Ultrastructural Localization of Actin in Nuclear Matrices from Mouse Leukemia L5178Y Cells.

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ABSTRACT. We examined the distribution of actin in isolated nuclear matrices from mouse leukemia L5178Y cells using an anti-actin antibody and protein A-conjugated colloidal gold particles. Before immunogold staining, we partially digested the surface lamina of the nuclear matrix with trypsin (Nakayasu and Ueda, *Exp. Cell Res.* **143**, 55-62, 1983) to allow penetration of the gold particles into the nuclear matrix. Trypsin digestion slightly modified the internal structure of the nuclear matrix, but did not affect the actin content in the nuclear matrix nor the reactivity of actin with the antibody. Many colloidal gold particles were present along fibrogranular structures in the nuclear matrix. The results reported here confirm the existence of actin in the interior of the nuclear matrices of L5178Y cells.

The isolated nuclear skeletal structure, the nuclear matrix (1), usually contains a considerable amount of actin (3, 9, 13). Little is known about the function of this nuclear actin, but several recent reports have revealed the involvement of nuclear actin in the synthesis (6, 15), processing (10, 12) and transport (13) of nascent nuclear RNAs. Therefore, we need to know what the architecture of this actin in the nucleus or nuclear matrix is. The nucleoplasm in the huge nuclei of amphibian oocytes is filled with numerous actin filaments (4, 5). Gounon and Karsenti (7) have reported nuclear RNP-like particles associated with these actin filaments in the newt oocyte nucleoplasm.

In contrast, there have been few studies done on the distribution of actin in nuclei of normal size. Recently, our research has suggested that actin is present in a fibrogranular structure, which corresponds to the nucleoplasm, in nuclear matrices of bovine lymphocytes (11). To confirm this, we set out to determine the architecture of the actin in the nuclear matrix by using colloidal gold staining. We found that actin is associated with the fibrogranular structure present in nuclear matrices of mouse leukemia L5178Y cells.

MATERIALS AND METHODS

Protein A-conjugated colloidal gold (about 25 nm in diameter) was prepared by reducing tetrachloroauric acid with sodium citrate as described by Roth *et al.* (14). But, because

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the gold particles might be excluded from the nuclear matrix because of nuclear lamina, the surface lamina was partially removed by trypsin treatment as described in (11) to permit penetration of both the anti-actin antibody and the colloidal gold into the sphere.

Nuclear matrices were prepared from L5178Y cells as described in (13). Briefly, cells were disrupted by the use of an isotonic buffer containing 1 % Triton X-100. The cytoplasmic fraction was separated from the nuclei by low speed centrifugation. The isolated nuclei were digested with DNase I (the supernatant, DNase extract), after which the chromatin was extracted with a high salt buffer (the supernatant, high salt extract). The resulting pellet (nuclear matrix, 1 mg protein) was suspended in 0.5 ml of Buffer A (10 mM PIPES buffer, pH 6.9, containing 2 mM MgCl_2 and 0.1 M NaCl) then digested with 5 μg of trypsin at 30°C for 2 min. The reaction was stopped by the addition of 10 μg of trypsin inhibitor. Little actin was released from the nuclear matrix by this digestion. Immunogold staining was carried out as described by Langanger *et al.* (8) with the following modifications:

Because trypsin-treated nuclear matrices tend to be broken easily by a mechanical force (e.g., centrifugation or pipetting), they first were fixed with glutaraldehyde (0.32 %) for 5 min, after which the remaining active aldehyde groups were blocked by the addition of sodium borohydride (0.1 %, 5 min) to prevent any non-specific binding of the anti-actin antibody. This mixture was centrifuged at 2,000 rpm for 20 min then washed twice with Buffer B (10 mM PIPES buffer, pH 6.9, containing 0.1 % ovalbumin, 2 mM MgCl_2 and 0.1 M NaCl). The trypsin-treated nuclear matrices were treated with 0.7 mg protein/ml of anti-actin antibody (obtained from Biomedical Technologies Inc.) for 2 h with mild shaking then washed twice with Buffer B. Next, the sample was mixed with protein A-conjugated colloidal gold ($\text{O.D.}_{520}=1.0$) for 2 h then washed twice with Buffer B. The immunogold-stained nuclear matrices obtained were fixed at 0°C for 5 h with 3.2 % glutaraldehyde and 0.2 % tannic acid (0.1 M sodium phosphate buffer, pH 7.2), after which they were fixed with 1 % osmium tetroxide at 4°C overnight then dehydrated with ethanol and embedded in an Epon 812 mixture (11).

RESULTS AND DISCUSSION

The intracellular and intranuclear distribution of actin in L5178Y cells is shown in Fig. 1. Western blotting and an enzyme-linked immunosorbent assay showed the presence of actin both in the cytoplasm and in the nucleus. It was clear that the isolated nuclear matrices did contain actin. Among the subnuclear fractions the actin content was remarkably high in the DNase extract (lane 3) and the nuclear matrices (lane 5), the values of the specific contents of actin in both being higher than the value of the cytoplasmic fraction (lane 2). From the intranuclear distribution of protein, we roughly estimated that the nuclear matrix contains about 30 % of the nuclear actin. As described elsewhere (11, 12), actin is resistant to trypsin; but, there was the possibility of actin's reactivity with the anti-actin antibody being decreased by some alteration during trypsin digestion. Western blots of trypsin-treated and untreated nuclear matrices revealed, however, that trypsin digestion affected neither the actin content in the nuclear matrix nor actin's reactivity with the antibody (data not shown).

The localization of actin in the nuclear matrix was examined by immunogold staining (Fig. 2). Slight cytoplasmic contamination was present on the surfaces of isolated nuclei (Fig. 2A). The nuclear matrix (Fig. 2B) has three structurally distinct parts in its sphere: the surface lamina, the internal fibrogranular structure and the

Fig. 1. Detection of actin by Western blotting. Each fraction (lane 2, cytoplasmic fraction; lane 3, DNase extract; lane 4, high salt extract; lane 5, nuclear matrix: 1 μ g protein each) was prepared as described in the text. Chicken gizzard actin (lane 1, 10 ng protein) was purified by the method of (13). After electrophoresis (9), Western blotting was performed as described previously (13). Actin bands were made visible by an enzyme-linked immunosorbent assay (2) using anti-actin antibody (rabbit) from Biomedical Technologies Inc., and alkaline phosphatase-conjugated anti-rabbit IgG antibody from Miles-Yeda Ltd. The arrowhead indicates the position of actin on the blots.

residual nucleoli. Using weak trypsin digestion, we partially removed the surface lamina (Fig. 2C); neither the fibrogranular structure nor the residual nucleoli was damaged by this digestion (Fig. 2B and 2C). Many gold particles were found in the spheres of the trypsin-treated nuclear matrices (Fig. 2C). This confirms the presence of actin in the interior of the nuclear matrix, as previously suggested (11, 13). Control experiments were done with a control serum from another rabbit instead of the anti-actin antibody. In this case, few colloidal gold particles were present in the nuclear matrices (data not shown).

A photograph taken at high magnification (Fig. 2D) shows the distribution of actin in more detail. Many gold particles are located along the fibrogranular structure, which suggests that actin is a constituent of this structure. This agreed with our previous findings (13) which indicated that the fibrogranular structure is altered remarkably by depolymerization of the nuclear matrix actin. Scarcely any colloidal gold particles were detected in the residual nucleoli of the nuclear matrix, which indicate the absence of actin in the nucleoli. But, it is possible that the colloidal gold could not penetrate into the nucleoli because of the high density of these residual nucleoli. Therefore, our results do not necessarily rule out the presence of actin in the nucleoli.

There was the possibility that the nuclear matrix actin might be contaminated with cytoplasmic actin. But, as shown in Fig. 1, the specific actin content in the nuclear matrix fraction was higher than that in the cytoplasmic fraction. Even if cytoplasmic actin had entered the nucleus and been non-specifically adsorbed on its chromatin during nuclear isolation, it should have been removed from the nuclear matrix by

Fig. 2. Electron microscopy of isolated nuclei (A), nuclear matrices (B) and trypsin-treated nuclear matrices (C, D). Samples C and D were fixed after immunogold staining as described in the text; samples A and B were not treated. The arrowheads indicate the nucleoli. Bar, 2 μ m.

the high salt extraction procedure. Therefore, we believe that the labeling of nuclear matrix actin with immunogold particles was not due to contamination by cytoplasmic actin; rather, it shows actin to be a characteristic constituent of the nuclear matrix.

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(Received for publication July 16, 1985)