ACTIN-LIKE PROTEIN OF THE CYTOPLASM IN THE CHROMATIN OF *DICTYOSTELIUM DISCOIDEUM*

Elisabeth WEISS and Volkmar BRAUN
Lehrstuhl Mikrobiologie II, Universität Tübingen, Auf der Morgenstelle 28, D-7400 Tübingen 1, FRG

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1. Introduction

The cellular slime mould, *Dictyostelium discoideum*, is an attractive eukaryotic microorganism for studies on cellular development and differential gene expression [1]. The genome has a low complexity, only 7 times that of *Escherichia coli* [2]. A large fraction of the chromatin is active as template [3] and the developmental cycle is determined, at least in part, by differential gene transcription [4]. Here we describe the purification of chromatin from different stages of development. Two methods were used for the isolation of nuclei, one applying 1% Triton X-100 and the other without use of a detergent. Washing a crude nuclear fraction with 1% Triton X-100 buffer resulted in an enrichment of two polypeptides among the chromatin proteins with mol. wt 44 000 and 180 000, respectively (designated 44 k and 180 k). The 44 k polypeptide was selectively absorbed from the cytoplasm. Partial peptide mapping (not shown) revealed similarity with rabbit muscle actin. The results suggest that recent publications which report actin and myosin as chromatin constituents, dealt with cytoplasmic contaminations since they all employed a nonionic detergent for lysing cells and purifying nuclei.

2. Methods

Dictyostelium discoideum, strain Ax-2 (obtained from G. Gerisch, Basel) was grown on the nutrient medium in [5] supplemented with 1.8% maltose. Cells were harvested in the logarithmic growth phase ($6–9 \times 10^6$ cells/ml). In order to study aggregation competent cells, exponentially growing cells were washed 3 times in 17 mM Soerensen phosphate buffer (pH 6.0), resuspended at 1×10^7 cells/ml in the same buffer and shaken at 23°C for 8 h [6]. For in vivo labelling 10^9 cells were shaken for 11 h in 100 ml phosphate buffer (pH 6.0) containing 4 mCi [^{35}S]-methionine.

2.1. *Isolation of nuclei*

Procedure A Nuclei were isolated from amoebae as in [7] except that a 48–54% sucrose–TKM gradient was used. The TKM buffer consisted of 50 mM Tris–HCl (pH 7.5), 25 mM KCl, 5 mM $MgCl_2$, 5 mM $NaHSO_3$, 0.1 mM PMSF (phenylmethyl sulfonylfluoride), 50 mg/ml each of penicillin and streptomycin. Cells were disrupted by N_2 cavitation at 45 Atm pressure for 20 min [8] at 1×10^8 cells/ml in a buffer containing 0.01 M Hepes (pH 7.5), 5 mM $MgCl_2$, 0.25 M sucrose, 0.1 mM PMSF and 5 mM $NaHSO_3$. The lysate was centrifuged at $500 \times g$ for 5 min to sediment the intact cells. A crude nuclear fraction was obtained by 15 min centrifugation of the supernatant at $2000 \times g$. All isolation procedures were carried out at 4°C. The nuclei were further purified as in [7]. The ratio of protein/DNA obtained was 6:1 (g/g).

Procedure B Washed amoebae were lysed by shaking in 0.32 M sucrose–TKM, 1% Triton X-100 for 5 min (1×10^8/ml). Intact cells were removed by centrifugation ($300 \times g$, 5 min) and nuclei sedimented at $2000 \times g$ for 15 min. They were washed once with 0.32 M sucrose–TKM buffer and further

purified by centrifugation through the sucrose gradient described in procedure A or by repeated washes with 0.32 M sucrose–TKM buffer.

2.2. *Isolation of chromatin*

The purified nuclei (2×10^{10}) were washed once with cold 20 ml 0.32 M sucrose–TKM buffer, containing 1% Triton X-100. After 5 min the suspension was centrifuged at 2000 × *g* for 15 min. The nuclei were washed then twice with 20 ml cold 75 mM NaCl, 25 mM EDTA (pH 7.5), 0.1 mM PMSF, 5 mM $NaHSO_3$, and sedimented for 15 min at 3000 × *g* and 4000 × *g*, respectively. Chromatin was washed once with 20 ml cold 50 mM Tris–HCl, 0.1 mM PMSF, and sedimented at 20 000 × *g* for 20 min. It was finally washed with 10 ml cold 10 mM Tris–HCl (pH 8.0) and spun down at 40 000 × *g* for 30 min. The ratio of protein/DNA obtained was 2.5:1 (w/w). Three preparations showed a A_{260}:A_{280} ratio of 1.45 ± 0.12.

The cytoplasmic fraction was obtained by freezing and thawing 10^9 cells in 20 ml 10 mM Tricin buffer (pH 7.5), 10% sucrose, 0.5 mM PMSF. The lysate was centrifuged at 800 × *g* for 20 min and the supernatant at 15 000 × *g* for 30 min. The resulting cytoplasmic fraction (supernatant) was dialysed against distilled water in the cold and lyophilized.

2.3. *Analytical gel methods*

Proteins were separated on polyacrylamide–SDS gels with 10–15% acrylamide [9]. Chromatin was dissolved in sample buffer and heated for 2 min at 100°C. Gels were stained by the method in [10]. Radioactive gels were infiltrated with PPO, dried and fluorographed with preflashed Kodak RP X-Omat film as described [11].

Protein was determined by the Lowry method [12].

3. Results

Using the modified method [7], clean nuclei were isolated with an intact outer membrane, which were devoid of any cytoplasmic contaminants. To isolate the chromatin, the outer membrane was removed by washing the nuclei with 1% Triton X-100 sucrose buffer. They were then lysed by treatment with NaCl–EDTA buffer. It was found that washing the crude nuclear fraction with sucrose buffer containing Triton X-100 or lysing the cells in this buffer (procedure B) resulted in a dramatic increase of chromosomal proteins with mol. wt 44 000 and 180 000, respectively (fig.1, cf. rows 2,3 with 4–7). The amount of these proteins was much less when the cells were disrupted and the nuclei purified without the use of any detergent. No differences were found in the chromatin proteins from cells of different stages of development (fig.1, row 2,3).

A reconstitution experiment was done to test if the 44 k and 180 k proteins were true chromatin proteins or chromatin contaminations. The crude nuclear fraction from cells in the growth phase was divided, 50% was treated with ^{35}S-labeled cytoplasm in sucrose–TKM buffer containing 1% Triton X-100, and the other half was incubated with ^{35}S-labeled cytoplasm in sucrose–TKM buffer without detergent. The nuclear fractions were purified and chromatin was isolated.

Based on the specific activities of the cytoplasmic fraction and the chromatin protein, 0.26% of the cytoplasmic protein contaminated the chromatin from the nuclei when isolated by the method without Triton X-100 (table 1). The contamination of chromosomal proteins of nuclei that had been washed with the detergent was 3-times higher, 0.87%.

Since a cytoplasmic fraction with a high specific activity was used in the reconstitution experiment, it was possible to identify the chromosomal polypeptides of cytoplasmic origin by autoradiography (fig.1, row 7,8). It is evident that most of the 44 k protein is of cytoplasmic origin. This band is only faintly detectable in pure chromatin after prolonged exposure and is not visible in fig.1. In the cytoplasmic fraction this polypeptide was shown to be only a minor constituent by SDS–polyacrylamide gel electrophoresis. Therefore the 44 k protein was apparently selectively adsorbed to chromatin by the use of Triton X-100.

4. Discussion

The data show that if chromatin from *Dictyostelium discoideum* was isolated from nuclei which have been purified without the use of Triton X-100 (procedure

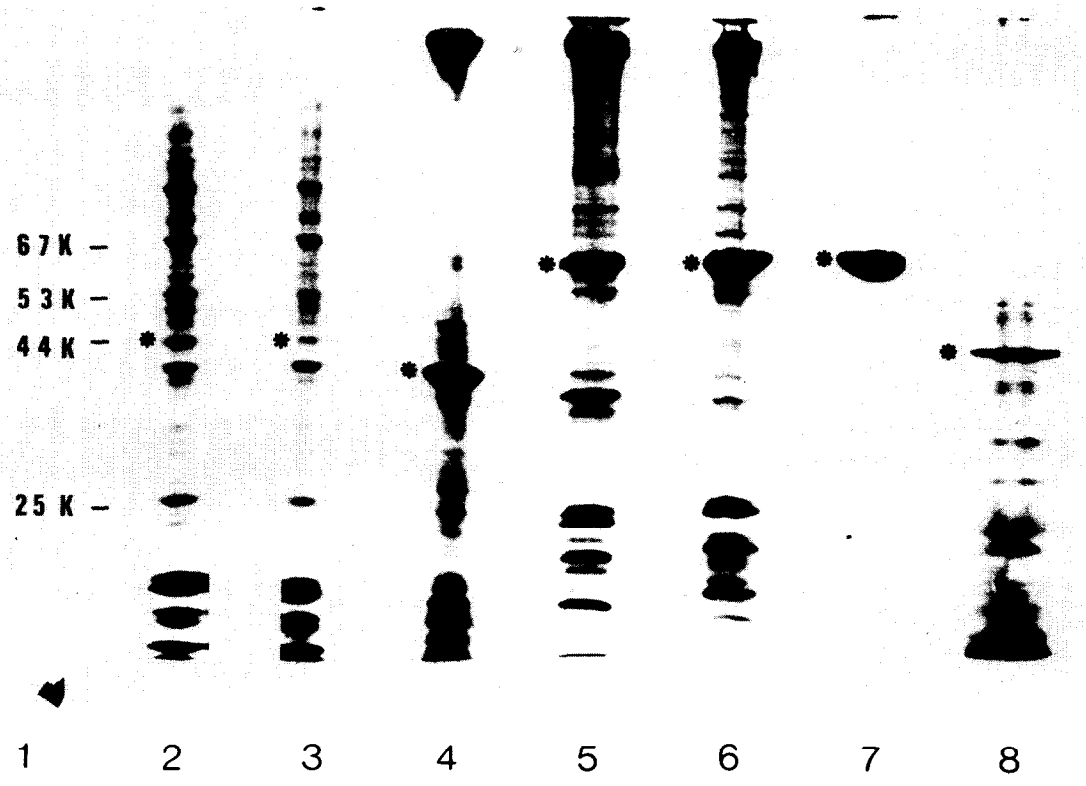

Fig.1. Analysis of chromatin proteins of *Dictyostelium discoideum* by SDS–polyacrylamide gel electrophoresis. (1) Standard proteins (from top to bottom: bovine serum albumin, γ-globulin, ovalbumin, chymotrypsinogen). The numbers denote the molecular weights expressed in mol. wt × 10^{-3}. (2) Chromatin from vegetative cells isolated by procedure A. (3) Chromatin from aggregation competent cells (isolation procedure A). (4) Chromatin proteins isolated from nuclei treated with 1% Triton X-100 sucrose–TKM buffer. (5) Chromatin from vegetative cells lysed with 1% Triton X-100–sucrose–TKM buffer. The nuclei were purified by sucrose gradient centrifugation. (6,7) Chromatin prepared as in (5) but the nuclei were purified by repeated washes with 0.32 M sucrose–TKM buffer instead of sucrose gradient centrifugation. Cells were lysed in the presence of a [^{35}S]methionine-labeled cytoplasmic fraction (2000 × *g* supernatant of procedure B). (8) Reconstitution experiment as described in table 1 under B (adsorption of radioactive cytoplasmic proteins during isolation of nuclei in the presence of Triton X-100). (1–6) Staining of proteins with Coomassie blue; (7,8) autoradiograms. The gels of (2,3) and (6,7) are from the same experiments, (4,5) and (8) from different experiments. The star indicates the positions of the 44 k protein in the different electrophoreses.

Table 1
Extent of chromatin contamination by cytoplasmic proteins

Fraction	Spec. act. (cpm/μg protein)
Cytoplasmic proteins	23 000
Chromatin proteins A	60 = 0.26%
Chromatin proteins B	200 = 0.87%

The total radioactivity of the cytoplasmic fraction was 150 × 10^6 cpm in A and 101 × 10^6 cpm in B. (A) Crude nuclear fraction washed with sucrose–TKM buffer. (B) Crude nuclear fraction washed with 1% Triton X-100–sucrose–TKM buffer

A) the content of actin-like and myosin-like proteins was very low compared to preparations where detergents had been used [13,14]. The concentration of these proteins was significantly raised if Triton X-100 was present during lysis of cells (procedure B) or during the washing of the crude nuclear fraction. Triton X-100 removes the outer nucleus membrane and detaches microfilaments from the plasma membrane [15]. Perhaps it also affects the intranuclear actin. Intranuclear actin in *Dictyostelium* was shown by DMSO-induced polymerisation of G-actin in nuclei

[16,17]. Washing the isolated nuclei with buffer of low ionic strength removed predominantly a 44 k protein (data not shown), demonstrating that actin in nuclei is presumably a nucleoplasmic protein rather than a chromatin constituent.

The reconstitution experiment shows that the high concentration of this polypeptide in the chromatin of nuclei, purified by washes with detergent–sucrose buffer, was due to the adsorption of cytoplasmic protein. Lysing cells or washing a crude nuclear fraction with Triton X-100 selectively induced attachment of the polypeptide presumed actin or actin filaments, to chromatin. It is of interest that nuclei isolated by procedure B contained an increased content of a myosin-like protein in chromatin but the corresponding chromosomal polypeptide was not labeled in the reconstitution experiment (fig.1). Myosin in the cytoplasmic fraction used in that study was labeled.

The peptide pattern obtained by proteolytic degradation of the 44 k protein by the method in [18] was similar to the cleavage pattern of rabbit actin, so it is likely that the 44 k protein was actin. In studies demonstrating a large amount of actin and other contractile proteins as chromatin constituents from various eukaryotes [13,14,19], a non-ionic detergent was applied for lysing cells or purifying nuclei. In the light of the artificial adsorption of an actin-like protein to chromatin, as shown here, it should be considered that the isolation procedure employed for the other eukaryotes yielded high concentrations of actin in chromatin which is, at least to this extent, not naturally present in chromatin.

References

[1] Loomis, W. F. (1975) *Dictyostelium discoideum:* A developmental system, Academic Press, London, New York.
[2] Firtel, R. A. and Bonner, J. (1972) J. Mol. Biol. 66, 339–361.
[3] Firtel, R. A. and Lodish, H. F. (1973) J. Mol. Biol. 79, 295–314.
[4] Alton, T. H. and Lodish, H. F. (1977) Dev. Biol. 60, 180–206.
[5] Watts, D. J. and Ashworth, J. M. (1970) Biochem. J. 119, 171–174.
[6] Beug, H., Katz, F. E. and Gerisch, G. (1973) J. Cell Biol. 56, 647–658.
[7] Rogge, H. and Risse, H.-J. (1974) Hoppe Seyler's Z. Physiol. Chem. 355, 1467–1470.
[8] Odenthal, H. (1973) PhD Thesis, University of Freiburg.
[9] King, J. and Laemmli, U. K. (1971) J. Mol. Biol. 62, 465–477.
[10] Fairbanks, G., Steck, T. L. and Wallach, D. F. H. (1971) Biochemistry 10, 2606–2617.
[11] Laskey, R. A. and Mills, A. D. (1975) Eur. J. Biochem. 56, 335–341.
[12] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[13] Pederson, T. (1977) Biochemistry 16, 2771–2777.
[14] Douvas, A. S., Bakke, A. and Bonner, J. (1977) in: The Molecular Biology of the Mammalian Genetic Apparatus (Ts'o, P.O.P. ed) vol. 1, pp. 143–162, Elsevier/North-Holland, Amsterdam, New York.
[15] Tuchman, J., Alton, T. and Lodish, H. F. (1974) Dev. Biol. 40, 116–128.
[16] Fukui, J. (1978) J. Cell Biol. 76, 146–157.
[17] Fukui, J. and Katsumaru, H. (1979) Exp. Cell Res. 120, 451–455.
[18] Cleveland, D. W., Fischer, S. G., Kirschner, M. W. and Laemmli, U. K. (1977) J. Biol. Chem. 252, 1102–1106.
[19] Douvas, A. S., Harrington, C. A. and Bonner, J. (1975) Proc. Natl. Acad. Sci. USA 72, 3902–3906.