resemble either the large four-in-one sodium channel gene, a single one of the homology domains, or, as in the case of the acetylcholine receptor, four separate homologous genes.

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Cloning of Genomic and Complementary DNA from *Shaker*, a Putative Potassium Channel Gene from *Drosophila*

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On the basis of electrophysiological analysis of *Shaker* mutants, the *Shaker* locus of *Drosophila melanogaster* has been proposed to encode a structural component of a voltage-dependent potassium channel, the A channel. Unlike sodium channels, acetylcholine receptors, and calcium channels, K^+ channels have not been purified biochemically. To facilitate biochemical studies of a K^+ channel, genomic DNA from the *Shaker* locus has been cloned. Rearrangements in five *Shaker* mutants have been mapped to a 60-kilobase segment of the genome. Four complementary DNA clones have been analyzed. These clones indicate that the *Shaker* gene contains multiple exons distributed over at least 65 kilobases of genomic DNA in the region where the mutations mapped. Furthermore, the gene may produce several classes of alternatively spliced transcripts. Two of the complementary DNA clones have been sequenced and their sequences support the hypothesis that *Shaker* encodes a component of a K^+ channel.

EXCITABLE MEMBRANES OF NERVE AND MUSCLE ARE ENdowed with a variety of potassium channels that are activated by voltage or calcium, or controlled by neurotransmitters (*1*). These channels conduct several kinetically distinct types of K^+ currents, which have been characterized electrophysiologically. They determine, in large part, the rate of repolarization after action potentials and whether a cell will be quiescent or spontaneously active (*1*). Since even small alterations in the duration of action potentials can lead to large variations in the amount of transmitter released from nerve terminals, the regulation of K^+ channels is likely to modulate the strength of synaptic interactions in the nervous system (*2*). This regulation may be involved in complex behaviors, such as learning and memory. A well-studied example is the involvement of a serotonin-sensitive K^+ channel in sensitization of the gill withdrawal reflex in *Aplysia* (*3*). Potassium channels also function in cells and tissues not generally considered to be excitable. For example, mitogenesis in T lymphocytes may involve a delayed rectifier-type K^+ channel (*4*).

Although K^+ channels are widely distributed, they have not yet been purified because no tissues rich in K^+ channels have been identified, and until recently (*5*) no high-affinity ligands for them were known. Until now, genes for ion channels have been isolated only after channel proteins have been purified and characterized. For example, partial amino acid sequences of the Na^+ channel and the subunits of the acetylcholine receptor have been used to design oligonucleotide probes for identifying the corresponding complementary DNA (cDNA) clones (*6*). This strategy has not been applicable to genes encoding K^+ channels.

The genetics of *Drosophila melanogaster* provides an alternative approach for the molecular analysis of rare or uncharacterized proteins. This approach requires that mutations in the gene encoding the protein have been identified, and that the precise chromosomal location of the gene is known. Cloned genomic DNA from that region is isolated, and the gene is localized by mapping the molecular positions of mutations. Coding sequences can then be identified by using the genomic DNA to identify transcripts on

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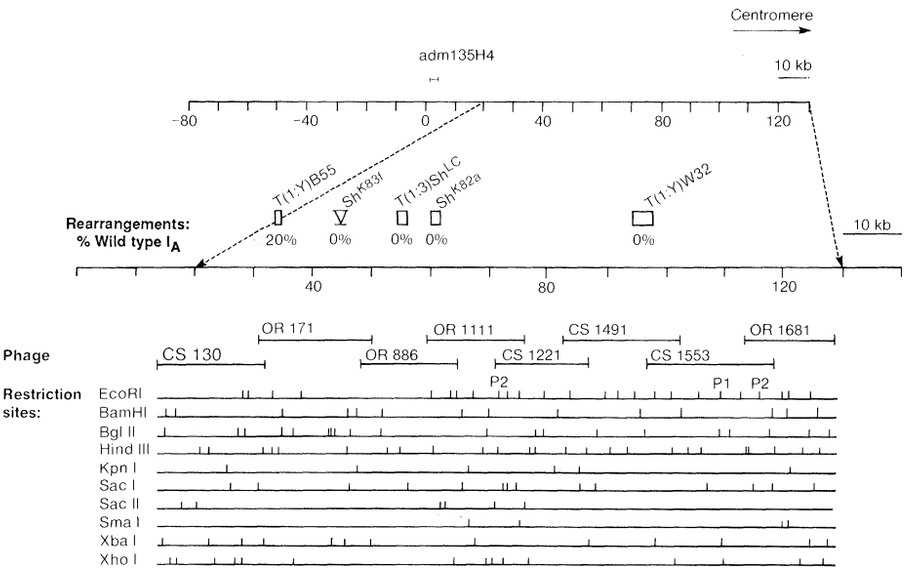

Fig. 1. Isolation of genomic DNA from the region of 16F. A schematic representation of 210 kb of genomic DNA isolated from 16F in a chromosomal walk that started with adm135H4 (near 0 kb) (**top**). The walk included 15 steps (reiterative isolations of overlapping clones) in phage lambda libraries (*20*) and was oriented relative to the centromere by in situ hybridization (*21*). Eight of these steps have been analyzed in detail (expanded scale, positions +20 kb to +130 kb); representative phage clones from Canton S (CS) and Oregon R (OR) genomic libraries are shown. In situ hybridization confirmed that these phage were from 16F on the X chromosome. These clones and additional clones isolated at each step were mapped with various restriction enzymes (**bottom**). At each step, the restriction maps for a subset of the enzymes shown were confirmed by probing Southern blots of uncloned genomic DNA with the phage clones. A few polymorphic Eco RI sites are shown: P1, present in CS, absent in OR; P2, present in OR, absent in CS. Additional strain-dependent polymorphisms exist (*28*). Between positions +34 kb and +95 kb, rearrangements were found in five *Shaker* mutations that reduce or eliminate I_A in pupal muscle (**middle**). The molecular positions of these mutations have been mapped by genomic DNA blot analysis and in situ hybridization (see text and Fig. 2). Other aspects of the walk are summarized in (*31*).

RNA blots or to isolate cDNA clones. Although this strategy has been used extensively in *Drosophila*, genes for ion channels have not previously been isolated by a genetic approach.

To analyze a K^+ channel genetically and molecularly, we and others (*7*) have been studying the *Shaker* locus of *Drosophila*. *Shaker* mutants were originally isolated because the flies shake their legs when anesthetized with ether (*8*). Voltage-clamp experiments in pupal and larval muscle show that *Shaker* mutations affect a fast, transient K^+ current, I_A (*9–11*), similar to one first recorded in the somata of molluscan neurons (*12*). Other currents are not affected (*9–11*). *Shaker* mutations may affect I_A in neurons as well as in muscle, since both neuronal action potentials (*13*) and transmitter release from motor nerve terminals (*14*) are prolonged in mutant compared to normal flies.

Several lines of evidence suggest that the *Shaker* gene encodes a structural component of the A channel (*9–11, 15*). (i) *Shaker* mutations do not cause a general membrane defect, since they do not affect currents other than I_A. (ii) In *Drosophila*, the amount of a product of a structural gene is generally proportional to gene dosage (*16*). Flies with fewer than the normal number of copies of the *Shaker* gene have reduced I_A. (iii) Different *Shaker* mutations affect I_A in different ways. Many *Shaker* alleles and a deficiency of the X chromosome at the site of the *Shaker* locus eliminate I_A. Several alleles reduce the amplitude of I_A. Most significantly, one allele, Sh^5, alters the kinetics and voltage dependence of the current. This last phenotype in particular can be explained most readily if the *Shaker* gene product is part of the A channel per se.

We report here the isolation of genomic DNA from the *Shaker* locus, the mapping of alterations in the DNA of mutants, and the characterization of cDNA clones from the region. In an accompanying paper (*17*), we report the nucleotide sequences of two cDNA clones and discuss the properties of a protein product predicted by conceptual translation of the cDNA sequences. Since the predicted *Shaker* product contains several potential membrane-spanning segments and a region homologous to vertebrate voltage-dependent Na^+ channels, our results support the hypothesis that *Shaker* codes for a structural component of the A channel.

Isolation of *Shaker* genomic DNA and localization of *Shaker* mutations. Several *Shaker* mutations are caused by chromosomal translocations in which the chromosome has been broken at or near the gene and then attached by its new ends to another broken chromosome. These mutations have been used to localize the *Shaker* gene cytologically to a region on the X chromosome [polytene bands 16F(1–4)] (*13*). The cDNA clone adm135H4, which had been isolated for purposes unrelated to *Shaker* but which hybridized to band 16F (*18*), was used to begin a chromosomal "walk" (*19*) in 16F, that is, to obtain from libraries of genomic DNA a set of overlapping clones containing DNA from this region (*20*). Genomic clones that hybridized to adm135H4 were isolated first; the ends of these clones were then used to identify clones containing adjacent pieces of the genome. By repeating this process, the walk was extended bidirectionally until it included 210 kilobases (kb) of genomic DNA (Fig. 1). The walk was oriented by in situ hybridization of cloned DNA to polytene chromosomes (*21*). We refer to locations in the walk relative to the Eco RI site nearest adm135H4, which has been assigned to position 0 kb. From this site, 80 kb extended toward the telomere (distally, negative direction in Fig. 1), and 130 kb extended toward the centromere (proximally, positive direction in Fig. 1).

We examined mutants by Southern analysis and in situ hybridization in order to localize the *Shaker* gene within the walk. Voltage-clamp experiments have shown that the X-Y chromosomal translocation T(1;Y)B55 reduces the amplitude of I_A in pupal muscle. The remaining I_A can be conferred by the proximal chromosomal fragment of T(1;Y)B55 alone (*9, 11*). Therefore, we expected that most or all of the structural information for the gene product should be proximal (relative to the centromere) to the location of the breakpoint in T(1;Y)B55. This breakpoint was localized, by in situ hybridization of genomic clones to translocation chromosomes and by analysis of genomic DNA, to a 1.0-kb Bgl II–Hind III fragment at position +34 kb in the walk (Fig. 1) (*22*).

We proceeded to analyze the DNA between positions +31 and +130 kb. The DNA from this region of the walk was used to probe blots containing genomic DNA from 16 *Shaker* mutants and the appropriate wild type parental strains (*23*). Four mutants contained detectable rearrangements absent in their parental lines. Two of these were the chromosomal translocations T(1;3)Sh^{LC} and T(1;Y)W32, which, along with T(1;Y)B55, had previously been used to identify the cytological position of the *Shaker* locus (*13*). The breakpoint of T(1;3)Sh^{LC} disrupted a 1.6-kb Hind III–Sac I

fragment at position +55 kb without affecting the adjacent fragments (Fig. 1). The breakpoint of T(1;Y)W32 fell within a 3.3-kb Eco RI fragment at position +95 kb (*24*) (Fig. 1). In situ hybridization of cloned DNA to translocation chromosomes confirmed that these rearrangements corresponded to the physical breaks observed cytologically (*24*).

Two other mutants, Sh^{K83f} and Sh^{K82a}, contained rearrangements not present in their parental lines (*25*). The Sh^{K83f} mutant contained 1.8 kb of DNA inserted into a 2.1-kb Bgl II–Xba I fragment at position +44 kb (Figs. 1 and 2A). Although Sh^{K83f} arose in a cross designed to mobilize P-element transposons (*26*), P-element DNA did not hybridize in situ to 16F on the Sh^{K83f} X chromosome. Therefore, we concluded that the insertion contained little, if any, P-element DNA. The Sh^{K82a} mutation, induced by gamma-irradiation, was mapped to a 1.3-kb Hind III–Sac II fragment at position +61 kb (*25*) (Figs. 1 and 2B). Probably Sh^{K82a} is an inversion of the proximal portion of the X chromosome with one breakpoint in 16F and one in the centromeric heterochromatin. (i) The cytology of Sh^{K82a} differed from that of wild type in that the region of 16F was drawn into the centromere, causing the proximal portion of the X chromosome to form a loop. The DNA proximal to the alteration shown in Fig. 2B hybridized in situ to the tip of this loop as it entered the centromere, an indication that this alteration corresponded to the cytological breakpoint in 16F. (ii) The mutation was not genetically linked to any autosome, nor did it alter the autosomes cytologically. (iii) The mutation suppressed recombination between *Shaker* and two flanking loci, *Bar* and *outstretched–small eye*, as expected of inversions or other large rearrangements (*27*).

No alterations were detected on Southern blots of genomic DNA of 12 other *Shaker* mutants (*23*); single base changes and small insertions or deletions might have escaped detection. In comparing wild-type strains, we observed two insertions and a small deletion in this region that did not appear to alter the A current in pupal muscle (*28*). The breakpoint of a translocation at 16F(5–8), T(1;Y)V7, was not covered by the chromosomal walk. Although T(1;Y)V7 flies show some physiological abnormalities (*13*), they do not shake, and they contain I_A in pupal muscle (*11*). The rearrangements found in the mutants Sh^{K83f}, T(1;3)Sh^{LC}, Sh^{K82a}, and T(1;Y)W32 all mapped proximal to the breakpoint in T(1;Y)B55, consistent with the expectation that the *Shaker* gene should be in this region of the genome. The mutations were not clustered at a particular location; rather, they were spread over 60 kb of DNA. Sh^{K83f}, T(1;3)Sh^{LC}, Sh^{K82a}, and T(1;Y)W32 were located at 10, 20, 27, and 60 kb proximal to the T(1;Y)B55 breakpoint, respectively (Fig. 1).

Isolation of cDNA clones from the *Shaker* locus. Because the mutations were widespread, cDNA libraries were screened with a large expanse of DNA to find the *Shaker* coding regions. Restriction fragments 1 to 6 kb long, containing all the genomic DNA from positions +30 kb to +120 kb, were isolated from phage or from plasmid subclones. These fragments were used individually or in mixtures to probe several cDNA libraries including one prepared from adult head polyadenylated RNA (*29*). From this library, three clones, ShA1, ShA2, and ShB1, were isolated on the basis of homology to a 1.7-kb Bam HI–Eco RI fragment at position +65 kb. A fourth clone, ShC1, was isolated on the basis of homology to a mixture of fragments spanning positions +72 kb to +88 kb. Clones ShA1, ShA2, ShB1, and ShC1 had inserts of approximately 2.9 kb, 2.5 kb, 2.6 kb, and 2.6 kb, respectively.

To locate potential exons, we probed Southern blots containing the DNA from positions +5 to +130 kb with the inserts of ShA1, ShA2, ShB1, and ShC1. All of the cDNA clones hybridized to four sites between positions +36 kb and +49 kb. Outside this cluster,

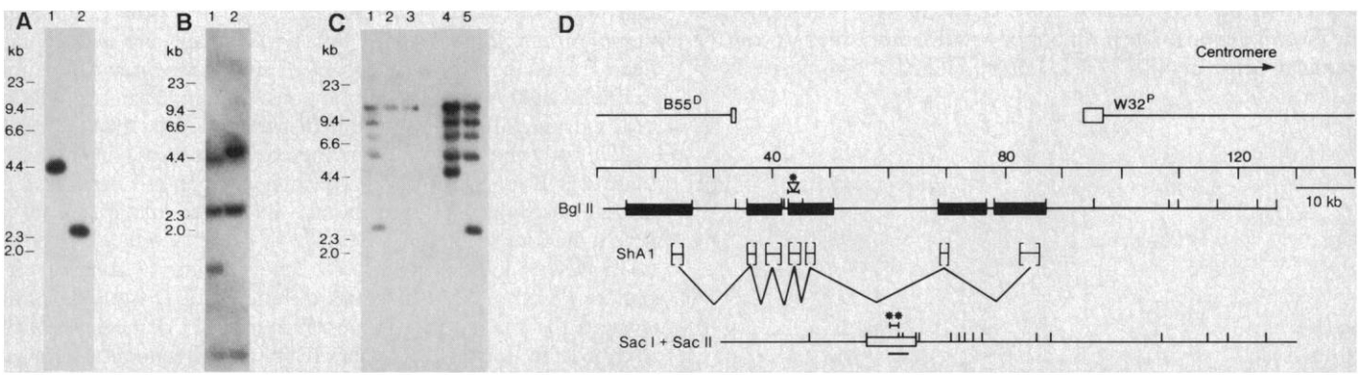

Fig. 2. Analysis of *Shaker* mutants and cDNA clone ShA1 on genomic Southern blots. (**A**) Sh^{K83f} contains an insert at position +42 kb. Genomic DNA from Sh^{K83f} flies (lane 1) and *garnet scalloped forked* flies (*g sd f*) (parental line, lane 2) was digested with Bgl II, blotted, and hybridized with a 2.5-kb Bgl II fragment near position +42 kb in the chromosomal walk. In *g sd f* DNA (lane 2), the 2.5-kb fragment labeled itself. In Sh^{K83f} DNA (lane 1), this fragment was shifted to 4.3 kb because of a 1.8-kb insertion. Genomic blots with other enzymes further narrowed the site of the insertion to the 2.1-kb Bgl II–Xba I fragment at position +42 kb [see asterisk in (D)]. (**B**) Mapping the breakpoint of Sh^{K82a}. Genomic DNA's from Sh^{K82a} flies (lane 1) and Oregon R flies (lane 2) were digested with Sac I and Sac II, blotted, and hybridized with a 3.2-kb Eco RI fragment near position +60 kb. For reference, a restriction map of Sac I and Sac II sites is provided at the bottom of (D); below the map, a line indicates the Eco RI fragment used as probe. As predicted by the map [open box in (D)] this probe labeled three fragments in wild-type DNA (lane 2), while in Sh^{K82a} DNA (lane 1) the 4.6-kb band was replaced by two new bands of 4.3 kb and 1.3 kb. These new bands represent *Shaker* DNA fused to heterologous flanking sequences at the two breakpoints of the inversion (see text). (**C**) To determine whether the cDNA ShA1 was encoded at 16F, a pUC subclone of ShA1 was used as the probe on a blot containing genomic DNA digested with Bgl II from (lane 1) Oregon R flies, (lanes 2 and 3) a deficiency of 16F (genotype $B55^D/W32^P$), (lane 4) Sh^{K83f}, and (lane 5) *g sd f* (parental line for Sh^{K83f}). In wild-type DNA (lanes 1 and 5), ShA1 hybridized to five fragments while in deficiency DNA (lanes 2 and 3) all that remained was a 12-kb band that was predicted to fall outside the deficiency [see (D)]. One 6-kb band that had been predicted from the map [see (D)] was not detected in wild-type DNA. This fragment was not strongly labeled by ShA1 even in cloned genomic DNA (it probably contains only small exons) and was therefore below the level of detection in the blot of total genomic DNA shown. In Sh^{K83f} DNA (lane 4), ShA1 hybridized to the same bands as in wild type, except that the 2.5-kb band was replaced by one of 4.3 kb, an indication that the insertion in Sh^{K83f} disrupted the same 2.5-kb Bgl II fragment that also contained a putative exon. (**D**) A schematic diagram is provided for reference. Genomic DNA is designated with the same system as in Fig. 1. The pattern of ShA1 hybridization to the walk is as in Fig. 3 and the six Bgl II fragments predicted to hybridize to ShA1 are shown as black boxes. The diagram also shows: (*), site of Sh^{K83f} insertion; (**), site of Sh^{K82a} breakpoint; a Sac I and Sac II restriction map; and (—) the 3.2-kb Eco RI fragment used as probe in (B). The distal fragment of T(1;Y)B55 ($B55^D$) and the proximal fragment of T(1;Y)W32 ($W32^P$) are also shown to indicate the region between them that is absent in the deficiency.

the cDNA clones were homologous to different, additional fragments so that the four clones showed different patterns of hybridization with potential exons between +24 and +89 kb (Fig. 3). Clones ShA1 and ShA2 appeared similar, differing only in their extent; ShA2 was shorter, lacking hybridization to the most proximal potential exon of ShA1. Both ShB1 and ShC1 exhibited distinct patterns that cannot be explained by differences in length; each of them lacked sites of hybridization shown by ShA1, and each hybridized to one or more additional sites not labeled by the other clones. ShB1 deviated from the ShA1 pattern only at its proximal end, whereas ShC1 differed from the ShA1 pattern only at its distal end (Fig. 3). Thus the four clones fell into three classes.

Three experiments were done to determine whether these cDNA clones were encoded in the region of the *Shaker* locus. (i) The cDNA clones were used as probes on Southern blots of total genomic DNA from wild-type flies and from flies containing a synthetic deficiency of the region between T(1;Y)B55 and T(1;Y)W32 (genotype $B55^{D}/W32^{P}$). The genomic fragments in wild-type DNA recognized by the cDNA clones had sizes appropriate to the patterns of hybridization in Fig. 3. Those fragments that should fall within the deficiency were absent from deficiency flies (Fig. 2C). (ii) In situ hybridization of ShA1 to polytene chromosomes labeled 16F exclusively (Fig. 4). (iii) The nucleotide sequences of the cDNA clones and partial sequences of genomic DNA from the *Shaker* locus have been determined, compared, and found to agree (*17, 30*). Therefore, we concluded that these four cDNA clones were encoded at the *Shaker* locus.

These results suggest that the *Shaker* gene has a large, primary transcript of at least 65 kb that may be alternatively spliced to produce mRNA's of several different classes. From the number and size of transcripts encoded at the *Shaker* locus, however, it appears that our cDNA clones do not represent all the *Shaker* transcripts and they may not be full length (*30*).

Relation between the locations of the *Shaker* mutations and the putative exons. The positions of putative exons suggest why the rearrangements in Sh^{K83f}, T(1;3)Sh^{LC}, and Sh^{K82a} eliminate I_A in pupal muscle (*11*) while other rearrangements are not correlated with changes in A current (*31, 32*). The insertion in Sh^{K83f} disrupted a 2.5-kb genomic fragment that contained a putative exon (Fig. 2C, lanes 4 and 5). Although revertants of Sh^{K83f} that have lost the insertion have not been isolated, the proximity of the insertion to a putative coding region supports the idea that this rearrangement was the cause of the *Shaker* phenotype. T(1;3)Sh^{LC} and Sh^{K82a} contained translocations that separated putative exons of the gene (Fig. 3).

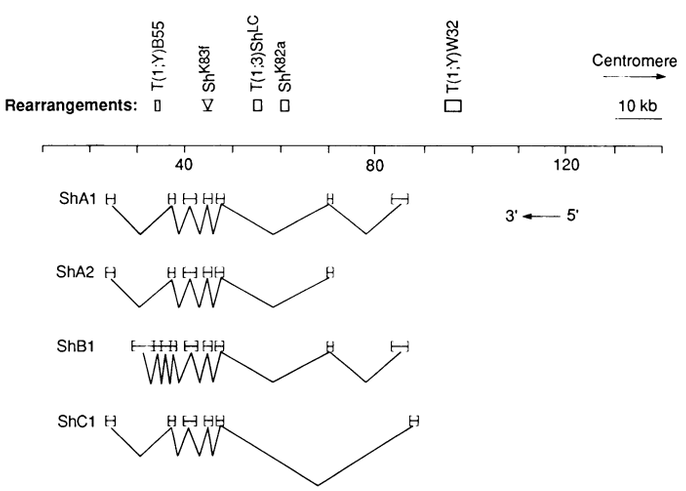

Fig. 3. Locations of putative exons. Southern blots containing cloned genomic DNA digested with restriction enzymes were probed with pUC subclones of ShA1, ShA2, ShB1, and ShC1. The four cDNA clones had different patterns of hybridization to the walk (scale as in Fig. 1); areas of hybridization ([—]) have been localized to the nearest identified restriction sites in the genome (Fig. 1). The restriction maps of the cDNA clones [figure 1 in (*17*)] were useful in identifying the locations of some potential introns (downward deflections). The bracketed fragments, however, do not represent a precise map of the exon structure of the cDNA clones. The potential exons were distributed over the same region that contained rearrangements in *Shaker* mutations (top).

Since electrophysiological experiments show that T(1;Y)B55 reduces but does not eliminate I_A in pupal muscle, it was surprising to find that all four of the cDNA clones hybridized to DNA distal to this breakpoint (Fig. 3). The carboxyl terminal 25 percent of a protein predicted by the sequences of ShA1 and ShA2 (*17*) would be encoded distal to the T(1;Y)B55 breakpoint. In contrast, the product corresponding to ShC1 may be encoded primarily or entirely proximal to the breakpoint (*30*). Therefore, the residual A current present in T(1;Y)B55 flies may be due to partially functional, truncated *Shaker* proteins, or to a subset of *Shaker* products encoded proximal to the breakpoint.

The breakpoint of T(1;Y)W32 was located about 6 kb beyond the most proximal putative exon we detected in the cDNA clones. Several explanations are possible for why this translocation eliminates I_A. (i) The cDNA clones may not be full length, so there may be additional exons proximal to the breakpoint. (ii) Since this is the 5′ end of the *Shaker* transcripts (*17*), 5′ elements required for expression of the gene may be affected by the rearrangement. (iii) Proximity to heterochromatin in translocations similar to T(1;Y)W32 has been shown to inhibit transcription at other loci (*33*).

Alternative splicing of the *Shaker* transcription unit. Because the cDNA clones hybridized to different patterns of genomic sites (Fig. 3), we suggest that the *Shaker* gene is a complex transcription unit subject to alternative exon splicing. For other genes, the multiple transcripts produced by alternative splicing may contain different protein coding sequences (*34*). In the *Shaker* gene, multiple products could represent homologous subunits that combine to form a channel. It is also possible that the splicing variants are part of different channel subtypes and thereby create subtle differences in A currents. If splicing variants are expressed in different tissues, they may also account for tissue-specific differences in the severity of some mutations (*11*).

The *Shaker* locus has provided an opportunity to apply the traditional and molecular genetic techniques of *Drosophila melanogaster* to the study of a voltage-dependent K^+ channel. We have described the isolation and characterization of genomic DNA and cDNA clones from the locus. The sequences of two of these cDNA

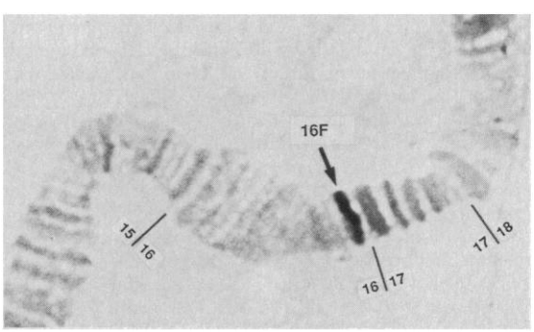

Fig. 4. The cytological location of cDNA ShA1. A biotinylated probe made to a pUC subclone of ShA1 was hybridized to polytene chromosomes from Oregon R flies (*21*). The only staining detected was at band 16F, the known cytological location of *Shaker* (*13*). The boundaries between regions 15, 16, 17, and 18 are indicated for reference.

clones (*17*) support the hypothesis that the gene product is a structural component of the A channel.

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20. Four libraries were used to isolate phage clones for the chromosomal walk at 16F. They contained genomic DNA cloned in lambda vectors as follows. (i) Canton S DNA in Charon 4A [T. Maniatis *et al.*, *Cell* **15**, 687 (1978)]. (ii) and (iii) Canton S and Oregon R DNA in EMBL4 (prepared in the laboratory of V. Pirrotta). (iv) Sh^{SB5} DNA in EMBL3 (prepared in our laboratory). The libraries were screened by standard procedures [T. Maniatis, E. F. Fritsch, J. Sambrook, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982)]. Library filters were hybridized with nick-translated probes in 50 percent formamide, 5× SSC, salmon sperm DNA (0.25 mg/ml), 5× Denhardt's 50 m*M* sodium phosphate (*p*H 6.5), and 0.1 percent SDS, at 42°C for 20 hours. Filters were washed at 50°C in three changes of a solution of 0.1 percent ×SSC, 0.1 percent sodium pyrophosphate, and 0.1 percent SDS for 1 hour each time. At position +30 kb, just distal to the T(1;Y)B55 breakpoint, there was a short stretch of genomic DNA underrepresented (reason undetermined) in genomic libraries. However, an Oregon R library in EMBL4 contained one phage, OR171 (Fig. 1), that allowed us to continue the walk.
21. For in situ hybridization to polytene chromosomes, we prepared chromosome squashes by the method of J. G. Gall and M. L. Pardue [*Methods Enzymol.* **21**, 470 (1971)]. Nick-translated, biotinylated probes [P. R. Langer-Sofer, M. Levine, D. C. Ward, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 4381 (1982)] were made with bio-16-dUTP (ENZO). Hybridization was carried out overnight at 37°C in 45 percent formamide, 2× SSC, 10 percent dextran sulfate. The squashes were subsequently washed at 37°C in 2× SSC, developed with the DETEK-HRP detection kit (ENZO), and counterstained with 5 percent Giemsa solution.
22. The position of the breakpoint in T(1;Y)B55 was determined by Southern analysis of genomic DNA from male flies hemizygous for T(1;Y)B55 or for a synthetic deficiency from this breakpoint to the breakpoint of T(1;Y)W32. T(1;Y)B55 flies lacked a 4.3-kb Eco RI–Hind III fragment (+34 to +38) and a 5.8-kb Bgl II fragment (+28 to +35). The breakpoint, therefore, appears to lie where these two fragments overlap (+34 to +35). We confirmed that all the DNA from this point proximal to the T(1;Y)W32 breakpoint (*24*) was absent in the synthetic deficiency but present in T(1;Y)B55 males. Frequently the new fragments that might be anticipated at the breakpoints of translocations were not observed or appeared very large. We presume that this is due to the absence of appropriate restriction sites in the heterochromatin adjoining the *Shaker* locus in these translocations. A Bgl II digest of T(1;Y)B55 DNA, however, revealed two new fragments: a 3.3-kb Bgl II fragment formed by the fusion of the proximal part of the X chromosome to the Y chromosome, and a fragment of more than 12 kb similarly formed from the distal part. In an X;Y translocation such as T(1;Y)B55, the Y chromosome causes the tip of each half of the X chromosome (the 16F region on either side of the breakpoint) to be drawn into the centromere. In situ hybridization to the translocated chromosomes (*21*) confirmed that the DNA rearrangement described above corresponded to the cytological breakpoint. A subclone of the distal-most 4 kb of OR171, which straddles the breakpoint, labeled the tips of each half of the X chromosome as they entered the centromere. Adjacent fragments labeled either the distal side or the proximal side, as appropriate.
23. Besides T(1;Y)B55, T(1;Y)W32, T(1;3)Sh^{LC}, T(1;Y)V7, Sh^{K82a}, and Sh^{K83f}, the other mutants examined were: (i) Sh^{rK0120} (*11*); (ii) Sh^{102}, Sh^{5}, and Sh^{K82b} (*25*) which are all derived from Canton S; (iii) Sh^{P} (*25*); (iv) Sh^{X}, isolated by B. Ganetzky in a *para*^{ts} background; (v) Sh^{SB1}, Sh^{SB2}, Sh^{SB3}, $Sh^{SB6\text{-}7}$, $Sh^{SB6\text{-}12}$, and Sh^{SB13} which were isolated in a π_2 background [L. Y. Jan *et al.*, *Cold Spring Harbor Symp. Quant. Biol.* **48**, 233 (1983)]. The physiology and genetics of these mutants have been described (*11*). The control strains whose DNA was also analyzed were Oregon R; Canton S; π_2; *para*^{ts}; *g sd f*; and two attached-X strains, one of which carried the *shi*^{ts} mutation and the other was marked with *y w f*.
24. DNA from male flies hemizygous for T(1;Y)W32 lacked the 3.3-kb Eco RI fragment (+95 to +98 kb) present in the X chromosomes of Oregon R, Canton S, and T(1;Y)V7 [a strain closely related to T(1;Y)W32]. The adjacent predicted Eco RI fragments were all present. The synthetic deficiency $B55^{D}/W32^{P}$ confirmed that this fragment is the site of the breakpoint in that the deficiency lacked all the DNA distal to this site until the T(1;Y)B55 breakpoint, but contained the appropriate fragments proximal to position +98 kb. Furthermore, by in situ hybridization to polytene chromosomes a cosmid derived probe corresponding to map positions +88 kb to +102 kb was shown to label both sides of the T(1;Y)W32 breakpoint.
25. Sh^{K83f}, Sh^{K82a}, Sh^{P}, and Sh^{K82b} were isolated by B. Ganetzky. The parental line for Sh^{K83f} and Sh^{P} was *g sd f*. We compared Sh^{K82a} and Sh^{K82b} DNA to each other and to DNA of Canton S flies from which both these alleles were derived. In Sh^{K82a}, besides the altered fragment at position +57 to +62 kb (Fig. 2B), a 5.8-kb Bam HI–Hind III fragment (position +61 to +67 kb) was replaced by a band or bands whose size was too large to measure accurately. This restricted the site of the breakpoint to the 1.3-kb Hind III–Sac II fragment at position +61 to +62 kb.
26. W. R. Engels, *Annu. Rev. Genet.* **17**, 315 (1983).
27. P. A. Roberts, in *The Genetics and Biology of Drosophila*, M. Ashburner and E. Novitski, Eds. (Academic Press, New York, 1976), vol. 1a, pp. 67–184.
28. An insert of approximately 4 kb was seen in T(1;Y)W32 and in T(1;Y)V7 in the 2.2-kb Eco RI–Hind III fragment at position +29 to +32 kb. Another insert was found on the *g sd f* chromosome and its derivatives Sh^{K83f} and Sh^{K82b}, and on the *shi*^{ts} attached-X chromosome. This insert was 2 kb and fell within a 5.6-kb Hind III fragment at positions +55 to +61 kb. A small deletion in these same chromosomes may occur at position +115, as indicated by the absence of a Sac I site and 0.5 kb of DNA from a 3.1-kb Eco RI fragment.
29. Two cDNA libraries were screened extensively: a pupal library [S. J. Poole, L. M. Kauvar, B. Drees, T. Kornberg, *Cell* **40**, 37 (1985)] and an adult head library [N. Itoh, P. Salvaterra, K. Itakura, *Drosophila Information Service* **61**, 89 (1985)]. Plaques (2×10^6) were screened from each library with individually labeled fragments [2.5×10^5 cpm per fragment per milliliter of 2× SSC, salmon sperm DNA (0.25 mg/ml), 10× Denhardt's, 0.1 percent SDS, 65°C for 16 hours; filters were washed at 65°C with four or five 1-hour rinses in a solution of 0.5× SSC and 0.1 percent SDS].
30. T. L. Schwarz, B. L Tempel, D. M. Papazian, Y. N. Jan, L. Y. Jan, in preparation.
31. Between positions −20 kb and 0 kb, the Oregon R genome contained a 7-kb segment of DNA repeated in tandem. The two copies were homologous to one another and had identical restriction maps. This region appeared to be a hot spot for rearrangement of genomic DNA. Nine strains of *Shaker* and wild-type flies were compared. Among these, five variations of the tandem repeat region were found, including insertions of transposable elements and deletion of one copy of the repeat. In general, the patterns of alterations were not correlated with the *Shaker* phenotype, but in one case a recently derived allele, Sh^{X}, contained a 3-kb insert absent from its parental strain, *para*^{ts}. This insertion may reflect the susceptibility of this region to rearrangements, or the insertion may be related to the Sh^{X} phenotype.
32. Two of the three polymorphic rearrangements that do not seem to correlate with a change in pupal A current (*28*) fall in regions that are not likely to affect the predicted transcripts. One (at +115 kb) is 26 kb more proximal than the most proximal predicted exon and the other (at +55 to +61 kb) fell within a large predicted intron. However, the third rearrangement, a 4-kb insert at +29 to +32 kb, falls close to an apparent exon at the end of ShB1. This insert was observed in both T(1;Y)W32 and T(1;Y)V7 flies (*28*) and, although it cannot explain the absence of A current in T(1;Y)W32, it could account for the more subtle physiological abnormalities of T(1;Y)V7 (*11, 13*).
33. J. B. Spofford, in *The Genetics and Biology of Drosophila*, M. Ashburner and E. Novitski, Eds. (Academic Press, New York, 1976), vol. 1C, pp. 955–1018.
34. S. E. Leff, M. G. Rosenfeld, R. M. Evans, *Annu. Rev. Biochem.* **55**, 1091 (1986).

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