Purification and characterization of a DNA-binding heterodimer of 52 and 100 kDa from HeLa cells

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In studies of protein binding to the upstream region of the human proliferation-associated antigen p120 gene, a heterodimer of 52 and 100 kDa proteins was purified from HeLa cells. A 1:1 ratio of p52 and p100 was constant throughout the purification. The heterodimer was localized to cell nuclei, as shown by immunofluorescence. The pI values of the p52 and p100 were 7.8 and 8.6 respectively. The peptide sequences obtained for p52 (QSNKTFNLEKQNHTPRKKHQ and PLRGKQLRVRF AA-HSASLTVR) and for p100 (PGGPKPGGGPGLSTPGGHPK-PPHRGGGEPPRGRQ and GPGPGQSGPKPIPPPPPHQQ)

were not found in the computer databanks. One p52 peptide sequence, PLRGKQLRVRF A, shows considerable sequence similarity to a conserved motif in topoisomerase II of multiple species. The p52/100 heterodimer bound to different DNA probes. The binding was competed by poly(dI-dC), sonicated salmon sperm DNA, and circular or linearized plasmid DNA. The optimal DNA binding for the heterodimer was at pH 7–9 with low salt. The DNA-binding subunit of the heterodimer was the p100 polypeptide, as shown by u.v.-cross-linking assays and Southwestern blots.

INTRODUCTION

The interactions of proteins with DNA underlie many processes in a living cell: gene transcription, DNA replication, repair, cleavage and modification. Sequence-specific DNA-binding proteins (Wingender, 1988; Mitchell and Tjian, 1989; Johnson and McKnight, 1989) were shown to play specific roles in cellular gene expression and regulation (Latchman, 1990; Evan, 1990). Sequence-non-specific DNA-binding proteins, such as TFIIC (Slattery et al., 1983; Kurl and Jacob, 1985), topoisomerases (Osheroff, 1989), c-Myc (Eisenman et al., 1985; Watt et al., 1985), Rb protein (Wang et al., 1990), simian-virus-40 T-antigens (Prives et al., 1980; Auborn et al., 1989), Ku antigen (Mimori and Hardin, 1986; Yaneva and Busch, 1986), also play important roles in many cellular processes associated with DNA.

In the course of identification and purification of *trans*-acting factors that reacted with the *cis*-elements of human proliferation-associated nucleolar antigen p120 gene (Busch, 1990), we found several new DNA-binding proteins in HeLa nuclear extracts (Zhang et al., 1991; Chatterjee et al., 1991). A heterodimer containing proteins with molecular masses of 100 and 52 kDa was purified by conventional chromatography coupled with a DNA-affinity column. The heterodimer was localized to cell nuclei, as shown by immunofluorescence. It bound to DNA in a sequence-non-specific manner. Peptide sequencing of the dimer and searching for sequence similarity in databanks showed that it was a novel heterodimer.

MATERIALS AND METHODS

DNA probe and radioactive labelling

The DNA probe for band mobility shift and Southwestern assays was a 125 bp fragment (F1) including the 5'-flanking region (–1430/–1327) of the p120 gene (Zhang et al., 1991). The probe was end-labelled by using the Klenow fragment of

Escherichia coli polymerase I and [α -³²P]dATP. Competitor DNAs were the unlabelled probes, poly(dI-dC) (Pharmacia), sonicated salmon sperm DNA (Sigma), pBluescript II KS +/– plasmic DNA (Stratagene). All DNA manipulations followed the general procedures outlined by Sambrook et al. (1989).

Nuclear-extract preparation and protein fractionation

Nuclear extracts were prepared from fresh HeLa cells essentially as described by Dignam et al. (1983). The crude nuclear extract (Fraction I) was fractionated by adding (dropwise, 10 ml/min) an equal volume of saturated (NH₄)₂SO₄ (pH 7.0) in an ice bath with constant stirring. After being allowed to settle for 2 h at 4 °C, the mixture was centrifuged for 20 min at 25000 *g*. The pellet was resuspended in buffer D [20 mM Hepes (pH 7.9)/20 % glycerol/0.2 mM EDTA/1 mM dithiothreitol (DTT)/1 mM phenylmethanesulphonyl fluoride (PMSF)/100 mM KCl] and dialysed extensively against the same buffer. This was followed by centrifugation at 25000 *g* for 20 min to remove insoluble materials. The clear supernatant (Fraction II) in buffer D was loaded on to a DEAE-Sepharose column, and the flow-through (Fraction III) was applied to a heparin-Sepharose column. The bound proteins were eluted with a linear gradient of KCl (100–600 mM) in buffer D. Fractions containing the DNA-binding activity were pooled, dialysed against buffer D containing 50 mM KCl (Fraction IV) and loaded on to an oligo-affinity column containing the concatenated oligonucleotide (–1385/–1344 of the p120 gene; (Zhang et al., 1991), according to the method of Kadonaga and Tjian (1986). The bound proteins were eluted with a linear KCl gradient (50–300 mM). The active fractions were pooled and dialysed against buffer D containing 50 mM KCl and reloaded on the column. The bound proteins were eluted, resulting in the final fraction (Fraction V). The purified proteins were concentrated in a Centricon-30 (Amicon),

Abbreviations used: PHA, phytohaemagglutinin; homopipes, homopiperazine-*NN'*-bis-2-(ethanesulphonic acid); DTT, dithiothreitol; PMSF, phenylmethanesulphonyl fluoride; PBS, phosphate-buffered saline; ds, double-stranded.

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dialysed in buffer D containing 50% glycerol, and stored at -20°C .

Band-mobility-shift assay and competition assay

The band-mobility-shift assay was modified from the method of Prywes and Roeder (1987). Nuclear extracts ($0.5\text{--}1\text{ }\mu\text{g}$) or chromatographic fractions ($0.01\text{--}0.5\text{ }\mu\text{g}$) were incubated for 15 min at room temperature with $0.1\text{ }\mu\text{g}$ of poly(dI-dC) (Pharmacia) in $20\text{ }\mu\text{l}$ of binding buffer [20 mM Hepes (pH 7.5)/50 mM KCl/5 mM MgCl_2 /0.5 mM EDTA/1 mM DTT/5% glycerol]. Then, 1 ng of ^{32}P -labelled DNA probe was added. After 20 min incubation, the reaction mixture was loaded on to a 5%-polyacrylamide gel in $0.25\times\text{TBE}$ ($1\times\text{TBE}$ is 25 mM Trizma base/25 mM boric acid/1 mM EDTA, pH 8.3) and electrophoresed at 200 V for 1.5–2 h at room temperature. The gel was dried and analysed by autoradiography. In competition assays, a 10–100 molar excess of competitor DNA was mixed with proteins and incubated for 10 min before the radioactive DNA probe was added.

Protein–DNA u.v.-cross-linking assay

These experiments essentially followed the method of Gilmour et al. (1990). Reaction mixtures (30 μl) contained 2 μl of Fraction V (0.5 μg of protein) with 0.2 μg of poly(dI-dC) and 2×10^5 c.p.m. of uniformly labelled F1 fragment in (final concentrations) 20 mM Hepes (pH 7.9)/40 mM KCl/5 mM MgCl_2 /0.2 mM EDTA/2 mM DTT/10% glycerol buffer. After 20 min of incubation at room temperature, the reactions mixtures were irradiated with short-wavelength u.v. light (15 W) for 10 min at 4°C . This was followed by adding 45 μl of nuclease digestion buffer [20 mM Tris/HCl (pH 8.0)/1% Nonidet P40, 10 mM MgCl_2 /5 mM CaCl_2 /DNAase I (1 μl ; 5 unit)/micrococcal nuclease (1 μl ; 5 unit)]. The digestion was incubated at 37°C for 30 min and terminated by adding 25 μl of 4 $\times$ SDS-loading buffer, boiled for 5 min and electrophoresed on an SDS/10%-PAGE. Autoradiograms were prepared by exposing dried gels to XAR-5 film with intensifying screens at -70°C for 20 h.

SDS/PAGE and Southwestern assay

Protein concentrations were determined by a Coomassie Blue protein assay (Pierce, Rockford, IL, U.S.A.). SDS/PAGE was performed as described by Laemmli (1970). The gels for protein profiles were stained with 0.1% Coomassie Blue R-250 in 40% (v/v) methanol and 10% (v/v) acetic acid, and destained in the same solvent. For Southwestern blotting, modified from Mangalam et al. (1989), the gels were transferred to nitrocellulose (Schleicher and Schuell) in transfer buffer [25 mM Tris/192 mM glycine/20% (v/v) methanol, pH 8.3] for 1 h at 120 V. After transfer was complete, proteins on the membrane were denatured with 6 M guanidinium chloride in binding buffer [20 mM Hepes (pH 7.5)/50 mM KCl/5 mM MgCl_2 /0.5 mM EDTA/10% glycerol/herring sperm DNA (5 $\mu\text{g}/\text{ml}$)] for 10 min at room temperature, then renatured stepwise by washing with 3 M, 1.5 M, 0.75 M, 0.37 M, and 0.18 M guanidinium chloride in binding buffer for 5 min at each step. The membrane was rinsed in distilled water for 5 min and blocked with blocking buffer [10 mM Tris (pH 7.4)/0.15 M NaCl/3% BSA/10% chicken serum/herring sperm DNA (20 $\mu\text{g}/\text{ml}$)] for 15 min. This was followed by rinsing the membrane in binding buffer for 5 min. The membrane was sealed into a polyethylene bag with binding buffer (1 ml/10 cm^2) containing ^{32}P -labelled DNA probe (5×10^5 c.p.m./ml) and herring sperm DNA (5 $\mu\text{g}/$

ml). After tumbling for 16 h on a rotating wheel at 4°C , the membrane was washed at room temperature with several changes of binding buffer for up to 30 min, blotted dry, covered with Saran Wrap (clingfilm), and exposed to XAR-5 film with an intensifying screen at -70°C for 3–4 h.

Protein microsequencing

The subunits of the heterodimer p52 and p100 were first separated by SDS/PAGE and individually purified by electroelution in Elutrap apparatus (Schleicher and Schuell). Since both N-termini of p52 and p100 were blocked, peptides of both polypeptides were prepared using Probe-Design Peptide Separation Kit (Promega). Portions (100 pmol) each of p52 and p100 were digested with CNBr and fractionated by SDS/PAGE and transferred to ProBlott membrane (Applied Biosystems). After staining with Coomassie Blue R-250, the peptide bands were excised and sequenced by Dr. R. G. Cook, Baylor College of Medicine, using an Applied Biosystems model 477A protein sequencer with a 120A PTH analyser.

Computer databank searches

Sequence comparisons were carried out using the FASTA program (Pearson and Lipman, 1988). The National Biomedical Research Foundation Protein Database (PIR) was searched using the EuGene 3.2 interface in a SUN workstation, at the Molecular Biology Information Resource, Baylor College of Medicine. Swiss-Prot (release 14.0) and GenPept (release 63.0) databanks were accessed through the GenBank on-line service.

Cell culture and immunological assays

HeLa cells were cultured in 10% (v/v) foetal-bovine serum-supplemented Dulbecco's modified Eagle's medium (GIBCO-BRL) at 37°C with 5% CO_2 . Polyclonal antibodies against heterodimer were prepared using the purified protein in BALB/c mice (Charles River Laboratories). Indirect immunofluorescence assays were carried out as previously described (Jhiang et al., 1990). The primary antibodies were used in 1:50 dilution in phosphate-buffered saline (PBS). The fluorescein isothiocyanate-conjugated goat anti-mouse IgG (Cappel) was used in 1:20 dilution in PBS.

Figure 1 Heterodimer purification

Fractions [lanes 1–5: nuclear extract, $(\text{NH}_4)_2\text{SO}_4$ precipitation, DEAE-Sepharose, heparin-Sepharose and DNA-affinity chromatographies] were analysed on a SDS/7.5%-PAGE, followed by Coomassie Blue staining. M, prestained molecular-mass (M) markers (26–180 kDa range; Sigma).

Table 1 Recovery of p52–p100 DNA-binding activity at various steps in the purification scheme

Step	Volume (ml)	[Protein] (mg/ml)	Total protein (mg)	Total activity (units)*	Specific activity (units/mg)	Yield (%)	Purification (fold)
Nuclear extract	250	10	2500	27 000	10.8	100	1.0
50% Satd. (NH ₄) ₂ SO ₄	140	8.6	1200	25 500	21.3	94	2.0
DEAE-Sepharose	140	4.2	585	24 000	41.0	89	3.8
Heparin-Sepharose	20	0.9	17.7	14 500	810	52	75
DNA-affinity column	10	0.19	1.9	11 000	5800	41	537

* One unit is the amount of protein needed to shift 50% of ³²P-labelled F1 (1 ng) in the presence of 100 ng of poly(dI-dC) in band-mobility-shift assays.

RESULTS

Purification of the DNA-binding heterodimer

The heterodimer was first identified during purification of the *trans*-acting factors that bound to the F1 fragment (–1430/–1327) of the human proliferation-associated antigen p120 gene (Zhang et al., 1991). This protein dimer was greatly enriched in eluates from the heparin-Sepharose column. The dimer also bound to the double-stranded (ds)DNA–cellulose column (Pharmacia) and the DNA-affinity column, which contained the concatenated oligonucleotide (–1385/–1344) of the p120 gene (Zhang et al., 1991). Purification of the heterodimer was accomplished through a combination of chromatographic steps. The protein profiles at various stages of the purification are shown in Figure 1. During the purification, the heterodimer was monitored by SDS/PAGE and band-mobility-shift assays. The overall enrichment of the DNA-binding activity of the heterodimer is summarized in Table 1.

DNA-binding properties of the heterodimer

(a) Sequence non-specificity of DNA binding

Besides binding to the F1 fragment, the purified heterodimer bound to other DNA probes, such as the DNA fragments from simian-virus-40 and pBS plasmid DNA. The DNA binding to the F1 fragment was easily competed by 25–50-fold molar

Figure 2 Effects of circular or linearized plasmid on DNA binding

The plasmid pBluescript II (KS +/–) was used as competitor DNA either in circular form or linearized by endonuclease *Hind*III. The purified heterodimer (40 ng) was allowed to react with 1 ng of ³²P-labelled F1 fragment in the presence of increasing amounts (as indicated) of plasmid DNA at room temperature. After 20 min incubation, the reactions were analysed in 5%-polyacrylamide gels. C1–C3 indicate the protein–DNA complexes. F1 is the free probe.

Figure 3 Effect of pH and salt concentration on DNA-binding activity of the heterodimer

(a) A series of buffers (pH 4.5–11) with increments of 0.5 pH unit was prepared using 20 mM Homopipes, Mes, Pipes, Mops, Hepes, Tris, Ches, and Caps according to their pK_a values. Portions (50 ng) of the proteins were allowed to react with 1 ng of ³²P-labelled F1 fragment in the buffer at room temperature for 20 min and resolved on 5%-polyacrylamide gels. (b) Portions of the proteins (50 ng) allowed to react with 1 ng of ³²P-labelled F1 fragment in a buffer (pH 8) containing various amounts of KCl (0–200 mM) with increments of 20 mM. After 20 min incubation at room temperature, the reaction mixtures were analysed on 5%-polyacrylamide gels.

excesses of linearized or circular pBS plasmid DNA (Figure 2). When the dimer was assayed in DNAase I footprint analysis, only non-specific protection was detected for both the F1 and simian-virus-40 probes (results not shown). Thus far, no sequence specificity was found for the heterodimer. In addition, the purified heterodimer produced multiple band patterns in band-mobility-shift assays (Figures 2 and 3), suggesting that more than one heterodimer may bind to one DNA probe.

(b) Effects of pH and ionic strength on the DNA binding

A series of buffers with different pH values were used. The optimal pH range for binding of the heterodimer to DNA is from 7.0 to 9.0 (Figure 3a). When the binding activity was titrated with

Figure 4 Analysis of the DNA-binding subunits of the heterodimer

(a) U.V.-cross-linking assay. Proteins of HeLa nuclear extract (30 μ g) and 0.5 μ g of the purified proteins were incubated with uniformly labelled F1 fragment (2×10^5 c.p.m.) at room temperature for 20 min. After u.v. irradiation and DNAase digestion, the samples were fractionated by SDS/7.5%-PAGE. The gel was stained with Coomassie Blue, dried, and exposed to X-ray film for autoradiograms. Lanes 1 and 2 are nuclear extracts and the purified proteins respectively (SDS/PAGE). Lanes 3 and 4 are autoradiograms from the SDS/PAGE gels. (b) Southwestern assay. Proteins from HeLa nuclear extracts (10 μ g) and 5 μ g each of the flow-through and the eluted proteins of the heparin-Sepharose column were subjected to SDS/7.5%-PAGE and transferred to nitrocellulose membranes. After treatment with 6 M guanidinium chloride and washed, the membrane was probed with F1 fragment at 4 °C overnight. The membrane was washed, air-dried and exposed to X-ray film to produce autoradiograms. Lanes 1–3 are the nuclear extract, the heparin flow-through and heparin eluates respectively subject to SDS/PAGE; lanes 4–5 are the corresponding autoradiograms.

different KCl concentrations at pH 8.0, the heterodimer was optimally bound to DNA at low salt concentrations (Figure 3b).

(c) DNA-binding subunit of the heterodimer

UV-crosslinking experiments were used to determine whether one or both subunits of the complex contacted DNA in non-denaturing state. HeLa nuclear extracts and the purified heterodimer were incubated with the ³²P-uniformly-labelled F1 fragment. After u.v. irradiation, nuclease digestion and SDS/PAGE fractionation, the autoradiogram showed that the radioactivity was bound to the p100 subunit (Figure 4a). In Southwestern assays, in which the complex was separated in subunits and immobilized on nitrocellulose membrane, it was also shown that

p52: Sequence 1 (from a peptide of 15 kDa)

1 Gln-Ser-Asn-Lys-Thr-Phe-Asn-Leu-Glu-Lys-10
11 Gln-Asn-His-Thr-Pro-Arg-Lys-Lys-His-Gln-20

Sequence 2 (from a peptide of 9 kDa)

1 Pro-Leu-Arg-Gly-Lys-Gln-Leu-Arg-Val-Arg-10
11 Phe-Ala-Ala-His-Ser-Ala-Ser-Leu-Thr-Val-20
21 Arg-21

p100: Sequence 1 (from a peptide of 18 kDa)

1 Pro-Gly-Gly-Pro-Lys-Pro-Gly-Gly-Gly-Pro-10
11 Gly-Leu-Ser-Thr-Pro-Gly-Gly-His-Pro-Lys-20
21 Pro-Pro-His-Arg-Gly-Gly-Gly-Glu-Pro-Pro-30
31 Arg-Gly-Arg-Gln-31

Sequence 2 (from a peptide of 5 kDa)

1 Gly-Pro-Gly-Pro-Gly-Gln-Ser-Gly-Pro-Lys-10
11 Pro-Pro-Ile-Pro-Pro-Pro-Pro-Pro-His-Gln-20
21 Gln-21

Figure 5 Peptide sequences of p62 and p100 proteins

A portion (10 pmol) each of the gel-purified p52 and p100 proteins were treated with CNBr and fractionated by SDS/20%-PAGE. The peptides were transferred to ProBlott membrane (Promega), stained with Coomassie blue and excised for protein microsequencing. Four peptide sequences (two from each of the polypeptides) are presented; amino acids of uncertain identity are italicized.

the p100 subunit bound to DNA probe (Figure 4b). Accordingly, p100 is the DNA-binding subunit.

Biochemical properties of the heterodimer

(a) Stoichiometry of p52/p100

During chromatography, p52 and p100 were in an approximate 1:1 ratio in the heparin column fractions and the DNA-affinity column fractions (Figure 1).

(b) Molecular masses of the heterodimer and its subunits

The heterodimer is stable in buffer D containing 1 M KCl. At this ionic strength, the heterodimer had a molecular mass of 280 kDa as determined by Sephacryl S-300 gel filtration (results not shown). The subunit molecular masses determined in SDS/PAGE were 100 kDa and 52 kDa respectively, which suggests the heterodimer is a tetramer in physiological solutions.

(c) Two-dimensional gel analysis of the heterodimer

To determine the purity of the purified heterodimer and pI of the subunits, the DNA-affinity column fraction was subjected to gel analysis. The 100 kDa and 52 kDa subunits were localized at pH 8.6 and pH 7.8 respectively (results not shown).

Microsequencing of the p52 and p100 proteins

To analyse the primary structures of p52 and p100 proteins and

Figure 6 Immunofluorescence staining of HeLa cells with anti-(p52–p100) antiserum

HeLa cells were fixed with formaldehyde, permeabilized with acetone, and probed with 1:50-diluted primary antiserum, followed by addition of fluorescein-conjugated goat anti-mouse second IgG. (a) Phase-contrast image; (b) fluorescence image. Magnification $\times 320$. Arrows indicate cells in the mitotic phase.

to compare the similarity between them, protein microsequencing was done. The polypeptides of p52 and p100 were first separated by SDS/PAGE and the excised gel pieces were electroeluted. Each protein was cleaved by CNBr in 70% formic acid. The resulting peptides were fractionated on SDS/20%-PAGE, transferred to ProBlott membrane (Promega), and stained with Coomassie Blue. Four major bands (20, 15, 9, and 5 kDa) from p52 and five major bands (38, 32, 18, 17, and 12 kDa) from p100 were generated. The individual bands were excised and sequenced. The partial peptide sequences of p52 and p100 are shown in Figure 5. The sequences obtained were subjected to similarity searches in the database network. Since no sequence identities were found for either protein when compared with known proteins, p52 and p100 are novel proteins.

Cellular localization of the heterodimer

Mouse polyclonal antibodies against the heterodimer were prepared using the purified proteins as antigens. Indirect immunofluorescence was performed to examine the cellular localization of the heterodimer. HeLa cells were cultured on slides, fixed with paraformaldehyde, treated with acetone, probed with the antiserum against the heterodimer, and stained with fluorescein-conjugated second antibody. The fluorescence was localized within cell nuclei in interphase cells and diffusely extrachromosomally in mitotic-phase cells (Figure 6).

DISCUSSION

The present study reports the purification of a heterodimer from HeLa-cell nuclear extracts. The subunits of the heterodimer with molecular masses of 52 and 100 kDa were in an equal stoichiometric ratio throughout the protein purification process (Figure 1). The dimer was stable in 1 M KCl. In Sephacryl S-300 chromatography the dimer was eluted at molecular mass of 280 kDa, which suggests that the functional form may be a tetramer. The pI values of the two subunits p52 and p100 were pH 7.8 and pH 8.6 respectively.

No sequence specificity was detected in the binding of the

heterodimer to DNA. The dimer bound to different DNA probes with different sequences and the binding of the dimer to DNA was competed for by different competitor DNAs, such as non-labelled DNA probes, sonicated salmon sperm DNA and linearized or circular plasmid DNA (Figure 2). Other examples of abundant nuclear proteins with little or no sequence specificity are TFIIC (Slattery et al., 1983; Kurl and Jacob, 1985), Ku antigen (Mimori and Hardin, 1986; Yaneva and Busch, 1986), and topoisomerase I (Osherooff, 1989). In addition to the lack of sequence specificity, the ionic strength for binding of the heterodimer to DNA was low (Figure 4).

The heterodimer was compared with the known nuclear proteins with similar molecular masses. Although some sequence-specific DNA binding proteins or transcription factors have similar molecular masses, none was in a heterodimer like the p52/100 (Wingender, 1988; Mitchell and Tjian, 1989; Johnson and McKnight, 1989; Evan, 1990). Adlakha et al. (1985) partially purified a 50 kDa mitotic factor from HeLa cells. Meerovitch et al. (1989) identified a 52 kDa RNA-binding protein in HeLa cells that bound to the 5'-non-coding region of poliovirus RNA. Previously, we purified a 55 kDa DNA-binding protein from HeLa nuclear extracts that reacted with the upstream region (–1400) of the p120 gene (Zhang et al., 1991). Recently, Millar

Table 2 Partial sequence comparison between p52 and topoisomerase II (Topo II)

Protein/enzyme	Sequence											
p52 sequence 2	P	L	R	G	K	Q	L	R	V	R	F	A
Topo II (human)	485P	L	R	G	K	I	L	N	V	R	E	A ⁴⁹⁶
Topo II (baker's yeast)	475P	L	R	G	K	M	L	N	V	R	E	A ⁴⁸⁶
Topo II (fission yeast)	476P	L	R	G	K	L	L	N	V	R	E	A ⁴⁸⁷
Topo II (<i>Drosophila melanogaster</i>)	466P	L	R	G	K	L	L	N	V	R	E	A ⁴⁷⁷
Topo II (<i>Trypanosoma brucei</i>)	463P	L	R	G	K	L	L	N	V	R	N	K ⁴⁷⁴

et al. (1991) reported the identification of nuclear 52 and 55 kDa proteins required for the initiation of mitosis in human cells. However, none of these proteins were associated with any 100 kDa proteins. Yamaski et al. (1989) identified four proteins (55, 70, 100 and 140 kDa) that may be involved in nuclear localization of proteins. Patton et al. (1991) reported a 57 kDa protein, which along with a 100 kDa protein was part of a complex required to rescue splicing in depleted nuclear extracts. The relationship of the p52/100 dimer to any of these proteins is not clear.

When the partial sequences of p52 and p100 (Figure 5) were analysed in database networks (GenBank, Swiss-Prot, and GenPept) for sequence identity, no identical proteins were found. Thus, the p52-p100 protein complex is a novel DNA-binding heterodimer. Interestingly, one sequence of the p52 peptide, PLRGKQLRVRFA, showed considerable sequence similarity to a conserved motif in topoisomerase II of several species (Table 2). Although topoisomerase II activity was associated with the heterodimer during the purification process, topoisomerase II activity was not detected in the purified heterodimer (results not shown).

The p52-p100 heterodimer is relatively abundant in HeLa nuclear extracts and readily purified. However, it was not previously reported, which may result from its unknown function and non-specific DNA-binding property. Our preliminary data showed that the expression levels of the heterodimer, determined by Western blotting and e.l.i.s.a. analysis, were 20–50 times higher in human cancer cell lines than that in resting cells (results not shown). The heterodimer was also shown to be induced by the mitogen phytohaemagglutinin in normal human lymphocytes (results not shown). These suggest that the heterodimer may be in part associated with cell proliferation.

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