stress sensitive B Encodes an Adenine Nucleotide Translocase in *Drosophila melanogaster*

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Manuscript received March 31, 1999
Accepted for publication July 2, 1999

ABSTRACT

Adenine nucleotide translocases (ANT) are required for the exchange of ADP and ATP across the inner mitochondrial membrane. They are essential for life, and most eukaryotes have at least two different *Ant* genes. Only one gene had been described from Drosophila, and this had not been characterized genetically. We show that mutations in this gene correspond to the previously described loci, *sesB* and *l(1)9Ed*. Immediately adjacent to this gene is another encoding a second ANT protein, which has 78% identity to that encoded by *sesB/l(1)9Ed*. These two genes are transcribed from a common promoter, and their mRNAs are produced by differential splicing. Hutter and Karch suggested that the *sesB* ANT gene corresponded to *Hmr*, a gene identified by an allele that rescues otherwise inviable interspecific hybrids between *Drosophila melanogaster* and its sibling species. This hypothesis is not supported by our study of the ANT genes of *D. melanogaster*.

HYBRIDS between *Drosophila melanogaster* and its sibling species, *D. simulans*, *D. mauritiana*, and *D. sechellia*, are either sterile or inviable. Male hybrids from crosses between *D. melanogaster* females and sibling species males die as late larvae or early pupae, while female hybrids from the reciprocal crosses die as embryos. Mutations or strains of *D. melanogaster* and *D. simulans* that rescue either the embryonic or the larval/pupal lethality of interspecific hybrids have been characterized. One of these is *Hybrid male rescue* (*Hmr*), a mutation of *D. melanogaster* that rescues to viability otherwise inviable male hybrids from the cross of *D. melanogaster* females to sibling species males (Hutter and Ashburner 1987; Hutter *et al.* 1990; Sawamura *et al.* 1993; Hutter 1997). *Hmr* was mapped to 9D1-9E4 on the polytene chromosomes (Hutter *et al.* 1990), close to the distal breakpoint of *In(1)AB*, an inversion that also rescues interspecific hybrid inviability (Hutter *et al.* 1990). Hutter and Karch (1994) mapped this *In(1)AB* breakpoint just distal to a gene encoding a protein very similar to adenine nucleotide translocases (ANT, ADP/ATP translocase) of other organisms, and they considered this gene to be a candidate for *Hmr*/*In(1)AB*. We have restudied the relationship between the adenine nucleotide translocase gene, *Hmr*, and *In(1)AB*. We cannot confirm any effect of either *Hmr* or *In(1)AB* on ANT. Moreover, we show that mutations in two previously characterized genes, *sesB* and *l(1)9Ed*, map to the adenine nucleotide translocase, and neither these nor transformants carrying extra copies of the wild-type *sesB* gene have any effect on the viability of interspecific hybrids.

sesB is one of a series of stress-sensitive mutants (*sesA*–*sesH*) characterized by Homyk (Homyk and Sheppard 1977; Homyk *et al.* 1980); other bang-sensitive mutants had been recovered previously in Benzer's laboratory (Benzer 1971). Some of these have already been cloned (Royden *et al.* 1987; Pavlidis *et al.* 1994; Schubiger *et al.* 1994; Andrews *et al.* 1996), but the mechanisms underlying their phenotypes are largely unknown (Pavlidis and Tanouye 1995). The cloning of *sesB* demonstrates that a bang-sensitive phenotype can result from a defect in energy metabolism. The Drosophila adenine nucleotide translocase genes are duplicated tandemly. They are 72% identical in nucleotide sequence and 78% identical in amino acid sequence. Remarkably, these two proteins are produced by alternative splicing of a dicistronic primary transcript; these mRNAs share a 5′ untranslated exon. It is clear that the adenine nucleotide translocase gene is not *Hmr*, which remains to be clarified molecularly, but is *sesB*.

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MATERIALS AND METHODS

Stocks and chromosomes: $sesB^{1}$ was obtained from T. Homyk and *l(1)DC701* ($sesB^{9Ed\text{-}6}$) from N. Perrimon. *l(1)A17* ($sesB^{9Ed\text{-}1}$), *l(1)G16* ($sesB^{9Ed\text{-}2}$), *l(1)H22* ($sesB^{9Ed\text{-}3}$), *l(1)M27* ($sesB^{9Ed\text{-}4}$), *l(1)Q10* ($sesB^{9Ed\text{-}5}$), *l(1)H6* (ras^{l17}), *l(1)J5* (ras^{l19}), *l(1)E20* ($l(1)9Ec^{2}$), and

l(1)G4 (*l(1)9Ec*^{3}), as well as the chromosome aberrations *Df(1)ras59* and *Df(1)RJ7*, were from the Edmonton collection. Both the *sesB*$^{9Ed\text{-}1}$ and *sesB*$^{9Ed\text{-}2}$ chromosomes carried extraneous *X*-linked lethal mutations; that from *sesB*$^{9Ed\text{-}2}$ was removed by recombination. Zhimulev *et al.* (1987) showed that *l(1)S12* (Lefevre 1971) is an allele of *sesB*. The only allele of "*l(1)9Ee*" available to us, *Q21*, was lethal with all *sesB* alleles; we do not know whether this is because of a stock mix-up at some stage or some other reason, but we note that Janca *et al.* (1986) recovered only one allele of *l(1)9Ee* and were unable to separate it from alleles of *l(1)9Ed* by deletion mapping. The chromosome aberrations *In(1)AB*, *In(1)AC2*, *Df(1)v64f*, *Df(1)HC133*, *Dp(1;2)v*$^{+75d}$, *Dp(1;3)v*$^{+74c}$, and *Dp(1;2)v*$^{+63i}$ were obtained from the Bloomington Stock Center or were in the Cambridge collection. All lethal alleles and aberrations are described in FlyBase (1999). The synthetic duplication *Dp(1;1)AB*^{L}*AC2*^{R} (1A-9E|13E-9D|13B-20F) and its reciprocal deletion, *Df(1)AC2*^{L}*AB*^{R} (1A-9D|13B-9E|13E-20F), were made as recombinants between *In(1)AC2* and *In(1)AB*, and were verified by polytene chromosome cytology. The *ras*$^{-}$ deletions *ras-TM3* (new order: 1-9A3.5|52F3.9-21; 60-52F3.9|13D3.4-9E6|13E-20), *ras-TM5* (new order: 1-9E2.3|13B3.4-9E7.8|13E1.2-20), and *ras-TM6* (new order: 1-9C1|13C3-9E7.8|13E1.2-20) were induced by X rays on *In(1)AB* (D. Coulson, personal communication). The deletion *Df(1)B13* (Df(1)9D1-2;9E4-10) was identified by D. Coulson (personal communication) in the *l(1)B13* stock of D. Nash.

A new *sesB* allele, *sesB*^{6}, was isolated from a *P*-element screen for lethal mutations on *In(1)AB* in which *P{ry*$^{+t7.2}$*=HBDelta-23}9E* (from the Bloomington Stock Center) was mobilized; the chromosomes were screened for lethals against *Df(1)HC133*.

Crosses: All crosses were done at 25°, unless stated otherwise, and flies were reared on standard yeast-cornmeal medium. Care was taken to ensure that all eclosed flies were scored when scoring lethal complementation tests.

Cytology: Polytene chromosome cytology was performed on propionic carmine-orcein squash preparations of salivary gland chromosomes, which were interpreted using the revised maps of Bridges (see Lefevre 1976).

Bang-sensitivity assay: Bang sensitivity was assayed essentially according to Ganetzky and Wu (1982). Individual flies were transferred to an empty vial and immediately vortexed for 10 sec using a Vortex-Genie 2 (Scientific Instruments Ltd.) at its highest setting. The time for each fly to right itself after vortexing was recorded.

Clones: Cosmid 152F6 was obtained from the European *Drosophila* Mapping Consortium (Madueño *et al.* 1995). A *sesB* cDNA (HE50) and other clones were the gift of P. Hutter.

DNA sequencing: The sequence of the wild-type *ANT* genes was determined from cosmid 152F6. Mutant alleles were sequenced from adult genomic DNA PCR products amplified with Vent DNA polymerase (New England Biolabs, Beverly, MA). For the sequencing of *sesB*, PCR reactions were done with primers S5 and S3, corresponding to the nucleotides 5168–5187 and 6270–6291, respectively, of the sequence EMBL:Y10618 (all sequence coordinates are with respect to this record). The PCR products were cloned into pBluescript by PCR-introduced *Hin*dIII and *Xho*I sites in S5 and S3, respectively. Commercial forward and reverse primers for pBluescript and a synthesized internal primer, SM (nucleotides 5464–5484), were used for sequencing the full-length coding region of *sesB*.

To sequence the gene immediately distal to *sesB* (*Ant2*), two primers flanking the coding region were designed, SU (nucleotides 7291–7308) and SD (nucleotides 8646–8664). PCR products were cloned in pBluescript by primer-introduced *Eco*RI and *Xba*I sites. Two internal primers, S (nucleotides 7797–7817) and 2.8A (nucleotides 7823–7842), were used to obtain the full sequence of the 1.4-kb *Eco*RI-*Xba*I fragment.

To sequence the *P*-element insertion associated with *sesB*^{6}, we used a protocol and primers designed for the *PZ* vector (Rehm 1998). Total DNA was digested with *Cfo*I, ligated, amplified with the primers Plac4/Plac1 and sequenced with primer SP1 (for 5′-end rescue), and amplified with primers Pry1/Pry4 and sequenced with primer Spry3 (for 3′-end rescue).

The breakpoints of two chromosome aberrations were sequenced by inverse PCR. To sequence the distal breakpoint of *In(1)AB*, DNA from a homozygous stock was digested with *Pst*I, ligated, and amplified with primers corresponding to (1) the reverse complement of Y10681:7514–7533 (AB1) and (2) bases 8830–8849 of this sequence (AB2). The 0.5-kb PCR fragment was purified and sequenced. DNA was prepared from *Df(1)v64f/FM7* females, digested with *Eco*RI, ligated, and amplified with primer AB1 and one corresponding to bases 10250–10270 of Y10168. The expected 0.8-kb band was gel purified and sequenced (amplification from the balancer chromosome gave the expected 1.7-kb product).

Sequencing was performed by using an ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer, Norwalk, CT) and an ABI 377 sequencer (Applied Biosystems, Foster City, CA).

Sequence analysis: General sequence analyses were done with the GCG package running on a Silicon Graphics workstation on the University of Cambridge Molecular Biology server. Sequence alignments were done using CLUSTAL W (version 1.74) from the EBI server (http://www2.ebi.ac.uk/clustalw/), and gene trees were built using the PHYLIP package (version 3.5c; Felsenstein 1988) on the Cambridge Molecular Biology server.

Rapid amplification of cDNA ends: Drosophila cDNA libraries were the gift of N. Brown. Adenine nucleotide translocase cDNA ends were amplified with one primer homologous to the vector pNB40 (GGTGACACTATAGAATACAAG for 5′-end amplification, TTAATGCAGCTGGCTTATCG for 3′-end amplification) and another homologous to an internal sequence of the adenine nucleotide translocase cDNA. For the amplification of the 5′ ends of *sesB*, a vector primer and primer Rb (complementary to the nucleotides 5442–5460) were used. The 3′ ends of *sesB* were amplified with a vector primer and primer Ra (nucleotides 5191–5210), introducing a *Bam*HI site just before the initiation codon ATG. For the amplification of the 3′ ends of *Ant2*, primer 2.8A (described above) was used. For the amplification of the 5′ end of *Ant2*, primers corresponding to nucleotides 7393–7413 and 7427–7444 were used.

Germline transformation: The genomic transformation construct was made as follows: The 10.3-kb *Eco*RI-*Hin*dIII fragment (see Figure 2) was cloned into pP{CaSper-hs}. This plasmid, *pP{Ant2*$^{+}$ *sesB*$^{+}$*10.3}* (at 1 μg/μl), was coinjected with plasmid pHSΔ2-3 (at 0.5 μg/μl) in Spradling buffer into embryos from a *y w* strain.

General molecular methods: General methods for handling nucleic acids were according to protocols in Ashburner (1989) and Sambrook *et al.* (1989). Southern and Northern filters were washed at low stringency (2× SSC, 0.5% SDS at 65°).

Sequence data: The genomic sequence is deposited in the EMBL Nucleic Acid Sequence Data Library under accession no. Y10618. The sequences across the breakpoints of *Df(1)v64f* and *In(1)AB* are deposited as EMBL:AJ236836 and EMBL:AJ236835, respectively. We also used the two existing cDNA sequences of *sesB*, those of Louvi and Tsitilou (1992, EMBL:S43651) and Hutter and Karch (1994, EMBL:S71762).

The expressed sequence tag (EST) sequence data of the Berkeley *Drosophila* Genome Project (BDGP) have been very useful. These partial cDNA clone sequences have been computationally clustered into "clots" on the basis of their sequence overlaps. These clots are available from http://www.fruitfly.org/EST/. The sequence of any one clot is not necessarily stable; it may change as new EST sequences are analyzed. Moreover, at the moment, there is not any stable way to refer to a particular clot sequence. We refer to them by the name of one of their constituent clones, and this will ensure that the latest version of any particular clot can be recovered by this clone name. We have also archived the clot sequences recovered from the Berkeley server for analysis on April 11, 1998, and these are available on request from M.A. For the ANT genes, there were three EST clots on this date. These are consensus sequence 1 (represented by clone GM13259.5′, EMBL:AA803475), a 700-bp sequence of only one clone; consensus sequence 2 (represented by GM14781.5′, EMBL: AA821173), a 550-bp consensus of four clones; and consensus sequence 3 (represented by LD14425.5′, EMBL:AA439770), a 1500-bp consensus of 59 different EST clones.

RESULTS

This study began with the working hypothesis that the two mutations known to rescue the lethality of interspecific hybrids between *D. melanogaster* and its sibling species, *Hmr* and *In(1)AB*, were allelic (see Hutter *et al.* 1990) and that the corresponding gene was that encoding an adenine nucleotide translocase (Hutter and Karch 1994). *Hmr* had been mapped genetically to *1*-31.84, approximately corresponding to region 9D1-9E4 on the polytene chromosome map (Hutter *et al.* 1990). The distal breakpoint of *In(1)AB* maps to 9E7-8. A gene encoding an adenine nucleotide translocase had been characterized from *D. melanogaster* by Louvi and Tsitilou (1992) and mapped to 9E by *in situ* hybridization to polytene chromosomes. Hutter and Karch (1994) showed that the distal breakpoint of *In(1)AB* was just distal (*i.e.*, 3′) to this gene.

***Hmr* maps distal to the distal *In(1)AB* breakpoint:** Hutter and Ashburner (1987) originally mapped *Hmr* to *1*-31.84, relative to *lz* and *v*. A small-scale experiment recovered *Hmr-ras* recombinants (Hutter *et al.* 1990), although none were recovered in a second experiment reported by Hutter and Karch (1994). To resolve the issue, we scored 2687 rescued male hybrids from the cross of *D. melanogaster y Hmr v*/*ras dy* females to *D. mauritiana* males (at 18°). Six phenotypically *ras* males (crossovers in the *Hmr-ras* interval) and 14 males that were neither *ras* nor *v* (crossovers in the *ras-v* interval) were recovered, placing *Hmr* at *1*-32.0, unambiguously distal to *ras* (at *1*-32.35 in 9E4) and, therefore, distal to the distal *In(1)AB* breakpoint (at 9E7-8).

***sesB* maps proximal to the distal breakpoint of *In(1)AB* and corresponds to *l(1)9Ed*:** There have been several screens for lethal mutations mapping to region 9-10 of the *X* chromosome in addition to several mutations recovered by Lefevre (Lefevre 1981; Lefevre and Watkins 1986) from more general screens for *X*-linked lethals. Janca *et al.* (1986) defined eight lethal complementation groups within bands 9E1-9F13. We have obtained all extant mutations and aberrations in the 9D-9F chromosome region and have mapped these by complementation (Figure 1). For our present purposes, the critical data are (i) that the stress-sensitive allele, $sesB^{1}$ (Homyk and Sheppard 1977) is allelic to mutations that define *l(1)9Ed* of Lindsley and Zimm (1992), which is group B of Janca *et al.* (1986; Table 1) and (ii) that this locus (which we call *sesB*) is proximal to the distal breakpoint of *In(1)AB*. The last fact is most convincingly shown by the complementation of *sesB* alleles by the synthetic deletion $Df(1)AC2^{L}AB^{R}$ (Table 1) and by the fact that both ras^{-} deletions that we have induced with X rays on *In(1)AB* are $sesB^{+}$ (Figure 1).

Cytologically, the distal breakpoint of *In(1)AB* maps to 9E7-8. We place *sesB* at the same cytogenetic position, not only from the molecular mapping of this inversion breakpoint (Hutter and Karch 1994; see below), but also because *sesB* is included in both *Df(1)v64f* (Df(1)9E7-8;10A1-2) and *Df(1)ras217* (Df(1)9A;9E7-8), (data of Janca *et al.* 1986).

The *sesB* phenotypes: A total of 13 mutant alleles of *sesB* have been described (data from FlyBase), although we have been able to find only 7 now remaining in stock. Of the original 13, 11 were recovered as *X*-linked lethal mutations and 2 as viables with a stress-sensitive phenotype (Homyk and Sheppard 1977; Homyk *et al.* 1980). The original behavioral mutation $sesB^{1}$ was recovered on the basis of its reduced flight ability (Sheppard 1974) and was then shown to be stress sensitive, *i.e.*, reversibly paralyzed when subjected to a mechanical shock (Homyk 1977; Homyk and Sheppard 1977). Even in the absence of any external shock, $sesB^{1}$ flies may knock themselves out if they fall down (Homyk 1977). After "vortexing" for 10 sec, 1- to 2-day-old $sesB^{1}$ adult males are paralyzed for 38 ± 51 sec ($n = 29$); for 6-day-old males, paralysis lasts >100 sec; and 6-day-old homozygous adult females are paralyzed for 71 ± 53 sec ($n = 20$). After recovery from paralysis, the flies are not hyperactive, as is seen in some "bang-sensitive" mutations (Pavlidis and Tanouye 1995), but they are refractory to further paralysis for a period of 5–10 min, as observed in other bang-sensitive mutations (Ganetzky and Wu 1982). From our observations on $sesB^{1}$, the flies are generally rather hypoactive and have delayed development. $sesB^{9Ed-4}$ flies are very hypoactive and, as a result, are difficult to assay for stress sensitivity by observing their recovery from paralysis. However, we think that this allele is stress sensitive, since females heterozygous for $sesB^{9Ed-4}$ and *Df(1)HC133* are extremely sensitive to shock and are paralyzed for >100 sec after vortexing. Neither of the two other male-viable alleles ($sesB^{9Ed-2}$ and $sesB^{9Ed-5}$) is stress sensitive.

We have examined eight *sesB* alleles. Three of these, $sesB^{9Ed-2}$ (*G16*), $sesB^{9Ed-4}$ (*M27*), and $sesB^{9Ed-5}$ (*Q10*), were described by Nash and Janca (1983) as "haplo-specific,"

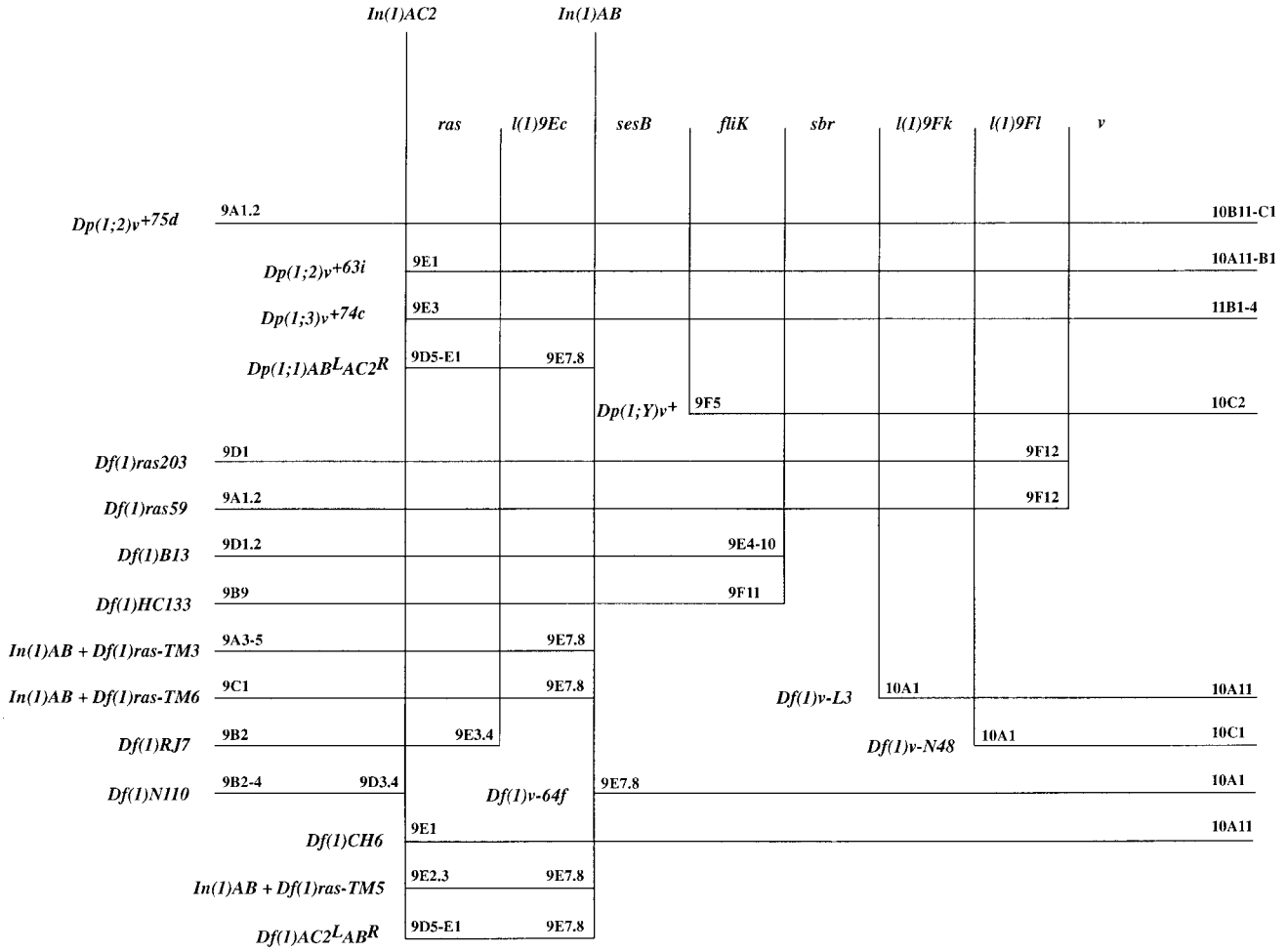

Figure 1.—Genetic map of the *sesB* region of the *X* chromosome. The genetic extents of duplications and deficiencies, as determined by complementation analysis, are shown with their cytological extents [data from FlyBase (1999) and this study].

i.e., lethal as hemizygous females but viable as homozygous females; these were interpreted by Nash and Janca (1983) as hypomorphic alleles. We have confirmed these data and have shown in Table 1 that $sesB^{1}$ is itself in this class, and when hemizygous with *Df(1)HC133*, it is semilethal. The viability data for homozygous females and hemizygous males (Table 1) suggest an allelic series with $sesB^{9Ed\text{-}2}$ being the weakest of the "lethal" alleles and $sesB^{9Ed\text{-}1}$, $sesB^{9Ed\text{-}3}$, and $sesB^{9Ed\text{-}6}$ being the strongest. When hemizygous with the deficiency *Df(1)HC133*, most *sesB* alleles are lethal in females, with only $sesB^{1}$ and $sesB^{9Ed\text{-}4}$ showing any escapers (Table 1).

***sesB* encodes an adenine nucleotide translocase:** The facts that the *sesB* locus maps proximal to the distal breakpoint of *In(1)AB* and that this breakpoint maps just distal to the 3′ end of genes encoding an adenine nucleotide translocase (see below) led us to examine whether or not the *sesB* mutations mapped to the *Ant* translocase genes. This was done both by sequencing the gene from four mutant alleles and by transformation rescue of the mutant phenotypes.

To avoid the introduction of errors during PCR reactions, multiple PCR products amplified with the high-fidelity polymerase Vent were cloned independently, and several clones were sequenced. Three EMS-induced alleles sequenced showed substitutions in *sesB*. For $sesB^{1}$, all six sequenced clones (from three independent PCR reactions) showed a missense mutation in codon 289, CTT to TTT, which would result in a leucine-to-phenylalanine substitution in the putative sixth transmembrane domain of the protein. L289 is conserved in 30/35 available adenine nucleotide translocase sequences; the exceptional five are all from yeasts. The lethal allele $sesB^{9Ed\text{-}1}$ has an alanine-to-threonine missense mutation in codon 144, GCT to ACT (seven clones from two independent PCR reactions were sequenced, and four of them showed the mutation; as the DNA amplified was from a balanced stock, this is expected). A144 is conserved in 34/35 available adenine nucleotide translocase sequences [curiously, the exception is the protein from another insect, *Anopheles gambiae* (Beard *et al.* 1994)]. The lethal allele $sesB^{9Ed\text{-}6}$ has a nonsense muta-

TABLE 1

Complementation between *sesB* alleles and deletions

	Female																			
	sesB^{1}		*sesB*^{6}		*9Ed-1*		*9Ed-2*		*9Ed-3*		*9Ed-4*		*9Ed-5*		*9Ed-6*		*AC2*^{L}*AB*^{R}		*v64f*		*HC133*	
Male	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*
sesB^{1}	29.3	123	—		11.6	172	42.0	172	4.9	204	42.3	111	31.9	395	3.5	143	52.9	376	9.0	167	25.2	318
sesB^{6}; v^{+75d}/*CyO*	1.9	479	—		0	552	0	382	0	324	15.8	527	0.6	433	6.9	836	24.7	594	0	637	1.7	558
9Ed-2	27.8	331	—		20.4	255	29.9	167	0	258	36.7	229	26.1	436	13.1	175	49.8	596	37.9	240	2.2	946
9Ed-3; *sesB*$^{+}$/*CyO*	5.0	111	—		0	139	0	76	—		0	57	0	235	0	148	50.7	442	0	459	0	169
9Ed-4; *sesB*$^{+}$/*CyO*	24.6	186	—		0	246	4.0	245	0	274	—		10.5	330	—		51.1	451	43.4[a]	304	13.3	379
9Ed-5; *sesB*$^{+}$/*CyO*	36.3	346	—		0	262	0	263	0	190	28.6	321	—		0	173	46.5	336	17.2	278	0	168
9Ed-6; *sesB*$^{+}$/*CyO*	4.9	210	—		0	210	7.9	212	0	134	8.8	188	42.9	99	—		52.1	267	—		0	170
v64f; v^{+75d}/*CyO*	7.0	839	—		0	422	22.8	609	0	434	0	206	0[b]	448	0	481	31.0	169	—		0	194
HC133; v^{+75d}/*CyO*	9.5	353	—		0	426	0	479	0	375	4.5	324	0	94	0	788	0	288	0	386	—	
Male viability	61.3	287	0	720	—		59.7	501	0	161	30.6	108	43.5	138	0	250	—		—		—	

Complementation data are the results of crosses of *sesB* alleles *inter se* and *sesB* alleles to deletions. All females were balanced over *FM6* or *FM7*. Males were of three classes: (1) semiviable males from the *sesB*^{1} and *sesB*$^{9Ed-2}$ stocks, (2) males carrying a *sesB* allele or deletion rescued by *Dp(1;2)v*$^{+75d}$ balanced over *CyO*, and (3) males carrying a *sesB* allele rescued by the *sesB* transgene balanced over *CyO*.

The viability shown is the number of *sesB*^{X}/*sesB*^{Y} Cy females over the total number of Cy females expressed as a percentage; or in the case of crosses with *sesB*^{1} and *9Ed-2* males, the number of *sesB*^{X}/*sesB*^{Y} females over the total females. In both cases, full viability is 50%. In every case, *n* is the total number of females.

The male viability data are from *sesB*/*FM6* or *FM7* females × *FM6* or *FM7* males under low-density larval conditions. The viability shown is the number of *sesB* males as a percentage of the total males; *n*, total number of males. Escaper males generally eclose later than their balancer-carrying sibs. *sesB*^{1} males are bang sensitive; other viable males are not bang sensitive, with the possible exception of *sesB*$^{9Ed-4}$ males, which are very sluggish.

Note that *sesB*$^{9Ed-1}$ carries a second-site *X*-linked lethal mutation outside of the 9E region; it is not rescued by *Dp(1;2)v*$^{+75d}$.

Dashes indicate no data.

[a] *9Ed-4*/*v64f* flies eclosed late.

[b] Many *9Ed-5*/*v64f* flies died soon after eclosion, before inflating their wings.

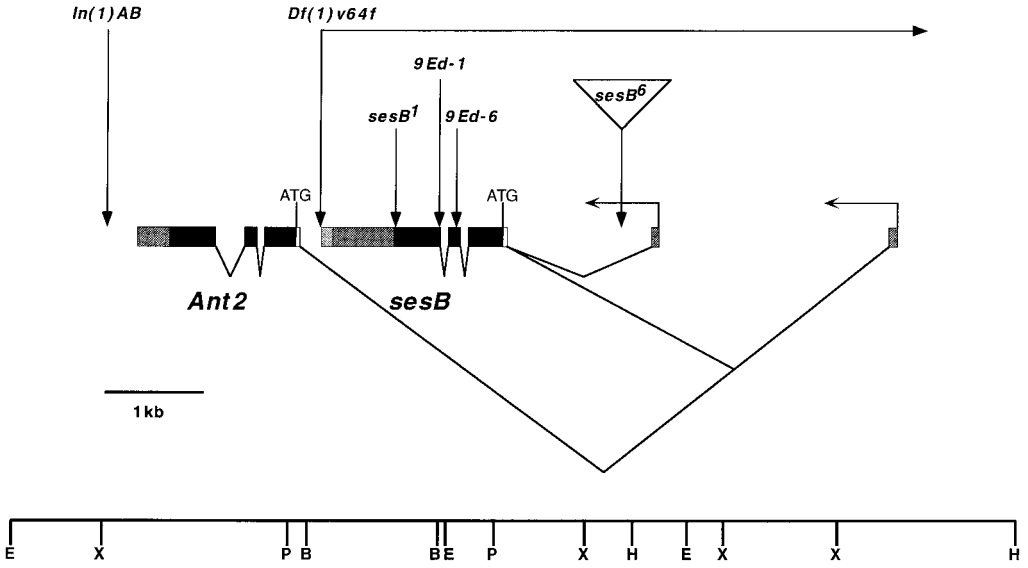

Figure 2. The organization and structure of adenine nucleotide translocase genes in *D. melanogaster*. A restriction map of the 10.3-kb region that was sequenced and used for transformation is shown at the bottom of the figure. Abbreviations for restriction enzymes are as follows: B, *Bgl*II; E, *Eco*RI; H, *Hin*dIII, P, *Pst*I; X, *Xba*I. The structures of the two tandemly arrayed adenine nucleotide translocase genes are shown above the restriction map; the chromosomal-distal gene is *Ant2*, and the chromosomal-proximal gene is *sesB*. The two arrows above the 5′ untranslated region (UTR) exons indicate the direction of transcription from the two promoters. The solid boxes are coding regions of exons, and the shaded boxes represent the UTR regions of exons; the two different 3′ UTRs of *sesB* are indicated by a difference in shading. The positions of the distal breakpoints of *In(1)AB* and *Df(1)v64f* are indicated, as determined by sequencing. The positions of the four mutations of *sesB* that we have sequenced are indicated. The sequence of this DNA is under EMBL accession no. Y10618. The Hutter and Karch (1994) sequence (EMBL:S71762) is of the distal *sesB* transcript, with the more distal 3′ end. It starts at base 1076 (relative to the right-hand *Hin*dIII site) and ends at base 6988; the Louvi and Tsitilou (1992) cDNA sequence is (after removal of the terminal *Eco*RI recognition site sequences from its end) of the proximal *sesB* transcript, with the more proximal 3′ end; it starts at base 3585 and ends at base 6827. The BDGP LD14425.5′ sequence is of the distal *sesB* transcript, starting at base 1086 and ending (prematurely, we presume) at base 6749 within the 3′ UTR. The BDGP GM14781.5′ sequence is of the proximal *sesB* transcript; it starts at base 3529 and ends (prematurely) within the third coding exon at base 5800. The BDGP GM13259.5′ sequence is of *Ant2*; it starts at base 1100 and ends (prematurely) at base 8336, within the third coding exon of *Ant2*. The PCR product of *Ant2* amplified from an imaginal disc cDNA library (see materials and methods) corresponds to bases 1076–1174 and 7338–7392 of the genomic sequence with three mismatches, presumably errors introduced by the PCR reaction.

tion at codon 112, TGG to TGA (6 of 12 clones from 3 independent PCR reactions). These two lethal alleles, $sesB^{9Ed\text{-}1}$ and $sesB^{9Ed\text{-}6}$, were recovered from the same *y cv v f* progenitor stock after EMS mutagenesis (Nash and Janca 1983).

The insertion site of the *P* element in the newly induced *P*-element allele, $sesB^{6}$, was also sequenced (see materials and methods). The *P* element is inserted in the first intron (between nucleotides 3917 and 3919) of *sesB*/*Ant2*, with the element in the opposite orientation to *sesB*/*Ant2*. In addition to the deletion of nucleotide 3918 of *sesB*, this insertion is associated with 47 bp of unidentified sequence between the *sesB* sequence and the 3′ end of the *P* element, as well as 58 bp of unidentified sequence between *sesB* and the 5′ end of the *P* element.

Transformation rescue: A 10.3-kb *Eco*RI-*Hin*dIII genomic DNA fragment (cloned in pP{CaSper-hs}) that contains the entire adenine nucleotide translocase gene as well as its distal relative (Figure 2; see below) was transformed. Two independent insertions, $P\{Ant2^{+}\ sesB^{+}10.3\}42A$ and $P\{Ant2^{+}\ sesB^{+}10.3\}102E$, were recovered. Both rescue the male lethality of lethal alleles of *sesB* (Table 2) and the stress-sensitive and hypoactive phenotypes of $sesB^{1}$ and $sesB^{9Ed\text{-}4}$.

Taken together, the sequencing and transformation rescue data unambiguously show that mutations in the adenine nucleotide translocase are responsible for both the behavioral and lethal *sesB* phenotypes.

Transformants for *sesB* do not affect interspecific

TABLE 2

Rescue of *sesB* alleles by two independent $Ant2^{+}$ $sesB^{+}$ transformation lines

	42A			*102E*		
	$Cy^{+}\ B^{+}$	$Cy\ B^{+}$	Total	$ey^{+}\ B^{+}$	$ey\ B^{+}$	Total
$sesB^{1}$	110[a]	5[b]	249	134[a]	5[b]	241
$sesB^{9Ed\text{-}2}$	192	16	253	159	43	277
$sesB^{9Ed\text{-}3}$	95	0	279	38	0	107
$sesB^{9Ed\text{-}4}$	83	37	244	61	20	265
$sesB^{9Ed\text{-}5}$	52	0	137	39	0	154
$sesB^{9Ed\text{-}6}$	129	0	251	69	0	130

sesB/*FM7* females were crossed to *y w;* $P\{Ant2^{+}\ sesB^{+}10.3\}42A/CyO$ or $P\{Ant2^{+}\ sesB^{+}10.3\}102E/ey^{D}$ males; numbers of sons are shown. The Cy^{+} or ey^{+} males are rescued by the transgene; the $Cy\ B^{+}$ or $ey\ B^{+}$ males are *sesB* escapers.

[a] Not bang sensitive.

[b] Bang sensitive.

TABLE 3

***sesB* and *Ant2* transgenes do not suppress the rescue of interspecific hybrid males**

Male	*w* males	w^+ males	Total flies
A. *w In(1)AB/y w; P{Ant2$^+$sesB$^+$ 10.3}42A/+* females crossed to sibling species males at 25°[a]			
D. mauritiana	67	72	375
D. sechellia	10	10	118
D. simulans	34	40	243
B. *y Hmr v; P{Ant$^+$sesB$^+$ 10.3}42A/CyO* females × *D. mauritiana* males at 18°[b]			
y v; Cy$^+$ males		48	
y v; Cy males		14	
Total flies		208	

[a] Rescued hybrid males are w^+ if they carry the transgene and white if not.

[b] Rescued males are Cy^+ if they carry the transgene and *Cy* if not.

hybrid viability: Hutter *et al.* (1990) showed that *Dp(1;2)v^{+75d}* suppressed hybrid rescue by *Hmr*, presumably because this duplication carries *Hmr$^+$*. To determine whether an extra copy of *sesB$^+$* would suppress the rescue of *D. melanogaster* × sibling hybrid males by either *Hmr* or *In(1)AB*, we compared the viability of rescued males with or without *P{Ant2$^+$sesB$^+$ 10.3}42A*. No differences were found (Table 3); in contrast, *Dp(1;2)v^{+75d}* (which carries *Hmr$^+$*) has been shown to completely suppress the rescue of hybrid males by *Hmr* (Hutter *et al.* 1990; J. Roote and M. Ashburner, unpublished observations). In addition, we crossed *D. melanogaster* females carrying both *P{Ant2$^+$sesB$^+$ 10.3}42A* and *P{Ant2$^+$sesB$^+$ 10.3}102E* to *D. mauritiana* males (at 18°); no hybrid male adults were recovered, although the experiment was small (64 females). These data indicate that neither *sesB* nor *Ant2* interact with the genetic factors that influence interspecific male viability.

A second adenine nucleotide translocase-like gene is contiguous to *sesB*: Both Louvi and Tsitilou (1992) and Hutter and Karch (1994) had suggested, on the basis of Southern DNA blots, that there was more than one gene encoding adenine nucleotide translocase in *D. melanogaster*. If so, then the *in situ* hybridization data indicate that these must both be in region 9E, since neither these studies nor our own showed any indication of other sites of hybridization with ANT cDNA probes. Indeed, on the basis of Southern blot data, Hutter and Karch (1994) considered the possibility of tandemly duplicated ANT genes in Drosophila. We sequenced both the 4.4- and 2.5-kb *Eco*RI fragments that include *sesB* (Figure 2) and thereby discovered a second open reading frame 1104 bp distal (that is, 3′) to *sesB*. This ORF is similar in sequence (and in intron/exon structure, see below) to *sesB*. The coding regions of the two genes show 74% nucleic acid sequence identity and 78% protein sequence identity (Figure 3). Our evidence (below) is that this new gene is functional, and we named it *Ant2* (adenine nucleotide translocase 2). By hybridization of whole genomic DNA with probes to these genes, there is no indication of further genes of similar sequence in the genome of *D. melanogaster*.

The relationship between *Ant2* and *sesB*: Despite their sequence similarity, *sesB* and *Ant2* appear to be functionally distinct. The two genes cannot, of course, be fully functionally redundant; otherwise, the frequent recovery of lethal mutations by EMS in *sesB* would not be possible. We have sequenced *Ant2* in four *sesB* alleles (*sesB^1*, *sesB$^{9Ed\text{-}1}$*, *sesB$^{9Ed\text{-}3}$*, and *sesB$^{9Ed\text{-}5}$*) and found no amino-acid substitutions in these.

Df(1)v64f ends within the 3′-untranslated region of *sesB*, removing the 5′ untranslated exon of *Ant2* but not affecting the *Ant2*-coding region. The viabilities of *sesB* alleles, when heterozygous with this deletion, are similar to those seen when they are heterozygous for the longer deletion *Df(1)HC133*, which removes both *sesB* and *Ant2* in their entirety (Table 1).

The distal breakpoint of *In(1)AB* was also mapped by Southern blot hybridization; it is in the distal part of the 1.8-kb *Xba*I-*Pst*I fragment (see Figure 2). The breakpoint was sequenced by inverse PCR and was found to be 314 bp 3′ to the end of the *Ant2* transcript (at position 9288 on EMBL:Y10618). *In(1)AB* is fully viable when heterozygous with deletions of this chromosome region or when homozygous. The synthetic deletion *Df(1)AC2^{L}AB^R*, which extends distally from the *In(1)AB* distal breakpoint, is completely viable when heterozygous with lethal alleles of *sesB* (Table 1). The distal breakpoint of *Df(1)v64f* was also determined by inverse PCR; it is at position 7033 on the sequence EMBL:Y10618, *i.e.*, in the short "intergenic" region between *sesB* and *Ant2*.

The structure of the two ANT genes and their expression: Comparison of cDNA and genomic sequences show that *sesB* has two alternative transcription starts, and that each transcript is interrupted by an intron in its 5′ UTR (Figure 2). The coding region of *sesB* is interrupted by small introns within codons 99 and 142. The genomic structure of the coding region of *Ant2* is similar to that of *sesB*, with two introns interrupting at positions identical to those within the coding sequence of *sesB*. The cDNAs previously sequenced by Louvi and Tsitilou (1992) and Hutter and Karch (1994) are clearly derived from *sesB*, although there are some differences between these and our sequences (see legends to Figures 2 and 3). The Hutter and Karch (1994) cDNA and BDGP LD14425.5′ sequences are of the distal transcript, and the Louvi and Tsitilou (1992) cDNA and GM14781.5′ sequences are of the proximal transcript.

No cDNA of *Ant2* was recovered in our screens of cDNA libraries. The BDGP EST collection, however, includes a partial 5′ cDNA of this gene, GM13259.5′ (the fact that there was only one cDNA of *Ant2 vs.* 63

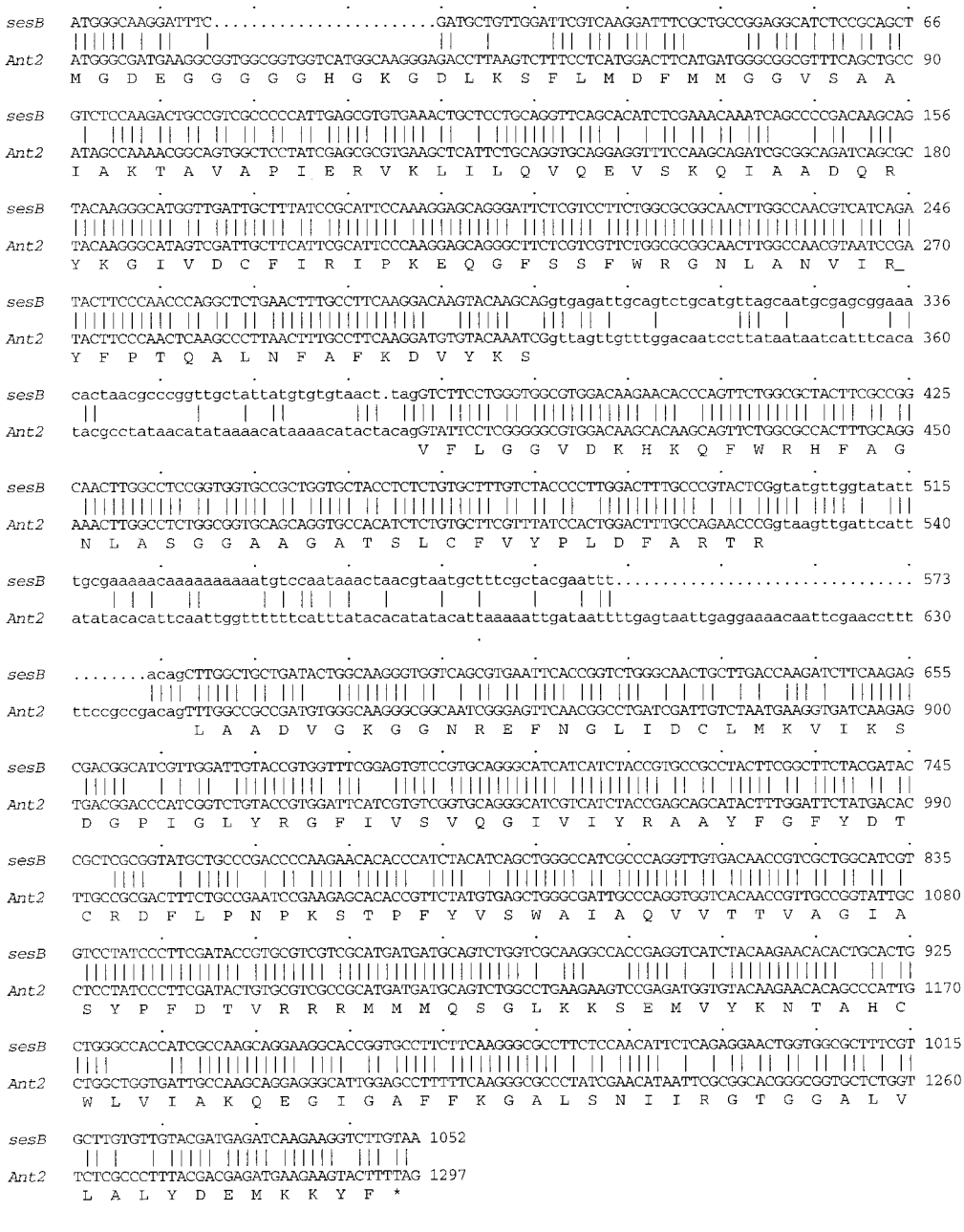

Figure 3.—Sequence alignment of the protein-coding regions and internal introns of *sesB* and *Ant2*. The exons are in capital letters, the introns in lowercase; gaps are indicated by periods, stop codons by asterisks. The translation of the ANT2 protein is shown. The single-letter amino-acid code is used here and in Figure 6. There are seven amino-acid differences between this *sesB* sequence and those of Louvi and Tsitilou (1992, EMBL: S43651) and Hutter and Karch (1994, EMBL: S71768); in five of these cases, the two latter sequences are in agreement. In addition to checking our own data, we have studied the full-length cDNA sequence assembled from 59 independent EST sequences by the BDGP (LS14425.5′, see materials and methods). In all cases except one, our sequence agrees with this assembled cDNA sequence; the exception is residue 270, Gly in our sequence and Asp in the BDGP sequence. Residue 270 is one of two (the other is residue 202) that are absent from both S43651 and S71768.

for *sesB* gives some clue as to the relative abundance of *Ant2* and *sesB* transcripts). Bases 76–700 of GM13259.5′ match the corresponding region of the predicted *Ant2* cDNA sequence with only five base mismatches. We were puzzled to find that the first 75 bp of the GM13259.5′ sequence matched no sequence in the 1.1-kb *sesB-Ant2* intergenic region, where the *Ant2* promoter might reasonably be expected to be found. Remarkably, a sequence with 100% identity to these first 75 bp is found in the 5′ untranslated exon sequence of the distal *sesB* transcript (see Figure 2). We conclude that *sesB* and *Ant2* are transcribed from the same promoter and share a common 5′ untranslated exon. The dicistronic structure of the 5′ end of this transcript was confirmed by PCR amplification of the predicted sequence from an imaginal disc cDNA library (see materials and methods and legend to Figure 2).

Using a 4.4-kb genomic *Eco*RI fragment containing *Ant2* and the 3′ end of *sesB*, two major transcripts of 1.6 and 1.2 kb are seen in the Northern blots (see also Louvi and Tsitilou 1992). Both are seen at all developmental stages assayed, although there are clear variations in their levels. These two transcripts are also seen using a *sesB* cDNA probe from Hutter and Karch (1994). With a probe corresponding to the 3′-untranslated region of *Ant2*, a single transcript of 1.6 kb is seen; its abundance in adult flies is considerably less than that of the 1.6-kb *sesB* transcript (Figure 4). We have not detected any sign of a 2.8-kb transcript, as seen in the wild type by Hutter and Karch (1994). It was the absence of this

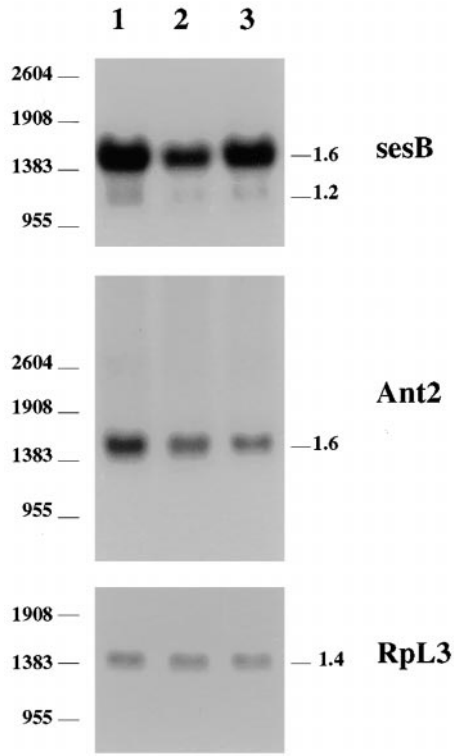

Figure 4.—Northern blot hybridization of total RNA from adult flies with probes to *sesB* (HE50 cDNA), *Ant2* (3′ UTR), and *RpL3* (loading control; Chan *et al.* 1998). The lanes are as follows: (1) Canton-S, (2) *Hmr*, and (3) *In(1)AB*. The approximate sizes of the transcripts are indicated in kilobases on the right; the RNA size markers (Promega) are indicated on the left.

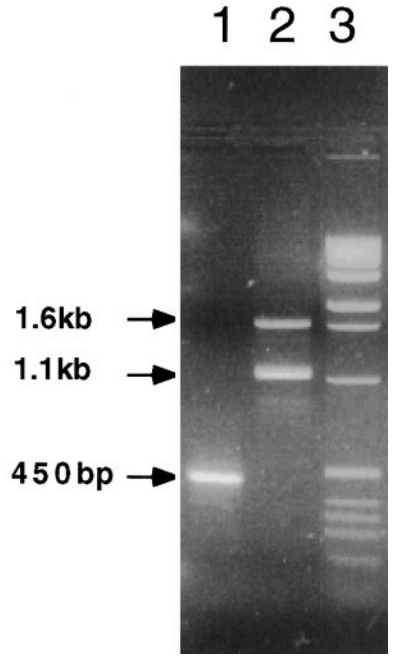

Figure 5.—RACE analysis of a *sesB* cDNA isolated from an imaginal disc cDNA library. Lanes 1 and 2 represent 5′ and 3′ products, respectively. Sizes are marked by arrows. The primers used for 5′- and 3′-end amplification are described in materials and methods. Lane 3 is a 1-kb DNA ladder from GIBCO BRL (Gaithersburg, MD).

transcript in *In(1)AB* and its reduced level in *Hmr* that led these authors to conclude that *In(1)AB* was mutant for the adenine nucleotide translocase.

The two transcripts seen by the Northern blot analysis result, at least in part, from the alternative 3′ ends of *sesB*, as shown by RACE analysis of *sesB* cDNAs. Only a single 450-bp product was found from the amplification of the 5′ end of *sesB* cDNA, but two products of 1.1 and 1.6 kb were found from amplification of the 3′ end (Figure 5). All three RACE bands were sequenced, producing the expected results. When used as a probe to a Northern blot, the 1.6-kb RACE product only hybridizes to the 1.6-kb transcript.

DISCUSSION

The relationship between adenine nucleotide translocase and interspecific hybrid lethality rescue: This study began with the working hypothesis (Hutter and Karch 1994) that some mutations that rescue the lethality of hybrids between *D. melanogaster* and its sibling species may be the result of changes in an adenine nucleotide translocase gene. Several lines of evidence are now consistent in suggesting that is not the case: *Hmr* itself maps distal to both ANT genes and to the *In(1)AB* breakpoint. Furthermore, we cannot confirm any effect of the distal *In(1)AB* breakpoint or of *Hmr* on ANT gene expression, although the *In(1)AB* breakpoint is indeed very close to, or perhaps even within, the 3′ end of *Ant2*. Finally, neither *sesB* mutant alleles nor transformants carrying extra copies of *sesB* and *Ant2* have any effect on interspecific viability. The molecular nature of these hybrid-lethality-rescuing mutations remains to be discovered.

ANT genes in *D. melanogaster*: We show that there are two genes in *D. melanogaster* encoding proteins very similar in sequence to the adenine nucleotide translocases of other metazoa. These genes are in a tandem array, and the terminator codon of *sesB* and the initiator ATG codon of *Ant2* are separated by only 1104 bp. They show similarities not only in sequence, but also in intron/exon structure, suggesting that they arose from a single gene by an event such as unequal exchange. There is no evidence, *e.g.*, from Southern blot hybridization or *in situ* hybridization to polytene chromosomes, of any more genes similar in sequence in the genome of *D. melanogaster* (see Louvi and Tsitilou 1992). Tandem pairs of related genes are quite common in *D. melanogaster*; other examples include *Adh* and *Adhr* and pairs of *polyhomeotic*, *gooseberry*, *engrailed*, *sloppy-paired*, *zen*, and *knirps* genes (see FlyBase 1999). These vary in the degree of similarity between members of a pair and the extent to which they are functionally similar. This variation presumably reflects both the time of duplication and functional constraints.

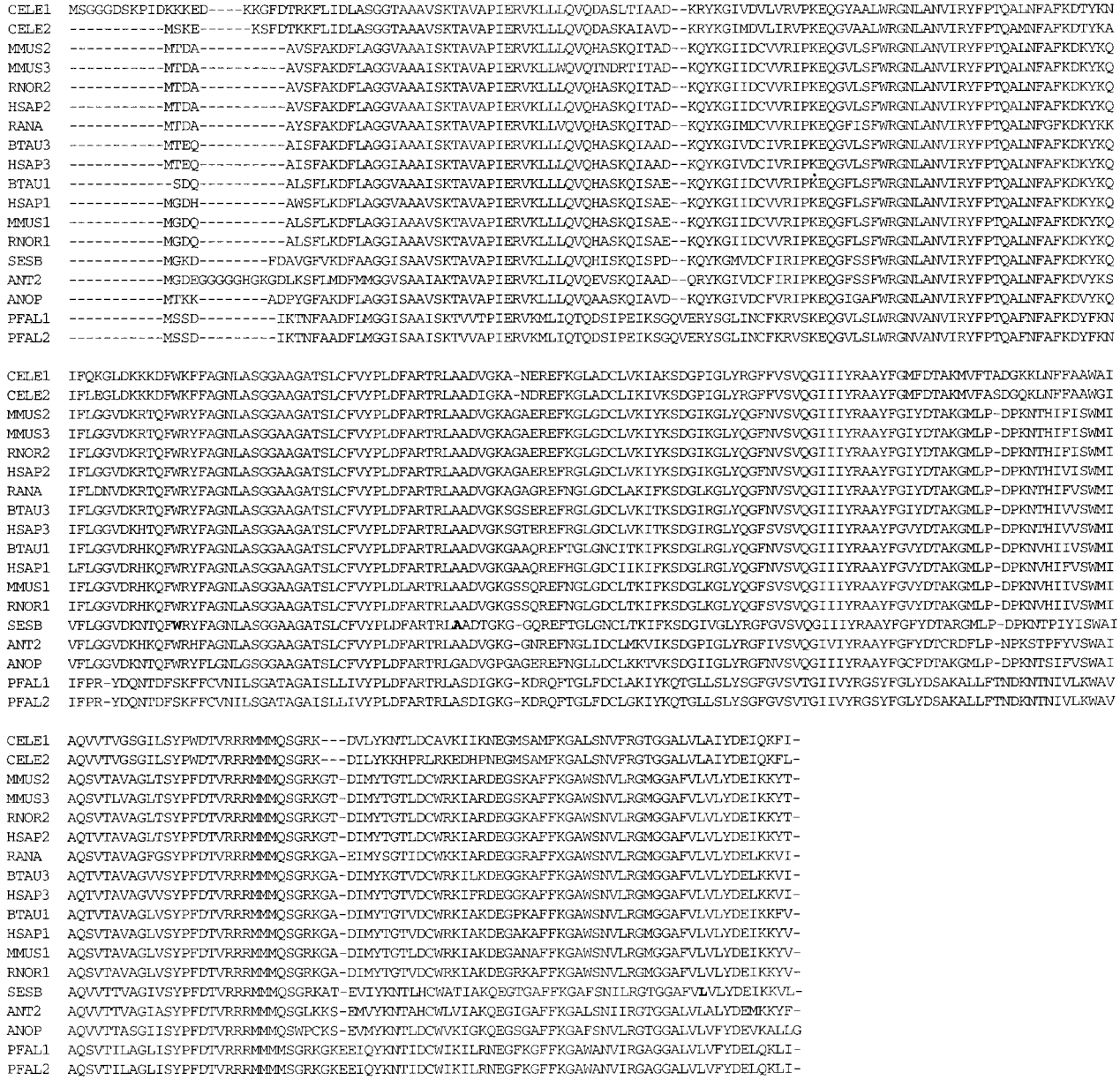

Figure 6.—An alignment of adenine nucleotide translocase proteins from protozoa and animals. The sites of three point mutations in *sesB* are highlighted in bold. In *sesB*$^{9Ed\text{-}6}$, W112 is changed to a stop codon; in *sesB*$^{9Ed\text{-}1}$, there is the change A144T, and in *sesB*^{1} the change L289F. All sequences are from either the SWISSPROT or SPTREMBL database, and are those available on a search for adenine nucleotide translocase through the EBI SRS server (http://srs.ebi.ac.uk/). PFAL1:Q26006 and PFAL2:Q25692 (*Plasmodium falciparum*), CELE1:P91410 and CELE2:Q17407 (*C. elegans*), ANOP:Q27238 (*A. gambiae*), SESB:P91614 (*D. melanogaster sesB*), ANT2:O62526 (*D. melanogaster Ant2*), RANA:Q91336 (*Rana sylvatica*), BTAU1:P02722 (*Bos taurus* ANT1), BTAU3:P32007 (*B. taurus* ANT3), MMUS1:P48962 (*Mus musculus* ANT1), MMUS2:P51881 (*M. musculus* ANT2), MMUS3:Q61311 (*M. musculus* ANT3); RNOR1:Q05962 (*Rattus norvegicus* ANT1), RNOR2:Q09073 (*R. norvegicus* ANT2), HSAP1:P12235 (*Homo sapiens* ANT1), HSAP2:P05141 (*H. sapiens* ANT2), and HSAP3:P12236 (*H. sapiens* ANT3). The sequence of the Rana protein (Q91336) was corrected for the obvious errors in annotation of its nucleic acid sequence record and for a missed base (clearly seen if this nucleic acid sequence is aligned with those from mammals), which resulted in a frame-shifted protein sequence.

In the majority of examples of tandemly duplicated genes, each gene is transcribed independently. This is not the case for *Adh* and *Adhr*, which are transcribed as a functional dicistronic mRNA, with internal translation initiation required for the synthesis of the ADHR protein (Brogna and Ashburner 1997). *sesB* and *Ant2* would also appear to be dicistronically transcribed, but here, alternate splicing results in separate mRNAs for each protein. These mRNAs share a 5′ untranslated exon. The circumstantial evidence is that the *Ant2* mRNA is much rarer than the *sesB* mRNA—indeed, this conclusion (made from the relative abundance of their

ESTs in the BDGP collection) is supported by Northern blots. This situation is a remarkable variant on the quite familiar situation of alternative splicing that results in two or more protein isoforms. In the case of *sesB* and *Ant2*, the two proteins share no coding sequences at all, and they differ in their primary sequence by >20%. The most similar case of which we are aware, in Drosophila, is that of the gene encoding the UB80 protein (a ubiquitin-fusion protein) and a conserved protein of unknown function called IP259 (Mottus *et al.* 1997). The mRNAs for these proteins share a 5′ untranslated exon and are derived by alternative splicing. The single exon encoding IP259 is wholly contained within the first intron of *Ub80*. The *Su(var)3-9* gene of Drosophila has a structure that is similar, but with the difference that the shared 5′ exons are coding with respect to two different proteins (Tschiersch *et al.* 1994). Mutations of this gene are dominant suppressors of position-effect variegation. *Su(var)3-9* encodes two different mRNAs by alternative splicing. One of these encodes a protein with chromo- and SET-domains; the other is quite unrelated in sequence and is of an unknown functional class. These two proteins share their N-terminal 80 amino acids, encoded by two exons.

There are two genes known in *Caenorhabditis elegans* with an organization similar to that of *sesB* and *Ant2*. One is the *unc-60* locus, which encodes two actin-binding proteins that are 38% identical in amino-acid sequence. Both genes, *unc-60A* and *unc-60B*, share a common 5′ untranslated exon, and the former gene is entirely contained within the first intron of the latter (McKim *et al.* 1994). Even more remarkable is the organization of the genes coding choline acetyl transferase (*Cha*) and the vesicular acetyl choline transporter (*VAChT*). In *C. elegans*, mammals, and Drosophila, the *VAChT* gene is contained within the first intron of *Cha*, and the two share a 5′ exon. In *C. elegans* (Alfonso *et al.* 1994) and mammals (Erickson *et al.* 1994; Berrard *et al.* 1995), this exon is noncoding; in Drosophila (Kitamoto *et al.* 1998), the 5′ common exon codes for the N terminus of the CHA and probably the VACHT proteins. Another example of this type of gene organization of which we are aware is that for the human and murine leptin receptor and leptin receptor gene-related protein (Bailleul *et al.* 1997). Here, the mRNAs encoding these two unrelated proteins share two 5′ untranslated exons.

Figure 6 is an alignment of the Drosophila ANT proteins with those sequenced from a variety of animals (those of algae and plants are very clearly related, but they form a distinct sequence subfamily). Many other organisms, such as *D. melanogaster*, have two (or more) ANT genes. A gene tree of these, constructed by DRAWTREE, is shown in Figure 7. In mammals, there are usually three different functional ANT genes encoding proteins with 88–89% amino-acid sequence identity. These show differential tissue expression (Powell *et al.* 1989; Stepien *et al.* 1992). The *sesB* protein is equally

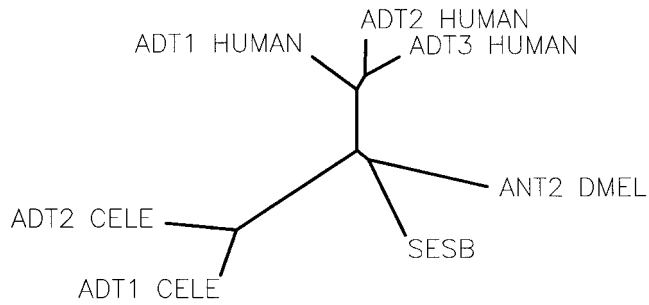

Figure 7.—Relationships of adenine nucleotide translocases from Drosophila, *C. elegans*, and human (as typical of mammals). The tree was constructed with DRAWTREE (PHYLIP), with sequences aligned by CLUSTAL W.

similar to all three mammalian proteins (78% amino-acid sequence identity); similarly, the *Ant2* protein is equally similar to the three mammalian proteins (72% amino-acid sequence identity). The predicted ANT2 protein has, relative to mammalian ANT proteins, a rather hydrophilic 10-amino-acid insertion immediately before the first predicted transmembrane domain.

Adenine nucleotide translocases are members of a larger family of mitochondrial carrier proteins that are located in the inner mitochondrial membrane (Walker 1992; Kuan and Saier 1993; Walker and Runswick 1993). These transport a variety of substances across this membrane, including organic acids and phosphate. Mitochondrial carrier proteins are characterized by three ~100-amino-acid domains, each with two α-helical transmembrane regions (Saraste and Walker 1982). [Nonhomologous proteins of similar function to adenine nucleotide translocases are found in the intracellular prokaryotic symbiont *Rickettsia prowazekii* (Winkler 1976; Plano and Winkler 1991; Andersson *et al.* 1998)]. The genome of *Saccharomyces cerevisiae* includes >20 genes encoding members of this family (data from PROSITE Release 14.0; see also Yeast Proteome 1998), and there are many in *C. elegans* (*e.g.*, Runswick *et al.* 1994). The *sesB* and *Ant2* proteins of Drosophila are very distant (<25% amino-acid sequence identity) to any known members of this protein family other than the ANT proteins. We know of only one other member of the mitochondrial carrier protein family in Drosophila. This is *colt*, which is required for tracheal function (Hartenstein *et al.* 1997). The *colt* protein is similar to the *C. elegans dif-1* protein (Ahringer 1995); the solute carried by these proteins is not known. There is also a clear *colt* homolog in *D. grimshawi* (EMBL:U87812; SPTREMBL:P92028).

Adenine nucleotide translocase is the most abundant integral protein of the inner mitochondrial membrane (Klingenberg 1985). It is an electrogenic protein and catalyzes the exchange of cytoplasmic ADP for ATP across the membrane in a 1:1 stoichiometry. Because this protein links the processes of ADP production to

those of ATP utilization, it can exert a control on the rate of oxidative phosphorylation, as well as on the rates of energy-consuming processes. So far, no phenotypes have been associated with mutation of any of the three adenine nucleotide translocase genes in humans, though several myopathies with abnormal mitochondria remain to be characterized at the molecular level. A knockout mutation of mouse ANT1, the heart/muscle specific protein, is not lethal, but it does cause severe mitochondrial hypertrophy in these tissues and obvious disease in adult mice (Graham *et al.* 1997). The features of Luft's disease, the first myopathy described in humans, are abnormally large mitochondria containing packed cristae in muscle fibers and defective respiratory control with normal phosphorylation ("loose coupling," Dimauro *et al.* 1976). Defective adenine nucleotide translocase might lead to this type of loose coupling. It would be interesting to study the ultrastructure of the mitochondria in *sesB* mutant alleles. This could indicate whether or not these mutations would be a useful model for the study of some human mitochondrial diseases.

The *sesB* phenotypes and ANT: Stress- or bang-sensitive phenotypes are quite common in *D. melanogaster*, since they are easily recovered in screens, especially if *X*-linked; at least 21 loci with alleles showing this phenotype have been described (Benzer 1971; Homyk and Sheppard 1977; Ganetzky and Wu 1982; FlyBase 1999). It would seem probable that there are at least 100 genes in *D. melanogaster* that can mutate to this general phenotype.

Four other bang-sensitive mutants have already been identified molecularly, *tko*, *eas*, *stn*, and *Atpα*. *technical knockout* (*tko*) encodes a protein homologous to the prokaryotic ribosome protein S12; this is presumably a mitochondrial ribosomal protein in Drosophila (Royden *et al.* 1987; Shah *et al.* 1997). Most mutant alleles of *tko* are lethal, but one, tko^{25t}, is male viable (but hemizygous female lethal) and results in a bang-sensitive phenotype. This is a single-amino-acid substitution in a highly conserved residue (Shah *et al.* 1997). *easily shocked* (*eas*) encodes an ethanolamine kinase involved in membrane phospholipid synthesis. It seems that *eas* is associated with an increased membrane excitability caused by an altered membrane phospholipid composition (Pavlidis *et al.* 1994). Interpretation of the molecular basis for the *stoned* (*stn*) phenotype is complicated by the discovery that this locus encodes two proteins (from a dicistronic transcript); one is novel and the other has similarity to AP50 family clathrin assembly proteins and to the UNC-41 protein of *C. elegans* (Andrews *et al.* 1996; Blumenthal 1998). Finally, some hypomorphic mutations of the sodium/potassium-transporting ATPase gene (*Atpα*) have a bang-sensitive phenotype (Schubiger *et al.* 1994).

It is interesting that two genes with bang-sensitive mutant phenotypes, *tko* and *sesB*, encode mitochondrial proteins. The bang-sensitive alleles show other similarities, *e.g.*, delayed development, but they differ behaviorally, *e.g.*, tko^{25t}, but not $sesB^{1}$, shows hyperexcitability during recovery from stress-induced paralysis. For both of these genes and for *stn* (originally recovered as *sesC*), viable bang-sensitive mutations are relatively rare, and most alleles are lethal.

We thank Pierre Hutter for his encouragement to start this work and for clones. We also thank Igor Zhimulev, Norbert Perrimon, and Kathy Matthews of the Bloomington *Drosophila* Stock Center for stocks. We thank Carol McKimmie for making the transformants, and our colleagues Darin Coulson, Tim Fergestad, Glynnis Johnson, Terri Morley, Esther Betran, Simon Collier, Siegrun Herrmann, and Steve Russell for both practical help and discussion. We are most grateful to Suzanna Lewis of the Berkeley *Drosophila* Genome Project for her help, as well as Howy Jacobs and Julie Ahringer for stimulating discussions. This work was supported by a Biotechnology and Biological Science Research Council Project Grant to M.A. S.B. was also supported by grants from the European Union, D.A.B. was supported by a National Science Foundation/Sloan Foundation Fellowship and Y.Q.Z. by a grant to M.A., D. M. Glover, and R. D. C. Saunders from the Medical Research Council. D.N. was supported by the National Science and Engineering Research Council (Canada, grant no. A3269).

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Communicating editor: V. G. Finnerty