Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 6354–6358, July 1994
Biochemistry

A DNA-binding activity, TRAC, specific for the TRA element of the transferrin receptor gene copurifies with the Ku autoantigen

MARGO R. ROBERTS*, YOUNG HAN, ALAN FIENBERG, LISA HUNIHAN, AND FRANCIS H. RUDDLE

Department of Biology, Yale University, New Haven, CT 06511

Contributed by Francis H. Ruddle, March 11, 1994

ABSTRACT **We have previously described purification and characterization of a nuclear protein, TREF, which interacts specifically with the transcriptional control element, TRA, of the human transferrin receptor (TR) gene. In this report we show that TREF can be separated into two functionally distinct DNA-binding activities. The first DNA-binding activity (TRAC) is highly specific for the 8-bp element TRA and the related *Escherichia coli* cAMP receptor binding site. This motif is homologous to the phorbol 12-tetradecanoate 13-acetate- and cAMP-responsive elements of eukaryotic genes and the regulatory proximal sequence elements of the U1 small nuclear RNA gene and is also present in the promoter of the *Drosophila melanogaster* yolk protein factor 1 gene. In striking contrast, the second activity exhibits high affinity for the ends of double-stranded DNA in a sequence-unspecific manner and is attributable to the heterodimeric Ku autoantigen. Notably, transcription of Ku is induced during mid-late G_0/G_1 with kinetics similar to the TR gene. Ku is a highly abundant nuclear protein possessing nonspecific affinity for the ends of DNA, whose biological role remains to be elucidated. A transcriptional role for this protein has been proposed, however, on the basis of studies attributing DNA sequence-specific binding activity, notably for TRA-like sequences described above, directly to the Ku heterodimer. The observation that Ku-mediated nonspecific DNA-binding activity copurifies with the TRA-specific activity, TRAC, clearly has implications for these and related studies. The unusual properties of TRAC activity and its relationship, if any, with the enigmatic Ku protein, are discussed.**

The transferrin receptor (TR) is a transmembrane glycoprotein expressed on the cell surface of nearly all cell types of higher organisms, where it mediates iron uptake by binding and internalizing iron-loaded transferrin. Increased TR expression in response to a mitogenic stimulus is observed in many systems: for example, T-cell activation (1–4), stimulation of quiescent fibroblasts to reenter the cell cycle (5), and transformation (6, 7). A regulatory region of the TR promoter necessary for increased expression in response to proliferation (8–10) contains an 8-bp element (TRA; nucleotides −77 to −70) that is essential for transcriptional activity of the TR promoter, as assayed either by transient transfection of HeLa cells (10, 11) or by *in vitro* transcription employing HeLa cell nuclear extract (M.R.R., unpublished observations). The TRA element shares seven of eight bases with the proximal sequence element (PSE) of the U1 small nuclear RNA gene which is essential for U1 gene transcription (12, 13).

We have previously reported the purification and characterization of nuclear preparations from HeLa cells which bind specifically to the region of the TR promoter encompassing the 8-bp transcriptional control element of the TR gene, TRA, described above (14). Analysis of these nuclear fractions, designated TREF, revealed the presence of a heterodimeric complex composed of an 82-kDa protein and a 62-kDa protein, TREF1 and TREF2, respectively, which copurify with the TRA-specific activity (14). In this report, we show that the nucleotide sequences of cDNAs encoding TREF1 and TREF2 are identical to the regions encoding the Ku autoantigen subunits p86 and p70, respectively (15–18). Furthermore, upon release of human fibroblasts from quiescence, Ku mRNA is induced during mid-late G_0/G_1 with kinetics similar to those observed for TR gene mRNA.

Although these data are consistent with a role for Ku in transcription, Ku exhibits a number of unusual properties which appear difficult to reconcile with such a function. These include the ability to bind with lack of specificity, but with high affinity, to the ends of double-stranded DNA (19, 20). In addition, it is one of the most abundant DNA-binding proteins detectable in the nucleus (M.R.R., unpublished observations). These properties have led to speculation that Ku may play a role in cellular processes such as DNA replication, repair, or recombination. Despite these data demonstrating nonspecific DNA binding and extreme abundance, investigators have also implicated Ku as a possible transcriptional regulator. Evidence corroborating such a role includes a study in which a heterodimeric protein, PSE1, which shares physical, functional and immunological characteristics with Ku, appears to interact specifically with the PSE of the human U1 promoter (12, 21), which shares sequence identity with the TRA element. Another study reports specific binding the Ku p70 subunit to the enhancer of the T-cell receptor β-chain gene (22), a sequence which also is similar to the TRA element. It has also been shown that nuclear fractions enriched for a heterodimeric protein related to Ku, Ku-2, bind to a sequence unrelated to the TRA/PSE, the octamer motif (23). Intriguingly, more recent studies have shown that Ku functions as the DNA-binding component of the DNA-dependent protein kinase, a serine/threonine kinase whose ability to phosphorylate Sp1 and other nuclear proteins (24) is suggestive of a transcriptional role.

In this report, we present evidence that the nuclear TREF activity can be separated into two functionally distinct DNA-binding activities: the nonspecific DNA-binding activity mediated by Ku, and another DNA-binding activity, TRAC. In contrast to the sequence-nonspecific binding behavior displayed by Ku, TRAC is highly specific for the eight-nucleotide TRA sequence AAGTGACG which encompasses the transcriptional control element of the TR gene. The apparent instability of TRAC that we observe under standard assay conditions may explain, at least in part, the inability of previous studies to distinguish between the TRA-specific activity described here and the nonspecific activity mediated by Ku. In addition to the TRA element, the TRAC factor(s)

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: TR, transferrin receptor; TRA, TR transcriptional control element; PSE, proximal sequence element; CRP, cAMP-receptor protein or related oligonucleotide; CRE, cAMP-responsive element; TRE, phorbol 12-tetradecanoate 13-acetate-responsive element.

*To whom reprint requests should be addressed at: Cell Genesys, 322 Lakeside Drive, Foster City, CA 94404.

also recognizes the core sequence of the *Escherichia coli* cAMP-receptor protein (CRP) binding site. TRA shares seven of eight bases with the bacterial cAMP-responsive element (CRE) and is also homologous to the phorbol 12-tetradecanoate 13-acetate-responsive element (TRE) and CRE known to be regulated by mammalian and yeast transcription factors. The possible impact of these data on interpretation of previous studies implicating Ku as a transcription factor and the relationship, if any, between TRAC and Ku are discussed.

MATERIALS AND METHODS

Molecular Cloning of TREF. The two subunits of the TREF heterodimer were purified from HeLa cell nuclear extract by successive fractionation steps involving ion-exchange and oligonucleotide affinity chromatography as previously described (14). The two proteins were then purified to homogeneity by preparative SDS/PAGE. Degenerate sets of oligonucleotides were then generated from the informative amino acid sequences (25) derived from the amino acid sequence of either the N terminus of TREF1 or tryptic peptides of TREF2 and used as probes to screen a cDNA library derived from the simian virus 40-transformed human fibroblast cell line GM637 (26). The identity of candidate clones encoding either TREF1 or TREF2 was then established by sequence analysis of nested 5′ and 3′ deletions generated with exonuclease III (27) and by comparison of the deduced open reading frame with amino acid sequences derived from the respective purified protein.

Gel Mobility-Shift Assays. Preparation of nuclear extracts and subsequent analysis of protein–DNA complexes by low ionic strength polyacrylamide gel electrophoresis was carried out as previously described (14). Assays were all performed under conditions of probe excess.

Northern Analysis. Total RNA was isolated from cell lines by a guanidinium thiocyanate method (28) and analyzed by Northern hybridization as previously described (29). Individual transcripts were quantitated by scanning laser densitometry.

Cell Culture and Synchronization. Cell lines were grown in Dulbecco's modified Eagle's medium (DMEM) plus 10% fetal bovine serum (DMEM-10). Quiescent populations of WI38 cells were obtained by placing cells in serum-free medium 24 hr after plating and were maintained in the same medium for four days. Serum stimulation was carried out by changing the medium to DMEM-10. Flow cytophotometric DNA histogram analysis was carried out on propidium-iodide-stained cells (30) to determine the relative DNA content of the cell population at intervals after release from quiescence.

RESULTS

TREF1 and TREF2 Are Identical to the Ku Heterodimer. We have previously described studies in which fractions obtained from HeLa cell nuclear extracts exhibit DNA-binding activity specific for the transcriptional control element of the TR gene, as determined by DNase 1 footprinting experiments (14). This nuclear TREF activity copurifies through a number of chromatographic steps with two proteins of 82 and 62 kDa, TREF1 and TREF2, respectively, which behave as a heterodimer (14). cDNAs encoding TREF1 and TREF2 were isolated as described in *Materials and Methods*. Sequence comparisons with current data bases revealed the 82- and 62-kDa proteins to be identical to the two subunits of the human autoantigen Ku and confirmed published sequences (15–18). The apparent molecular mass of the purified 82-kDa subunit observed upon SDS/PAGE corresponds well with the value of 82.7 kDa predicted from the deduced protein sequence. The predicted mass of the smaller subunit is 69.8 kDa, which correlates with the mass of 70 kDa observed for the small subunit of Ku upon SDS/PAGE (16, 17). However, purified TREF2 migrates with an apparent mass of 62 kDa (14), perhaps reflecting proteolytic cleavage of the full-length protein during the purification procedure. Similarly, others have observed that the small subunit of PSE1 purified from human placental extract has an apparent mass of 63 kDa (21).

Ku1 and Ku2 Are Both Induced in Human Embryonic Cells After Release from G_0 Arrest. Studies from our laboratory have shown that when quiescent murine fibroblasts are stimulated to enter the cell cycle, TR mRNA levels are induced in mid-late G_0/G_1 prior to S-phase entry (5), an observation consistent with the correlation between TR expression and proliferative status found in other systems (1–8). It has also been demonstrated that expression of the TR gene is essential for traversal through the S phase of the cell cycle (31, 32). We therefore sought to determine the relationship, if any, between the proliferative status of the cell and the kinetics of both TR and Ku gene expression. Prior to carrying out such a study, we investigated the expression profile of Ku in a number of human cell lines. Five human cell lines were examined for Ku expression by employing either TREF1 (Ku1)- or TREF2 (Ku2)-derived cDNA fragments as probes in Northern analysis (Fig. 1). WI38 is derived from normal human embryonic lung tissue, whereas the remaining four lines are derived from human tumors: A549 from a lung carcinoma, NGP and SK from neuroblastomas expressing high and low levels of myc, respectively, and BeWo from a trophoblast-derived choriocarcinoma. The Ku1- and Ku2-encoded mRNA species observed in these five cell lines are also detected in the cell lines GM637 (a human fibroblast cell line from which the TREF cDNAs were isolated) and HeLa (data not shown). The relative levels of the Ku1 and Ku2 transcripts vary significantly between the different cell lines assayed, with the neuroblastoma line SK exhibiting a greatly reduced expression of Ku2 relative to that of Ku1.

The relationship between Ku gene expression and proliferation was then investigated in the human cell line WI38. Quiescent populations of the human cell line WI38 were stimulated to reenter the active cell cycle by the addition of serum to the medium. Subsequently, samples were removed at regular intervals and assayed for Ku and TR mRNA expression by Northern analysis (Fig. 2*A*). Evaluation of β_2-microglobulin mRNA levels served as an internal control. The percentage of G_0/G_1, S, or G_2/M-phase cells at each time was determined by flow cytophotometric DNA histogram analysis as described in *Materials and Methods*, and the kinetics of cell cycle progression are shown in Fig. 2*B*. The percentage of the cell population with a chromosomal content of $2n$ does not change significantly for at least 12 hr after release from G_0, with entry of cells into S phase first observed

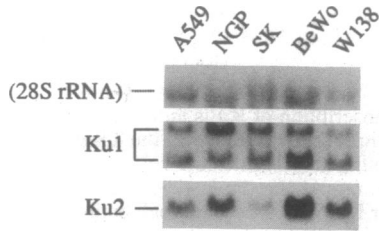

FIG. 1. Northern blot analysis of Ku1 and Ku2 expression in human cell lines. Total RNA was purified from the five cell lines indicated, and formaldehyde/agarose gel electrophoresis and Northern transfer were carried out as previously described (29). Northern analysis was carried out on duplicate RNA samples, and the mRNA transcripts detected with Ku1- or Ku2-specific DNA probes are indicated. The nonspecific signal observed for 28S rRNA is also shown.

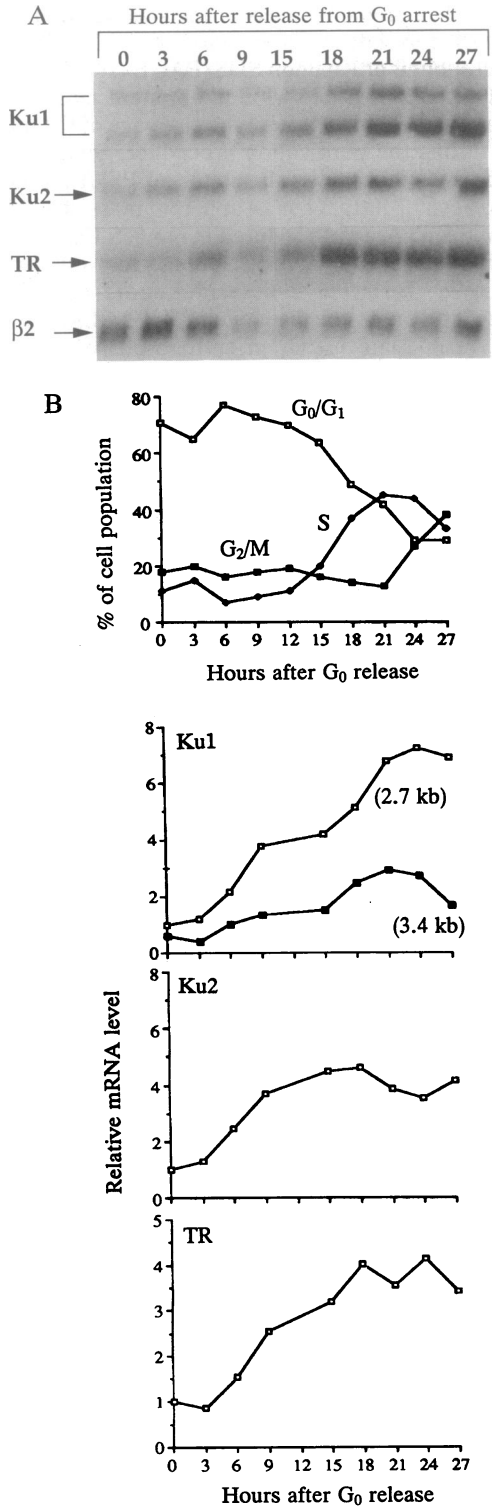

FIG. 2. Transcription of Ku is induced during the G_0/G_1 transition with kinetics similar to those of the TR gene. Quiescent WI38 cells were stimulated to reenter the cell cycle by addition of serum to the medium. (*A*) At the times indicated, samples were removed and assayed for Ku, TR, and β_2-microglobulin mRNA expression by Northern analysis. (*B*) The percentage of G_0/G_1, S, or G_2/M-phase cells at each time point was determined by flow cytometry (30) and is summarized in the top panel. The lower three panels show the relative levels of Ku1, Ku2, and TR mRNA transcripts at each time point assayed in *A* after quantitation by scanning laser densitometry.

at about 15 hr after stimulation, the point of G_1/S-phase transition. This trend continues, with the cell population gradually moving into G_2/M with time. Fig. 2*B* summarizes the data obtained from Northern analysis and shows the kinetics of expression of the Ku mRNA transcripts (relative to that of β_2-microglobulin RNA) after release of the cell population from G_0 arrest. Levels of both mRNA species encoded by Ku1 begin to increase between 3 and 6 hr after addition of serum. The 2.7-kb transcript reaches a plateau of expression at about 21–24 hr after serum stimulation, attaining an ≈7-fold increase over basal levels. Similar kinetics of expression are observed for the 3.4-kb transcript, although the magnitude of the increase observed is much lower than that of the 2.7-kb transcript. The kinetics of Ku2 mRNA induction follow a similar pattern, mRNA levels reaching a 4-fold maximum ≈15 hr after serum addition. When TR mRNA levels are followed during the same experiment, induction is first observed between 3 and 6 hr after G_0 release, increasing to a plateau during S phase. The kinetics of mRNA induction are therefore rather similar for the three genes encoding Ku1, Ku2, and TR: induction is initiated during G_0/G_1, ≈6–9 hr prior to S-phase entry, and transcript levels rise steadily as cells enter the proliferative state.

Detection of the TRA-Specific Activity TRAC, which Copurifies with Ku-Mediated Nonspecific DNA-Binding Activity. We have previously demonstrated that highly purified nuclear preparations containing the p82/p62 heterodimer exhibit TRA-specific binding activity, as determined by DNase 1 footprinting (14). In contrast, these same nuclear fractions exhibit a relative lack of sequence specificity when assayed by DNA mobility assays (ref. 14; M.R.R., unpublished observations). The contradictory DNA-binding properties exhibited by the TREF nuclear activity, and the lack of specificity attributed to the Ku heterodimer, prompted us to further investigate the DNA-binding activities present in these purified nuclear fractions.

Nuclear fractions highly enriched for Ku were prepared from HeLa cell nuclear extract by ion-exchange chromatography as previously described (14). DEAE-5PW fractions enriched for Ku were detected by gel mobility-shift analysis employing the copolymer poly(dI-dC) as nonspecific competitor DNA and the TRA-containing double-stranded oligonucleotide TR56 (14) as labeled probe (Fig. 3*A*). Previous studies have shown that Ku is present in the DNA–protein complex (K) observed under these conditions (14, 19, 20, 23). The participation of Ku in complex K is further confirmed by the observation that preincubation of the nuclear fractions with anti-Ku monoclonal antibodies (kindly provided by

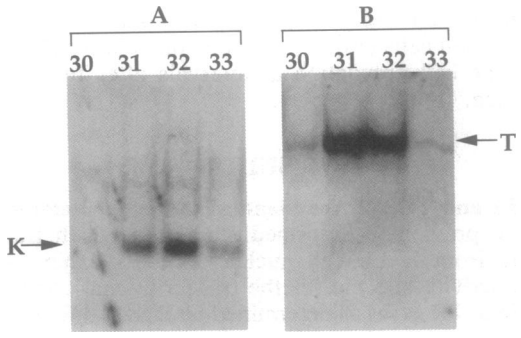

FIG. 3. DNA mobility-shift analysis of nuclear DEAE fractions with TREF activity. Nuclear extract was prepared from HeLa cells as previously described (14) and fractionated by DEAE chromatography (14). DEAE fractions 30–33 inclusive were analyzed by gel mobility shift employing the double-stranded oligonucleotide TR56 (14), which contains the TRA element as labeled probe. (*A*) The DNA–Ku protein complex K is detected in the presence of the poly(dI-dC). (*B*) Analysis of the same DEAE fractions in the presence of a 1000-fold excess of TRA-unrelated double-stranded oligonucleotide, and in the absence of poly(dI-dC), reveals a new DNA–protein complex TRAC (T), of lower mobility.

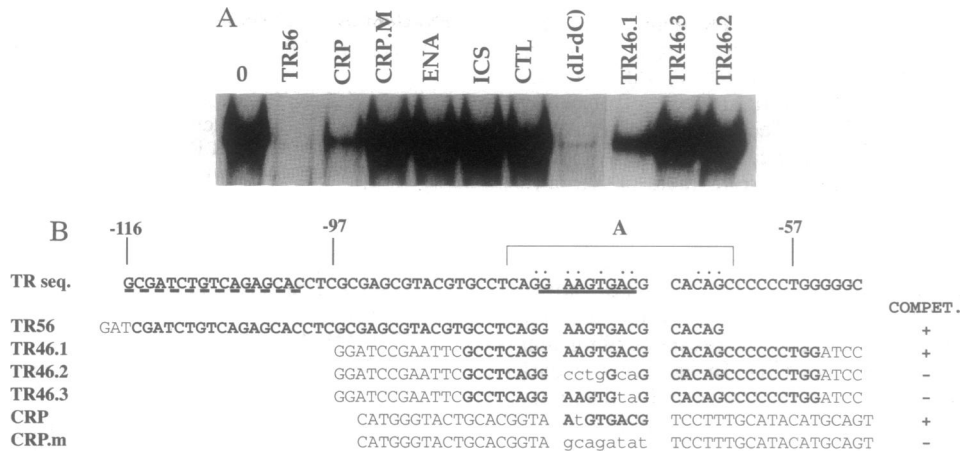

FIG. 4. TRAC is highly specific for the TRA element of the TR gene. DEAE fractions 31 and 32 shown in Fig. 3 were pooled and subjected to competitive DNA mobility-shift analysis. TR56 was employed as labeled probe, and unlabeled TRA-related and unrelated double-stranded oligonucleotides [ENA, ICS, and CTL correspond to sequences from the regulatory element of the *HLA-B2* gene (14); others are discussed in the text] were added as competitor DNA, using a competitor-to-probe molar ratio of 200:1. The results are shown in *A* and the sequences of the double-stranded oligonucleotides employed in the competition study are shown in *B*, with the successful competitors indicated by + in the column on the right. The sequence of the TR promoter (TR seq.) is shown, with regions A (solid underline) and B (broken underline) identified as sequences previously shown to be protected from DNase 1 digestion with TREF nuclear fractions (14). Dots indicate sites of diminished cleavage. Region A (under brace) is a sequence previously shown to be protected from DNase 1 digestion with unfractionated HeLa cell nuclear extract (5). Bold typeface identifies bases identical to the TR sequence. Lowercase identifies bases which differ from the 8-nucleotide TRA sequence located within region A.

W. H. Reeves of Rockefeller University) inhibits formation of this complex (data not shown). As would be predicted from the DNA end-specific binding properties of Ku, the addition of a high molar excess of DNA ends in the form of a 1000-fold molar excess of TRA-unrelated double-stranded oligonucleotide probe, in the presence of the poly(dI-dC), readily out-competes the Ku complex. However, when poly(dI-dC) is not present, addition of the TRA-unrelated double-stranded oligonucleotide not only results in loss of complex K as expected but also, surprisingly, facilitates the appearance of a new complex, T (Fig. 3*B*). These studies, therefore, reveal a DNA-binding activity not previously detected due to instability in the presence of poly(dI-dC), which copurifies with the Ku complex during DEAE chromatography (see fractions 31 and 32 in Fig. 3).

TRAC Is Specific for the TRA Element. The sequence specificity of the DNA–protein complex T was investigated by gel mobility-shift analysis, employing a variety of TRA-related and -unrelated double-stranded oligonucleotides as competitor DNA (Fig. 4*A*), the results of which are summarized in Fig. 4*B*. Oligonucleotide TR46.1 contains TRA, sharing a 30-bp sequence with the TR promoter. TR46.2 and TR46.3 bear mutations in TRA, specifically in the transcription control element, and in contrast to TR46.1 and TR56, are unable to compete for binding. The 45-mer oligonucleotide referred to as CRP, which is homologous to *crp*, the CRP-binding site of the *E. coli lac* operon, shares 7 of 8 base pairs with TRA and efficiently competes for binding. When this 8-bp sequence is replaced by an unrelated motif (CRP.m), competition is no longer observed. Thus, the 8-bp sequence AAGTGACG of the TRA region, the TRA element, which encompasses the transcriptional control element of the TR gene, is sufficient for specific interaction with this factor(s). These studies also confirm the instability of the TRA-specific complex (TRAC) in the presence of poly(dI-dC) (Fig. 3). We have also been unable to detect TRAC when other forms of nonspecific competitor DNA such as purified plasmid or salmon sperm DNA have been employed, an observation which may explain the difficulty in identifying this sequence-specific complex in previous studies. In marked contrast to TRAC, formation of the Ku complex is readily blocked by competition by all of the above double-stranded oligonucleotides (data not shown). This observation is consistent with the previously described property of Ku to bind with high affinity to the ends of double-stranded DNA (14, 19, 20, 23).

The TRA Motif Is Related to CRP, PSE, TRE, and CRE. Fig. 5 shows the high degree of sequence similarity of the 8-bp TRA element to a number of different cis-acting elements found in other genes. The TRA motif shares sequence identity with the prokaryotic CRP consensus sequence, which regulates expression of a large number of cAMP-responsive genes, including the *lac* operon and the ferric uptake (*fur*) gene of *E. coli* (33). The TRA element is also homologous to the CRE and TRE elements found in a variety of mammalian genes (ref. 34, and references therein). Whether the TRAC factor(s) is related to the mammalian Fos/Jun and ATF/CREB families of transcription factors known to interact with these regulatory sequences is not yet known. The PSE or element B of the human U1 small nuclear RNA gene also shares eight of nine nucleotides with the TRA sequence (21).

DISCUSSION

We have demonstrated that the Ku heterodimer copurifies with a second DNA-binding activity, TRAC, which is highly specific for a sequence coincident with a previously defined

TRA element TR gene	**CAGG AAGTGACG CACAGCC**
E. coli crp: lac operon	GGTA **ATGTGACG** TCCTTTG
E. coli crp: fur gene	TAAG **CTGTGCCA** CGTTTTT
proximal sequence element U1 snRNA gene	GGGC **AAGTGACC** GTGTGTG
E3 enhancer of T-cell receptor beta-chain	**CAGG ATGTG**GTT TG
YPF1 element yolk protein 1 gene	GCCG **AAGTTACG** GACCAA
TPA responsive element, TRE	**GTGACT CA**
cAMP responsive element, CRE	**GTGAC**G TC
Consensus TRAC binding site	**AAGTGACG** **T**

FIG. 5. Homology of the TRA motif to known regulatory elements of other genes. The sequence of the TRA element of the TR gene is compared to the sequences of bacterial and eukaryotic regulatory elements. Bold typeface identifies bases identical to the consensus TRAC motif.

transcriptional control region of the TR gene, the TRA element. The identity of the protein(s) involved in formation of this complex, and its relationship, if any, with Ku, are particularly intriguing, especially in light of data from other laboratories. Knuth *et al.* (21) purified a heterodimeric protein, PSE1, possessing immunological and amino acid sequence properties indistinguishable from those of Ku (14). Footprinting assays employing nuclear fractions highly enriched for Ku suggested that Ku binds specifically to the PSE of the human U1 gene, an element which shares eight of nine nucleotides with the TRA element of the TR gene. Subsequent studies showed that a minimal TR promoter retaining the TRA sequence was dependent on the presence of nuclear fractions highly enriched for Ku (21). In light of the data shown here, it is highly likely that the transcriptional activity observed was due to TRAC activity which copurified with Ku in the nuclear fractions employed in these studies. A DNA-binding protein isolated from *Drosophila melanogaster*, yolk protein factor 1 (YPF1) (35), exhibits a number of characteristics which bear a striking resemblance to those of Ku. Nuclear extracts highly enriched for YPF1 exhibit sequence-specific binding activity for a 31-bp sequence in the yolk protein 1 gene encompassing a TRA element (35). It is tempting to speculate that YPF1 may be the *D. melanogaster* equivalent of TREF, a nuclear activity in which Ku copurifies with TRAC-like factor(s) specific for the TRA sequence motif. A more recent study reports binding of the p70 subunit of Ku to the E3 enhancer motif of the T-cell receptor β-chain gene (22), a sequence which also shares sequence identity with the TRA element (Fig. 5). Interestingly, the p70 protein appears to exhibit both sequence-nonspecific and E3-specific DNA-binding activities as assayed by DNA mobility-shift analysis (22). We have previously observed that Ku does exhibit some limited specificity for certain unrelated DNA sequences, as determined by competition DNA mobility-shift analysis of the Ku complex, K (14).

Ku has recently been identified as the DNA-binding component of the DNA-dependent protein kinase (DNA-PK), which phosphorylates several DNA binding proteins, including Oct-1 and Oct-2, c-Myc, and Sp1 (24). Furthermore, Ku and Sp1 must be localized on the same molecule for efficient phosphorylation to occur. On the basis of the data in the studies outlined above, a transcriptional role for Ku, perhaps by phosphorylation, has been proposed. Our observation that Ku mRNA levels correlate with the proliferative status of the cell, increasing during the G_0/G_1 transition and S phase, although consistent with a direct or indirect role in the transcription of genes such as the TR whose expression is required during this period, is also consistent with a role in the regulation of cellular replication, for example. Although the studies described above suggest that Ku acts directly as a sequence-specific binding protein, it is clear that care must be taken when ascribing such properties to as highly abundant a DNA-binding protein as Ku, since the true identity of proteins directly responsible for the specific activity observed may be readily masked. A challenge for future studies will be to determine whether Ku is functionally associated, directly or indirectly, with the TRAC activity observed, or merely copurifies with this activity. The instability of the TRA-specific complex observed in the presence of various forms of nonspecific competitor DNA other than double-stranded oligonucleotides warrants further investigation, to determine whether this phenomenon results from direct destabilization of the complex by the noncompetitor DNAs employed or whether it is an indirect effect associated with the high-affinity binding of Ku to the ends of double-stranded DNA. This report provides the framework for future investigations aimed at identification of the nuclear factor(s) which make up the TRAC activity and will enable clarification of the physical or functional relationship, if any indeed exists, between the TRAC factor(s) and the enigmatic Ku protein.

We thank Dean Kedes, Keith Miskimins, Paul Stroobant, and Sue Klapholz for helpful discussions, and Suzy Pafka and Veronica Lubeck for artwork. M.R.R. was a Special Fellow of the Leukemia Society of America during the course of this work.

1. Reed, J. C., Alpers, J. C., Nowell, P. C. & Hoover, R. G. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 3982–3986.
2. Trowbridge, I. S. & Omary, M. B. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 3039–3043.
3. Pauza, C. D., Bleil, J. D. & Lennox, E. S. (1984) *Exp. Cell Res.* **154**, 510–520.
4. Depper, J. M., Leonard, W. J., Drogula, C., Kronke, M., Waldmann, T. A. & Greene, W. C. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4230–4234.
5. Miskimins, W. K., McClelland, A., Roberts, M. P. & Ruddle, F. H. (1986) *J. Cell Biol.* **103**, 1781–1788.
6. Hamilton, T. A. (1982) *J. Cell. Physiol.* **113**, 40–46.
7. Larrick, J. W. & Cresswell, P. (1979) *J. Supramol. Struct.* **11**, 579–586.
8. Miskimins, W. K. (1992) *J. Cell. Biochem.* **49**, 349–356.
9. Ouyang, Q., Bommakanti, M. & Miskimins, W. K. (1993) *Mol. Cell. Biol.* **13**, 1796–1804.
10. Owen, D. & Kuhn, L. C. (1987) *EMBO J.* **6**, 1287–1293.
11. Casey, J. L., Di Jeso, B., Rao, K. K., Rouault, T. A., Klausner, R. D. & Harford, J. B. (1988) *Nucleic Acids Res.* **16**, 629–646.
12. Gunderson, S. I., Knuth, M. W. & Burgess, R. R. (1990) *Genes Dev.* **4**, 2048–2060.
13. Murphy, J. T., Steinberg, T., Skuzeski, J. T., Burgess, R. R., Lund, E. & Dahlberg, J. E. (1987) *J. Biol. Chem.* **262**, 1795–1803.
14. Roberts, M. R., Miskimins, W. K. & Ruddle, F. H. (1989) *Cell Regul.* **1**, 151–164.
15. Yaneva, M., Wen, J., Ayala, A. & Cook, R. (1989) *J. Biol. Chem.* **264**, 13407–13411.
16. Reeves, W. H. & Sthoeger, Z. M. (1989) *J. Biol. Chem.* **264**, 5047–5052.
17. Mimori, T., Ohosone, Y., Hama, N., Suwa, A., Akizuki, M., Homma, M., Griffith, A. J. & Hardin, J. A. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 1777–1781.
18. Chan, J. Y. C., Lerman, M. I., Prabhakar, B. S., Isozaki, O., Santisteban, P., Kuppers, R. C., Oates, E. L., Notkins, A. L. & Kohn, L. D. (1989) *J. Biol. Chem.* **264**, 3651–3654.
19. de Vries, E., van Driel, W., Bergsma, W. G., Arnberg, A. C. & van der Vliet, P. C. (1989) *J. Mol. Biol.* **208**, 65–78.
20. Stuiver, M. H., Coenjaerts, F. E. J. & van der Vliet, P. C. (1990) *J. Exp. Med.* **172**, 1049–1054.
21. Knuth, M. W., Gunderson, S. I., Thompson, N. E., Strasheim, L. A. & Burgess, R. R. (1990) *J. Biol. Chem.* **265**, 17911–17920.
22. Messier, H., Fuller, T., Mangal, S., Brickner, S., Igarashi, S., Gaikwad, J., Fotedar, R. & Fotedar, A. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 2685–2689.
23. May, G., Sutton, C. & Gould, H. (1991) *J. Biol. Chem.* **266**, 3052–3059.
24. Gottlieb, T. M. & Jackson, S. P. (1993) *Cell* **72**, 131–142.
25. Lathe, R. (1985) *J. Mol. Biol.* **183**, 1–12.
26. Okayama, H. & Berg, P. (1983) *Mol. Cell. Biol.* **3**, 280–289.
27. Henikoff, S. (1987) *Methods Enzymol.* **155**, 156–165.
28. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18**, 5294–5299.
29. Violette, S. M., Shashikant, C. S., Salbaum, J. M., Belting, H. G., Wang, J. C. & Ruddle, F. H. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 3805–3809.
30. Raveche, E. S., Steinberg, A. D., DeFranco, A. L. & Tjio, J. H. (1982) *J. Immunol.* **129**, 1219–1226.
31. Trowbridge, I. S. & Lopez, F. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 1175–1179.
32. Lesley, J. F. & Schulte, R. J. (1985) *Mol. Cell. Biol.* **5**, 1814–1821.
33. DeLorenzo, V., Herrero, M., Giovannini, F. & Neilands, J. B. (1988) *Eur. J. Biochem.* **173**, 537–546.
34. Hai, T. & Curran, T. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 3720–3724.
35. Mitsis, P. G. & Wensink, P. C. (1989) *J. Biol. Chem.* **264**, 5188–5194.