattgattcgg; and h5-5, 5′-gatgtgcgtggcgagtagcaccc. For UFW 3′ *hobo* mapping, the following primers were used: h3-1, 5′-ccgaatcaatacggtgtttttgcgt; h3-2, 5′-cgagtggtctcgtaggtact(n_{10}); h3-3, cacttgtgggtataccgtta; h3-4, 5′-gatcgttgactgtgcgtccactca; h3-5, 5′-acacaacgtcggtaaaacactcga.

Complementation analysis and fertility tests: Complementation analysis was performed by crossing heterozygous virgin females of one genotype to heterozygous males of the other genotype. All genotypes were balanced over *SM6a* or *CyO* second chromosome balancers, and the Cy^{+} phenotype was used to identify *trans*-heterozygous individuals resulting from the cross ($n > 100$ adults scored in all cases). Whenever possible, additional markers were scored. For example, for *P{3′wHy}02B10* crosses to *P{5′wHy}14H10* lines (balanced with *SM6a* in all cases), it was possible to identify *trans*-heretozygotes as the w^{+}, y^{+}, Cy^{+} class. All viable *trans*-heterozygous combinations were tested for female fertility by crossing *trans*-heterozygous females to wild-type males; all homozygous viable genomic disruption strains were also tested for female fertility.

RESULTS

Three insertions define a 101-kb region in polytene region 54: Using iPCR, several independent insertions of the *P{wHy}* hybrid transposable element (Figure 1A) were molecularly mapped from a collection of autoso-

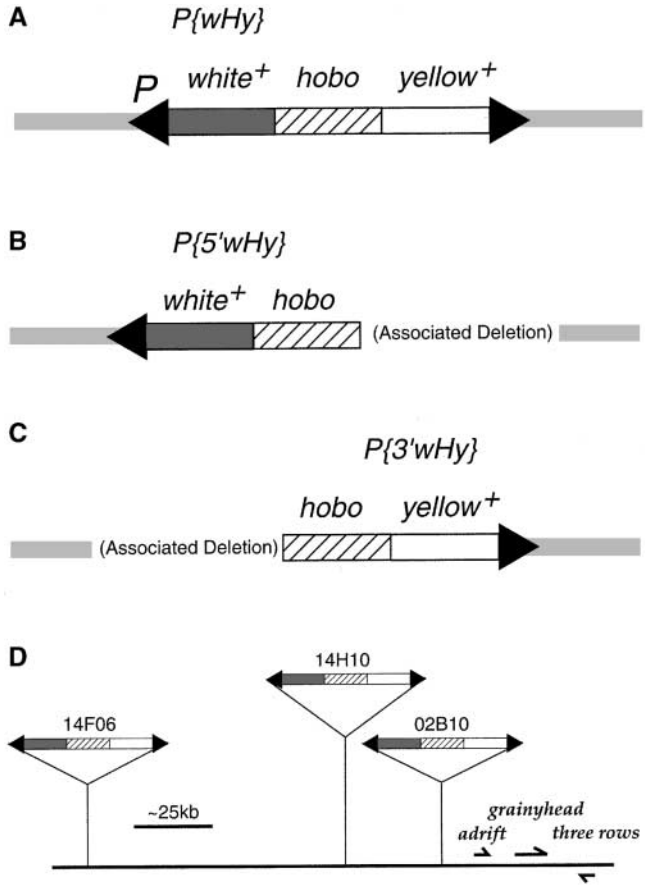


FIGURE 1.—The *P{wHy}* element. (A) The *P{wHy}* element is made up of *P*-element ends sufficient for *P*-transposase-mediated transposition (solid arrowheads) enclosing the *white*$^+$ gene (solid box), a transposase-deficient Hobo transposon (boxed diagonals), and the *yellow*$^+$ gene (open box). The shaded box indicates genomic DNA into which the *P{wHy}* element is inserted. After transposition with Hobo transposase, insertion of *hobo* into a new location followed by recombination between the original and newly inserted *hobo* transposons can result in B, the *P{5′wHy}* element, or C, the *P{3′wHy}* element. (D) The orientations of *P{wHy}14F06*, *14H10*, and *02B10* relative to the previously identified and genetically characterized genes in the region (see Figure 2 for a complete map of transcripts in the region). Proximal is to the left and distal is to the right.

mal insertions. Sequencing of flanking DNA revealed that three inserts, *P{wHy}14F06*, *P{wHy}14H10*, and *P{wHy}02B10* (hereafter, 14F06, 14H10, and 02B10), are in a region in which little genetic data are connected with molecularly defined genes in the region (Figure 1D; GenBank accession nos. BH836458–BH836469). The three inserts have the same orientation of insertion and are within 101 kb of one another between the cytogenetically defined loci *abero* (54E; KANIA *et al.* 1995) and *grainyhead* (54F1-5; DYNLACHT *et al.* 1989) and, at the molecular level, between *rhino* (VOLPE *et al.* 2001) and *CG6370* (Figure 1D; Figure 2; ADAMS *et al.* 2000). We compared the genomic sequence of the region to cDNAs, expressed sequence tags, and computationally predicted genes to obtain a map of the genes in the region (ADAMS *et al.* 2000; RUBIN *et al.* 2000). Twenty-five protein-coding transcription units are predicted to be present between 14F06 and 02B10 (Figure 2).

Each of the three *P{wHy}* inserts is predicted to disrupt a transcript (Figure 2). Whereas the 14F06 insertion chromosome is homozygous lethal, the 14H10 and 02B10 insertion chromosomes are homozygous viable. The 14F06 insertion disrupts the *eIF3-S8* gene, which encodes a highly conserved translation factor (LASKO 2000). The transcript disrupted by 14H10, *CG4996*, encodes a conserved protein of unknown function (Figure 3). The transcript disrupted by 02B10, *CG6370,* encodes a homolog of the human ribophorin II protein, a dolichyl-diphosphooligosaccharide-protein glycosyltransferase (Figure 3; CRIMAUDO *et al.* 1987). The inserts represent new alleles of the genes and thus can be designated *eIF-3S8*^{14F06}, *CG4996*^{14H10}, and *CG6370*^{02B10}; for convenience, we will continue to refer to the insertions as 14F06, 14H10, and 02B10.

Mobilization of *hobo* in 14F06, 14H10, and 02B10 results in disruption of genomic DNA: To generate and isolate unidirectional deletions from each of the three starting *P{wHy}* insertions, we introduced *hobo* transposase to generate deletions (see MATERIALS AND METHODS; HUET *et al.* 2002). After introduction of *hobo* transposase, local transposition followed by recombination is thought to result in deletion of flanking DNA. Deletions of the marker genes or of the marker genes and flanking genomic DNA are detected by loss of the *white*$^+$ or *yellow*$^+$ marker genes. Next, disruption of only the marker gene (both *P* ends retained) is distinguished from disruption of flanking DNA (one *P* end retained; Figure 1, B and C) by assaying for the presence or absence of the *P*-element ends. Phenotypically w^+ y^- strains or w^- y^+ strains in which only the appropriate *P*-element end is present were retained for further study. We refer to this class of events as "genomic disruptions" and term the newly formed *P{wHy}* derivatives *P{5′wHy}* (phenotypically w^+ y^-) and *P{3′wHy}* (phenotypically w^- y^+; Figure 1, B and C).

Since the 14H10 insertion is between 02B10 and 14F06, events in both directions are useful for obtaining overlapping deletions. Therefore, about twice as many crosses were performed with 14H10 as with the others. With 14F06, 273 fertile crosses resulted in 78 w^+ y^- and w^- y^+ strains; 35 of these are genomic disruptions. With 14H10, 559 fertile crosses resulted in identification of 174 w^+ y^- and w^- y^+ strains; 78 of these are genomic disruptions. With 02B10, 204 fertile crosses resulted in identification of 55 w^+ y^- and w^- y^+ strains; 25 of these are genomic disruptions.

To determine the extent of the putative deletions in each genomic disruption, the new 5′ or 3′ *hobo* insertion points in *P{5′wHy}* and *P{3′wHy}* strains were sequenced by iPCR or UFW. Of the 96 genomic disruptions analyzed, 67 unambiguously map to the 54D-55B region

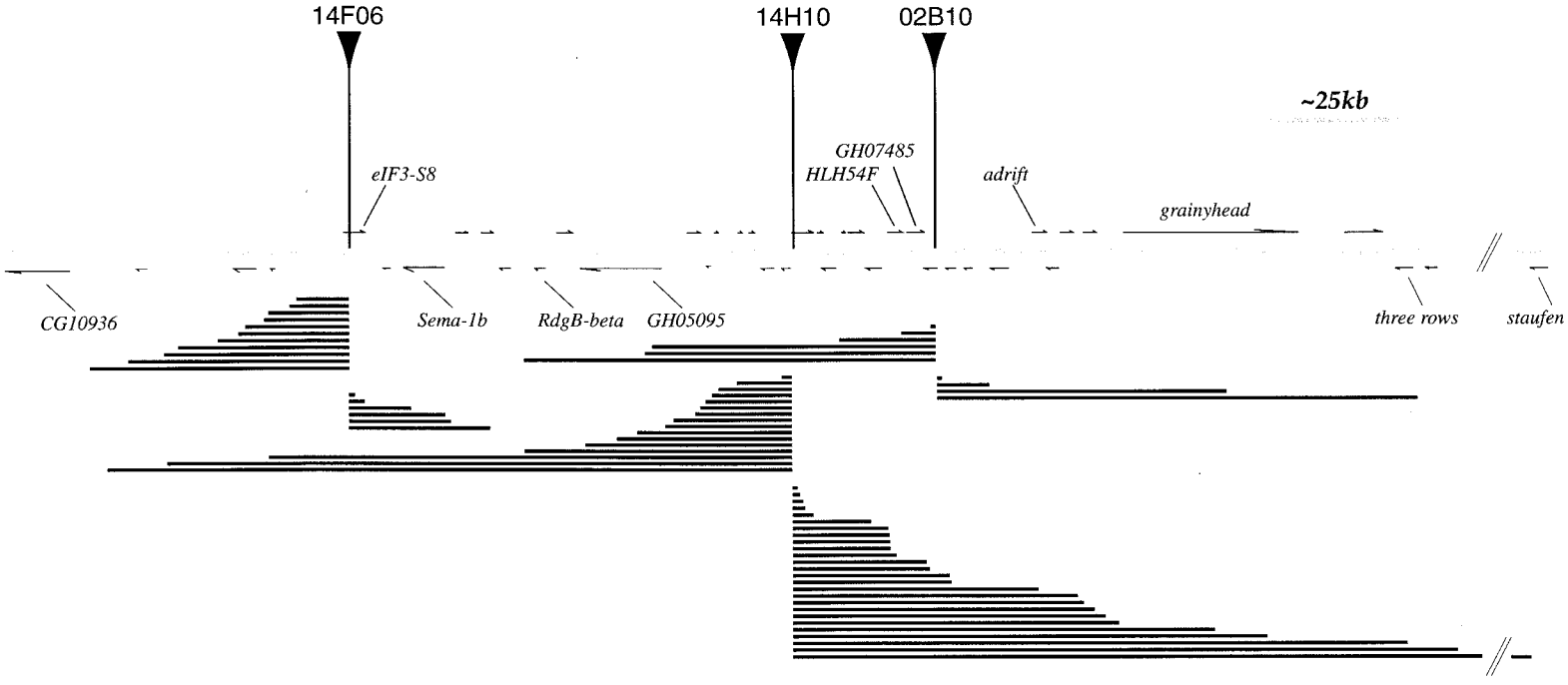
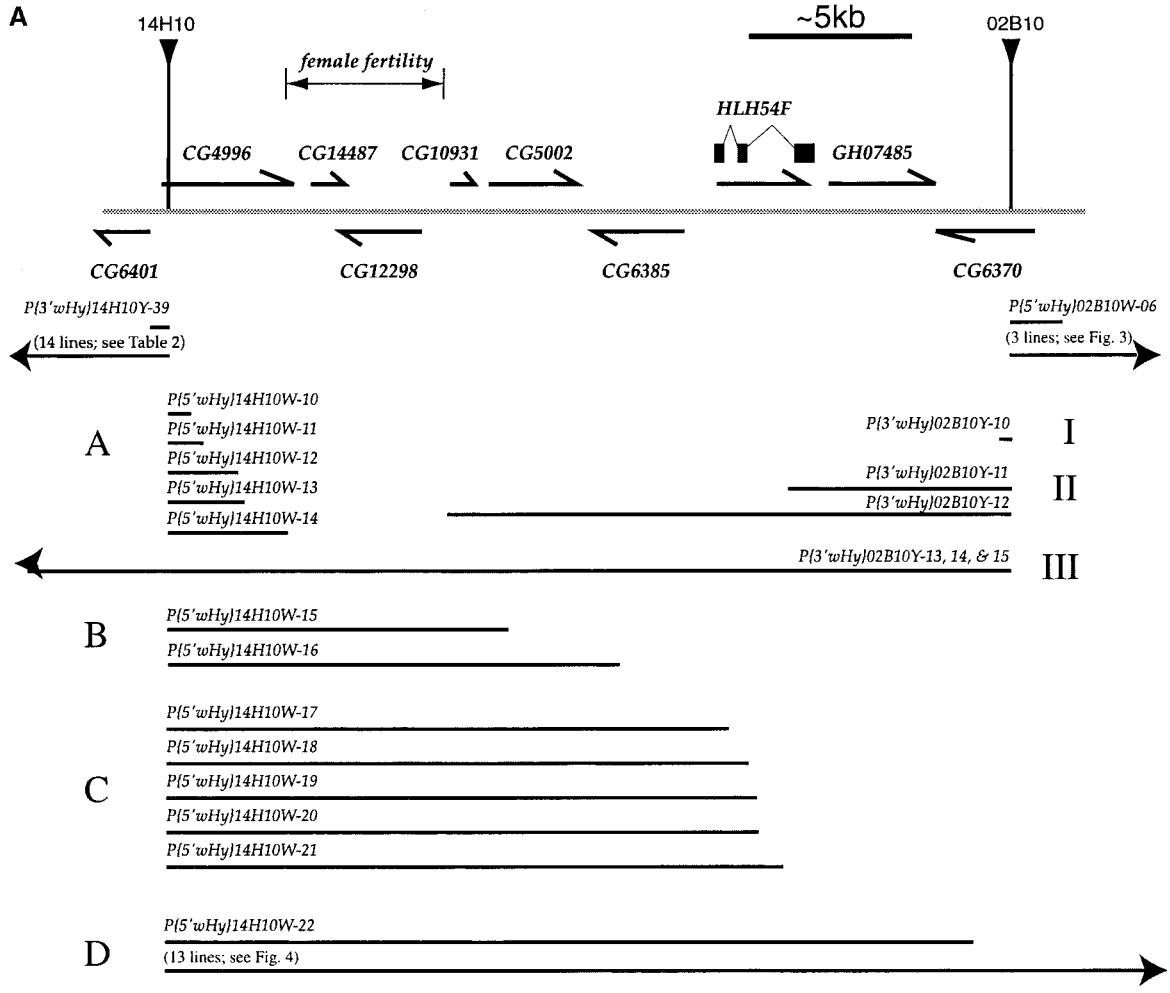


FIGURE 2.—*P{wHy}*-generated deletions and transcripts in the 54D-55A region. Proximal is to the left and distal is to the right in this and all subsequent figures. Inverted triangles, sites of insertion of 14F06, 14H10, and 02B10. Shaded line, genomic DNA. Arrows show the location of known and predicted transcripts and indicate the direction of transcription; exon/intron boundaries are not shown. The names of a subset of transcripts are indicated for reference. Solid lines, the region deleted in each *P{wHy}*-generated deletion that was molecularly mapped.



B

	I	II	III
A	+	+	+
B	+	+	Female Sterile
C	+	Semi-Lethal	Semi-Lethal
D	+	-	-

FIGURE 3.—Overlapping deletions between 14H10 and 02B10. (A) Map of the 14H10-02B10 region. All known and predicted transcripts in the region are indicated with arrows. For *HLH54F*, the exon/intron structure is indicated. Solid lines, the region removed in *P{wHy}*-generated deletions extending from 14H10 and 02B10. An arrowhead on a solid line indicates that the deletion extends in the direction of the arrow beyond the region depicted in the figure. (B) Complementation analysis between *P{5′ wHy} 14H10* deletions and *P{3′ wHy} 02B10* deletions. +, viable and fertile; −, inviable.

and carry single insertions of *hobo*. Genetic data with the unmapped lines are consistent with disruption of adjacent genomic DNA, but these lines may have multiple *hobo* insertions or rearrangements that preclude molecular mapping, and thus were excluded from the analysis presented below. The GenBank accession numbers corresponding to the flanking DNA sequence from *hobo* mapping are provided for each genomic disruption in Table 1 (14F06), Table 2 (14H10), and Table 3 (02B10).

Two observations based on the sequence data support the idea that the genomic disruptions are deletions. First, the *hobo* flanking sequence is in the orientation expected for a simple deletion. Second, the positions of the putative deletion breakpoints relative to the starting

TABLE 1

Deletions generated from 14F06

Insertion	Associated deletion[a]	Size of deletion (bp)	GenBank accession no.
Proximally extending			
P{3′wHy}14F06Y-18	*eIF3-S8$^{14F06Y-18}$*	4,965	BH758878
P{3′wHy}14F06Y-19	*eIF3-S8$^{14F06Y-19}$*	6,699	BH758879
P{3′wHy}14F06Y-20	*Df(2R)14F06Y-20*	12,504	BH758880
P{3′wHy}14F06Y-21	*Df(2R)14F06Y-21*	12,611	BH758881
P{3′wHy}14F06Y-22	*Df(2R)14F06Y-22*	17,125	BH758882
P{3′wHy}14F06Y-23	*Df(2R)14F06Y-23*	18,091	BH758883
P{3′wHy}14F06Y-24	*Df(2R)14F06Y-24*	19,712	BH758884
P{3′wHy}14F06Y-25	*Df(2R)14F06Y-25*	27,158	BH758885
P{3′wHy}14F06Y-26	*Df(2R)14F06Y-26*	27,164	BH758886
P{3′wHy}14F06Y-27	*Df(2R)14F06Y-27*	39,676	BH758887
P{3′wHy}14F06Y-28	*Df(2R)14F06Y-28*	49,073	BH758888
Distally extending			
P{5′wHy}14F06W-05	*eIF3-S8$^{14F06W-05}$*	737	BH758908
P{5′wHy}14F06W-06	*eIF3-S8$^{14F06W-06}$*	3,168	BH758909
P{5′wHy}14F06W-07	*Df(2R)14F06W-07*	11,780	BH758910
P{5′wHy}14F06W-08	*Df(2R)14F06W-08*	16,562	BH758911
P{5′wHy}14F06W-09	*Df(2R)14F06W-09*	18,117	BH758912
P{5′wHy}14F06W-10	*Df(2R)14F06W-10*	21,850	BH758913

[a] As per convention, deletions that disrupt only one transcript are indicated as alleles of that transcript, and deletions that remove more than one transcript are indicated as deficiencies (FlyBase, 1999).

insertion sites are consistent with simple deletions in the direction predicted from the marker gene phenotype ($w^+ y^-$ or $w^- y^+$). Thus, together with the original insertion point of the *P{wHy}* element, the *hobo* mapping data indicate the predicted extent of the deletions. The putative deletions range in size from 412 to 377,752 bp (Tables 1, 2, and 3; Figure 2). Together, the deletions extend over >0.5 Mb between *CG10936* (Adams *et al.* 2000) and *stau* (St. Johnston *et al.* 1991) in cytogenetic region 54D-55B (Figure 2).

Genomic disruptions fail to complement previously identified loci in 54D-55B: If the genomic disruptions are deletions, then they should fail to complement existing mutations in the 54D-55B region in a predictable pattern. Therefore, we tested whether the genomic disruption strains have complementation patterns consistent with deletions of the predicted sizes. Mutations in several previously identified loci were useful for this analysis—namely, the recessive lethal loci *grainyhead* (*grh*; Dynlacht *et al.* 1989), *three rows* (*thr*; D'Andrea *et al.* 1993), and *eIF3-S8* (this work; Lasko 2000) and the female sterile locus *stau* (St. Johnston *et al.* 1991).

Some of the genomic disruptions extending distally from 02B10 and 14H10 are predicted to delete *grh* and *thr* (Figure 4A). We observe the expected complementation pattern between deletions of specific predicted sizes and mutant alleles of these genes (Figure 4B). Similarly, three deletions extending proximally from 14H10 that are predicted to remove *eIF3-S8* (*P{3′wHy}14H10Y-51*, *P{3′wHy}14H10Y-52*, and *P{3′wHy}14H10Y-53*; Table 2) fail to complement the lethality of *eIF3-S8^{14F06}*. The distal breakpoint of the longest deletion, *P{5′wHy}14H10W-35*, is 1424 bp 3′ of the end of the *stau* coding region and thus is not expected to disrupt *stau* function (Figure 4A). As expected, the *P{5′wHy}14H10W-35* deletion complements *stau* and *eIF3-S8^{14F06}* but fails to complement *grh*, *thr*, and *Df(2R)Pcl7B* (Duncan 1982; Dynlacht *et al.* 1989; St. Johnston *et al.* 1991; D'Andrea *et al.* 1993; Englund *et al.* 1999). Lastly, *Df(2R)30W*, a deletion that disrupts *EG:52C10.5* but not *adrift* (C. Englund and C. Samakovlis, personal communication), complements *P{5′wHy}02B10W-06* but fails to complement longer deletions. Therefore, the molecular and genetic data support the idea that the genomic disruptions are deletions, making them useful for further genetic analysis.

Overlapping deletions define regions required for viability: We were particularly interested in the region between 14F06 and 02B10, since no previously identified mutations have been associated with the genes in this interval. To test the requirement for specific regions of DNA between 14F06 and 02B10, strains with deletions extending distally from 14F06 and 14H10 were crossed to those with deletions extending proximally from 02B10 and 14H10 (Figure 3A). The gene proximal to the 14H10 insertion (*CG6401*) is essential, since deletions that extend proximally from 14H10 that disrupt this gene or larger regions (for example, *P{3′wHy}14H10Y-39*) are inviable *in trans* to deletions that remove the entire region (Figure 3). No further phenotypic information

TABLE 2

Deletions generated from 14H10

Insertion	Associated deletion[a]	Size of deletion (bp)	GenBank accession no.
Proximally extending			
P{3′ wHy} 14H10Y-39	*Df(2R) 14H10Y-39*	459	BH758889
P{3′ wHy} 14H10Y-40	*Df(2R) 14H10Y-40*	8,524	BH758890
P{3′ wHy} 14H10Y-41	*Df(2R) 14H10Y-41*	10,835	BH758891
P{3′ wHy} 14H10Y-42	*Df(2R) 14H10Y-42*	11,502	BH758892
P{3′ wHy} 14H10Y-43	*Df(2R) 14H10Y-43*	16,554	BH758893
P{3′ wHy} 14H10Y-44	*Df(2R) 14H10Y-44*	19,756	BH758894
P{3′ wHy} 14H10Y-45	*Df(2R) 14H10Y-45*	24,694	BH758895
P{3′ wHy} 14H10Y-46	*Df(2R) 14H10Y-46*	25,420	BH758896
P{3′ wHy} 14H10Y-47	*Df(2R) 14H10Y-47*	26,803	BH758897
P{3′ wHy} 14H10Y-48	*Df(2R) 14H10Y-48*	28,859	BH758898
P{3′ wHy} 14H10Y-49	*Df(2R) 14H10Y-49*	34,519	BH758899
P{3′ wHy} 14H10Y-50	*Df(2R) 14H10Y-50*	45,070	BH758900
P{3′ wHy} 14H10Y-51	*Df(2R) 14H10Y-51*	86,539	BH758901
P{3′ wHy} 14H10Y-52	*Df(2R) 14H10Y-52*	101,915	BH758902
P{3′ wHy} 14H10Y-53	*Df(2R) 14H10Y-53*	123,132	BH758903
Distally extending			
P{5′ wHy} 14H10W-10	*CG4996$^{14H10W-10}$*	893	BH758914
P{5′ wHy} 14H10W-11	*CG4996$^{14H10W-11}$*	1,316	BH758915
P{5′ wHy} 14H10W-12	*CG4996$^{14H10W-12}$*	2,315	BH758916
P{5′ wHy} 14H10W-13	*CG4996$^{14H10W-13}$*	2,644	BH758917
P{5′ wHy} 14H10W-14	*CG4996$^{14H10W-14}$*	4,313	BH758918
P{5′ wHy} 14H10W-15	*Df(2R) 14H10W-15*	10,140	BH758919
P{5′ wHy} 14H10W-16	*Df(2R) 14H10W-16*	14,806	BH758920
P{5′ wHy} 14H10W-17	*Df(2R) 14H10W-17*	18,403	BH758921
P{5′ wHy} 14H10W-18	*Df(2R) 14H10W-18*	19,734	BH758922
P{5′ wHy} 14H10W-19	*Df(2R) 14H10W-19*	19,734	BH758923
P{5′ wHy} 14H10W-20	*Df(2R) 14H10W-20*	19,736	BH758924
P{5′ wHy} 14H10W-21	*Df(2R) 14H10W-21*	20,084	BH758925
P{5′ wHy} 14H10W-22	*Df(2R) 14H10W-22*	25,638	BH758926
P{5′ wHy} 14H10W-23	*Df(2R) 14H10W-23*	32,450	BH758927
P{5′ wHy} 14H10W-24	*Df(2R) 14H10W-24*	33,279	BH758928
P{5′ wHy} 14H10W-25	*Df(2R) 14H10W-25*	48,605	BH758929
P{5′ wHy} 14H10W-26	*Df(2R) 14H10W-26*	54,697	BH758930
P{5′ wHy} 14H10W-27	*Df(2R) 14H10W-27*	56,671	BH758931
P{5′ wHy} 14H10W-28	*Df(2R) 14H10W-28*	60,716	BH758932
P{5′ wHy} 14H10W-29	*Df(2R) 14H10W-29*	62,257	BH758933
P{5′ wHy} 14H10W-30	*Df(2R) 14H10W-30*	63,648	BH758934
P{5′ wHy} 14H10W-31	*Df(2R) 14H10W-31*	83,361	BH758935
P{5′ wHy} 14H10W-32	*Df(2R) 14H10W-32*	99,928	BH758936
P{5′ wHy} 14H10W-33	*Df(2R) 14H10W-33*	100,816	BH758937
P{5′ wHy} 14H10W-34	*Df(2R) 14H10W-34*	138,890	BH758938
P{5′ wHy} 14H10W-35	*Df(2R) 14H10W-35*	377,752	BH758939

[a] As per convention, deletions that disrupt only one transcript are indicated as alleles of that transcript, and deletions that remove more than one transcript are indicated as deficiencies (FlyBase, 1999).

about the genes between *eIF3-S8* and *CG6401* can be uncovered because shorter deletions from 14F06 and 14H10 fail to overlap, and longer deletions from 14H10 delete the essential gene *eIF3-S8* (Figure 2). A larger sample size of deletions extending from 14F06 toward 14H10 would increase the chance that deletions that overlap to remove genes between *CG6401* and *eIF3-S8* could be identified.

The most informative analysis was in the region between the 14H10 and 02B10 insertions. As shown in Figure 3, deletions that disrupt *HLH54F* (Georgias *et al.* 1997) are semilethal (<30% of expected, $n > 500$) *in trans* to one another, including deletions that disrupt exons 1 and 2 *in trans* to a deletion that disrupts exon 3 (class C *vs.* classes II and III). However, deletions that extend distally from 14H10 beyond *HLH54F* (class D deletions) are inviable *in trans* to class II and III deletions. Class D deletions are viable *in trans* to the 02B10

TABLE 3

Deletions generated from 02B10

Insertion	Associated deletion[a]	Size of deletion (bp)	GenBank accession no.
Proximally extending			
P{3′wHy}02B10Y-10	*CG6370$^{02B10Y-10}$*	412	BH758872
P{3′wHy}02B10Y-11	*Df(2R)02B10Y-11*	7,330	BH758873
P{3′wHy}02B10Y-12	*Df(2R)02B10Y-12*	18,440	BH758874
P{3′wHy}02B10Y-13	*Df(2R)02B10Y-13*	52,484	BH758875
P{3′wHy}02B10Y-14	*Df(2R)02B10Y-14*	56,058	BH758876
P{3′wHy}02B10Y-15	*Df(2R)02B10Y-15*	70,233	BH758877
Distally extending			
P{5′wHy}02B10W-06	*Df(2R)02B10W-06*	1,700	BH758904
P{5′wHy}02B10W-07	*Df(2R)02B10W-07*	9,294	BH758905
P{5′wHy}02B10W-08	*Df(2R)02B10W-08*	56,075	BH758906
P{5′wHy}02B10W-09	*Df(2R)02B10W-09*	97,891	BH758907

[a] As per convention, deletions that disrupt only one transcript are indicated as alleles of that transcript, and deletions that remove more than one transcript are indicated as deficiencies (FlyBase, 1999).

insertion in *CG6370* and to a short deletion that disrupts *CG6370* (*P{3′wHy}02B10Y-10*; Figure 3). Together these data suggest that *CG6370* is dispensable, but that *BcDNA: GH07485* (*GH07485*; Rubin *et al.* 2000) is required for full viability. Finally, complementation between class B and class II defines a region dispensable for viability, fertility, and gross morphology (Figure 3).

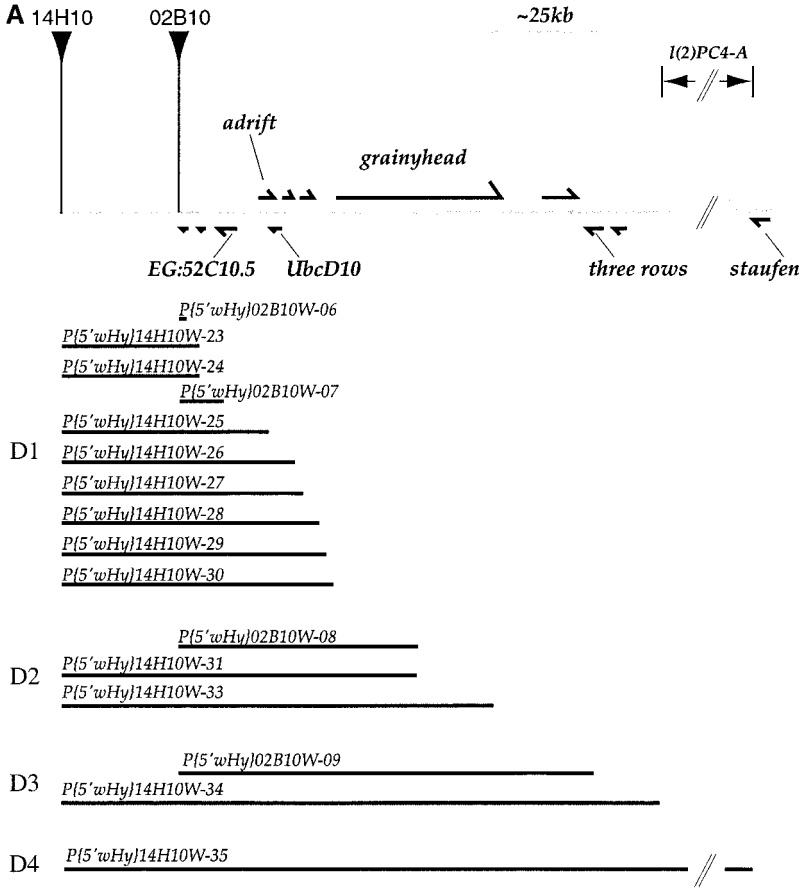
Overlapping deletions define a region required for female fertility: In addition to uncovering essential loci, the analysis shows that a locus is required for female fertility in the region between 14H10 and 02B10 (Figure 3). Females with class B deletions are sterile *in trans* to class III deletions, as are homozygous *P{5′wHy}14H10W-15* females and *trans*-heterozygous *P{5′wHy}14H10W-15/P{5′wHy}14H10W-16* females. These females fail to lay eggs, and their ovaries are smaller than those of wild type (data not shown). The locus is limited proximally by *P{5′wHy}14H10W-14*, the largest class A deletion tested that complements class III deletions for fertility, and distally by *P{3′wHy}02B10Y-12*, the largest class II deletion that complements class B deletions for fertility (Figure 3). The deletions complement a mutation in *haulted*, which maps genetically to the 54-55 region (Schüpbach and Wieschaus 1991).

Mapping anonymous EMS-induced mutations with *P{wHy}* deletions: In addition to helping to uncover knockout phenotypes of genes between 14F06 and 02B10, the deletions in our collection have proven useful for molecular mapping of existing lesions that map genetically to the 54D-55B region. We performed complementation analysis between distally extending deletions from 14H10 and 02B10 and strains from a collection of lethal mutations that were isolated in a screen for EMS-induced mutations that fail to complement *Df(2R)PC4* [55A1; 55F1-2] (FlyBase 1999). A subset of these mutations that map to the proximal end of the deletion in *Df(2R)PC4* were tested [*l(2)PC4-A*, *-B*, *-D*, *-E*, *-F*, *-G*, and *-M*].

The *l(2)PC4-B*, *-E*, *-F*, *-G*, and *-M* mutations that were tested complement the largest deletion in the region, *P{5′wHy}14H10W-35*, suggesting that the lesions are distal to *stau* in 55B. Both *l(2)PC4-D^{202}* and *l(2)PC4-A^{139}* fail to complement a subset of the *P{wHy}*-generated deletions (Table 4). The *l(2)PC4-A* locus is within an ~239-kb region, limited proximally by *P{5′wHy} 14H10W-34*, the longest deletion that complements *l(2)PC4-A^{139}*, and distally by *P{5′wHy}14H10W-35*, which fails to complement *l(2)PC4-A^{139}* (Table 4; Figure 4). At the level of known and predicted transcripts, this region extends from *Black cells* to *stau* and includes many candidate transcription units for the *l(2)PC4-A* locus. The *l(2)PC4-D* locus maps to a 24-kb region limited proximally by *P{5′wHy}14H10W-25*, which complements *l(2)PC4-D^{202}*, and distally by *P{5′wHy}14H10W-27*, which fails to complement *l(2)PC4-D^{202}* (Table 4; Figure 5). Three transcripts map to this interval: *UbcD10*, *EG:52C10.1*, and *EG:52C10.2* (Adams *et al.* 2000; Aguilera *et al.* 2000). These data suggest that *l(2)PC4-D^{202}* affects *UbcD10*, *EG52C10.1*, or *EG:52C10.2*.

DISCUSSION

***P{wHy}*-generated genomic disruptions behave as deletions:** We mobilized the *hobo* transposon in *P{wHy}* insertions in 54E/F to generate genomic disruptions and screened for events that remove flanking genomic DNA proximally or distally from the starting insertion. By mapping the new *hobo* insertion point, we were able to identify the breakpoint ends of putative deletions. Molecular evidence and results of complementation analyses with *eIF3-S8^{14F06}* and previously isolated mutations of *grh* and *thr* (Dynlacht *et al.* 1989; D'Andrea



B

	grh	*thr*	*stau*
D1	+	+	+
D2	-	+	+
D3	-	-	+
D4	-	-	+

FIGURE 4.—Complementation between deletions in the *grainyhead* region and *grainyhead* and *three rows*. (A) Map of the *grainyhead* region. Transcripts distal of the 02B10 insertion are indicated with arrows. Solid lines, the region removed in *P{wHy}*-generated deletions extending distal of 02B10 (see Figure 3 for transcripts and deletions between 14H10 and 02B10). An arrowhead on a solid line indicates that the deletion extends in the direction of the arrow beyond the region. (B) Complementation analysis between the deletions shown in A and *grainyhead*, *three rows*, and *staufen*. +, viable and fertile; −, inviable.

et al. 1993) are consistent with the idea that the majority of the genomic disruptions in the region are deletions. Thus, in a relatively small-scale screen (~1000 crosses), deletions that extend >0.5 Mb can be identified and recovered.

Overlapping *P{wHy}*-generated deletions reveal mutant phenotypes: A subset of the genomic disruptions from 14F06, 14H10, and 02B10 are predicted to remove overlapping regions of DNA (Figure 2). The 14H10 and 02B10 insertions disrupt *CG4996* and *CG6370*, respectively, but are fully viable and fertile. Deletion of *CG4996* or *CG6370* also has no discernible effect on viability, fertility, or gross morphology (Figure 3). Complementation of class B with class II deletions suggests that the following genes are similarly dispensable: *CG10931* and *CG5002*, which are completely removed in class B/class II animals, and *CG6385*, which is disrupted in class B/class II animals (Figure 3).

Clearly, these five genes do not provide nonredundant information required for overall survival to adulthood, gross morphology, or fertility. Obviously, tests for more subtle phenotypes need to be applied. Moreover, there is great value in knowing that deletion of a specific gene has no obvious visible lethal or sterile phenotype.

Combining the complementation data with the molecular map also reveals that the regions of DNA required for viability correlate to the *eIF3-S8*, *CG6401*, and *GH07485* genes (Figures 2 and 3; ADAMS *et al.* 2000; LASKO 2000). The *CG6401* gene encodes an *N*-acetyl glucosaminyl phosphatidylinositol synthesis protein similar to human PIG-A (MIYATA *et al.* 1993). The *GH07485* gene encodes a member of the acyl Co-A oxidase family of proteins with the greatest similarity to the human peroxisomal acyl Co-A oxidase (FOURNIER *et al.* 1994).

A subset of deletions define a region of DNA required for female fertility. The region is limited proximally by the *P{5′wHy}14H10W-14* breakpoint and distally by the *P{3′wHy}02B10Y-12* breakpoint. Therefore, it is likely to

TABLE 4

Complementation between deletions and *l(2)PC4-D* and *l(2)PC4-A* mutant alleles

	l(2)PC4-D^{202}	*l(2)PC4-A*^{139}
P{5′wHy}14H10W-23	+	+
P{5′wHy}14H10W-24	+	+
P{5′wHy}02B10W-07	+	+
P{5′wHy}14H10W-25	+	+
P{5′wHy}14H10W-26	ND[a]	+
P{5′wHy}14H10W-27	−	+
P{5′wHy}14H10W-28	−	+
P{5′wHy}14H10W-29	−	+
P{5′wHy}14H10W-30	−	+
All D2 class deletions[b]	−	+
All D3 class deletions[b]	−	+
P{5′wHy}14H10W-35	−	−

+, viable and fertile; −, inviable.

[a] ND, not determined; the *P{5′wHy}14H10W-26* stock was lost from our collection.

[b] See Figure 3.

result from the disruption of *CG12298* and/or *CG14487* (Figure 3). The *CG12298* gene encodes a kinesin-like protein. Conceptual translation of *CG14487* reveals no significant similarity to proteins outside of Drosophila, but does show significant similarity to a family of predicted proteins in the fly genome with the closest similarity to the CG12960, CG12961, CG17379, CG17381, CG13582, CG14584, and CG12525 proteins.

In the absence of genomic disruptions that separate these candidate genes, further experiments will be necessary to determine which gene(s) in the region are required for female fertility. Clearly, the set of ~70 deletions described is not sufficient to achieve the goal of removing each transcript individually as was accomplished for *HLH-54F*. This appears to reflect the fact

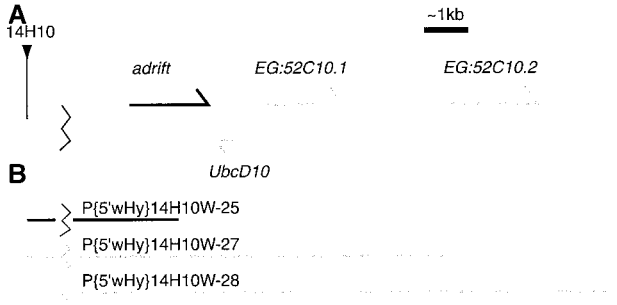


FIGURE 5.—The *l(2)PC4-D* mutation maps to the *Ubc10*, *EG:52C10.1*, and *EG52C10.2* regions. (A) Map of the ~12-kb *adrift* to *EG:52C10.2* region (exon/intron boundaries not shown). Solid lines, the *adrift* gene; shaded lines, candidate transcription units for the *l(2)PC4-D* locus. (B) A subset of the deletions tested for complementation with *l(2)PC4-D*^{202} (see Table 4). Solid lines, the longest deletion that complements *l(2)PC4-D*^{202}; shaded lines, the shortest deletions that fail to complement the mutation.

that our initial screen was fairly modest (~1000 chromosomes), rather than reflecting a general limitation of the *P{wHy}* approach. HUET *et al.* (2002) suggest that ~100 deletions from a single starting insertion would be needed to resolve every transcript in a 50-kb region. Thus, a larger-scale screen would likely provide useful reagents for targeting individual transcripts and enable us to limit the region required for fertility to a single transcription unit.

The *P{wHy}* method complements other methods for gene disruption: We have shown that *P{wHy}*-generated deletions from adjacent insertions can be used to remove specific genes. The advantages of this technique include: (1) transposon insertions into intergenic regions are not disadvantageous; (2) a given phenotype can be precisely mapped to a small interval on the genomic DNA independently of gene annotation in the region; (3) conversely, even in the absence of a phenotype, one can be confident that a gene or genes have been deleted; and (4) data for the 54D-55B region suggest that the method provides a relatively efficient approach for determining the mutant phenotypes of all the genes in a specific region.

Like *P*-element mobilization or PMR (ROBERTSON *et al.* 1988; PRESTON *et al.* 1996), the *P{wHy}* method relies on having an insertion (or in this case, at least two adjacent insertions) in the region of interest. However, the "reach" of the *P{wHy}* technique is fairly large, as deletions of up to a few hundred kilobase pairs can be isolated. Moreover, the presence of *hobo* immediately adjacent to the genomic disruptions enables precise molecular mapping of deletion endpoints. We anticipate that once a large collection of molecularly mapped insertions is available, many regions of the genome can be surveyed using the method presented here. For regions in which the adjacent insertions are farther apart (*i.e.*, farther than ~50 kb apart), larger screens for genomic disruptions will be necessary, as longer disruptions are less common than shorter ones (this work; HUET *et al.* 2002).

Combining *P{wHy}* with other approaches: The *P{wHy}* method seems particularly well suited for use in conjunction with EMS screens for mutations that fail to complement a deletion of the region of interest. In this case, we benefited from an EMS screen having been performed with *Df(2R)PC4* (FLYBASE 1999). The lethal loci identified in the screen had been mapped with existing deficiencies to within a few lettered units on the cytogenetic map. Complementation analysis with *P{wHy}*-generated deletions limits the regions in which the *l(2)PC4-A* and *l(2)PC4-D* loci reside to 239 and 24 kb, respectively (Table 4; Figure 4).

X- and gamma-ray-induced deletions commonly used for EMS screens can often have >100 kb of genomic DNA deleted. This is beyond the ~50- to 60-kb region well covered in a *P{wHy}* screen for deletions from a single starting insertion (HUET *et al.* 2002). Thus, it may

be necessary to start with several *P{wHy}* insertions in the region of interest to map all of the EMS alleles in a region. Alternatively, the largest of a set of *P{wHy}* deletions can be used in a screen for noncomplementation, and smaller *P{wHy}* deletions can be used to molecularly map the resultant noncomplementing lesions. The availability of a set of starting insertions, then, will be one limiting factor in using the *P{wHy}* approach to map anonymous mutations in a given region.

The advantages of combining overlapping *P{wHy}* deletion analysis with an EMS screen are threefold. First, *P{wHy}*-generated deletions can be used to determine loss-of-function phenotypes (or lack thereof), assisting a focused effort in screening for EMS-induced mutations. Second, *P{wHy}* deletions can be used to map EMS-generated mutations to particular regions of the DNA, as shown here for *l(2)PC4-A* and *l(2)PC4-D*. Third, the EMS alleles can then provide genetic reagents for analyses such as mosaic analysis that are not feasible with the overlapping *P{wHy}* deletion approach. Thus, both on its own and in conjunction with other approaches, the *P{wHy}* strategy for determining complete loss-of-function phenotypes with overlapping deletions can contribute to our understanding of gene function in Drosophila and other organisms.

The authors thank Adam Casterano, Phillip Chan, Madeline Crosby, Francois Huet, Yevgenya Kraytsberg, Todd Martin, Sara McKinley, Jaitra Murthy, and Kyl Myrick for technical support and many helpful discussions. We thank Kathy Matthews at the National Drosophila Stock Center and Christos Samakovlis at Umea University for providing us with mutant strains used for this study. Lastly, thanks go to Ines Alvarez-Garcia and Madeline Crosby for critical review of the manuscript. This work was supported by grant no. R37 GM28669 from the National Institutes of Health to W.M.G.

LITERATURE CITED

Adams, M. D., S. E. Celniker, R. A. Holt, C. A. Evans, J. D. Gocayne *et al.*, 2000 The genome sequence of *Drosophila melanogaster*. Science **287:** 2185–2195.

Aguilera, M., M. Oliveros, M. Martinez-Padron, J. A. Barbas and A. Ferrus, 2000 *Ariadne-1*: A vital Drosophila gene is required in development and defines a new conserved family of ring-finger proteins. Genetics **155:** 1231–1244.

Ashburner, M., S. Misra, J. Roote, S. Lewis, R. Blazej *et al.*, 1999 An exploration of the sequence of a 2.9-Mb region of the genome of *Drosophila melanogaster*: the *Adh* region. Genetics **153:** 179–219.

Blackman, R. K., R. Grimaila, M. M. D. Koehler and W. M. Gelbart, 1987 Mobilization of *hobo* elements residing within the *decapentaplegic* gene complex: suggestion of a new hybrid dysgenesis system in Drosophila melanogaster. Cell **49:** 497–505.

Calvi, B. R., 1993 The hobo transposable element in Drosophila: evolution, regulation, and use as a genetic tool. Ph.D. Thesis, Harvard University, Cambridge, MA.

Cooley, L., R. Kelley and A. Spradling, 1988 Insertional mutagenesis of the *Drosophila* genome with single P elements. Science **239:** 1121–1128.

Crimaudo, C., M. Hortsch, H. Gausepohl and D. I. Meyer, 1987 Human ribophorins I and II: the primary structure and membrane topology of two highly conserved rough endoplasmic reticulum-specific glycoproteins. EMBO J. **6:** 75–82.

D'Andrea, R. J., R. Stratmann, C. F. Lehner, U. P. John and R. Saint, 1993 The *three rows* gene of *Drosophila melanogaster* encodes a novel protein that is required for chromosome disjunction during mitosis. Mol. Biol. Cell **4:** 1161–1174.

Duncan, I. M., 1982 Polycomblike: a gene that appears to be required for the normal expression of the Bithorax and Antennapedia gene complexes of *Drosophila melanogaster*. Genetics **102:** 49–70.

Dynlacht, B. D., L. D. Attardi, A. Admon, M. Freeman and R. Tjian, 1989 Functional analysis of NTF-1, a developmentally regulated Drosophila transcription factor that binds neuronal cis elements. Genes Dev. **3:** 1677–1688.

Eggleston, W. B., D. M. Johnson-Schlitz and W. R. Engels, 1988 P-M hybrid disgenesis does not mobilize other transposable element families in *D. melanogaster*. Nature **331:** 368–370.

Englund, C., A. E. Uv, R. Cantera, L. D. Mathies, M. A. Krasnow *et al.*, 1999 *adrift*, a novel bnl-induced Drosophila gene, required for tracheal pathfinding into the CNS. Development **126:** 1505–1514.

FlyBase, 1999 The FlyBase database of the Drosophila genome projects and community literature. Nucleic Acids Res. **27:** 85–88 (http://flybase.bio.indiana.edu/).

Fournier, B., J. M. Saudubray, B. Benichou, S. Lyonnet, A. Munnich *et al.*, 1994 Large deletion of the peroxisomal acyl-CoA oxidase gene in pseudoneonatal adrenoleukodystrophy. J. Clin. Invest. **94:** 526–531.

Georgias, C., M. Wasser and U. Hinz, 1997 A basic-helix-loop-helix protein expressed in precursors of Drosophila longitudinal visceral muscles. Mech. Dev. **69:** 115–124.

Huet, F., J. T. Lu, K. V. Myrick, L. R. Baugh, M. A. Crosby *et al.*, 2002 A deletion-generator compound element allows deletion saturation analysis for genomewide phenotypic annotation. Proc. Natl. Acad. Sci. USA **99:** 9948–9953.

Kania, A., A. Salzberg, M. Bhat, D. D'Evelyn, Y. He *et al.*, 1995 *P*-element mutations affecting embryonic peripheral nervous system development in *Drosophila melanogaster*. Genetics **139:** 1663–1678.

Kennerdell, J. R., and R. W. Carthew, 1998 Use of dsRNA-mediated genetic interference to demonstrate that *frizzled* and *frizzled2* act in the Wingless pathway. Cell **95:** 1017–1026.

Lasko, P., 2000 The *Drosophila melanogaster* genome: translation factors and RNA binding proteins. J. Cell Biol. **150:** F51–F56.

Lim, J. K., and M. J. Simmons, 1994 Gross chromosome rearrangements mediated by transposable elements in *Drosophila melanogaster*. Bioessays **16:** 269–275.

Miyata, T., J. Takeda, Y. Iida, N. Yamada, N. Inoue *et al.*, 1993 The cloning of PIG-A, a component in the early step of GPI-anchor biosynthesis. Science **259:** 1318–1320.

Myrick, K. V., and W. M. Gelbart, 2002 Universal fast walking for direct and versatile method determination of flanking sequence. Gene **284:** 125–131.

Ochman, H., A. S. Gerber and D. L. Hartl, 1988 Genetic applications of an inverse polymerase chain reaction. Genetics **120:** 621–623.

Preston, C. R., J. A. Sved and W. R. Engels, 1996 Flanking duplications and deletions associated with *P*-induced male recombination in Drosophila. Genetics **144:** 1623–1638.

Robertson, H. M., C. R. Preston, R. W. Phillis, D. M. Johnson-Schlitz, W. K. Benz *et al.*, 1988 A stable genomic source of *P*-element transposase in *Drosophila melanogaster*. Genetics **118:** 461–470.

Rong, Y. S., and K. G. Golic, 2000 Gene targeting by homologous recombination in *Drosophila*. Science **288:** 2013–2018.

Rong, Y. S., and K. G. Golic, 2001 A targeted gene knockout in Drosophila. Genetics **157:** 1307–1312.

Rørth, P., K. Szabo, A. Bailey, T. Laverty, J. Rehm *et al.*, 1998 Systematic gain-of-function genetics in Drosophila. Development **125:** 1049–1057.

Rubin, G. M., L. Hong, P. Brokstein, M. Evans-Holm, E. Frise *et al.*, 2000 A Drosophila complementary DNA resource. Science **287:** 2222–2224.

Schüpbach, T., and E. Wieschaus, 1991 Female sterile mutations on the second chromosome of *Drosophila melanogaster*. II. Mutations blocking oogenesis or altering egg morphology. Genetics **129:** 1119–1136.

Sheen, F., J. K. Lim and M. J. Simmons, 1993 Genetic instability in *Drosophila melanogaster* mediated by *hobo* transposable elements. Genetics **133:** 315–334.

Spradling, A. C., D. Stern, A. Beaton, E. J. Rhem, T. Laverty *et al.*, 1999 The Berkeley Drosophila Genome Project gene disruption project: single *P*-element insertions mutating 25% of vital Drosophila genes. Genetics **153:** 135–177.

St. Johnston, R. D., D. Beuchle and C. Nusslein-Volhard, 1991 *staufen*, a gene required to localize maternal RNAs in the Drosophila egg. Cell **66:** 51–63.

Triglia, T., M. G. Peterson and D. J. Kemp, 1988 A procedure for in vitro amplification of DNA segments that lie outside the boundaries of known sequences. Nucleic Acids Res. **16:** 8186.

Volpe, A. M., H. Horowitz, C. M. Grafer, S. M. Jackson and C. A. Berg, 2001 Drosophila *rhino* encodes a female-specific chromodomain protein that affects chromosome structure and egg polarity. Genetics **159:** 1117–1134.

Communicating editor: T. Schüpbach