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The transcription of B2 repeated sequences is regulated during the transition from quiescent to proliferative state in cultured rodent cells

Luigi Lania*+, Antonio Pannuti*, Girolama La Mantia* and Claudio Basilico+

*Dipartimento di Genetica, Biologia Generale e Molecolare, University of Naples, Via Mezzocannone 8, 80134, Naples, Italy and +Department of Pathology, New York University School of Medicine, 550 First Avenue, New York, NY 10016, USA

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The RNA polymerase III-dependent transcription of B2 repeated sequences has been monitored during the transition from the quiescent to proliferative state in cultured rodent cells and after polyomavirus-induced transformation. The level of RNAs containing B2 sequences was found to be higher in both the proliferative state of normal cells and in polyomavirus-transformed cells. In both systems, nuclear run-off transcription assays indicated that high levels of B2 RNAs are due to an enhanced transcription rate. These results suggest the presence of a B2-specific RNA pol III transcription factor(s) whose activity is sensitive to cell cycle progression and oncogenic transformation.

B2 sequence; RNA polymerase III; Transcription; Cell cycle; Transformation

1. INTRODUCTION

The mouse genome contains several highly repeated families of DNA sequences [1]. These include the B2 sequences represented by a family of repeated elements of about 190 nucleotides, flanked by direct repeats and transcribed by RNA polymerase III [2–5]. Elevated expression of B2-containing RNAs appears to be associated with embryonic or transformed phenotypes. It has been reported that RNAs containing B2 sequences are present at low levels in unfertilized mouse eggs, and that their levels increase during early embryogenesis [6]. High levels of B2 RNA have also been found in embryonal carcinoma cells but not in their differentiated derivatives [7]. Oncogenic transformation of cultured mouse and rat fibroblasts by SV40 or polyomavirus results in the induction of elevated levels of B2 RNA transcripts [8–10]. Moreover, high levels of B2 RNA have been found in serum-stimulated quiescent mouse 3T3 cells [11].

The function of the small B2 RNAs and the mechanism by which their levels are increased are unknown. Here, we show that the transcription of B2 RNAs is regulated during the transition from the quiescent to proliferative state of cultured rodent cells, and that the induction of elevated levels of B2 RNAs in transformed cells is due to an enhanced transcription rate.

Correspondence address: L. Lania, Dipartimento di Genetica, Biologia Generale e Molecolare, University of Naples, Via Mezzocannone 8, 80134 Naples, Italy

2. MATERIALS AND METHODS

2.1. *Synchronization procedures*

2.1.1. Serum starvation

BHK-21 cells were plated and incubated at 37°C for at least 24 h. The medium was removed, the cells were washed with medium without serum, and incubated for 2 days in medium containing 0.25% calf serum [12]. Cells were released by changing to medium containing 10% serum.

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2.1.2. Isoleucine deprivation

Cells plated and incubated as before were washed once with isotonic buffer and incubated for 2 days in medium lacking isoleucine [13], supplemented with 5% dialysed calf serum. Cells were released by changing the medium to complete medium containing 10% serum. DNA synthesis was monitored by 30 min pulses with [^{3}H]thymidine (2 μCi/5 cm dish).

2.2. *Northern hybridization analysis*

Total RNA was isolated by the guanidine isothiocyanate method. For the analysis of B2 RNA transcripts, poly(A$^+$) was isolated using Hybond-MAP (Amersham), and aliquots of poly(A$^+$) RNA (1–3 μg) were fractionated by electrophoresis in 1.2% agarose-formaldehyde gels. Blotting and hybridization conditions were as described elsewhere [9]. The plasmid probes pB2-R [9] and pXls11 [14] were labelled by nick-translation to a specific activity of 1×10^8 cpm/μg.

2.3. *Nuclear run-off transcription assay*

Nuclear run-off assays were performed essentially as in [15,16]. The plasmids were linearized, denatured and spotted onto nitrocellulose filter using the Scheicher & Schüll slot-blot apparatus. The plasmids were: pB2-R [9], pXls11 [14], human β-actin [16] and pHh5B (histone-3 gene, provided by Dr N. Heintz).

3. RESULTS

To analyze the transcription of B2 repeated sequences during the cell cycle of cultured rodent cells, we synchronized the Syrian hamster cell line, BHK-21, with two different procedures. The cells were synchronized by either serum starvation or isoleucine deprivation, and in both procedures the cells were then released by supplying normal medium containing 10% serum. The synchronization induced by both serum starvation and isoleucine deprivation was reproducible, and both methods gave similar results by monitoring DNA synthesis over a period of 30 h after releasing the cells from the quiescent state (fig.1). Fig.2 (panels A,B) shows the Northern blot analysis of poly(A$^+$) RNAs isolated from BHK-21 cells at various times after releasing with normal medium the cells synchronized by serum starvation (panel A) or isoleucine deprivation (panel B). The Northern blots were hybridized with a nick-translated pB2-R DNA probe. This plasmid containing B2 repeated sequences has been described [9], and it contains a 137 bp *SalI-BglII* mouse fragment comprising 72 bp of B2 repeats and 65 bp of H2 class I intron sequences. The results reported in fig.2 clearly show the increase of B2 RNAs transcripts of 200–500 bp during progression from the quiescent to the S-phase. To determine whether the progression from the quiescent to proliferative state of cultured cells results in a general induction of RNA pol III transcription, we have studied the 5 S RNA gene expression during the cell cycle. Fig.2C shows that no significant difference in 5 S RNA gene expression was detected during the transition from the quiescent to proliferative state of the hamster cells. Thus, there is no generalized induction of pol III transcription after serum stimulation of G_0 synchronized cells.

3.1. *B2 RNA expression is regulated at the transcriptional level*

One interpretation of the results reported in fig.2 is that B2 RNA expression is controlled at the transcriptional level during the cell cycle. To determine the transcription rate of B2 repeated sequences, we performed nuclear run-off transcription assays [15,16] on nuclei isolated at various times after stimulation of quiescent cells with normal medium. Fig.3A shows that the B2

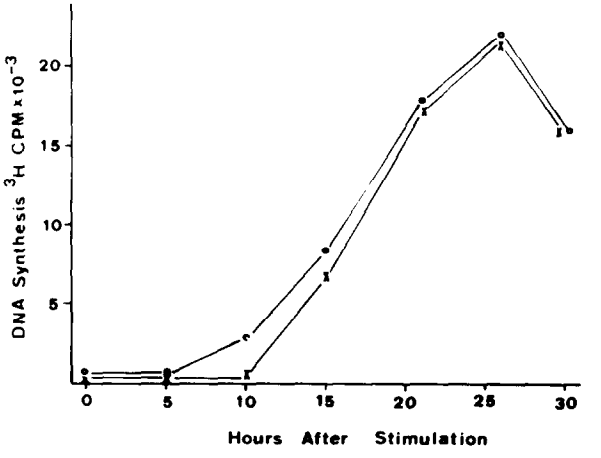

Fig.1. Thymidine incorporation following stimulation of cells made quiescent after serum starvation (○—○) or isoleucine deprivation (×—×) as described in the text.

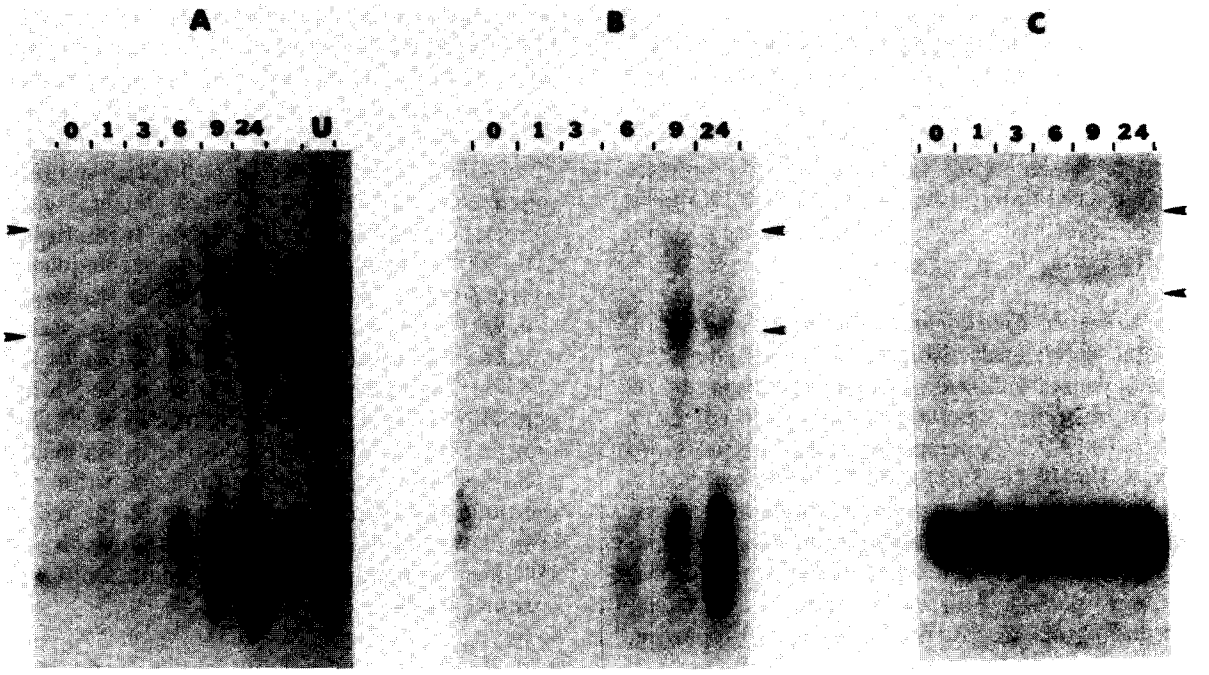

Fig.2. Time course of B2 expression following stimulation of quiescent BHK cells. Cells were synchronized by serum starvation (A) or isoleucine deprivation (B). 3 μg poly(A^+) RNA, isolated at the times indicated above the autoradiograms, were analyzed by Northern blot hybridization using a B2 probe as described in the text. U, RNA extracted from growing BHK-21 control cells. In the overexposed autoradiogram some hybridization to both 18 S and 28 S RNA becomes detectable. (C) 5 S RNA expression after serum stimulation of quiescent BHK cells. Cells were synchronized by serum starvation. 5 μg total RNA isolated at the times indicated above the autoradiograms were analyzed by Northern hybridization using a 5 S RNA probe. The arrows indicate the positions of the 18 S and 28 S RNA.

repeated sequences are transcribed at a high rate during the S-phase, hence the induction of the steady-state level of B2 RNAs shown in fig.2 is at least in part due to an elevated transcription rate of the B2 repeated sequences in the S-phase of the cell cycle. As control, we monitored the transcription rate of the 5 S RNA gene (Pol III) and the histone-3 gene (Pol II). In accord with published results [16,17], we found induction of the His-3 gene expression during the S-phase, whereas transcription of the 5 S RNA gene remains largely unaffected by the growth state of the cells (fig.3A). Next we investigated whether the transcription of B2 repeated sequences during the cell cycle was dependent on RNA pol III activity. We included various concentrations of α-amanitin (2 and 200 μg/ml) in nuclei isolated at 20 h after serum stimulation. The lower level of α-amanitin inhibits polymerase II transcription while the higher level abolishes pol III activity. Inclusion of α-amanitin at 2 μg/ml in the run-off reaction assay with nuclei isolated after serum stimulation had no effect on B2 transcription (fig.3B), whereas 200 μg/ml was completely inhibitory.

In this context is is interesting that Edwards et al. [11] found no difference in B2 transcription between quiescent and serum-stimulated NIH 3T3 cells. As discussed by others [18], they may have missed such induction either by a technical artifact or because they studied only total transcription (pol II and pol III) of B2 genes in isolated nuclei.

3.2. *B2 RNAs transcription in transformed cells*

We and others [8–10] have shown independently that the steady-state levels of B2 RNA transcripts of 200–500 bp are enhanced in papovavirus-transformed rodent cells. To address this point further we have measured the relative rate of

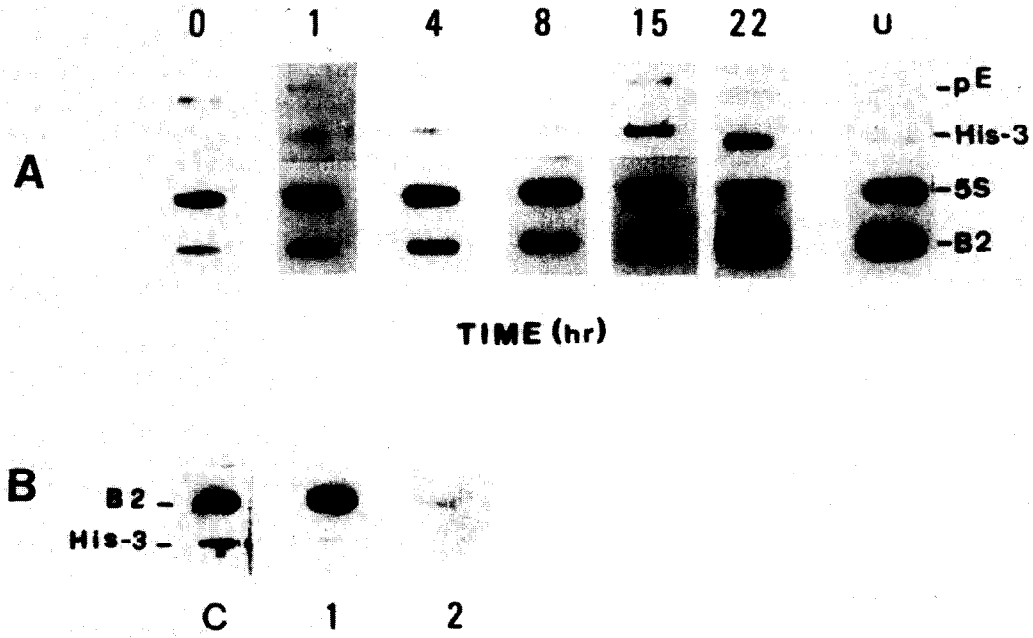

Fig.3. Transcription rate of B2 repeated sequences in BHK-21 cells following isoleucine deprivation and subsequent stimulation. Nuclei were isolated and assayed by nuclear run-off transcription as described in the text. (A) The numbers above the autoradiograms indicate the various time points (h) at which the nuclei were isolated. U, run-off result obtained with nuclei isolated from growing control cells. The cloned DNAs bound to nitrocellulose were: pB2-R (B2 repeat), 5 S (5 S RNA gene), His-3 (human histone-3 gene), pE (pEMBL8 plasmid DNA control). (B) Addition of 2 μg/ml of α-amanitin (1) to the run-off reaction eliminated hybridization to RNA pol II transcribed gene (His-3). Addition of 200 μg/ml of α-amanitin (2) eliminates the transcription of the B2 repeated sequences.

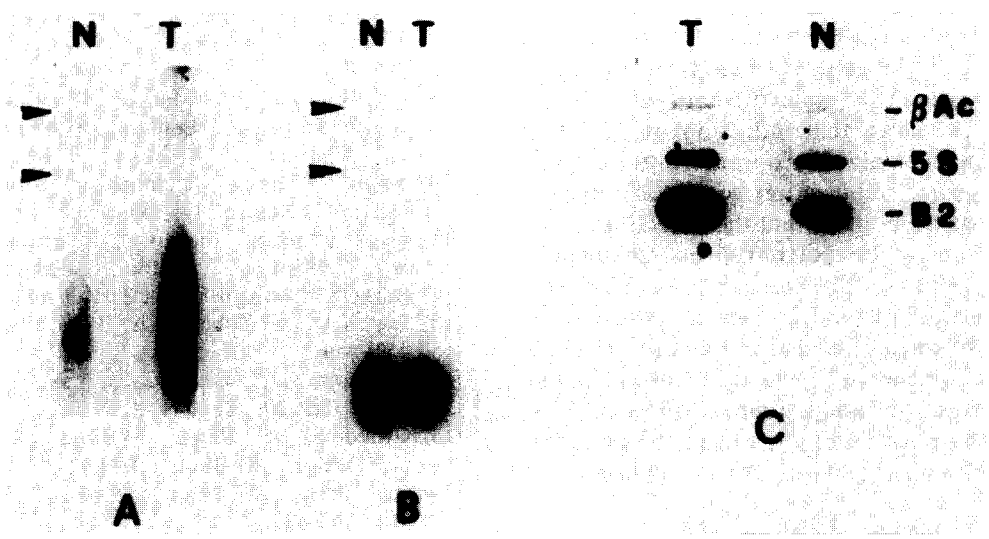

Fig.4. Expression of B2 repeated sequences in normal 3T3 (N) and Py-transformed 3T3 cells (T).(A) Northern analysis of total RNAs (10 μg) isolated from both normal and transformed cells and hybridized to a B2 probe. (B) Northern analysis of total RNA isolated from 3T3 (N) and Py-3T3 (T) cells after hybridization with a 5 S RNA probe. (C) Linear cloned DNAs bound to nitrocellulose were hybridized to ^{32}P-labelled run-off transcripts from nuclei isolated from 3T3 (N) and Py-3T3 (T) cells.

transcription of B2 sequences in transformed and normal cells, using the nuclear run-off transcription assay [15,16]. We have used a number of well-characterized polyomavirus transformed rat and mouse cell lines [9]. In all the transformed cell lines analyzed by nuclear run-off transcription assay, we found that the B2 transcription rate is enhanced (5–10-fold) compared to normal untransformed cells (not shown). An example of such an analysis is reported in fig.4 (panels A and C) which shows that the induction of the B2 RNA transcripts in transformed cells is at the level of transcription. Moreover, the induction of B2 transcription is not correlated to a generalized increase of pol III transcription activity in polyomavirus-transformed cells as the level of 5 S RNA expression in both normal and Py-transformed cells is not affected by this transformation system (fig.4B,C). A similar conclusion has been recently reached by others [19] for SV40-transformed cells.

4. DISCUSSION

We have found that the transcription of RNAs containing B2 sequences is regulated through the transition from the quiescent to proliferative state of cultured rodent cells, and upon polyomavirus-induced transformation. Accumulation of B2 transcripts in parallel with an enhanced transcription rate has been observed during the S-phase of synchronized rodent cells. Using run-off transcription analysis on nuclei isolated from synchronized cells we have shown that induction of B2 RNA transcription is mainly due to transcription activation rather than stabilization of the B2 RNAs. Moreover, we have found that the induction of B2 RNA transcripts in polyomavirus-transformed cells [9] is due to an enhanced level of the transcription rate of the B2 repeated sequences in the Py-transformed cells. Taken together our data suggest the presence of factor(s) involved in the RNA pol III transcription complex whose activity is affected by position of the cell in the cycle (S-phase) and by polyomavirus transformation.

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REFERENCES

[1] Schmid, C.W. and Jelinek, W.R. (1982) Science 216, 1065–1070.
[2] Kramerov, D.A., Grigoryan, A.A., Ryskov, A.P. and Georgiev, G.P. (1979) Nucleic Acids Res. 6, 697–713.
[3] Krayer, A.S., Markusheva, T.V., Kramerov, D.A., Ryskov, A.P., Skyabin, K.G., Bayev, A.A. and Georgiev, G.P. (1982) Nucleic Acids Res. 10, 7461–7475.
[4] Kramerov, D.A., Lekakh, I.V., Samarina, O.P. and Ryskov, A.P. (1982) Nucleic Acids Res. 10, 7477–7491.
[5] Ryskov, A.P., Ivanov, P.L., Kramerov, D.A. and and Georgiev, G.P. (1983) Nucleic Acids Res. 11, 6541–6558.
[6] Vasseur, M., Condamine, H. and Duprey, P. (1985) EMBO J. 4, 1749–1753.
[7] Benett, K.L., Hill, R.E., Pietras, D.F., Woodworth-Gutai, M., Kane-Haas, C., Houston, J.M., Heath, J.K. and Hastie, N.D. (1984) Mol. Cell. Biol. 4, 1561–1571.
[8] Murphy, D., Brickell, P.M., Latchman, D.S., Willison, K. and Rigby, P.W.J. (1983) Cell 35, 865–871.
[9] Majello, B., La Mantia, G., Simeone, A., Boncinelli, E. and Lania, L. (1985) Nature 314, 457–459.
[10] Singh, K., Carey, M., Saragosti, S. and Botcham, M. (1985) Nature 314, 553–556.
[11] Edwards, D.R., Parfelt, C.L.J. and Denhardt, D.T. (1985) Mol. Cell. Biol. 5, 3280–3288.
[12] Burk, R.R. (1970) Exp. Cell Res. 63, 309–316.
[13] Ley, K.D. and Tobey, R.A. (1970) J. Cell Biol. 47, 453–459.
[14] Peterson, R.C., Doering, J.L. and Brown, D.D. (1980) Cell 20, 131–141.
[15] Groudine, M., Peretz, M. and Weintraub, H. (1981) Mol. Cell. Biol. 1, 281–288.
[16] Greemberg, M.E. and Ziff, E.B. (1984) Nature 311, 433–438.
[17] Heintz, H. and Roeder, R.G. (1984) Proc. Natl. Acad. Sci. USA 81, 2713–2717.
[18] Carey, M.F., Singh, K., Botcham, M. and Cozzarelli, N.R. (1986) Mol. Cell. Biol. 6, 3068–3076.