ADP-ribosylation of metaphase and interphase nonhistones using [^{3}H]adenosine as a radioactive label

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ADP-ribosylation of HeLa nonhistone proteins was investigated by using [^{3}H]adenosine as an in vivo radioactive label. The aim was to determine basic differences in the patterns of modification of interphase and metaphase nonhistones. Fluorography revealed a relatively small number of modified proteins for isolated metaphase chromosomes. In addition to the core histones, a protein of 116 kDa, which is identified as poly(ADP-ribose) polymerase, was a primary acceptor of [^{3}H]adenosine. Two-dimensional gels revealed a profound difference in the modification of metaphase and interphase nonhistones. For interphase nuclei, ^{3}H label was distributed among a large number of nonhistone acceptors.

ADP-ribosylation *Nonhistone* *Metaphase chromosome* *Interphase nucleus* *[^{3}H]Adenosine* *HeLa cell*

1. INTRODUCTION

A significant role for ADP-ribosylation of nuclear proteins in the regulation of nuclear function has recently been recognized. Cellular differentiation, gene expression, malignant transformation and repair of DNA damage are all cellular events which involve ADP-ribosylation of proteins [1–5]. Poly(ADP-ribose) polymerase is tightly bound to chromatin, so that it may be considered a nonhistone protein. The histones are major protein acceptors of poly(ADP-ribose) in nuclei, and most previous investigations of the modification have been concerned with the histones as well as with poly(ADP-ribose) polymerase [6–10]. The levels of ADP-ribosylation of nuclear proteins have been characterized at different phases of the cell cycle. Most investigations have indicated that the highest degree of modification occurs for metaphase cells [11–13].

ADP-ribosylation of HeLa nonhistones was previously investigated by this laboratory through radioactively labeling the proteins of isolated nuclei and chromosomes with [^{32}P]NAD [14]. Here, the use of [^{3}H]adenosine as an in vivo label for ADP-ribosylation of nuclear and chromosomal proteins is examined.

Abbreviations: NP40, Nonidet-P40; PMSF, phenylmethylsulfonyl fluoride; TLCK, *N*-α-p-tosyl-L-lysine chloromethyl ketone; DTT, DL-dithiothreitol

2. MATERIALS AND METHODS

2.1. *Isolation of metaphase chromosomes and interphase nuclei*

HeLa S-3 cells were propagated in minimum essential medium at 37°C in suspension culture. The medium was supplemented with 5% fetal bovine serum.

HeLa cells were arrested in mitosis with colchicine (0.2 μg/ml, 12–18 h) and washed twice with chromosome isolation buffer (50 mM NaCl, 5 mM Hepes, pH 7.4, 5 mM $MgCl_2$, 0.5 mM $CaCl_2$, 0.1 mM PMSF) [15]. Cells were resuspended in buffer and disrupted with a Dounce tissue homogenizer after adding 0.5% NP40 and 0.1% sodium deoxycholate. Purification of chromosomes was accomplished with density gradients of Metrizamide (0.3–0.8 M).

Sucrose density gradients were used to purify nuclei obtained by a previously described pro-

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cedure [16]. Cells of unsynchronized cultures were suspended in hypotonic buffer (10 mM NaCl, 10 mM Tris, pH 7.4, 5 mM $MgCl_2$) and mechanically disrupted with the aid of detergents.

To prepare nuclear scaffolds, purified nuclei were treated with DNase I (100 μg/ml, 60 min, on ice) or with micrococcal nuclease (15 units/ml, 5 min, 37°C). The nuclei were mixed with an equal volume of high salt buffer (4 M NaCl, 20 mM Tris, pH 7.4, 20 mM EDTA, 0.2% Ammonyx LO), and kept on ice for 30 min.

2.2. *Radioactive labeling of proteins with [^{3}H]adenosine*

Interphase cells were suspended at a 5-fold concentration in medium and, after the addition of 0.5 μM [^{3}H]adenosine (48 Ci/mmol), the cells were incubated for 4 h at 37°C. Metaphase nonhistones were labeled by concentrating cells 5-fold, adding colchicine (0.2 μg/ml), and then incubating the cells with 0.5 μM [^{3}H]adenosine (48 Ci/mmol) for 12–18 h at 37°C.

2.3. *Treatment of samples with snake venom phosphodiesterase*

Isolated chromosomes, purified on Metrizamide density gradients, were resuspended in chromosome isolation buffer (50 mM NaCl, 5 mM Hepes, pH 7.4, 5 mM $MgCl_2$, 0.5 mM $CaCl_2$) containing 0.5% NP40, 1 mM PMSF, and 1 mM TLCK. Snake venom phosphodiesterase was added (5 units/ml), and samples were incubated at 37°C for 0.5, 2.0 and 18 h. Purified nuclei in nuclear isolation buffer supplemented with 0.5% NP40, 1 mM DTT, 1 mM PMSF, and 1 mM TLCK were similarly treated with enzyme.

2.4. *Polyacrylamide gel electrophoresis*

Two-dimensional separation of proteins employed the procedures of O'Farrell et al. [17], with the first dimension separation by nonequilibrium pH gradient electrophoresis. Separation in SDS-polyacrylamide gels used the discontinuous pH conditions of Laemmli [18].

The phosphate-buffered, pH 6.8 gel system of Weber and Osborn [19] was also used. Results similar to those with Laemmli gels were obtained.

3. RESULTS

3.1. *In vivo modification of nonhistone proteins: labeling of cells in culture with [^{3}H]adenosine*

Fig.1 includes autoradiograms of 12.5% gels containing the [^{3}H]adenosine-labeled proteins of metaphase chromosomes, nuclei and nuclear scaffolds. The distribution of modified species for isolated metaphase chromosomes is most explicable (fig.1A). Although long exposures during autoradiography reveal a number of minor bands, shorter exposures show only a single high molecular mass protein. Considering the gels for nuclei and metaphase chromosomes labeled with [^{32}P]NAD [14], the band is identified as poly(ADP-ribose) polymerase. Other bands are seen in the region of the nucleosome core histones. The major species are close to stained bands of core histones, though offset to higher molecular masses.

Of an uncertain nature are the major ^{3}H-labeled bands of nuclei and nuclear scaffolds shown in fig.1B. The patterns of incorporation of

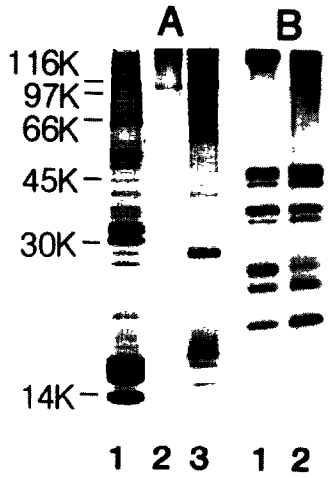

Fig.1. Modification of nonhistone proteins in vivo using [^{3}H]adenosine label. One-dimensional SDS-polyacrylamide gels (12.5%) of labeled proteins were processed by fluorography. (A) Lane 1 contains a stained gel of the proteins of isolated metaphase chromosomes, while lane 2 and, with a greater amount of material, lane 3 contain a fluorogram which reveals the distribution of ^{3}H-labeled proteins. (B) Fluorogram of nuclei (lane 1) and nuclear scaffolds (lane 2) labeled with [^{3}H]adenosine. K, kDa.

[^{3}H]adenosine are similar (fig.1B) with about a dozen major species. However, two-dimensional gels show that instead of forming discrete spots or series of spots, these major bands streak into 2-D gels from the acidic ends of the gels during isoelectric focusing.

Since nucleic acids extensively incorporate [^{3}H]adenosine, one-dimensional gels were not useful for resolving the modified nonhistones of chromatin because of the background radioactivity of DNA fragments produced by micrococcal nuclease digestion.

3.2. *Two-dimensional gel electrophoresis of cell cycle changes in ADP-ribosylation using cells labeled with [^{3}H]adenosine*

To examine the differences in ADP-ribosylation of nonhistone proteins during interphase and metaphase, two-dimensional gel electrophoresis was employed with samples labeled in culture with [^{3}H]adenosine. Fig.2 presents the results. Panel B is a fluorogram that reveals the proteins of interphase nuclei which have incorporated ^{3}H label. Panel C is a fluorogram showing the ^{3}H-labeled proteins of metaphase chromosomes. The difference between the patterns is striking. A large number of nonhistones of interphase nuclei have incorporated the ^{3}H label, but the labeling of a single species, poly(ADP-ribose) polymerase, dominates the fluorograms of metaphase chromosome nonhistones. The difference is not due to metaphase chromosomes having a much less complex nonhistone composition. Stained, two-dimensional gels of both interphase nuclei (panel A of fig.3) and metaphase chromosomes show the presence of hundreds of proteins. And for both metaphase chromosomes and nuclei, the polymerase is a minor component among the hundreds of nonhistone species.

Fig.3 contains autoradiograms of two-dimensional gels which show the effect of snake venom phosphodiesterase in removing [^{3}H]adenosine label from metaphase chromosomes and interphase nuclei. For isolated metaphase chromosomes, label was rapidly removed from the predominant band identified as poly(ADP-ribose) polymerase and from the minor species. Radioactivity incorporated by nuclear proteins was also largely sensitive to treatment with the enzyme. The relative resistance to digestion of some nuclear proteins (such as a group of around 32–40 kDa) may reflect the shielding of these species in the dense, nuclear structure.

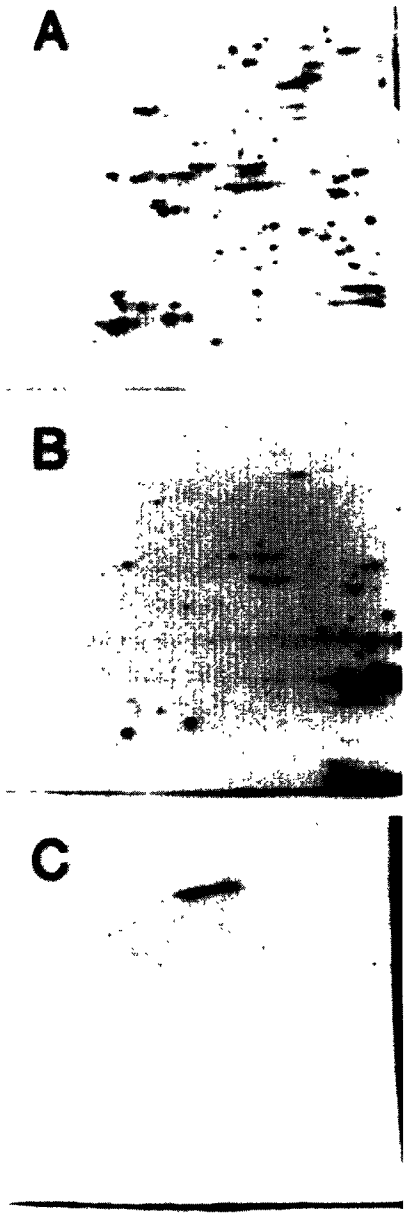

Fig.2. Two-dimensional gels of proteins from interphase nuclei and metaphase chromosomes labeled in culture with [^{3}H]adenosine. (A) Gel, stained with Coomassie brilliant blue, of total nuclear proteins. (B) Fluorogram showing the ^{3}H-labeled proteins of total nuclei. (C) Fluorogram of metaphase chromosome nonhistones labeled with [^{3}H]adenosine.

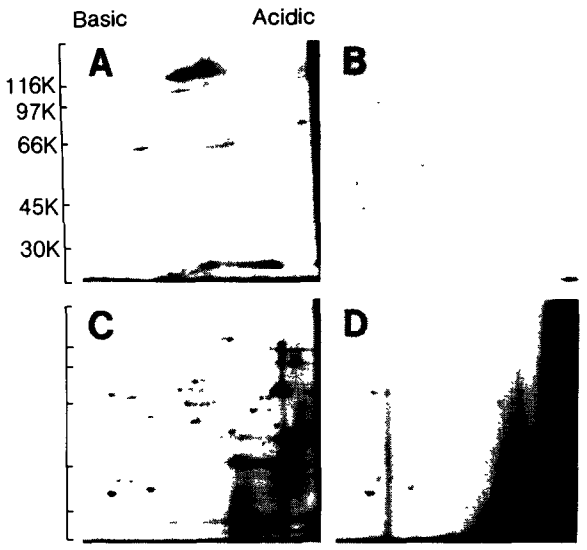

Fig.3. Effect of treatment of [^{3}H]adenosine-labeled proteins of metaphase chromosomes and interphase nuclei with snake venom phosphodiesterase. (A) Fluorogram of two-dimensional gel of control, ^{3}H-labeled metaphase chromosome proteins. (B) Chromosomal proteins treated with phosphodiesterase (5 units/ml, 37°C, 2.0 h). (C) Fluorogram of control, ^{3}H-labeled nuclear proteins. (D) Nuclear proteins incubated with enzyme (5 units/ml, 37°C, 22 h). K, kDa.

4. DISCUSSION

This investigation has revealed a profound difference in the pattern of ADP-ribosylation of nonhistones from interphase nuclei and mitotic chromosomes with [^{3}H]adenosine as an in vivo label. This general result with [^{3}H]adenosine supports the findings for in vitro labeling with [^{32}P]NAD [14].

What is the structural or functional role of the decrease in the modification of nonhistones between interphase and metaphase? ADP-ribosylation could have a structural influence upon the packaging of chromatin fibers and be directly involved in the condensation of metaphase chromosomes. The reduction between interphase and metaphase could also reflect changes in the activity or localization of poly(ADP-ribose) polymerase.

Some differences in the results with [^{32}P]NAD and [^{3}H] were observed. Two-dimensional gels of the proteins of total nuclei and chromatin were qualitatively similar in showing that the two labels were distributed among a large number of proteins. The patterns, however, were not identical. Another difference was that proteins labeled with [^{3}H]adenosine tended to carry fixed lengths of ADP-ribose chains.

Despite these differences, the experiments described here have demonstrated that [^{3}H]adenosine is a useful radioactive label for revealing a substantial decrease in ADP-ribosylation during mitosis.

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