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Requirement of protein co-factor for the DNA-binding function of the human *ski* proto-oncogene product

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ABSTRACT

We identified the human c-*ski* gene product (c-Ski) as a protein with the apparent molecular weight of 100,000, p100$^{c\text{-}ski}$, by using a c-Ski-specific polyclonal antibody. p100$^{c\text{-}ski}$ was a nuclear protein and p100$^{c\text{-}ski}$ in nuclear extracts of Molt4 cells bound to calf thymus DNA cellulose, but the bacterially synthesized c-Ski did not, suggesting that Ski was associated with another protein(s) and that the Ski complex had DNA-binding activity. This hypothesis was supported by the finding that the bacterially synthesized Ski bounds to DNA cellulose after being mixed with a nuclear extract of Molt4 cells. By use of a series of deletion mutants of Ski synthesized in an *in vitro* translation system, two portions in Ski were found to be necessary for the DNA binding of the Ski complex: the N-proximal portion containing a cystein/histidine-rich domain and the C-terminal portion including a region rich in basic amino acids.

INTRODUCTION

The v-*ski* oncogene is carried by Sloon-Kettering viruses (SKVs), which are acute transforming retroviruses (1). Chicken or quail embryo cells transformed by SKVs form foci in monolayer cultures and form colonies when suspended in soft agar (2). Analysis of fibroblasts transformed by v-*ski* indicated that most of the *gag-ski* fusion protein in these cells is in the nucleus (3), but the function of Ski is poorly understood.

As a first step in the examination of the structure and function of the *ski* gene, chicken and human *ski* cDNAs were isolated and the structure of c-Ski was compared with that of v-Ski (4–6). The recombination events that led to the incorporation of *ski* into the viral genome resulted in the truncation of *ski* sequences at both the 5′ and 3′ ends. In addition, the structures of cDNAs suggested that multiple c-*ski* mRNAs encoding different Ski proteins are produced from the c-*ski* locus by alternate splicing (4–6). cDNA clones of the *ski*-related gene, *sno*, have also been obtained (6), and the N-terminal domain of the *sno* protein had much homology with Ski, so this domain may be important for the function of the gene products of the *ski* gene family. To understand the role of Ski in the control of cell growth, we examined the cell location and the DNA-binding activity of human c-Ski. Our studies showed that c-Ski, p100$^{c\text{-}ski}$, was in the nuclei, and that the Ski complex bound to DNA, although DNA-binding activity is not an intrinsic property of c-Ski.

MATERIALS AND METHODS

Construction of c-Ski expression vector. For expression of c-Ski in *Escherichia coli*, the plasmid pAR2156*ski* was constructed as follows. A *Bam*HI linker was added to the *Nco*I site at nucleotide 71 and the *Dra*I site at nucleotide 2,562 of the human c-*ski* cDNA clone (6), and a 2,492-bp fragment containing the complete c-Ski-coding region was isolated. This was inserted into the *Bam*HI site of the pAR2156 expression vector (7) with the T7 late promoter and Shine–Dalgarno box to generate the plasmid pAR2156*ski*.

To express c-Ski in an *in vitro* translation system, the 2,492-bp *Bam*HI fragment containing the complete c-Ski-coding region was isolated from the plasmid pAR2156*ski* and inserted into the *Bam*HI site of the pSP65 expression vector (8) with the SP6 promoter to generate the plasmid pSP65*ski*. All plasmids designed to express mutant c-Ski proteins in the *in vitro* translation system were generated from the plasmid pSP65*ski*. To generate the plasmid pSP65*ski*Δ1 without the 690-bp *Apa*LI-*Eco*RI fragment (nucleotides 854-1,543; nucleotide numbers are as in ref. 6), the plasmid pSP65*ski* was digested with *Apa*LI and *Eco*RI, filled up by Klenow polymerase, and ligated after ligation with *Bgl*II linker (8 mer) and digestion with *Bgl*II. To obtain the plasmid pSP65*ski*Δ2 containing the deleted 647-bp *Stu*I-*Apa*LI fragment (nucleotides 207–853), the plasmid pSP65*ski* was digested with *Stu*I and *Apa*LI, filled up by Klenow enzyme, and joined by blunt-end ligation. To make plasmid pSP65*ski*CT2, which lacks the C-terminal 1,020-bp fragment downstream of the *Eco*RI site at nucleotide 1,543, the 1,491-bp *Eco*RI fragment containing the region between the *Eco*RI site in the pSP65 vector and the *Eco*RI site at nucleotide 1,543 was isolated from the plasmid pSP65*ski* and inserted into the *Eco*RI site of pSP65 plasmid.

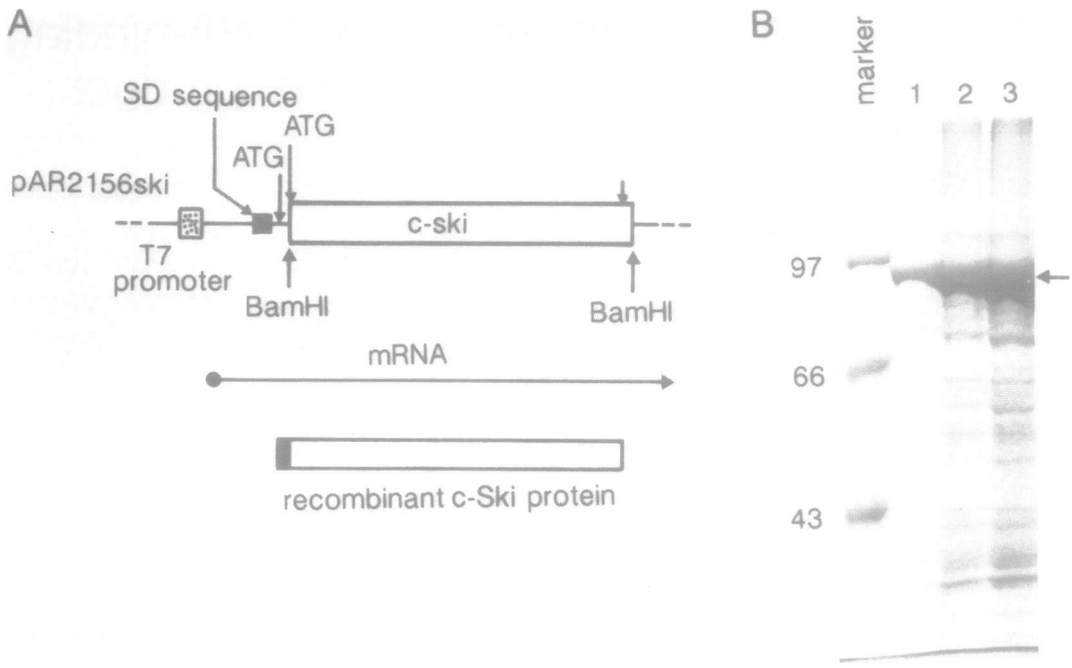

Fig. 1. Expression of c-Ski in *E. coli*. A. The structure of recombinant c-Ski synthesized in *E. coli*. The first ATG initiates translation of a short run of the T7 vector sequence fused to the c-*ski* cDNA. The composition of the hybrid protein and construction of pAR2156*ski* plasmid are described in the text. B. Analysis of bacterial protein. Insoluble proteins prepared from bacteria BL21(DE3) harboring the pAR2156*ski* expression vector were electrophoresed in a 10% SDS-polyacrylamide gel and stained with Coomassie brilliant blue. Lanes 1–3 contain material from 0.1, 0.3, and 0.5 ml of culture, respectively, grown in the presence of IPTG to induce expression of T7 RNA polymerase from the *lac* promoter. Sizes of molecular mass markers are shown in kDa on the left. C-Ski is indicated by the arrow.

Purification of bacterially synthesized c-Ski. Bacteria BL21(DE3) carrying pAR2156*ski* were grown and the expression of c-Ski was induced by addition of isopropyl-β-D-thiogalactopyranoside (IPTG) as described before (9). The insoluble material containing c-Ski was prepared as described elsewhere (9) and dissolved in urea buffer (50 mM Tris-HCl, pH 8.0, 1% Triton X-100, 8 M urea, 5% [v/v] glycerol, 0.1 mM phenylmethysulfonyl fluoride [PMSF], and 0.1 mM dithiothreitol [DTT]). After the sample was left on ice for 2 h, it was centrifuged at 100,000×*g* for 2 h; the supernatant was dialyzed against TDU buffer (10 mM Tris-HCl, pH 8.0, 1 mM DTT, and 2 M urea) for 1.5 h, and then against TDKM buffer (20 mM Tris-HCl, pH 7.4, 0.2 mM DTT, 70 mM KCl, 2 mM $MgCl_2$). The c-Ski protein in this dialyzed sample was about 60% of the total protein.

Preparation of antibody against human c-Ski and Western blot analysis. The bacterially synthesized c-Ski was purified by SDS-polyacrylamide gel electrophoresis as described previously (9) and used for immunization. Adult female rabbits (New Zealand White) were immunized s.c. with 500 μg of purified c-Ski emulsified in complete Freund's adjuvant. The immunization was repeated every 2 to 4 weeks with antigen in incomplete Freund's adjuvant. After proteins were separated on a 10% polyacrylamide gel containing SDS and electrotransferred to nitrocellulose filters, blots were blocked by incubation with 5% non-fat dried milk in TBS (20 mM Tris-HCl, pH 7.8, and 150 mM NaCl) for 30 min at room temperature. Then the filter was incubated with rabbit serum raised against the bacterially synthesized c-Ski diluted 1:100 in TBS containing 0.05% Tween 20 for 90 min at room temperature. Anti-Ski specific antibodies were detected by anti-rabbit IgG (BioRad) antibody conjugated with alkaline phosphatase as described in manufacturer's instructions.

Fractionation of nuclei. Nuclei were prepared from the human T-cells, Molt4, in hypotonic buffer (10 mM Hepes, pH 7.9, 5 mM KCl, and 2 mM $MgCl_2$) as described eleswhere (10). For the fractionation experiments, nuclear supernatants and residual nuclei were separated by centrifugation (10,000×*g*, 5 min).

Preparation of nuclear extracts and fractionation by heparin agarose. Proteins were extracted from 4.75×10^9 nuclei of Molt4 cells with 20 ml of HM buffer (10 mM Hepes, pH 7.9, and 2 mM $MgCl_2$) containing 0.4 M NaCl for 10 min at 4°C. The eluate was centrifuged at 10,000 × *g* for 10 min and diluted with the hypotonic buffer to a final concentration of 60 mM NaCl. This nuclear extract was put on a 4-ml column of heparin agarose equilibrated with buffer B (10 mM Hepes, pH 7.9, 1 mM KCl, 0.4 mM $MgCl_2$, and 0.8 mM EDTA) containing 60 mM NaCl. Bound proteins were eluted with a salt gradient of 0.1 to 1.1 M NaCl in buffer B and a portion of each fraction was mixed with the bacterially synthesized c-Ski and analyzed by DNA-cellulose column chromatography.

In vitro transcription and translation. Plasmid DNAs carrying various *ski* cDNAs, purified by equilibrium centrifugation in CsCl, were digested with the appropriate restriction enzymes, extracted with phenol/chloroform and precipitated with ethanol. To make the wild-type c-Ski, CT1, CT3, and CT4 mutants, pSP65*ski* DNA was digested with *Hind*III (in the pSP65 vector), *Mlu*I (at nucleotide 1,854), *Ava*II (at nucleotide 881), and *Pvu*II

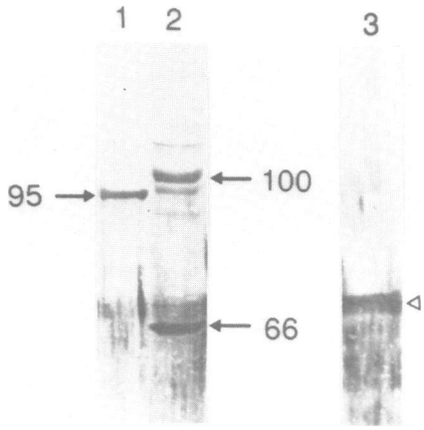

Fig. 2. Identification of human c-Ski by Western blot analysis. Insoluble proteins from 10 μl of cultured bacteria BL21(DE3) harboring pAR2156*ski* (lane 1) or nuclear extracts from 6 × 10^6 Molt4 cells (lanes 2 and 3) were fractionated in 8% SDS-polyacrylamide gel, blotted onto nitrocellulose, and stained with an antibody raised against the bacterially synthesized c-Ski (lanes 1 and 2) or with the preimmune serum (lane 3). The band indicated by an open triangle corresponds to the keratin protein. Masses are indicated in kilodaltons.

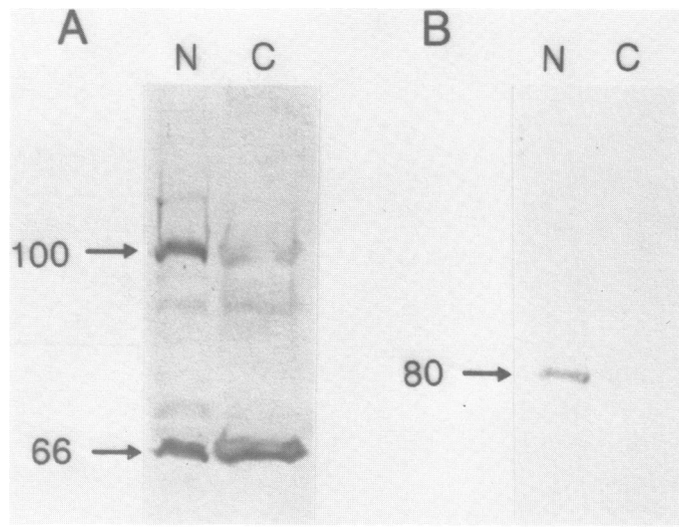

Fig. 3. Subcellular location of human c-Ski. Nuclear (N) and cytoplasmic fractions (C) of Molt4 cells were analyzed by electrophoresis in a 8% SDS-polyacrylamide gel, blotted onto nitrocellulose, and stained with an antibody raised against the bacterially synthesized c-Ski (A) or against the bacterially synthesized c-*myb* protein (B). Each lane contains material from 6 × 10^6 cells. Masses are indicated in kDa.

(at nucleotide 543). To obtain the CT2, Δ1, and Δ2 mutants, pSP65*ski*CT2, pSP65*ski*Δ1, and pSP65*ski*Δ2 DNAs were digested with *Hind*III (in the pSP65 vector), respectively. To generate the Δ3 mutant, pSP65Δ2 DNA was digested with *Mlu*I (at nucleotide 1,854). Five micrograms of linearized DNA template was transcribed *in vitro* by SP6 RNA polymerase in 50 μl of reaction mixture (8). For *in vitro* capping, 500 μM m^7GpppG (Pharmacia) and 50 μM GTP were used in the transcription reaction. RNA was extracted with phenol/chloroform and precipitated with ethanol. Five hundred nanograms of RNA was translated *in vitro* with use of a lysate of rabbit reticulocytes (25 μl of total reaction volume) with 25 μCi of ^{35}S-methionine (29.6 TBq/mmol) as directed by the supplier (New England Nuclear Corp.). ^{35}S-methionine-labeled translation products were analyzed by SDS-polyacrylamide gel (10% or 15%) electrophoresis followed by fluorography.

Analysis of DNA-binding activity by DNA-cellulose column chromatography. The bacterially synthesized c-Ski or the reticulocyte lysates containing ^{35}S-labeled c-Ski were put on a column (0.3−1.0 ml) of native calf thymus DNA cellulose (Sigma) equilibrated with TDE buffer containing 50 mM NaCl. To analyze the protein in the nuclear extract from Molt4 cells, which is required for the DNA binding of the c-Ski complex, the bacterially synthesized c-Ski was incubated on ice for 1 h with nulear extract prepared from Molt4 cells or a fraction separated by heparin agarose column chromatography and put onto the same kind of DNA-cellulose column. The column was washed with 10 bed volumes of equilibration buffer to remove unbound proteins. Bound proteins were eluted with a stepwise gradient of 0.05 to 1.0 M NaCl in TDE buffer. Proteins in each fraction were precipitated with trichloroacetic acid, washed with acetone, suspended in SDS sample buffer, separated on 8, 10, or 15% polyacrylamide gel containing 1% SDS and analyzed by Western blotting or fluorography. To measure the amount of mutant c-Ski bound to the DNA cellulose, autoradiograms were traced by a densitometer and the peak areas for each mutant c-Ski were measured.

RESULTS

Expression of c-Ski in E. coli. To obtain large amounts of c-Ski, the human c-*ski* cDNA (6) was inserted into a T7 expression vector (7) downstream of a ribosome binding site and its accompanying ATG initiation codon to generate pAR2156*ski* (Fig. 1A outlines the construction of this vector). The hybrid T7-c-Ski protein encoded by this gene contains the first 14 amino acid residues derived from the vector fused to the complete c-Ski protein. Large amounts of c-Ski fusion-protein, with the apparent molecular weight of 95,000 were produced, and they accumulated as insoluble material within the cells (Fig. 1B). The identity of c-Ski was confirmed by amino acid sequencing 30 amino-terminal residues and determination of amino acid composition (data not shown). By washing the insoluble material, we obtained highly enriched preparations of the bacterial Ski, and recovered 2 to 3 mg/l of c-Ski following detergent or urea treatment.

Identification of human c-Ski and its nuclear location. The bacterially expressed c-Ski was purified by preparative SDS-polyacrylamide gel electrophoresis and used as an antigen for polyclonal antibody production. To identify the human c-Ski, the human T-cell line Molt4, which expresses the c-*ski* mRNA, was chosen as a source of protein. The antiserum raised against the bacterially synthesized c-Ski recognized two protein species of Molt4 cells with the apparent molecular weight of 100,000 and 66,000 (Fig. 2). These observations suggested that the 100-kDa protein might be a product of the human c-*ski* gene and the 66-kDa protein also might be such a product, generated by alternative splicing or being the result of degradation. We therefore tentatively refer to this 100-kDa protein as p100$^{c\text{-}ski}$. To judge from the sequence of the human c-*ski* cDNA, the human c-*ski* gene encodes a protein of 728 amino acid residues with a calculated molecular weight of 80,004 (6). The difference of molecular weight between p100$^{c\text{-}ski}$ and the bacterially

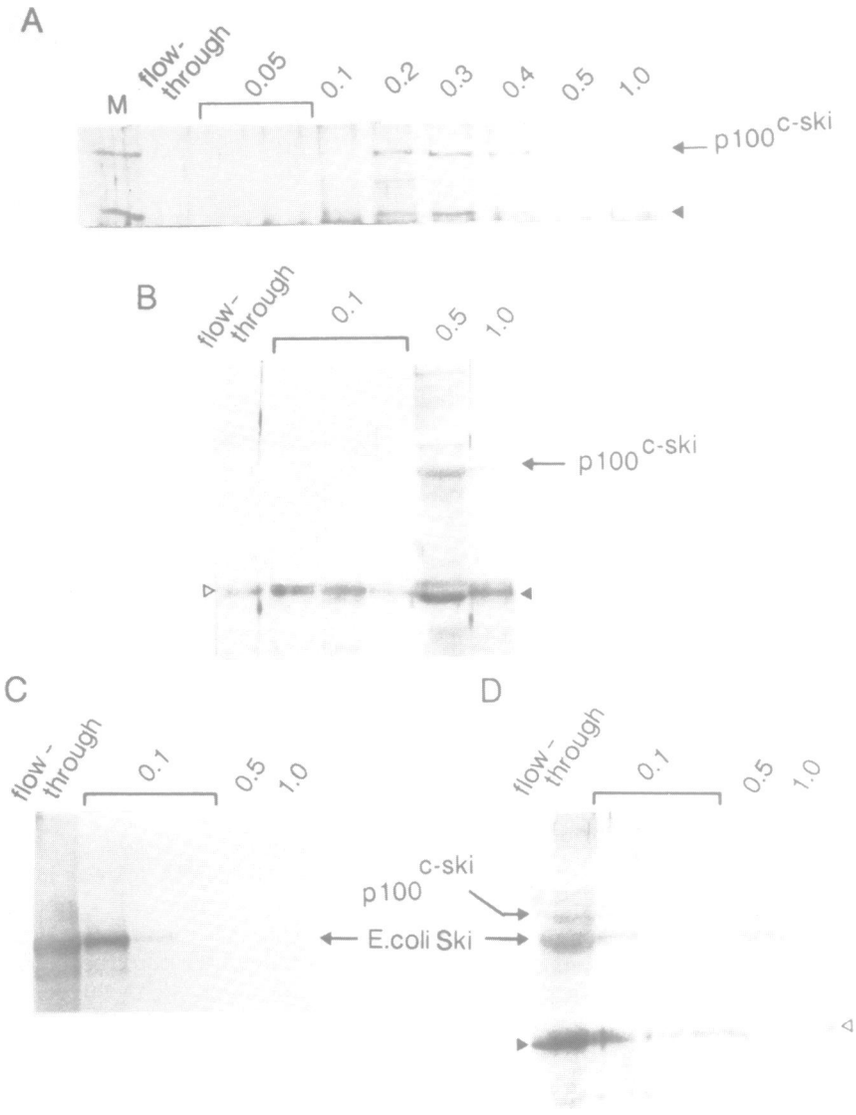

Fig. 4. DNA-cellulose chromatography of human c-Ski and bacterially synthesized c-ski. A nuclear extract prepared from 2×10^7 Molt4 cells (A and B), 10 μg of the bacterially synthesized c-Ski (C), or a mixture of the nuclear extract prepared from 2×10^7 Molt4 cells and 10 μg of the bacterially synthesized c-Ski (D) was put onto a double-stranded DNA-cellulose column, and bound proteins were eluted by a stepwise gradient of NaCl. The whole portion of each fraction was analyzed by 8% SDS-polyacrylamide gel electrophoresis and Western blot analysis with the antibody raised against the bacterially synthesized c-Ski. Numbers above each lane indicate the concentrations of NaCl (molar) in the elution buffer.

synthesized c-Ski could be caused by a post-translational modification such as glycosylation.

We expected $p100^{c\text{-}ski}$, like the *gag-ski* fusion protein found in v-*ski* transformed fibroblasts, to be located in the nucleus. Fractionation of Molt4 cells showed that $p100^{c\text{-}ski}$ was in the nuclei, not in the cytosol (Fig. 3A), indicating that $p100^{c\text{-}ski}$ is a nuclear protein. As a control, the subcellular location of the human c-*myb* protein, a nuclear protein (11), was checked by use of the polyclonal antibody raised against the bacterially synthesized c-*myb* protein. The c-*myb* protein was detected only in the nuclei (Fig. 3B). The 66-kDa protein was detected in both the nuclei and the cytosol (Fig. 3A), but the meaning of this finding is unclear.

DNA-binding properties of the c-Ski complex. The ability of c-Ski to bind to double-stranded DNA was assayed by its retention on native calf thymus DNA cellulose. When nuclear extracts prepared from Molt4 cells were put on a native DNA-cellulose column, most of the $p100^{c\text{-}ski}$ and most of the 66 kDa species were retained on double-stranded DNA cellulose and eluted with 0.2−0.5 M NaCl (Fig. 4, A and B). In contrast, the bacterially synthesized c-Ski had no DNA-binding activity, and almost all of the bacterially synthesized c-Ski put on the DNA cellulose column was collected in the flow-through fractions or the column buffer washes (Fig. 4C). These findings suggest that DNA binding is not an intrinsic property of c-Ski, and that it is dependent on associated protein(s). In fact, 30−40% of the bacterially synthesized c-*ski* protein bound to the DNA cellulose and about half of the $p100^{c\text{-}ski}$ and most of the 66 kDa species extracted from Molt4 cells were collected in the flow-through fractions when the bacterially synthesized c-Ski was mixed with nuclear extracts prepared from Molt4 cells (Fig. 4D), perhaps because protein(s) associated with $p100^{c\text{-}ski}$ and the 66 kDa species were transferred to the bacterially synthesized c-Ski.

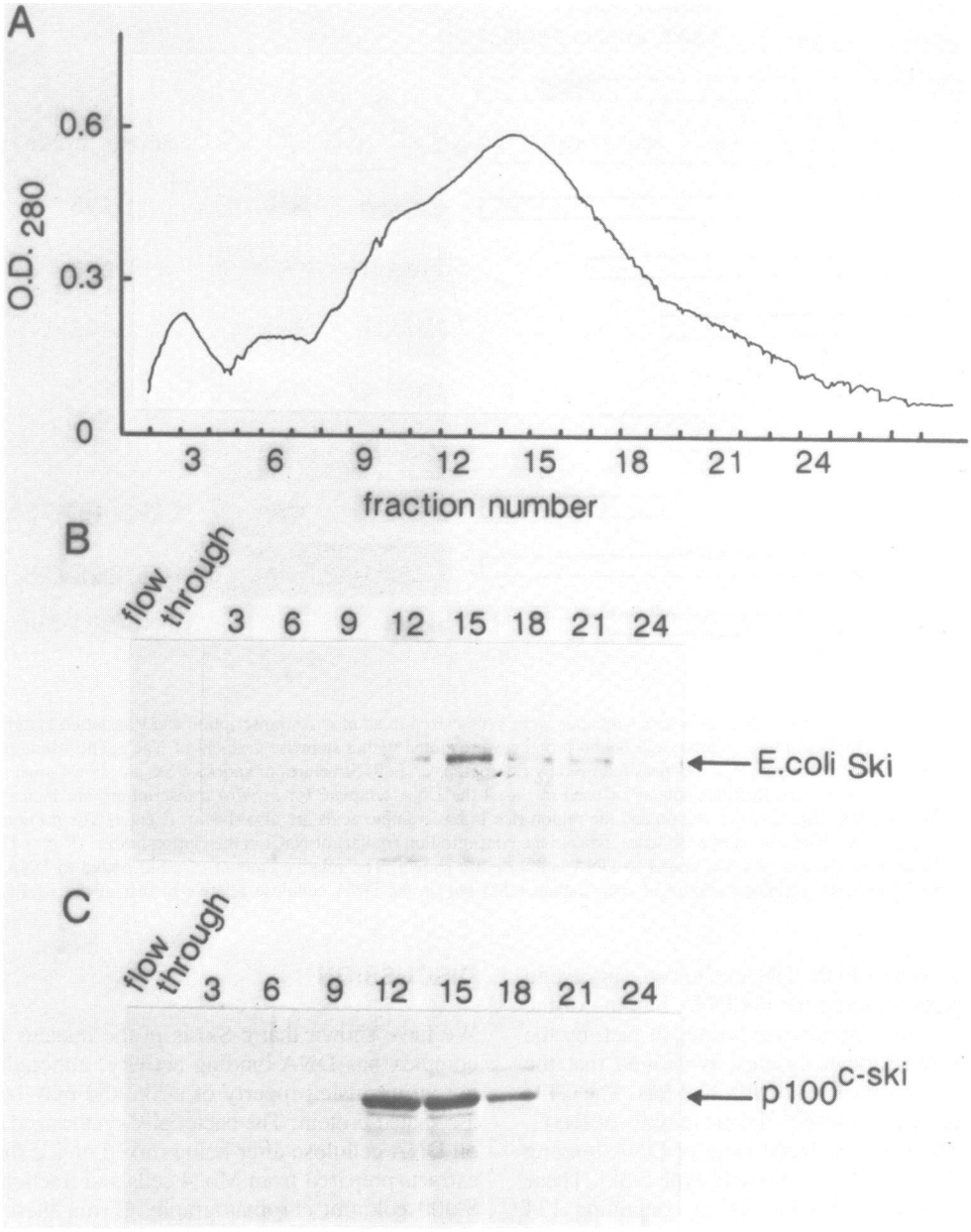

Fig. 5. Partial purification of the protein required for DNA binding of the c-Ski complex. A. Heparin agarose chromatography of Molt4 nuclear extracts. Nuclear extracts from Molt4 cells were fractionated by heparin agarose and their OD_{280} profile is shown here. B. Analysis for the protein required for DNA binding of the c-Ski complex. Fifty three micrograms of the bacterially synthesized c-Ski and 100 μl of a fraction separated by heparin agarose were mixed and put onto a DNA-cellulose column. After the column was washed, bound proteins were eluted with 1.0 M NaCl. The whole portion of a fraction eluted with 1.0 M NaCl was analyzed by 8% SDS-polyacrylamide gel electrophoresis followed by Western blot analysis with antibody against the bacterially synthesized c-Ski. The number above each lane shows the number of fraction used. C. Analysis for $p100^{c\text{-}ski}$. A hundred microliter of each fraction was analyzed by 8% SDS-polyacrylamide gel electrophoresis followid by Western blot analysis with the antibody against the bacterially synthesized c-Ski.

To fractionate the protein(s) that may associate with c-Ski and contribute the DNA-binding of the c-Ski complex, nuclear extracts prepared from Molt4 cells were fractionated by heparin agarose column chromatography (Fig. 5A). The fractions were mixed with the bacterially synthesized c-Ski and put onto the DNA-cellulose column. Fractions between 12 and 15 contained most of the activity of conferral of the DNA-binding function to the bacterially synthesized c-Ski (Fig. 5B). These fractions also contained the native $p100^{c\text{-}ski}$ (Fig. 5C). In Fig. 5B, the $p100^{c\text{-}ski}$ was not detected because the relative amount of $p100^{c\text{-}ski}$ was far less than the bacterially synthesized c-Ski.

Domains in c-Ski required for the DNA-binding of the c-Ski complex. To analyze the domain structure in c-Ski needed for the DNA binding of the c-Ski complex, various deletion mutants of c-Ski synthesized in an *in vitro* translation system with use of rabbit reticulocyte lysate were employed. The bacterially synthesized c-Ski was retained on a DNA-cellulose column after being mixed with the reticulocyte lysate (data not shown), indicating that the reticulocyte lysate contained the protein required for the DNA-binding of the c-Ski complex. The wild-type c-Ski was synthesized in the *in vitro* translation system and put onto the DNA-cellulose column. About half of the ^{35}S-

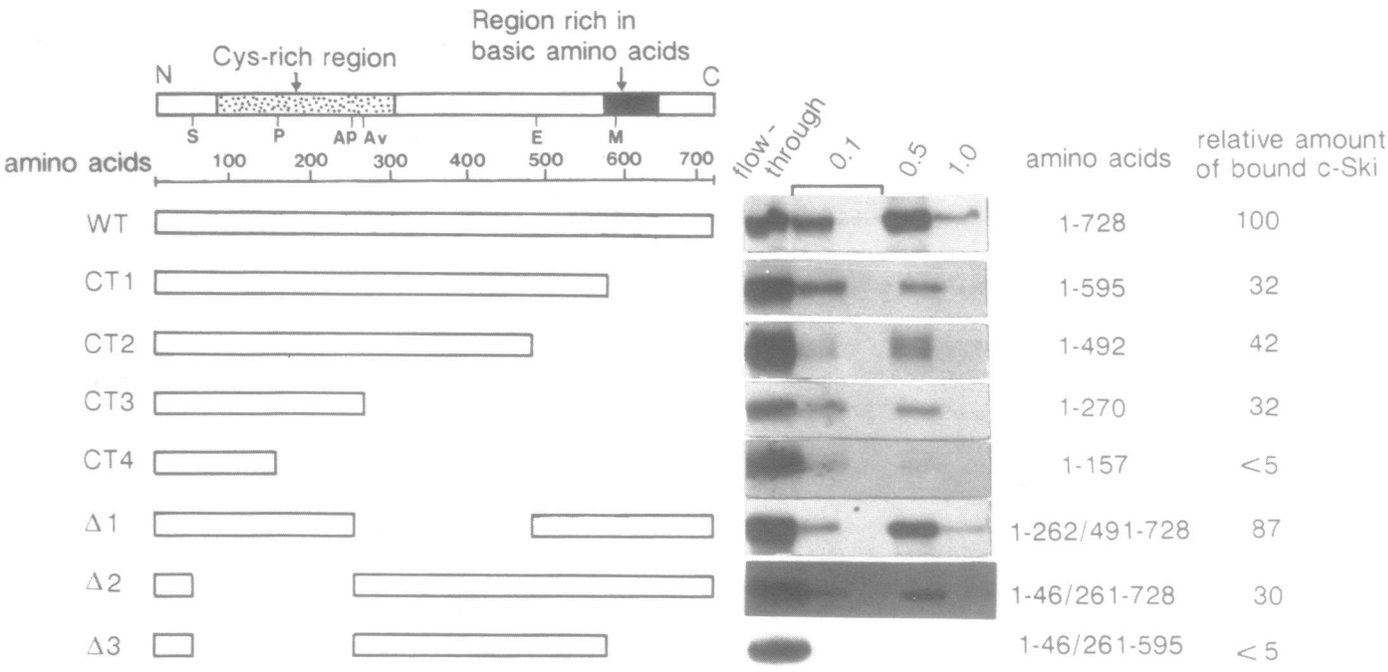

Fig. 6. Deletion analysis of c-Ski. The wild-type c-Ski and various mutants were synthesized in an *in vitro* transcription and translation system with ^{35}S-methionine. Translation products were put onto a DNA-cellulose column, and bound proteins were eluted with a stepwise gradient of NaCl. The whole portion of each fraction was analyzed by 10 or 15% SDS-polyacrylamide gel electrophoresis followed by fluorography. (Left) Structures of various c-Ski are shown schematically. The restriction enzyme sites, which were used to construct the deletion mutants or used to digest the DNA template for *in vitro* transcription, are indicated; S, *Stu*I; P, *Pvu*II; Ap, *Apa*I; Av, *Ava*II; E, *Eco*RI; M, *Mlu*I. The Cys-rich region and the region rich in basic amino acids are also shown. (Center) The portions containing the bands corresponding to various c-Ski are shown. Numbers above the lanes indicate the concentration (molar) of NaCl in the elution buffer. (Right) The amino acid residues contained in each c-Ski and the relative amount of c-Ski bound to DNA cellulose are shown. The relative amount of c-Ski bound to DNA cellulose is shown as the amount of mutant c-Ski bound to DNA cellulose/amount of each mutant c-Ski put on the DNA cellulose relative to that of the wild-type c-Ski.

methionine-labeled c-Ski bound to the DNA cellulose, suggesting that the amount of protein required for the DNA binding of the c-Ski complex is small in the reticulocyte lysate. In fact, by the use of various lots of reticulocyte lysates, we found that the amount of this protein co-factor varied in different lots. The CT1, CT2, and CT3 mutants, which lacked various lengths of the C-terminal portion of c-Ski, had a reduced level of DNA-binding activity, at 32−42% of the activity of the wild-type c-Ski. These findings suggested that the C-terminal region containing 134 amino acid residues (amino acids 595−728), which was deleted in the CT1 mutant, was partly responsible for the DNA-binding activity of the c-Ski complex. The CT4 mutant, which lacks a larger region (amino acids 158−728), was no longer retained on the DNA cellulose, indicating that the region between amino acids 158 and 270 also contributed to the DNA-binding activity of the c-Ski complex. To check these results, three mutants, Δ1, Δ2 and Δ3, were synthesized *in vitro* and their DNA-binding activities were examined. The Δ2 mutant, which lacked the N-proximal portion, the region between amino acids 46 and 260, had 30% of the DNA-binding activity of the wild-type c-Ski. The Δ3 mutant, containing the deletion of both this N-proximal portion (amino acids 46−260) and the C-terminal portion (amino acids 594−728) of c-Ski, was not retained on the DNA cellulose, but the Δ1 mutant, which lacks the central portion (amino acids 263−490) of c-Ski, had almost the same level of DNA-binding activity as the wild type. We concluded that two regions, the N-proximal portion (amino acids 157−270) and the C-terminal portion (595−728) of c-Ski, were required for the DNA-binding activity of the c-Ski complex.

DISCUSSION

We have shown that c-Ski is in the nucleus and that the c-Ski complex has DNA-binding activity, although DNA binding is not an intrinsic property of c-Ski and may be dependent on an associated protein. The bacterially synthesized c-Ski was retained on DNA cellulose after being mixed on ice for 1 h with nuclear extracts prepared from Molt4 cells and fractionated by Sephacryl S-300 columm chromatography. From these results, it seems unlikely that some chemical modification of the bacterially synthesized c-Ski such as phosphorylation or glycosylation is responsible for the DNA binding of c-Ski, because of the lack of a co-factor of low molecular weight such as ATP in the fractionated extracts. The findings that the apparent molecular weight of the bacterially synthesized c-Ski that bound to the DNA cellulose was the same as that of the unbound c-Ski, and that the DNA-binding activity of p100$^{c\text{-}ski}$ in nuclear extracts of Molt4 cells was lost upon mixing with the bacterially synthesized c-Ski, are also consistent with this idea. Fractionation of nuclear extracts by various resins and dialysis experiments on nuclear extracts with the use of various dialysis membranes suggested that the molecular weight of the protein that may be associated with c-Ski and is required for DNA binding of the c-Ski complex is more than 30,000 (data not shown). The kind of protein required for the DNA binding of the c-Ski complex is not known, but these features of c-Ski are similar to those of the c-*fos* gene product, which associates with c-jun/AP1 and binds to the TPA-response element, although c-*fos* protein itself has no DNA-binding activity (12−14).

Comparison of the sequence of the human c-*ski* cDNA that we used (6) with the sequences of the chicken c-*ski* cDNAs reported by two other groups (4,5) indicated that our human c-*ski* cDNA lacked a small region of 111 bp (exon 2; nucleotides 1,079−1,191; nucleotides numbers are as in ref. 5) that would account for 37 amino acids of the chicken c-Ski. Our human c-*ski* cDNA could be generated by alternative splicing, and it was also confirmed that both kinds of the chicken c-*ski* mRNA species, those with and without the second exon, are expressed in normal cells (5). To find whether this small portion affected the properfies of the c-Ski we characterized, the *Sac*I fragment of the human c-*ski* cDNA was replaced by the *Sac*I fragment of the v-*ski* DNA containing this region of 37 amino acids. The bacterially synthesized c-Ski derived from this hybrid cDNA also did not bind to DNA cellulose, but was retained there after being mixed with nuclear extracts prepared from Molt4 cells. These results indicated that this region did not affect the properties of c-Ski that have been identified so far.

Use of a series of deletion mutants of c-Ski synthesized in an *in vitro* translation system showed that two regions, the N-proximal portion (amino acids 157−270) and the C-terminal portion (amino acids 595−728), were required for DNA binding of the c-Ski complex. The N-proximal portion corresponded to the cystein/histidine-rich domain said to bind to metal (4−6). The C-terminal portion involves a region rich in basic amino acids. In the region between amino acids 598 and 662, 23 of the 65 residues are basic. *tat*, the transactivating protein from human immunodeficiency virus, forms a metal-linked dimer with metal ions bridging cystein-rich regions from each monomer, and this arrangement is distinct from the 'zinc finger' structure in the DNA-binding domain of other eukaryotic regulatory proteins (15). Therefore, the N-proximal portion of c-Ski also could contribute to interaction with other protein in a way similar to the cystein-rich region of *tat*.

ski is related to *myc* both in sequence and in the distribution of charged amino acids (4), but the function of c-*ski* is still unknown. In nuclear oncoproteins, the function of *jun*/AP1 (16,17), *fos* (18−21), and *myb* (22−27) as transcriptional regulatory proteins has been demonstrated, but the role of *myc* is unclear. Analyses of the protein, which may be associated with c-Ski and responsible for DNA binding of the c-Ski complex and identification of the DNA sequence recognized by the c-Ski complex may lead to more understanding of the mechanism of cellular transformation.

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