

The Autoantigen Ku Is Indistinguishable from NF IV, a Protein Forming Multimeric Protein-DNA Complexes

By Maarten H. Stuiver, Frank E. J. Coenjaerts,
and Peter C. van der Vliet

From the Laboratory for Physiological Chemistry, University of Utrecht, 3521 GG Utrecht, The Netherlands

Summary

We have isolated a cDNA encoding the 84-kD subunit of NFIV. Tryptic peptide sequences were identified within the coding sequences, confirming its proper identity. The primary sequence of the protein is identical to that of the large subunit of the Ku autoantigen. A missing NFIV peptide sequence was identified within the sequence of the small subunit of Ku. In addition, the proteins are identical in immunological aspects. We suggest that the Ku and NFIV proteins are identical. This connection adds new biochemical data to our knowledge of the Ku autoantigen.

Nuclear factor IV (NFIV)¹ is a dimeric protein with subunits of 72 and 84 kD. It is an abundant protein in HeLa cell nuclei. As shown by combined biochemical and electronmicroscopical methods (1), NFIV recognizes the molecular ends of any double-stranded DNA molecule and can subsequently move freely, without energy input, on the DNA, until it encounters a blockade. When the DNA is fully covered, a stable regular DNA-multimeric protein complex with a spacing of 27–32 bp is formed. NFIV also binds to single-stranded DNA but no such regularity is detected in this complex. Based upon its properties, a role for NFIV in DNA replication, repair, or recombination has been proposed (1). The unique DNA binding properties of NFIV prompted us to investigate the protein in more detail.

The molecular weight as well as some other properties of NFIV are remarkably similar to those of the auto-antigenic protein Ku (2–4). This protein is also referred to as p70/p80 (5) and the 86–70-kD protein complex (6, 7). These antigens were identified on the basis of their reactivity with antibodies from patients with scleroderma-polymyositis overlap syndrome as well as with systemic lupus erythematosus and scleroderma. Like NFIV, the immunopurified Ku complex binds to the ends of double-stranded DNA for which the 70-kD subunit seems to be primarily responsible (2). However, a regular footprint pattern on double-stranded DNA, resulting from a translocation step, was not observed. Moreover, binding to single-stranded DNA was much less efficient than with NFIV.

We used a polyclonal antiserum against NFIV to isolate a cDNA clone of the 84-kD subunit of NFIV. The coding region of this sequence completely overlapped with that of the recently published 86-kD Ku subunit (8). In addition, peptides obtained from NFIV overlapped in sequence with

the large Ku subunit as well as with the sequence of the Ku 70-kD DNA-binding subunit (9). In accordance with this, we show here that NFIV and Ku crossreact immunologically. These data indicate that NFIV and Ku are identical. This then provides new data on the mode of Ku antigen-DNA interaction as well as a detailed kinetic analysis of this interaction (1).

Materials and Methods

Protein Sequencing. NFIV was purified from HeLa cell nuclei using ion-exchange and DNA-cellulose affinity chromatography (1). The protein was found to be >95% pure as judged by SDS-gel electrophoresis and silverstaining. 50 μ g of NFIV (corresponding to \sim 300 pmol) was digested with trypsin-TPCK (Worthington Biochemical Corp., Freehold, NJ) in a 100:1 mass ratio for 16 h at 37°C. The resulting peptides were separated on a HPLC μ Bondapak C18 column using a linear acetonitrile gradient in 0.1% trifluoroacetic acid. Four peptide-containing fractions were analyzed on a gas-phase sequencer (Applied Biosystems Inc., Foster City, CA). The results are shown in Fig. 1.

Isolation of the cDNA Clone and DNA Sequencing. The rabbit antiserum was preincubated with a nitrocellulose-immobilized *Escherichia coli* extract as well as with 20 ng/ml β -galactosidase to inhibit nonspecific binding. The antiserum (diluted 1:500) was then used to screen (10) a cDNA library in λ gt11, made from polyadenylated RNA of NTera2D1 human teratocarcinoma cells (11) using horseradish peroxidase-conjugated second antibodies (SWARPO, DAKO immunoglobulins a/s, Denmark). From a screening of $\sim 3.5 \times 10^5$ recombinant phages, five immunoreactive clones were isolated, plaque-purified, and amplified. *E. coli* extracts from cells infected with the λ -clones were prepared according to Landschulz et al. (12). Total infected cells were lysed in SDS-containing sample buffer and loaded on a 7%-polyacrylamide SDS-gel. The proteins were analyzed by immunoblotting.

The 3.0-kb EcoRI insert of clone λ -1 was subcloned in pUC18.

¹ Abbreviation used in this paper: NFIV, nuclear factor IV.

peptide number	amino acids
I	K ₄ , A ₃₀ , S ₄₅ , F ₁₅ , E ₂₀ , E ₂₀ , A ₂₀ , S ₃₀ , N ₁₅ , Q ₁₃ , L ₁₅ , I ₁₀ , N ₆ , H ₄ , I ₅ , E ₇ , Q ₅ , F ₇ , L ₆ , D ₅ , T ₇ , N ₄ , E ₃ , T ₃
IIa	- K ₂₄ , P ₃₄ , S ₃₈ , G ₂₅ , T ₄₀ , A ₂₅ , A ₃₀ , V ₆ , F ₁₀ , E ₁₅ , E ₂₅ , G ₁₅ , G ₁₂ , D ₁₀ , V ₅ , D ₇ , D ₁₂ , L ₃ , L ₆ , D, M, I
IIb	- L ₃₁ , M ₃₅ , L ₃₃ , P ₄₂ , D ₁₀ , F ₃₂ , D ₃₅ , L ₂₀ , L ₂₀ , E ₁₅ , D ₂₀ , I ₂₀ , E ₅ , S ₂₀ , K ₅
IIIa	K ₃₀ , K ₃₀ , D ₃₀ , Q ₂₀ , T ₂₀ , A ₁₇ , Q ₁₅ , E ₁₀ , I ₆ , F ₇ , Q ₆ , D ₆ , N ₄ , E ₃ , E ₃ , D ₄ , G ₅ , V ₂₀
IIIb	T ₁₀ , W ₅ , T ₁₅ , V ₅ , D ₁₀
IVa	- G ₂₀ , L ₂₀ , E ₂₀ , I ₁₂ , V ₁₂ , K ₁₁
IVb	- E, Y, S, E, E, E, L, K, - - I S K

Figure 1. Sequence of the tryptic peptides obtained from NFIV. The amount of amino acids (in picomoles) observed during the sequencing steps are indicated in the subscript. HPLC peaks 2, 3, and 4 contained overlapping peptides that were aligned according to their match with the nucleotide sequence (Fig. 2).

Its sequence was determined by shotgun cloning of *TaqI* and *Sau3A* restriction fragments in M13mp18 and M13mp19. Dideoxy chain-termination sequencing was carried out using Sequenase components (U.S. Biochemical Corp., Cleveland, OH) according to the specifications of the manufacturer.

Antibodies and Immune Precipitation. A rabbit antiserum against NFIV was prepared by primary injection of antigen (100 µg) in the popliteal lymph nodes, followed by two intramuscular booster injections. The serum was highly specific for NFIV, as shown by immunoblotting. Subunit-specific antibodies were prepared as described (13). This procedure yielded antisera that were highly specific for each of the two subunits. The patient serum containing antibodies to the Ku autoantigen was kindly donated by Drs. Griffith and Hardin (Yale University, New Haven, CT). A hybridoma supernatant containing the mAb RN3 directed against the 80-kD Ku subunit, was a gift of R. Verheijen and W. van Venrooij (University of Nijmegen) (14). Immunoprecipitations were performed as described (3). For precipitation with the RN3 mAb the protein A-sepharose beads were coated with rabbit anti-mouse IgG (RAM, Nordic Sciences, Tilburg, The Netherlands) as described (14).

Results

Structural Identity Between the Large Subunits of NFIV and Ku. A teratocarcinoma λgt11 library was screened with a rabbit antiserum raised against purified NFIV. This revealed five reactive clones which were plaque-purified and amplified. An *E. coli* extract from phage-infected cells was then analyzed by SDS-gel electrophoresis and immunoblotting. One clone encoded a polypeptide that reacted with the NFIV antiserum. Immunoblotting with the subunit-specific antisera (see Materials and Methods) indicated that the clone contained information for the 84-kD subunit of NFIV (data not shown). The insert DNA was 3.0 kb in size and consisted of a single *EcoRI* fragment, which was subcloned in pUC18 and sequenced. The clone contained 2,969 bp consisting of a single 1,885-bp long open reading frame and a 1,084-bp 3'-untranslated region. Analysis of the open reading frame showed it to be completely identical to the region encoding amino acids 105-732 of the 86-kD subunit of the Ku protein (8). The identity extends to the 3'-untranslated region with one difference, G-A at position 2683. Compared with the

Ku cDNA we find a 263 bp extended 3' sequence (Fig. 2). In spite of this, no poly(A) tail or poly(A) signal was found, indicating that priming of the cDNA must have occurred internally. The combined information of the Ku and NFIV-encoding clones spans 3,313 bp, which is close to the 3.1-kb Ku-86 mRNA detected in Northern blots (8).

NFIV Peptides Map Both in the Large and Small Ku Subunit. To confirm the identity of our clone, we purified NFIV to apparent homogeneity and determined the amino acid sequence of tryptic peptides separated by reversed-phase chromatography. Due to the large size of NFIV, three of the four peaks contained a mixture of two peptides (Fig. 1). In two cases, IIIa/b and IVa/b, the amount of detected amino acids could be used to identify the two different peptides in the mixed sequences. Peptides I, IIa, IIIa, IIIb, and IVa matched the predicted amino acid sequence of Ku/NFIV, while peptide IIb was present in the first 104 amino acids of the Ku sequence, not present in the NFIV clone (see Fig. 2). All peptides are preceded by an arginine or lysine residue, confirming their proper tryptic origin. This indicates that we have cloned the cDNA encoding the 84-kD subunit of NFIV, confirming that the large subunits of the Ku and NFIV proteins are identical. The only unassigned NFIV-derived peptide, IVb, was found to match the 70-kD Ku subunit, at positions 557-570 (9). This suggests that the small subunits of Ku and NFIV are identical too.

Ku and NFIV Are Immunologically Indistinguishable. We examined the relationship of the Ku and NFIV proteins by immunoprecipitation. Using a rabbit antiserum against purified NFIV we immunoprecipitated both the 72- and 84-kD subunits from a HeLa extract. The migration of the radiolabeled proteins on the SDS-gel was indistinguishable from that of purified NFIV, as visualized by silverstaining (see Fig. 3, lanes 1 and 3). Immunoprecipitation with a human anti-Ku serum revealed polypeptides of identical size (lane 5). The mAb RN3 (14), which is specific for the 86-kD subunit of Ku, also precipitated proteins indistinguishable from NFIV (lane 7). These experiments indicate that NFIV and the Ku antigen are indistinguishable in electrophoretic behavior, but do not exclude the possibility that NFIV represents a immunologically distinct subpopulation of the Ku antigen or

Ku-86kDa

1
GGCGGGCGCAAAAGCCCTGAGGACCGGCAACATGGTCCGGTCGGGGAATAAGGCAGCTGTTGTGCTGTGTATGGACGTGGGCTTACCATGAGTAACTOCATTCTGGTATAGAATCC
MetValArgSerGlyAsnLysAlaAlaValValLeuCysMetAspValGlyPheThrMetSerAsnSerIleProGlyIleGluSer

121
CCATTTGAACAAGCAAAGAAGGTGATAACCATGTTTGTACAGCGACAGGTGTTTGTCTGAGAACAGGATGAGATTGCTTTAGTCTGTTTGGTACAGATGGCACTGACAATCCCTTTCT
ProPheGluGlnAlaLysLysValIleThrMetPheValGlnArgGlnValPheAlaGluAsnLysAspGluIleAlaLeuValLeuPheGlyThrAspGlyThrAspAsnProLeuSer

NFIV-84kDa

241
GGTGGGATCAGTATCAGAACATCACAGTGCACAGACATCTTATGCTACCAGATTTTATTGCTGGAGGACATGAAAGCAAATCCAACAGGTTCTCAACAGGCTGACTTCTGGAT
GlyGlyAspGlnTyrGlnAsnIleThrValHisArgHisLeuMetLeuProAspPheAspLeuLeuGluAspIleGluSerLysIleGlnProGlySerGlnGlnAlaAspPheLeuAsp
IIB

361
GCACATACTGAGCATGGATGTGATTCAACATGAAACAATAGGAAAGAAGTTTGAAGAAGGCATATGAAATATTCACTGACCTCAGCAGCCGATTGAGCAAAAGTCAGCTGGATATT
GCACATACTGAGCATGGATGTGATTCAACATGAAACAATAGGAAAGAAGTTTGAAGAAGGCATATGAAATATTCACTGACCTCAGCAGCCGATTGAGCAAAAGTCAGCTGGATATT
AlaLeuIleValSerMetAspValIleGlnHisGluThrIleGlyLysLysPheGluLysArgHisIleGluIlePheThrAspLeuSerSerArgPheSerLysSerGlnLeuAspIle

481
ATAATTCATAGCTTGAAGAAATGTGACATCTCCCTGCAATTTCTTCTGCTTTTCTCACTTGGCAAGGAAGTGAAGTGGGGACAGAGGAGATGGCCCTTTCCGTTAGGTGGCCATGGG
ATAATTCATAGCTTGAAGAAATGTGACATCTCCCTGCAATTTCTTCTGCTTTTCTCACTTGGCAAGGAAGTGAAGTGGGGACAGAGGAGATGGCCCTTTCCGTTAGGTGGCCATGGG
IleIleHisSerLeuLysLysCysAspIleSerLeuGlnPhePheLeuProPheSerLeuGlyLysGluAspGlySerGlyAspArgGlyAspGlyProPheArgLeuGlyGlyHisGly

601
CCTTCCTTTCCACTAAAAGGAATTACCGAACAGCAAAAAGAAGGCTTGAGATAGTGAATAATGGTGTATGATATCTTTAGAAGGTGAAGATGGGTTGGATGAAATTTATTCATTCACTGAG
CCTTCCTTTCCACTAAAAGGAATTACCGAACAGCAAAAAGAAGGCTTGAGATAGTGAATAATGGTGTATGATATCTTTAGAAGGTGAAGATGGGTTGGATGAAATTTATTCATTCACTGAG
ProSerPheProLeuLysGlyIleThrGluGlnGlnLysGluGlyLeuGluIleValLysMetValMetIleSerLeuGluGlyGluAspGlyLeuAspGluIleTyrSerPheSerGlu
IIVa

721
AGTCTGAGAAAACGTGCGTCTTCAAGAAAATTGAGAGGCATTCCATTCACTGGCCCTGCCGACTGACCATTGGCTCCAATTTGTCTATAAGGATTGCAGCCTATAAATCGATTCTACAG
AGTCTGAGAAAACGTGCGTCTTCAAGAAAATTGAGAGGCATTCCATTCACTGGCCCTGCCGACTGACCATTGGCTCCAATTTGTCTATAAGGATTGCAGCCTATAAATCGATTCTACAG
SerLeuArgLysLeuCysValPheLysLysIleGluArgHisSerIleHisTrpProCysArgLeuThrIleGlySerAsnLeuSerIleArgIleAlaAlaTyrLysSerIleLeuGln

841
GAGAGAGTTAAAAGACTTGGACAGTTGTGGATGCAAAAACCCATAAAAAAGAAGATATACAAAAGAAGCAGTTTATTGCTTAAATGATGATGATGAACTGAAGTTTTAAAAGAGGAT
GAGAGAGTTAAAAGACTTGGACAGTTGTGGATGCAAAAACCCATAAAAAAGAAGATATACAAAAGAAGCAGTTTATTGCTTAAATGATGATGATGAACTGAAGTTTTAAAAGAGGAT
GluArgValLysLysThrTrpThrValValAspAlaLysThrLeuLysLysGluAspIleGlnLysGluThrValTyrCysLeuAsnAspAspAspGluThrGluValLeuLysGluAsp
IIB

961
ATTATTCAAGGGTTCCTCTATGGAAGTGATATAGTTCCCTTTCTCTAAAGTGGATGAGGAACAAATGAAATATAAATCGAGGGGAAGTCTTCTCTGTTTTGGGATTTTGTAAATCTTCT
ATTATTCAAGGGTTCCTCTATGGAAGTGATATAGTTCCCTTTCTCTAAAGTGGATGAGGAACAAATGAAATATAAATCGAGGGGAAGTCTTCTCTGTTTTGGGATTTTGTAAATCTTCT
IleIleGlnGlyPheLeuTyrGlySerAspIleValProPheSerLysValAspGluGluGlnMetLysTyrLysSerGluGlyLysCysPheSerValLeuGlyPheCysLysSerSer

1081
CAGGTTTCAGAGAAGATTCTCATGGAAAATCAAGTTCTAAAGGCTTTGACGCAAGAGATGATGAGGCAGCTGCAGTTGCACTTTCTCCCTGATTCATGCTTTGGATGACTTAGACATG
CAAGTTTCAGAGAAGATTCTCATGGAAAATCAAGTTCTAAAGGCTTTGACGCAAGAGATGATGAGGCAGCTGCAGTTGCACTTTCTCCCTGATTCATGCTTTGGATGACTTAGACATG
GlnValGlnArgArgPhePheMetGlyAsnGlnValLeuLysValPheAlaAlaArgAspAspGluAlaAlaAlaValAlaLeuSerSerLeuIleHisAlaLeuAspAspLeuAspMet

1201
GTGGCCATAGTTCGATATGCTTATGACAAAAGAGCTAATCCTCAAGTCGGCGTGCTTTTCTCATATCAAGCATAACTATGAGTGTATTAGTGTATGTGCAGCTGCCTTTTCAATGGAAGAC
GTGGCCATAGTTCGATATGCTTATGACAAAAGAGCTAATCCTCAAGTCGGCGTGCTTTTCTCATATCAAGCATAACTATGAGTGTATTAGTGTATGTGCAGCTGCCTTTTCAATGGAAGAC
ValAlaIleValArgTyrAlaTyrAspLysArgAlaAsnProGlnValGlyValAlaPheProHisIleLysHisAsnTyrGluCysLeuValTyrValGlnLeuProPheMetGluAsp

1321
TTGCGGCAATACATGTTTTCATCCTTGAAAAACAGTAAGAAATATGCTCCACCGAGGCACAGTTGAATGCTGTGTATGCTTTGATTGACTCCATGAGCTTGGCAAAGAAAGATGAGAAG
TTGCGGCAATACATGTTTTCATCCTTGAAAAACAGTAAGAAATATGCTCCACCGAGGCACAGTTGAATGCTGTGTATGCTTTGATTGACTCCATGAGCTTGGCAAAGAAAGATGAGAAG
LeuArgGlnTyrMetPheSerSerLeuLysAsnSerLysLysTyrAlaProThrGluAlaGlnLeuAsnAlaValAspAlaLeuIleAspSerMetSerLeuAlaLysLysAspGluLys

1441
ACAGACACCCTTGAAGACTTGTTCACCAACCACAAAATCCCAATCCTCGATTTCAGAGATTATTCAGTGTCTGCTGCACAGAGCTTTACATCCCGGGAGCCTTACCCCAATTCAG
ACAGACACCCTTGAAGACTTGTTCACCAACCACAAAATCCCAATCCTCGATTTCAGAGATTATTCAGTGTCTGCTGCACAGAGCTTTACATCCCGGGAGCCTTACCCCAATTCAG
ThrAspThrLeuGluAspLeuPheProThrThrLysIleProAsnProArgPheGlnArgLeuPheGlnCysLeuLeuHisArgAlaLeuHisProArgGluProLeuProProIleGln

Figure 2. See legend on following page.

vice versa. To examine this possibility, we depleted radiola-
beled extracts of NFIV by immunoprecipitation with anti-
NFIV serum and incubated the supernatant with an anti-Ku
serum or mAb RN3 to detect any Ku-specific proteins. As
shown in Fig. 3 B, lanes 2 and 4, Ku polypeptides could not

be detected in these supernatants. The reverse experiment,
depletion of Ku polypeptides (lane 5) followed by immuno-
precipitation with anti-NFIV serum, also failed to reveal any
residual polypeptide (Fig. 3 B, lane 6). Thus we conclude
that NFIV and Ku antigen are immunologically crossreac-

1561
CAGCATATTTGGAATATGCTGAATCCTCCCGCTGAGGTGACAACGAAAAGTCAGATTCCTCTCTCTAAAATAAAGACCCCTTTTCCTCTGATTGAAGCCAAGAAAAGGATCAAGTGACT
CAGCATATTTGGAATATGCTGAATCCTCCCGCTGAGGTGACAACGAAAAGTCAGATTCCTCTCTCTAAAATAAAGACCCCTTTTCCTCTGATTGAAGCCAAGAAAAGGATCAAGTGACT
GlnHisIleTrpAsnMetLeuAsnProProAlaGluValThrThrLysSerGlnIleProLeuSerLysIleLysThrLeuPheProLeuIleGluAlaLysLysLysAspGlnValThr

1681
GCTCAGGAAATTTTCCAAGACAACCATGAAGATGGACCTACAGCTAAAAAATTAAGACTGAGCAAGGGGGAGCCCACTTCAGCGTCTCCAGTCTGGCTGAAGGCAGTGTCCACCTCTGTT
GCTCAGGAAATTTTCCAAGACAACCATGAAGATGGACCTACAGCTAAAAAATTAAGACTGAGCAAGGGGGAGCCCACTTCAGCGTCTCCAGTCTGGCTGAAGGCAGTGTCCACCTCTGTT
AlaGlnGluIlePheGlnAspAsnHisGluAspGlyProThrAlaLysLysLeuLysThrGluGlnGlyGlyAlaHisPheSerValSerSerLeuAlaGluGlySerValThrSerVal
IIa

1801
GGAAAGTGTGAATCCTGCTGAAAACCTCCGTGTTCTAGTGAAACAGAAGAAGGCCAGCTTTGAGGAAGCGAGTAACCAGCTCATAAATCACATCGAACAGTTTTTGGATACTAATGAAACA
GGAAAGTGTGAATCCTGCTGAAAACCTCCGTGTTCTAGTGAAACAGAAGAAGGCCAGCTTTGAGGAAGCGAGTAACCAGCTCATAAATCACATCGAACAGTTTTTGGATACTAATGAAACA
GlySerValAsnProAlaGluAsnPheArgValLeuValLysGlnLysLysAlaSerPheGluGluAlaSerAsnGlnLeuIleAsnHisIleGluGlnPheLeuAspThrAsnGluThr
I

1921
CCGTATTTTATGAAGAGCATAGACTGCATCCGAGCCTCCCGGAAGAAGCCATTAAGTTTTTCAGAAGAGCAGCGCTTTAACAACTTCTGAAAGCCCTTCAAGAGAAAAGTGGAAATTA
CCGTATTTTATGAAGAGCATAGACTGCATCCGAGCCTCCCGGAAGAAGCCATTAAGTTTTTCAGAAGAGCAGCGCTTTAACAACTTCTGAAAGCCCTTCAAGAGAAAAGTGGAAATTA
ProTyrPheMetLysSerIleAspCysIleArgAlaPheArgGluGluAlaIleLysPheSerGluGluGlnArgPheAsnAsnPheLeuLysAlaLeuGlnGluLysValGluIleLys

2041
CAATTAATCAATTTCTGGGAAATGTTGTCCAGGATGGAATTAATCTGTATCACCAGGAGGAAAGCCCTCGGAAGTCTGTGCACAGCTGAGGAAGCCAAAAAGTTTTCTGGCCCCAAGAC
CAATTAATCAATTTCTGGGAAATGTTGTCCAGGATGGAATTAATCTGTATCACCAGGAGGAAAGCCCTCGGAAGTCTGTGCACAGCTGAGGAAGCCAAAAAGTTTTCTGGCCCCAAGAC
GlnLeuAsnHisPheTrpGluIleValValGlnAspGlyIleThrLeuIleThrLysGluGluAlaSerGlySerSerValThrAlaGluGluAlaLysLysPheLeuAlaProLysAsp

2161
AAACCAAGTGGAGACACAGCAGCTGTATTTGAAGAAGTGGTGTATGTGGACGATTTATTGGACATGATATAGTTCGTGGATGTATGGGAAATCTAAGAGAGCTGCCATCGCTGTGATGCT
AAACCAAGTGGAGACACAGCAGCTGTATTTGAAGAAGTGGTGTATGTGGACGATTTATTGGACATGATATAGTTCGTGGATGTATGGGAAATCTAAGAGAGCTGCCATCGCTGTGATGCT
LysProSerGlyAspThrAlaAlaValPheGluGluGlyGlyValAspAspLeuLeuAspMetIleEnd
IIa

2281
GGGAGTCTAACAAAACAAGTTGGATGCGGCCATTCAAGGGGAGCCAAAATCTCAAGAAAATCCCAGCAGGTTACCTGCAGGCGGATCATCTAATCTCTGTGGAAATGAATACACACATA
GGGAGTCTAACAAAACAAGTTGGATGCGGCCATTCAAGGGGAGCCAAAATCTCAAGAAAATCCCAGCAGGTTACCTGCAGGCGGATCATCTAATCTCTGTGGAAATGAATACACACATA

2401
TATATTACAAGGATAATTTAGACCCCATACAAGTTTATAAAGAGTCATTGTTATTTCTGGTTGGTGTATTATTTTTCTGTGGTCTTACTGATCTTTGTATATTACATACATGCTTTG
TATATTACAAGGATAATTTAGACCCCATACAAGTTTATAAAGAGTCATTGTTATTTCTGGTTGGTGTATTATTTTTCTGTGGTCTTACTGATCTTTGTATATTACATACATGCTTTG

2521
AAGTTTCTGAAAGTAGATCTTTTCTTGACCTAGTATATCAGTGACAGTTGCAGCCCTTGTGATGTGATTAGTGTCTCATGTGGAACCATGGCATGGTTATTGATGAGTTCTTAACCCCT
AAGTTTCTGAAAGTAGATCTTTTCTTGACCTAGTATATCAGTGACAGTTGCAGCCCTTGTGATGTGATTAGTGTCTCATGTGGAACCATGGCATGGTTATTGATGAGTTCTTAACCCCT

2641
TTCCAGAGTCTCCTTTGCTGATCCTCCAACAGCTGTCACAACCTTGTGTTGAGCAAGCAGTAGCATTGCTTCTCCCAACAAGCAGCTGGGTTAGGAAAACCATGGGTAAGGACGGAC
TTCCAGAGTCTCCTTTGCTGATCCTCCAACAGCTGTCACAGCTTGTGTTGAGCAAGCAGTAGCATTGCTTCTCCCAACAAGCAGCTGGGTTAGGAAAACCATGGGTAAGGACGGAC
A

2761
TCACTTCTCTTTTAGTTGAGGCCTTCTAGTTACCACATTACTCTGCCTCTGTATATAGGTGGTTTTCTTTAAGTGGGTGGGAAGGGGAGCACAATTTCCCTTCATACTCCTTTAAGC
TCACTTCTCTTTTAGTTGAGGCCTTCTAGTTACCACATTACTCTGCCTCTGTATATAGGTGGTTTTCTTTAAGTGGGTGGGAAGGGGAGCACAATTTCCCTTCATACTCCTTTAAGC

2881
AGTGAGTTATGGTGGTGGTCTCATGAAGAAAAGACCTTTGGCCCAATCTCTGCCATATCAGTGAACCTTTAGAAAACCAAAAATTTACTACAGTAGTTAGAATTATATCAC
AGTGAGTTATGGTGGTGGTCTCATGAAGAAAAGACCTTTGGCCCAATCTCTGCCATATCAGTGAACCTTTAGAAAACCAAAAATTTACTACAGTAGTTAGAATTATATCAC

3001
TTCACCTGTTCTACTTTGCAAGCCCTCAAAGAGAGAAAAGTTTCGTTATATTAACAACTTAGGTAACCTTTTCGATCTTTCCCATTTCTACCTAAGTCAGCTTTCATCTTTGTTGGATGGTGT
TTCACCTGTTCTACTTTGCAAGCCCTCAAAGAGAGAAAAGTTTCGTTATATTAACAACTTAGGTAACCTTTTCGATCTTTCCCATTTCTACCTAAGTCAGCTTTCATCTTTGTTGGATGGTGT

3121
CTCCTTTACTAAATAAGAAAATAACAAAGCCCTTATTCTCTTTTTTCTTGTCTCATTCTTGCCTTGAGTCCAGTTCCTTTGGTGTACAGACTTCTTGGTACCCAGTCACCTCTGT

3241
CTTCAGCACCCCTCATAAGTCGTCATAATACACAGTTTTGTACATGTAACATTAAGGCATAAATGACTCAA
3313

Figure 2. Sequence similarity between the cDNAs encoding the large subunits of Ku and NFIV. NFIV 84 kD and Ku 86 kD cDNA sequences are shown in top and bottom strand, respectively. Note the single G-A difference at position 2683. The NFIV-derived peptides are indicated in the deduced amino acid sequence.

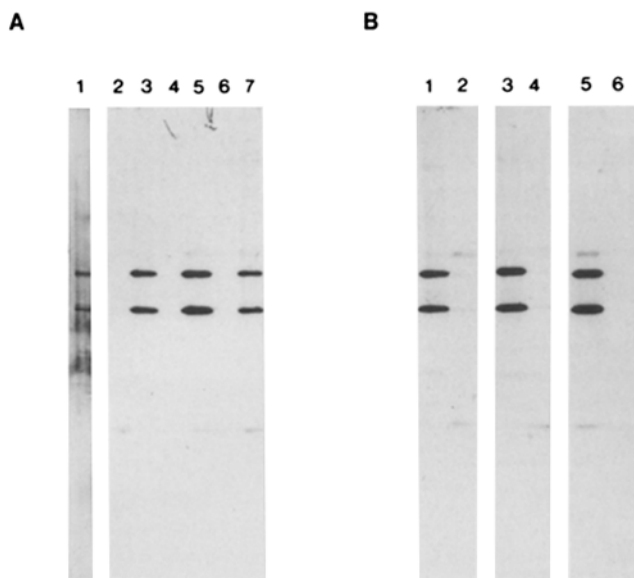


Figure 3. (A) Immunoprecipitation of Ku and NFIV polypeptides. Lane 1 shows a silver-stained part of the gel containing purified NFIV. Lanes 2 to 7 show the autoradiograph of immunoprecipitated [³⁵S]methionine-labeled proteins. (Lane 2) Pre-immune rabbit serum; (lane 3) anti-NFIV serum; (lane 4) normal human serum; (lane 5) patient anti-Ku serum; (lane 6) rabbit anti-mouse IgG serum; (lane 7) mAb RN3. (B) Radiolabeled HeLa extracts were depleted of NFIV by immunoprecipitation with anti-NFIV serum. The pellets were analyzed (lanes 1, 3). The supernatants were analyzed by immunoprecipitation using anti-Ku serum or RN3 (lanes 2, 4). Lanes 5 and 6 show the depletion of Ku polypeptides by the anti-Ku serum followed by immunoprecipitation with anti-NFIV serum.

tive and do not represent immunologically distinct subpopulations of each other. Taken together these results strongly indicate that Ku and NFIV are identical proteins.

Discussion

Based upon several criteria, NFIV and Ku appear to be indistinguishable. First, an 84-kD NFIV cDNA clone was identical to the Ku 86-kD cDNA sequence (8), except for a single point mutation. Second, all NFIV peptides that were analyzed fit the Ku protein sequence. In accordance with this, antibodies against NFIV quantitatively remove Ku polypeptides and vice versa. Northern blot experiments using the Ku 70-kD encoding cDNA as a probe reveal only one mRNA (9). This indicates again that also the small subunits of Ku and NFIV may be indistinguishable. However, we cannot exclude that NFIV and Ku have the same primary structure but are modified differently, since both Ku peptides can be phosphorylated at serine residues (7). Such a modifica-

tion may go undetected in immunological or electrophoretic analysis.

NFIV and Ku show differences in DNA-binding properties. Most interestingly, NFIV is able to translocate over dsDNA (1), whereas Ku is not (2). This difference may be related to a DNA-binding specificity of NFIV. We observed that A + T-rich DNA-ends are covered by NFIV with a higher affinity than G + C-rich DNA-ends. The use of a different DNA molecule may provide an explanation for the detection of only a terminal footprint with the Ku protein. These differences may of course also be due to a difference in modification of the proteins or to different isolation procedures.

Ku is isolated by immuno-affinity chromatography, necessitating elution with 3.5 M MgCl₂. Considering the limited stability of Ku (4), this procedure may functionally change the Ku molecule. During purification of NFIV, only buffers with neutral pH and moderate salt concentrations (up to 0.5 M NaCl) are used. Thus, we consider it possible that the differences between Ku and NFIV are due to different isolation procedures.

By gel filtration analysis, the Ku complex in a crude extract was shown to behave as a molecule of ~300 kD, suggesting a tetramer. Purified NFIV was shown by electron microscopy to behave as a 150-kD heterodimer in DNA-bound and unbound form (1). Possibly the Ku-containing, 300-kD particle represents a complex with another protein or contains Ku proteins that are linked together by DNA remaining from the isolation procedure. We noticed that most of the protein in the cell is present in a DNA bound form that can be extracted by 0.3 M NaCl. However, as we have not analyzed NFIV by gel filtration, we can not exclude that the different values are due to different methods used.

The function of the Ku/NFIV protein is presently unknown. Several other proteins, involved in DNA replication, repair, or recombination are able to recognize molecular ends. A remarkable similarity in DNA binding properties involving an ends-specific DNA interaction as well as a presumed translocation step was noted within the bacteriophage Mu pgam protein, that is involved in recombination control (15). However, several other functions are possible, like a role in DNA replication (1) or in chromatin structure (7). This would be in agreement with the high level of active protein found in HeLa cells (5×10^5 molecules per cell). The rapid inactivation of Ku/NFIV in the absence of DNA (4; and van Driel, W., personal communication), coupled to the high stability in the presence of DNA (1) also suggests that most of the molecules in the cell are present in a DNA-bound form.

While this article was under review, Mimori et al. (16) reported the cloning of cDNAs encoding the larger subunit of the Ku protein. Their sequence overlaps the sequences described in this paper.

We thank P. Burbach, A. van der Kleij, and R. Amons for help with the amino acid sequence analysis; J. Skowronski and W. van Venrooij for the λ gt11 library; R. Verheijen for the RN3 antibody; and J. A. Hardin for the anti-Ku serum. Discussions with E. de Vries and W. van Driel are gratefully acknowledged.

This work was supported in part by the Netherlands Foundation for Chemical Research (SON) with financial support from the Netherlands Organization for Scientific Research (NWO).

Address correspondence to Dr. Peter C. van der Vliet, Laboratory for Physiological Chemistry, Vondellaan 24A, 3521 GG Utrecht, the Netherlands.

Received for publication 20 April 1990 and in revised form 29 June 1990.

References

1. De Vries, E., W. van Driel, W.G. Bergsma, A.C. Arnberg, and P.C. van der Vliet. 1989. HeLa nuclear protein recognizing DNA termini and translocating on DNA forming a regular DNA-multimeric protein complex. *J. Mol. Biol.* 208:65.
2. Mimori, T., and J.A. Hardin. 1986. Mechanism of interaction between Ku protein and DNA. *J. Biol. Chem.* 261:10375.
3. Mimori, T., J.A. Hardin, and J.A. Steitz. 1986. Characterization of the DNA-binding protein antigen Ku recognized by autoantibodies from patients with rheumatic disorders. *J. Biol. Chem.* 261:2274.
4. Mimori, T., M. Akizuki, H. Yamagata, S. Inada, S. Yoshida, and M. Homma. 1981. Characterization of a high molecular weight acidic nuclear protein recognized by autoantibodies in sera from patients with polymyositis-scleroderma overlap. *J. Clin. Invest.* 68:611.
5. Reeves, W.H. 1985. Use of monoclonal antibodies for the characterization of novel DNA-binding proteins recognized by human autoimmune sera. *J. Exp. Med.* 161:18.
6. Yaneva, M., R. Ochs, D.K. McRorie, S. Zweig, and H. Busch. 1986. Purification of an 86-70 kDa nuclear DNA-associated protein complex. *Biochim. Biophys. Acta.* 841:22.
7. Yaneva, M., and H. Busch. 1986. A 10S particle released from deoxyribonuclease-sensitive regions of HeLa cell nuclei contains the 86-kilodalton-70-kilodalton protein complex. *Biochemistry.* 25:5057.
8. Yaneva, M., J. Wen, A. Ayala, and R. Cook. 1989. cDNA-derived amino acid sequence of the 86-kD subunit of the Ku antigen. *J. Biol. Chem.* 264:13407.
9. Reeves, W.H., and Z.M. Shoenberger. 1989. Molecular cloning of cDNA encoding the p70 (Ku) lupus autoantigen. *J. Biol. Chem.* 264:5047.
10. Mierendorf, R.C., C. Percy, and R.A. Young. 1987. Gene isolation by screening λ gt11 libraries with antibodies. *Methods Enzymol.* 152:458.
11. Sen Gupta, D.N., B.Z. Zmudzka, P. Kumar, F. Cobiainchi, J. Skowronski, and S.H. Wilson. 1986. Sequence of human DNA polymerase β mRNA obtained through cDNA cloning. *Biochem. Biophys. Res. Commun.* 136:341.
12. Landschulz, W.H., P.F. Johnson, E.Y. Adashi, B.J. Graves, and S.L. McKnight. 1988. Isolation of a recombinant copy of the gene encoding C/EBP. *Genes Dev.* 2:786.
13. Smith, D.E., and P.A. Fischer. 1984. Identification, developmental regulation, and response to heat shock of two antigenically related forms of a major nuclear envelope protein in *Drosophila* embryos: application of an improved method for affinity purification of antibodies using polypeptides immobilized on nitrocellulose blots. *J. Cell Biol.* 99:20.
14. Verheijen, R., H. Kuijpers, W. van Venrooij, and F. Ramaekers. 1988. The 80 kilodalton component of the Ku autoantigen complex is associated with the nuclear matrix. Ph.D. Thesis. University of Nijmegen, The Netherlands. 129-149.
15. Williams, J.G., and C.M. Radding. 1981. Partial purification of an exonuclease inhibitor induced by bacteriophage Mu-1. *J. Virol.* 39:548.
16. Mimori, T., Y. Ohosone, N. Hama, A. Suwa, M. Akizuki, M. Homma, A.J. Griffith, and J.A. Hardin. 1990. Isolation and characterization of cDNA encoding the 80-kDa subunit protein of the human autoantigen Ku (p70/p80) recognized by autoantibodies from patients with scleroderma-polymyositis overlap syndrome. *Proc. Natl. Acad. Sci. USA.* 87:1777.