by a Y at 245, and the Ts at 441 and 442 are conserved (252 and 253).

The region between amino acids 393 and 477 is homologous with mammalian ion channels (*10–12*) and protein kinases (*22*) that are regulated by cGMP or cAMP, and with the catabolite gene activator protein (CAP) of *Escherichia coli* (*23*) (Fig. 3A), which includes a cAMP binding site (*24*). Similarly, the corresponding regions of cyclic nucleotide–gated channels are thought to bind cAMP (*10, 11*). The significance of homologies to these regions in the protein encoded by AKT1 is not clear because there is as yet no conclusive evidence for the presence of cyclic nucleotides in higher plants (*25*).

The six imperfect repeating sequences of 32 or 33 amino acids between positions 517 and 712 of the protein encoded by AKT1 (Fig. 3B) show homology to a 33-residue motif repeated 22 times in tandem in erythrocyte ankyrin (*26*), a protein that attaches integral membrane proteins to cytoskeleton components. In brain, ankyrin links a voltage-dependent $Na^+$ channel to spectrin, and thus may restrict the channel to specific locations in the neuronal membrane (*27*). Similar repeats have been observed in a variety of proteins and are thought to tether the subunits of regulatory proteins (*28, 29*). Thus, the presence of ankyrin-like repeats in the protein encoded by AKT1 suggests that this transport system may interact with the cytoskeleton or with regulatory proteins.

The sequence homologies between the protein encoded by AKT1 and cyclic nucleotide–gated channels encompass the six putative transmembrane segments present in the channels between the $NH_2$-terminus and the cyclic nucleotide–binding region. These homologies with this region of the channels suggest that the protein encoded by AKT1 also has six transmembrane segments (Fig. 4), although only four (S1, S2, S5, and S6) may be inferred from the hydropathicity plot. Whatever the exact topography, the presence in a plant transport system of both the highly conserved S4 and H5 regions, typical of Shaker channels, and of a cyclic nucleotide–binding site supports the hypothesis of an ancient common origin of voltage-gated and cyclic nucleotide–gated channels (*14*).

It is unlikely that the protein encoded by AKT1 is an accessory polypeptide interacting with yeast transport proteins, because the protein encoded by AKT1 was not homologous to the yeast *TRK1* gene product (*4*), and expression of AKT1 in the mutant yeast strain was sufficient to form a functional $K^+$ uptake system. The homologous Shaker polypeptides form functional channels when expressed in *Xenopus* oocytes (*15*).

*Note added in proof*: Functional expression of another putative $K^+$ transport system cDNA (*KAT1*) from *Arabidopsis thaliana* in a yeast *trk1*Δ *trk2*Δ mutant has just been reported (*29a*). The predicted amino acid sequences of *AKT1* and *KAT1* share extensive identity but are not allelic.

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7. Flame emission assay of whole-cell HCl extract, corrected for a cytoplasmic volume equal to 50% of the packed cell volume.
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31. Yeast cells were grown to early stationary phase in a synthetic liquid (*3*), incubated for 3 hours at 28°C, centrifuged, and resuspended at 2.5 × $10^{10}$ cells per milliliter. A 20-μl aliquot of the yeast suspension was mixed with 20 μl of a medium (*3*) containing KCl and $^{86}Rb^+$ (12 KBq per ml). After 3 minutes, uptake was stopped by addition of 10 ml of ice-cold 2 mM $CaSO_4$. The cells were collected on filters, washed with 10 ml of ice-cold $CaSO_4$, dried, and assayed for radioactivity. Uptake was linear with time for the first 5 minutes of incubation.
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# DNA Hydrolyzing Autoantibodies


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**A DNA-nicking activity was detected in the sera of patients with various autoimmune pathologies and was shown to be a property of autoantibodies. The DNA hydrolyzing activity, which was purified by affinity and high-performance liquid chromatography, corresponded in size to immunoglobulin M (IgM) and IgG and had a positive response to antibodies to human IgG. The DNA hydrolyzing autoantibodies were stable to acid shock and yielded a DNA degradation pattern that was different from that of deoxyribonuclease (DNase) I and blood DNase.**


Patients with autoimmune diseases produce autoantibodies to nucleoprotein complexes (*1*), to DNA, and to enzymes that participate in nucleic acid metabolism (*2*). In autoimmune diseases, there can be spontaneous induction of anti-idiotypic antibodies (Abs), which are Abs elicited by a primary antigen. These anti-idiotypic Abs may have characteristics of the primary antigen, including catalytic activity. In some cases, the sera of patients with scleroderma, systemic lupus erythematosus (SLE), or rheumatoid arthritis have an


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increased titer of primary Abs to topoisomerase I and (3) anti-idiotypic Abs to topoisomerase I, which are characterized by a high affinity for DNA [dissociation constant ($K_d$) = 0.1 nM]. Antibodies capable of catalyzing cleavage of peptide bonds have also been demonstrated in human sera (4). Thus, we investigated whether autoanti-

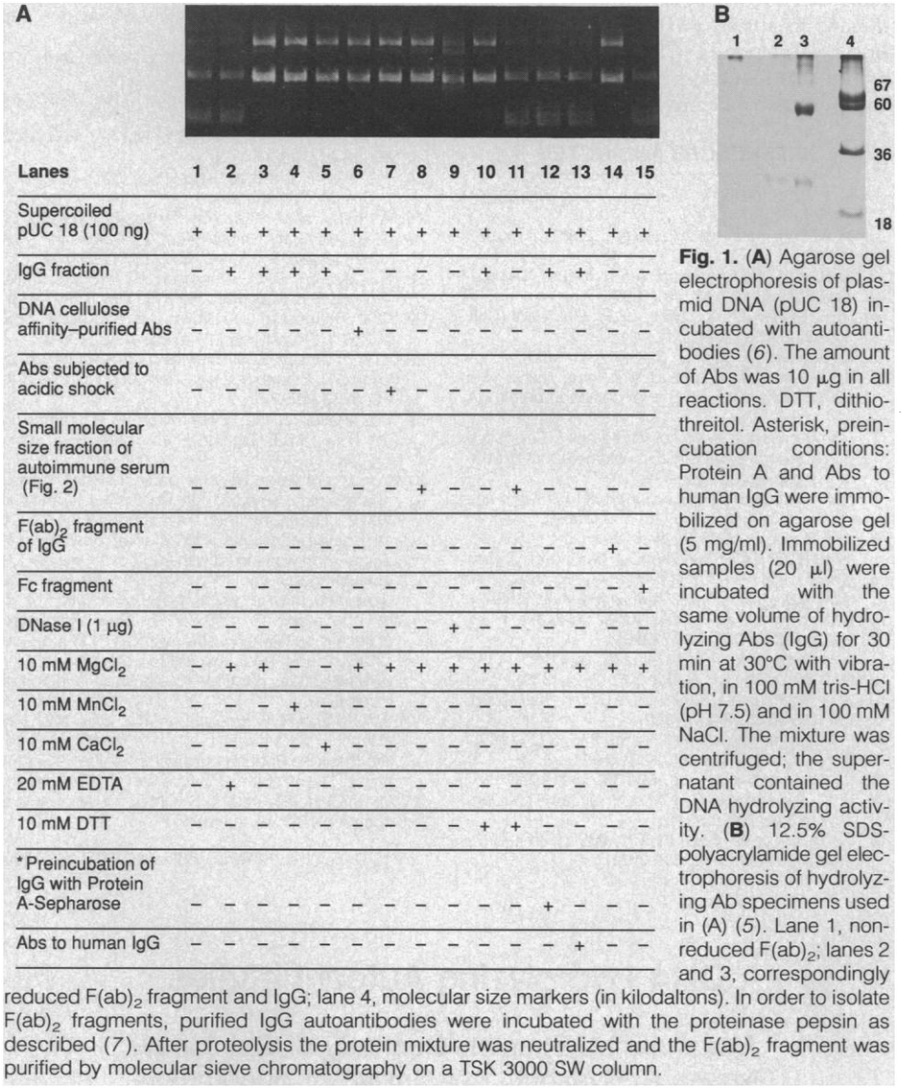


**Fig. 1.** (**A**) Agarose gel electrophoresis of plasmid DNA (pUC 18) incubated with autoantibodies (*6*). The amount of Abs was 10 μg in all reactions. DTT, dithiothreitol. Asterisk, preincubation conditions: Protein A and Abs to human IgG were immobilized on agarose gel (5 mg/ml). Immobilized samples (20 μl) were incubated with the same volume of hydrolyzing Abs (IgG) for 30 min at 30°C with vibration, in 100 mM tris-HCl (pH 7.5) and in 100 mM NaCl. The mixture was centrifuged; the supernatant contained the DNA hydrolyzing activity. (**B**) 12.5% SDS-polyacrylamide gel electrophoresis of hydrolyzing Ab specimens used in (A) (*5*). Lane 1, nonreduced $F(ab)_2$; lanes 2 and 3, correspondingly reduced $F(ab)_2$ fragment and IgG; lane 4, molecular size markers (in kilodaltons). In order to isolate $F(ab)_2$ fragments, purified IgG autoantibodies were incubated with the proteinase pepsin as described (*7*). After proteolysis the protein mixture was neutralized and the $F(ab)_2$ fragment was purified by molecular sieve chromatography on a TSK 3000 SW column.

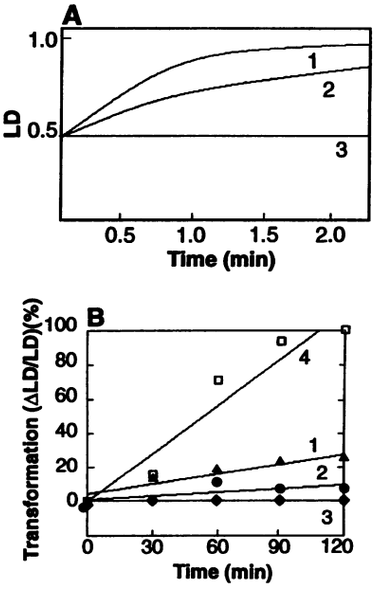


**Fig. 3.** (**A**) Kinetics of the increase in the number of nick sites in plasmid DNA pUC 18 treated with DNase I (line 1), hydrolyzing Abs (line 2), or untreated (line 3), recorded by the linear dichroism method (LD). (**B**) Activity assay of hydrolyzing Abs from different sera by the LD method. Plasmid DNA (1 μg) was incubated with the fixed amount of Abs (2 μg) in 200 μl of the reaction buffer that contained 50 nm tris-HCl (pH 7.5), 10 nm $MgCl_2$, and 10% glycerol. Curve 1, 10 μg of total IgG fraction from the serum of an SLE patient; curve 2, 10 μg of total IgG fraction from the serum of an SLE patient after treatment; curve 3, 10 μg of total IgG fraction from healthy donors; and curve 4, DNase I from bovine pancreas.

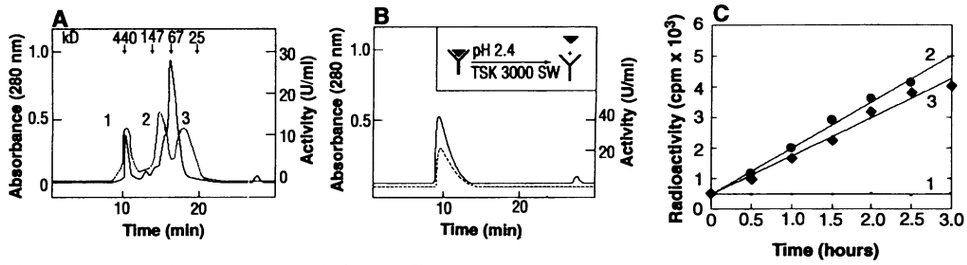


**Fig. 2.** (**A**) Nuclease activity assay of autoimmune serum elution profile after gel filtration on a TSK 3000 SW column. Solid line, elution profile, 200 μl of serum introduced; dotted line, DNA-hydrolyzing specific activity profile estimated by electrophoresis of plasmid DNA (pUC 18) incubated overnight with 10 μl of various fractions. Experimental conditions are as described (*6*); nicking activity was present in fractions with large molecular size (peaks 1 and 2) that corresponded to IgM and IgG, as well as in the small molecular size fraction that was ~30 kD (peak 3). The activity of this fraction was probably a result of blood DNase. The large molecular size fractions were subjected to further purification (*5*). (**B**) Chromatography of hydrolyzing autoantibodies at low pH values after acidic shock. The scheme of the experiment is given at the top. Autoantibodies were incubated at pH 2.4 for 2 hours, and gel filtration on a TSK 3000 SW column was performed. Solid line, elution profile; dotted line, specific activity profile. (**C**) Kinetics of nicking determined by the coupled DNA polymerase I reaction. DNA (pUC 18) and the following additions were incubated for the indicated times. After incubation, the mixture was supplemented with 2 U of DNA polymerase I and incubated for 5 min at 25°C; the reaction was then terminated with EDTA (20 mM). Line 1, DNA plus no addition; line 2, DNA incubated with DNase I (10 ng); line 3, DNA incubated with the $F(ab)_2$ fragment of autoantibodies (10 μg).

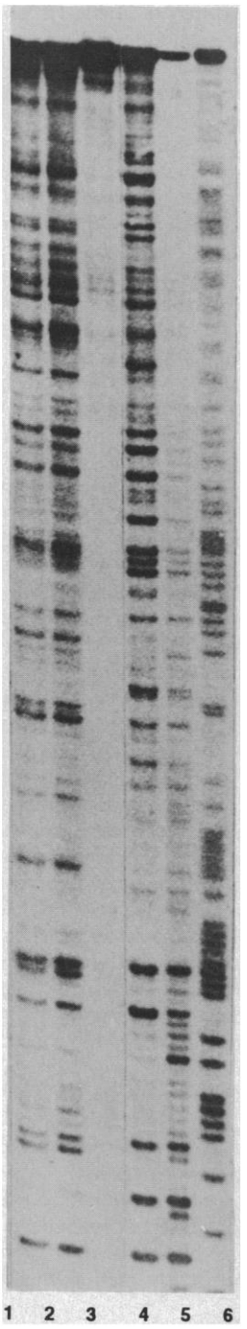


**Fig. 4.** Comparison of the degradation patterns of DNase I, DNase from human sera, and hydrolyzing autoantibodies. The end-labeled 300-bp fragment [including the promoter and part of the transcribed spacer of the rat ribosomal genes (20 ng)] was digested in the presence of 25 mM tris-HCl (pH 7.5), 100 mM NaCl, and 10 mM $MgCl_2$ with: lane 1, IgG autoantibodies, affinity purified, 1 μg (12 hours at 37°C); lane 2, IgM autoantibodies, crude fraction, 1 μg (12 hours at 37°C); lane 3, control (untreated); lane 4, the crude fraction of the DNase from human sera, 20 μg (12 hours at 37°C); lane 5, DNase I from bovine pancreas, 0.25 U (1 min at room temperature); lane 6, DNA sequence reaction (A + G).

bodies with DNA-cleaving activity could be generated.

Using plasmid DNA (pUC18) as the substrate, we tested the serum of a patient with SLE for DNA hydrolyzing activity. The reaction mixtures were analyzed by electrophoresis in agarose gels (Fig. 1A). When the supercoiled plasmid DNA was incubated overnight with the total Ab fraction from autoimmune serum (10 μg), supercoiled DNA decreased in amount with a concomitant increase in nicked DNA. In order to study the properties of this DNA-nicking activity, we purified it using the following protocol (5): (i) precipitation of fractions with ammonium sulfate, (ii) molecular sieve chromatography, (iii) affinity chromatography on Protein A–Sepharose, (iv) affinity chromatography on DNA cellulose in the absence of metal ions, and (v) a second molecular sieve chromatographic step.

The large molecular size fraction obtained from the second molecular sieve chromatographic step yielded protein bands on an SDS-polyacrylamide gel that corresponded in size to IgG. This fraction retained nicking activity and, in an enzyme-linked immunosorbent assay, yielded a positive response for Ab to human IgG. These observations suggest that the detected nicking activity was contained in the immunoglobulin fraction. However, it is possible that this nicking activity was a property of an IgG-associated DNase.

Because IgGs are stable to acid shock, we tested whether the DNA-nicking activity of the purified Ab fraction was stable in 1 M acetic acid (Fig. 2B). Noncovalent complexes dissociate under these conditions. However, 76% of the DNA hydrolyzing activity remained.

To further study the properties of these DNA hydrolyzing Abs, we isolated, from the IgG fraction of hydrolyzing Abs, a homogeneous $F(ab)_2$ fragment that retained nicking activity. No such activity was detected in the Fc fragment. The DNA-nicking activity of the $F(ab)_2$ fragment was dependent on the presence of metal ions ($Mg^{2+}$, $Mn^{2+}$, and $Ca^{2+}$) and was inhibited by EDTA (Fig. 1). Incubation of the reaction mixture with immobilized Abs to human IgG and protein A led to the disappearance of the DNA-nicking activity (Fig. 1).

We measured the kinetics of an increase in nick sites in DNA in the presence of the DNA hydrolyzing Abs (Fig. 2C). DNA polymerase I, which requires a nick to initiate DNA replication, incorporated more $^{32}P$-labeled nucleotides when replicating plasmid DNA in the presence of these Abs than in their absence. We also used the linear dichroism method (LD), which is sensitive to the integrity of flow-oriented DNA molecules, to measure the DNA hydrolyzing activity of these Abs compared to that of DNase I. The LD value of plasmid DNA increased with DNase I treatment and treatment with DNA-nicking Abs (Fig. 3A).

The LD method also allowed quantitation of the specific activity of various autoantibody preparations (Fig. 3B). From these data we conclude that the specific activity of hydrolyzing Abs from autoimmune sera was more than an order of magnitude higher than that from healthy donors and two orders of magnitude less than that of DNase I. Our investigation of the DNA cleavage patterns by the hydrolyzing Abs did not reveal any cleavage-site specificity (Fig. 4). The cleavage patterns of DNA by the IgG and IgM autoantibodies were similar, but differed from those produced by DNase from human sera and by DNase I.

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6. Purification of DNA hydrolyzing Abs was performed with 1 ml of serum from a patient with SLE. Plasma DNA from the reaction was subjected to electrophoresis on a 1% agarose gel with TAE buffer. We incubated 100 ng supercoiled DNA pUC 18 in 50 mM tris-HCl buffer (pH 7.5) and 100 mM NaCl for 1 hour at 37°C.
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