Nonhistone proteins of the transcriptionally active chromatin fraction of *Physarum polycephalum*, associated with nucleosome linker DNA instead of histone H1

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It was established that 2 nonhistone proteins preferentially associated with the transcriptionally active chromatin fraction of *Physarum polycephalum* (M_r = 37000 and 39000) are bound to nucleosome linker DNA instead of histone H1. This observation suggests the possibility that 37 and 39 kDa proteins are important structural elements of active chromatin, involved in maintaining its open conformation.

Physarum polycephalum *Nonhistone protein* *Histone H1* *Transcriptionally active chromatin*

1. INTRODUCTION

Nonrandom distribution of chromosomal proteins between transcriptionally active and inactive regions of animal genome has been reported by several authors (for reviews see [1,2]). Proteins specifically associated with transcribed chromatin have lately attracted a great deal of attention because of their possible involvement in gene regulation. Studying transcriptionally active chromatin of the primitive eukaryote *Physarum polycephalum*, we have shown that, like in animal cells, it is enriched in several nonhistone proteins, including those of the HMG class, and has a significantly reduced content of histone H1 [3–5]. Here we show that two of the nonhistone proteins specific for the active chromatin fraction of *Physarum* are bound to the nucleosome linker DNA instead of histone H1.

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Abbreviations: HMG, high mobility group; MNase, micrococcal nuclease; bp, base pairs

2. EXPERIMENTAL

Microplasmodia of *P. polycephalum* (strain M_3CIV) were grown in submerged shaken cultures in semidefined medium [6]. Isolation of nuclei and chromatin fractionation was as described previously [4].

Electrophoresis of chromosomal proteins was performed in the SDS-polyacrylamide gel system of Laemmli [7], using 15% polyacrylamide, 0.1% SDS · slab gels. The gels were stained with Coomassie blue and scanned with a densitometer.

MNase digestion products were electrophoresed on 4% polyacrylamide slab gels according to [8]. For analysis of DNA fragments of separated nucleoprotein particles, individual bands were cut from the gels, DNA extracted [9] and subjected to electrophoresis on 3.5% polyacrylamide gel as in [10]. As molecular mass markers AluI restriction fragments of pBR322 DNA were used.

To determine protein composition of nucleosomal species, gel strips were incubated in the sample buffer of [7], positioned in wells of SDS-polyacrylamide protein gel and electrophoresed as in [5]. Protein patterns were revealed by either Coomassie blue or silver [11] staining.

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3. RESULTS AND DISCUSSION

Fig.1 shows densitometer scans of SDS-polyacrylamide gel electrophoretograms of proteins associated with the transcriptionally active and inactive chromatin fractions of *P. polycephalum*, obtained by the MNase-$MgCl_2$ procedure of [12]. It can be seen that the active fraction, in accordance with the previous results [3–5], is depleted in histone H1 and enriched in a specific subset of nonhistone proteins.

In this work we focused our attention on proteins of M_r = 37000 and 39000, which are the most abundant nonhistone proteins of the active chromatin. To obtain some insight into the role of these proteins, we found it advisable to determine their location in the chromatin fiber. For this purpose, proteins associated with mononucleosomes and with nucleosome multimers of active and inactive fractions were analysed. The results are shown in fig.2. Nucleosomal particles liberated by MNase from both chromatins were fractionated into three different size classes, i.e., monomers, dimers and higher oligomers by SDS-PAGE under non-dissociating conditions, and afterwards protein composition of separated particles was analysed by running SDS-polyacrylamide gels. Two gels were always run in parallel, one for Coomassie blue

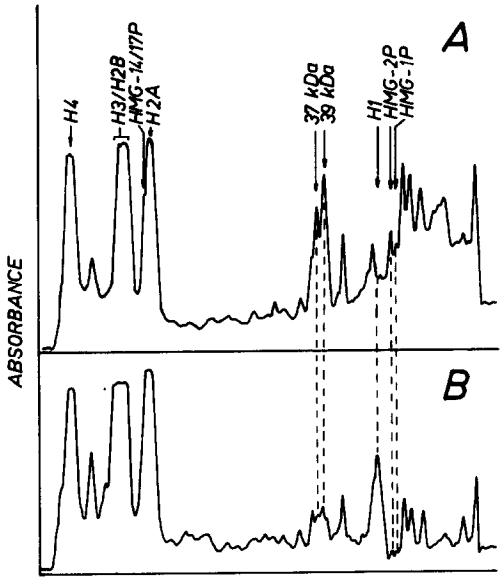

Fig.1. Electrophoretic profiles of proteins associated with transcriptionally active (A) and inactive (B) chromatin fractions of *P. polycephalum*. Densitometer scans of gel photographs are shown. The samples applied were from identical amounts of chromatin. Migration was from right to left.

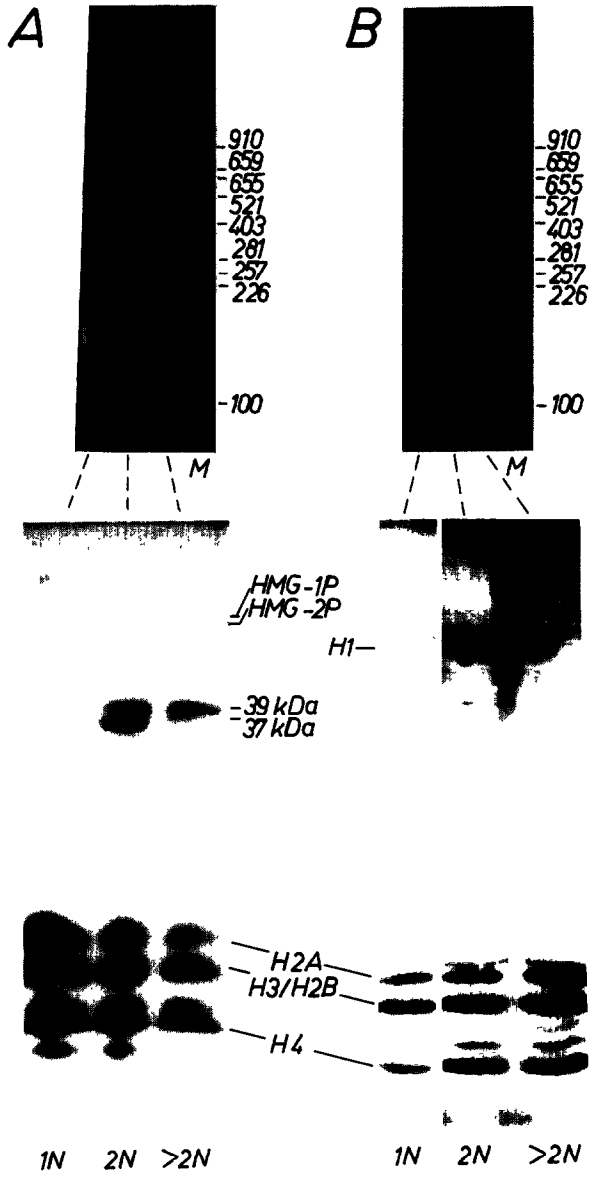

Fig.2. Composition of nucleosomal particles from the active (A) and inactive (B) chromatin fractions. Top panels, electrophoretic profiles of DNA fragments. M, marker pBR322/AluI restriction fragments, sizes in bp. Bottom panels, electrophoretic profiles of proteins. 1N, 2N and >2N designate nucleosome monomer, dimer and higher oligomers, respectively.

staining (better visualization of nonhistone proteins), the other for silver staining (better visualization of histone H1). Since nucleosomes of active and inactive chromatin fractions have different electrophoretic mobilities (not shown), nucleosomal classes were identified by determining the size of their DNA fragments (these are identical for the homologous particles from active and inactive chromatin; fig.2, top panels).

It is clear from fig.2 that 37 and 39 kDa proteins are absent in the monomer particles (average length of DNA fragment 154 ± 2 bp, from the mean ± SD from 3 independent gels) but are associated with dimers and higher oligomers of active chromatin fraction. This indicates that the studied nonhistone proteins are bound to the linker DNA fragment of the nucleosome, similarly as histone H1 in the inactive chromatin.

The above results strongly suggest that the process of transcriptional activation in *P. polycephalum* may involve both the removal of histone H1, as similarly postulated for animal cells (e.g. see [13,14]), as well as association of specific nonhistone proteins (M_r = 37000 and 39000) with nucleosome linker fragments in the activated region. It is conceivable that the binding of these proteins instead of histone H1 could be responsible for maintaining the chromatin fiber in an extended conformation. A possibility of the involvement of defined nonhistone proteins in conferring open conformation of nucleosomes on active ribosomal genes in *Physarum* has been suggested by Prior et al. [15] in their model of the lexosome.

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