

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

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SV40 transformed mouse cells (mKSA) were labeled in parallel with either [<sup>35</sup>S]methionine or [<sup>3</sup>H]palmitate and subfractionated. Nuclear extracts and solubilized plasma membranes were analyzed for the presence of either <sup>35</sup>S- or <sup>3</sup>H-labeled SV40 large tumor antigen by immunoprecipitation and SDS polyacrylamide gel electrophoresis. The majority of the [<sup>35</sup>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 [<sup>3</sup>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  
Protein acylation*

*Cell fractionation*

*Post-translational modification  
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.

## 2. MATERIALS AND METHODS

### 2.1. Radiolabeling and cell fractionation

Parallel cultures of  $5 \times 10^7$  mKSA cells (an SV40-transformed BALB/c mouse tumor line), grown in suspension culture in Ca<sup>2+</sup>-free minimum essential medium (Gibco, F-13), were labeled for 4 h with either 500  $\mu$ Ci [<sup>35</sup>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 [<sup>3</sup>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<sub>2</sub>) and swelling for 10 min in lysis buffer [9] the cells were homogenized in a stainless steel dounce homogenizer (clearance about 5  $\mu$ m). Cell frac-

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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;

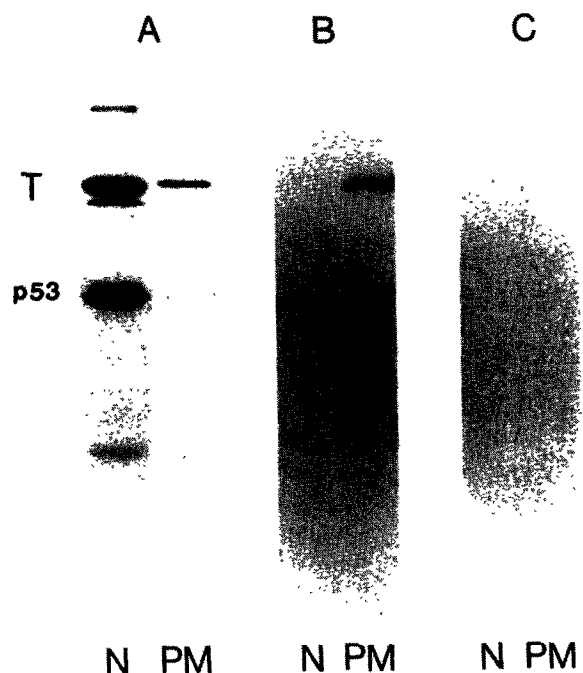


Fig.1. Distribution of [ $^{35}$ S]methionine and [ $^3$ H]palmitate-labeled SV40 large T in different subcellular fractions of SV40-transformed mKSA cells; SDS-polyacrylamide gel fluorograms. Extracts of nuclei (N) and plasma membranes (PM) isolated from [ $^{35}$ S]methionine or [ $^3$ H]palmitate-labeled 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].

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