Methylation of elongation factor 1α in mouse 3T3B and 3T3B/SV40 cells

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Received 19 October 1983

Two-dimensional gel electrophoretic (NEPHGE) analysis of proteins from mouse 3T3B and 3T3B/SV40 cells labelled with [*methyl*-^{3}H]methionine in the presence of cycloheximide have revealed that the elongation factor 1α (EF-1α) in these cells is methylated and that the extent of methylation is higher in the SV40 transformed cell type. It is suggested that methylation may account for differences in growth properties for the different cell types.

Elongation factor 1α *Two-dimensional gel electrophoresis* *Methylation* *3T3B* *3T3B/SV40*

1. INTRODUCTION

The eukaryotic protein elongation factor EF-1α, which is functionally equivalent to the bacterial factor EF-Tu, has been isolated from various tissues including pig liver [1,2], rabbit reticulocytes [3–5], wheat germ [6,7] and *Artemia salina* [8]. Two-dimensional gel electrophoresis and immunoblotting have revealed that EF-1α is a basic protein with an isoelectric point of between 9.0 and 9.5 and an M_r of ~53000 [9].

It has been shown previously that EF-Tu from *Salmonella typhimurium* and *Escherichia coli* is methylated [10]. In *E. coli*, the methylation occurs at lysine residue 56 [11]. The importance and significance of this modification is not clear, although it has been suggested that methylation might be correlated with the regulation of activity or synthesis or EF-Tu or alternatively might fulfill a structural requirement [10].

During the course of our studies on EF-1α in cultured somatic cells, we have found that the factor is methylated in mouse 3T3B cells and that the extent of methylation is higher in simian-virus SV40 transformed 3T3B cells.

Methylation of eukaryotic proteins has been reported previously in *Mucor* [12], yeast [13], chicken fibroblasts and BHK-21 [14], and in *Drosophila* [15]. Interestingly, in many cases, the methylation occurs in proteins which, like EF-1α, interact specifically with nucleic acids.

Abbreviation: NEPHGE, non-equilibrium pH gradient gel electrophoresis

2. MATERIALS AND METHODS

2.1. *Materials*

L-[*methyl*-^{3}H]Methionine (85 Ci/mmol) was purchased from Amersham (England). Mouse 3T3B and 3T3B/SV40 cells were the generous gift of Dr Donna Jovin of the Max-Planck-Institut for Plant Physiology, Göttingen.

2.2. *Cell cultures*

Mouse 3T3B and 3T3B/SV40 were grown in Dulbecco's modified Eagle's minimum essential medium (DMEM) supplemented with 10% foetal calf serum and containing 2.02 g/l $NaHCO_3$ and 50 units/ml penicillin and 50 μg/ml streptomycin (base medium). Prior to labelling, cells were rinsed once in fresh DMEM.

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00145793/83/$3.00

2.3. *Labelling of cells with L-[methyl-^{3}H]-methionine*

Cells for labelling were grown in 0.3 ml flat-bottomed microtitre plates. To label the cells, the base medium was drawn off and replaced with 0.1 ml base medium lacking methionine but containing 100 μCi of L-[*methyl*-^{3}H]methionine. The cells were routinely labelled for periods of between 1 and 20 h at 37°C. Following labelling, the cells were rinsed once in fresh DMEM and rapidly dissolved in dissociation buffer [16]. Procedures for [^{35}S]methionine labelling have been described in [24].

To resolve methylated proteins, the cells were labelled in the presence of 400 μg/ml cycloheximide. Medium was removed from cells grown in microtitre plates and replaced with base medium containing 1 mg/l cold methionine and 400 μg/ml cycloheximide. The cells were then incubated for 30 min at 37°C. The medium was then removed and replaced with base medium lacking cold methionine but containing 100 μCi L-[*methyl*-^{3}H]methionine. The cells were then incubated for a further 60 min at 37°C, after which time the medium was removed, the cells rinsed rapidly once in fresh DMEM and dissolved in dissociation buffer.

2.4. *2-D gel electrophoresis and fluorography*

Non-equilibrium pH gradient gel electrophoresis (NEPHGE) was used as the first dimension of two-dimensional gel electrophoresis, which was performed as in [16,17]. Gels were processed for fluorography as in [18] and exposed to pre-flashed Kodak X-omat RP X-ray film at −70°C. Typical exposure times were 10 days for two-dimensional fluorograms of cells labelled in the absence of cycloheximide and 25–30 days for those cells labelled in the presence of cycloheximide.

3. RESULTS AND DISCUSSION

The two-dimensional mobility of mouse liver EF-1α and its location in protein catalogues has been demonstrated in [9]. The position of EF-1α from mouse 3T3B/SV40 cells is indicated in the NEPHGE two-dimensional fluorogram of total protein isolated from asynchronous 3T3B/SV40 shown in fig.1. For reference, the positions of proteins catalogued [19] as N21, N15, N16d and N19 are also indicated. Under the labelling conditions employed, the [^{3}H]methyl group from L-[*methyl*-^{3}H]methionine is incorporated into proteins both as methionine during de novo protein biosynthesis and as methyl group during post-translational modification. This modification occurs presumably via methyl transfer from *S*-adenosylmethionine. Labelling of cells under the same conditions using [^{35}S]methionine gave similar results (not shown).

3T3B and 3T3B/SV40 cells were labelled with [*methyl*-^{3}H]methionine both in the presence and absence of cycloheximide. The results are shown in fig.2. Fig.2A and C show fluorograms of NEPHGE two-dimensional separations of total proteins from 3T3B and 3T3B/SV40 labelled in the absence of cycloheximide. Analysis and quantitation of the labelled polypeptides reveal that although no new polypeptides appear in the transformed cells, some polypeptide species show significant changes in abundance. Radioactive species were excised from gels and counted. The radioactivity incorporated into each was then expressed as a percentage of the total applied to the gel (see legend to table 1). The results of these studies show that whilst the percentages of the

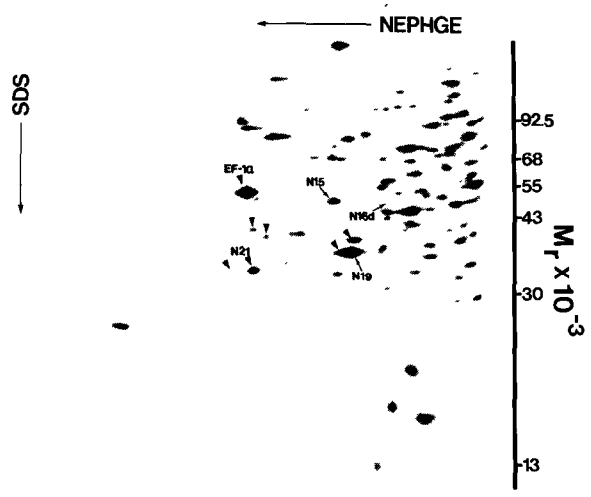

Fig.1. NEPHGE two-dimensional fluorogram of [*methyl*-^{3}H]methionine-labelled total protein from 3T3B/SV40 cells. The cells were labelled in the absence of cycloheximide for 20 h at 37°C. The positions of EF-1α, N21, N15, N16d and N19 [19] are indicated. Polypeptides methylated in 3T3B/SV40 cells are marked with arrow heads.

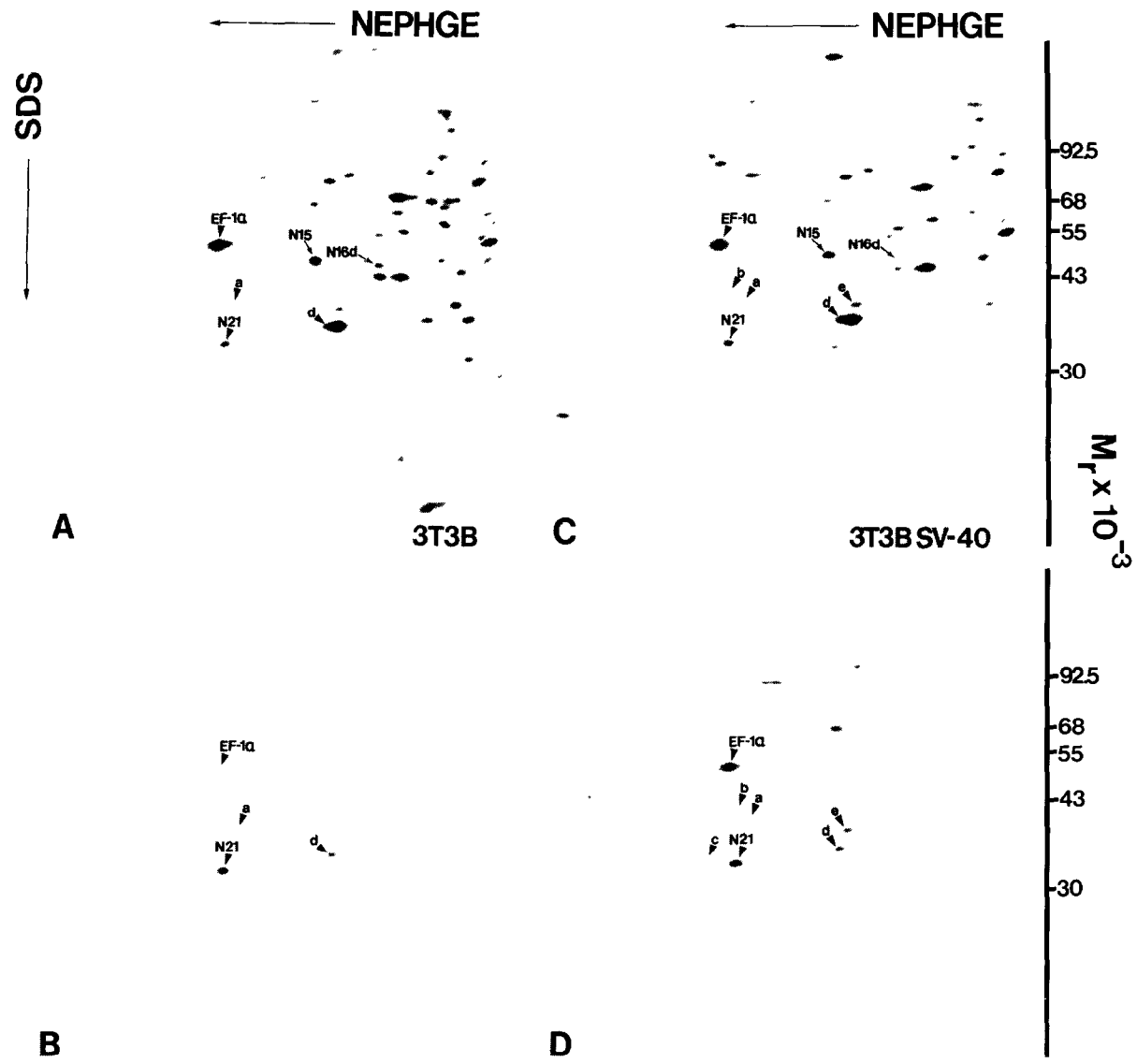

Fig.2. NEPHGE two-dimensional fluorograms of 3T3B and 3T3B/SV40 proteins labelled for 1 h at 37°C with [*methyl*-^{3}H]methionine both in the absence (A,C) and presence (B,D) of 400 μg/ml cycloheximide as described in section 2. (A,C) 3T3B and 3T3B/SV40 cells respectively, labelled in the absence of cycloheximide. EF-1α, N21 and methylated polypeptide species a, b, c, d and e are indicated. Visual inspection would indicate that a, d and e correspond to N16v, N19a and N19z$_6$ in the protein catalogues, respectively [19]. (B,D) 3T3B and 3T3B/SV40, respectively, labelled as for A and C, except that the labelling medium contained 400 μg/ml cycloheximide. Major methylated species are indicated.

total label incorporated into EF-1α and N15 remain relatively constant, N21 shows a 37% increase and N16d a 91% decrease in the SV40 transformed cells. Whilst these results are in good agreement with those in [20], it must be borne in mind that estimates can vary with cell passage number and expression in high passage 3T3B tends towards the levels seen in the SV40 transformed cell type [23]. When asynchronous cells were labelled with [*methyl*-^{3}H]methionine in the presence of 400 μg/ml of cycloheximide, conditions which resulted in the complete inhibition of

[^{35}S]methionine incorporation into protein (not shown), label was incorporated into only a few polypeptides (fig.2B,C), including EF-1α and N21.

Since cycloheximide treatment results in the complete inhibition of protein biosynthesis in 3T3B and 3T3B/SV40, label incorporation from [*methyl*-^{3}H]methionine must occur via methyl group transfer to previously synthesized protein. Indeed, post-translational methylation of heat shock protein (HSP) 83 and the Thermins A and B in chicken embryonic fibroblasts labelled under similar conditions with [*methyl*-^{3}H]methionine is completely suppressed by the methylation inhibitors homocysteine and thiolactone [14].

Whilst the extent of post-translational methylation in 3T3B and 3T3B/SV40 is similar for polypeptides N21 and polypeptide a, EF-1α is more heavily methylated in the SV40 transformed cells (fig.2B,D). The relative intensities of these spots can be conveniently measured directly from fluorograms of two-dimensional gels by densitometric analysis. The spot quantitation data for methylated polypeptides from 3T3B and 3T3B/SV40 are presented in table 1. Only EF-1α, N21 and polypeptides a, b and c were quantitated. These measurements show that there is an almost 10-fold increase in EF-1α methylation in the SV40 transformed 3T3B cells over the non-transformed cell type. Since the high and low methylated species co-migrate under the NEPHGE conditions employed here, it has not been possible to determine what proportion of the factor is methylated. Immunoprecipitation or double labelling experiments will be necessary to answer this question.

The methylation of 3 other basic polypeptides also increases markedly in the SV40 transformed cells. These are indicated in fig.2D. Other methylated species remain relatively constant in both cell types. Of the methylated polypeptides shown in fig.2B,D, those marked a, d and e probably correspond to polypeptides catalogued as N16v; N19a and N19z_6, respectively [19]. Polypeptides b and c are not present in the current mouse catalogue of mouse primary kidney fibroblast.

Whilst it has also been shown that the amount of EF-1α present in 3T3B cells does not increase in SV40 transformed cells [20], our results indicate that a change does occur in the level of post-translational methylation of the factor. Whether

Table 1

NEPHGE polypeptides	Total protein % compared with total [^{35}S]-methionine (radioactivity) counts incorporated[a]		Methylated protein % of total *methyl*-^{3}H radioactivity incorporated[b]	
	3T3B (1)	3T3B/SV40 (2)	3T3B (3)	3T3B/SV40 (4)
EF-1α	1.71	1.85	5.52[b]	52.2[b]
N15	1.05	1.00	0	0
N16d	0.50	0.043	0	0
N21	0.35	0.46	32.8[b]	34.4[b]
a	ND	ND	4.03	3.72
b	ND	ND	0	3.03
c	ND	ND	0	0.86

[a] Expressed as % of total counts present in NEPHGE gels

[b] Data are corrected for the variations in abundance of individual species (columns 1 and 2) and are expressed relative to N21, for which the extent of methylation was constant in 3T3B and 3T3B/SV40 and closely reflected variation in abundance. ND, not done. Incorporations were extremely low and in some cases at background level

Fluorograms of 3T3B and 3T3B/SV40 cells labelled with [^{35}S]methionine or with [*methyl*-^{3}H]methionine in the presence of cycloheximide were subjected to radioactive spot quantitation (columns 1 and 2) and to computer densitometric analysis, respectively (columns 3 and 4). Polypeptide a probably corresponds to N16v on current mouse kidney catalogues [19]

this change is concomitant with viral transformation itself or is related to the increased rate of cell division associated with the viral transformation is unclear. In this connection, we are currently investigating the timing of the methylation event in the cell cycle. Furthermore, it will be of interest to compare the enzymic activities of EF-1α in the high and low methylated forms since post-translational modifications such as phosphorylation have been suggested to play a role in the control of the activity of other factors such as eIF-2α [21,22].

ACKNOWLEDGEMENTS

We thank W. Müller for excellent technical assistance and O. Jensen for photography. We are also indebted to Dr H. Kronberg for help with the densitometric scanning and to Drs J.E. Celis and I. Thrippleton for helpful discussions. This work was supported by the Danish Natural Science Research Council, The Danish Medical Research Council, The British Science and Engineering Research Council (SERC), The Carlsberg Foundation, P. Carl Petersens Fund, Kong Christian den Tiendes Fund, Novo Fund and Aarhus University Research Fund. N.C. is a recipient of an overseas studentship from the SERC in England.

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