Volume 232, number 1, 99–102 FEB 05822 May 1988

Multi-site binding of human nuclear protein to the Alu-family repeated DNA

O.I. Podgornaya, L.M. Perelygina and N.V. Tomilin

Institute of Cytology, The Academy of Sciences of the USSR, Tikchoretskii Av.4, Leningrad 194064, USSR

Received 1 March 1988.

Nuclear protein which selectively binds to the Alu-family DNA repeat (AFR, Blur8) is partially purified from human HeLa cells using a gel retention assay. At low protein concentrations only a single complex of the protein with AFR is formed (CII). Increasing protein concentrations lead to the gradual disappearance of CII, being replaced by complexes with higher (CI) and lower (CIII, CIV) electrophoretic mobilities. Differential binding of AFR restriction subfragments indicates that multiple protein-binding sites are present within AFR. We discuss two models explaining the anomalous electrophoretic mobility of CII by DNA bending or looping upon cooperative multi-site binding of the protein to AFR.

DNA-binding protein; Alu-family DNA repeat

1. INTRODUCTION

The human genome contains $3–5 \times 10^5$ dispersed Alu-family DNA repeats (AFRs) which have a variable length of about 300 base pairs and vary in nucleotide sequence, having evolved from a large pool of precursors [1–3]. AFRs are suggested to be mobile genetic elements transposed when reverse-transcript AFR-precursors integrate preferentially into AT-rich regions [4–6]. AFRs contain RNA polymerase III promoter [4] and 10–20% of all genomic AFRs are capable of being transcribed [3], but in HeLa cells AFRs are transcriptionally silent [7]. It was also suggested that AFRs contain the origin of replication and are involved in the initiation of DNA synthesis [2,8]. The SV40 virus protein A (T-antigen) stimulates the initiation of DNA replication starting from AFR both in vivo and in vitro [9,10]. T-antigen interacts with the pentanucleotide 5′-GAGGC-3′ [11–13] which is present in AFR [3,14].

In our previous report [15] the Alu-family repeat binding protein (ABP) from HeLa cells was described which also interacts with the regulatory region of the SV40 virus genome. In this paper data will be presented suggesting that AFR contains several binding sites for ABP, and that the major binding site is situated within the 35 bp *Hin*fI subfragment which is homologous with the T-antigen binding site II at the SV40 replication origin.

Correspondence address: O.I. Podgornaya, Institute of Cytology, The Academy of Sciences of the USSR, Leningrad 194064, Tikchoretskii Av.4, USSR

2. MATERIALS AND METHODS

Methods for the growth of HeLa cells, isolation of 0.4 M NaCl nuclear extract, purification of plasmid DNAs, isolation and end-labelling of restriction fragments with the help of DNA polymerase have been described before [15].

For this study Alu-binding protein (ABP) was purified as follows: 0.4 M NaCl nuclear extract from 3 g of wet cells was diluted to obtain 50 mM concentration of NaCl and applied to a column of DEAE-cellulose (1.5 × 13.0 cm) equilibrated with buffer A (15 mM Tris-Cl, pH 8, 0.1 mM EDTA, 0.5 mM dithiothreitol, 5% glycerol) containing 50 mM NaCl, and then the column was washed with the same buffer. Adsorbed proteins were eluted with a stepwise gradient of NaCl in buffer A: 150 mM, 250 mM (DE_{250} fraction) and 350 mM. The DE_{250} fraction containing ABP was dialysed against 20 mM Tris-HCl buffer, pH 8, and passed through the FPLC column MonoQ (HR 5/5, Pharmacia) which was then washed with the same buffer and with a linear gradient of NaCl (0–1 M) in the same buffer. ABP is eluted at 0.4 M NaCl (MQ_{400} fraction).

Incubation mixtures for the gel retention assay of DNA-binding proteins contained 1–10 ng of end-labelled [^{32}P]DNA

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

of short *Bam*HI AFR fragment (or its subfragments produced by endonuclease *Hin*fI) from plasmid Blur8 [14], 0.1% Triton X-100, 4% glycerol, 1 mM EDTA, 10 mM dithiothreitol, 10 mM Tris-HCl buffer, pH 7.5), 150 mM NaCl, and different quantities of non-labelled non-Alu DNAs as indicated in legends to the figures. After incubation for 30 min at room temperature complexes were run in low salt 4% polyacrylamide gel [16,17], then gel was dried and radioautographed.

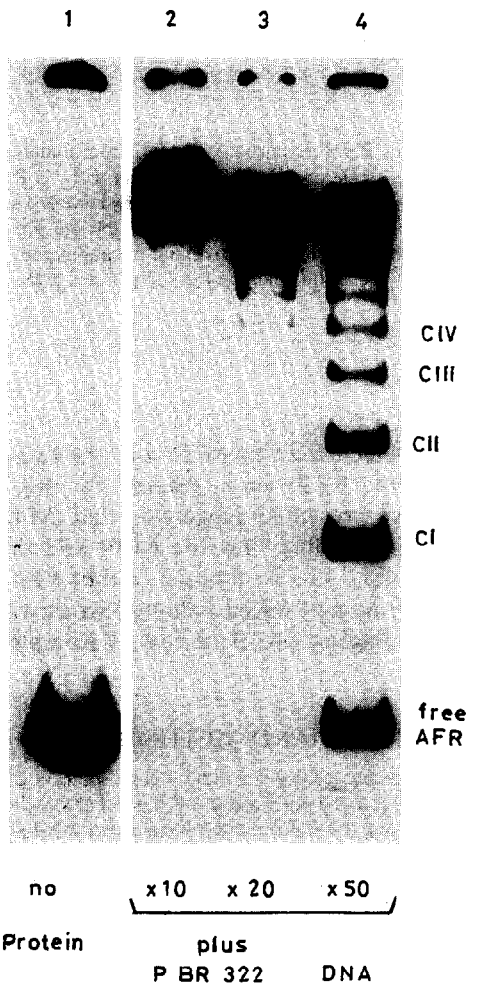

Fig.1. Complexes formed by the protein (ABP) and Alu-family repeat. 0.5 ng of the short end-labelled (about 260 bp) *Bam*HI fragment from the plasmid Blur8 [14] was incubated with ABP (MQ_{400} fraction, 1 μg of protein) in the presence of 80 mM NaCl and increasing amounts (10-, 20- and 50-fold molar excess, lanes 2, 3, and 4, respectively) of pBR322 DNA. After 30 min samples were run in low salt polyacrylamide 4% gel [16]. Complexes CI, CII, CIII and CIV have electrophoretic mobilities relative to the free Alu-family repeat (lane 1) equal to 0.73, 0.57, 0.45, and 0.34, respectively. 50-fold molar excess of pBR322 DNA corresponds to about 700-fold excess of the DNA (expressed in ng).

3. RESULTS

The gel retention assay allows one to detect stable and specific protein-DNA complexes which appear as discrete bands in low salt polyacrylamide gel [16,17]. When ABP is added to the end-labelled full-length AFR *Bam*HI fragment mixed with increasing amounts of non-specific DNA (pBR322 plasmid DNA), a characteristic series of bands is observed (fig.1, lane 4) of regularly decreasing mobility: complexes I, II, III, IV, etc. (CI, CII, CIII, CIV). In 4% polyacrylamide gel the complexes show a reproducibly decreased electrophoretic mobility (R_A) relative to the free AFR fragment equal to 0.73 (CI), 0.57 (CII), 0.45 (CIII), 0.34 (CIV). Low-mobility complexes apparently contain multiple molecules of bound protein: a similar pattern was observed in experiments with lac-operator DNA and lac-repressor [17].

The relative amount of each complex depends on the concentration of ABP (fig.2). At low protein concentration only CII is seen, and the increase of protein concentration leads to a gradual disappearance of CII and formation of CI, CIII, CIV, etc.; CII with $R_A = 0.57$ apparently contains a lower number of bound protein molecules as compared to CI which shows (figs 1,2) a higher

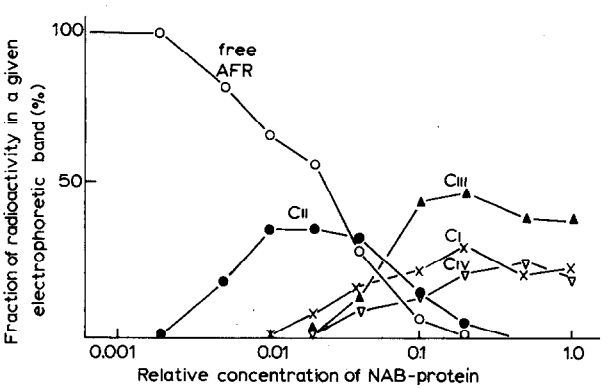

Fig.2. Formation of complexes between ABP and AFR at different protein concentrations. 2 ng of the mixture of end-labelled *Bam*HI restriction fragments of Blur8 (short AFR and pBR322 vector fragment) were incubated in the presence of 80 mM NaCl and 400 ng of non-labelled *E. coli* chromosomal DNA and increasing amounts of ABP (MQ_{400} fraction). The samples were processed as described in section 2 and in the legend to fig.1, and the radioautograph was analysed by densitometry. Maximal amount of added protein was 1 μg; the abscissa shows the extent of dilution (log scale).

electrophoretic mobility (R_A = 0.73). It is known that decreased electrophoretic mobility is characteristic of protein-DNA complexes with the ends of the DNA molecule drawn together which might be caused by DNA bending or looping [17]. DNA looping promoted by the protein molecule bound at two widely separated sites permits cooperative binding at low protein concentrations [18].

To examine if AFR contains single or multiple binding sites for ABP we have isolated restriction subfragments of AFR produced by the cleavage of full *Bam*HI AFR fragment from Blur8 with nuclease *Hin*fI, and used these subfragments as substrates for ABP. It was found that AFR subfragments B, C, and D (fig.3) are able to interact with ABP forming discrete bands in the retardation gel. Preference in binding measured as the percent of DNA in a complex shows that the 35 bp *Hin*fI fragment B (fig.3) contains the sequence 5′-GAGGCTGAGAC-3′ [14] homologous to the sequence 5′-GAGGCCGAGGC-3′ present in the T-antigen binding site II of the SV40 genome [11,12]. The data on the binding of AFR subfragments suggest that AFR contains multiple binding sites for ABP and different sites vary in their affinity. The major binding site is situated in the middle part of AFR: nucleotides 163–197 [3]. It should be noted that *Hin*fI subfragments C and D (fig.3) contain a GAGGC motif [3,14].

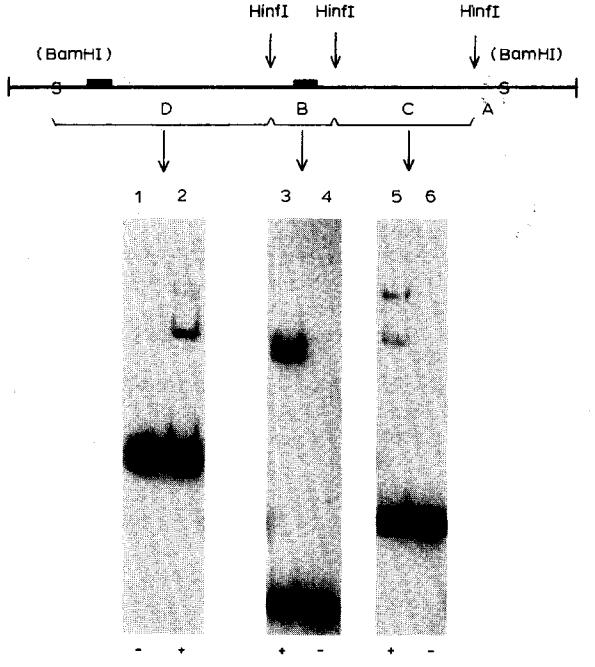

Fig.3. Gel retention assay of differential binding of restriction subfragments of full-length AFR produced by nuclease *Hin*fI. Short *Bam*HI fragment from the plasmid Blur8 (full-length AFR) was cleaved with *Hin*fI, subfragments were end-labelled with ^{32}P using Klenow fragment of DNA polymerase and isolated by electrophoresis in polyacrylamide gel. DNA binding and gel retention assays were performed as described in section 2 and in the legends to figs 1 and 2 using equimolar quantities of subfragments B, C and D (0.1–0.5 ng) incubated with a fixed amount of ABP (fraction MQ_{400}, 1 μg of protein) in the presence of 100 ng of non-labelled *E. coli* DNA and 80 mM NaCl. S——S, borders of Blur8 AFR flanked with *Bam*HI sites; ⊢—⊣, borders of consensus AFR [3]. Boxed are 5′-GAGGC-3′ pentanucleotide tandems with 1 bp spacing. Lanes: 1 and 2, subfragment D without (−) and with (+) ABP; 4 and 3, subfragment B without (−) and with (+) ABP; 5 and 6, subfragment C without (−) and with (+) ABP. Upper arrows show the positions of *Hin*fI restriction sites [14] present in Blur8 AFR.

4. DISCUSSION

Characteristic properties of Alu-repeat binding protein (ABP) described in this study and in our previous report [15] are its ability to form multiple protein-DNA complexes which have reproducible mobilities in retardation gels, as well as its ability to bind the 660 bp SV40 restriction fragment containing the replication origin [15]. It is shown here that AFR contains multiple binding sites for ABP and that the major binding site is situated within a 35 bp restriction fragment with the sequence 5′-GAGGCTGAGAC-3′ which could potentially explain the high-affinity interaction of ABP with the SV40 replication origin and with AFR. Binding specificity of ABP is clearly different from that of Sp1 [19] and of nuclear factor I [20]. Mouse nuclear protein was recently described [21] as enhancing T-antigen dependent replication of SV40 DNA in vitro and this protein might be similar to human ABP.

The high-affinity complex formed between AFR and ABP at the lowest protein concentration (CII, figs 1 and 2) showed an anomalous electrophoretic mobility in retardation gels as compared to CI which should contain more bound protein molecules but showed a higher electrophoretic mobility than CII. Two models could explain this effect. First, ABP might induce DNA bending at the major binding site in the central part of AFR which should decrease the electrophoretic mobility

of CII [17], and CI is formed upon additional binding to CII of ABP or another protein possibly present in the MQ_{400} fraction [15]. However, it is difficult to understand from this point of view the disappearance of DNA bent at the CII $\longrightarrow$ CI transition.

The second model suggests that CII is formed upon high-affinity cooperative binding of a single molecule of ABP to multiple sites within a single AFR molecule which induces DNA looping [18] and also should decrease the electrophoretic mobility [17]. The increase in concentration of ABP leads to the competitive displacement of cooperatively bound protein from some of the multiple binding sites and to the disappearance of the loop.

Acknowledgements: We thank Drs V. Malev, V. Bozhkov and B. Zhestopalov for helpful discussions of the data presented in this paper.

REFERENCES

[1] Houck, C.M., Rinehard, F.P. and Schmid, C.W. (1979) J. Mol. Biol. 132, 289–306.
[2] Jelinek, W.R. and Schmid, C.W. (1982) Annu. Rev. Biochem. 51, 823–844.
[3] Bains, W. (1986) J. Mol. Evol. 23, 189–199.
[4] Jagadeeswaran, P., Forget, B.G. and Weissman, S.M. (1981) Cell 26, 141–142.
[5] Daniels, G.R. and Deininger, P.L. (1985) Nucleic Acids Res. 13, 8939–8954.
[6] Rogers, J. (1985) Int. Rev. Cytol. 93, 187–279.
[7] Paulson, E.K. and Schmid, C.W. (1986) Nucleic Acids Res. 14, 6145–6158.
[8] Krayev, A.S., Kramerov, D.A., Skryabin, K.G., Ryskov, A.P., Bayev, A.A. and Georgiev, G.P. (1980) Nucleic Acids Res. 8, 1201–1217.
[9] Johnson, E.M. and Jelinek, W.R. (1986) Proc. Natl. Acad. Sci. USA 83, 4660–4664.
[10] Ariga, H. (1984) Mol. Cell. Biol. 4, 1476–1482.
[11] DeLucia, A.L., Lewton, B.A., Tjian, R. and Tegtmeyer, P. (1983) J. Virol. 46, 143–150.
[12] Tegtmeyer, P., Lewton, B.A., DeLucia, A.L., Wilson, W.G. and Ryder, K. (1983) J. Virol. 46, 151–161.
[13] Wright, P.J., DeLucia, A.L. and Tegtmeyer, P. (1984) Mol. Cell Biol. 4, 2631–2638.
[14] Rubin, C.M., Houck, C.M., Deininger, P.L., Friedmann, T. and Schmid, C.W. (1980) Nature 284, 372–374.
[15] Perelygina, L.M., Tomilin, N.V. and Podgornaya, O.I. (1987) Mol. Biol. Rep. 12, 111–116.
[16] Strauss, F. and Varshavsky, A. (1984) Cell 37, 889–901.
[17] Crothers, D.M. (1987) Nature 325, 464–465.
[18] Ptashne, M. (1986) Nature 322, 697–701.
[19] Gidoni, D., Dynan, W.S. and Tjian, R. (1984) Nature 312, 409–413.
[20] Gronostajski, R.M., Nagata, K. and Hurwitz, J. (1984) Proc. Natl. Acad. Sci. USA 81, 4013–4017.
[21] Ariga, H. (1986) Nucleic Acids Res. 14, 9457–9470.