RESEARCH COMMUNICATION

Prothymosin α is a chromatin-remodelling protein in mammalian cells

Jaime GÓMEZ-MÁRQUEZ[1] and Pilar RODRÍGUEZ

Departamento de Bioquímica y Biología Molecular, Facultad de Biología, Universidad de Santiago de Compostela, 15706 Santiago de Compostela, Galicia, Spain

Prothymosin α (ProTα) is an abundant mammalian acidic nuclear protein whose expression is related to cell proliferation. Here we report that in HL-60 cells overexpressing ProTα, the accessibility of micrococcal nuclease to chromatin is strongly increased. In the DNA ladder generated by the nuclease activity, the sizes of the mononucleosome (146 bp, the DNA fragment that is bound to the histone octamer) and its multimers correspond to nucleosomes lacking histone H1. The percentage of histone-H1-depleted chromatin (active chromatin) is also higher in the cells overexpressing ProTα. On the basis of these and previous findings, we propose a biological role for ProTα in the remodelling of chromatin fibres through its interaction with histone H1.

INTRODUCTION

Eukaryotic chromatin consists of DNA complexed with histones and other proteins. This association is essential for some of the most important cellular processes, such as DNA replication and transcription, therefore the mechanisms involved in chromatin remodelling are of basic interest. The elemental structural unit of chromatin is the nucleosome, a bead-like structure composed of a short length of DNA wrapped around a core histone octamer [1]. The repeating nucleosome cores further assemble into a second level of organization, the 30 nm chromatin fibre, which is stabilized by histone H1 via its association with the internucleosomal linker DNA [2].

Prothymosin α (ProTα) is an abundant small acidic nuclear protein widely distributed in mammalian cells and tissues [3]. The expression of the ProTα gene is related to cell proliferation [4–8] and is activated by c-*myc* genes [9,10]. Nevertheless, in spite of its abundance and wide distribution, until now the biological function of ProTα has remained obscure, although accumulating experimental evidence points to a role for this protein in chromatin organization. In support of this view are its localization and abundance inside the cell nucleus [4,11–13] as well as its highly acidic character (pI 3.5). In this regard, anionic regions have been identified in several chromatin and chromosomal proteins [14]. Moreover, ProTα shares significant homology with nucleoplasmin, a chromatin-remodelling protein that is very abundant in the nucleus of *Xenopus* [15] and is capable of interacting with histones *in vitro* [16,17].

The present work was an attempt to determine whether or not ProTα has a role in chromatin structure. For this purpose, we used as a biological system HL-60 cells stably transfected with plasmids expressing the ProTα cDNA in either the sense or the antisense orientation. With this system, we have previously shown that overexpression of ProTα stimulates cell proliferation, shortening the G_1-phase of the cell cycle, which interferes with differentiation [4].

MATERIALS AND METHODS

HL-60 cell culture and nuclei isolation

Untransfected and transfected [with the pMAM*neo* vector, with the pMAM*neo*-short sense ProTα cDNA (sense clone) or with the pMAM*neo*-short antisense ProTα cDNA (antisense clone)] HL-60 cells were obtained and cultured as described previously [4]. With this system, we have previously demonstrated that in the sense clones (overexpressing ProTα) there is an increase in the levels of this protein inside the nucleus [4]. Actively growing HL-60 cell clones were induced with 5 μM dexamethasone 24 h before nuclei preparation. Nuclei were then isolated from the different HL-60 cell clones according to the method of Levy-Wilson et al. [18]. Nuclei concentration was adjusted to 0.6 mg DNA/ml.

Micrococcal nuclease (MNase) digestion of isolated nuclei and analysis of MNase digests

Digestion of nuclei with MNase was carried out following the protocol of Keene and Elgin [19]. Briefly, parallel aliquots of nuclei were incubated in digestion buffer at 37 °C for 10 min with various concentrations (0.25–1.5 units/ml, final concentration) of MNase (Boehringer Mannheim). Reactions were terminated by lysing the nuclei, incubation with proteinase K at 55 °C for 4 h and then extraction with organic solvents. Samples were treated with a mixture of RNases A and T (50 μg/ml and 2 μg/ml respectively). After incubation at 37 °C for 2 h, the DNA was precipitated and resuspended in 10 mM Tris/HCl, 1 mM EDTA, pH 7.4. Aliquots of the various MNase digests were analysed by electrophoresis in 1 % (w/v) agarose gels. For the purification of DNA fragments (generated by the MNase digestion) from the agarose gels, a QIAEX II Gel Extraction kit (Qiagen) was used. Gel-purified fragments were also analysed in 1 % (w/v) agarose gels.

Chromatin fractionation

The fractionation of chromatin was by the method of Zhao et al. [20]. Two fractions were obtained: a supernatant corresponding to the histone-H1-depleted active chromatin and a pellet that contained the bulk chromatin. To verify the depletion in histone H1, aliquots from both types of chromatin were analysed by standard SDS/PAGE (15 % gels). Gels were stained with Coomassie Brilliant Blue. The relative amount of each type of chromatin was established by measuring the A_{260}.

Abbreviations used: ProTα, prothymosin α; MNase, micrococcal nuclease.

[1] To whom correspondence should be addressed (e-mail bnjgm@usc.es).

RESULTS AND DISCUSSION

For analysis of chromatin structure, digestion with MNase was used. This enzyme releases mono- and oligonucleosomes because DNA in chromatin is protected from digestion, except at particular sites where the linker DNA joins the nucleosomes. Thus when histone H1 is present on the nucleosomes, 166 bp are protected from the nuclease digestion. However, if histone H1 is absent, digestion with the nuclease is greatly facilitated and a protected fragment of 146 bp is generated, which represents the DNA associated with the histone octamer that forms the nucleosome core particle.

As shown in Figure 1(a), after incubation of isolated nuclei with different concentrations of MNase, we found that in the HL-60 cells transfected with the sense ProTα cDNA the overexpression of ProTα clearly facilitated accessibility to chromatin of MNase compared with the control HL-60 cells (untransfected, or transfected with either the pMAM*neo* vector or the antisense ProTα cDNA). Increasing amounts of nuclease produced a parallel shortening of the DNA ladder and, consequently, an increase in the number of smaller DNA fragments (Figure 1a). Remarkably, in the HL-60 cells overexpressing ProTα, the MNase digestion generated a ladder in which all DNA fragments were slightly shorter than those observed with the controls. Thus in the cells overexpressing ProTα, the size of the monomer was approx. 146 bp (Figure 1b), the longest DNA fragment that was associated with mononucleosomes lacking histone H1 (the nucleosome core particle). In contrast, in all control HL-60 cells, digestion with the MNase generated a ladder in which the smallest DNA fragment had a size (Figure 1b) corresponding to the unit of one nucleosome plus one bound histone H1, a particle known as the chromatosome. Furthermore, other fragments in the ladder (dimers, trimers, etc.) were also smaller in the cells overexpressing ProTα compared with the controls (Figures 1 and 2). The difference between the equivalent multimers in the chromatin of the sense and antisense clones was practically constant, the size of each nucleosome multimer always being smaller in the clones overexpressing ProTα (Figure 2). This result can only be explained if we assume that ProTα is removing histone H1 from the chromatin without changing the size of the repeating unit. Therefore the nature of the difference between the chromatin of the cells overexpressing ProTα and the chromatin of the control cells appears to be caused by the latter being associated with histone H1, yielding the chromatosome on extensive digestion, whereas the chromatin of the sense clone lacks histone H1 and is digested by the MNase, giving rise to the core particle. It is noteworthy that the absence of monomer degradation, even in the presence of a high MNase concentration (Figure 1a), indicates that the structure of the nucleosome protein core (histone octamer) is not disassembled by the presence of high levels of ProTα.

To determine the percentage of histone-H1-depleted chromatin (active chromatin) in the different HL-60 cell clones, the fractionation of chromatin was carried out in two pools [20] and the depletion in histone H1 was confirmed by gel electrophoresis. We

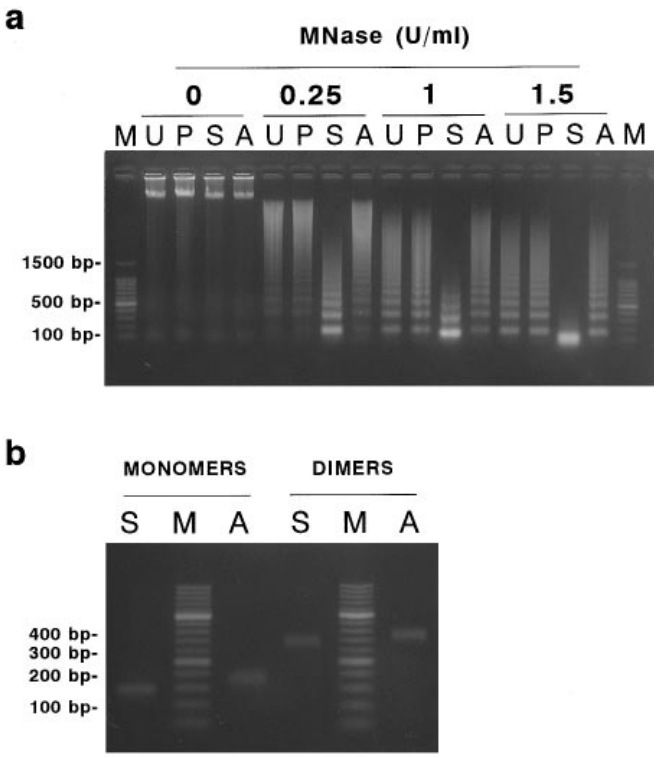

Figure 1 Overexpression of ProTα facilitates chromatin opening

(a) Chromatin digestion with MNase. Isolated nuclei from the different HL-60 cell clones were incubated with the indicated concentrations (0.25, 1, 1.5 units/ml) of MNase; 0 indicates incubation without nuclease. DNA fragments were separated in 1% (w/v) agarose gels. **(b)** A comparative size analysis between monomers and dimers. Monomers and dimers derived from the MNase digestion of the sense (S) clone (HL-60 cell clone transfected with pMAM*neo*-ProTα sense) and antisense (A) clone (HL-60 cell clone transfected with pMAM*neo*-ProTα antisense) were gel-purified and separated as described in the Materials and methods section. Other clones used: U, untransfected HL-60 cell clone; P, HL-60 cell clone transfected with pMAM*neo*. M represents the molecular mass markers, a 100-bp DNA ladder (MWM XIII) in (**a**) and a 50-bp DNA ladder (MWM XIII) in (**b**), both from Boehringer Mannheim. The estimated sizes of the monomers/dimers are $\approx 146/\approx 342$ bp (S clone) and $\approx 171/\approx 369$ bp (A clone) respectively.

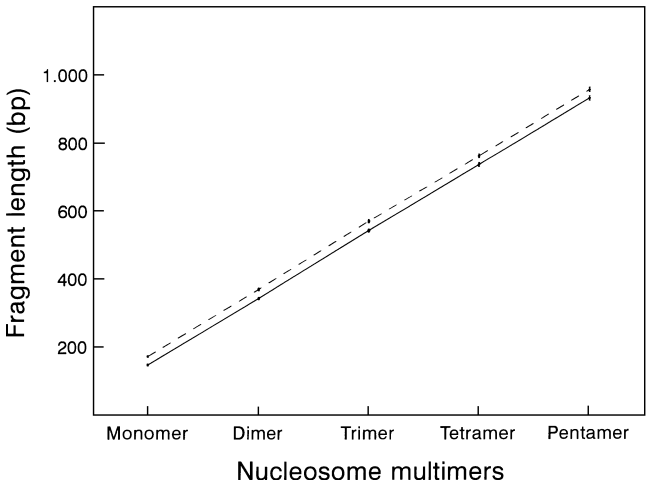

Figure 2 Relationship between nucleosome multimers and their fragment length

The graph represents multimers derived from the MNase digestion of the chromatin obtained from the sense (continuous line) and antisense (discontinuous line) clones against fragment length. The sizes of the DNA fragments associated with the multimers (from the monomers to the pentamers) were estimated with the SeqAid II program using as molecular mass markers the 50-bp and 100-bp ladders; with these markers, the computed fragment size deviates from actual size $\pm$ 0.34 bp. Graphical representation was obtained using the Harvard Graphics software. Values represent means $\pm$ S.D. of the DNA length of each multimer measured in four different gels.

found that the clones overexpressing ProTα contained approximately 72% more active chromatin than the controls (results not shown). Moreover, run-off experiments indicate that the rate of general transcriptional activity was higher in the cells overexpressing ProTα (J. Gómez-Márquez and P. Rodríguez, unpublished work). Again, these results provide strong evidence for an interaction between ProTα and histone H1 *in vivo*. There are two additional pieces of experimental evidence favouring the existence of an interaction between both proteins: ProTα is present in amounts equivalent to those of histone H1 in mammalian cells [21] and, more importantly, it binds specifically to histone H1 through its acidic domain *in vitro* [16].

A function of some anionic regions might be to unfold the condensed higher-order chromatin fibre and this unfolding could result from electrostatic interactions with the basic domains of the core histones and/or histone H1 [14]. The unusual pattern of chromatin organization found in the cells overexpressing ProTα, together with its nuclear localization, acidic nature and ability to bind to histone H1, led us to propose that ProTα is capable of promoting the decondensation of the 30 nm fibre of chromatin through its interaction with histone H1. Interestingly, it has been recently reported that ProTα is an energy-rich molecule due to the presence of glutamyl phosphate [22], and that the activity of ProTα involves the turnover of its acyl phosphates [23]. Hence it is possible that the interaction between ProTα and histone H1 is regulated by phosphorylation of one or both molecules. As far as we know, the present study is the first one demonstrating that the overexpression of a protein can produce a dramatic alteration of chromatin structure in isolated nuclei, and ProTα would be the first known protein that appears to be directly involved in the unfolding of the 30 nm chromatin fibre.

Understanding the mechanisms by which eukaryotic chromatin is remodelled is of fundamental importance in biology. Changes in the levels of chromatin organization are required for very essential cellular processes, such as recombination, replication, transcription and chromosome packaging. Experiments with different organisms from yeast to human cells have uncovered proteins that work as chromatin-remodelling machines participating in chromatin assembly and/or transcriptional regulation [24,25]. According to our results, ProTα seems to have a role in disassembling chromatin fibres without altering the nucleosome structure. Since the degree of compaction of eukaryotic chromatin varies as the cell cycle progresses and ProTα is present throughout all the stages of cell cycle, it is reasonable to assume that the activity of this protein could be necessary for several chromatin-related events, such as transcription and/or replication. In conclusion, our findings demonstrate an involvement of ProTα in the remodelling of chromatin and open up new pathways in the fascinating study of some of the basic processes that involve chromatin reorganization during the life of the eukaryotic cell.

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