box," which presumably bears an intrinsic function in principal sigma factors.

All of the 13 *Streptomyces* strains examined had multiple *rpoD* signals (*16*). Multiple *rpoD* genes may be common in divergent eubacterial strains. The previously identified protein of *S. coelicolor*, which has a functional homology with the principal sigma factor of *B. subtilis* (*17*), may be one of the *rpoD* gene products reported here. More recently, three different RNA polymerase holoenzymes were identified in *S. coelicolor* A3(2) by using the agarase gene (*dagA*) (*18*). Conceivably the biochemical and morphological complexity of differentiating *Streptomyces* cells are guaranteed by the temporal and topological expression of genes through the heterogeneity of the principal sigma factors.

The characterization of *rpoD* homologs from bacteria gives us insights into the gene control mechanisms as well as the transcriptional machinery of divergent bacteria. The study of *rpoD* gene constituents in prokaryotic cells will also give the molecular base for phylogenetic relations.

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Cloning of a Membrane Protein That Induces a Slow Voltage-Gated Potassium Current

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A rat kidney messenger RNA that induces a slowly activating, voltage-dependent potassium current on its expression in *Xenopus* oocytes was identified by combining molecular cloning with an electrophysiological assay. The cloned complementary DNA encodes a novel membrane protein that consists of 130 amino acids with a single putative transmembrane domain. This protein differs from the known ion channel proteins but is involved in the induction of selective permeation of potassium ions by membrane depolarization.

ION CHANNELS EXHIBIT A HIGH DEgree of diversity, varying in their electrophysiological and pharmacological properties (*1*). However, the molecular basis for the different properties of the ion channels remained to be clarified. We previously developed a new strategy to characterize receptors, ion channels, and modulatory proteins by combining molecular cloning in an RNA expression vector with an electrophysiological assay in *Xenopus* oocytes (*2*). Because the kidney is involved in controlling various types of ion permeations to maintain the electrolyte homeostasis (*3*), we began this investigation by characterizing ion currents elicited by membrane polarization after injection of rat kidney polyadenylated [poly(A)$^+$] RNA into *Xenopus* oocytes (Fig. 1A). In the control oocytes injected with distilled water, an outward current appeared as the oocyte membrane potential was stepped from −80 mV to more positive levels. This current probably results from activation of a voltage-dependent K^+ channel as reported (*4*). In the oocytes injected with the kidney mRNA, depolarization induced a more markedly activating outward current, and this current was five to ten times as large as that of the control oocytes. To characterize the mRNA responsible for the induction of the outward current, we size-fractionated the kidney mRNA by centrifugation on sucrose gradient. The activity of the mRNA was found in a fraction with an average mRNA size of ~700 nucleotides, which was considerably smaller than those of other ion channel mRNAs (*5*). We therefore initiated the isolation of a functional cDNA clone from a kidney cDNA bank (*6*) and finally obtained a single cDNA clone that was capable of eliciting a markedly elevating outward current by membrane depolarization.

The currents induced by the mRNA synthesized in vitro from the cloned cDNA were characterized in more detail (Fig. 1B). Depolarization from a holding potential of −80 mV elicited large voltage- and time-dependent outward currents. On repolarization to −80 mV, slow outward tail currents were observed. The outward current lasted for at least 20 min, suggesting that the channel responsible for the outward current does not undergo inactivation. Currents with similar amplitude and properties could not be seen in water-injected oocytes or oocytes injected with tRNA.

A series of experiments were conducted to determine ionic mechanisms underlying the outward current. In the external medium containing 2 m*M* K^+, reversal of the tail currents occurred at −101 mV (mean ± 2, $n = 4$) (Fig. 1C), similar to the estimated reversal potential of −103 mV for K^+ in *Xenopus* oocytes (*7*). At the 20 m*M* K^+ concentration, the tail currents reversed at −42 mV (mean ± 3, $n = 3$), in agreement

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with the shift predicted by the Nernst equation for K^+. In addition, the current was reduced by the K^+ channel blockers tetraethylammonium (10 to 20 m*M*) and Ba^{2+} (10 m*M*). Substituting external chloride ions with aspartate ions had no effect on the reversal potential of the tail currents; neither did halving the external NaCl concentration with *N*-methyl-D-glucamine. The reversal potential was also not affected by removal of Ca^{2+} ions from the medium (the medium was deficient in Ca^{2+} and contained 0.1 m*M* EGTA), indicating that Ca^{2+} entry is not necessary for inducing the outward current. On the basis of these results, we conclude that the slowly activating outward current is carried selectively by K^+ ions.

The sequence determination of the cloned cDNA indicates that it consists of 585 nucleotides (Fig. 2). Only one frame of the nucleotide sequence encodes a large protein sequence composed of 130 amino acids. The estimated molecular weight of this protein (14,698) is very close to that (15,000) obtained by urea–SDS–gel electrophoresis analysis of the mRNA-directed translation product of the reticulocyte lysate system. The nucleotide sequence surrounding the predicted initiation codon is similar to the consensus sequence (*8*). We thus conclude that the protein sequence encoded by the cDNA is composed of 130 amino acids as indicated in Fig. 2.

Computer-aided analysis of the predicted protein does not show any obvious sequence similarity to the proteins in the National Biomedical Research Foundation/European Molecular Biology Laboratory (NBRF/EMBL) database or to any other known ion-channel protein (*5*). The hydropathicity profile analysis (*9*) indicates a clear hydrophobic segment consisting of 23 uncharged amino acid residues in the middle portion of the protein. Consistent with this finding, SDS–gel electrophoresis analysis of the membrane fraction derived from the mRNA-injected oocytes gave rise to an [^{35}S]methionine-labeled translation product that corresponded exactly to that synthesized in vitro from the mRNA (Fig. 3). In contrast, this product was not observed in the soluble fraction. We thus conclude that the slow voltage-gated K^+ current is induced by a novel membrane protein that differs from the conventional ion channel proteins.

Several structural characteristics of this protein can be discussed from its primary structure. The moderately hydrophobic NH_2-terminus with nine nonpolar residues (residues 9 to 17) probably cannot serve as a signal peptide, because cleavage of the primary translation product was not observed in the oocyte translation system (Fig. 3) or in the reticulocyte translation system containing a membrane signal peptidase (*10*). With some exceptions, such as rhodopsin-type receptors (*11*), the transmembrane proteins with no signal sequence have their NH_2-termini on the cytoplasmic side of the membrane (*12*). However, the presence of two potential *N*-glycosylation sites (*13*) in the NH_2-terminal region suggests a reversed orientation. Interestingly, as observed for some other ion channel proteins (*5*), negatively and positively charged residues predominate in the NH_2-terminal and COOH-terminal regions, respectively, that flank the putative transmembrane domain. In the COOH-terminal region a single cysteine residue is present and may be involved in forming a dimeric or oligomeric structure of this protein. Thus, although the protein discussed here is small and apparently simple, the topology of this protein may be more complex than expected.

On blot hybridization analysis, poly(A)$^+$ RNAs from the kidney, duodenum, stomach, pancreas, and submandibular gland showed a common band with an estimated mRNA size of ~700 nucleotides. We detected no appreciable amount of the mRNA in the brain or liver. The mRNA appears to be distributed in tissues where epithelial cells are actively involved in conducting the K^+ permeations (*3*).

The protein identified in this study is considerably smaller than the Na^+-, K^+-, or Ca^{2+}-channel proteins that have been cloned (*5*) and contains only a single putative transmembrane domain. The K^+ current elicited by this protein is also unusually slow in activation and deactivation after electrical polarization. These characteristics differ from those of the conventional ion channels and resemble those of simple channel-forming peptide ionophores such as alamethicin and synthetic amphiphilic peptides (*14–16*). Although these peptides are small and are made up solely or almost solely of uncharged amino acids, they are able to produce a pore by forming an oligomeric structure to allow selectively monovalent cations to flow down along their electrochemical gradient. It has been proposed that a series of oxygen molecules lining the inside of peptide channels can form ligands with cations and govern ionic selectivity and per-

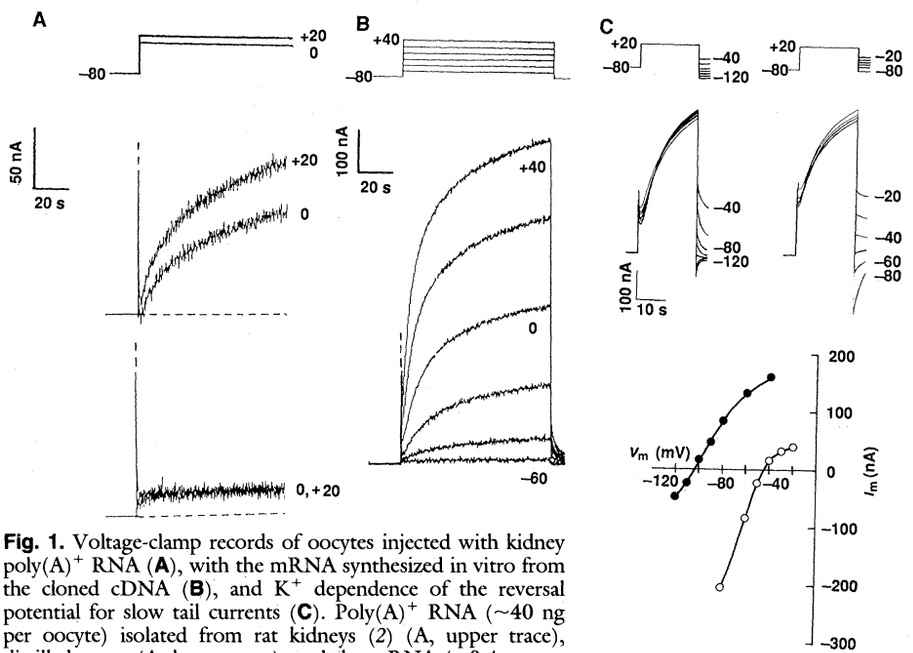

Fig. 1. Voltage-clamp records of oocytes injected with kidney poly(A)$^+$ RNA (**A**), with the mRNA synthesized in vitro from the cloned cDNA (**B**), and K^+ dependence of the reversal potential for slow tail currents (**C**). Poly(A)$^+$ RNA (~40 ng per oocyte) isolated from rat kidneys (*2*) (A, upper trace), distilled water (A, lower trace), and the mRNA (~0.4 ng per oocyte) derived from the cloned cDNA (pKI27) (B and C) were injected into oocytes. The pKI27 cDNA clone was isolated according to the procedures described (*2*) with some modifications (*6*). The oocytes were incubated for 3 days at 19°C in Barth's medium. Conventional two-microelectrode voltage-clamp techniques were used (*2*) and all recordings were made at 22° to 24°C. In (A) and (B), the oocytes were held at −80 mV and depolarized stepwise to test potentials for 90 s. The bathing medium consisted of 96 m*M* NaCl, 2 m*M* KCl, 1.8 m*M* $CaCl_2$, 1 m*M* $MgCl_2$, and 5 m*M* Hepes, *p*H 7.6. Leakage currents were subtracted from the recording currents by appropriate multiples of the current elicited by a hyperpolarizing pulse to −90 mV. A peak observed immediately after depolarization was due to a transient outward current elicited by activation of an oocyte Ca^{2+}-dependent Cl^- channel (*4*) and an artifact. In (C), an oocyte injected with the mRNA was held at −80 mV, depolarized to +20 mV for 20 s, and then repolarized to various potentials (top traces). The currents were recorded in the bath solution containing either 2 m*M* K^+ (upper left trace, same bath solution as described above) or 20 m*M* K^+ (upper right trace). The amplitudes of the tail currents (I_m) are plotted as a function of voltage (V_m) in 2 m*M* K^+ (●) or 20 m*M* K^+ (○) (lower).

```
                    CGTCCATCCAGGTCCAGGCGTGCAGAGTTTTGCTCCACATCAGGGAAACCGTGAAGCCCCAGG   -1

Met Ala Leu Ser Asn Ser Thr Thr Val Leu Pro Phe Leu Ala Ser Leu Trp Gln Glu Thr    20
ATG GCC CTG TCC AAT TCC ACA ACT GTT CTG CCT TTT CTG GCC TCG CTG TGG CAG GAA ACA    60

Asp Glu Pro Gly Gly Asn Met Ser Ala Asp Leu Ala Arg Arg Ser Gln Leu Arg Asp Asp    40
GAT GAG CCG GGT GGC AAT ATG TCG GCG GAC TTG GCT CGT AGG TCC CAG CTC CGA GAT GAC   120

Ser Lys Leu Glu|Ala Leu Tyr Ile Leu Met Val Leu Gly Phe Phe Gly Phe Phe Thr Leu    60
AGC AAG CTG GAG GCT CTC TAT ATC CTC ATG GTG CTG GGT TTC TTC GGC TTC TTC ACC CTG   180

Gly Ile Met Leu Ser Tyr Ile|Arg Ser Lys Lys Leu Glu His Ser His Asp Pro Phe Asn    80
GGC ATC ATG CTG AGT TAC ATC CGA TCC AAG AAG CTG GAA CAC TCG CAC GAC CCT TTC AAC   240

Val Tyr Ile Glu Ser Asp Ala Trp Gln Glu Lys Gly Lys Ala Leu Phe Gln Ala Arg Val   100
GTG TAC ATC GAG TCG GAC GCC TGG CAG GAG AAA GGC AAG GCC CTC TTC CAG GCC CGC GTT   300

Leu Glu Ser Phe Arg Ala Cys Tyr Val Ile Glu Asn Gln Ala Ala Val Glu Gln Pro Ala   120
CTG GAG AGC TTC AGA GCT TGC TAT GTC ATT GAA AAC CAG GCG GCC GTA GAA CAA CCT GCC   360

Thr His Leu Pro Glu Leu Lys Pro Leu Ser                                           130
ACA CAC CTT CCT GAA CTG AAG CCA CTG TCA TGA ACCCCATAGTTAATTAATAGACAAGTGATAAGTGG   428

GTCTTTCTAGTCAAATGCCTGCCAGTCTTTATTGTAGAGGTACCCTTGAGTTTTTATAAGGGGTGAGTTAATAACACCA   507

GTTTTCTGAAATTGC                                                                   522
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Fig. 2. The cDNA sequence and the deduced amino acid sequence of its coding protein. The amino acid sequence deduced from the longest open reading frame and the position of the putative transmembrane domain are indicated above the nucleotide sequence; ➧, potential *N*-glycosylation sites; ⊕ and ⊖, positively and negatively charged residues at the membrane-flanking regions; *, a cysteine residue. The cDNA sequence was determined by the chain termination method (*20*).

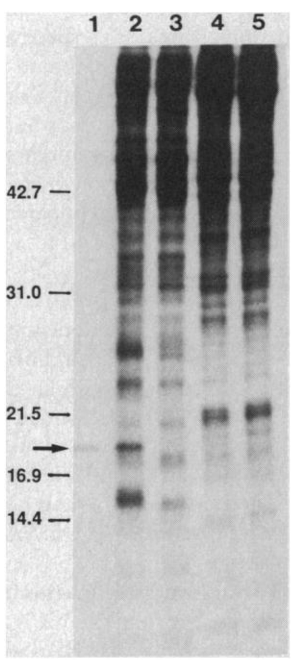

Fig. 3. Membrane localization of the translation product of the mRNA. A protein synthesized in vitro from the mRNA in the reticulocyte lysate system (lane 1); labeled proteins in the membrane and soluble fractions of the mRNA-injected oocytes (lanes 2 and 4, respectively) and of the control oocytes (lanes 3 and 5). Twenty-two oocytes with and 22 without mRNA injection were incubated in Barth's medium for 7.5 hours, followed by further incubation in the medium containing [^{35}S]methionine (120 μCi) for 21 hours. Membrane and soluble fractions were obtained by fractionation of oocyte homogenates (*21*). The in vitro translation product of the mRNA was prepared with the reticulocyte lysate translation kit (Du Pont, Biotechnology Systems). The labeled protein samples were reduced and analyzed by SDS–polyacrylamide gel electrophoresis (15% acrylamide), followed by fluorography; arrow, the translation product derived from clone pKI27 (*22*).

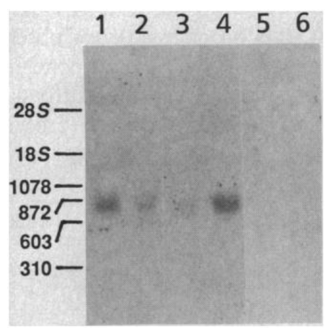

Fig. 4. Blot hybridization of poly(A)$^+$ RNAs from various rat tissues. Lanes 1 to 6, kidney, stomach, duodenum, pancreas, brain, and liver, respectively. Poly(A)$^+$ RNAs (30 μg) obtained from various tissues were separated by electrophoresis on 1.5% agarose and transferred to a nylon filter; the probe used, the 552-bp Bam HI–Kpn I cDNA fragment; the molecular markers, rat ribosomal RNA (18*S*, 28*S*) and the Hae III–digested φXDNA.

meation (*14, 16*). These oxygen ligands are supplied by hydroxyl side chains and charged carboxylates (*14*). In addition, glycine, by causing a kink, may provide a carbonyl oxygen from the peptide backbone (*14*). In the proposed transmembrane domain of the protein, there are four hydroxyl side chains and three glycine residues, and some of these residues could function as ligands that coordinate the permeation of K^+ ions. Thus, the simplest and most likely explanation for the function of the novel membrane protein is that it per se acts as a discrete K^+-conducting ion channel. Alternatively, because the K^+ channel activity can be modulated by various intracellular signals (*1, 17*), it is possible that this membrane protein may subserve as a modulatory protein that activates endogenous K^+ channels.

Boyle *et al.* (*18*) reported that expression of the rat uterine mRNA that induces a voltage-dependent K^+ current in the oocyte system is regulated in accordance with changes in electrical excitability in the myometrium during the estrous cycle and estrogen treatment (*18*). Because the K^+ current we characterized seemed to resemble that reported by Boyle *et al.* (*18*), it will be interesting to see whether this protein is involved in controlling electrical excitability in smooth muscle cells (*19*) such as the myometrium. The tissue distribution of the mRNA, however, shows that the mRNA is expressed not only in the kidney but also in some other tissues comprising glandular epithelial cells. In these tissues, various types of epithelial cells are able to permeate K^+ ions into both the interstitial space and the lumen, when the Na^+- and K^+-dependent adenosine triphosphatase pump creates high intracellular K^+ concentrations (*3*). These K^+ permeations are thought to be mediated through K^+ channels (*3*), but the function of the K^+ permeation in nonexcitable epithelial cells may be different from that of excitable nerve and skeletal muscle cells, where a rapid activation-deactivation of the K^+-channel activity must occur to control cell excitability and synaptic transmission. In contrast, in epithelial cells, the rapid response of the K^+ channel activity is not necessarily needed to control intracellular K^+ concentrations. Thus, this membrane protein may be involved in the permeability of nonexcitable epithelial cells to K^+ ions and thus control K^+ homeostasis.

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~90,000, ~20,000, ~2,000, and 60 cDNA clones. A single clone (pKI27) was finally obtained.
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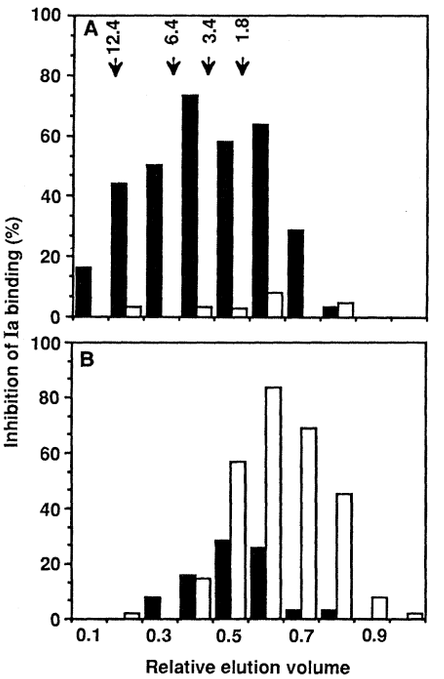

Fig. 1. One of two experiments showing the Ia binding capacity of material obtained from acid-treated Ia. Acid-treated I-A^d (**A**) and I-E^d (**B**) were gel-filtered and assayed for I-A^d (filled bars) and I-E^d (open bars) binding activity. I-A^d–derived material bound exclusively to I-A^d and not I-E^d. I-E^d–derived material bound predominantly but not exclusively to I-E^d. Ia molecules were affinity-purified as described (*3*) and either treated with acid or left untreated for peptide binding assays. Briefly, 200 μg of I-A^d or I-E^d in phosphate-buffered saline was exposed to 2.5*M* acetic acid for 20 min at 37°C, and then separated according to size by Sephadex G-50 chromatography (buffer: 0.04*M* ammonium acetate and 0.02*M* acetic acid). Fractions (10 ml) were collected and lyophilized four times. Each fraction was taken up in 50 μl of buffered saline solution, and 10 μl was tested for its capacity to inhibit the binding to Ia of a radiolabeled peptide [Ova (Y 323-339) for I-A^d and lambda repressor C1 (Y 12-26)], which binds to I-E^d (*5*). The relative elution volume was calculated as $(V_e - V_o)/V_t - V_o)$, where V_e is elution volume, V_o is void volume, and V_t is total volume. The positions of markers of known molecular weights are indicated in (A).

Autologous Peptides Constitutively Occupy the Antigen Binding Site on Ia

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Low molecular weight material associated with affinity-purified class II major histocompatibility complex (MHC) molecules of mouse (Ia) had the expected properties of peptides bound to the antigen binding site of Ia. Thus, the low molecular weight material derived from the I-A^d isotype was efficient in inhibiting the binding of ^{125}I-labeled I-A^d–specific peptide to I-A^d, but did not significantly inhibit the binding of an I-E^d–specific peptide to I-E^d; the reciprocal isotype-specific inhibition was demonstrated with low molecular weight material derived from I-E^d. The inhibitory material was predominantly peptide in nature, as shown by its susceptibility to protease digestion. It was heterogeneous as measured by gel filtration (mean molecular weight ~3000), and when characterized by high-performance liquid chromatography, it eluted over a wide concentration of solvent. Such self peptide–MHC complexes may have broad significance in the biology of T cell responses, including generation of the T cell repertoire, the specificity of mixed lymphocyte responses, and the immune surveillance of self and nonself antigens in peripheral lymphoid tissues.

IN GENERAL, T CELLS OF THE HELPER subset recognize complexes of altered—that is, denatured or fragmented—protein antigen and MHC Ia molecules (*1–3*). Ia molecules act as receptors (*4*) with a single binding site for "processed" antigen (*5, 6*). The permissive specificity of Ia—probably through the broad recognition of peptide "motifs"—allows each Ia to bind many different peptides (*5, 7*). Ia does not distinguish between peptides representing self and nonself (*8*).

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Circumstantial evidence suggests that Ia may bind peptides derived from endogenous, as well as exogenous proteins. First, Babbitt *et al.* (*8*) demonstrated that I-A^k was capable of binding the autologous mouse lysozyme peptide 46-61 and the homologous xenogenic hen egg lysozyme peptide equally well. Second, Björkman *et al.* (*9, 10*) examined the x-ray crystallographic structure of a human MHC class I molecule, HLA-A2, and found evidence of electron-dense material occupying the putative antigen binding site. Finally, we found that only 5% to 10% of affinity-purified Ia molecules are capable of binding peptide and have speculated that this could be caused by the presence of self peptides constitutively present in the binding site of Ia (*11*).

To evaluate whether peptides constitutively bound to Ia would likely remain bound during the affinity purification of Ia, we exposed radiolabeled peptide-Ia complexes to the same conditions as used in the affinity purification procedure. Our purification procedure involved a high *p*H (*p*H 10 to 10.5) elution step at 4°C lasting 5 to 10 min. At least for the interaction of ovalbumin (Ova) peptide 323-339 and I-A^d, only a minor fraction (approximately 2%) of peptide-Ia complexes was dissociated under these conditions. These results are consistent with the possibility that affinity-purified Ia might have a low peptide binding capacity because its antigen binding site is already occupied to a large extent with self peptides.