DIFFERENTIAL STABILITY OF α- AND β-GLOBIN mRNAs AFTER INFECTION WITH HERPES SIMPLEX VIRUS

Yutaka NISHIOKA*, Uri NUDEL**, Francesco RAMIREZ** and Saul SILVERSTEIN*

**Departments of Microbiology and **Human Genetics and Development, Columbia University, College of Physicians and Surgeons, 701 West 168th Street, New York, NY 10032, USA*

Received 31 May 1978
Revised version received 27 June 1978

1. Introduction

Friend erytholeukemia (FL) cells can be induced to undergo erythroid differentiation following exposure to dimethyl sulfoxide (Me_2SO) [1] or hemin [2]. The differentiation process involves a series of events which include, production of globin mRNA [3–5] expression of erythrocyte membrane antigens [6] and a decrease in the ability of induced cells to divide [7]. In cells exposed to either Me_2SO or hemin, both α- and β-globin mRNAs are synthesized [2,5]. It has been shown that the relative rate of accumulation of α- and β-globin mRNAs differs with different inducing agents [8]. We have utilized this system to examine the fate of cellular mRNAs during the course of lytic infection with herpes simplex virus (HSV). We demonstrated that the de novo synthesis of globin was rapidly shut off early after infection with HSV and that the abundance of globin mRNA sequences decreased as the infection proceeded [9]. In a subsequent analysis of total infected cell mRNA we provided evidence which suggested that total polyadenylated cellular mRNA was degraded at 4 h postinfection and that the degradation of globin mRNA was a stochastic process as judged by the failure to detect a shift in the kinetics of hybridization of total polyadenylated mRNA extracted at various times postinfection to globin cDNA [10].

To further explore these findings, specific cDNA probes to α- and β-globin mRNA were prepared and used to analyze the relative stability of α- and β-globin mRNAs following infection with HSV. The results demonstrate that degradation of α-globin mRNA is more refractile to HSV infection than β-globin mRNA in cells fully induced with Me_2SO and hemin.

2. Materials and methods

The conditions for cell culture, induction of globin, propagation of virus and infection with HSV were as described [7,9]. Total cytoplasmic RNA was isolated as described [9], and stored under ethanol until it was ready to be used. RNA was resuspended in water and hybridized as described [11]. Specific cDNA probes for α- and β-globin mRNA were prepared by reverse transcribing purified α- and β-globin mRNA. The two mRNAs were separated by preparative polyacrylamide gel electrophoresis in formamide according to Nudel et al. [12].

3. Results

Cultivation of FL cells in the presence of 2% Me_2SO results in the synthesis of both α- and β-globin mRNAs which code for both α- and β-globins [8]. The relative rate of accumulation is not the same for the two messengers. In fact the α-globin mRNA is detectable after 16 h, whereas, increase in β-globin mRNA sequences is not detected until 20–24 h after exposure to inducer [8]. After 3 days exposure to this compound cells contained approximately equimolar amounts of α- and β-globin, mRNAs as judged by the relative $C_r t_{\frac{1}{2}}$ after hybridization to specific α- and β-globin cDNAs (table 1). These cells were infected with 10 pfu cell of HSV-1 and at 4 h post-

Table 1
Fate of α- and β-globin mRNA

	Time post-infection	$C_r t_{\frac{1}{2}}$ α-mRNA	$C_r t_{\frac{1}{2}}$ β-mRNA	β/α	Percent remaining α	Percent remaining β
Experiment 1[a]	0	4.6	4	1.15	–	–
	4	6.6×10^1	5×10^2	0.13	7	0.8
Experiment 2[a]	0	1.2×10^1	1.4×10^1	0.86	–	–
	1	1.2×10^1	5.6×10^1	0.21	100	25
	2	3.0×10^1	2×10^2	0.15	40	7
	3	1.2×10^2	4×10^2	0.30	10	3
	4	2.0×10^2	4×10^2	0.50	6	3

[a] Exponentially growing FL cells were exposed to 2% Me_2SO and diluted to a constant cell density in medium containing 2% Me_2SO. After 72 h in culture the cells were concentrated 10-fold and infected with 10 pfu/cell of HSV-1. After 1 h the cells were diluted in fresh medium and samples were withdrawn at the indicated times. RNA was extracted from cytoplasm prepared as previously described [9] and hybridized to specific α- and β-globin cDNA [8]

infection the level of cytoplasmic α- and β-globin mRNA was analyzed. Our results (fig.1) demonstrate that both globin mRNAs are degraded during the course of infection. At 4 h postinfection only 7% of α-globin and 0.8% of β-globin mRNA sequences persisted (table 1). The ratio of β- to α-globin mRNA changed from 1.15 to 0.13 suggesting that α-globin mRNA is more stable in the face of HSV infection than the mRNA coding for β-globin.

In order to determine the time course of degradation of the two messengers, the amount of each globin mRNA species was determined throughout the early course of infection with HSV. A single culture of 3-day Me_2SO induced FL cells was infected at an moi=10 and aliquots were withdrawn at 1-h intervals postinfection. Total cytoplasmic RNA extracted from each sample was hybridized to α- and β-globin cDNAs and the relative amount of each mRNA which persisted was determined from the $C_r t_{\frac{1}{2}}$ of the reaction. The mRNA coding for α-globin becomes more abundant relative to that coding for β-globin during the course of infection (table 1, experiment 2). The β-globin mRNA is rapidly and preferentially degraded during HSV infection. By 2-h postinfection only 7% of β-globin mRNA persists whereas, 40% of the original α-globin mRNA sequences remain. In this experiment, the relative ratio of β- to α-mRNA sequences has decreased from 1.15 to 0.13. Thereafter, the rate of degradation of β-globin mRNA decreases and α-globin mRNA is preferentially degraded. At 4-h postinfection 3% of the β- and 6% of the original α-globin mRNAs remain. The kinetics of degradation of α- and β-globin following infection with HSV demonstrate that the α-globin mRNA is initially more stable in the infected cell than β-globin mRNA. As the infection proceeds and after the bulk of β-globin mRNA is degraded the α-globin mRNA is also destroyed

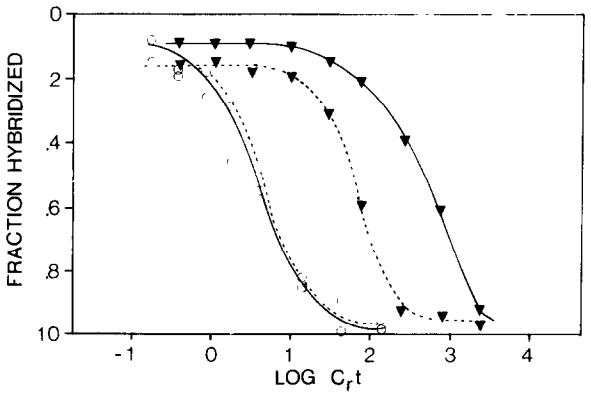

Fig.1. Hybridization of purified α- and β-globin cDNA probes with total cellular RNA. Between 1 mg and 20 mg of RNA was hybridized to 178 pg of human globin cDNA (specific activity 14×10^3 cpm/ng). The time for incubations for all the experiments reported was 4 h. (o- -o- - -o) α-cDNA at zero time postinfection; (o—o—o) β-cDNA at zero time postinfection; (▼- -▼- - -▼) α-cDNA at 4-h postinfection; (▼- -▼—▼) β-cDNA at 4-h postinfection.

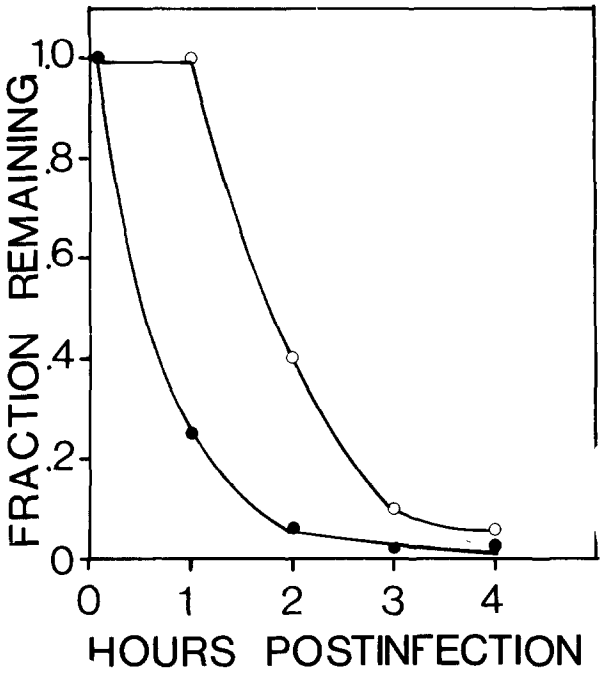

Fig.2. Kinetics of degradation of α- and β-globin mRNAs from induced FL cells after infection with HSV. The fraction remaining was calculated from the ratio of the $C_r t_{\frac{1}{2}}$ of α- or β-cDNA at zero time. (o——o——o) α-mRNA, (•——•——•) β-mRNA.

(fig.2). Similar results were observed when FL cells induced with hemin were infected with HSV (data not shown).

4. Discussion

In this communication we have analyzed the fate of the mRNAs which code for α- and β-globin in FL cells exposed to 2% Me_2SO after infection with HSV.

In differentiating fetal mouse erythroid cells there is a balanced accumulation of globin mRNAs [13] as in FL cells exposed to 2% Me_2SO for 72 h (fig.1). The results of Aviv [14] demonstrate that total globin mRNA has a half-life of 17 h in cultured FL cells. While there is no available data on the stability of the individual α- and β-globin mRNAs, it is reasonable to assume that individually these messengers have a half-life approximating that of the total globin mRNA population which is far in excess of the duration of these experiments. In previous studies, we utilized cDNA prepared from total unfractionated globin mRNAs and demonstrated that the globin mRNA was degraded throughout the course of infection with HSV with a half-life of approximately 2 h [9,10]. These same studies showed that polyribosomes were rapidly dissociated into monoribosomes and that the synthesis of cell specific polypeptides was turned off in favor of synthesizing virus specified polypeptides. We have employed a similar approach in this study and observed that the mRNAs coding for α- and β-globin are degraded at different rates in FL cells fully induced with either Me_2SO or hemin. In each instance, the relative amount of α-globin mRNA that persisted after HSV infection was greater than the amount of persisting β-globin mRNA.

The mechanism responsible for preferential degradation of the β-globin mRNA and the apparent refractile nature of the α-globin mRNA is at present unknown. However, there are a number of interesting observations which might be considered to help account for this phenomenon. The data of Lodish [15–17] demonstrate that α- and β-globin mRNAs possess different affinities for ribosome binding sites. These studies suggest that β-globin mRNA has a stronger affinity for ribosome binding sites. Thus a ribosome mediated protection mechanism cannot be invoked to account for the refractile nature of α-globin mRNA. Alternatively, our observation might be a reflection of the fact that β-globin mRNA resides on larger polyribosomes [18] and that these larger polyribosome structures appear to be more rapidly dissociated after HSV infection than the smaller α-globin containing polyribosomes [10]. In either case, the rapid decrease in the absolute content of the globin mRNAs, and the ability of HSV to shut off cell-specific transcription provides us with an interesting model system with which we can examine the stability rather than the turnover of the two messenger species.

Acknowledgements

We wish to thank Drs A. Bank and M. Terada for their helpful comments in preparing this manuscript, and Dr J. Beard for providing AMV reverse transcriptase. This research was supported by grants to Saul Silverstein from the N.I.H., #CA 17477 and to

Dr A. Bank #GM 14552. Francesco Ramirez is on leave of absence from the Istituto Anatomia Comparata, Palermo, Italy.

References

[1] Friend, C., Scher, W., Holland, J. G. and Sat, T. (1971) Proc. Natl. Acad. Sci. USA 68, 378–382.
[2] Ross, J. and Sautner, D. (1976) Cell 8, 513–520.
[3] Ross, J., Ikawa, Y. and Leder, P. (1972) Proc. Natl. Acad. Sci. USA 69, 3620–3623.
[4] Boyer, S. H., Wuu, K. D., Noyes, A. N., Young, R., Scher, W., Friend, C., Preisler, H. and Bank, A. (1972) Blood 40, 823–835.
[5] Ostertag, W., Melderis, H., Steinheider, G., Kluge, N. and Dube, S. (1972) Nature New Biol. 239, 231–234.
[6] Ikawa, Y., Furusawa, M. and Sugano, H. (1973) Bibl. Haematol. 39, 955–967.
[7] Nishioka, Y. and Silverstein, S. (1978) J. Cell Physiol. 95, 323–332.
[8] Nudel, U., Salmon, J., Fiback, E., Terada, M., Rifkind, R., Marks, P. A. and Bank, A. (1977) Cell 12, 463–469.
[9] Nishioka, Y. and Silverstein, S. (1977) Proc. Natl. Acad. Sci. USA 74, 2370–2374.
[10] Nishioka, Y. and Silverstein, S. (1978) J. Virol. in press.
[11] Ramirez, F., Gambino, R., Maniatis, G. M., Rifkind, R. A., Marks, P. A. and Bank, A. (1975) J. Biol. Chem. 250, 6054–6058.
[12] Nudel, U., Ramirez, F., Marks, P. A. and Bank, A. (1977) J. Biol. Chem. 252, 2181–2186.
[13] Maniatis, G. M., Ramiriz, F., Nudel, U., Rifkind, R. A., Marks, P. A. and Bank, A. (1978) FEBS Lett. 85, 43–46.
[14] Aviv, Voloch, A., Bastos, R. and Levy, A. (1976) Cell. 8, 495–503.
[15] Lodish, H. F. (1971) J. Biol. Chem. 246, 7131–7138.
[16] Lodish, H. F. and Jacobsen, M. (1972) J. Biol. Chem. 247, 3622–3629.
[17] Lodish, H. F. (1974) Nature 251, 385–388.
[18] Temple, G. F. and Housman, D. E. (1972) Proc. Natl. Acad. Sci. USA 69, 1574–1577.