Parathymosin Affects the Binding of Linker Histone H1 to Nucleosomes and Remodels Chromatin Structure*

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Linker histone H1 is the major factor that stabilizes higher order chromatin structure and modulates the action of chromatin-remodeling enzymes. We have previously shown that parathymosin, an acidic, nuclear protein binds to histone H1 in vitro and in vivo. Confocal laser scanning microscopy reveals a nuclear punctuate staining of the endogenous protein in interphase cells, which is excluded from dense heterochromatic regions. Using an in vitro chromatin reconstitution system under physiological conditions, we show here that parathymosin (ParaT) inhibits the binding of H1 to chromatin in a dose-dependent manner. Consistent with these findings, H1-containing chromatin assembled in the presence of ParaT has reduced nucleosome spacing. These observations suggest that interaction of the two proteins might result in a conformational change of H1. Fluorescence spectroscopy and circular dichroism-based measurements on mixtures of H1 and ParaT confirm this hypothesis. Human sperm nuclei challenged with ParaT become highly decondensed, whereas overexpression of green fluorescent protein- or FLAG-tagged protein in HeLa cells induces global chromatin decondensation and increases the accessibility of chromatin to micrococcal nuclease digestion. Our data suggest a role of parathymosin in the remodeling of higher order chromatin structure through modulation of H1 interaction with nucleosomes and point to its involvement in chromatin-dependent functions.

Eukaryotic DNA is packaged into chromatin (1), a structural framework that plays a key role in the regulation of the transcriptional status of genes and genetic loci (2–4). The basic level of chromatin organization is the nucleosome that consists of DNA wrapped around an octamer of histones (5). Linker histones (e.g., H1 and H5) seal the entry/exit of DNA and stabilize the folding of 10-nm nucleosomal arrays into higher order chromatin structures (6, 7). Binding of histone H1 to nucleosomal arrays affects the spacing of nucleosomes (8), restricts their mobility (9), and reduces the transient exposure of DNA on the nucleosome surface (10). Consistent with its structural role as a major determinant of chromatin folding, histone H1 was found to inhibit the action of enzymes that enhance the accessibility of DNA, such as histone acetyltransferases and ATP-dependent remodeling complexes. For example, changes in linker histone stoichiometry modulate the levels of core histone acetylation (11) and regulate the activity of all classes of ATP-dependent remodeling enzymes (12, 13). Collectively, these findings support the notion that H1 “locks down” chromatin regions and acts as a general inhibitor of transcription (14, 15). However, genetic studies have shown that, rather than being global repressors of transcription, linker histones affect the expression of specific genes (16). Multiple isoforms of linker histone H1 exist in mammalian somatic cells (17) and play differential roles in gene expression (18). Recently, two independent studies highlight the important role of H1 subtypes in development and apoptosis. H1b, a specific subtype of H1, which is expressed in undifferentiated cells, specifically represses the expression of MyoD gene, and thus restricts muscle development (19). Furthermore, in response to DNA double-strand breaks, only H1.2 (H1c) translocates from the nucleus into the cytoplasm and triggers the onset of apoptosis through the release of cytochrome c from mitochondria (20).

Collectively, the properties of linker histone H1 suggest that local H1 displacement and/or rearrangement must occur for efficient re-organization of chromatin during several DNA-dependent processes. Therefore, the roles of specific H1-protein interactions appear to be important for H1 function and chromatin structure. We have previously reported a specific in vitro and in vivo interaction between parathymosin (ParaT) and histone H1 (21). ParaT is expressed in all cell types (22) and is widely distributed in mammalian tissues with the highest concentrations found in liver, heart, and kidney (23). It contains a functionally active bipartite nuclear localization signal and is actively translocated into the nuclei of undifferentiated cells (24). Inspection of the amino acid sequence of ParaT (101 amino acids, pI 4.15) reveals that it is predominantly composed of hydrophilic amino acids, typically found on protein surfaces. Aromatic, sulfur, histidine, isoleucine, and leucine amino acids are totally absent, whereas the central region of the protein contains runs of aspartic and glutamic acid residues, occasionally interspersed with glycine, alanine, threonine, and asparagine (25). This unusual sequence shares significant similarity

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** The abbreviations used are: ParaT, parathymosin; CMV, cytomegalovirus; GFP, green fluorescent protein; TAF, template-activating factor; GST, glutathione S-transferase; BSA, bovine serum albumin; PBS, phosphate-buffered saline; TRITC, tetramethyl rhodamine isothiocyanate; CD, circular dichroism; NKL, nucleosome repeat length.

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with the amino acid composition of prothymosin α, a histone H1-binding protein associated with cell proliferation, chromatin remodeling, and gene transcription (26–29).

Data on the biological function of ParaT suggest functions in both the cytoplasm and nucleus. Early studies have identified ParaT as a zinc-binding protein, which interacts with several enzymes involved in carbohydrate metabolism (30, 31). Recently, ParaT was found to inhibit the binding of the activated glucocorticoid receptor to nuclei, suggesting its involvement in the regulation of glucocorticoid steroids action (32).

In the present study we analyzed the effect of ParaT on the interaction of histone H1 with chromatin using an in vitro chromatin reconstitution system on plasmid and bead-immobilized DNA. We also monitored the binding of H1 and ParaT by circular dichroism and fluorescence spectroscopy to detect potential changes of H1 conformation in the presence of ParaT. Furthermore, we studied the potential involvement of this acidic polypeptide in chromatin decondensation. Our results indicate that ParaT modulates the interaction of histone H1 with chromatin and induces global chromatin decondensation.

MATERIALS AND METHODS

Plasmids—To generate pCMVParaT the coding region of the rat ParaT cDNA (23) was amplified and subcloned into the EcoRI-Xhol sites of a CMV expression cassette pBluescript KS(−), where the H2 promoter was exchanged with the CMV promoter. For pFLAG-ParaT, the coding region of ParaT was amplified and subcloned into the EcoRI-SalI sites of pFLAG-CMV2 vector (Eastman Kodak Co.). To generate pGST-ParaT, the coding region of the protein was removed from pFLAG-ParaT and cloned into the EcoRI-SalI sites of pGEX-6P3 (Amersham Biosciences). GFP-ParaT was generated by inserting ParaT gene into EcoRI-Smal cleavage sites of pEGFP-C1 expression vector (Clontech). pFLAG-ParaT and pCMVParaT were grown for 24 h in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum and antibiotics. For transient transfections, HeLa cells were grown in 10-cm dishes to 50–60% confluency and transfected with 15–20 μg of DNA using the calcium phosphate method. pFLAG or pEGFP-ParaT-transfected cells were detected through cotransfection of 2 μg of pCMV-β-galactosidase vector. For microinjection experiments the calcium method was modified as follows: Cells were transfected with 16 μg of DNA containing 1.5 μg of pPUR (Clontech), a plasmid that confers resistance to puromycin. After 6 h the medium was changed and cells were grown for 24 h in Dulbecco’s modified Eagle’s medium containing 0.25 μg/ml puromycin. The transfection efficiency ranged from 75 to 90%.

Indirect Immunofluorescence and Confocal Microscopy—A polyclonal antibody recognizing the NH terminus of ParaT (peptide P1, residues 5–30) was raised in rabbits (Davids Biotechnology, Germany), and the serum was purified over P1-agarose affinity column. HeLa cells grown on coverslips were fixed with methanol at −20 °C for 5 min, followed by incubation in 3.8% paraformaldehyde for 20 min at room temperature and quenched in 50 mM ammonium chloride for 15 min. Nonspecific sites were blocked with 10% fetal calf serum in PBS, and antibody incubations were carried out for 1 h in a humidified chamber. Primary and secondary antibodies were diluted in 10% fetal calf serum. Fluorescein isothiocyanate- or TRITC-conjugated anti-rabbit IgG secondary antibodies were purchased from DianoVa and used at 1:200 dilution. Coverslips were mounted in Mowiol containing 100 mg/ml 1,4-diazabicyclo[2.2.2]octane (Sigma) and viewed using a Leica TCS-SP confocal microscope equipped with a HeNe laser and Leica TCS software. The 488 and 568 nm wavelengths were used to excite fluorescein isothiocyanate and TRITC, respectively. Images were exported to Adobe Photoshop.

Chromatin Fractionation—Isolation of S1, S2, and P chromatin fractions from HeLa nuclei was performed according to the method of Rose and Edgar (37) with minor modifications. Brains were transfected with GFP vector or GFP-ParaT and incubated in hypotonic buffer for 10 min. After incubation, cells lysates were centrifuged at 3000 × g for 10 min. The nuclear fraction was resuspended in 200 μl of buffer B (20 mM HEPES, pH 7.9, 20 mM NaCl, 5 mM MgCl2, 1 mM ATP) on ice for 15 min. After incubation, cell lysates were centrifuged at 3000 × g for 10 min. The nuclear fraction was resuspended in 200 μl of buffer B (20 mM HEPES, pH 7.9, 0.5 mM MgCla, 1 mM ATP, 0.3 mM sucrose, 2 mM CaCl2, 150 mM NaCl), and DNA content was determined in 2 μl of NaCl. Nuclei suspension (100 μl) containing 100 μg of DNA was incubated with 8 units of micrococcal nuclease for 10 min at 15 °C and cooled on ice for 10 min. The mixture was centrifuged at 3000 × g for 5 min and 10 μl of 10× stop buffer (20 μl EDTA, 10 μg EGTA, 5% SDS) was then added to the supernatant (S1 fraction). The nuclear pellet was resuspended in 100 μl of 8 μM EDTA for 15 min at 4 °C and centrifuged as above. The supernatant and the pellet were designated as S2 and P fractions, respectively. For DNA analysis, S1, S2, and P fractions were treated with 1 μg/ml RNase for 10 min at 25 °C and deproteinized with 1 μg/ml proteinase K for 2 h at 37 °C. DNA was phenol-chloroform purified, ethanol-precipitated, and analyzed on 1.3% agarose gels and ethidium bromide staining.

Sperm Chromatin Decondensation Assays—Human semen was obtained from fertile healthy donors and was processed in the Contraception Unit of the University Hospital of Ioannina. Sperm was kept frozen at −80 °C until use. Membrane-bound human sperm nuclei were prepared as described (38) and incubated with 6 μM of protein at room temperature in 10 μl of reaction mixture containing 8 mM Hepes, pH 7.5, 5 mM KCl, 2 mM MgCl2, 200 mM sucrose, and an ATP-regeneration system. After the incubation, 1 μl of the reaction mixture was added to 1 μl of PBS containing 100 mM KCl, 7.4% formaldehyde, and 5 μg/ml Hoechst 33342.
FIG. 1. Subnuclear localization of ParaT. A, schematic representation of ParaT. The polyglutamic acidic domain and the bipartite nuclear localization signal (NLS) of rat ParaT. B, indirect immunofluorescence and confocal scanning microscopy. HeLa cells grown on coverslips were fixed and labeled with affinity-purified anti-ParaT antibody. ParaT, confocal optical sections of cells labeled for endogenous ParaT at different cell cycle phases: interphase (a), prophase (b), and metaphase (c). PI, DNA staining with propidium iodide. Merge, overlay of ParaT and PI staining. Scale bar, 2 μm.
containing traces of $^{32}$P-phosphorylated H1 in the absence or in the presence of increasing concentrations of ParT. As depicted in Fig. 2B, in all cases the assembled chromatin contained the full complement of core histones. However, the binding of H1 to chromatin beads was significantly inhibited in the presence of ParT (Fig. 2B, lanes B), with a concomitant retention of H1 in the supernatant (lanes S). Quantitation of the intensity of the bands of the autoradiography by densitometry revealed that this effect was dose-dependent (Fig. 2C).

We next investigated the possibility that the alteration in NRL and the inhibition of H1 binding to chromatin beads in the presence of ParT might be due to a structural change of H1 resulting from its interaction with ParT. To this aim, we used CD spectroscopy, a technique sensitive to conformational changes and well established in the field of protein folding (42). CD spectra of purified histone H1 and recombinant ParT were recorded (a) before and (b) after they were mixed in several molar ratios. In all cases the CD spectrum of the mixture was different from the sum of the CD spectra of the individual proteins. More specifically, the CD pattern observed for the mixture of the two proteins was distinctly indicative of a higher content of secondary structure (possibly of an α-helical type) in comparison to the CD patterns of the individual proteins and to their arithmetic sum (Fig. 3A). Such a difference could only result from a change in conformation of one or both proteins upon mixing. These results suggest that the interaction of the two proteins is accompanied by a conformational change, but they cannot indicate whether the conformation of one or both proteins is affected. To address this specific question we turned to fluorescence spectroscopy measurements. Both proteins are poor in aromatic amino acids: There is no such amino acid in ParT, the nuclei displayed approximately a 2-fold increase in fluorescence intensity of H1 upon addition of ParT for a quantitative fluorescence-based binding assay. A binding curve was obtained by monitoring the change in fluorescence intensity at 306 nm as a function of ParT concentration at constant temperature (Fig. 3C). Fitting of the fluorescence data resulted in a $K_d$ value of 19.6 ± 11.3 μM for the H1-ParT interaction. Such a modest in vitro affinity is not unusual for interactions between chromatin and chromatin-remodeling factors (44–46) and could be due to several reasons (see “Discussion”).

**ParT Induces Global Chromatin Decondensation**—Because ParT affects the binding of H1 to nucleosomes and histone H1 is the major determinant of higher order chromatin folding (7), we investigated the effect of ParT on chromatin structure. We used two different experimental systems: sperm chromatin as an *in vitro* system and ParT-overproducing cells as an *in vivo* system. Sperm has an exceptionally dense chromatin packing compared with that of nuclei from somatic cells and has been used extensively in chromatin decondensation studies (38, 47). In our study, we used human sperm nuclei, which are approximately spherical compared with the spiral shape of *Xenopus* nuclei, to quantitate more accurately the extent of sperm decondensation. Demembraned human sperm nuclei were incubated with purified ParT and the sperm decondensation factor TAF-Iβ (38) or BSA, which were used as positive and negative controls, respectively. After incubation, the DNA was stained with Hoechst 33258 and visualized under a fluorescence microscope. As shown in Fig. 4A, upon challenging sperm nuclei with ParT, the nuclei displayed approximately a 2-fold increase in their surface. Using the AutoCAD 2000 software, the average surface area of 50 nuclei treated with BSA, ParT, or TAF-Iβ was found to be 2.0, 3.1, and 3.2, respectively. To discriminate among different subgroups, the values of each experiment were classified arbitrarily into three clusters (1–1.5, 1.5–2, and >2),
corresponding to small, medium, and large nuclear surface area. The results shown in Fig. 4B indicate that 50% of the nuclei treated with ParaT displayed large surface area, an effect comparable with that of TAF-1β.

We next examined whether ParaT can function as a chromatin decondensation factor in vivo. GFP-ParaT and FLAG-tagged ParaT expression plasmids were transiently transfected into HeLa cells and 36 h post-transfection, cells were fixed and stained with Hoechst 33258. Overexpression of ParaT binding was followed by monitoring the intensity of the fluorescence emitted at 306 nm by the single tyrosine residue of H1. H1-ParaT binding was titrated with increasing amounts of a 270 μM solution of H1 in PBS buffer (top) and of the arithmetic sum of the H1 and ParaT spectra is also shown (dashed-dotted line). Excitation was at 276 nm. C, fluorescence-based binding assay. H1-ParaT binding was followed by monitoring the intensity of the fluorescence emitted at 306 nm by the single tyrosine residue of H1 after excitation at 276 nm. H1 at an initial concentration of 15 μM was titrated with increasing amounts of a 270 μM ParaT stock solution in PBS buffer. The solid line represents the binding curve derived by non-linear regression analysis of the fluorescence intensity values at 306 nm, corrected for dilution and for buffer contribution.

transfected with empty vectors (pGFP and pFLAG) displayed an average surface nuclei area of 6.3, 5.9, and 5.8, respectively. Classification of the values arbitrarily into three groups (1–1.5, 1.5–2, and >2) to represent the extent of sperm chromatin decondensation.

In another attempt to study the effect of ParaT on chromatin structure, we tried to partition between active and inactive chromatin, using an established fractionation procedure in which nuclei are subjected to mild digestion with micrococcal nuclease and subsequent extraction with EDTA (37). This method sequentially yields three fractions that contain materials that is soluble in divalent metals (S1) and soluble in EDTA (S2) and a remaining insoluble fraction (P). The detailed analysis of the protein components of these fractions has shown that S1 is highly depleted of H1 relative to S2, which contains more compact chromatin, whereas the nuclease-resistant fraction P is enriched in actively transcribed gene sequences and contains nuclear matrix components (37, 48). In accordance with this protocol, HeLa cells overexpressing GFP-ParaT or GFP alone were incubated with micrococcal nuclease and centrifuged to obtain the supernatant fraction (S1). Subsequently, the pellet was resuspended in an EDTA-containing buffer and centrifuged to prepare S2 and the remaining insoluble fraction P. As shown by DNA analysis on agarose gels, the profile of micrococcal nuclease digestion of S1 and S2 fractions was similar in both cell populations (Fig. 5C, lanes 1–5). However, the DNA extracted from the nuclease-resistant chromatin fraction (P) of ParaT-overproducing cells yielded a typical nucleosomal ladder with a strong mono-nucleosomal band (Fig. 5C, lane 7). On the contrary, the corresponding fraction of DNA obtained from control cells was more heterogenous and exhibited a smeared profile (Fig. 5C, lane 6). Taken together, these results suggest the involvement of ParaT in the decondensation of chromatin fibers.

**DISCUSSION**

ParaT belongs to a group of acidic proteins that interact specifically with linker histone H1 in vitro and in vivo (21). In the present study, the effect of ParaT on the interaction of histone H1 with chromatin was studied using a cell-free chromatin reconstitution system on bead-immobilized DNA (36). This chromatin system assembles regularly spaced nucleosomes on DNA attached to paramagnetic beads with or without
H1 and offers the opportunity to study the interaction of H1 with nucleosomes under different salt conditions and in the presence of several factors (36). Our results show that ParaT was able to inhibit the association of H1 with the chromatin template. Remarkably, the presence of this acidic protein during chromatin assembly resulted in the organization of H1-containing chromatin with reduced nucleosomal spacing.

To provide interpretation for these data, one should take into consideration the binding determinants of H1 interaction with chromatin and the sequence characteristics of ParaT. Previous studies have clearly established that the interaction of H1 with chromatin is dynamic and the overall affinity is governed by a set of interactions mediated by the globular domain (49, 50) and the positively charged COOH-terminal tail (51, 52). The folded globular domain of H1 binds preferentially to crossovers of duplex strands in superhelical DNA (40), contains two binding sites for DNA (49), and protects an additional 20 bp from micrococcal nuclease digestion (6, 41). The highly basic COOH-terminal domain of H1 adopts secondary α-helical structure upon binding to DNA (51) and has been identified as the primary determinant of H1 binding to chromatin in vitro (51, 53) and in vivo (52). Interestingly, molecular modeling of the chromatosome particle predicts that the location of the COOH-terminal domain on the particle is directed by the positioning of the globular region (54). Based on our results, it is appealing to propose that the binding of ParaT within the globular domain of H1 affects the conformation of the linker histone in such a way that the binding affinity of H1 for chromatin is altered. The CD-based results indicate a higher content of secondary structure when the two proteins are mixed, whereas the fluorescence data confirm that the conformation of H1 is definitely affected by the interaction of the two proteins. This increased amount of helicity that seems to result from the binding is highly reminiscent of the behavior of the H1 C terminus that becomes folded (α-helical) when it binds to linker DNA and it might provide a clue for the possible mechanism of inhibition of H1-chromatin interaction by ParaT. On the other hand, ParaT exhibits all the characteristics of "natively disordered" proteins (55), which have structural plasticity and can accommodate different shapes upon association with several partners; it is highly polar, showing low structural complexity at neutral pH and becomes partially folded at low pH (data not shown), possibly due to the decrease of charge and minimization of charge/charge interactions under acidic conditions. Such a protein is reasonably expected to be involved in highly dynamic interactions. Our model implies that ParaT might be associated with chromatin transiently through cycles of binding and release. Such a dynamic interaction might not be detectable with our experimental system, and this provides a possible explanation to the fact that the protein was not detected on chromatin beads (not shown). In addition, the relatively moderate affinity of the H1-ParaT interaction, estimated from our fluorescence measurements, which is not unusual for interactions between chromatin and nuclear proteins (44–46), points to two nonmutually exclusive possibilities. First, it could suggest that other additional factors might be involved in stabilizing these interactions in vivo. Second, it might indicate that the biologically important but suboptimal H1-ParaT interaction can occur only if the local concentration is high enough. Interestingly, both possibilities are perfectly consistent with
the ample evidence for the localization of ParaT in specific subnuclear domains. In short, taken together, our data suggest that ParaT may regulate the mode of H1 association with chromatin regions.

There are several potential consequences of reducing H1 content in chromatin, either at the level of the nucleosome or globally, at the level of the cell nucleus, which arise from the ability of H1 to compact chromatin (7). We have obtained experimental evidence for a role of ParaT in chromatin decondensation using human sperm chromatin as an in vitro model system. Sperm chromatin represents a physiological highly condensed chromatin template, compared with the in vitro reconstituted chromatin substrates, which do not reproduce the higher order nature of chromatin of the interphase nuclei. Early experiments have investigated in detail the decondensation of sperm chromatin upon incubation with acidic proteins. For example, substantial swelling of nuclei was observed when sperm was challenged with TAF-I β sperm decondensation factor (38) or nucleoplasmin, a highly acidic, histone-binding protein in Xenopus egg extracts (47). Interestingly, ParaT resembles both nucleoplasmin and TAF-I β with respect to its central region that contains the polyglutamtic stretch, which suggests similar functions of these proteins. In our study we used human sperm nuclei, which have round morphology and allow an accurate quantification of nuclear surface. Our results indicated that ~50% of the sperm nuclei were highly decondensed upon challenging with ParaT.

Consistent with the in vitro data, overexpression of ParaT in HeLa cells resulted in an increase of cell nuclei surface, suggesting global chromatin decondensation. Interestingly, and in agreement with these results, disruption of the gene encoding macronuclear histone H1 of Tetrahymena resulted in enlarged macronuclei, presumably due to lower levels of chromatin compaction (56). The decondensation state of chromatin in the cells that overproduce ParaT was further confirmed by micrococcal nuclease digestion of chromatin fractions. It is well established that partial digestion of chromatin by micrococcal nuclease yields a “ladder” of oligonucleosome-sized fragments that reflects the extent of chromatin accessibility to enzymatic digestion. In our experimental system, micrococcal nuclease “ladders” were evident in the fraction that contained nucleoacid-resistant chromatin from ParaT-overproducing cells. Interestingly, nucleoacid-resistant chromatin has been proposed to be a component of the nuclear matrix that contains active genes, RNA polymerase (37, 48), and the human SWI/SNF chromatin remodeling complex (57), suggesting a potential function of ParaT in active chromatin.

In summary, the results reported here introduce ParaT as a new member of the group of proteins that modulate H1 interaction with chromatin and affect the condensation state of chromatin fibers. ParaT localizes in subnuclear domains, indicating that its local concentration is significantly higher at specific chromatin regions. Recent evidence has shown that some of the subnuclear bodies contain factors involved in the processing and transcription of RNA, whereas others function either as deposits supplying factors to active gene loci, or platforms accumulating factors involved in replication or transcription (58). Therefore, identification of functionally relevant neighboring proteins that reside at ParaT domains will provide further insight into the molecular mechanism of this nuclear polypeptide. Nevertheless, the punctuated nuclear pattern guarantees a temporally and spatially coordinated availability of ParaT to perform optimally its function. Interestingly, fluorescence recovery after photobleaching experiments reveal that ParaT moves very fast throughout the cell nucleus (not shown). Because H1 has rapid dynamics of association and dissociation (59), ParaT may access the linker histone in nucleosomes rapidly and modulate the dynamic interaction of H1 with chromatin, either globally or locally. It is worth mentioning at this point that such a dynamic association is perfectly consistent with the modest affinity detected by our fluorescence binding experiments. A similar modest affinity (10 μM) has been reported for an intramolecular interaction closely resembling that of ParaT-H1, namely the one between the acidic tail and a part (boxA) of high mobility group box 1 (HMG1) protein (46). The acidic tail is believed to modulate the interaction of HMG1 with nucleosomes and chromatin remodeling machines (60, 61). This function of the tail is due to its extended and flexible structure that interacts with specific residues within and between high mobility group boxes, shielding part of the protein surface from other interactions in a dynamic way (46).

The structural and functional significance of varying histone H1 stoichiometry in chromatin has been recently the focus of intensive work. Because linker histone binding to chromatin stabilizes folded secondary structures (7, 62), the H1-nucleosome interactions are greatly altered in active chromatin (7, 63). Consistent with this, accessible chromatin at a glucocorticoid-responsive enhancer was found to be associated with a decreased interaction of linker H1 with DNA (64). Furthermore, mouse embryos lacking three mouse H1 subtypes (H1c, H1d, and H1e) die by mid gestation, suggesting that changes in H1 stoichiometry are crucial for proper embryonic development (65). Therefore, variations in H1 stoichiometry may indicate distinct chromatin higher order structures and functional states. Competition between H1 and other nucleic proteins containing long acidic stretches, such as high mobility group proteins (66) and prothymosin α (26), has been recently reported. The effects of these proteins on the binding of H1 to chromatin might be either additive or synergistic depending on temporal/spatial localization to specific chromatin loci and the tissue-specific or developmental-specific protein expression patterns. We speculate that ParaT may participate in global chromatin remodeling during gene activation and perhaps the transcription initiation process itself. Further studies using small interfering RNAs and chromatin immunoprecipitation assays will shed light on the role of ParaT in gene regulation and may lead to the identification of specific genes that are regulated by changes of H1 stoichiometry.

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