Volume 281, number 1,2, 272–274 FEBS 09603 April 1991

ADONIS 001457939100312I

A 53 kDa protein binds to the negative regulatory region of JC virus early promoter

Arun K. Sharma[1] and Gyanendra Kumar[1,2]

[1]*Department of Molecular Biology and Genetics* and [2]*Center for Molecular Biology, Wayne State University, 540 E. Canfield, Detroit, MI 48201, USA*

Received 7 January 1991; revised version received 13 February 1991

Using gel retardation and photocrosslinking experiments, we have identified proteins of about 53 kDa in size in both rat glioma (C6) and cervical carcinoma (HeLa) cell extracts which bind to the negative regulatory region of JC virus (JCV) early promoter. The glial cell protein binds to its cognate promoter element with lesser affinity when compared to the protein present in HeLa cells. Further, these proteins interacted differentially to an oligonucleotide, containing the neighboring *cis*-acting domain, which is recognized by nuclear factor I (NFI). These findings suggest that the interactions of the 53 kDa HeLa protein may contribute to the negative regulation of JCV early promoter in HeLA cells.

JC virus; Transcription factor; Glial cell; HeLa cell; T-antigen promoter

1. INTRODUCTION

The human papova virus, JC (JCV), causes a fatal demyelinating disease, progressive multifocal leucoencephalopathy (PML) [1]. Although, JCV infects most of the human population in early childhood, antibodies against it have been detected in 60–80% of adults [2]. It remains inactive in most cases while causing PML only in immunocompromised individuals [3]. The regulatory region of the prototype (Mad1) strain of JCV, which consists of a 98 bp tandem repeat (Fig. 1), has been demonstrated to determine the tissue specificity of the expression of viral early genes [4,5]. The tissue-specific expression of JCV T antigen has been presumed to be determined by an interplay of positive and negative regulatory factors [6,7]. The early promoter has a consensus sequence for the NF1 binding domain which has been found to affect its transcription positively in brain nuclear extract [6]. Both, glial and HeLa cells have been found to have proteins which bind to the NF1 domain [6,8]. A 45 kDa protein which binds to this region has been purified from calf brain extract [7]. This protein stimulated viral RNA synthesis in vitro when complemented with glial or HeLa cell nuclear extracts. However, experiments involving formation of heterokaryons between JCV-transformed hamster glial cells, expressing JCV T antigen, and mouse fibroblasts suggest that cellular factor(s) present in non-glial cells may negatively regulate viral gene expression and thus contribute to the restricted tissue specificity of JCV transcription [9]. The binucleate heterokaryons formed in this study did not express T-antigen. Subsequently, some of the subclones that lost murine chromosomes started expressing T-antigen. The negative regulation of JCV promoter in non-glial cells has also been shown to be regulated by the *cis*-acting domain of JCV promoter [10]. In these studies, synthetic oligonucleotides spanning the JCV early region, inserted into expression vectors were used in transfection assays with glial and non-glial cells. Two of the oligonucleotides used in this study (A and B, Fig. 1) stimulated the activity of the reporter gene in glial cells but decreased the basal activity in non-glial cells. JCV early promoter has a tandem repeat of AGGGA, present between TATA box and NF1 consensus sequence. A careful look at the two oligonucleotides reveals that both of them had an AGGGA pentanucleotide repeat divided between them (see Fig. 1), suggesting a negative role of AGGGAAGGGA in the regulation of JCV early promoter in non-glial cells. This conclusion is supported by the presence of similar domains in the negative regulatory regions of other genes. Rat embryonic myosine heavy chain gene and c-myc gene regulatory regions contain AGGGAAGGAGA and AGGGAAAGGGA domains respectively [11,12]. Both of these regions have been shown to have silencer activity. In the present study we have used a synthetic oligonucleotide containing an intact AGGGA pentanucleotide repeat for gel retardation and photocrosslinking experiments to identify the protein factors interacting with this region.

Correspondence address: G. Kumar, Center for Molecular Biology, Wayne State University, 540 E. Canfield, Detroit, MI 48201, USA

2. MATERIALS AND METHODS

Nuclear extracts from rat glioma (C6) and human HeLa cells were made using a previously described method [13]. DNA-binding conditions were as described [14], except that the final reaction volume of 20 μl contained 50 fmol probe, 3 μg (HeLa) or 6 μg (C6) nuclear pro-

Published by Elsevier Science Publishers B.V.

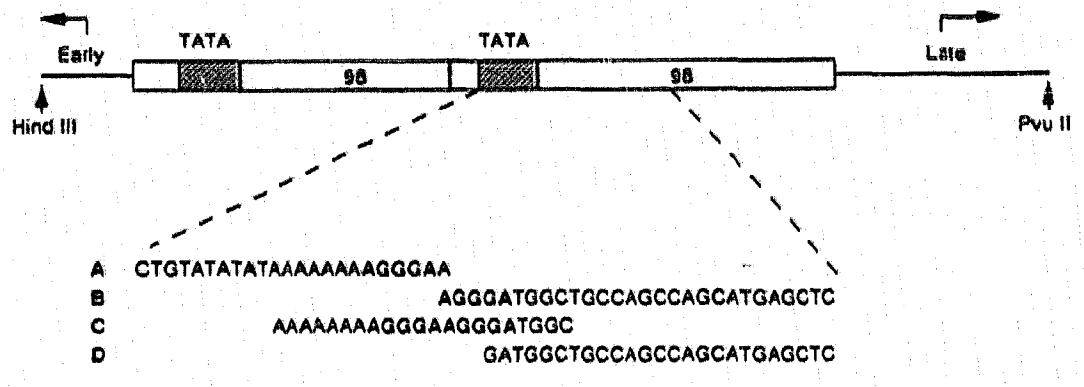

Fig. 1. JC virus regulatory region indicating the sequences of the DNA duplexes used to characterize the regulatory region.

tein and 1 μg poly dI:dC. For photocrosslinking, samples were exposed to short wave length UV light, using a hand held UV illuminator for 30 min, after the 30 min DNA-binding reaction.

3. RESULTS AND DISCUSSION

A previous study has shown that the region of JCV early promoter, including the AGGGA repeat, could down-regulate the promoter in non-glial cells [10]. In the present study, we used a synthetic oligonucleotide representing this part of the JCV regulatory region (Duplex C, Fig. 1), to identify cellular proteins from both glial and non-glial cells which bind to this *cis* element. Duplex C showed a strong band (complex A, Fig. 2a, lane 2) when used with a HeLa cell nuclear extract but no complex formation was observed with glial cell nuclear extracts in mobility shift assays (Fig. 2a, lane 1). In order to check the possibility that a glial cell protein may be interacting with duplex C with lower affinity resulting in a protein-DNA complex that is too weak to survive electrophoresis, we crosslinked any bound protein to the duplex by UV-light irradiation. The resulting mixture was run on a native polyacrylamide gel. A protein-DNA complex closely matching complex A formed with HeLa cell extract was now observed with the glial cell extract (Fig. 2b, lane 1), suggesting that, indeed, proteins present in the glial cell extract also bind to this negative regulatory element of JCV early promoter, albeit with less affinity when compared to proteins present in HeLa cells. An additional, weaker, slower moving complex was observed with HeLa cell extract (complex B). Complex A observed with glial or HeLa cell extract was competed away by 20-fold excess of homologous competitor (Duplex C) but it was unaffected by similar excess of heterologous competitor (Duplex D). Complex B appeared to be non-specific as it was competed by both, homologous and heterologous competitors. When the crosslinked products were run on SDS-polyacrylamide gel, to determine the molecular mass of proteins binding directly to duplex C, proteins of about 53 kDa were observed with both glial and HeLa cell extracts (Fig. 3).

The putative negative regulatory region is flanked by a TATA box on one side and an NF1 binding consensus sequence on the other side (Fig. 1). Since the duplex containing the TATA box (Duplex A, Fig. 1) has been found to interact with proteins of similar molecular weight in both glial and HeLa cell extract whereas the

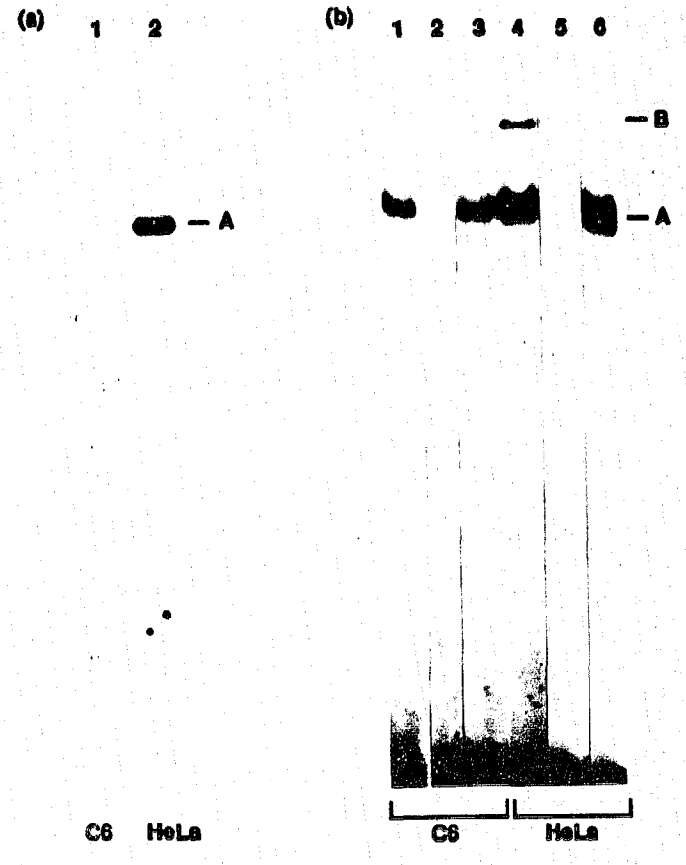

Fig. 2. (a) Gel retardation of duplex C in the presence of (1) C6 glioma nuclear extract and (2) HeLa cell nuclear extract. (b) Gel retardation of duplex C was done in the presence of (1 and 4) no competitor, (2 and 5) 20-fold excess of duplex C, and (3 and 6) 20-fold excess of duplex D. After the binding reaction, the proteins bound to DNA were crosslinked and electrophoresed on 10% native polyacrylamide gels.

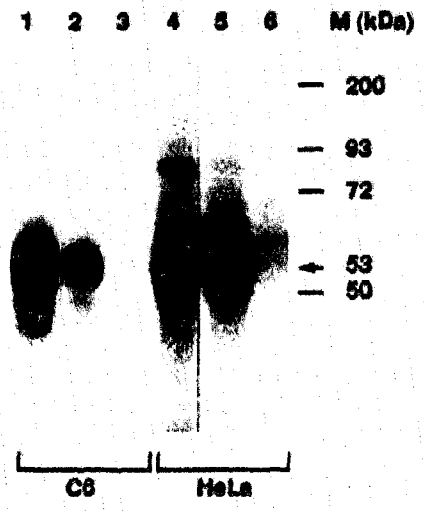

Fig. 3. Photocrosslinking of duplex C to nuclear extract in the presence of (1 and 4) no competitor, (2 and 5) 100-fold excess of duplex D, and (3 and 6) 100-fold excess of unlabeled duplex C. The samples were electrophoresed on 12% SDS-polyacrylamide gels.

duplex containing NF1 binding domain (duplex B, Fig. 1) has been found to interact with very distinct proteins in glial and HeLa cells [7,15], we tried to study the interaction of the 53 kDa proteins with the NF1 domain to get an insight into the tissue-specific expression of JCV. Whereas the binding of the 53 kDa protein to duplex C was not affected by a 100-fold excess of duplex D (NF1 domain) in HeLa cell extracts, the binding of glial protein was affected to some extent by similar excess of duplex D (Fig. 3). Both proteins were competed by a 100-fold excess of duplex C.

The present study suggests that both glial and HeLa cells have a 53 kDa protein which binds to the putative negative regulatory region of JCV early promoter. The glial protein has poor affinity for its binding domain as compared to the HeLa protein which binds very tightly and may, therefore, contribute to the negative regulation of JCV early promoter in HeLa cells. These proteins were found to interact differentially to the NF1 binding domain (present in duplex D), suggesting that an interplay of the 53 kDa protein and NF1 may contribute, at least in part, to the tissue-specific expression of JCV early genes. It is not clear how the interaction of the 53 kDa protein to the JCV promoter could alter promoter activity. Perhaps, the close proximity of the binding site of the 53 kDa protein to the potential positive regulatory motif (NF1) could alter positive protein-protein interactions. We are currently purifying these factors to study protein-protein interactions in detail.

In a recent study, which appeared during the revision of this manuscript, the AGGGAAGGGA domain has been shown to be responsible for the negative regulation of the JCV late promoter [16].

Acknowledgements: This work was supported by NIH Grant GM 38228 to G.K. and also by funds from the Center for Molecular Biology, Wayne State University, Detroit.

REFERENCES

[1] Padgett, B.L., Walker, D.L., ZuRhein, G.M., Hodach, A.E. and Chow, S.M. (1976) J. Infect. Dis. 133, 686-690.
[2] Padgett, B.L. and Walker, D.L. (1983) in: Polyoma viruses and human neurological diseases (Sever, J.L. and Madden, D.L. eds) pp. 107-118, Alan R. Liss, New York.
[3] Padgett, B.L. and Walker, D.L. (1976) Prog. Med. Virol. 22, 1-35.
[4] Kenney, S., Natarajan, V., Strike, D., Khoury, G. and Salzman, N. (1984) Science 226, 1337-1339.
[5] Small, J.A., Scangos, G.A., Cork, L., Jay, G. and Khoury, G. (1986) Cell 46, 13-18.
[6] Tamura, T., Inoue, T., Nagata, K. and Mikoshiba, K. (1988) Biochem. Biophys. Res. Commun. 157, 419-425.
[7] Ahmed, S., Rappaport, J., Tada, H., Kerr, D. and Khalili, K. (1990) J. Virol. 265, 13899-13905.
[8] Amemiya, K., Traub, R., Durham, L. and Major, E.O. (1989) J. Biol. Chem. 264, 7025-7032.
[9] Beggs, A.H., Frisque, R. and Scangos, G.A. (1988) Proc. Natl. Acad. Sci. USA 85, 7632-7636.
[10] Tada, H., Lashgari, M., Rappaport, J. and Khalili, K. (1989) J. Virol. 63, 463-466.
[11] Kakkis, E., Riggs, K.J., Gillepsie, W. and Calama, K. (1989) Nature 339, 718-721.
[12] Bouvagrt, P.F., Strehler, E.E., While, G.E., Strehler-Page, M.A., Nadal-Ginard, B. and Madhavi, V. (1987) Mol. Cell Biol. 7, 4377-4389.
[13] Dignam, J.D., Lebovitz, R.M. and Roeder, R.G. (1983) Nucleic Acids Res. 11, 1475-1489.
[14] Ahn, J.K., Gavin, B.J., Kumar, G. and Ward, D.C. (1989) J. Virol. 63, 5425-5439.
[15] Khalili, K., Rappaport, J. and Khoury, G. (1988) EMBO J. 7, 1205-1210.
[16] Tada, H., Lashgari, M.S. and Khalili, K. (1991) Virology 180, 327.