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Localization of proteins forming the outer surface of isolated metaphase chromosomes

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The outer surface of isolated metaphase chromosomes has been investigated by a method of thermally activated tritium labelling. We show that both chromosomal proteins and DNA are tritium-labelled. Fractionation of the chromosomal proteins reveals that scaffold proteins are the most labelled in condensed and EDTA-decondensed chromosomes. Exposition of some scaffold proteins on the outer surface of metaphase chromosomes is suggested.

Metaphase chromosome; Non-histone protein; Scaffold

1. INTRODUCTION

Investigations of metaphase chromosome structure, intensive as they are, have not elucidated in full the topography of chromosomal proteins. It is generally accepted that some non-histone proteins form a mitotic chromosome framework, the scaffold, but scaffold is an operational term only, which designates a structure maintaining a high level of organization after treatment with high salt concentrations and nuclease hydrolysis [1]. Determination of the proteins forming the outer surface of metaphase chromosomes holds interest to provide a more precise definition of the structure of chromosomes and scaffold. Here, the bombardment of isolated chromosomes with thermally activated tritium atoms was used as a tool [2]. This method allows labelling of the molecules exposed on the structure surface only, since the depth of the tritium path in condensed phase is very low (3–5 Å).

The fractionation of chromosomal proteins has revealed that scaffold proteins are the most labelled, and hence probably take part in the formation of the outer surface of metaphase chromosomes.

Abbreviations: TCA, trichloroacetic acid; EDTA, ethylendiaminetetraacetic acid; topo II, topoisomerase II; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

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2. MATERIALS AND METHODS

2.1. *Cell culture and chromosome isolation*

Chromosomes were isolated from a culture of mouse fibroblasts (line A9) according to [3] followed by 0.5% octylglycoside treatment (Sigma, USA) and centrifugation through 0.3 M sucrose on conventional buffer at 4,000 × *g* for 10 min.

2.2. *Chromosome labelling*

Chromosomes were frozen in a thin layer on the inner surface of a reaction flask (previously cooled with liquid nitrogen), lyophilized and labelled with tritium activated on scorching (2,000 K) tungsten (tritium pressure in the system was 5–8 × 10^{-3} mmHg) according to [2]. The unbound and labile tritium was removed by 5-fold centrifugation of the chromosomes in 0.01 M HEPES, 0.003 M $CaCl_2$ (pH 7.4) at 4,000 × *g* for 5 min.

2.3. *Electron microscopy*

Labelled and unlabelled chromosomes were prepared according to [3] and examined with a JEM-100 C electron microscope (Japan).

2.4. *Tritium distribution in metaphase chromosomes*

Labelled chromosomes were sequentially fractionated with 0.3 M and 2 M NaCl. Proteins from extracts were precipitated by addition of TCA. Residual material was hydrolyzed with DNase I to prepare scaffolds. The protein content in the fractions was determined by the method of Lowry [4].

DNA was isolated from metaphase chromosomes according to [5] and its concentration was determined spectrophotometrically.

Radioactivity in the chromosome fractions was measured using a Mark II scintillation counter (Nuclear Chicago).

2.5. *Decondensation of the chromosomes*

Chromosomes were decondensed by incubation with 0.01 M HEPES, 0.005 M EDTA (pH 7.4) at room temperature for 30 min, washed with the same buffer and labelled as described above.

2.6. *Protein analysis*

The total chromosomal proteins, the proteins extracted with 2 M

NaCl, 0.01 M EDTA and DNAse I hydrolysis and scaffold proteins were analyzed by a 12.5% SDS-PAGE according to Laemmli [6] followed by blotting on nylon membrane and fluorography.

The fraction of the total chromosomal proteins was obtained by exposing isolated metaphase chromosomes to 20% TCA, followed by washing of the pelleted proteins with acetone.

The scaffolds were prepared by DNAse I hydrolysis of the chromosomes, with subsequent incubation in 2 M NaCl, 0.01 M EDTA for 30 min at room temperature. The proteins, extracted with 2 M NaCl, 0.01 M EDTA and DNAse I hydrolysis were precipitated from supernatants by addition of TCA.

3. RESULTS

As was shown by electron microscopy neither lyophilization nor labelling appreciably changed the metaphase chromosome structure (Fig. 1). Obviously, it should be taken into account that this test of structural integrity is not accurate enough to detect fine disruptions.

Fractionation of the labelled chromosomes revealed tritium in all fractions analyzed, but scaffold proteins were the most labelled (Table I).

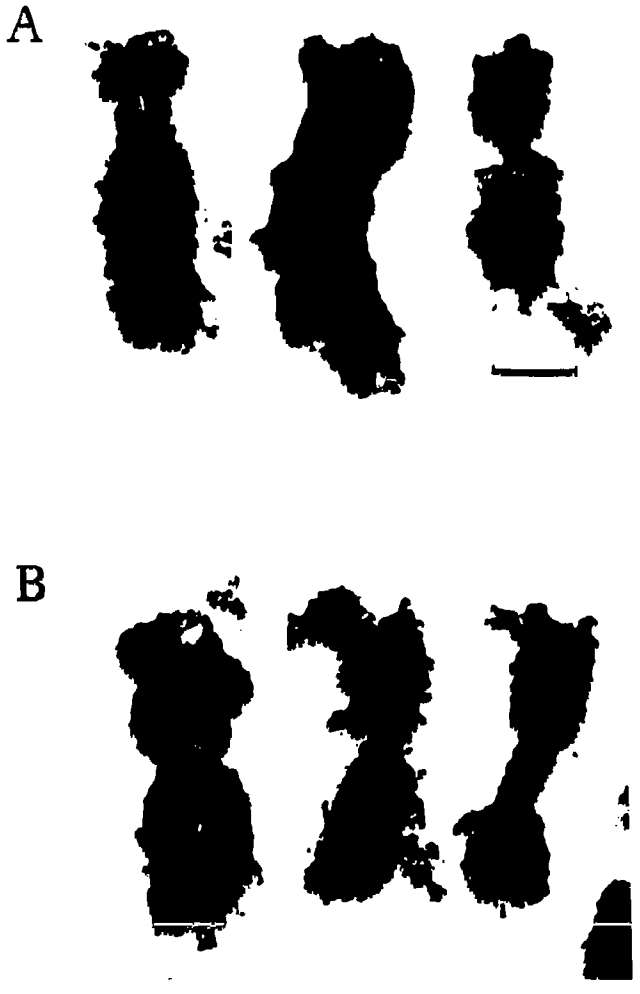

Fig. 1. Electron micrographs of isolated metaphase chromosomes (A) before labelling and (B) after labelling. Bar represents 1 μm.

Table I

The distribution of radioactivity in chromosomal fractions

Fraction	Specific radioactivity (cpm/μg)
Non-histone proteins (extracted with 0.3 M NaCl)	785
Histones (extracted with 2 M NaCl)	1,391
Scaffold proteins	5,480
DNA	1,399

The distribution of tritium in the chromosomal proteins is shown in Fig. 2. Five labelled polypeptides (76, 74, 45, 34 and 28 kDa) were found in the fraction extracted by nuclease hydrolysis and 2 M NaCl, 0.01 M EDTA (Fig. 2, lane 3, indicated by arrows). This fraction included non-histone proteins and histones, but there were no H2B, H2A, H3 and H4 among labelled proteins, although the 28-kDa band may be attributed to the histone, H1. In the fractions of the total chromosomal and scaffold proteins more than 10 labelled polypeptides were found (Fig. 2, lane 2,4).

Since, in the course of tritium labelling, the chromosomal proteins could have covalently bound to DNA, the DNA was isolated after labelling, treated with nuclease and analyzed electrophoretically (not shown). As the proteins were not found in the DNA fraction the probability of their covalent binding is very low.

Apparently, the most exposed metaphase chromosome proteins were the proteins of the scaffold. In order to verify whether these proteins form the outer cover of metaphase chromosomes we treated the latter with

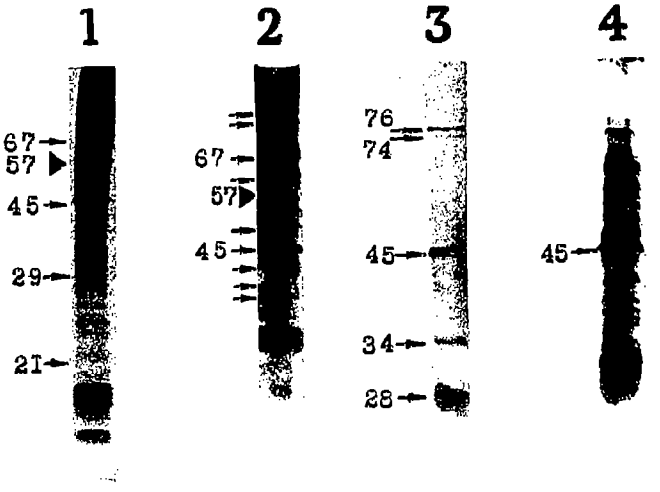

Fig. 2. Coomassie blue staining pattern (lane 1) and fluorographic profiles (lanes 2–4) of tritium-labelled proteins of condensed metaphase chromosomes. (Lanes 1,2) fraction of total chromosomal proteins: (lane 3) fraction of the proteins extracted with 2 M NaCl, 0.01 M EDTA and nuclease digestion; (lane 4) fraction of scaffold proteins. The mol. wts. of the labelled chromosomal proteins were determined according to the markers: phosphorylase B (92.5 kDa), bovine serum albumin (67 kDa), ovalbumine (45 kDa), carboanhydrase (29 kDa), soybean trypsin inhibitor (21 kDa).

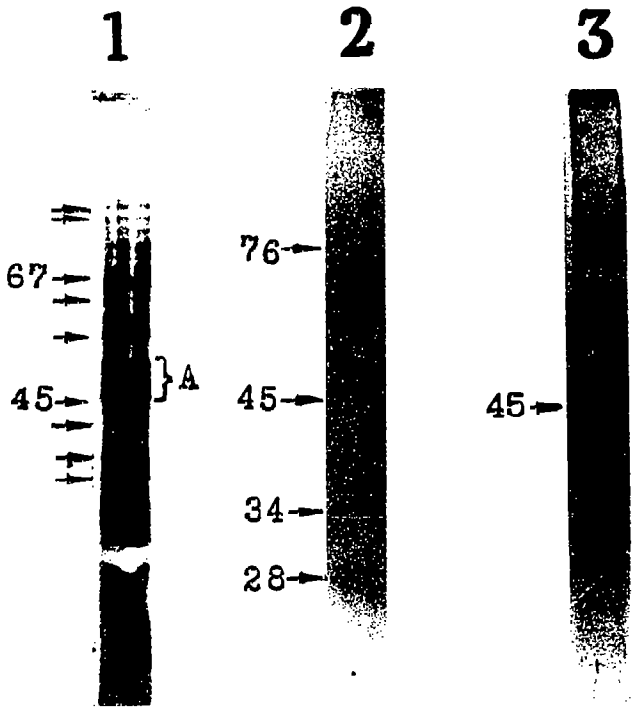

Fig. 3. Fluorographic profiles of the tritium-labelled proteins of decondensed metaphase chromosomes. (Lane 1) fraction of total chromosomal proteins; (lane 2) fraction of the proteins extracted with 2 M NaCl, 0.01 M EDTA and nuclease digestion; (lane 3) scaffold fraction.

0.005 M EDTA. Removal of Ca^{2+} ions induces decondensation of chromosomes to the level of chromatin-like structures and release of some chromosomal proteins [7].

EDTA-decondensed chromosomes were also subjected to tritium labelling, followed by electrophoretic analysis of their proteins. The fraction of the total chromosomal proteins, salt-extracted fraction and scaffold were found to contain the major labelled bands of the same mol. wt. as that from condensed chromosomes, while the extent of their labelling differed (Figs. 2 and 3, indicated by arrows).

4. DISCUSSION

Only a few chromosomal non-histone proteins have been identified so far, so it is difficult to assign our labelled polypeptides to any particular class. The most prominent scaffold protein, topoisomerase (topo) II, has a mol. wt. of 170 kDa. Since none of the labelled polypeptides had mol. wts. above 100 kDa, topo II is probably shielded. This is in accordance with the data of Gasser et al. [8], who showed that topo II was difficult to access by antibodies in condensed chromosomes.

Also, we have found that the major Coomassie-stained band of 57 kDa in condensed chromosomes is not labelled, and hence is completely shielded (Fig. 2, lane 1,2, indicated by arrowheads).

In condensed chromosomes, the most labelled are the polypeptides with mol. wts. of 45–55 kDa (Fig. 3, lane 1,A), which apparently become more accessible to tritium in the course of decondensation.

Lewis et al. [1] reported earlier on cytoskeleton protein admixtures: mainly actin (45 kDa), vimentin (57 kDa) and prekeratin (52 kDa) in the chromosomes isolated according to the method of Wray and Stubblefield (cited in [1]). Since the polypeptides with the same mol. wts. were observed among labelled chromosomal proteins (particularly the 45-kDa major band), it is highly likely that they are cytoskeleton proteins.

In summary, on the one hand the outer surface of decondensed chromosomes is formed by chromatin fibers [7], and on the other, the most exposed proteins proved to be those of the scaffold. Hence, one can assume that some scaffold proteins form the outer surface of chromatin fibers in decondensed chromosomes. In support of this is the fact that in condensed chromosomes the mol. wts. of the major labelled bands coincide with those of the decondensed chromosomes. Consequently, the outer surface of metaphase chromosomes is not a cover of the chromosomes as such, but apparently, is the outer surface of tightly packed chromatin fibers.

Thus, if the exposed proteins are labelled by tritium equally it is likely that some scaffold proteins form the outer surface of isolated metaphase chromosomes.

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