Volume 258, number 2, 255–258 FEB 07874 December 1989

HSV infection induces increased transcription of Alu repeated sequences by RNA polymerase III

K.L. Jang and D.S. Latchman

Medical Molecular Biology Unit, Department of Biochemistry, University College and Middlesex School of Medicine, The Windeyer Building, Cleveland Street, London W1P 6DB, England

Received 30 August 1989

The Alu family of repeated sequences is transcribed by both RNA polymerase II and RNA polymerase III In cells infected with HSV, transcription by polymerase III increases while transcription by polymerase II decreases By using virus strains carrying mutations in the genes encoding individual regulatory proteins, we have shown that this effect is dependent upon the immediate-early protein ICP27 and occurs by a process distinct from those which regulate viral gene expression This is the first example of increased transcription of endogenous cellular sequences by RNA polymerase III during infection with a DNA virus

Herpes simplex virus infection, Alu repeat, RNA polymerase III, Transcriptional control

1. INTRODUCTION

Although in eukaryotes RNA polymerase II is responsible for the transcription of protein-encoding genes, the activity of RNA polymerase III is essential for the transcription of several vital cellular transcription units such as those encoding the transfer RNAs and the 5S RNA of the ribosome (reviewed in [1]).

To investigate the regulation of transcription by this polymerase further, several investigators have studied the effects of viral proteins on the expression of genes transcribed by RNA polymerase III. These studies have shown that both the E1A protein of adenovirus [2] and the immediate-early protein of pseudorabies virus [3] can increase the expression of a co-transfected 5S or tRNA gene by increasing the activity of transcription factor IIIC [4] whilst having no effect on the transcription of the endogenous 5S or tRNA genes.

To characterize further the interaction of viral genes with RNA polymerase III-transcribed genes, we have studied the effect of herpes simplex virus (HSV) proteins on the Alu family of repeated sequences, several 100 000 copies of which are present in the human genome [5]. Many of these 300 base pair sequences are found within the 3′ untranslated region or in the intervening sequences of protein-coding genes and are hence transcribed by RNA polymerase II as part of the large primary transcripts of such genes. In addition however, many of these repeats contain functional promoters recognized by RNA polymerase III and are hence transcribed in isolation by this polymerase producing small RNA molecules [16].

This system thus provides a unique opportunity to investigate the effects of viral proteins on transcription of the same sequence by two different RNA polymerases. Recently Panning and Smiley (manuscript in preparation) showed that although large RNA species containing Alu sequences decreased in abundance upon HSV infection, smaller Alu transcripts were present at increased abundance in the infected cells. Such observations are consistent with a differential effect of HSV infection on transcription of Alu sequences by RNA polymerases II and III but this possibility could not be confirmed since inhibitors which distinguish between transcription by these two polymerases (see below) also inhibit HSV infection.

Here we report that HSV infection does indeed stimulate transcription of Alu repeated elements by RNA polymerase III and that this effect is dependent on the viral immediate-early protein ICP27. This is the first report of the stimulation of and endogenous as opposed to a transfected polymerase III transcription unit by a viral protein.

2. MATERIALS AND METHODS

2 1. *Viral infection*

HeLa cells were infected at a multiplicity of infection of 10pfu per cell with HSV-1 strain F [7] or with the HSV-1 mutant strains tsLB2 (which has a mutation in the gene encoding ICP4:–8), 17X2D (which has a mutation in the gene encoding ICP27:–9), d11403 (mutant in the ICPO gene·–10), R325 (mutant in the ICP22 gene:–11) or N38

Correspondence address: D.S. Latchman, Medical Molecular Biology Unit, Department of Biochemistry, University College and Middlesex School of Medicine, The Windeyer Building, Cleveland Street, London W1P 6DB, England

Published by Elsevier Science Publishers B V (Biomedical Division)
00145793/89/$3 50

(mutant in the ICP47 gene:-12). Cells were harvested 8 h after infection for the preparation of RNA or nuclei. Mock-infected cells were similarly treated without addition of virus.

2.2 *RNA analysis*

Total RNA was prepared from cells by the guanidinium isothyocyanate procedure [13] and analyzed by Northern blotting [14] using the Alu probe Blur 11 [15] which had been radiolabelled by the method of Feinberg and Vogelstein [16].

2.3 *Nuclear run-on*

Nuclear run-on assays of transcription were carried out as previously described [17] and the labelled products used to probe dot blots onto which the Alu probe Blur 11 [13], a control probe which hybridizes to ribosomal RNA [18] and a histone H2B probe [19] had been spotted. α-Amanitin was added to the reactions as indicated at a final concentration of 2.5 μg/ml.

3. RESULTS AND DISCUSSION

To confirm the effect of HSV infection on Alu containing RNA species, HeLa cells were infected with HSV-1 and total RNA was isolated 8 h after infection. The expression of Alu RNA in infected cells was compared to that in mock-infected cells by hybridization with the radio-labelled Alu probe, Blur 11 [15]. As shown in fig.1, a smear of high molecular weight Alu containing RNAs was detected, the abundance of which decreased slightly upon infection. In contrast, small RNA species of approximately 200–300 bases in size increased approximately five fold in abundance upon infection.

We next investigated whether these effects occurred at the transcriptional level by carrying out a nuclear run-on assay [17] using nuclei prepared from mock-infected or infected cells 8 h after infection. In these experiments (fig.2a,b), decreased transcription of the Alu repeated sequence was detected in the infected cell nuclei indicating that overall Alu transcription falls in infected cells. The fact that the nuclear run-on assay measures transcription by assaying the in vitro elongation of transcripts initiated in vivo allowed us to use inhibitors to dissect out the effects of infection on Alu transcription by polymerases II and III. In particular the fungal toxin α-amanitin inhibits RNA polymerase II when it is present at very low levels (1 μg per ml) whereas RNA polymerase III is only inhibited at much higher levels (over 10 μg per ml:-20). We therefore added α-amanitin to the nuclear extracts at a concentration of 2.5 μg per ml and carried out nuclear run-on experiments as before. In this case, increased transcription of Alu repeated sequences was observed in the infected cell extract (fig.2, c and d), indicating that elevated transcription of these repeated sequences by RNA polymerase III does occur in infected cells against a background of declining transcription by RNA polymerase II. No increased transcription of other genes transcribed by RNA polymerase III including those encoding 5S RNA, transfer RNA and 7SL RNA was observed in these experiments (fig.3) indicating

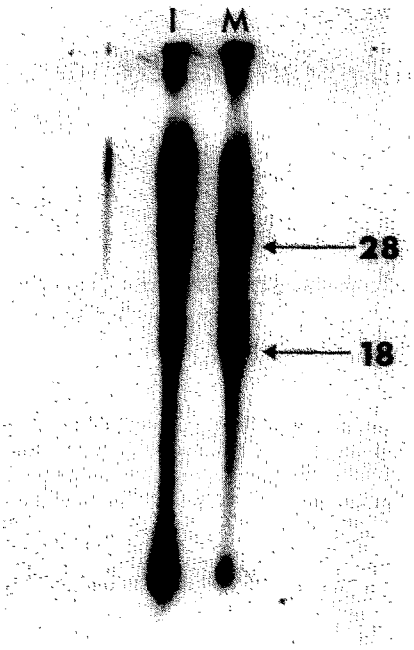

Fig.1. Northern blot of total RNA isolated from mock infected (M) or HSV-1 infected (I) HeLa cells and hybridized with the Alu specific probe Blur 11. Labelled arrows indicate the positions of the 28S and 18S ribosomal RNAs.

that the effect is specific for Alu repeated sequences. Such a conclusion is of particular interest in the case of 7SL RNA which has sequence homology to Alu [21] and indicates that increased transcription of this gene is not responsible for the increased transcription detected with Alu probes. As expected the transcription of the viral immediate-early 4 gene and the cellular histone H2B gene by RNA polymerase II was abolished by the α-amanitin treatment (fig.2, c and d) confirming that this treatment differentially affected polymerase II and polymerase III transcription.

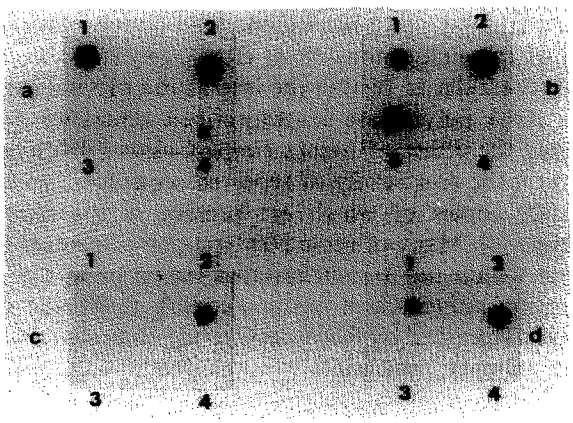

Fig.2. Nuclear run-on assay of transcription in mock-infected (a and c) or infected (b and d) HeLa cells. In c and d the assay was carried out in the presence of α-amanitin at a concentration of 2.5 μg/ml. Key to spots: 1, Alu clone Blur 11; 2, control ribosomal clone; 3, HSV-1 immediate-early 4 gene; 4, histone H2B gene.

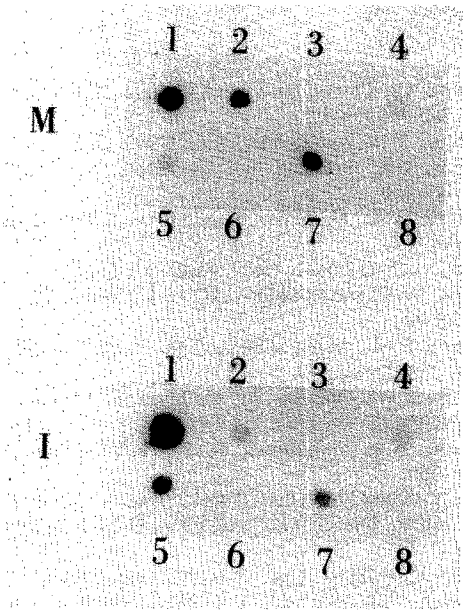

Fig.3. Nuclear run-on assay of transcription (in the presence of α-amanitin) in mock infected (M) or HSV-1 infected (I) HeLa cells. Key to spots: 1 and 5, Alu clone; 2, control ribosomal clone; 3, 5SRNA gene; 4, tRNA gene; 6, histone H2B gene; 7, 7SL gene; 8, 7SK gene.

Having established that HSV infection stimulates polymerase III transcription of Alu sequences we wished to determine the relationship of this process to the various phases of viral gene expression which occur in the infected cell.

To do this we infected HeLa cells with the HSV-1 mutant ts LB2 which carries a temperature-sensitive lesion in the gene encoding the viral immediate-early protein ICP4 [8]. Because this protein plays an essential role in the transcription of the viral early and late genes [22] when it is non-functional infection is aborted at an early stage, only the 5 viral immediate-early proteins including defective ICP4 being synthesized in the infected cell [23]. Despite this, in infections carried out with tsLB2 at the non-permissive temperature of 39°C increased transcription of Alu repeats was observed in the infected cells when compared to mock infected cells incubated at the same temperature (fig.4). Similar increased transcription was also observed in infections with another virus carrying a temperature-sensitive mutation in the ICP4 gene, tsK ([23], data not shown) indicating that this effect is not unique to tsLB2. Indeed the increase in Alu transcription observed in tsK and tsLB2 infection was actually greater than that observed in infections with wild type virus paralleling the over-production of the immediate-early proteins which occurs in infections with these viruses [23].

To investigate the possibility that one of the 4 other viral immediate-early proteins synthesized in tsLB2 infected cells was responsible for the induction of Alu transcription, we carried out infections with viral strains each of which contained a mutation in the gene

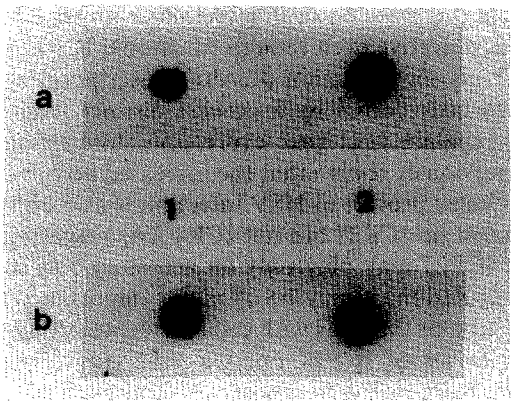

Fig.4. Nuclear run-on assay of transcription (in the presence of 2.5 μg/ml α-amanitin) in HeLa cells either mock infected (a) or infected with HSV-1 strain tsLB2 (b) at the non-permissive temperature of 39°C. Key to spots: 1, Alu clone Blur 11; 2, control ribosomal clone.

encoding one of these proteins. In these experiments (fig.5), no effect on Alu induction was observed in infections with strains carrying mutations in the genes encoding ICPO, ICP22 and ICP47 (see for example fig.5c) but induction of Alu transcription was abolished in the HSV-1 mutant 17X2D which carries a mutation that eliminates the production of functional ICP27 (fig.5b).

Hence the induction of Alu transcription by RNA polymerase III requires the presence of a functional ICP27 protein. This protein is also involved in the induction of viral late gene expression in infected cells [24,25] but only in conjunction with ICP4. Unlike the processes described here, such viral late gene induction

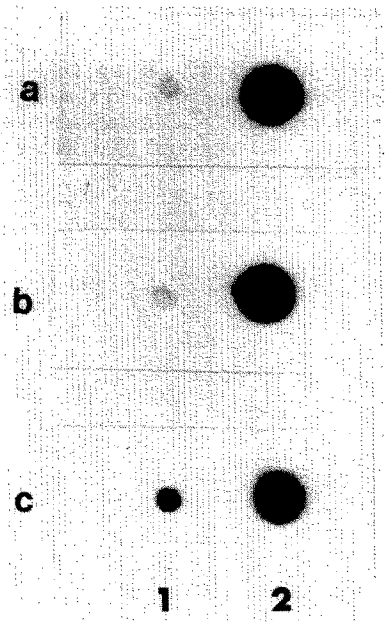

Fig.5. Nuclear run-on assay of transcription (in the presence of 2.5 μg/ml α-amanitin) in HeLa cells either mock infected (a) or infected with HSV-1 strain 17X2D (b) or N38 (c). Key to spots: 1, Alu clone Blur 11; 2, control ribosomal clone.

does not therefore occur in infections with tsLB2 where ICP4 is defective even though functional ICP27 is present. Thus the induction of Alu transcription in HSV infected cells occurs by a process distinct from those which regulate viral gene expression. Interestingly we have previously shown that the increased accumulation of a cellular protein in HSV infection is also obligately dependent on ICP27 but not ICP4 [26] suggesting that whilst ICP27 may play only an accessory role in viral gene regulation, it may be of major importance in the interaction of the virus with cellular regulatory systems.

The effect of ICP27 on Alu gene transcription is distinct from previous reports of viral proteins acting on polymerase III transcription units in that in these cases both the E1A protein of adenovirus [2] and the immediate-early protein of pseudorabies virus [3] increased the expression of cotransfected 5S and tRNA genes transcribed by RNA polymerase III but as in our experiments, no increased transcription of endogenous 5S or tRNA genes was observed in the infected cells [2, 3]. This was attributed to the organization of such genes into stable transcription complexes unable to respond to increased levels of transcription factor III C (for review see [27]). If a similar mechanism operates in HSV infection this would suggest that the Alu repeats like recently transfected genes may be more accessible to cellular transcription factors than other endogenous polymerase III genes. Whatever the case, the work described here represents the first description of the increased transcription of endogenous RNA polymerase III transcription units during infection by a large DNA virus, such transcription being induced by a process distinct from those which activate the expression of the different classes of viral genes.

Acknowledgements. We thank Moira Brown, Ian Haliburton, Chris Preston, Bernard Roizman, Nigel Stow and Kenichi Umene for gifts of virus strains and Jim Smiley for discussing his results with us prior to publication K L J was supported by a studentship from the Government of Korea

REFERENCES

[1] Cilberto, G , Castagnoli, L and Cortese, R (1983) Curr Top Dev Biol 18, 59–88
[2] Hoeffler, W.K. and Roeder, R.G (1985) Cell 41, 955–963
[3] Gaynor, R B , Feldman, L T and Berk, A.J. (1985) Science 230, 447–450
[4] Hoeffler, W.K., Kovelman, R. and Roeder, R G (1988) Cell 53, 907–920
[5] Rogers, J.H. (1985) Int. Rev Cytol. 93, 188–279
[6] Jelinek, W R and Schmid, C W (1982) Annu Rev Biochem 51, 813–844.
[7] Ejercito, P M , Kieff, E D. and Roizman, B (1986) J Gen Virol. 2, 357–364.
[8] Honess, R W., Buchan, A , Hallıburton, I W. and Watson, D H. (1980) J. Virol. 34, 716–742
[9] Maclean, A.R. and Brown, S.M (1987) J Gen Virol 68, 1339–1350.
[10] Stow, N D. and Stow, E.C (1986) J Gen Virol. 67, 2571–2585
[11] Sacks, W R., Greene, C.C., Aschman, D P and Schaffer, P A (1985) J Virol 55, 796–805.
[12] Umene, K. (1986) J. Gen Virol. 67, 1035–1048
[13] Chirgwin, J M , Przybyla, A.E , MacDonald, R J and Rutter, W J. (1979) Biochemistry 18, 5294–5299
[14] Murphy, D , Brickell, P M., Latchman, D S , Willison, K and Rigby, P.W.J. (1983) Cell 35, 865–871
[15] Deininger, P L , Jolly, D J , Rubin, K M., Friedmann, T and Schmid, C W (1981) J. Mol Biol 151, 17–23
[16] Feinberg, A P and Vogelstein, B (1983) Anal. Biochim 132, 6–13
[17] Patel, R , Chan, W.L , Kemp, L M , La Thangue, N B and Latchman, D.S (1986) Nucleic Acids Res 14, 5629–5640
[18] Kemp, L.M , Brickell, P M., La Thangue, A B and Latchman, D.S (1986) Biosci Rep. 6, 945–951.
[19] Marash, F , Prukopff, K , Stein, G. and Stein, J (1984) Proc Natl. Acad Sci USA 81, 1936–1940.
[20] Lewis, M K and Burgess, R R (1982) In· The Enzymes Vol. 15, (Boyer, P ed) pp. 109–153, Academic Press, New York.
[21] Ullu, E and Tschudi, C. (1984) Nature 312, 171–172.
[22] Watson, R.J. and Clements, J B. (1980) Nature 285, 329–330
[23] Preston, C.M (1979) J Virol 29, 275–285
[24] Sacks, W R., Greene, C C , Aschman, D P and Schaffer, P A (1985) J Virol 55, 796–805
[25] Everett, R D. (1986) J. Gen Virol 67, 2507–2513
[26] Estridge, J.K., Kemp, L.M., La Thangue, N.B , Mann, B S , Tyms, A.S. and Latchman, D S (1989) Virology 168, 67–72
[27] Brown, D D. (1984) Cell 37, 359–365