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A Family of Three Mouse Potassium Channel Genes with Intronless Coding Regions

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To understand the molecular mechanisms responsible for generating physiologically diverse potassium channels in mammalian cells, mouse genomic clones have been isolated with a potassium channel complementary DNA, MBK1, that is homologous to the *Drosophila* potassium channel gene, *Shaker*. A family of three closely related potassium channel genes (*MK1*, *MK2*, and *MK3*) that are encoded at distinct genomic loci has been isolated. Sequence analysis reveals that the coding region of each of these three genes exists as a single uninterrupted exon in the mouse genome. This organization precludes the generation of multiple forms of the protein by alternative RNA splicing, a mechanism known to characterize the *Drosophila* potassium channel genes *Shaker* and *Shab*. Thus, mammals may use a different strategy for generating diverse K^+ channels by encoding related genes at multiple distinct genomic loci, each of which produces only a single protein.

SEVERAL CLASSES OF K^+ CHANNELS are involved in maintaining membrane potential and regulating cell volume in diverse cell types, as well as modulating electrical excitability in the nervous system (*1*). The molecular mechanisms generating K^+ channel diversity are best understood in the *Shaker* locus from *Drosophila*, which contains 21 exons spanning 130 kb and generates four different K^+ channel proteins through alternative splicing of a single primary transcript (*2*). Expression of these cDNAs in *Xenopus* oocytes gives rise to voltage-dependent K^+ currents with distinct physiological properties (*3*). The related *Drosophila* K^+ channel gene *Shab* also exhibits alternative splicing of a primary transcript, giving rise to two distinct proteins (*4*). To determine if similar mechanisms are responsible for K^+ channel diversity in mammals, we have isolated and characterized eight mouse genomic clones that contain three related K^+ channel genes (*5–7*), all of which hybridize with the cDNA MBK1 (mouse brain K^+ channel 1) (*8*).

Southern (DNA) transfers (*9*) of Hind III–digested liver DNA from BALB/c mice were hybridized with a 1695-bp probe derived from the MBK1 cDNA that contained the entire 1485-bp coding region (*8*). Three Hind III fragments (8.6 kb, 3.4 kb, and 2.0 kb) hybridized strongly with this probe (Fig. 1). These bands may represent multiple exons, as in the *Shaker* locus (*2*), or each band could represent a unique K^+ channel gene. Shorter probes representing the 5′ and the 3′ portions of the MBK1 coding region (0.5 and 1.1 kb, respectively) yielded a hybridization pattern identical to that obtained with the full-length 1.6-kb probe, suggesting that each of the multiple bands may contain a sequence corresponding to the full-length MBK1 coding region.

To further study the genomic organization of these putative K^+ channel genes, we screened a mouse genomic library (*5, 6*) with the full-length MBK1 cDNA probe (*8*). Of the eight genomic clones characterized thus far (*5, 6*), four, containing hybridizing Hind III fragments identical in size to those in Fig. 1, are described here: KC250, which contains a 2.0-kb hybridizing fragment; overlapping clones KC238 and KC255 (KC238/255), which contain a 3.4-kb hybridizing fragment; and identical clones KC236 and KC237, which contain an 8.6-kb hybridizing fragment within a 12-kb insert.

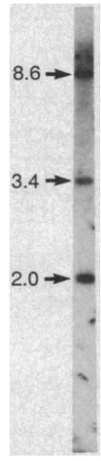

Fig. 1. Southern blot analysis of genomic DNA from BALB/c mice. Liver DNA (10 μg) from BALB/c mice was digested with Hind III, separated on a 0.9% agarose gels, and transferred to nitrocellulose papers by standard procedures (*9, 22*). Southern blots were hybridized with ^{32}P-labeled MBK1 cDNA probes. We used the 0.5-kb Sma I fragment and the 1.1-kb Sma I–Hind III fragments of the MBK1 cDNA (*8*), labeled to a specific activity of 1×10^9 cpm/μg with the random-primer method of Feinberg *et al.* (*23*). Hybridization was performed at 55°C in hybridization buffer for 16 to 18 hours. Hybridization buffer is 5× SSC (*5*), 10× Denhardt's solution [0.2% bovine serum albumin, 0.2% Ficoll and 0.2% polyvinyl pyrrolidone], and 0.1% SDS. The blot was washed at 55°C at a final stringency of 0.2× SSC and 0.1% SDS for 60 min. Exposure time for this autoradiogram was 10 days with an intensifying screen.

We determined the order of restriction fragments of these genomic clones (Fig. 2) by partial digests, by sequencing (*10*), and by aligning the genomic sequence with that of their homologous cDNA sequences. The restriction map of clone KC250 is identical to that of the MBK1 cDNA (*8*) and is similar to the map of the RCK1 (*11*) (the rat cortical K^+ channel 1, a homolog of MBK1) cDNA, in the coding region as well as in the nearby noncoding regions (NCR). The restriction map of the KC238/255 insert is very similar to the map of the RBK2 (rat brain K^+ channel 2) cDNA (*12*). This suggests that KC250 and KC238/255 encode *MK1* and *MK2*, respectively; KC237 appears to encode a related K^+ channel gene.

Partial nucleotide sequences (*10*) of clones KC250 (2222 bp), KC238/255 (3383 bp), and KC237 (2000 bp) were determined. The KC250 sequence is virtually identical to the published MBK1 sequence (*8*) throughout its length (there are additional G's at positions 162 and 442 in the 5′ untranslated region), and is 97.3% identical to RCK1 within the putative coding region (*11*). The entire coding region for this gene, *MK1*, is contained in a single uninterrupted exon of 1485 bp. The 5′ and 3′ NCRs of *MK1* are 91.4% similar to RCK1 (*11*), indicating that the NCRs of *MK1/RCK1* are fairly well conserved. Comparison of KC238/255 with the coding region of RBK2 cDNA reveals 97% sequence identity and shows that the entire coding region of this second gene (*MK2*) is also contained in a single exon of

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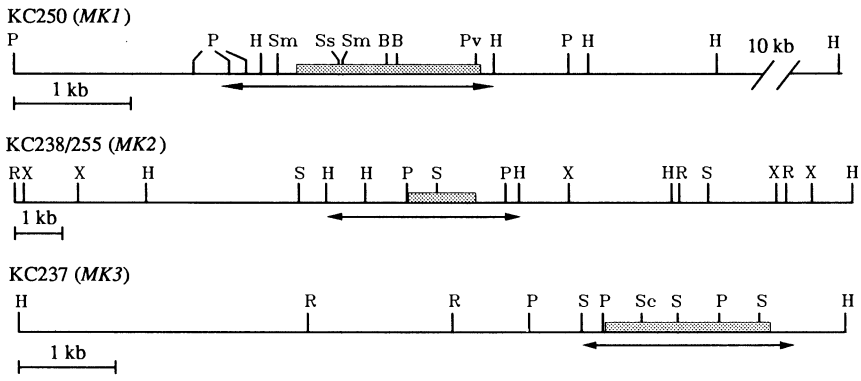

Fig. 2. Restriction maps of the mouse genomic clones containing the genes *MK1*, *MK2*, and *MK3*. The top line represents a portion of the KC250 genomic clone (containing *MK1*). The restriction maps of mouse genomic clones KC238/255 (containing *MK2*) and KC237 (containing *MK3*) are shown in the middle and bottom lines, respectively. B, Bal I; H, Hind III; P, Pst I; Pv, Pvu II; R, Eco RI; S, Sac; Sca, Sca I; Sm, Sma I; Ss, Sst I; and X, Xba I. Additional regions of DNA are present in each clone between the ends of the maps shown and the lambda J1 Mbo I cloning sites. One additional Pst I site is present in the 1.1-kb KC250 Hind III fragment, but has not been mapped.

1497 bp; the NCR sequences of *MK2* and RBK2 cDNA (*12*) share 94.1% sequence identity. Alignment of the KC237 sequence with *MK1* and *MK2* indicates that the coding region of the third member of this family (*MK3*) is also encoded by a single exon. Thus, *MK1*, *MK2*, and *MK3* represent a family of K^+ channel genes with intronless coding regions; such intronless mammalian genes have been described for the muscarinic, 5-hydroxytryptamine (5-HT_{1A}), and β-adrenergic receptors (*14*).

These three K^+ channel genes are closely related; *MK2* and *MK3* share 72.1% and 71.6% coding region sequence identity with *MK1*, and 68.9% with each other. However, the nearby NCRs of *MK1*, *MK2*, and *MK3* do not share any appreciable sequence identity with each other, although they are fairly well conserved when compared with their rat counterparts.

The deduced *MK1*, *MK2*, and *MK3* proteins contain 495, 499, and 530 amino acids, respectively (Fig. 3). Interestingly, MK3 closely resembles the amino acid sequence of the RCK3 cDNA (*12*). The protein sequences and the hydropathy profiles of *MK1*, *MK2*, and *MK3* proteins suggest that these proteins include six membrane-spanning segments. These putative transmembrane helices are designated S1 through S6, in keeping with the terminology proposed for the *Shaker* K^+ channels (*2, 15*) and the voltage-gated Na^+ channel (*16*). In this model, the NH_4- and COOH-termini are located on the cytoplasmic side of the cell membrane, and the loops linking S1 with S2, S3 with S4, and S5 with S6 are extracellular. Comparison of the amino acid sequences of *MK1, MK2*, and *MK3* reveals remarkable conservation within the transmembrane helices as well as in the S4/S5 cytoplasmic loop (Fig. 3). Sequences of the other four loops as well as the intracellular NH_4- and COOH-termini show greater differences among these three genes (Fig. 3), although a substantial portion of the NH_4-terminus appears to be highly conserved.

Alignment of the genomic restriction map and sequence of *MK1* with MBK1 (*8*) and RCK1 (*11*) cDNA suggests that at least 5.5-kb of the 8-kb *MK1* transcript (*8*) is uninterrupted in the genome, including 1.5-kb of 5′ NCR and 2.1-kb of 3′ NCR [this represents the overlapping region from position −530 of MBK1 cDNA to the 3′ end of RCK1 cDNA (*8, 11*)]. The region from −898 to −530 in *MK1* appears to represent an intron in the 5′ NCR that yields both spliced and unspliced mature mRNA (*8*) RBK2 has a large 9.5-kb transcript (*12*), indicating that this gene also has extensive 5′ and 3′ NCRs. Comparison of the *MK2* sequence with that of RBK2 cDNA suggests that *MK2* also may contain an intervening sequence in its 5′ NCR. Splice donor and acceptor sites (*17*) for the putative intron in *MK2* are present at positions −591 and −159, and an additional possible splice acceptor is located at position −920. *RGK5* (a rat homolog of *MK3*) also has a large 9.5-kb transcript that is abundantly expressed in the thymus, and when expressed in *Xenopus* oocytes (*18*) has biophysical properties closely resembling the T lymphocyte voltage-gated type *n* K^+ channels we have previously described (*19*).

The diversity of vertebrate K^+ channels must be generated in a manner different from that described in flies. In *Drosophila*, multiple transcripts are generated by alternative splicing of more than 21 exons spanning at least 130 kb of genomic DNA (*2*) within the *Shaker* K^+ channel locus, generating at least four distinct proteins (*3*). The related K^+ channel gene *Shab* also exhibits alternative splicing of a primary transcript giving rise to two proteins (*4*). In contrast, the vertebrate K^+ channel genes *MK1* to *3*, *RGK5* [rat genomic homolog of *MK3* (*18*)], and *Xsha1* [*Xenopus* homolog of *MK2* (*20*)] are encoded by single exons and cannot

```
MK1  MTVMSGENADEASTAPGHPQDGSYPRQADHD                          DHECCERVVINISGLRFET  70
MK2  ---AT-DPV---AAL------TYD-EA                            --------------------
MK3  ---VP-DHLL-PEA-G-GGG-PPQGGCGSGGGGGGCDRYEPLPPALPAAGEQDC-G--------------

MK1  QLKTLAQFPNTLLGNPKKRMRYFDPLRNEYFFDRNRPSFDAILYYYQSGGRLRRPVNVPLDMFSEEIKFY 140
MK2  ---------E----D-------------------------------------------I-----R--
MK3  -----C---E----D--R-----------------------------------I-------I-I-----R--
                                                          S1
MK1  ELGEEAMEKFREDEGFIKEEERPLPEKEYQRQVWLLFEYPESSGPARVIAIVSVMVILISIVIFCLETLP 210
MK2  --------M------Y----------N-F-----------------I--------------S-------
MK3  Q---------------LR-------RRDF-----------------G------L---------------
                                                 S2
MK1  ELKDDKDFTGTIHRIDNTTVIYTSNI        FTDPFFIVETLCIIWFSFELVVRFFACPSKTDFFKNI 280
MK2  IFR-ENEDMHGGGVTFH-YSNS-IGYQQST  S-------------------FL---------AG--T--
MK3  -FR-E--YPASPSQDVFEAANNSTSGAPSGASS-S----V-------------L---------AT-SR--
           S3                                        S4
MK1  MNFIDIVAIIPYFITLGTEIAEQ EGNQKGEQATSLAILRVIRLVRVFRIFKLSRHSKGLQILGQTLKAS 350
MK2  --I----------------L--KP-DA-Q-Q--M-----------------------------------
MK3  --L----------------L--RQGNG-    --M-----------------------------------
              S5
MK1  MRELGLLIFFLFIGVILFSSAVYFAEAEEAESHFSSIPDAFWWAVVSMTTVGYGDMYPVTIGGKIVGSLC 420
MK2  -------------------------D-RD-Q-P--------------------V-T-----------
MK3  ---------------------A-----DDPS-G-N-----------T---------H-----------
       S6
MK1  AIAGVLTIALPVPVIVSNFNYFYHRETEGEEQAQLLHV SSPNLASDSDL  SRRSSSTISKSEYMEIEE 490
MK2  ---------------------------------Y-Q-T-C-KIP-SP-- KKS--A------D----Q-
MK3  ---------------------------------YM--G-CQH-S-SAEELRKA--N--L------V---

MK1  D MNNSIAHYRQANIR  TGNCTTADQN  CVNKSKLLTDV*
MK2  G V---NEDF-EE-LK  -A---L-NT-  Y--IT-M----*
MK3  GG--Q-AFPQTPFKTGNS-AT---NNNPNS---IK-IF---*
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Fig. 3. Alignment of the deduced amino acid sequences of MK1, MK2, and MK3. Gaps have been introduced for alignment. Putative transmembrane segments, S1 to S6, are indicated by solid lines. The extent of each segment was based on the models for *Shaker* and mammalian K^+ channels (*2, 8, 11, 12, 15*).

therefore produce multiple forms of the protein by alternative RNA splicing. From an evolutionary standpoint, it is interesting that for both K^+ channels and muscarinic acetylcholine receptors, the *Drosophila* protein-coding regions are interrupted by introns (*2, 21*), whereas the corresponding vertebrate proteins are encoded by single exons (*10, 18, 20*).

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Transplanted Suprachiasmatic Nucleus Determines Circadian Period

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The pacemaker role of the suprachiasmatic nucleus in a mammalian circadian system was tested by neural transplantation by using a mutant strain of hamster that shows a short circadian period. Small neural grafts from the suprachiasmatic region restored circadian rhythms to arrhythmic animals whose own nucleus had been ablated. The restored rhythms always exhibited the period of the donor genotype regardless of the direction of the transplant or genotype of the host. The basic period of the overt circadian rhythm therefore is determined by cells of the suprachiasmatic region.

THERE IS CONSIDERABLE EVIDENCE to suggest that the suprachiasmatic nucleus (SCN) of the hypothalamus is the site of circadian pacemaker cells that generate overt circadian rhythms in mammals. The evidence that supports this view is diverse. (i) The SCN is the target of direct and indirect retinal projections required for entrainment of circadian rhythms to environmental cycles (*1, 2*). (ii) The SCN exhibits strong circadian rhythms of glucose utilization in vivo (*3*). (iii) Ablation of the SCN or its surgical isolation within the brain eliminates overt behavioral rhythmicity (*4–6*) and rhythmic electrical activity in the brain (*7*). (iv) Tissue explants containing the SCN continue to express circadian rhythms in electrical activity (*8, 9*) and vasopressin release (*10*) in vitro. (v) Circadian rhythmicity can be restored to SCN-lesioned arrhythmic hosts by implantation of fetal brain tissue containing SCN cells (*11–14*).

Despite this evidence, however, the pacemaker role of the SCN circadian oscillator has not been confirmed. In addition, the role of the nucleus has come into question because methamphetamine given on a long-term basis to arrhythmic, SCN-lesioned rats will restore circadian rhythmicity (*15*). Moreover, in the rat (*16*) and in lower vertebrates (*17*), structures outside the SCN are able to generate circadian rhythms.

Although in the aggregate the evidence is compelling, final proof that the SCN is the site of a central driving oscillator for mammalian circadian systems requires that characteristics of the overt rhythm such as phase and period be unambiguously attributable to the activity of SCN cells. The discovery of the τ mutation in hamsters provided the opportunity to test directly the pacemaker role of the SCN by tissue transplantation. The mutation has the primary behavioral effect of reducing the period of the circadian rhythm from 24 hours to about 22 hours in heterozygotes and to about 20 hours in homozygotes (*18*). If the SCN drives overt behavioral rhythmicity in hamsters, then the period of the rhythm that is restored by SCN transplantation should reflect the genotype of the donor tissue and not that of the lesioned host.

All animals used in these experiments were raised in our colony, and only male animals were used as hosts. These were placed in running wheel cages for activity recording after reaching 8 weeks of age and were kept in constant dim light or constant dark for the duration of the experiment. After the period of the host rhythms had been established (7 to 21 days), animals were anesthetized and placed in a Kopf model 900 stereotaxic instrument for SCN ablation. Lesions were made by current in-

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