Published in final edited form as:
FEBS Lett. 2010 July 2; 584(13): 2833–2836. doi:10.1016/j.febslet.2010.04.065.

Prothymosin α is a component of a linker histone chaperone

Eric M. George and **David T. Brown***
Department of Biochemistry, University of Mississippi Medical Center, Jackson, MS 39216.

Abstract

Linker histone H1 binds with high affinity to naked and nucleosomal DNA *in vitro* but is rapidly exchanged between chromatin sites *in vivo* suggesting the involvement of one or more linker histone chaperones. Using permeabilized cells, we demonstrate that the small acidic protein prothymosin α (ProTα) can facilitate H1 displacement from and deposition onto the native chromatin template. Depletion of ProTα levels *in vivo* by siRNA-mediated mRNA degradation resulted in a decreased rate of exchange of linker histones as assayed by photobleaching techniques. These results indicate that ProTα is a component of a linker histone chaperone.

Keywords

Prothymosin α; Histone H1; linker histone; histone chaperone; chromatin

1. Introduction

The linker or H1 histones stabilize metazoan chromatin by binding to DNA as it enters and exits the nucleosome and through interactions with the inter-nucleosomal linker DNA [1,2]. *In vitro* evidence indicates that H1 binds tightly to both naked and nucleosomal DNA primarily through electrostatic interactions [3,4]. However, *in vivo* photobleaching studies demonstrate that linker histones remain bound to nucleosomal sites for only about a minute before translocation to a new binding site [5,6]. These observations suggest the existence of one or more linker histone chaperones that mediate exchange *in vivo*.

Core histone chaperones have been identified with defined roles in nucleosome assembly, histone removal, histone shuttling, and histone sequestration [7,8]. Thus, it is conceivable that a linker histone chaperone is at least partially responsible for the dynamic behavior *in vivo* although none have been unequivocally identified. One attractive candidate for a linker histone chaperone component is the small acidic molecule prothymosin α (ProTα). A complex containing ProTα and H1 was immunoisolated from extracts of cultured mouse cells and ligand binding assays revealed direct interaction between these proteins [9]. In this study it was also shown that ProTα could promote the release of a fraction of H1 from chromatin reconstituted in vitro. Mammalian cells transfected with a ProTα expression construct are more susceptible to digestion of chromatin by micrococcal nuclease, a hallmark of chromatin decondensation [10,11]. Metaphase chromosomes incubated *in vitro* with recombinant ProTα displayed a time

*Address for manuscript correspondence: Dr. David T. Brown, Department of Biochemistry, University of Mississippi Medical Center, 2500 N. State St., Jackson, MS 39216. TEL:601-984-1849, FAX: 601-984-1501, dbrown@biochem.umsmed.edu.

dependent unraveling of the chromosomes [12]. Based on these and other observations, it has been proposed that ProTα is a chromatin-remodelling protein in mammalian cells [9,11].

Here we have exploited mouse fibroblasts that stably express low levels of an H1 protein fused to the enhanced Green Fluorescent Protein (H1-GFP). Stringent biochemical and molecular assays demonstrate that these fusion proteins bind to chromatin with properties indistinguishable from that of the endogenous linker histones [13]. This system allowed us to examine the effect of ProTα on H1 binding to native chromatin templates.

2. Materials and methods

2.1 Purification of Bacterially Expressed His-Tagged ProTα and H1cGFP

Plasmids containing the mouse ProTα gene were obtained from the American Type Culture Collection. Reconstructed DNA fragments containing the intact coding region and the coding region containing a deletion of residues 3–13 (ProTα(Δ3–13)) were inserted into pET28a (Novagen) to generate His-tagged fusions. The coding region of the H1cGFP fusion protein [5] was also inserted into pET28a (Novagen) to generate a C-terminal His-tagged fusion. All constructs were verified by sequencing and transfected into *E. coli* Rosetta pLysS (Novagen). Crude cell lysates prepared from exponentially growing cultures were sonicated, and purification was performed with a one ml His-Trap nickel column (GE Healthcare) per the manufacturer's instructions. Purified preparations were run on 17% polyacrylamide gels. ProTα and ProTα(Δ3–13) were stained with InVision His-tag (Invitrogen). Relative quantification of wild type and mutant proteins was determined with a Bio-Rad Molecular Imager.

2.2 Cell culture, siRNA transfection and immunofluorecence assays

Mouse BALB/c 3T3 fibroblast cells and the MTH1cGFPneo cell line [5] were propagated in DMEM-low glucose supplemented with 10% heat inactivated bovine serum. For immunofluorescence assays, cells were plated on 4 cm^2 Labtek II (Nalgene) chamber slides at 10–15% confluence. The siRNA duplex targeted to the prothymosin accession number NM_008972 was designed by Dharmacon (Sense: GCGGGUAGCUGAGGAUGAUUU, Antisense AUCAUCCUCAGCUACCCGCUU). Transfection of annealed siRNA (40 pmol/chamber) was performed with Lipofectamine 2000 reagent (Invitrogen) using the manufacturer's modified protocol designed specifically for siRNA transfection. At 48 h post-transfection, cells were paraformaldehyde fixed, washed with PBS and then incubated with 2F11 anti-prothymosin (Alexis Biochemicals) at a 1:300 dilution which was subsequently detected by Alexa Red-conjugated goat anti-mouse secondary antibody. Cells were counterstained with 1 ng/ml Hoechst 33258 in PBS and wet mounted before imaging on a Nikon Eclipse E600 microscope with a Photometrics Coolsnap FX CCD camera.

2.3 In vitro fluorescence exchange assays

The *in vitro* exchange assays were performed according a previously published protocol [14] with minor modifications. Fibroblasts were plated at 75% confluence on 12 mm coverslips in 24-well cell culture plates one day prior to the experiment. The media was aspirated and each well was rinsed twice with 0.5 ml of Physiological Buffer with Ficoll (PBF) (100 mM CH_3CO_2K, 30 mM KCl, 10 mM Na_2HPO_4, 1 mM DTT, 1 mM $MgCl_2$, 5% w/v Ficoll 400 and 1 mM ATP). Each well was then incubated with 0.5 ml of PBF-T (PBF supplemented with 1% Triton X-100) for ten min on ice. Each well was then incubated at 30°C for 30 min in 300 μl of PBF supplemented with the appropriate exogenous proteins. Wells were then washed twice with 0.5 ml of PBF for five min each. Cells were then incubated for ten min in Hoechst stain. After a final five min rinse in PBF, the coverslips were wet mounted and observed as described above for epifluorescence.

2.4 Fluorescent Recovery after Photobleaching (FRAP) assays

Fibroblasts were plated in LabTekII chambered cover slips (Nalgene) and cotransfected with 40 pmol of ProTα siRNA and 400 ng of plasmid dsRED-C1 (Clontech) with Lipofectamine 2000 (Invitrogen) as per the manufacturer's instructions. Cells were visually selected by epifluorescence for the presence of dsRED expression at 48 h post transfection. FRAP assays were performed as previously described [13]. For details see Supplementary Material. All datasets consisted of at least eight cells per experiment.

3. Results

3.1. Recombinant ProTα facilitates displacement of H1 from the native chromatin template

The coding regions of wild type ProTα and a mutant construct containing a deletion of the residues encoding amino acids three through thirteen (ProTα(Δ3–13)) were cloned in frame with a 6x-histidine tag located at the C-terminus. The particular region deleted was chosen because it removes approximately 10% of the protein without substantially changing the overall charge (Fig. 1A). Wild type and mutant proteins were purified from *E. coli* by binding and elution from a nickel resin column. Because ProTα binds Coomassie Blue poorly, visualization and relative quantification were carried out with a His-tag based fluorescent stain (Fig. 1B).

To determine if ProTα is capable of displacing H1 from a native chromatin template, we adapted a previously described exchange assay [14]. Mouse fibroblasts stably expressing H1-GFP were grown on glass coverslips and permeabilized in a physiological buffer containing Triton X-100 under conditions that do not significantly disrupt the native chromatin conformation (Fig. 2A, Supplementary Fig. S1). Incubation of permeabilized cells with purified recombinant ProTα resulted in a significant loss of endogenous fluorescence, indicating that the H1-GFP protein was displaced from the cellular chromatin and lost during the subsequent wash step (Fig. 2B). We were not able to determine the relative stoichiometry of added ProTα to endogenous H1-GFP; however, the effect was dose-dependent as increasing amounts of ProTα lead to greater loss of fluorescence (Fig. S2). For all displayed experiments, three arbitrary units of ProTα were used. Remarkably, incubation with an equivalent amount of the deletion mutant ProTα(Δ3–13) showed no loss of fluorescence, indicating that this construct is incapable of facilitating H1-GFP displacement (Fig. 2C).

3.2 ProTα facilitates deposition of H1-GFP onto chromatin

A second activity often associated with histone chaperones is the ability to facilitate incorporation of histones into native chromatin, either during or outside of DNA replication (7,8). We modified the previous protocol to ask whether ProTα possesses this attribute. Permeabilized wild type BALB/c 3T3 fibroblasts (not expressing H1-GFP) were incubated with exogenous H1-GFP alone, or in combination with purified ProTα. Incubation with H1-GFP alone resulted in dim, diffuse nuclear staining (Fig. 3A,B). However, incubation of H1-GFP and ProTα simultaneously led to bright nuclear staining (Fig. 3C,D). Importantly, the staining pattern was indistinguishable from that observed in cell lines expressing endogenously synthesized H1-GFP, which is characterized by enrichment in heterochromatin and exclusion from nucleoli (Fig. 3E–G, Fig. S1). We considered the possibility that the incorporation of exogenous H1-GFP into chromatin might be due to ProTα-mediated displacement of endogenous H1 thereby making previously occupied binding sites available for the exogenous protein. To address this, we pre-incubated wild type fibroblasts with ProTα alone to facilitate endogenous H1 release using the same conditions as in Fig. 2, washed the cells with buffer, and then incubated with exogenous H1-GFP alone (Fig. 3 H,I). Pre-incubation of chromatin with ProTα did not facilitate subsequent H1-GFP deposition, indicating that ProTα and H1-GFP must be present simultaneously to facilitate incorporation into chromatin. Pre-incubation of chromatin with ProTα followed by addition of ProTα and H1-GFP gave results nearly

identical to Fig. 3C,D (data not shown). Simultaneous incubation of permeabilized cells with H1-GFP and ProTα(Δ3–13) resulted in slightly more nuclear GFP staining than that observed for H1-GFP alone (Fig. 3J,K). However, the staining was dim and diffuse and no evidence of heterochromatin accumulation was observed (Fig. 3L–M).

3.3 Reduction of ProTα levels in vivo slows intranuclear linker histone exchange

We next considered the possibility that in intact living cells, ProTα might be a component of the mechanism that mediates the exchange of linker histones between chromatin binding sites. To test this, we introduced siRNAs specific for ProTα mRNA into fibroblasts expressing H1-GFP. We obtained an approximately 80% reduction of ProTα protein levels after 48 h of treatment (Figs. 4A). We then measured the *in vivo* mobility of H1-GFP by Fluorescence Recovery After Photobleaching (FRAP) (Fig. 4B). The time to 50% recovery value (t_{50}) for mock treated cells was ~18 sec, identical to that observed for untreated cells (manuscript submitted). Depletion of ProTα levels resulted in a significantly longer t_{50} time of ~30 sec, indicating a slowing of the H1-GFP exchange rate (Figure 4B).

4. Discussion

Despite extensive study, the function of ProTα is still not completely clear. The body of evidence suggests that ProTα modulates the interaction of linker histones with chromatin to promote chromatin remodeling or decondensation and, in so doing, to promote transcription [9–12,15,16]. ProTα has also been shown to be up-regulated in proliferating cells and aggressive tumors [17,18], to induce transformation in rat fibroblasts [19], to retard differentiation [20] and to act as an inhibitor of apoptosis [21]. Thus it seems likely that it is an essential player in the replicative program as well. Here, we have investigated the possibility that ProTα functions as a linker histone chaperone, defined as any factor which associate directly with histones and facilitate their transfer without remaining a part of the final complex.

The results from the permeabilized cell assays indicate that ProTα can promote both the removal and deposition of H1 in the context of a native chromatin structure. These observations are consistent with previous studies utilizing predominantly in vitro reconstituted chromatin templates [9,11]. The inability of the ProTα(Δ3–13) mutant to mediate either removal or deposition is an important control as it demonstrates that these effects are not mediated simply by charge displacement. Although ProTα has been shown to influence nucleosome spacing during reconstitution experiments [11], the results presented here suggest that this protein may be a necessary component for H1 deposition *in vivo*, consistent with a role in cell proliferation.

The dynamic nature of linker histone binding has been an unresolved enigma since it was first observed [5,6]. The regulation of H1 binding is likely to have long ranging effects on all chromatin associated processes; including transcription and replication [6]. Modulating linker histone dynamics is functionally important. In undifferentiated stem cells, the majority of H1 is transiently bound to chromatin, creating an open chromatin conformation, but as the cells became lineage committed binding became more static [22]. Introduction of H1 artificially restricted in its mobility causes disruption of the differentiation pathway [22]. Similar observations were made in an erythroid differentiation model, and it was also demonstrated that changes in the dynamics of a small subset of H1 molecules could have a dominant effect on the dynamics of the remaining H1 pool [23]. Here, we show that depletion of ProTα in intact cells results in a slower rate of exchange of H1-GFP between chromatin sites. We suggest that ProTα, possibly in the context of a larger complex, acts as a linker histone chaperone to promote the exchange of linker histones and possibly to facilitate H1 deposition during replication. Such a role is consistent with all of the proposed functions of this protein.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations used

ProTα	Prothymosin α
FRAP	Fluorescence Recovery after Photobleaching
GFP	Green Fluorescent Protein

Acknowledgments

FRAP studies were performed at the Mississippi Functional Genomics Network Imaging Facility, University of Southern Mississippi and we are grateful to G. Shearer and B. Kang for their assistance. We thank T. Smith and P. Vyas for technical assistance and D. Sittman for critical reading of the manuscript and valuable discussions. This work was supported by grant MCB0235800 from the National Science Foundation (to D.T.B.) and NIH grant RR016476 from the MFGN INBRE Program of the National Center for Research Resources.

References

1. Wolffe, A. Chromatin : structure and function. San Diego: Academic Press; 1998.
2. Happel N, Doenecke D. Histone H1 and its isoforms: contribution to chromatin structure and function. Gene 2009;431:1–12. [PubMed: 19059319]
3. Mamoon NM, Song Y, Wellman SE. Binding of histone H1 to DNA is described by an allosteric model. Biopolymers 2005;77:9–17. [PubMed: 15558656]
4. Clark DJ, Kimura T. Electrostatic mechanism of chromatin folding. J Mol Biol 1990;211:883–896. [PubMed: 2313700]
5. Misteli T, Gunjan A, Hock R, Bustin M, Brown DT. Dynamic binding of histone H1 to chromatin in living cells. Nature 2000;408:877–881. [PubMed: 11130729]
6. Catez F, Ueda T, Bustin M. Determinants of histone H1 mobility and chromatin binding in living cells. Nat Struct Mol Biol 2006;13:305–310. [PubMed: 16715048]
7. Park YJ, Luger K. Histone chaperones in nucleosome eviction and histone exchange. Curr Opin Struct Biol 2008;18:282–289. [PubMed: 18534842]
8. De Koning L, Corpet A, Haber JE, Almouzni G. Histone chaperones: an escort network regulating histone traffic. Nat Struct Mol Biol 2007;14:997–1007. [PubMed: 17984962]
9. Karetsou Z, Sandaltzopoulos R, Frangou-Lazaridis M, Lai CY, Tsolas O, Becker PB, Papamarcaki T. Prothymosin alpha modulates the interaction of histone H1 with chromatin. Nucleic Acids Res 1998;26:3111–3118. [PubMed: 9628907]
10. Karetsou Z, Martic G, Tavoulari S, Christoforidis S, Wilm M, Gruss C, Papamarcaki T. Prothymosin alpha associates with the oncoprotein SET and is involved in chromatin decondensation. FEBS Lett 2004;577:496–500. [PubMed: 15556635]
11. Gomez-Marquez J, Rodriguez P. Prothymosin alpha is a chromatinremodelling protein in mammalian cells. Biochem J 1998;333(Pt 1):1–3. [PubMed: 9639554]
12. Boan F, Vinas A, Buceta M, Dominguez F, Sanchez L, Gomez-Marquez J. Prothymosin alpha, a mammalian c-myc-regulated acidic nuclear protein, provokes the decondensation of human chromosomes in vitro. Cytogenet Cell Genet 2001;93:171–174. [PubMed: 11528108]
13. Brown DT, Izard T, Misteli T. Mapping the interaction surface of linker histone H1(0) with the nucleosome of native chromatin in vivo. Nat Struct Mol Biol 2006;13:250–255. [PubMed: 16462749]
14. Kimura H, et al. A novel histone exchange factor, protein phosphatase 2Cgamma, mediates the exchange and dephosphorylation of H2A-H2B. J Cell Biol 2006;175:389–400. [PubMed: 17074886]
15. Trumbore MW, Berger SL. Prothymosin alpha is a nonspecific facilitator of nuclear processes: studies of run-on transcription. Protein Expr Purif 2000;20:414–420. [PubMed: 11087681]

16. Gomez-Marquez J. Function of prothymosin alpha in chromatin decondensation and expression of thymosin beta-4 linked to angiogenesis and synaptic plasticity. Ann N Y Acad Sci 2007;1112:201–209. [PubMed: 17495247]

17. Leys CM, Nomura S, LaFleur BJ, Ferrone S, Kaminishi M, Montgomery E, Goldenring JR. Expression and prognostic significance of prothymosin-alpha and ERp57 in human gastric cancer. Surgery 2007;141:41–50. [PubMed: 17188166]

18. Suzuki S, et al. Expression of prothymosin alpha is correlated with development and progression in human prostate cancers. Prostate 2006;66:463–469. [PubMed: 16353248]

19. Orre RS, Cotter MA 2nd, Subramanian C, Robertson ES. Prothymosin alpha functions as a cellular oncoprotein by inducing transformation of rodent fibroblasts in vitro. J Biol Chem 2001;276:1794–1799. [PubMed: 11036085]

20. Rodriguez P, Vinuela JE, Alvarez-Fernandez L, Buceta M, Vidal A, Dominguez F, Gomez-Marquez J. Overexpression of prothymosin alpha accelerates proliferation and retards differentiation in HL-60 cells. Biochem J 1998;331(Pt 3):753–761. [PubMed: 9560301]

21. Letsas KP, Frangou-Lazaridis M. Surfing on prothymosin alpha proliferation and anti-apoptotic properties. Neoplasma 2006;53:92–96. [PubMed: 16575463]

22. Meshorer E, Yellajoshula D, George E, Scambler PJ, Brown DT, Misteli T. Hyperdynamic plasticity of chromatin proteins in pluripotent embryonic stem cells. Dev Cell 2006;10:105–116. [PubMed: 16399082]

23. Yellajoshyula D, Brown DT. Global modulation of chromatin dynamics mediated by dephosphorylation of linker histone H1 is necessary for erythroid differentiation. Proc Natl Acad Sci U S A 2006;103:18568–18573. [PubMed: 17124174]

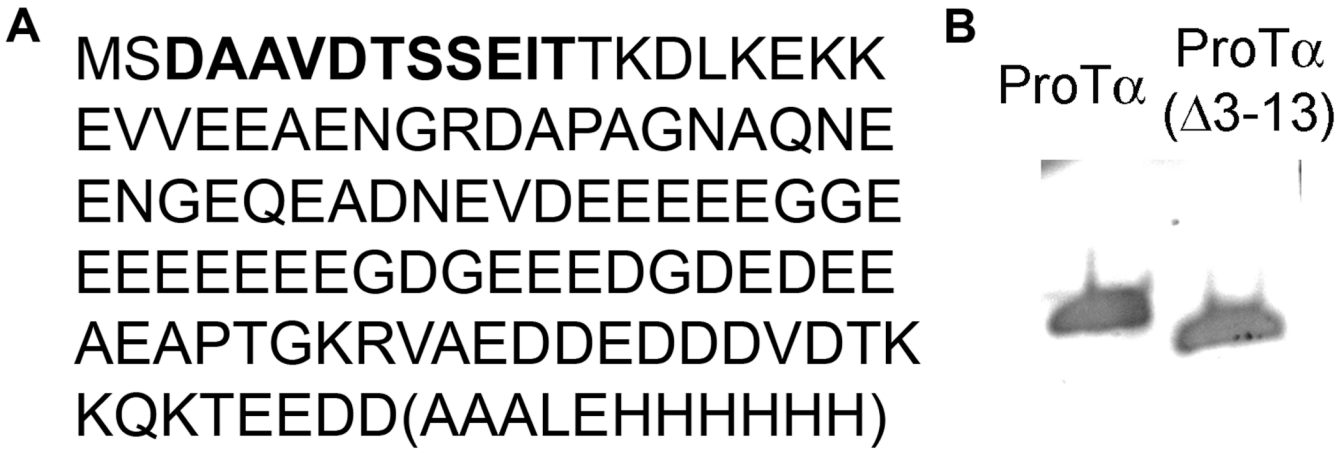

Fig 1.

Expression of recombinant ProTα. (A) Primary sequence of mouse ProTα. The residues removed in the ProTα(Δ3–13) construct are in bold, and the residues added for the His-tag are in parentheses. (B) Final protein preparations used in this study were electrophoresed on a 17% polyacrylamide gel and stained with InVision His-tag (Invitrogen).

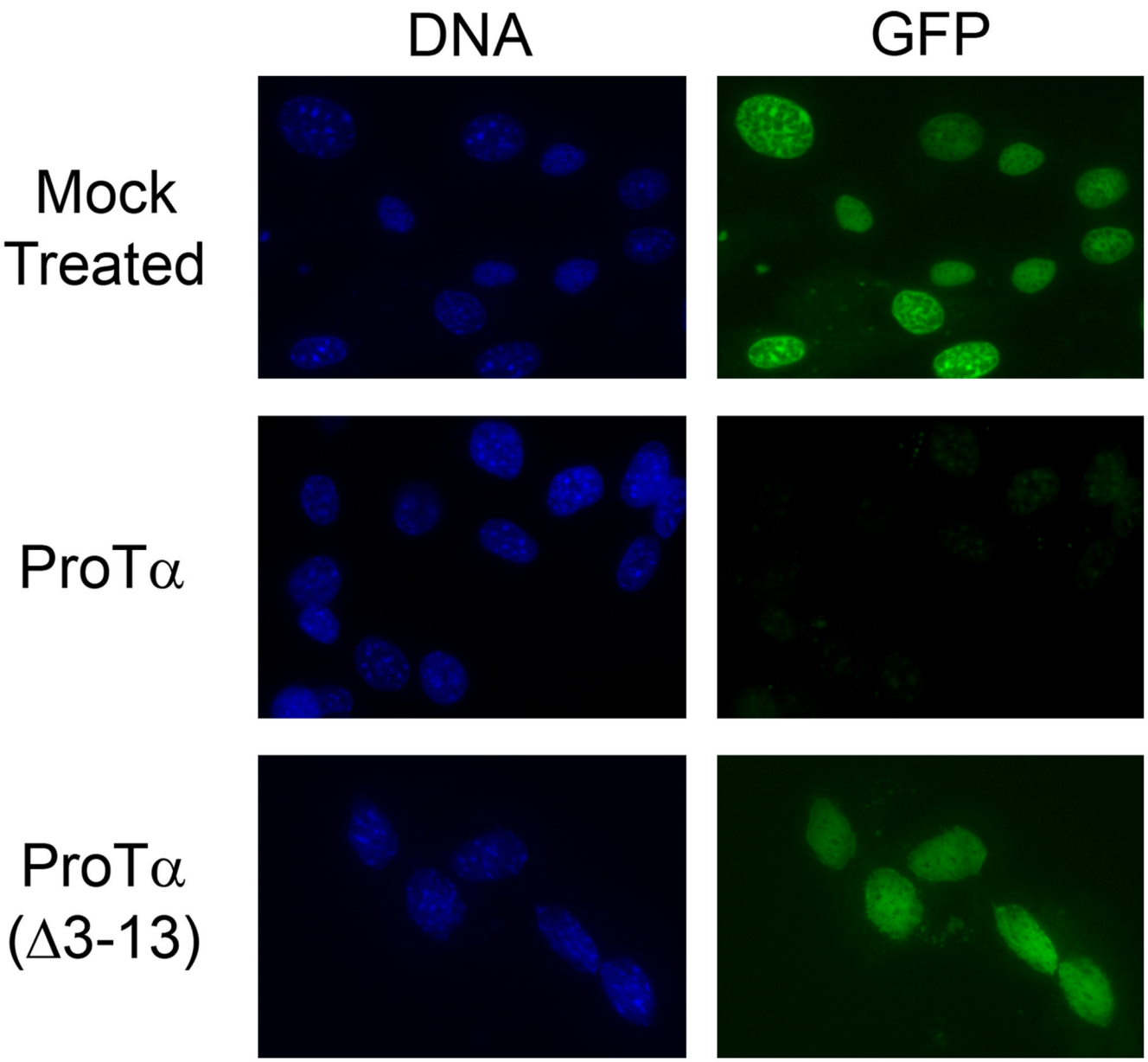

Fig 2.

ProTα displaces H1-GFP from mouse fibroblasts. 3T3 fibroblasts expressing low levels of endogenously synthesized H1-GFP were permeabilized and then incubated for 30 min with PBF buffer (Mock Treated) or with PBF supplemented with three units of recombinant ProTα or an equivalent amount of ProTα(Δ3–13). Cells were then washed, stained with Hoechst and processed for epifluorescence microscopy.

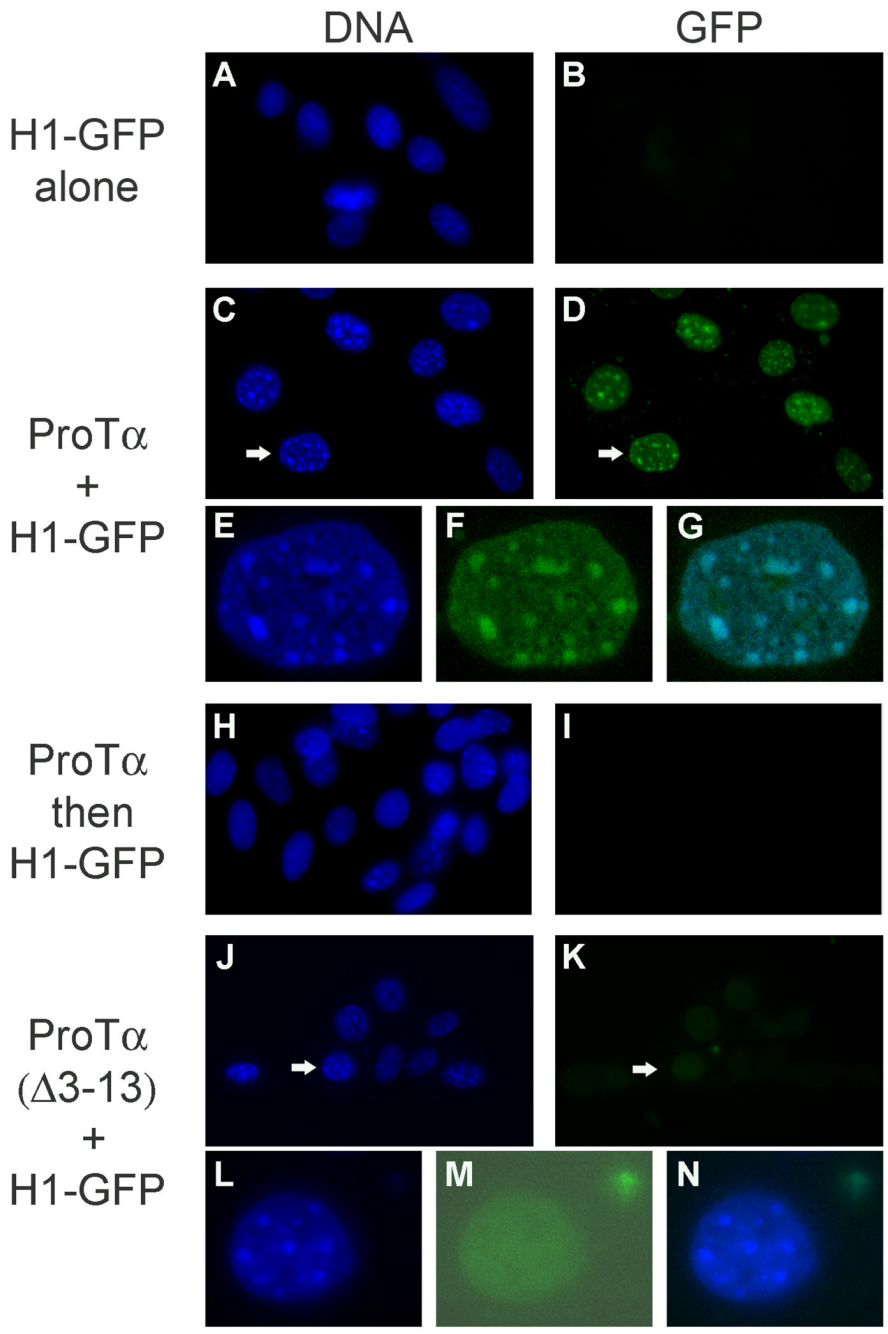

Fig 3.

ProTα promotes loading of H1-GFP onto chromatin. Permeabilized BALB/c 3T3 fibroblasts were incubated with H1-GFP alone (A,B) or with H1-GFP and three units of ProTα simultaneously (C–G). Panels E and F are higher magnifications of the cell indicated by the arrow in panels C and D; panel G is the merged image. (H,I) Permeabilized cells were incubated with three units of ProTα for 30 min, washed and then incubated with H1-GFP. (J–N) Simultaneous treatment with H1-GFP and three units of ProTα(Δ3–13). Panels L and M are higher magnifications of the cell indicated by the arrow in panels J and K; panel N is the merged image. The brightness of the image in panel M was deliberately increased to show the lack of H1-GFP organization into heterochromatin.

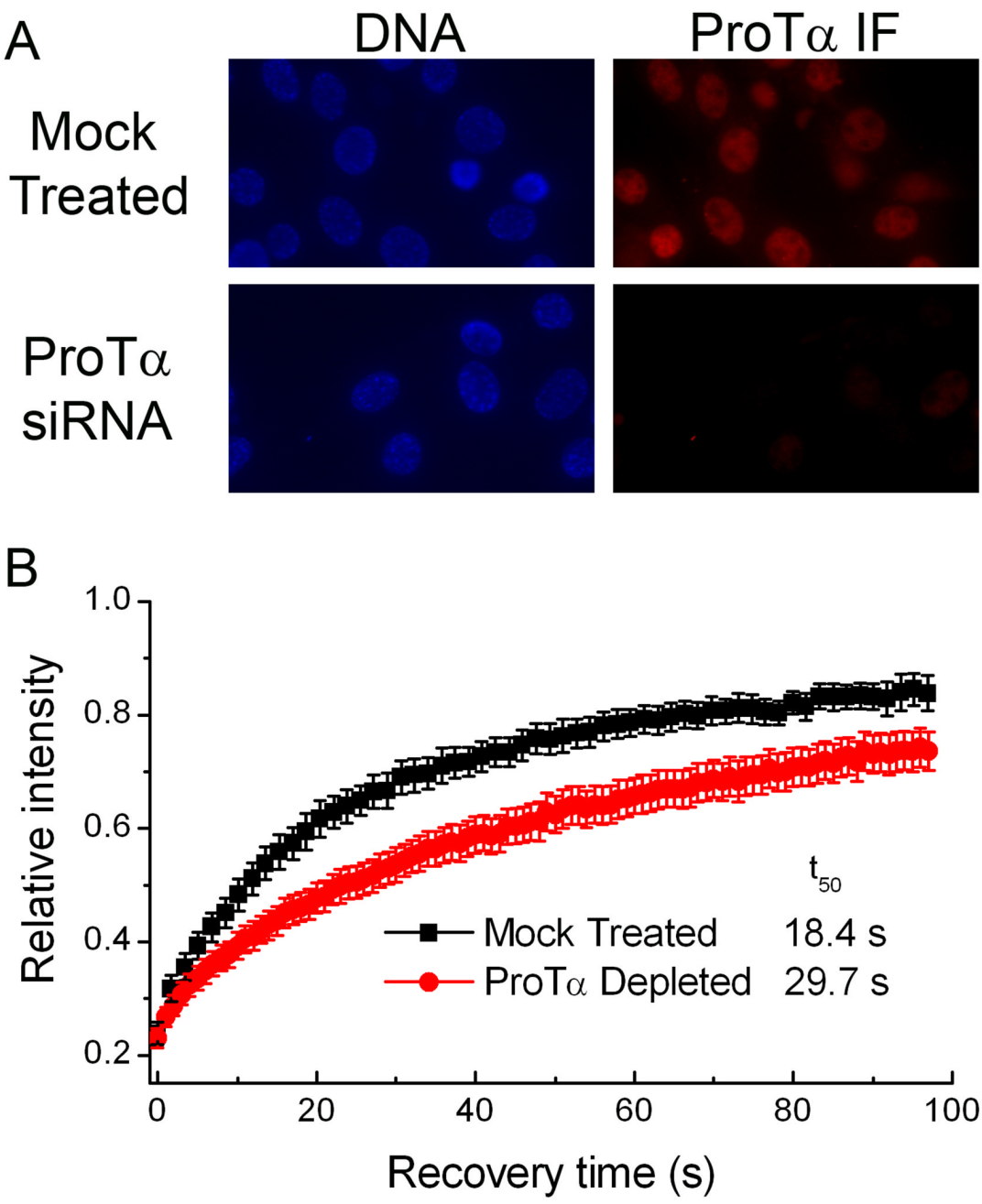

Fig 4.

ProTα depletion significantly decreases H1c-GFP mobility *in vivo*. (A) Analysis by immunofluorescence of ProTα protein levels after siRNA treatment. (B) FRAP analysis of H1c-GFP mobility. Average recovery curves from ten siRNA treated cells and eight mock treated cells are shown.