Vol. 83, No. 3, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 14, 1978 Pages 800-805

METABOLIC STABILITY OF NUCLEAR SELF-COMPLEMENTARY RNA IN CELLS INFECTED WITH HERPES SIMPLEX VIRUS

Marie-Elbine Mouttet and Jean-Marie Béchet
Unité d'Oncologie Virale, Institut Pasteur, Paris, France

Received April 20,1978

SUMMARY - The self-complementary fraction of RNA labeled late in infection with herpes simplex virus was quantitated as a function of labeling and chase times. The labeled RNA that became double-stranded upon self-annealing was a stable fraction of labeled total cell RNA, and was present almost exclusively in the nucleus. The relative amount of complementary sequences in viral RNA was evaluated by hybridization of total and double-stranded RNA to viral DNA, and was found to remain stable with increasing labeling times. The data suggest that post-transcriptional selection of the coding strand occurs during or after transport from the nucleus.

INTRODUCTION

Animal cells have been shown to contain complementary RNA sequences of both mitochondrial and nuclear origin (1-3). Virus-specific self-complementary RNA has also been found in cells infected with members of all major groups of DNA viruses (4-9). The high molecular weight (1, 4, 5) and kinetic complexity (9) of complementary sequences indicate that they do not represent only the short "hairpin" structures known to exist in nuclear RNA (10), but suggests that they result from the symmetrical transcription of complementary DNA strands.

Owing to the restricted complexity of their genomes and to the relatively large amounts of self-complementary RNA found in infected cells (8), herpesviruses provide useful systems for analysis of the cellular mechanisms involved in the synthesis and metabolism of complementary RNA transcripts.

One important question is the fate of the complementary sequences. Since presumably only one of two complementary RNA strands can specify the synthesis of a protein, symmetrical transcription of both strands of DNA

0006-291X/78/0833-0800$01.00/0

must be followed by post-transcriptional selection of the messenger strand. The experiments described here were aimed at defining the level at which this selection occurs. The results suggest that viral non-messenger sequences are conserved in the nucleus but not transported to the cytoplasm.

MATERIALS AND METHODS

Human HEK (11) and rat RL (12) cells were infected with herpes simplex virus type 1, strain A 44 (8) and incubated at 32°, the optimal temperature for virus growth (13), for the times indicated, prior to labeling with ^{3}H-uridine (CEA, France).

Extraction of total cell RNA, self-annealing of RNA and RNA-DNA hybridization on filters were as previously described (8). Cell fractionation and DNase-digestion of nuclei were as described by Penman (14). The nuclear and cytoplasmic fractions were precipitated with two volumes of ethanol in the presence of 0.5% sodium dodecylsulphate. The precipitates were dissolved in 100 mM NaCl, 0.5% sodium dodecylsulphate, 10 mM EDTA, pH 7.4 and extracted twice with a phenol-chloroform-isoamyl alcohol (80:20:0.2) mixture and once with chloroform-isoamyl alcohol (99:1) at room temperature. The RNA contained in the aqueous phase was precipitated with ethanol, digested with RNase-free DNase (Worthington; 10 µg/ml, 1 hr at 37°), extracted with phenol and precipitated with ethanol.

Self-annealed RNA was digested at 37° either with 10 µg/ml of pancreatic RNase (Worthington) for 1 hr or, when the RNA was to be subsequently hybridized to DNA, with 0.01 µg/ml for 4 hr. The RNase-digested RNA was then extracted with phenol and chromatographed on a 1 ml cellulose column (15). A preliminary experiment showed that the material eluted in ethanol-free buffer from the cellulose column was similar in amount, DNase and RNase-resistance and melting behaviour to the material isolated by exclusion chromatography on Sephadex G-200, which had previously been characterized as double-stranded RNA (8).

RESULTS AND DISCUSSION

If symmetric transcription was quickly followed by degradation of non-functional ("anti-messenger") sequences, the yield of double-stranded RNA upon self-annealing of pulse-labeled RNA should decrease as the length of the pulse is increased. As seen in Table 1, this is not the case in HSV-infected cells. In experiments with two different cell lines, the fraction of total incorporated label that was recovered as double-stranded RNA after annealing did not change significantly when labeling time varied from 15 minutes to 2 hours, nor when labeling for 1 hour was followed by a 2-hour chase in the presence of 5 µg/ml of Actinomycin D. Although this dose of the drug reduced the rate of incorporation of label into RNA by more than 98% (data not shown), the amount of previously incorporated label decreased by only 15 to 20% in a 2 hr chase (Table 1), indicating

Table 1 - Effect of labeling and chase times on the yield of double-stranded RNA from herpes simplex virus-infected cells .

host cells	labeling time (min)	chase time (min)	double-stranded RNA (% of total) expt. 1	expt. 2
RL	15	0	6.8	-
	30	0	7.9	5.6
	60	0	7.1	-
	30	120[a]	-	5.8
HEK	30	0	7.2	-
	60	0	5.8	-
	120	0	6.8	7.0
	120	120[a]	-	5.4
	120	120[b]	-	5.0

Cells were labeled 16 hr after infection
(a) chased in the presence of 5 µg/ml of Actinomycin D
(b) chased in the presence of 100 µM cold uridine
- : not done

that the bulk of the RNA synthesized in infected cells is stable under these conditions.

In order to establish whether complementary RNA sequences were transported to the cytoplasm, infected cells were labeled for different periods of time and fractionated into nucleus and cytoplasm. Nuclear and cytoplasmic RNA were extracted and annealed separately. The results (Table 2) show that only the nuclei contained significant amounts of self-complementary RNA, since the low amounts found in the cytoplasmic fraction might be due to some leakage from the fragile nuclei of infected cells.

During infection with herpes simplex virus, the transcription of cellular genes, although inhibited, is not arrested. Thus, even late in

Table 2 - Double-stranded RNA obtained by self-annealing of nuclear and cytoplasmic RNA from infected cells.

Expt.no.	labeling time (min)	chase time (min)	double-stranded RNA after annealing (%)	
			nuclear	cytoplasmic
1	60	0	5.4	0.3
	60	60	4.0	0.2
	60	120	2.9	0.1
2	60	0	3.8	0.5
	180	0	5.2	0.4
	60	120	4.0	0.5

HEK cells were labeled 15 hr after infection. The label was chased in the presence of 5 µg/ml of Actinomycin D.

infection, only a fraction of labeled RNA is virus-specific (17). Although we find that self-complementary RNA is as stable as total labeled RNA, this may not be true when viral sequences alone are considered, because viral and cellular RNAs may have different stabilities.

In order to compare the stability of viral and cellular RNAs and of the complementary strands of viral RNA, total and double-stranded nuclear RNA labeled for different periods of time were hybridized to viral DNA. Table 3 shows the results obtained in one representative experiment. The percentage of nuclear RNA hybridizing to viral DNA did not decrease when labeling was prolonged or followed by a chase in the presence of 5 µg/ml of Actinomycin D, indicating that, in cells infected with herpes simplex virus, in contrast to SV40 (18), virus-specific RNA is at least as stable as cellular RNA. By comparing the figures obtained for hybridization of total and double-stranded nuclear RNA to viral DNA, assuming the same efficiency of hybridization in both cases, it was possible to

Table 3 - Hybridization of total and double-stranded nuclear RNA to viral DNA.

labeling time (min)	30	120	30
chase time (min)	0	0	120
double-stranded RNA (% of nuclear RNA)	2.3	3.2	2.5
% of nuclear RNA hybridized to viral DNA	5.4	7.8	8.2
% of double-stranded RNA hybridized to viral DNA	28	28	23
viral double-stranded RNA (% of viral RNA)	11.2	11.5	7.0

HEK cells were labeled 9 hr after infection. The label was chased in the presence of 5 µg/ml of Actinomycin D. Hybridization values are the mean of two filters with 5 µg of viral DNA per filter. The percentage of viral double-stranded RNA relative to total viral nuclear RNA (last column) was calculated by multiplying the percentage of double-stranded RNA relative to nuclear RNA by the ratio of their hybridization to viral DNA.

evaluate the fraction of viral RNA that is self-complementary, hence the relative amounts of messenger and anti-messenger viral sequences. The self-complementary fraction of viral RNA remained constant when labeling time was increased from 30 to 120 minutes. After a 2 hour-chase in the presence of Actinomycin D, this fraction decreased by less than a half.

The relative stability of self-complementary RNA in cells infected with herpes simplex virus is in contrast to the situation found in other systems in which symmetric transcription occurs. Thus, in mitochondria (2) and in cells infected with SV 40 (5) and adenovirus 2 (16), the

amount of labeled self-complementary sequences was found to decrease quickly with increasing labeling times. In the case of SV40, however, the decrease was shown to be due to the rapid decay of viral RNA as a whole compared to cellular RNA, while the relative amounts of messenger and anti-messenger sequences remained constant (18).

The fact that viral complementary sequences are stable in the nucleus of herpesvirus-infected cells indicates that, contrary to cellular heterogeneous nuclear RNA, viral non-coding sequences are not rapidly degraded in the nucleus. The virtual absence of self-complementary RNA in the cytoplasm suggests that post-transcriptional selection of the coding strand occurs at the level of transport from the nucleus to the cytoplasm.

ACKNOWLEDGMENTS

We thank Dr. Luc Montagnier, in whose laboratory this work was performed, for advice and criticism, and Denise Guétard for skilful technical assistance. This work was supported by grant no. 76.4.125.2 from the Institut National de la Santé et de la Recherche Médicale.

REFERENCES

1. Montagnier, L. (1968) C.R. Acad. Sc. Paris 267, 1417-1420.
2. Aloni, Y. and Attardi, G. (1971) J. Mol. Biol. 55, 251-270.
3. Harel, L., Riou, G. and Montagnier, L. (1975) Biochimie 57, 227-233.
4. Colby, C. and Duesberg, P.H. (1969) Nature 222, 940-944.
5. Aloni, Y. (1972) Proc. Nat. Acad. Sci. USA, 69, 2404-2409.
6. Lucas, J. and Ginsberg, H. (1972) Biochem. Biophys. Res. Commun. 49, 39-44.
7. Petterson, U. and Philipson, L. (1974) Proc. Nat. Acad. Sci. USA, 71, 4887-4891.
8. Béchet, J.M. and Montagnier, L. (1975) C. R. Acad. Sc. Paris 280, 217-220.
9. Jacquemont, B. and Roizman, B. (1975) J. Virol. 15, 707-713.
10. Jelinek, W. and Darnell, J.E. (1972) Proc. Nat. Acad. Sci. USA 69, 2537-2541.
11. Nelson-Rees, W.A., Flandermeyer, R.R. and Hawthorne, P.K. (1974) Science 184, 1093-1096.
12. Bomford, R. and Weinstein, I.B. (1972) J. Nat. Canc. Inst. 49, 379-386.
13. Crouch, N.A. and Rapp, F. (1972) J. Virol. 9, 223-230.
14. Penman, S. (1966) J. Mol. Biol. 17, 117-130.
15. Franklin, R.M. (1966) Proc. Nat. Acad. Sci. USA, 70, 3400-3404.
16. Zimmer, S.G. and Raskas, H.J. (1976) Virology 70, 118-126.
17. Stringer, J.R., Holland, L.E., Swanstrom, R.I., Pivo, K. and Wagner, E.K. (1977) J. Virol. 21, 889-901.
18. Laub, O. and Aloni, Y. (1975) J. Virol. 16, 1171-1183.