Since the phenomenon of self-MHC restriction was first described, positive selection had been inferred to be the mechanism, but the evidence was always indirect. Now positive selection has been formally demonstrated. The mechanism involves the interaction of TCR chains, accessory molecules, and MHC during thymus development.

Note added in proof: Recently, positive selection has been proposed to explain the correlation between levels of T cells bearing $V_\beta 6^+$ TCR and MHC class II I-E molecules in MHC congenic and F_1 mice (*24*).

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32. We thank R. Kubo, T. Potter, S. Kimura, and B. J. Fowlkes for antibodies and advice; A. Pullen for discussion; E. Kushnir, R. Richards and W. Townend for technical assistance; and D. Thompson for assistance in preparation of the manuscript.

29 November 1988; accepted 16 February 1989

Chromosome 17 Deletions and p53 Gene Mutations in Colorectal Carcinomas

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Previous studies have demonstrated that allelic deletions of the short arm of chromosome 17 occur in over 75% of colorectal carcinomas. Twenty chromosome 17p markers were used to localize the common region of deletion in these tumors to a region contained within bands 17p12 to 17p13.3. This region contains the gene for the transformation-associated protein p53. Southern and Northern blot hybridization experiments provided no evidence for gross alterations of the p53 gene or surrounding sequences. As a more rigorous test of the possibility that p53 was a target of the deletions, the p53 coding regions from two tumors were analyzed; these two tumors, like most colorectal carcinomas, had allelic deletions of chromosome 17p and expressed considerable amounts of p53 messenger RNA from the remaining allele. The remaining p53 allele was mutated in both tumors, with an alanine substituted for valine at codon 143 of one tumor and a histidine substituted for arginine at codon 175 of the second tumor. Both mutations occurred in a highly conserved region of the p53 gene that was previously found to be mutated in murine p53 oncogenes. The data suggest that p53 gene mutations may be involved in colorectal neoplasia, perhaps through inactivation of a tumor suppressor function of the wild-type p53 gene.

RECENT STUDIES HAVE ELUCIDATED several genetic alterations that occur during the development of colorectal tumors (*1–3*), the most common of which are deletions of the short arm of chromosome 17. While some genetic alterations, such as *RAS* mutations, appear to occur relatively early during colorectal tumor development, chromosome 17p deletions are often late events associated with the transition from the benign (adenomatous) to the malignant (carcinomatous) state (*1*). Because carcinomas are often lethal, while the precursor adenomas are uniformly curable, the delineation of the molecular events mediating this transition are of considerable importance. The occurrence of allelic deletions of chromosome 17p in a wide variety of cancers besides those of the colon, including those of the breast and lung, further emphasizes the importance of identifying genes on 17p that are involved in the neoplastic process (*4*).

Our approach to this identification was based on first defining a small region of chromosome 17p that is commonly lost in different colorectal carcinomas. Twenty DNA probes detecting restriction fragment length polymorphisms (RFLPs) on chromosome 17p were used to examine the patterns of allelic losses in colorectal tumors. These probes have been mapped to seven discrete regions of 17p on the basis of their hybridization to human-rodent somatic cell hybrids containing parts of chromosome 17p (*5*). DNA was obtained from 58 carcinoma specimens and compared to DNA from adjacent normal colonic mucosa. Allelic losses were scored if either of the two alleles present in the normal cells was absent in the DNA from the tumor cells. Allelic deletions can be difficult to detect in DNA prepared from whole tumors because most solid tumors contain a significant number of non-neoplastic stromal and inflammatory cells. For this reason, regions of tumors containing a high proportion of neoplastic cells were isolated, and DNA was prepared from cryostat sections of these regions as described (*6*).

The two parental alleles could be distinguished in the normal mucosa of each patient by at least 5 of the 20 RFLP markers (the "informative" markers for each case). Seventy-seven percent of the tumors exhibited allelic losses of at least three markers. Studies of eight tumors that retained heterozygosity for some but not all markers on

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chromosome 17p enabled us to define a common region of deletion. For example, the tumor from patient S51 had retained both parental alleles of three markers from the distal region of 17p, but had lost one allele of all the more proximal markers that were informative (Fig. 1, A to C, and Fig. 2). This implied that the target of the allelic loss in this tumor was proximal to the three retained markers. The tumor from patient S103 had retained both parental alleles at all informative loci proximal to EW505, but had allelic deletions of several more distal markers (Fig. 1, D to F, and Fig. 2). The combined data depicted in Fig. 2 indicated that the smallest common region of deletion extended between markers within band 17p12 to those within band 17p13.3. This localization is based on the assumption that the same 17p locus was the target of deletion in all of the tumors.

Allelic deletions are thought to signal the presence of a tumor suppressor gene within the affected region of the chromosome (*7*). The tumor suppressor gene represents the critical gene ("target") of the deletion event. When both the maternal and paternal copies of such a gene are inactivated, suppression may be relieved and abnormal proliferation ensue. One scenario for the functional loss of tumor suppressor genes involves the inactivation of one allele through an inherited or somatic mutation (*7*). This inactivation is accompanied by loss of the remaining normal allele through a gross chromosomal change such as loss of a whole chromosome. An obligatory feature of this scenario is that the suppressor gene allele remaining in the tumor should contain a mutation.

The gene encoding p53 has been previously localized to region D_1 of chromosome 17p (*5*), which is within the common region of deletion observed in colorectal tumors (Fig. 2). As the p53 gene product has been implicated in the process of neoplastic transformation (*8*), we attempted to determine whether this gene might be a target of the deletions in colorectal tumors.

First, p53 cDNA probes detecting exons spread over 20,000 bp [including all protein encoding exons (*9*)] were used to examine the DNA of 82 colorectal carcinomas (50 primary specimens and 32 cell lines) in Southern blotting experiments. No rearrangements of the p53 gene were observed in Eco RI or Bam HI digests, nor were deletions of both alleles seen (*10*). As p53 expression might be affected by gross genetic alterations further removed from p53 coding sequences, pulsed-field gel electrophoresis was used to examine large restriction fragments encompassing the p53 gene. The restriction endonucleases Eco RV, Pae R7I, Not I, and Sal I generated p53 gene–containing fragments of 45 to 350 kb from the DNA of normal cells. No alterations were detected in the DNA from any of 21 colorectal tumor cell lines examined with each of these four enzymes (*10*).

We next considered the possibility that p53 gene inactivation could occur through interference with mRNA expression in the absence of gross changes in gene structure. To assess this possibility, we performed Northern blot experiments on RNA from 22 colorectal tumors (six primary tumors and 16 cell lines). The expression of p53 has been correlated with cellular growth and/or transformation (*11*); for this reason, other genes whose expression is similarly regulated were used as controls (*12*). The size of p53 mRNA was normal (2.8 kb) in all 22 tumors (*10*). Moreover, the relative abundance of p53 gene mRNA was usually at least as great in colorectal tumor cells as in normal colonic mucosa, confirming the results of Calabretta *et al.* (*12*). However, in four tumors, relatively little expression of p53 mRNA was observed compared to that in the other tumors. This low level of expression of p53 was specific in that c-*myc*, histone H3, and phosphoglycerate kinase mRNA's were expressed in these four tumors at levels similar to those seen in other colorectal tumors and at least as high as in non-neoplastic colonic mucosa (*10*).

The absence of gross alterations in p53 gene structure and expression in most colorectal carcinomas did not exclude the presence of subtle alterations of the p53 gene in these cases. To test for such subtle alterations, a tumor was chosen that had an allelic deletion of chromosome 17p yet expressed significant quantities of p53 mRNA. A cDNA clone originating from the remaining p53 allele was isolated and sequenced to determine whether the gene product was abnormal.

For practical reasons, a nude mouse xenograft (Cx3) of a primary tumor was selected

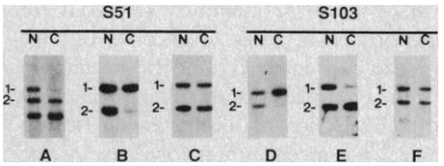

Fig. 1. Allelic deletions on chromosome 17p. DNA from normal (N) and carcinoma (C) tissue of patients S51 and S103 was digested with restriction endonucleases and the fragments separated by electrophoresis. After transfer to nylon filters, the DNA was hybridized to radiolabeled probes. Autoradiographs of the washed filters are shown. The alleles designated "1" and "2" refer to the larger and smaller polymorphic alleles, respectively, present in the normal DNA samples. The probes used were (**A**) MCT35.2; (**B**) EW301; (**C**) YNH37.3; (**D**) YNZ22.1; (**E**) MCT35.1; and (**F**) EW505. Deletions of allele 1 can be seen in panels A and E; deletions of allele 2 in panels B and D. Areas of tumors containing a high proportion of neoplastic cells were identified histopathologically in cryostat sections, and 12-μm-thick cryostat sections of these areas were used to prepare DNA (*6*). Grossly normal colonic mucosa adjacent to the tumors was obtained from each patient and used to prepare control DNA. DNA purification, restriction endonuclease digestion, electrophoresis, transfer, and hybridization were as described (*1, 6*). Taq I digestion was used for panels A, B, C, and F; Bam HI for panel D; and Msp I for panel E.

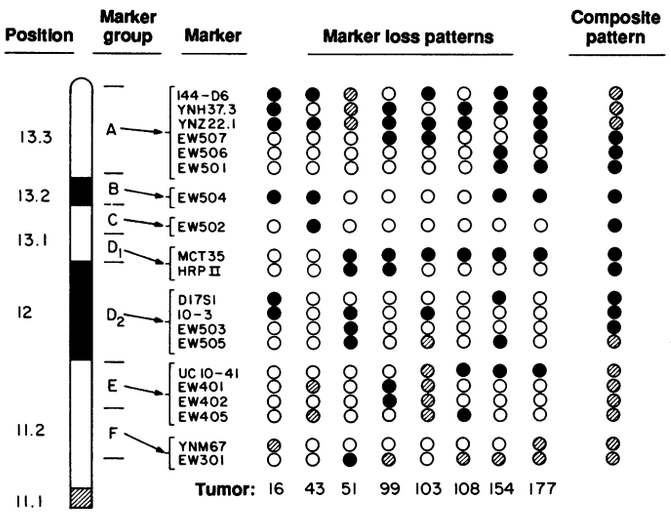

Fig. 2. Map of the common region of 17p deletion in colorectal tumors. Chromosomal positions of 20 markers from chromosome 17p are indicated. The markers were previously localized (*5*) to seven subchromosomal regions (A to F). Hybridization results for eight tumors are shown on the right, with patient identification numbers indicated at the bottom. For each of the 20 markers, a filled circle indicates that one parental allele was lost in the tumor; a cross-hatched circle indicates that both parental alleles were retained in the tumor; an open circle indicates that the marker was not informative (the patient's normal tissue was not heterozygous for the marker). The composite pattern (far right) assumes that there was only one target gene on chromosome 17p, so that markers for which heterozygosity was retained in any of the eight tumors would be outside the target gene locus. The region between probes YNZ22.1 and EW505 was deleted in every tumor in which markers in this region were informative.

for this test. Primary tumors contain non-neoplastic cells that could contribute p53 mRNA, while in xenografts the non-neoplastic cells (derived from the mouse) could not be the source of a human p53 cDNA clone. Cx3, like over 75% of colorectal carcinomas, had allelic deletions of several RFLP markers on chromosome 17 and expressed significant amounts of p53 mRNA (*10*).

A nearly full-length p53 cDNA was cloned from Cx3 mRNA by standard techniques (*13*). The clone extended 2567 nucleotides (nt) from position −198 relative to the translation initiation site to the polyadenosine tail. The clone was sequenced by the dideoxy chain-termination method and one nucleotide difference was identified in comparison with published p53 cDNA sequences (*9*). A transition from T to C had occurred within codon 143 (GTG to GCG), resulting in a change of the encoded amino acid from valine to alanine. To ensure that the sequence change was not an artifact of cDNA cloning, the polymerase chain reaction [PCR, (*14*)] was used to amplify a 111-bp sequence surrounding the presumptive mutation from genomic DNA of Cx3. Analysis of the PCR product was facilitated by the observation that the presumptive mutation created a new Hha I site (GCGC at nt 427 to 430). The 111-bp PCR product from tumor Cx3 was cleaved with Hha I to produce the expected 68- and 43-bp subfragments (Fig. 3, lanes 5 and 6). The 111-bp PCR product from the DNA of normal cells of the patient providing Cx3 was not cleaved with Hha I (Fig. 3, lanes 7 and 8), nor were the PCR products of 37 other DNA samples prepared from the normal tissues, primary colorectal tumors, or xenografts of other patients (examples in Fig. 3, lanes 1 to 4). Therefore, the valine to alanine substitution present in this tumor was the result of a specific point mutation not present in the germline of the patient.

A similar strategy was applied to the analysis of the remaining p53 allele of a colorectal tumor (Cx1) from another patient (*15*). A single point mutation was identified, which resulted in the substitution of histidine for arginine at codon 175 (transition from CGC to CAC). To ensure that the sequence change represented a mutation rather than a sequence polymorphism, PCR was used to amplify a fragment containing codon 175 from the genomic DNA of tumor Cx1 and normal cells. The presumptive mutation abolished the Hha I site normally present at codon 175 (GCGC at nt 522 to 525). Thus, Hha I cleavage of the PCR products from DNA of the normal cells of the patient providing Cx1 (Fig. 4, lanes 3 and 4) or from the tumor of another patient (Fig. 4, lanes 5 and 6) produced only the 48-bp product expected if codon 175 was wild type (see legend to Fig. 4). In contrast, the PCR product from tumor Cx1 was not cleaved at nt 524 (corresponding to codon 175) and exhibited only a larger 66-bp fragment resulting from cleavage at a normal downstream Hha I site at nt 542.

Thus, most colorectal tumors contained

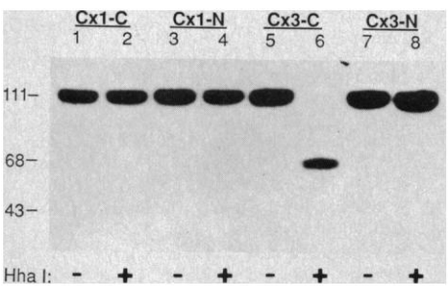

Fig. 3. Polymerase chain reaction analysis of p53 codon 143. A 111-bp fragment surrounding p53 codon 143 was amplified from genomic DNA by means of Taq polymerase (*14*). Half of the preparation was cleaved with Hha I (lanes marked "+"); the other half was not treated further (lanes marked "−"). After electrophoresis, PCR DNA fragments were detected by hybridization to a labeled p53 cDNA probe. The DNA samples used for PCR were derived from lanes 1 and 2, colorectal tumor (C) xenograft Cx1; lanes 3 and 4, normal (N) fibroblasts from the patient providing Cx1; lanes 5 and 6, colorectal tumor xenograft Cx3; lanes 7 and 8, normal fibroblasts from the patient providing Cx3. Only in tumor xenograft Cx3 (lane 6) did Hha I cleave the 111-bp fragment to the expected 68- and 43-bp subfragments (the 43-bp subfragment hybridized only weakly because of its small size). DNA was incubated in the presence of Taq polymerase with primer oligomers complementary to sequences 68 bp upstream and 43 bp downstream of codon 143. The upstream primer used was 5′-TTCCTC-TTCCTGCAGTACTCC-3′; all but six nucleotides of this primer were derived from the p53 intron 4 sequence determined by Buchman *et al.* (*9*). The downstream primer was 5′-GACGCGG-GTGCCGGGCGG-3′. After 35 cycles of denaturation (1 min, 93°), annealing (2 min, 55°) and elongation (2 min, 70°) amplified DNA fragments of 111 bp were generated. After electrophoresis, the 111-bp amplified fragments were eluted from a polyacrylamide gel and purified by extraction with phenol and chloroform. A small amount of a contaminating 73-bp PCR product was present in most of the eluates; the contaminant was not cleaved by Hha I, however, so that it did not interfere with the analysis. A portion of each of the purified DNA fragments was digested with Hha I, separated by electrophoresis on a nondenaturing polyacrylamide gel, and electrophoretically transferred to nylon filters. The fragments were hybridized with a radioactive probe generated from a 1.8-kb Xba I fragment of a p53 cDNA clone provided by D. Givol (*9*).

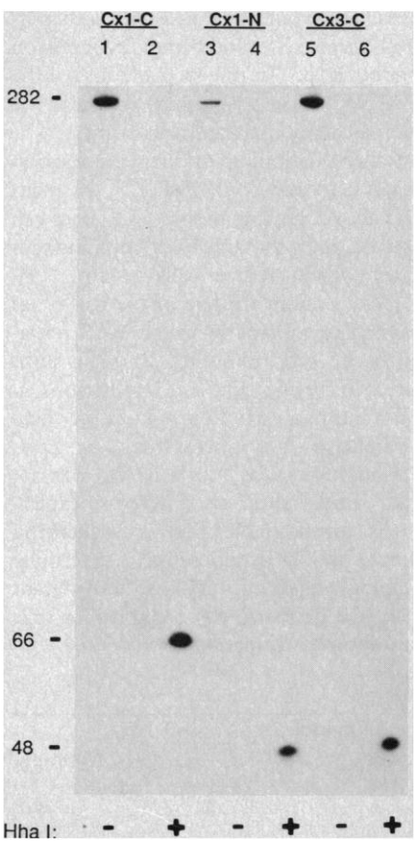

Fig. 4. Polymerase chain reaction analysis of p53 codon 175. A DNA fragment containing p53 codon 175 was amplified from genomic DNA by means of Taq polymerase and radioactively labeled at one end. A portion of the preparation was cleaved with Hha I (lanes marked "+"); another portion was not treated further (lanes marked "−"). The labeled fragments were then separated by electrophoresis and visualized through autoradiography. The DNA samples were derived from lanes 1 and 2, colorectal tumor (C) xenograft Cx1; lanes 3 and 4, normal (N) fibroblasts from the patient providing Cx1; lanes 5 and 6, colorectal tumor xenograft Cx3. A 48-bp Hha I fragment is produced if codon 175 is wild-type; a 66-bp Hha I fragment (present only in tumor Cx1) is produced if codon 175 is mutated. PCR was used to amplify a 319-bp fragment containing intron 5 and surrounding exon sequences. The upstream primer was the same as used for primer set 3 (*15*) and the downstream primer was 5′-CGGAATTCAGGCGGCTCATA-GGGC-3′; PCR was performed as described in the legend to Fig. 3. After electrophoresis through a 2% agarose gel, the 319-bp fragment was purified by binding to glass beads (*30*). The DNA fragments were cleaved with Sty I at nt 477 and end-labeled by fill-in with the Klenow fragment of DNA polymerase I and ^{32}P-labeled dCTP. After electrophoresis of the reaction mixture through a non-denaturing polyacrylamide gel, the 282-bp Sty I fragment (nt 477 to 758), labeled at the proximal end and containing codon 175, was eluted and purified by extraction with phenol and chloroform. A portion of the eluted DNA was cleaved with Hha I and the fragments separated by electrophoresis on a 6% sequencing gel. Hha I digestion of the 282-bp Sty I fragment produces a labeled 48-bp fragment (comprising nt 477 to 524) if codon 175 is wild type. If codon 175 is mutated, a labeled 66-bp fragment (comprising nt 477 to 542) is produced by Hha I as a result of cleavage at the first Hha I site downstream of codon 175.

deletions of the region containing the p53 gene, and the p53 gene was mutated in both tumors subjected to detailed analysis. There are many potential explanations for these findings: (i) p53 is not involved in colorectal tumorigenesis and the p53 abnormalities identified were coincidental epiphenomena, (ii) p53 is a target of the 17p deletions in all colorectal tumors, (iii) p53 is a target in some tumors, such as Cx3 and Cx1, but a different chromosome 17p gene is the target in other tumors, or (iv) p53 is not a target of deletion in any tumor, but mutations of p53 can provide a selective growth advantage complementing that derived from an unidentified tumor suppressor gene on 17p.

We cannot differentiate among these possibilities at present, but feel that the first explanation is unlikely for several reasons. Most importantly, the mutations in Cx1 and Cx3 were clonal, that is, they occurred in all of the neoplastic cells of the tumors (Figs. 3 and 4). As has been noted previously (*16*), such clonal mutations indicate that the mutation either provided a selective growth advantage to the cell or occurred coincidentally with another mutation that was responsible for the clonal expansion. Such a coincidence is unlikely, because point mutations are generally considered rare events. In one study of colorectal carcinomas, for example, no point mutations were observed at over 10,000 restriction endonuclease recognition sites encompassing more than 40,000 bp (*16*). Thus, the finding of independent clonal mutations within the 1179 bp of the p53 coding sequences in two different tumors probably did not represent random events unrelated to tumorigenesis. The position of these mutations in a highly conserved region of the protein also suggested a functional change, as noted below.

Although the gene encoding p53 has been considered an oncogene (*8*), several studies have suggested that the normal p53 gene might have suppressor activity. First, it has recently been shown that normal p53 genes do not function as oncogenes during in vitro transformation; only mutated forms have this capacity (*17–19*). The mutations in colorectal tumors Cx1 and Cx3 both occurred in highly conserved positions of the p53 gene. Mutations in this region have been shown to confer in vitro oncogenicity to murine p53 genes (*17–19*). Such mutant p53 gene products can form complexes with normal p53 proteins, perhaps inhibiting their function (*19*). Second, the only other candidate tumor suppressor gene so far identified is the retinoblastoma susceptibility (Rb) gene (*20*). Both the Rb and p53 gene products interact with the large T-antigen of SV40 (*21, 22*), and it has been suggested that the large T-antigen gene functions as an oncogene because the binding of its gene product inactivates the suppressor function of the Rb protein or p53 (or both) (*22, 23*). Similarly, the adenovirus E1A and E1B gene products may contribute to viral oncogenicity by binding the Rb protein and p53, respectively (*24*). Third, p53 genes are often inactivated through proviral integration in Friend virus–induced mouse leukemias (*25, 26*). Fourth, rearrangements of the p53 gene occur in the human leukemia cell line HL60 and in some osteosarcomas, and no p53 gene product is detectable in HL60 cells (*27*).

On the basis of these observations, it is reasonable to speculate that the normal p53 gene interacts with other macromolecules (DNA or proteins) to result in suppression of the neoplastic growth of colorectal epithelial cells. This suppression is relieved if p53 expression is extinguished or if p53 mutations prevent the normal interaction of p53 with other cell constituents. Mutant p53 gene products might compete with normal p53 proteins and so act in a "dominant negative" fashion (*17–19, 23, 25, 28*), but a more pronounced effect of a mutated p53 gene might be realized when the normal allele is lost from the tumor. This hypothesis could explain why allelic deletions on chromosome 17p are so common in colorectal tumors, and would be consistent with postulated mechanisms of tumor progression (mutation of p53 at one step and loss of the normal p53 allele at another step near the adenoma-carcinoma transition point).

Another possibility concerns the relation between activated *RAS* and p53. As mentioned above, mutant mouse p53 genes can cooperate with mutant *RAS* to transform primary rodent embryo cells in vitro (*29*). Colorectal tumors are one of the few types of human neoplasms in which *RAS* mutations occur commonly (*1, 2*). The joint occurrence of p53 and *RAS* mutations in colorectal tumors would provide a provocative parallel with in vitro systems.

Although there is much to be learned, several of the issues raised here are experimentally approachable. Further sequencing studies as well as experiments to determine the biologic effect of wild-type and mutant human p53 genes on colorectal tumor cells should prove informative.

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cloned in the Eco RI site of Bluescript SK and sequenced as described (*13*). Only one sequence change was identified (see text) and this change at codon 175 was found in two independent clones.
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6 February 1989; accepted 15 March 1989

Expression of a Cloned Rat Brain Potassium Channel in *Xenopus* Oocytes

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Potassium channels are ubiquitous membrane proteins with essential roles in nervous tissue, but little is known about the relation between their function and their molecular structure. A complementary DNA library was made from rat hippocampus, and a complementary DNA clone (RBK-1) was isolated. The predicted sequence of the 495–amino acid protein is homologous to potassium channel proteins encoded by the *Shaker* locus of *Drosophila* and differs by only three amino acids from the expected product of a mouse clone MBK-1. Messenger RNA transcribed from RBK-1 in vitro directed the expression of potassium channels when it was injected into *Xenopus* oocytes. The potassium current through the expressed channels resembles both the transient (or A) and the delayed rectifier currents reported in mammalian neurons and is sensitive to both 4-aminopyridine and tetraethylammonium.

POTASSIUM CHANNELS ARE MEMbrane proteins that are selectively permeable to K^+ ions (*1*). Some kinds of K^+ channels are opened by depolarization; outward K^+ currents limit the duration of single action potentials (delayed rectifier) or set the pattern of bursts of action potentials (transient or A current). Other K^+ channels are opened or closed by second messengers to mediate the actions of synaptic transmitters (*1, 2*). We have determined some functional properties of a mammalian K^+ channel of known primary structure by expression in *Xenopus* oocytes of a cDNA clone isolated from rat brain.

A cDNA library made from rat hippocampus polyadenylated RNA was screened with two radiolabeled oligonucleotide probes (*3*). The sequences of the probes were based on conservation of amino acid sequences between the *Shaker* A (*4–6*) and MBK-1 (*7*) predicted proteins. *Shaker* is a family of *Drosophila* mutants that expresses abnormal transient or A-type K^+ channels; at least four distinct proteins encoded by the *Shaker* locus form K^+ channels when expressed in *Xenopus* oocytes. MBK-1 is a clone isolated from a mouse brain cDNA library having a homologous nucleotide sequence, but MBK-1 has not been shown to direct the formation of functional channels. One of the rat brain clones (RBK-1) that gave positive hybridization with both probes was purified, and the nucleotide sequence of the 1.7-kb cDNA insert was determined (Fig. 1). The sequence contains one long open-reading frame that encodes a protein of 495 amino acids (molecular mass, 54.6 kD). The translation product predicted from RBK-1 is 69% homologous to the 453 residues that constitute the core common to the different proteins that could be formed from transcripts of *Shaker* cDNA. Significantly greater homology is found within the predicted membrane-spanning regions H1 to H6, and in the arginine-rich amphipathic helix (S4), which may represent the channel voltage sensor. The putative translation products of RBK-1 and MBK-1 differ by only three of their 495 amino acids, and there are no differences in the H1 to H6 or S4 regions; the sequence is identical to the expected product of a cDNA clone (RCK-2) isolated from rat cortex (*8*).

The RBK-1 sequence was subcloned into a plasmid expression vector (*3*), and the mRNA transcripts synthesized in vitro were injected into *Xenopus* oocytes (*9*). Voltage-clamp recordings made 24 to 96 hours later showed that the oocytes that had been injected produced large outward currents in response to depolarizing voltage commands. The currents were dependent on the amount of mRNA injected (*10*) and were not seen in uninjected cells. Some of the properties of the current are shown in Fig. 2; from a holding potential of −80 mV the current was first observed with depolarizations to −50 mV and was maximally activated at 0 or +10 mV.

The current resulted from the movement of K^+ ions, as shown by measurement of its reversal potential (*11*) in different K^+ ion concentrations (Fig. 3). The reversal potential (E_{rev}) was linearly related to the extracellular potassium concentration ($[K]_o$) by $E_{rev} = m\log([K]_o/[K]_i)$ (the Nernst equation) where $[K]_i$, the intracellular potassium concentration, was assumed to be 110 m*M*. The slope of the relation (m) was 55 ± 2 mV per decade (SEM, n = 5 oocytes; theoretical value is RT/F = 58), providing convincing evidence that the channels expressed in the oocyte membrane are highly selective for K^+ ions.

We also determined whether the properties of the expressed channels were similar to those of the known classes of K^+ channels in mammalian neurons. The current developed (activated) within a few milliseconds of the applied depolarization (*12*), and the voltage dependence of activation of the K^+ conductance was well fit by a Boltzmann function centered at −30 mV (Fig. 2C). When the depolarizing pulse was terminated, the current subsided within a few milliseconds (*12*). Steady-state inactivation was studied by changing the holding potential for 5 s before applying the depolarizing command to +10 mV; the midpoint of the inactivation curve was at about −30 mV (n = 3). The current inactivated only partially during a maintained depolarization of up to 10 s in duration. The inactivation had a fast component (time constant, 50 to 100 ms) and a much slower component (time constant, 5 to 10 s), neither of which was strongly

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