STRAND SWITCH DURING *IN VIVO* POLYOMA TRANSCRIPTION

N. MUELLER, J. ŽEMLA* and G. BRANDNER
Department of Virology, Institute of Hygiene, D 78 Freiburg, Germany

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1. Introduction

Attempts [1] to develop a sensitive detection method for minute amounts of SV40 cRNA led us to the observation that the strand orientation of the SV40 *in vivo* transcript is different from that of the SV40 transcript made *in vitro* with RNA polymerase from *E. coli* [2]. This observation was in agreement with the findings of other laboratories [3–5].

In this paper we describe investigations on the strand orientation during transcription of polyoma (Py) virus.

2. Materials and methods

Secondary mouse embryo cells used for virus production were cultured in Eagle's MEM with 15% calf serum. They were infected with polyoma virus essentially according to Crawford [6]. The polyoma DNA was isolated according to Hirt [7].

The separation of the viral DNA into the supercoiled [8] and open circular form [9], the transcription of the supercoiled form by RNA polymerase from *E. coli* and the RNA–RNA annealing procedures followed methods described for SV40 [1, 2]. The RNA polymerase, a gift from Dr. B. Puschendorf and Dr. H. Grunicke, had been prepared according to Burgess [10].

* On leave from the Institute of Virology, Czechoslovak Academy of Sciences, Bratislava, Czechoslovakia.

3. Results and discussion

3.1. *Saturation hybridization of in vitro [^{3}H]Py cRNA with Py DNA*

In order to prove the template fidelity during the synthesis of Py cRNA by RNA polymerase from *E. coli* we tested the hybridization of a constant amount of the ^{3}H-labelled transcript with increasing amounts of filter-bound Py DNA. We found that up to 63% of the input *in vitro* [^{3}H]Py cRNA was hybridized with Py DNA (fig. 1). This figure corresponds to the rate of saturation hybridization obtained for SV40 [1].

The reverse experiment, hybridization of a constant amount of filter-bound Py DNA with increasing amounts of *in vitro* [^{3}H]Py cRNA, resulted in the hybridization of up to 20% of the DNA. The factors influencing this type of saturation experiment have been discussed for the analogous SV40 hybridization [1]. These criteria

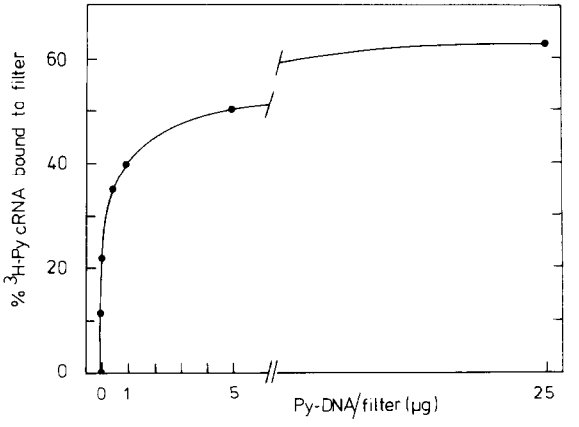

Fig. 1. Hybridization of 1 ng [^{3}H]Py cRNA with increasing amounts of Py DNA immobilized together with 5 µg of calf thymus DNA per filter.

prove the specificity of the *in vitro* Py transcript used for the further experiments.

3.2. *Self annealing of in vitro [^{3}H]Py cRNA*

The double-stranded DNA's from SV40 and Py virus (supercoiled molecules) are transcribed by RNA polymerase from *E. coli* mainly asymmetrically [11]. For Py this was confirmed by measurement of the extent of self annealing of the labelled *in vitro* transcript in the presence of tRNA from yeast or of RNA from uninfected mouse cells. RNAase was added to half the aliquots of the incubation mixture taken before and after the annealing and cell samples were treated with trichloroacetic acid to precipitate the RNA (fig. 2). We found that during this incubation up to 16% of the precipitable radioactivity became RNAase resistant, indicating an annealing of 8% symmetrically transcribed RNA.

3.3. *Annealing of in vitro [^{3}H]Py cRNA with late in vivo Py cRNA*

Incubation of *in vitro* [^{3}H]Py cRNA with unlabelled RNA from Py-infected mouse embryo cells (isolated 72 hr post infection) increased the percentage of RNAase resistant radioactivity up to 30% as compared with the amount obtained in an incubation with RNA from uninfected cells (fig. 2). This increase represents the portion of late *in vivo* Py cRNA which is transcribed from the strand complementary to the strand transcribed *in vitro*. In a corresponding experiment with SV40, we had found an increase of 55% [2].

3.4. *Annealing of in vitro [^{3}H]Py cRNA with early in vivo Py cRNA*

Unlabelled early *in vivo* Py cRNA was obtained from mouse embryo cells infected for 24 hr in the presence of cytosine arabinoside (20 μg/ml). The supression of the late genome functions by this inhibitor of DNA synthesis was shown by the absence of Py capsids, as demonstrated by means of an immunofluorescence test with anti-serum from rabbits.

The annealing of *in vitro* [^{3}H]Py cRNA with unlabelled early *in vivo* RNA led to no increase of RNAase resistant [^{3}H]cRNA as compared with the amount obtained by annealing of *in vitro* [^{3}H]Py cRNA with unlabelled RNA from uninfected cells (fig. 2). This means that the early *in vivo* and the *in vitro* Py cRNA are transcribed from the same DNA strand.

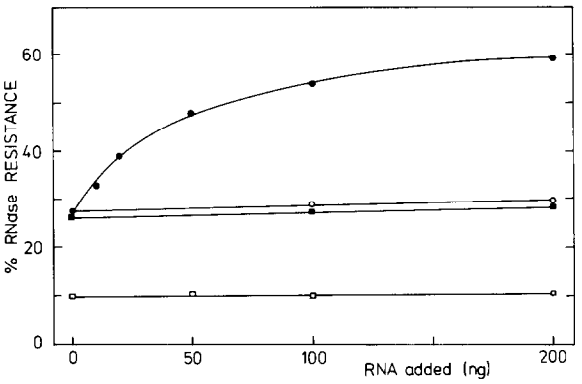

Fig. 2. Annealing of a mixture consisting of increasing amounts of unlabelled early (○—○—○) or late (●—●—●) RNA from Py infected cells plus RNA from uninfected cells (total RNA 200 ng) with ^{3}H *in vitro* Py cRNA (18 ng; 3.6×10^4 cpm) in a volume of 390 μl containing 900 mM NaCl, 90 mM Na-citrate and 5 mM EDTA, pH 7.4, for 20 hr at 66°. The *in vivo* cRNA's contained approx. 0.05% early and 0.5% late Py-specific RNA (calculated from [^{3}H]uridine labelled RNA of the infected cells which had been hybridized with Py DNA). Influence of increasing amounts of unlabelled RNA from uninfected cells on the self annealing rate of ^{3}H *in vitro* Py cRNA after 20 hr of incubation (■—■—■) and without incubation (□—□—□).

The above results provide evidence that the transcription of the late Py genes, like that of SV40 genes, occurs after a strand switch. However, the amount of complementarity between *in vitro* and late *in vivo* Py RNA is lower than the corresponding complementarity in the case of SV40 transcription.

Such a difference demonstrates a difference in the number of genome sequences transcribed from the early DNA strand. The question arises whether the additional sequences are transcribed early or late. A further question is whether considerable amounts of the *in vivo* Py cRNA are transcribed primarily symmetrically as claimed for SV40 [12].

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References

[1] N. Mueller, E.E. Petersen and G. Brandner, Med. Microbiol. Immunol. 158 (1972) 83.
[2] E.E. Petersen, N. Mueller, M. Heufer and G. Brandner, Arch. ges. Virusforsch. 39 (1972) 381.
[3] D.M. Lindstrom and R. Dulbecco, Proc. Natl. Acad. Sci. U.S. 69 (1972) 1517.
[4] G. Khoury and M.A. Martin, Nature New Biol. 238 (1972) 4.
[5] J. Sambrook, P.A. Sharp and W. Keller, J. Mol. Biol. 70 (1972) 57.
[6] L.V. Crawford, in: Fundamental Techniques in Virology, eds. K. Habel and N.P. Salzmann (Academic Press, New York, London, 1969) p. 75.
[7] B. Hirt, J. Mol. Biol. 26 (1967) 365.
[8] J. Vinograd, J. Lebowitz, R. Radloff, R. Watson and P. Laipis, Proc. Natl. Acad. Sci. U.S. 53 (1965) 1104.
[9] R. Dulbecco and M. Vogt, Proc. Natl. Acad. Sci. U.S. 50 (1963) 236.
[10] R.R. Burgess, J. Biol. Chem. 244 (1969) 6160.
[11] H. Westphal, J. Mol. Biol. 50 (1970) 407.
[12] Y. Aloni, Proc. Natl. Acad. U.S. 69 (1972) 2404.