TRANSFORMATION-ASSOCIATED INCREASE OF PHOSPHORIBOSYL PYROPHOSPHATE CONCENTRATION IN CHICK EMBRYO FIBROBLASTS

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1. Introduction

5-Phosphoribosyl-1-pyrophosphate (PRPP) is a high-energy compound which serves as a common precursor for both the de novo and the 'salvage' pathways of purine nucleotide biosynthesis, as well as for the synthesis of pyrimidine and pyridine nucleotides [1]. Cellular concentration of PRPP is considered to be one of the most important regulators of the de novo biosynthesis of purine nucleotides [2,3]. Increased availability of PRPP and the subsequent enhanced rate of the de novo synthesis of purine nucleotides have been observed within the few first hours of the mitogenic activation of lymphocytes [4,5]. Pool sizes of purine nucleoside triphosphates, ATP and GTP, may be limiting factors in the initiation of DNA synthesis in certain cultured cell lines [6,7]. Availability of PRPP and purine nucleotides may thus have an as yet undefined important role in the regulation of cellular growth processes [8].

Here we report that the measurable concentration of PRPP is higher in chick embryo fibroblasts transformed by Rous sarcoma virus than in uninfected cultures or in cultures infected with the corresponding non-transforming helper virus. Moreover, experiments with the temperature-sensitive NY68 mutant suggest that this change is associated with the transformed state of the cells.

2. Materials and methods

2.1. *Cell cultures*

Secondary cultures of leukosis-free chick embryo fibroblasts, prepared as in [9], were infected with wild-type Rous sarcoma virus (RSV; Schmidt-Ruppin strain, subgroup A), its NY68 mutant, temperature sensitive for transformation [10], or with the non-transforming RAV-1 virus, and incubated at 39°C. From day 3 onwards the culture medium was changed daily and in 2–3 days the cells infected with the RSV were fully transformed while those infected with RAV-1 showed a normal fibroblast-like morphology. Cultures infected with the NY68 virus showed intermediate morphology at 39°C, but were fully transformed after overnight incubation at 35°C. Tertiary cultures were prepared by seeding 6×10^6 trypsinized cells/53 cm^2 plastic Petri dish in 15 ml medium 199 supplemented with 10% tryptose phosphate broth and 5% calf serum. The cultures were used for the experiments on the following day, when the cells were still in the logarithmic phase of growth.

2.2. *Measurement of PRPP concentration*

A direct enzymatic method with radioactive co-substrate [11] was used. Tertiary chick embryo fibroblast cultures in 53 cm^2 Petri dishes to be analyzed for PRPP concentration were placed on crushed ice and washed once with ice-cold Dulbecco's phosphate-buffered saline (PBS). The cells were scraped into 3–4 ml PBS and collected by centrifugation at 4°C. The pellet was resuspended in 200 μl 1 mM ethylenediamine tetra-acetic acid (EDTA) (pH 7.0) and frozen at −20°C. Immediately after thawing the samples were heated in boiling water for 30 s. Chilled samples were then centrifuged for 10 min at 8000 × g and the slightly opalescent supernatant was assayed

for PRPP. The assay mixture (90 μl) contained 50 μl unknown sample or standard PRPP and 30 μl reagent mixture consisting of 0.03 μmol (1 μCi) [^{14}C]adenine (Radiochemical Centre, Amersham), 0.63 μmol $MgSO_4$, 9 μmol Tris–HCl buffer (pH 7.4, 23°C) and 0.45 μmol dTMP (to saturate possibly contaminating 5′-nucleotidase). The reaction was initiated by adding 10 μl purified adenine-phosphoribosyltransferase solution (a gift from Dr K. O. Raivio) and transferring the tubes to 37°C. After 60 min incubation the reaction was stopped by adding 10 μl 0.2 M EDTA and 10 μl 0.1 M AMP and chilling the tubes. Aliquots (5 μl) were then applied on cellulose sheets (Polygram MN300, Macherey and Nagel, FRG) and AMP was separated from adenine by a 20 min run in 1.6 M LiCl. Spots containing AMP were localized under ultraviolet light, cut out and dried, and radioactivity measured in a liquid scintillation spectrometer. Quantity of PRPP present in the assay was assumed to equal the fraction of [^{14}C]adenine converted to [^{14}C]AMP [11]. Known amounts of PRPP added into cell suspensions immediately before heating produced in subsequent assays [^{14}C]AMP counts equal to 60% of those obtained with the same amount of PRPP heated in EDTA alone. Values of PRPP presented in this paper are corrected for recovery. Protein was measured according to [12].

3. Results

Cultures transformed by Rous sarcoma virus showed levels of PRPP 2–3-times higher than those observed in the uninfected cultures, while cells infected with the non-transforming RAV-1 virus had PRPP levels comparable to or lower than those of uninfected cultures (table 1). It should be noted that under the conditions used all the different types of cell cultures analyzed for PRPP were subconfluent and actively proliferating. This is important as the rate of cell growth as such may influence the cellular PRPP levels [4,5].

Difference in PRPP concentration between SR-A and RAV-1-infected cells suggested that the increased PRPP concentration is associated with the transformed state of the cells and not with an infection by a retrovirus as such. Evidence for this view was substantiated by experiments with the NY68 virus [10]. This virus, like other temperature-sensitive transformation mutants of Rous sarcoma virus, is able to transform chick

Table 1
5-phosphoribosyl-1-pyrophosphate (PRPP) levels in normal and retrovirus-infected chick embryo fibroblasts

Cell culture	PRPP (μmol/mg protein)
Normal fibroblast	0.35 ± 0.08
RSV	0.74 ± 0.07
RAV-1	0.38 ± 0.05

RSV, fibroblasts infected with the Schmidt-Ruppin strain, subgroup A, of Rous sarcoma virus; RAV-1, cells infected with the respective non-transforming virus. All cells were from tertiary cultures. For experimental details, see section 2. Mean and SE of 3–8 experiments

embryo fibroblasts efficiently at 35°C but fails to induce or maintain transformation at 41°C, in spite of normal virus replication [10,13,14]. At 41°C cells infected with NY68 have a phenotype indistinguishable from that of normal fibroblasts. After shift-down of the incubation temperature to 35°C several transformation-associated characteristics of the cultures appear within hours [9,10,13–16].

Tertiary cultures of chick embryo fibroblasts, prepared as above and infected with NY68, were incubated overnight at 41°C or at 35°C. The cultures showed normal or transformed morphology, respectively. Transformed NY68-infected cultures had PRPP levels as high as those in the wild-type-infected cells, while cultures at 41°C had PRPP concentrations approximately equalling those of normal fibroblasts (table 2). Samples of two cultures were taken before and 1 h or 2 h after shift-down or shift-up of the temperature, and the levels of PRPP in the cells were measured. An increase of PRPP concentration was detected within 1 h after shift-down. Shift-up of NY68-transformed cells to 41°C affected PRPP concentration only slightly during that time (table 2).

4. Discussion

These results show that higher than normal levels of PRPP are associated with virus-induced transformation of fibroblast cultures and that the increase of PRPP concentration is one of the earliest signs of transformation. The mechanism by which this change is induced in transformed cells is not known. Cellular

Table 2
Association of high levels of PRPP with the transformed state of NY68-infected chick embryo fibroblasts

Exp.	Incubation temperature		Time after shift	Culture morphology	PRPP (μmol/mg protein)
	Overnight	Shifted to			
1.	35°C			Transformed	0.98 ± 0.10
	41°C			Normal	0.39 ± 0.07
	41°C	35°C	1 h	Normal	1.07 ± 0.08
2.	35°C			Transformed	0.99 ± 0.10
	41°C			Normal	0.48 ± 0.08
	41°C	35°C	1 h	Normal	0.85 ± 0.17
	41°C	35°C	2 h	Normal	0.94 ± 0.04
	35°C	41°C	1 h	Transformed	0.92 ± 0.01

Tertiary chick embryo fibroblast cultures were infected with NY68 and incubated overnight at 35°C or at 41°C. Then part of the cultures was transferred to the other temperature. Concentration of PRPP was measured after the time indicated. Mean and range of duplicate samples are given

PRPP concentrations are affected by several factors, which either influence the synthesis of PRPP or modify its utilization or degradation [1,2]. Although reduced PRPP utilization may result in increased PRPP concentrations under certain conditions [2] it is more likely that higher than normal levels of PRPP in transformed cells result from increased synthesis of the compound. There is some evidence that the transforming protein, and in the case of NY68 virus, probably the temperature-sensitive viral gene product, is a protein kinase [17,18]. It is conceivable that the latter could directly modify the structure and thereby possibly the activity of the enzyme PRPP synthetase. Another possible way to increase PRPP levels in transformed cells would be to increase, in one way or another, the intracellular concentration of phosphate ions, a potent activator of PRPP synthetase [19]. These possibilities can be tested experimentally. Further work is also required to find out whether the observed high levels of PRPP in transformed cells only reflect a non-specific general activation of the metabolism or whether the availability of PRPP could have a more specific role in the process of viral transformation of animal cells.

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References

[1] Murray, A. W., Elliot, D. C. and Atkinson, M. R. (1970) Progr. Nucl. Acid Res. Mol. Biol. 10, 87–119.
[2] Fox, I. and Kelley, W. N. (1971) Ann. Int. Med. 74, 424–433.
[3] Green, G. A. and Martin, D. W., jr (1973) Proc. Natl. Acad. Sci. USA 70, 3698–3702.
[4] Hovi, T., Allison, A. C. and Allsop, J. (1975) FEBS Lett. 55, 291–293.
[5] Hovi, T., Allison, A. C., Raivio, K. O. and Vaheri, A. (1977) in: Purine and Pyrimidine Metabolism, Ciba Found. Symp. 48, pp. 225–248, Elsevier/Excerpta Medica/North-Holland, Amsterdam, New York.
[6] Grummt, I. and Grummt, F. (1976) Cell 7, 447–453.
[7] Grummt, F., Paul, D. and Grummt, I. (1977) Eur. J. Biochem. 76, 7–12.
[8] Hovi, T. (1978) in: Growth Factors. Cellular Growth Processes, Growth Factors, and Hormonal Control of Growth (Kastrup, K. W. and Nielsen, J. H. eds) Proc. 11th FEBS Meet. pp. 13–22, Pergamon Press, Oxford.
[9] Vaheri, A. and Ruoslahti, E. (1974) Int. J. Cancer 13, 579–587.
[10] Kawai, S. and Hanafusa, H. (1971) Virology 46, 470–479.

[11] Hershko, A., Razin, A. and Mager, J. (1969) Biochim. Biophys. Acta 184, 64–76.
[12] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[13] Martin, G. S. (1970) Nature 227, 1021–1023.
[14] Bader, J. P. (1971) Nature New Biol. 234, 11–12.
[15] Robbins, P. W., Wickus, G. G., Branton, P. E., Gaffrey, B. J., Hirschberg, C. B., Fuchs, P. and Blumberg, P. M. (1975) Cold Spring Harbor Symp. Quant. Biol. 39, 1173–1180.
[16] Rifkin, D. B., Beal, L. P. and Reich, E. (1975) Cold Spring Harbor Conf. Cell Prolif. vol. 2, pp. 841–847, Cold Spring Harbor Lab., New York.
[17] Collett, M. S. and Erikson, R. L. (1978) Proc. Natl. Acad. Sci. USA 75, 2021–2024.
[18] Levinson, A. D., Oppermann, H., Levintow, L., Varmus, H. E. and Bishop, J. M. (1978) Cell 15, 561–572.
[19] Fox, I. and Kelley, W. N. (1971) J. Biol. Chem. 246, 5739–5748.