There's a Whole Lot of Shaking Going On

Molecular neurobiology is a field whose time has come. Thanks to advances in recombinant DNA techniques, investigators are for the first time getting a glimpse at the molecular hardware responsible for electrical signaling in the nervous system. Considerable interest has focused on ion channels—transmembrane proteins that permit the selective permeation of particular species of ions (e.g., Na⁺, Ca²⁺ or K⁺, depending on the channel type), thereby mediating action potentials and synaptic transmission in neurons. Until recently, the molecular details of the structure and function of these proteins were largely inaccessible. Now, by using toxins and other ligands that bind to certain types of ion channels with very high affinity and specificity, it has been possible to purify some of these proteins, obtain a partial amino acid sequence and subsequently isolate cDNAs from which the entire primary sequence has been deduced. This method has worked well for the study of several different channels, including sodium and calcium channels (see review by Catterall 1988). But what about those channels that cannot be readily purified?

A biochemical method that can overcome some of these difficulties takes advantage of the expression of functional channels in Xenopus oocytes injected with the appropriate mRNA. Using this system it is possible, though laborious, to identify a cDNA whose transcript when injected into oocytes directs the production of the channel of interest (Lübbert et al. 1987; Julius et al. 1988).

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Fortunately, genetics offers still another option. In an organism such as Drosophila melanogaster, mutations can be identified that disrupt the structure or function of ion channels and the corresponding genes can be cloned without any a priori information about the encoded product. Another important feature that distinguishes the genetic approach from all biochemical strategies is that the analysis of mutant phenotypes can provide insight into the biological role of a particular channel type in the nervous system. For example, what are the in vivo consequences for the function and development of the nervous system when the activity of a particular ion channel is eliminated or altered?

The recent cloning and characterization of the Drosophila Shaker (Sh) locus represents the first successful application of this genetic strategy to the study of ion channels. The success of this approach, the demonstration that Sh represents a potassium channel structural gene and the surprises that fell out of these studies have therefore generated considerable excitement among neurobiologists (including many who harbored initial doubts about the potential contribution of Drosophila genetics to neurobiology).

In February of 1969, when Kaplan and Trout published a description in Genetics of four behavioral mutants of D. melanogaster whose most distinctive phenotype was a rapid leg shaking under ether anesthesia, they could hardly have anticipated all the fanfare that would surround one of these genes 20 years later. The mutations, which were discovered fortuitously in a screen for X-linked lethals, identified three different loci: Shaker (Sh), Hyperkinetic (Hk) and ether à go-go (eag). Hk and eag represented previously undescribed genes. The first Sh mutation in D. melanogaster had been isolated earlier by Catsch (1944) and resembled one first described by Luers in Drosophila funebris (1936). However, there was little work on these mutants and the stocks appear to have been lost in the years before Kaplan and Trout reported their studies.

By 1969 a fledgling field fusing genetics with neurobiology was beginning to emerge in which Sh and related mutants would soon find a happy niche. In 1967, Benzer was charting the way by isolating behavioral mutants in Drosophila using a countercurrent apparatus. Also in Drosophila, PAK was embarking on a genetic dissection of visual transduction (PAK, 1989).
and pharmacological evidence suggested a defect in adigm permitted more straightforward assays by an extracellular electrode. This experimental paradigm suggested that
potassium channels (referred to as isolated by KAPLAN and TROUT, is a unique allele that OUYE, FERRUS and FUJITA 1981). Further experiments showed that IKEDA and KAPLAN (1970) pioneered the application of electrophysiological techniques to Drosophila to characterize abnormal electrical activity in the motor neurons in Hk mutants. Although these experiments helped to demonstrate that Drosophila was not hopelessly small for such studies, the methods were technically demanding; few if any investigators could match IKEDA’s skill in penetrating the small neurons of the adult thoracic ganglion with an intracellular electrode. In another breakthrough, JAN and JAN (1976) introduced the use of the Drosophila larva for electrophysiological studies. The body wall muscles in mature third-instar larvae are large single cells that can readily be impaled with glass microelectrodes and the nerves innervating these muscles can be stimulated by an extracellular electrode. This experimental paradigm permitted more straightforward assays of electrical signaling in the Drosophila nervous system in mutant and wild-type larvae (JAN, JAN and DENNIS 1977; WU et al. 1978).

The first mutant analyzed was an allele of the Sh locus isolated in BENZER’s laboratory. JAN, JAN and DENNIS (1977) demonstrated abnormally prolonged release of neurotransmitter at the larval neuromuscular junction in this mutant, owing to the failure of the nerve terminal to repolarize normally following an electrical stimulus. A variety of electrophysiological and pharmacological evidence suggested a defect in potassium channels (JAN, JAN and DENNIS 1977; TANOUYE, FERRUS and FUJITA 1981). Further experiments using more sophisticated electrophysiological methods provided direct evidence that a particular class of potassium channels (referred to as I\textsubscript{\alpha} channels) was altered in Sh mutants (SALKOFF and WYMAN 1981; WU and HAUGLAND 1985).

Phenotypic analysis of a collection of mutant alleles suggested that Sh was probably a structural gene for I\textsubscript{\alpha} potassium channels. Several alleles cause the total elimination of the A current, others a partial loss. Sh\textsuperscript{\beta}, isolated by KAPLAN and TROUT, is a unique allele that alters the kinetic and voltage-dependent properties of the current (SALKOFF and WYMAN 1981; WU and HAUGLAND 1985). The genetic and electrophysiological studies of Sh mutants thus provided a strong impetus to pursue molecular analysis of this gene.

Chromosome rearrangements that are now known to disrupt the Sh coding region (including an inversion and several translocations) and a Sh deletion provide additional information about the Sh phenotype (TANOUYE, FERRUS and FUJITA 1981; TIMPE and JAN 1987). Although these mutations completely eliminate the A current in muscle cells, the flies are viable when homozygous. Thus, Sh is not an essential gene. When heterozygous, these null mutations partially reduce I\textsubscript{\alpha} and produce a dominant leg-shaking phenotype. That known loss-of-function Sh alleles have a dominant effect on leg shaking permits the inference that the dominance or semidominance of all other Sh alleles with respect to this phenotype also results from complete or partial loss of function. Nonetheless, how a reduction or loss of the A current produces the leg-shaking behavior remains unexplained.

Most importantly, the chromosome rearrangements with breaks in the Sh region enabled precise cytological mapping of the Sh locus to polytene bands 16F1-4 on the X chromosome (TANOUYE, FERRUS and FUJITA 1981). This information proved essential for cloning Sh. Three groups independently cloned the genomic region containing this locus by chromosome walking using an unrelated cDNA that mapped to region 16F (KAMB, IVERSON and TANOUYE 1987; PAPAZIAN et al. 1987; BAUMANN et al. 1987). Sh alleles associated with chromosome rearrangements or transposon insertions were localized on the molecular map and found to span a region of about 60 kb, indicating that the Sh transcription unit was potentially quite large. Isolation of cDNAs from the Sh region confirmed this conclusion. When genomic DNA from the chromosome walk was probed with the Sh cDNAs, the cDNAs hybridized to portions that spanned more than 65 kb and encompassed the sites of all the Sh mutations that had been localized on the molecular map.

The deduced amino acid sequence left little doubt that Sh encoded a structural component of potassium channels (KAMB, IVERSON and TANOUYE 1987; TEMPEL et al. 1987; BAUMANN et al. 1987). The predicted protein contains six potential membrane-spanning domains, a characteristic feature of other channel proteins. Remarkably, the Sh protein also contains one region of high sequence similarity to the vertebrate sodium and calcium channels. This region (the “S4 domain”) consists of a stretch of 22 amino acids in which positively charged arginine residues are spaced at every third position and are separated by hydrophobic residues. It has previously been suggested that the S4 domains represent the voltage sensors by which the sodium and calcium channels respond to voltage changes across the membrane (see review by CATTERALL 1988). The discovery that potassium channels share this motif with sodium and calcium channels supports the notion that all voltage-dependent ion channels share a common mechanism of activation as well as a common evolutionary origin, as originally
proposed by Hille (1984). One important distinction between the Sh protein vs. sodium and calcium channels is that the latter comprise four internally homologous domains each containing six apparent membrane spanning regions (see review by Catterall 1988). In contrast, the Sh product does not contain these internally duplicated regions and appears to correspond to just a single homology unit of the sodium or calcium channel polypeptides. Proof that Sh cDNAs encode a potassium channel was provided by the demonstration that expression of these cDNAs in Xenopus oocytes results in the production of a voltage-dependent potassium current with appropriate kinetic and voltage-dependent properties (Timpe et al. 1988; Iverson et al. 1988).

Perhaps the most surprising result is the discovery that differential splicing of the primary Sh transcript apparently generates at least ten, and perhaps more, distinct products (Schwarz et al. 1988; Kamb, Tseng-Crank and Tanouye 1988; Pongs et al. 1988). The general pattern consists of variable 3' and 5' ends spliced onto a constant central portion (which contains all the membrane-spanning regions) to yield distinct cDNAs encoding different but functionally related proteins. Several of these cDNAs have been individually expressed in Xenopus oocytes and each gives rise to an I_A-type potassium current but with somewhat different physiological properties (Timpe et al. 1988; Iverson et al. 1988). It thus appears that alternative splicing is one mechanism generating the diversity of potassium channels in the nervous system.

It remains unknown when and where these potentially different forms of Sh protein are expressed in vivo and whether they each subserve distinct biological roles. Also unknown is the subunit composition of I_A channels in vivo. Because the Sh products, in contrast to sodium and calcium channels, do not contain four repeat segments within the same polypeptide, it is likely that a multimeric (perhaps tetrameric) assembly of polypeptide subunits is required to produce functional I_A channels. Whether such a multi-subunit assembly is homomultimeric or heteromultimeric in composition remains to be determined.

The Sh cDNAs have also provided the first molecular probes for potassium channel genes in vertebrate organisms. By screening with a probe derived from a Sh cDNA, Temple, Jan and Jan (1988) isolated a cDNA from mouse brain that specified an amino acid sequence very similar to that of the Sh protein. Similarly, five cDNAs, which have distinct but closely related nucleotide sequences and apparently represent different genes, were isolated from rat brain on the basis of their homology to Sh probes (A. Baumann and O. Pongs, personal communication). The amino acid sequences specified by these cDNAs show remarkably high conservation to each other and to the Sh protein. So far there is no evidence that the transcripts from any of these mammalian genes undergo alternative processing to generate a number of distinct products, as occurs with Sh. One possibility is that the diversity of I_A channels in mammalian systems is generated by a large multigene family rather than by multiple products from one or a few genes.

Although they have yet to achieve the superstar status of Sh, the other loci identified by Kaplan and Trout are also proving to be interesting. Electrophysiological studies of eag (Wu et al. 1983; Ganetzky and Wu 1988) and Hk (Stern and Ganetzky 1989) indicate that they, too, disrupt potassium currents, leading to neuronal hyperexcitability. Molecular analysis of these loci is now in progress (R. Drysdale, K. Schlimmen and B. Ganetzky, unpublished data) and should further advance our understanding of potassium channels in Drosophila and other organisms.

In the opening paragraph of their paper, Kaplan and Trout remark that the leg-shaking mutants were recovered serendipitously. Serendipity in this case was probably essential because it is doubtful that anyone back in 1969 would have thought to screen deliberately for so peculiar a mutant phenotype. Yet, it is to their credit that Kaplan and Trout were sufficiently observant to notice these mutants while looking for something else and sufficiently prescient to recognize their potential importance. Were it not for the attention they drew to their funny flies, we might still have no idea what a potassium channel looks like or how to get our hands on one.

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LITERATURE CITED


