Acylation: A new post-translational modification specific for plasma membrane-associated simian virus 40 large T-antigen

Ulrich Klockmann and Wolfgang Deppert*

Department of Biochemistry, University of Ulm, PO Box 4066, 7900 Ulm, FRG

Received 29 November 1982

SV40 transformed mouse cells (mKSA) were labeled in parallel with either [^{35}S]methionine or [^{3}H]palmitate and subfractionated. Nuclear extracts and solubilized plasma membranes were analyzed for the presence of either ^{35}S- or ^{3}H-labeled SV40 large tumor antigen by immunoprecipitation and SDS polyacrylamide gel electrophoresis. The majority of the [^{35}S]methionine labeled large T was recovered from the nuclear fraction, only minor amounts were detected in plasma membranes. In contrast, large T labeled specifically with [^{3}H]palmitate was found only in the plasma membrane fraction. Our results demonstrate a specific acylation of large T associated with plasma membranes, suggesting that the membrane location of this predominantly nuclear protein is specific.

Simian virus 40 *Large T-antigen* *Cell fractionation* *Post-translational modification* *Protein acylation* *Membrane-association of proteins*

1. INTRODUCTION

Simian virus 40 (SV40) large tumor antigen (large T) is a multi-functional protein involved in many regulatory processes in SV40-infected and -transformed cells [1]. Consequently, it is found in different subcellular locations: most of large T is recovered from the nucleus [2], but a small amount can also be detected in the plasma membrane [3] and on the cell surface [4,5]. One can assume that the known post-translational modifications of large T, phosphorylation [6] and ADP-ribosylation [7] play an important role in regulating the different activities of large T and, thereby, might influence its actual subcellular location. Nevertheless, it is difficult to conceive how a predominantly nuclear protein might achieve a specific plasma membrane location.

In this report we describe that plasma membrane-associated large T is specifically modified insofar as it can be labeled with fatty acid, whereas nuclear large T is not labeled. Since acylation is a post-translational modification specific for membrane proteins [8], our results may indicate that this modification mediates the specific plasma membrane association of large T antigen.

* To whom correspondence should be addressed

2. MATERIALS AND METHODS

2.1. *Radiolabeling and cell fractionation*

Parallel cultures of 5×10^7 mKSA cells (an SV40-transformed BALB/c mouse tumor line), grown in suspension culture in Ca^{2+}-free minimum essential medium (Gibco, F-13), were labeled for 4 h with either 500 μCi [^{35}S]methionine (NEN) in 20 ml of methionine-free Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal calf serum, or with 5 mCi [^{3}H]palmitate (NEN) in 20 ml DMEM, supplemented with 5% fetal calf serum and 5 mM pyruvate, respectively. After washing once with hypotonic buffer (10 mM MES (pH 6.2), 10 mM NaCl, 10 mM $MgCl_2$) and swelling for 10 min in lysis buffer [9] the cells were homogenized in a stainless steel dounce homogenizer (clearance about 5 μm). Cell frac-

Published by Elsevier Biomedical Press
00145793/83/0000—0000/$3.00

tionation was performed essentially as in [9].

2.2. *Extraction of large T, immunoprecipitation and SDS–polyacrylamide gel analysis*

Nuclei obtained by cell fractionation were extracted as in [10], plasma membranes were solubilized in RIPA buffer (10 mM sodium phosphate (pH 7.2), 150 mM NaCl, 1% Triton X-100, 1% deoxycholate, 0.1% sodium dodecyl sulfate [11]). Large T extracts were immunoprecipitated with rabbit anti-SDS–T serum [12], and analyzed by SDS–polyacrylamide gel electrophoresis (SDS–PAGE) as in [12]. Labeled proteins were detected by fluorography [13].

3. RESULTS AND DISCUSSION

mKSA cells grown in spinner culture were labeled in parallel with either [^{35}S]methionine or [^{3}H]palmitate and subfractionated. Large T was extracted from the nuclear and the plasma membrane fraction, immunoprecipitated and the immunoprecipitates analyzed by SDS–PAGE (see section 2). Fig.1A,B show the distribution of [^{35}S]methionine and [^{3}H]palmitate-labeled large T in the different subcellular fractions, respectively. As reported, the majority of the [^{35}S]methionine-labeled large T, as well as the large T associated cellular phosphoprotein p 53 were extracted from the nuclear fraction, and only a small amount of large T (about 1%) was recovered from the plasma membrane fraction [3] (fig.1A). In contrast, [^{3}H]palmitate-labeled large T was not found in the nuclear fraction, whereas large T specifically labeled with [^{3}H]palmitate was solubilized from the plasma membrane fraction (fig.1B). This demonstrated a specific acylation of membrane-associated large T.

Several criteria indicated that the ^{3}H-labeling found in immunoprecipitates of large T solubilized from plasma membranes reflected the covalent binding of fatty acid to this molecule:

(1) The fatty acid was still associated with large T after extraction with RIPA buffer containing several nonionic and ionic detergents [11];
(2) The fatty acid could not be removed from large T by boiling in 3% SDS for 5 min;
(3) The fatty acid was also still bound to large T after SDS–PAGE;

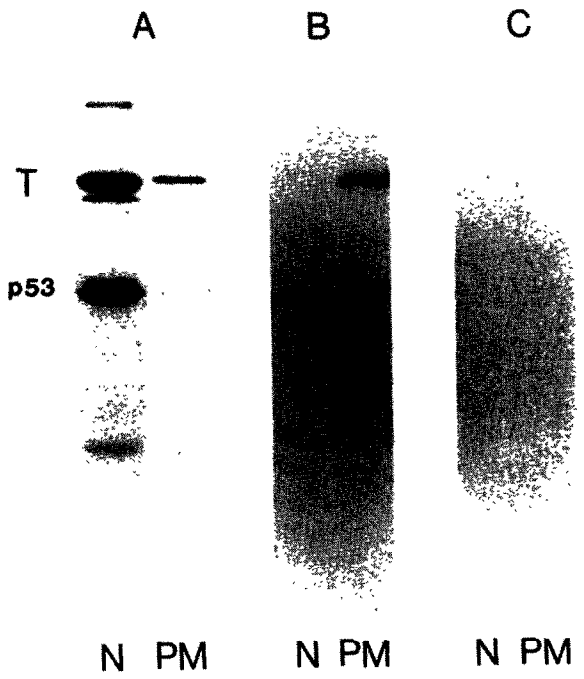

Fig.1. Distribution of [^{35}S]methionine and [^{3}H]palmitate-labeled SV40 large T in different subcellular fractions of SV40-transformed mKSA cells; SDS–polyacrylamide gel fluorograms. Extracts of nuclei (N) and plasma membranes (PM) isolated from [^{35}S]methionine or [^{3}H]palmitate-labeled mKSA cells, respectively, were immunoprecipitated with anti-SDS–T-serum and analyzed on 11.5% SDS–polyacrylamide slab gels: (A) distribution of [^{35}S]methionine-labeled large T in the nuclear (N) and plasma membrane (PM) fraction; (B) distribution of [^{3}H]palmitate-labeled large T in these fractions; (C) fluorogram of the gel shown in fig.1B, after treatment with 1 M hydroxylamine for 1 h.

(4) Most importantly, the ^{3}H-label could be quantitatively removed from large T, when the gel shown in fig.1B was treated with hydroxylamine (fig.1C). Since hydroxylamine treatment has been shown to result in a nucleophilic cleavage of many acyl derivatives [14], this quantitative removal of the ^{3}H-label indicated that the ^{3}H-labeling of plasma membrane-associated large T did not arise from metabolic conversion of the [^{3}H]palmitate into [^{3}H]amino acids which then were incorporated into the polypeptide backbone of large T.

Fatty acid labeling (acylation) in recent years has been recognized as a widespread post-translational modification of membrane proteins of viral and cellular origin [8]. Its biological role is not yet understood, but it is hypothesized that acylation might provide anchorage for otherwise hydrophilic proteins in the hydrophobic environment of the membrane [15]. In this way acylation might mediate the stable plasma membrane association of large T, since this molecule does not contain any prominent hydrophobic amino acid sequences [16,17].

ACKNOWLEDGEMENTS

This study was supported by grant De 212/5-3 from the Deutsche Forschungsgemeinschaft.

REFERENCES

[1] Tooze, J. (1980) Molecular Biology of Tumor Viruses, pt 2, DNA Tumor Viruses, Cold Spring Harbor Laboratory, New York.
[2] Anderson, J.L., Martin, R.G., Chang, Ch., Mora, P.T. and Livingston, D.M. (1977) Virology 76, 420–425.
[3] Soule, H.R. and Butel, J.S. (1979) J. Virol. 30, 523–532.
[4] Soule, H.R., Lanford, R.E. and Butel, J.S. (1980) J. Virol. 33, 887–901.
[5] Deppert, W., (1980) Virology 104, 497–501.
[6] Tegtmeyer, P., Rundell, K. and Collins, J.K. (1977) J. Virol. 16, 647–657.
[7] Goldman, N., Brown, M. and Khoury, G. (1981) Cell 24, 567–572.
[8] Schmidt, M.F.G. (1982) Trends Biochem. Sci. 7, 322–324.
[9] Deppert, W., Walter, G. and Linke, H. (1977) J. Virol. 21, 1170–1186.
[10] Schwyzer, M. (1977) Colloq. INSERM 69, 63–68.
[11] Gilead, Z., Jeng, Y., Wold, W.S.M., Sugawara, K., Mo Rho, H., Harter, M.L. and Green, M. (1976) Nature 264, 263–266.
[12] Deppert, W. and Pates, R. (1979) J. Virol. 31, 522–536.
[13] Bonner, W.M. and Laskey, R.A. (1974) Eur. J. Biochem. 46, 83–88.
[14] Omary, M.B. and Trowbridge, J.S. (1981) J. Biol. Chem. 256, 4715–4718.
[15] Schmidt, M.F.G. (1982) Virology 116, 327–338.
[16] Fiers, W., Contreras, R., Haegeman, G., Rogiers, R., Van de Voorde, A., Van Heuverswyn, H., Van Herreweghe, J., Volckaert, G. and Ysebaert, M. (1978) Nature 273, 113–120.
[17] Reddy, V.B., Thimmappaya, B., Dhar, R., Subramanian, K.N., Zain, B.S., Pan, J., Ghosh, P.K., Celma, M.L. and Weissman, S.M. (1978) Science 200, 494–502.