DIFFERENCES BETWEEN NUCLEUS AND CYTOPLASM IN THE DEGREE OF ACTIN POLYMERIZATION

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ABSTRACT

For purposes of studying the degree of polymerization of actin in nuclei, nuclei from 35S-labeled amoebas (Amoeba proteus) were transplanted into unlabeled cells, which were immediately lysed and extracted under conditions considered to stabilize preexisting fibrous actin. The enucleated 35S-donor cells were similarly treated for analysis of cytoplasmic actin. The extraction conditions permitted separation of soluble (unpolymerized or G) actin from pelletable (polymerized or F) actin, and the radioactivity of each was determined after the actin was separated from other proteins by polyacrylamide gel electrophoresis. We found that about 2/3 of the actin within the nucleus is pelletable, whereas only about 1/3 of the cytoplasmic actin is pelletable. We speculate that polymerized actin in the nucleus is involved in the condensation of chromatin.

KEY WORDS nuclear actin cytoplasmic actin nonmuscle actin polymerized (F) actin unpolymerized (G) actin SDS gel electrophoresis nuclear transplantation amoebas

Because actin has been found in certain nuclear preparations (compare, e.g., references 4, 5, 11, and 13), some investigators have hypothesized that actin within cell nuclei may be involved in such processes as chromosome movement and chromosome condensation. Indeed, some workers (e.g. references 16 and 2) have shown that actin probably plays a role in anaphase movement of chromosomes, but whether nuclear actin acts in any way differently than cytoplasmic actin in this process is not evident. On the basis of some recent work of ours with amoebas (8)—in which actin was shown (a) to have a very "loose" association with nuclei, (b) to be present in nucleus and cytoplasm at equal concentrations although present in 50-fold greater amount in cytoplasm, and (c) to equilibrate rapidly between the two compartments—we speculated that actin is present in the nucleus only for the trivial reason that the nuclear envelope is no barrier to the free movement of that protein through the cell. This implies that nuclear actin is in no way different from that in the cytoplasm and therefore its function is presumably independent of which compartment it happens to be located within at any particular time.

To explore this matter further, we decided to investigate whether the state of the actin in the nucleus is different from that of the actin in the cytoplasm. To do this, we employed the method of Bray and Thomas (1) for determining the degree to which actin may be polymerized. We report here our finding that, despite the earlier suggestion that the actins of nucleus and cytoplasm do not differ, the actin in the nucleus
apparently is substantially more insoluble than that in the cytoplasm.

MATERIALS AND METHODS

Cells, Nuclear Transplantation, and Labeling of Cells

The protozoan *Amoeba proteus* was used throughout this study. The cells were cultured and manipulated as described previously (6). Nuclear transplantations were performed by the method of Jeon and Lorch (10). The proteins of amoebas were 35S-labeled (followed by extensive chases with unlabeled food) as described previously (8).

Assay of "Polymerized" and "Unpolymerized" Actin in Nucleus and Cytoplasm

A total of 150-200 nuclei was transplanted from well-chased 35S-labeled cells into unlabeled recipient cells as follows: A set of five pairs (one donor plus one recipient) of cells was operated upon, and between 30 s (for the fifth transplantation) and 150 s (for the first transplantation) of the completion of the operation, the set of recipient cells was placed in 60 μl of the Nonidet P40 cell lysis and actin stabilization buffer solution of Bray and Thomas (1) and then the next set was operated upon. After accumulation of 40-50 of such sets, 1,000 cold carrier cells were added to the pooled sets, and the total preparation in an ice bath was subjected to homogenization by ultrasonic at 0°C. Bray and Thomas (1) have shown that ultrasonic homogenization provides values for the percent polymerized actin indistinguishable from values obtained by more gentle homogenization methods. Also, note that since transplanted nuclei were at no time outside either the donor or recipient cells, there is no possibility that nuclear proteins are lost from these preparations. Simultaneously with the lysis of the recipient cells, the enucleate 35S-donor cells were lysed to provide material for analysis of cytoplasms. Each lysate was centrifuged at 100,000 g for 3 h at 4°C; the supernatant solutions, considered to contain unpolymerized actin, were separately solubilized in 2% sodium dodecyl sulfate (SDS)-5% mercaptoethanol (12), as were the materials that were pelleted. The solubilized preparations were frozen and thawed in preparation for electrophoresis. For that purpose, 30-100% of each sample was heated at 100°C for 2 min and then run on slab polyacrylamide gels as described previously (8). After staining with Coomassie Blue R, the actin bands, recognized by methods described previously (8), were cut from the gels and the amount of 35S present was assayed by liquid scintillation spectrometry (15, 17). The values obtained (less background) ranged from 70 to 712 cpm/band for the nuclear fractions and from 666 to 8,590 for the cytoplasmic fractions, depending on the labeling conditions.

RESULTS

We have taken advantage of the ability to "isolate" labeled nuclei in unlabeled cytoplasms by nuclear transplantation and to use simultaneously the labeled enucleate donor cells as a source of undisturbed cytoplasms to compare the proportion of pelletable actin—defined by Bray and Thomas (1) as that which is polymerized—in nuclei and in cytoplasms. Table I shows the proportion of total actin that is ostensibly polymerized in each compartment and reveals that the proportion is consistently higher in nuclei. In fact, in nuclei about 2/3 of the actin is polymerized, whereas only about 1/3 of cytoplasmic actin is.

DISCUSSION

The results show an unmistakable difference between nucleus and cytoplasm in the proportion of actin that is insoluble. Whether the sedimentable material is entirely fibrous actin is not clear; conceivably, the actin within the nucleus could be complexed with other insoluble material and, for that reason, is pelletable under the extraction conditions we used. This is highly unlikely, however, since virtually all of the actin is leached out of nuclei during isolation in standard salt-nonionic detergent-spermidine solution (8). This isolation procedure does not employ strong homogenization or freezing procedures, yet the actin diffuses out within the few minutes required to isolate the nuclei.

It has been argued that the nuclear transplantation operation itself might be responsible for a

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<th>Exp</th>
<th>Total actin that is insoluble in:</th>
<th>Nuclei</th>
<th>Cytoplasms</th>
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<tr>
<td>1</td>
<td>59</td>
<td>26</td>
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<tr>
<td>2</td>
<td>71</td>
<td>34</td>
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<td>3</td>
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The percentages are based on the radioactive counts found in the actin bands cut from the electrophoretic gels for the pelleted and supernatant fractions extracted from nuclei and cytoplasms as described in the text.
reduced level of actin polymerization in the cytoplasm. We consider this possibility unlikely because enucleate cytoplasms have been found to possess at least as high a proportion of polymerized actin as whole cells that have never been subjected to micromanipulation. (The nuclei comprise only 2% of the volume of amoebas, and thus make little contribution to whole cell values.) Also of concern in interpreting the data reported here is the fact that some movement of labeled actin from transplanted nucleus to cytoplasm undoubtedly occurs in 30-150 s between the time that nuclear transplantation is effected and the time that the recipient cells are extracted. Since actin in the cytoplasm is less likely to be polymerized than that in the nucleus, the values reported for the proportion of nuclear actin that is insoluble are likely to be lower than is actually the case. Thus, the observed difference between nucleus and cytoplasm is probably underestimated.

Regardless of the reason for the actin insolubility, clearly some (apparently submicroscopic) structural difference exists between the pools of actin in nucleus and cytoplasm. This contradicts our previous speculation (8) that because actin is (a) freely exchangeable between the two compartments, (b) present in equal concentration in both compartments, and (c) not firmly bound in the nucleus under certain extraction conditions—there is little difference between the actins of nucleus and cytoplasm. Now we must conclude that, while freely exchangeable between nucleus and cytoplasm, actin in amoebas apparently is more highly organized within the nucleus. Since it is impossible to rule out the possibility that some polymerization or depolymerization occurs at the moment of cell lysis, and since, as noted above, the proportion of nuclear actin that is polymerized is likely to be underestimated, the values we report should be considered to represent only the relative degree of organization of actin. A recent report (3) provides entirely different kinds of evidence that a substantial proportion of the actin within Xenopus germinal vesicle nuclei is associated with a relatively insoluble structural matrix, a conclusion basically in agreement with our findings.

What is the biological significance of actin's being part of the structural organization inside the nucleus? We believe that too little information is available for drawing strong conclusions, but we cautiously suggest that the polymerized actin is involved in the condensation of chromatin into heterochromatin. Although some investigators (compare, e.g., references 5, 13, and 14) have reported the presence of significant amounts of actin in chromatin, others (4) were unable to detect differences between euchromatin and heterochromatin in the amount of actin; we, however, are concerned with the state of actin. Our suspicion that actin may be involved in chromatin condensation is based on a recent finding that actinomycin D, which causes a marked condensation of chromatin into heterochromatinlike material (9), is responsible for a remarkable increase in the amount of actin that is tightly bound within the nucleus (7). We recognize that even this observation tells us nothing about possible cause-and-effect relationships between actin binding and chromatin condensation, but we believe that the relationship deserves further study.

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REFERENCES