Role of Template Activating Factor-I as a chaperone in linker histone dynamics

Kohsuke Kato¹, Mitsuru Okuwaki¹,²,³ and Kyosuke Nagata¹,*

¹Department of Infection Biology, Graduate School of Comprehensive Human Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba 305-8575, Japan
²Initiative for the Promotion of Young Scientists’ Independent Research, University of Tsukuba, 1-1-1 Tennodai, Tsukuba 305-8575, Japan
³PRESTO, Japan Science and Technology Agency, 4-1-8 Honcho, Kawaguchi, Saitama 332-0012, Japan

*Author for correspondence (knagata@md.tsukuba.ac.jp)

Accepted 6 June 2011
Journal of Cell Science 124, 3254–3265
© 2011. Published by The Company of Biologists Ltd
doi: 10.1242/jcs.083139

Summary
Linker histone H1 is a fundamental chromosomal protein involved in the maintenance of higher-ordered chromatin organization. The exchange dynamics of histone H1 correlates well with chromatin plasticity. A variety of core histone chaperones involved in core histone dynamics has been identified, but the identity of the linker histone chaperone in the somatic cell nucleus has been a long-standing unanswered question. Here we show that Template Activating Factor-I (TAF-I, also known as protein SET) is involved in histone H1 dynamics as a linker histone chaperone. Among previously identified core histone chaperones and linker histone chaperone candidates, only TAF-I was found to be associated specifically with histone H1 in mammalian somatic cell nuclei. TAF-I showed linker histone chaperone activity in vitro. Fluorescence recovery after photobleaching analyses revealed that TAF-I is involved in the regulation of histone H1 dynamics in the nucleus. Therefore, we propose that TAF-I is a key molecule that regulates linker histone-mediated chromatin assembly and disassembly.

Key words: Chromatin, FRAP, Histone chaperone, Linker histone H1, Nuclear dynamics, TAF-I

Introduction
Eukaryotic genomic DNA is associated with chromatin proteins including histones and non-histone proteins, which form the chromatin structure. The repeating unit of chromatin is a nucleosome core particle (NCP) that consists of 147 base pair (bp)-long DNA wrapped around a histone octamer consisting of two copies each of core histone H2A, H2B, H3 and H4 (Luger et al., 1997). NCPs are flanked by linker DNA, and NCP repeats are folded into the 10 nm chromatin fiber. Linker histone H1 seals the entry and exit of nucleosomal DNA and is involved in folding and stabilization of the 30 nm chromatin fiber (Tremethick, 2007).

Chromatin remodeling is a crucial step associated with the expression of genomic functions such as transcription, replication, repair and recombination. In addition, deposition and replacement of histones are also important to maintain and alter the overall chromatin structure. ATP-dependent chromatin-remodeling factors and histone modification enzymes such as histone acetyltransferases and methyltransferases have important roles in the dynamic regulation of the chromatin structure and the gene activity. Furthermore, the histone chaperone family is also involved in regulation of chromatin structure and histone metabolism (De Koning et al., 2007). A variety of histone chaperones for core histones have been identified. Core histone chaperones bind to core histones and facilitate assembly and disassembly of the nucleosome structure in an ATP-independent manner. During cell-cycle-, development- and differentiation-specific exchange and assembly of histones, core histone chaperones and/or assembly factors have central roles (De Koning et al., 2007). For instance, histone H3.3 is suggested to be incorporated into transcription-active chromatin by the HIRA–Asf1 histone chaperone complex in a replication-independent manner (Ahmad and Henikoff, 2002; Tagami et al., 2004), whereas the canonical histone H3.1 is suggested to be incorporated into chromatin by the chromatin assembly factor 1 p55 subunit (CAF1)–Asf1 histone chaperone complex in a replication-dependent manner (Tagami et al., 2004). In the case of histone H2A–H2B, in vitro studies revealed that Nucleosome Assembly Protein-I (NAP-1) is involved in the exchange of histone H2A variants such as H2AZ and H2A-Bbd (Okuwaki et al., 2005; Park et al., 2005).

In addition to the dynamics of core histones, the linker histone dynamics should be considered in the context of global regulation of the genomic chromatin, as well as chromatin remodeling. Mammalian cells have at least 11 histone H1 variants, which are classified into three sub-groups: germ cell type, somatic cell type and replacement type (Happel and Doenecke, 2009). The composition of histone H1 variants on chromatin is altered dramatically during early embryogenesis and throughout cell differentiation (Godde and Ura, 2009). A knockout (KO) mouse with three somatic histone H1 variants (H1c, H1d and H1e) disrupted shows embryonic lethality, indicating that histone H1 is essential for mammalian development (Fan et al., 2003). Fluorescence recovery after photobleaching (FRAP) analyses have revealed that linker histone H1 is rapidly exchanged within a few minutes, whereas core histone species are more stably bound to chromatin (Kimura and Cook, 2001; Lever et al., 2000; Misteli et al., 2000; Th’ng et al., 2005). The kinetics of histone H1 exchange is altered in association with cell differentiation and...
nuclear reprogramming (Jullien et al., 2010; Meshorer et al., 2006; Yellajoshyula and Brown, 2006). The ectopic expression of less-mobile H1 mutants inhibits differentiation of lineage-committed cell lines and mouse embryonic stem cells (Mesher et al., 2006; Yellajoshyula and Brown, 2006). In such histone H1 dynamics, covalent modifications such as phosphorylation, acetylation and methylation of both N- and C-terminal tails of histone H1 are crucial as epigenetic molecular signatures (Trojer et al., 2009; Vaquero et al., 2004; Wisniewski et al., 2007).

In addition to the phosphorylation by cyclin-dependent kinases (CDKs), it is suggested that high mobility group (HMG) proteins are involved in the regulation of histone H1 dynamics by their competitive binding to the linker DNA for histone H1 (Catez et al., 2005). However, the detailed mechanism and involvement of other trans-acting factors in histone H1 dynamics is not clear. More importantly, the identity of a histone chaperone(s) specific for histone H1 is not known. It has been reported that NAP-1 functions as a linker histone H1 chaperone in Xenopus egg extracts (Shintomi et al., 2005). In mammalian cells, nuclear autoantigenic sperm protein (NASP) has been reported as a linker histone chaperone candidate (Alekseev et al., 2003). NASP associates with histone H1 and promotes its nuclear import in vitro (Alekseev et al., 2005). In addition, prothymosin-α (ProTα) was shown to have the modulatory activity of histone H1 binding to chromatin (Karetsov et al., 1998 George and Brown, 2010).

To reveal the regulatory mechanism of histone H1 dynamics, we aimed to identify a linker histone chaperone(s) in mammalian somatic cells. Here, we have identified Template Activating Factor-I (TAF-I, also known as protein SET), a histone H3–H4 chaperone (Kawase et al., 1996), as a linker histone binding protein. Results presented here propose that TAF-I, rather than NAP-1 and NASP, is involved in regulation of the plasticity of chromatin in higher eukaryotes through its linker histone chaperone activity.

Results
TAF-I binds to histone H1 in the mammalian somatic cell nucleus

To identify a mammalian linker histone chaperone, we first examined the in vivo interaction between histone H1 and putative linker histone chaperone candidates, NAP-1 and NASP (Shintomi et al., 2005; Alekseev et al., 2005). We also examined the binding activity to linker histones of well-characterized histone chaperones for histone H2A and histone H3. To this end, we established HeLa cell lines stably expressing C-terminally FLAG-tagged histones (supplementary material Fig. S1A,B) (Okuwaki et al., 2010) and performed immunoprecipitation and western blotting analyses (Fig. 1A,B). We found the pronounced co-precipitation of H1.0–FLAG with TAF-I, one of histone H3–H4 chaperones, in the nuclear soluble fraction (Fig. 1A). TAF-I has the core histone chaperone activity in vitro (Kawase et al., 1996), although the amount of TAF-I co-precipitated with H3.2–FLAG was less compared with that with H1.0–FLAG. Given that primary structure and the cell-cycle-dependent expression pattern of histone H1.0 (replacement subtype) differ from those of other five somatic H1 variants (Happel and Doenecke, 2009), we next examined the interaction between TAF-I and somatic type linker histone H1.1. TAF-I was also co-precipitated with H1.1–FLAG in the nuclear soluble fraction (Fig. 1B). TAF-Iβ associated with H1.0–FLAG strongly compared with TAF-Iα (Fig. 1A, lane 16). Similarly, H1.1–FLAG was preferentially associated with TAF-Iβ but not with TAF-Iα (Fig. 1B, lane 8). TAF-Iα and TAF-Iβ differ by a short N-terminal segment (Miyaji-Yamaguchi et al., 1999). Thus, the N-terminal region of TAF-I might be involved in regulating histone H1 binding (discussed later). By contrast, the p150 subunit of chromatin assembly factor-I (CAF-I) and anti-silencing function 1b (Asf1b) co-precipitated with histone H3.2 (Tagami et al., 2004), but not with histone H1.0. NAP-1 and Spt16, a subunit of the FACT complex, were co-precipitated with histone H2A (Okuwaki et al., 2010). Note that neither NAP-1 nor NASP were co-precipitated with histone H1.0 in either cytoplasmic or nuclear fractions. Rather, NASP was efficiently co-precipitated with histone H3.2, as previously reported (Tagami et al., 2004).

Because it is possible that an artificial interaction between TAF-I and excess FLAG-tagged histone H1 occurs, we next examined by liquid chromatography and mass spectrometry (LC-MS/MS) whether TAF-I interacts with endogenous histone H1. Fig. 1C shows that TAF-Iα and TAF-Iβ were efficiently precipitated with anti-TAF-I antibody, and proteins with molecular masses of 32 kDa were also co-precipitated. Trypsin digestion and LC-MS/MS analysis of these proteins revealed common peptides to the cell-cycle-dependent histone H1 variants (H1.1–H1.5), and specific peptides to H1.2, H1.4, H1.5 and H1.X (supplementary material Tables S1 and S2). Western blotting analyses verified the interaction between histone H1X and TAF-I (Fig. 1D). These results support our conclusion that TAF-I is associated with histone H1 in the nuclear soluble fraction. It is possible that the interaction between histone H1.0 and TAF-I cannot be detected because of the low expression level of histone H1.0 in HeLa cells (Wisniewski et al., 2007).

TAF-I has linker histone chaperone activity

The association of TAF-I with histone H1 in the nuclear soluble fraction suggests that TAF-I functions as a linker histone chaperone to regulate histone H1 binding to linker DNA in chromatin. We examined whether TAF-I can facilitate chromatosome (nucleosome core particle-histone H1 complex) assembly, using purified recombinant His-tagged histone H1.1 and GST-tagged TAF-Iβ (Fig. 2A), and nucleosome core particles (NCPs) (Okuwaki et al., 2005). NCPs resolved by polyacrylamide gel electrophoresis were detected by anti-histone H3 antibody (Fig. 2B, compare lanes 1 and 6, and Fig. 2C, lane 6). When His–H1.1 alone was added to naked DNA or NCPs, the high molecular weight aggregates were formed by non-specific association between histone H1.1 and DNA (Fig. 2B, lanes 2 and 7). However, when GST–TAF-Iβ was incubated with His–H1.1 before addition of DNA, TAF-Iβ suppressed aggregate formation (Fig. 2B, compare lanes 2 and 3 with 4 and 5). A novel complex (H1.1–NCPs) was formed when GST–TAF-Iβ was incubated with His–H1.1 before the addition of NCPs (Fig. 2B, lanes 8–10). To determine whether this novel complex contains His–H1.1, we carried out super-shift assays by incubating His–H1.1–NCPs with anti-His antibody (Fig. 2D). A band generated by the incubation of NCPs with His–H1.1 and GST–TAF-Iβ ceased to exist upon addition of anti-His antibody, whereas a novel super-shifted band appeared (Fig. 2D, compare lanes 3 and 4). Western blotting analyses revealed that this super-shifted band contains histone H3, similarly to NCPs and H1.1–NCPs (Fig. 2D, right panel, compare lanes 3 and 4). We therefore concluded that this novel complex consists of NCPs and His–H1.1 and TAF-Iβ facilitates the formation of H1.1–NCP complexes (Fig. 2B, lanes 9 and 10).
To verify that the H1.1–NCP complex forms proper chromatosome structures, micrococcal nuclease (MNase) digestion assays were performed essentially as described (Shintomi et al., 2005). The MNase digestion of nucleosomes assembled on plasmid DNA generates DNA fragments with a repeat length of about 147 bp. If proper chromatosome is formed, the linker DNA portion is additionally protected from MNase digestion and about 165 bp-long DNA is generated. When reconstituted chromatin with only core histones was subjected to MNase digestion, the typical nucleosomal DNA ladder pattern was observed in a MNase dose-dependent manner (Fig. 2E, lanes 1–3). However, in the presence of His–H1.1, the efficiency of MNase digestion was reduced (Fig. 2E, lanes 4–9), and the length of DNA protected from MNase digestion was slightly longer. TAF-Iβ facilitated the efficiency of MNase digestion in the presence of His–H1.1, and the protected DNA length was slightly extended compared with that in the absence of TAF-Iβ (Fig. 2E,F, compare lanes 4–6 with lanes 7–9). These results indicate that the observed H1.1–NCP complex corresponds to the proper chromatosome structure and that TAF-Iβ can facilitate stable chromatosome structure with NCPs compared with histone H1 alone. We conclude that TAF-I has the linker histone chaperone activity.

**Chromatosome assembly by TAF-I and disassembly by NAPs**

*Xenopus* NAP-1 was identified as a B4 (oocyte-specific histone H1) binding protein in *Xenopus* egg extracts and shown to function in chromatosome assembly in vitro (Shintomi et al., 2005). Furthermore, it was also reported that *Xenopus* NAP-1 facilitates both assembly and disassembly of chromatosome structure when somatic type linker histone was used (Saeki et al., 2005). Because TAF-I and NAP-1 adopt similar three-dimensional structures (Muto et al., 2007; Park and Luger, 2006), we hypothesized that TAF-I also facilitates chromatosome disassembly. To test this, recombinant His-tagged TAF-I, NAP-1 (NAP1L1) and NAP-2 (NAP1L4) proteins were prepared (Fig. 3A). To examine whether TAF-I facilitates chromatosome disassembly similarly to NAP-1, the chromatosome was pre-assembled by incubation of NCPs with histone H1.1, followed by
incubation with or without TAF-I or NAPs (molar ratio of chaperones to His–H1.1 were 6:1 or 24:1) (Fig. 3B). When His–H1.1 and naked DNA were incubated under non-saturated conditions (molar ratio of His–H1.1 to DNA was 0.5:1) without linker histone chaperones, a small fraction of aggregate was formed (which hardly entered the polyacrylamide gel) (Fig. 3B, lane 2). By contrast, when TAF-Iβ, NAP-1 and NAP-2 were added, this aggregate was dissociated. We also found that the ability of TAF-Iβ to dissociate this aggregate was significantly lower than that of other chaperone proteins (Fig. 3B, lanes 3–6). Chromatosome samples pre-assembled without chaperones contained a low level of NCPs, chromatosomes and aggregates between naked DNA and His–H1.1 (Fig. 3B, lane 8). Upon addition of TAF-Iβ, chromatosome formation increased and the DNA–His–H1.1 aggregates dissociated in a dose-dependent manner (Fig. 3B, lanes 11–12). These results suggest that TAF-Iβ can dissociate a minor population of His–H1.1–DNA aggregates and transfer His–H1.1 to NCPs to form

Fig. 2. In vitro linker histone chaperone activity of TAF-I. (A) Purified recombinant proteins. Recombinant GST, GST–TAF-Iβ, His–H1.1 proteins were separated by 10% SDS-PAGE and visualized with Coomassie Brilliant Blue staining. Lane M contains molecular size markers. (B) Formation of histone H1.1-NCPs complexes was examined by nucleoprotein gel analyses. The 196 bp 5S rRNA gene fragments or NCPs (0.4 pmol of DNA) assembled on the same DNA with core histones were incubated without (lanes 1 and 6) or with His–H1.1 (0.6 pmol, lanes 2–5 and 7–10) pre-incubated without (lanes 2 and 7), with GST (30 pmol, lanes 3 and 8) or GST–TAF-Iβ dimer (1.7 pmol, lanes 4 and 9; 6.8 pmol, lanes 5 and 10). The complexes were separated by 6% non-denaturing PAGE, and DNA was visualized by GelRed staining. Positions of gel well, naked DNA and H1.1-NCPs are indicated by arrowheads. (C) Confirmation of histone H3 inclusion in NCPs and shifted complexes. After GelRed staining (shown in B), the gel piece was cut out, and proteins were blotted to membrane and subjected to western blotting using anti-histone H3 antibody. (D) Formation of histone H1.1–NCPs complexes was examined by super-shift assay. NCPs (0.4 pmol of DNA) were incubated without (lanes 1, 3 and 5) or with anti-His antibody (lanes 2, 4 and 6). DNA was separated by 6% non-denaturing PAGE and visualized by GelRed staining (left panel). Positions of well, free DNA, NCPs, NCPs-H1.1 complexes and antibody-bound complexes are indicated. Asterisk also indicates super-shifted band. (E) Chromatosome formation was examined by MNase assays. The chromatin assembled on the plasmid pUC119-ML2 (200 ng of DNA) was incubated without (lanes 1–3) or with His–H1.1 (60 ng, lanes 4–9) pre-incubated without (lanes 4–6) or with His–TAF-Iβ (800 ng, lanes 7–9). Then, 5 mM CaCl2 was added to samples followed by digestion with MNase (0.004 U/μl, lanes 1,4,7; 0.02 U/μl, lanes 2,5,8; 0.1 U/μl, lanes 3,6,9) at 37°C for 5 minutes. After stopping the reaction, DNA was purified and subjected to 1.5% agarose gel electrophoresis in 1× TBE, and visualized by staining with GelRed. Lane M contains DNA size markers, and DNA sizes are indicated. (F) Graphical representation of MNase assays. DNA length of each nucleosomal ladder (shown by bullets in E) is plotted.
chromatosomes. TAF-I also showed a similar activity, but its activity was lower than that of TAF-Iβ (Fig. 3B, compare lanes 9–10 and lanes 11–12). By contrast, NAP-1 and NAP-2 can dissociate not only DNA–His–H1.1 aggregates, but also chromatosome structure (Fig. 3B, compare lanes 8 and 13–16).

We obtained similar results when the same experiments were performed in the presence of lower amounts of chaperones (supplementary material Fig. S2). Our results were in good agreement with the previous report (Saeki et al., 2005) that an excess amount of free NAP-1 facilitates the dissociation of histone H1 from chromatin. These in vitro studies indicate that both TAF-I and NAP proteins suppress and/or dissociate the non-specific aggregation formed by naked DNA and histone H1.1, and that TAF-I preferentially enhances chromatosome assembly, whereas NAP proteins enhance chromatosome disassembly.

We next examined which domain of histone H1.1 was required for the association with TAF-I and NAP-1. The structure of histone H1 is divided into N-terminal (N), globular (G) and C-terminal (C) domains (Raghuram et al., 2009). We prepared a series of six GST-tagged H1.1 deletion proteins (Fig. 3C), and the interaction between histone H1.1 domains and TAF-Iβ was assayed by GST pull-down (Fig. 3D). His-tagged TAF-Iβ could be precipitated with GST-H1.1 wild type (WT) but not with GST (Fig. 3D, lanes 2–3). When other H1.1 variants were used, both GST-H1.1 GC and C derivatives were precipitated with TAF-Iβ (Fig. 3D, lanes 5 and 8). Similar results were obtained using His-NAP-1 (Fig. 3D, lanes 9–16). These results indicate that both TAF-Iβ and NAP-1 bind to the highly basic C-terminal domain (CTD) of histone H1.1.

TAF-I affects the exchange kinetics of histone H1.1 in living HeLa cells

To investigate whether TAF-I and NAP-1 are involved in the exchange of histone H1 in living cells, we performed FRAP analyses using HeLa cells stably expressing H1.1–EGFP (Fig. 4A). When the cells were treated with siRNA to knock down TAF-I, the expression levels of TAF-Iα and TAF-Iβ were reduced to 10% of normal levels (Fig. 4B). Indirect immunofluorescence analyses showed that TAF-I is depleted in >90% of cells (Fig. 4C). When the cells were treated with NAP-1-specific siRNA, the expression level of NAP-1 was reduced to <25% of the normal level (Fig. 4B). We found that the recovery kinetics of H1.1–EGFP decreased in cells treated with TAF-I-specific siRNA, but not NAP-1-specific siRNA (Fig. 4D), suggesting that TAF-I, but not NAP-1, is involved in histone H1 dynamics as a histone chaperones in vivo.

In the above experiment, the expression level of H1.1–EGFP was approximately fivefold higher than that of endogenous total histone H1 (supplementary material Fig. S1C). To examine whether TAF-I-mediated alteration of H1.1–EGFP dynamics depends on the expression level of H1.1–EGFP, we further performed FRAP analyses using another cell line (clone #10).
whose expression of H1.1–EGFP is <10% of that of clone #1 (supplementary material Fig. S3A). We found that the H1.1–EGFP dynamics also decreased in clone #10 cells treated with TAF-I-specific siRNA (supplementary material Fig. S3B–E). The expression level of endogenous histone H1 or H1.1–EGFP was unaffected by depletion of all chaperones (supplementary material Fig. S4A). The cell cycle profiles of cells treated with these siRNAs were not altered compared with control cells (supplementary material Fig. S4B,C). Therefore, we conclude that the alteration of H1.1–EGFP dynamics caused by TAF-I depletion is independent of the expression levels of histone H1 and the cell cycle.

Previously, it has been proposed that at least three distinct kinetic pools of chromatin-bound histone H1 exist: a weakly chromatin-bound pool, a strongly chromatin-bound pool and possibly, a stably bound (immobile) pool (Phair et al., 2004; Raghuram et al., 2009).
Journal of Cell Science

indicated. Four point mutations in its hydrophobic surface of the coiled-coil region of TAF-I and histone H1 binding activity of TAF-I chromatin remodeling activity (Miyaji-Yamaguchi et al., 1999). GST pull-down assays showed that both TAF-IβAC3 and PME mutants bound to GST–H1.1 as efficiently as the wild type (Fig. 5B, compare lane 12 with lanes 14,15). By sharp contrast, neither TAF-IβAC3 nor TAF-IβPME was found to have chromatosome assembly activity (Fig. 5C, lanes 9–12), indicating that the acidic region and its proper conformational orientation by dimer formation are essential for the linker histone chaperone activity of TAF-Iβ.

**TAF-Iβ enhances the histone H1 fluidity depending on its acidic region**

To examine whether an increase in the amount of TAF-I enhances histone H1 dynamics, we overexpressed TAF-I and
measured the exchange kinetics of H1.1–EGFP in living cells. FRAP analyses were performed using the H1.1–EGFP cell line overexpressing hemagglutinin (HA)-tagged TAF-I\(\beta\), the linker histone chaperone activity-deficient TAF-I\(\beta\)\(\Delta\)C3 mutant (Fig. 5B) and NAP-1. Cells were transfected with plasmids expressing HA–TAF-I\(\beta\), HA–TAF-I\(\beta\)\(\Delta\)C3 and HA–NAP-1. HA–TAF-I\(\beta\), HA–TAF-I\(\beta\)\(\Delta\)C3 or HA–NAP-1 was expressed in approximately 80% of cells (supplementary material Fig. S6), and the each expression level was approximately 25 times higher than their respective endogenous levels (Fig. 6A). The dynamics of H1.1–EGFP was robustly enhanced in cells overexpressing HA–TAF-I\(\beta\), whereas both HA–TAF-I\(\beta\)\(\Delta\)C3 and HA–NAP-1 affected the recovery kinetics only slightly (Fig. 6B). The exchange rate constant of the fast fraction in cells expressing HA–TAF-I\(\beta\) was increased compared with that in mock-transfected cells, whereas there was no significant difference in the each fraction size and the exchange rate constant of the slow fraction (Fig. 6C,D). We also observed that the exchange rate constant of the fast fraction was slightly enhanced by HA–sNASP expression, although this effect was less significant (\(P = 2.50 \times 10^{-2}\)) (supplementary material Figs S7A–D and S8). It is possible that HA–sNASP affects the dynamics of H1.1–EGFP indirectly because it was not efficiently co-precipitated with H1.1–FLAG compared with TAF-I (supplementary material Fig. S7E). Neither HA–TAF-I\(\beta\)\(\Delta\)C3 nor HA–NAP-1 showed significant change in the exchange rate constant.

FRAP analyses suggested that TAF-I\(\beta\) enhances the dissociation of H1.1 from chromatin in vivo. To address this further, we examined the association level of H1.1–EGFP with chromatin under several biochemical extraction conditions in combination with overexpression of TAF-I\(\beta\) (Fig. 7A–C). The expression level of both H1.1–EGFP and histone H3 was little affected by the overexpression of HA–TAF-I\(\beta\) and HA–TAF-I\(\beta\)\(\Delta\)C3 (Fig. 7A). The expression levels of HA–TAF-I\(\beta\) and HA–TAF-I\(\beta\)\(\Delta\)C3 were approximately 25 times higher than that of endogenous TAF-I\(\beta\) (Fig. 7B). In mock-transfected cells, small amount of H1.1–EGFP or histone H3 was recovered in a supernatant (sup) fraction (Fig. 7C, both are approximately <10% of those in pellet fractions). Significant amount of H1.1–EGFP (approximately 30% of pellet) was recovered in the sup fraction of cells when HA–TAF-I\(\beta\) was overexpressed, independently of MgCl\(_2\) concentrations, which influence chromatin compaction (Arya and Schlick, 2009) (Fig. 7C). By contrast, the amount of histone H3 in the sup fraction was significantly less than that of H1.1–EGFP even when TAF-I\(\beta\) was overexpressed. These results suggest that overexpression of TAF-I\(\beta\) enhances the dissociation
Fig. 7. TAF-I facilitates the dissociation of histone H1 from native cellular chromatin. (A) The expression levels of histone H1.1–EGFP and histone H3 were examined by western blotting analyses using indicated antibodies. Loaded cell numbers are shown above figure. Two images taken by different exposure durations (short and long) are shown. (B) The expression levels of endogenous TAF-Iβ, exogenous HA–TAF-Iβ and HA–TAF-IβΔC3 were examined by western blotting analyses using indicated antibodies. (C) Subcellular fractionation was carried with cells expressing exogenous TAF-Iβ and TAF-IβΔC3. The amounts of histones H1.1–EGFP and H3 in supernatant and pellet fractions were examined. β-actin serves as a loading control. Loaded cell numbers were shown above panels. Two photos taken by different exposure durations (short and long) are shown. Additional western blotting analyses were performed using high titer-GFP antibody and smaller cell numbers for pellet samples (lanes 19–27). (D) TAF-Iβ overexpression induces nuclear morphological changes. Images of HeLa cells stably expressing H1.1–EGFP transfected with plasmid DNA encoding HA–TAF-Iβ (II) or HA–TAF-IβΔC3 (III) and control plasmid (I) are shown. Scale bar: 10 μm. IV and V are magnified images of indicated nuclei by arrows in images I and II, respectively. The nucleolar peripheral heterochromatin region is also indicated by arrowhead in image IV. (E) H1.1–EGFP dynamics was significantly enhanced in morphologically aberrant nuclei generated by TAF-Iβ overexpression. FRAP was performed in mock-transfected cells, and cells overexpressing TAF-Iβ including morphologically normal and aberrant nuclei (V as shown in D). Relative fluorescent intensities (measure before bleaching is set to 1) were plotted. The time point of bleaching is indicated by arrow. Each data set consists of at least ten cells per experiment. Three independent experiments were performed for each cell. Error bars represent s.d.
of histone H1 but not histone H3 from chromatin. This notion is in good agreement with the result obtained from FRAP analyses. We found that the cell population with aberrant nuclear morphology was increased by overexpression of TAF-1β. Nuclei with aberrant morphology in cells overexpressing TAF-1β became relatively enlarged compared with that of normal cells (Fig. 7D and supplementary material Fig. S9A). Furthermore, the localization of H1.1–EGFP became homogenous, and higher-ordered nuclear structures such as the nucleolus were invisible in those nuclei (Fig. 7D). Approximately 25% of total cells overexpressing TAF-1β showed morphologically aberrant nuclei (supplementary material Fig. S9B). In morphologically aberrant nuclei, the H1.1–EGFP mobility was extremely rapid, suggesting that H1.1–EGFP is greatly diffused (Fig. 7E). The cell cycle profiles of cells expressing HA–TAF-1β, HA–TAF-1βC3 and HA–NAP-1 were similar to that of mock-transfected cells (supplementary material Fig. S10A,B). However, we found that the DNA content becomes lower in approximately 20% of cells by overexpression of TAF-1β (supplementary material Fig. S10A,B). These cells would not be apoptotic because they were negative in Annexin-V staining (data not shown). It is possible that cells with aberrant nuclei are alive, but the chromatin (and/or nuclei themselves) in these cells becomes fragile. Collectively, these results suggest that TAF-1β enhances the dissociation of histone H1 and that its C-terminal acidic region is crucial for the histone H1 chaperone activity.

Discussion

Here, we have demonstrated that TAF-I functions as a linker histone chaperone, which is involved in the regulation of histone H1 exchange in the mammalian somatic cell nucleus. We observed that TAF-I is associated with histone H1 in the HeLa cell nuclear soluble fraction (Fig. 1A–D). More importantly, NAP-1 and NASP, previously reported linker histone chaperone candidates, are poorly associated with histone H1 in somatic cells (Fig. 1A,B). From immunoprecipitation and MS/MS analyses, we found that TAF-I associates with at least three cell-cycle-dependent variants and one cell-cycle-independent histone H1 variant (H1.2, H1.4, H1.5 and H1X) in the nuclear soluble fraction of HeLa cells (Fig. IC,D, and supplementary material Tables S1 and S2). We found that TAF-I associates with histone H1.0 and H1.1 when exogenously expressed in HeLa cells (Fig. 1A,B), suggesting that TAF-I has intrinsic ability to bind histone H1.0 and H1.1 in vivo. Histone H1 variants have similar primary structures, but histone H1.0 and H1X are different from the five somatic histone H1 subtypes (H1.1–H1.5). Because TAF-I binds not only to three cell-cycle-dependent histone H1 subtypes, but also H1X, it is possible that overall structure, but not the primary structure of histone H1 CTD (in the case of H1.1 in Fig. 3D), is important for the interaction between TAF-I and histone H1. In contrast to the high homology of histone H1 variants, their expression levels differ among organs or cell types. It was suggested that the expression levels of histone H1.0 and H1.1 are relatively low compared with that of four somatic histone H1 subtypes (H1.2–H1.5) in HeLa cells, whereas the expression levels of histone H1.2, H1.3 and H1.4 have not been reported (Wisniewski et al., 2007). Therefore, it is possible that we were unable to detect histone H1.0 and H1.1 (as well as H1.3) by MS/MS analyses, whereas TAF-I was found to bind to a variety of histone H1 variants.

Both TAF-I and NAP-1 have modulatory activity in histone H1 binding to chromatin in vitro (Fig. 3B). However, it is not clear how these proteins function differently. TAF-1β and NAP-1 are folded into a similar structure, whereas their backbone helices are shaped differently, and NAP-1 has an extra inserted helix (Muto et al., 2007). Such structural differences between TAF-1β and NAP-1 might contribute to the difference in the modulation of histone H1 dynamics. It has been speculated that the chromatosome is formed through interaction between histone H1 and the C-terminal tail of histone H2A (Luger et al., 1997). Because NAP-1, but not TAF-I, has binding specificity for histone H2A, NAP-1 might modulate the binding between histone H1 and histone H2A through its interaction with histone H2A. Alternatively, this interaction could have a role in NAP-1-mediated dissociation of chromatosome structure. Although our observation suggests that NAP-1 binds poorly to histone H1 in mammalian somatic cells, we cannot exclude the possibility that NAP-1 is also involved in histone H1 metabolism in a cell-lineage-specific manner. In support of this view, it was reported that histone H1 dissociates from chromatin when NAP-1 is transiently relocalized in the nucleus in mouse primordial germ cells on embryonic day 11.5 (Hajkova et al., 2008), suggesting that NAP-1 removes histone H1 to alter the chromatin structure for epigenetic reprogramming of germ cell lines.

NASP preferentially associates with histone H3.2, rather than H1, in the nuclear soluble fraction (Fig. 1A). FRAP analyses also revealed that NASP is not significantly involved in the exchange of histone H1 (supplementary material Figs S4 and S6). Therefore, it is probable that a major fraction of NASP forms the multi-chromatosome complex with Asf1, CAF-1 and histone H3.1 (Tagami et al., 2004), although we cannot rule out the possibility that a small fraction of NASP is associated with histone H1.

Previous studies have shown that HMG proteins affect the in vivo dynamics of histone H1 through their competitive binding to the nucleosomal binding sites of histone H1 (Catez et al., 2004; Rochman et al., 2009). These studies suggest the possible involvement of competition among linker histone, regulatory protein and nucleosomal DNA in the linker histone dynamic equilibrium. We have shown that TAF-I is a novel trans-acting factor involved in histone H1 dynamics by its chaperone activity but not competitive binding to chromatin. FRAP analyses indicated that TAF-I affects the exchange kinetics of the fast mobile (thus, low affinity for chromatin) histone H1 population (Figs 4 and 6). Although the biological significance of the two kinetic fractions of histone H1 is still unclear, it is likely that the fast mobile histone H1 population represents a non-specific DNA-binding and/or locus-specific but unstable chromatosome structure. Previously, it was suggested that weak binding between histone H1 and chromatin occurs through the interaction between its CTD and DNA (Raghuram et al., 2009). Because TAF-I preferentially localizes in euchromatic regions, it is possible that TAF-I modulates the DNA-binding affinity of euchromatic (probably loosely chromatin-bound) histone H1 through its interaction with CTD, and thus is involved in a certain nuclear reaction(s) such as transcription.

TAF-I could not efficiently dissociate histone H1 from chromatin in vitro even when present in excess (Fig. 3B), whereas FRAP analyses strongly suggested the involvement of TAF-I in the histone H1 assembly and disassembly in vivo (Figs 4, 6 and 7). Currently, it is not clear what causes such functional differences. We speculate that phosphorylation of histone H1 is cooperatively involved in its dissociation by TAF-I in vivo, because CDK-dependent phosphorylation of histone H1 is


involved in the regulation of its dynamics (Yellajoshuya et al., 2006). Alternatively, TAF-Iβ forms a protein complex including ProTzx in vivo (Karetou et al., 2004). ProTzx also has linker histone chaperone activity and is involved in the nuclear dynamics of histone H1 (George and Brown, 2010; Karetou et al., 1998). Our in vitro analyses revealed that TAF-I and NAP-I preferentially bind to the histone H1 CTD, whereas ProTzx binds to its globular domain (Karetou et al., 1998). Furthermore, ProTzx preferentially mediates histone H1 dissociation from chromatin in vitro (Karetou et al., 1998). It is therefore possible that a putative TAF-I–ProTzx complex efficiently regulates the exchange of histone H1 on chromatin using their functional differences in vivo.

Histone-H1-dependent chromatin dynamics is important for a variety of biological phenomena, including early embryogenesis, cell differentiation and cell senescence (Funayama et al., 2006; Godde and Ura, 2009; Mesheror et al., 2006; Yellajoshuya and Brown, 2006). Our results indicate that TAF-I could modulate the higher-ordered chromatin structure (Fig. 7D,E). It could be worthwhile to examine whether TAF-I is involved in nuclear reprogramming by re-organizing the whole chromatin structure through its linker histone chaperone activity, and thus is widely involved in cell fate.

Materials and Methods

Cell culture, construction of cell lines and FACS analyses

Maintenance of HeLa cells expressing H2A-FLAG and H1.0-FLAG, and construction of HeLa cells expressing H3.2-FLAG, H1.1-FLAG, H1.2-FLAG and H1.1-EGFP, were followed as described (Okuwaki et al., 2010). Stably expressed samples were selected in growth medium containing 1 mg/ml G418, and single colonies were picked to generate clonal cell lines. FACS analyses were performed by following standard protocols.

Antibodies and indirect immunofluorescence analyses

Antibodies used in this study were as follows: anti-CAF-Ip150 (clone SS1) and anti-Asf1b antibodies gifts from Alain Verreault (University of Montreal, Canada); anti-nAP-1, anti-Spt6 and anti-histone H1 (clone AE4) antibodies from SantaCruz Biotech; anti-NASP antibody from ProteinTech Group; anti-histone H3 and anti-histone H1X antibodies from Abcam; anti-β-actin, anti-FLAG-tag and anti-His-tag antibodies from Sigma; anti-GFP antibody from Nacalai tesque; anti-HA-tag antibody (clone 3F10) from Roche; anti-TAF-Iβ antibody (monoclonal antibody KM1720; Kirin-Kyowa Hakko) (Nagata et al., 1998); anti-TAF-Iβ polyclonal antibody, which was generated in rabbits using recombinant TAF-IβAC as an antigen. For indirect immunofluorescence analyses, antibodies conjugated to Alexa Fluor 488 and Alexa Fluor 568 were from Invitrogen. Images were obtained using microscopes (LSM EXCITER or Axioplan2 imaging; Carl Zeiss Microimaging).

Immunoprecipitation, liquid chromatography and mass spectrometry analyses (LC-MS/MS)

Preparation of cytoplasmic and nuclear extracts from HeLa cells and immunoprecipitation were performed as described (Okuwaki et al., 2010). Samples for LC-MS/MS analyses were prepared by cutting out immunoprecipitated proteins from silver-stained gel and in-gel trypsin digestion. Mass spectrometry data were obtained using LC-MS/MS apparatus (ZAPLOUS; AMR) and analyzed with Mascot software.

Nucleosome reconstitution and chromatosome assembly-disassembly assay

Reconstituent chaperone proteins were prepared as described previously (Matsumoto et al., 1999; Miyaji-Yamaguchi et al., 1999; Okuwaki et al., 2005). His-tagged H1.1, GST-tagged H1.1, and its mutant derivatives, were also expressed in E. coli BL21 (DE3). Core histones were purified from HeLa S3 cells. NCPs were assembled with 196 bp sea urchin SS rRNA gene fragment and core histones by the salt dilution method as described previously (Okuwaki et al., 2010). Chromatosome assembly-disassembly assays were performed essentially as follows: Briefly, naked 5S DNA or NCPs were incubated at 30°C for 30 minutes with His-H1.1 pre-incubated at 30°C for 30 minutes with or without histone chaperone proteins. Samples were then subjected to a 5% non-denaturing polyacrylamide gel electrophoresis in 0.5 × TBE buffer, and then DNA was visualized by staining with GelRed (Biotium). For super-shift assays, samples were incubated with anti-His antibody at 30°C for 15 minutes just before loading on the polyacrylamide gel. For MNase assays, chromatin was assembled with plasmid pUC19-ML2 (Karo et al., 2007) and subjected to the salt dilution method. The reconstituted chromatin (200 ng DNA-equivalent) was incubated at 30°C for 30 minutes with or without His-H1.1 pre-incubated at 30°C for 30 minutes with or without His-TAF-Iβ. DNA was purified, subjected to 1.5% agarose gel electrophoresis in 0.5 × TBE buffer and visualized by staining with GelRed. DNA images were analyzed with LAS-4000UV mini image analyzer (Fuji Film).

siRNA and plasmid transfection

All Stealth siRNAs were obtained from Invitrogen. siRNAs were transfected with Lipofectamine2000 (Invitrogen). Cells were re-plated at 72 hours after transfection and then incubated for 12 hours. Cells were transfected with siRNAs once again and further incubated for 48 hours after the second transfection. Cells were transfected with plasmid DNAs expressing HA-tagged proteins using GenElute (Novagen) in combination with plasmid phape-buro expressing pyruvycin-resistant gene. At 24 hours after transfection, 2 μg/ml of puromycin dihydrochloride was directly added to the culture medium, and cells were further incubated for 24 hours to remove untransfected cells.

FRAP analyses

HeLa cells stably expressing H1.1–EGFP grown on 35-mm-diameter glass base dishes were transfected with Stealth siRNA or plasmid DNAs. The dish was set on an inverted microscope (LSM EXCITER, Carl Zeiss Microimaging, Inc.) in an air chamber at 37°C, and the mobility of EGFF-tagged histone H1.1 was analyzed by photobleaching with a Plan-Apochromat 63 × 1.4 oil objective. A 2 μm spot was bleached using 85% transmittance of 488-nm Ar laser with 70% output power) every 2 seconds. The fluorescence intensity of the bleached area was measured. Subtracting the background, normalizing by fluorescent decay during scan and exponential recovery curve fitting were performed using FRAP for ZEN software (Carl Zeiss Microimaging). The double exponential recovery curve is described by the equation, \( F(t) = A_1(1 - e^{-kt}) + A_2(1 - e^{-kt}) \) (Sprague et al., 2004). \( F(t) \) indicates the normalized fluorescent intensity at each time point where the measurement just before bleaching is set to be 0 and that just after bleaching is set to be 0. \( A_1 \) and \( A_2 \) indicate the ratio of fast and slow mobile fraction each, and the ratio of total mobile fraction (A) is calculated by the equation, \( A = A_1 + A_2 \). Conversely, the ratio of immobile fraction is calculated by a formula: 1–A. K1 and K2 indicate the rate constants for the fluorescent exchange between bleached and un-bleached regions. FRAP recovery curves were drawn in Excel2003 where the intensity was normalized to the initial intensity before bleaching.

Subcellular fractionation

The basis of fractionation methods was essentially as described (Capco et al., 1982). Cells transfected with plasmids and selected with puromycin were incubated in an extraction buffer (10 mM PIPES-NaOH, pH 6.8, 100 mM NaCl, 1 mM EGTA, 300 mM sucrose, 1 mM PMSF, 1 mM NaF, 10 mM β-glycerophosphate, 1 mM NaVO3, and 0.1% NP40) with or without 3 mM MgCl2 on ice for 10 minutes. Supernatant and pellet fractions were separated by centrifugation, and subjected to 15% SDS-PAGE followed by western blot analyses.

Acknowledgements

We thank Alain Verreault (University of Montreal, Canada) for anti-CAF-Ip150 and anti-Asf1b antibodies, Takashi Minowa, Ayumu Yosida (National Institute for Materials Science, Japan), and Masamitsu N. Asaka (University of Tsukuba, Japan) for kind support with LC-MS/MS analyses, Shinichi Tate (Hiroshima University, Japan) for human histone expression plasmids, Miharu Hisaoka (University of Tsukuba) for purification of HeLa core histones, Shoko Saito and Kiong Ho (University of Tsukuba) for purification of HeLa core histones, Shoko Saito and Kiong Ho (University of Tsukuba) for discussion and proofreading of this manuscript.

Funding

This work was supported by Grants-in-aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (to M.O. and K.N.), in part by PRESTO from Japan Science and Technology Agency and Special Coordination Funds for Promoting Science and Technology (to M.O.).

Supplementary material available online at http://jcs.biologists.orglookup/suppl/doi:10.1242/jcs.083139/-/DC1
References


Fig. S1. Characterization of cell lines expressing histone H1. (A) The expression level of endogenous histone H1 and H1-Flag was examined by western blotting using anti-histone H1 antibody. Histone H3 is a loading control. Asterisks indicate H1-Flag. (B) Cell populations expressing H1-Flag were examined by immunofluorescence analyses. HeLa cells stably expressing H1.0-Flag, and H1.1-Flag were subjected to indirect immunofluorescence analyses using anti-Flag antibody. DNA was stained with DAPI. Scale bar, 100 μm. (C) The expression level of endogenous histone H1 and H1.1-EGFP was examined by western blotting using anti-histone H1 antibody. Cell line #1 was used. Histone H3 is a loading control. Asterisks indicate non-specific bands.
Fig. S2. Comparison of the linker histone chaperone activity between TAF-I and NAPs. DNA and NCPs (0.4 pmol of DNA), His-H1.1 (0.2 pmol), each chaperone dimer (0.06, 0.2, 0.6, and 2 pmol) were used. The positions of naked DNA, NCPs, and chromatosome (Chr) were indicated. TAF-Iα and β enhanced chromatosome assembly when molar ratios of chaperone to His-H1.1 were 3 and 10 (lanes 5-6, and 9-10). In contrast, NAPs enhanced the chromatosome disassembly when a molar ratio of chaperone to His-H1.1 was 10:1 (lanes 14 and 18).
Fig. S3. Effect of TAF-I KD on histone H1.1 dynamics was examined by FRAP analyses using other cell line (clone #10). (A) Expression levels of histones in clonal HeLa cell lines expressing H1.1-EGFP were examined by western blotting. Histone H3 is a loading control. (B) The KD efficiency of TAF-I was confirmed by western blotting. β-actin is a loading control. Asterisks indicate non-specific bands. (C) FRAP recovery curve. The means of the relative fluorescent intensity in a bleached area are indicated. The examined cell numbers are shown in inlet (n). Error bars, SD. (D and E) Exponential recovery curve fitting is performed, and calculated fraction sizes (C) and exchange rate constants (D) are shown. Error bars, SD.
Fig. S4. Effects of depletion of histone chaperones on protein expression levels and cell cycle profiles. (A) Expression levels of endogenous histone H1s and H1.1-EGFP in siRNA-mediated histone chaperone KD cells. Western blotting analyses using anti-H1 and anti-GFP antibodies were performed using cell extracts derived from TAF-I KD, NAP-1 KD, and NASP KD cells. β-actin is a loading control. Asterisks indicate non-specific bands. (B and C) Cell cycle profiles of histone chaperone-depleted cells. HeLa cells stably expressing H1.1-EGFP were transfected with control siRNA and either TAF-I, NAP-1, or NASP-specific siRNA, and subjected to FACS analyses using FACSCalibur (Becton Dickinson). Collected cells were fixed with 70% ethanol, treated with RNaseA, and DNA was stained with propidium iodide. For each sample, at least 30,000 cells were counted. Histograms after gating out doublets and debris were represented. In parallel, we performed this FACS analyses with western blotting analyses, showing the expression level of each chaperone protein in Fig. S3. Distribution of cells in cell cycle was analyzed using Cell Quest pro software (Becton Dickinson) and graphically represented in panel C.
Fig. S5. Effect of NASP KD on histone H1.1 dynamics was examined by FRAP analyses. (A) The KD efficiency of TAF-I or NASP was confirmed by western blotting. β-actin is a loading control. Asterisks indicate non-specific bands. (B) FRAP recovery curve. The means of the relative fluorescent intensity in a bleached area are indicated. The examined cell numbers are shown in inlet (n). Error bars, SD. (C and D) Exponential recovery curve fitting was performed, and calculated fraction sizes (C) and exchange rate constants (D) are shown. Error bars, SD.
Fig. S6. Populations of cells expressing exogenous TAF-Iβ and NAP-1 were examined by immunofluorescence analyses using anti-HA antibody. HeLa cells stably expressing H1.1-EGFP and over-expressing HA-TAF-Iβ, βΔC3, or NAP-1 were subjected to indirect immunofluorescence analyses using anti-HA antibody. DNA was stained with DAPI. Scale bar: 50 μm.
Fig. S7. Effect of over-expression of NASP on histone H1.1 dynamics was examined by FRAP analyses. 
(A) The expression level of HA-sNASP or HA-tNASP was examined by western blotting. β-actin is a loading control. 
(B) FRAP recovery curve. The means of the relative fluorescent intensity in a bleached area are indicated. The examined cell numbers are shown in inlet (n). Error bars, SD. 
(C and D) Exponential recovery curve fitting is performed, and calculated fraction sizes (C) and exchange rate constants (D) are shown. Error bars, SD. 
(E) Immunoprecipitation assay. Histone chaperones co-immunoprecipitated with Flag-tagged histone H1.1 from cell extracts were detected by western blotting analyses. Cytoplasmic extracts (CE) and nuclear extracts (NE) prepared from HeLa cell lines expressing Flag-tagged histone H1.1 were incubated with (lanes 3 and 4) or without (lanes 5 and 6) anti-Flag antibodies. Input extracts (1% of total extract volume) and immunoprecipitated proteins (IP, 20% of total elution volume) were loaded on 10% SDS-PAGE followed by western blotting with antibodies against NASP, TAF-I, and Flag-tag. Positions of proteins were indicated by arrowheads.
Fig. S8. Populations of cells expressing exogenous sNASP and tNASP were examined by immunofluorescence analyses using anti-HA antibody. HeLa cells stably expressing H1.1-EGFP and over-expressing HA-sNASP, or tNASP were subjected to indirect immunofluorescence analyses using anti-HA antibody. DNA was stained with DAPI. Scale bar: 50 mm.
Fig. S9. Over-expression of HA-TAF-1β induced aberrant nuclei in a dose-dependent manner. (A) Immunofluorescence analyses. HA-TAF-1β, βΔC3, and NAP-1 were over-expressed in HeLa cells expressing H1.1-EGFP, and cells were subjected to indirect immunofluorescence analyses using anti-HA antibody. Relatively high amount of HA-TAF-1β was expressed in cells including aberrant nuclei (indicated by arrows), while relatively low amount of that was expressed in cells including normal nuclei (indicated by arrow heads). Scale bar, 10 μm. (B) Populations of cells including aberrant nuclei (indicated by arrows in panel A) were counted. The ratio of the number of aberrant nuclei to that of total nuclei were plotted. The numbers of total nuclei are shown (n).
**Fig. S10.** **Cell cycle profiles of cells over-expressing histone chaperones.** (A) HeLa cells stably expressing H1.1-EGFP were transfected with plasmid DNAs expressing HA-TAF-Iβ, HA-TAF-IβΔC3, or HA-NAP-1, and subjected to FACS analyses using FACSCalibur (Becton Dickinson). Collected cells were fixed with 70% ethanol, treated with RNaseA, and DNA was stained with propidium iodide. Analyses were performed using FACSCalibur (Becton Dickinson). For each sample, at least 20,000 cells were counted. Histograms after gating out doublets were represented. Fraction M2-M4 indicate cells in the regular cell cycle, whereas fraction M1 indicate the cells whose DNA content becomes lower. In parallel, we performed this FACS analyses with FRAP analyses (Fig. 6), expression level of each chaperone protein was shown in Fig. 6A. (B) Cell cycle distribution (M1-M4) was analyzed using Cell Quest pro software (Becton Dickinson).
<table>
<thead>
<tr>
<th>Histone H1</th>
<th>Measured</th>
<th>Theoretical</th>
<th>Δ</th>
<th>Peptide sequence</th>
<th>Start-End (H1.2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1.1, 2, 3, 4, 5</td>
<td>532.3127</td>
<td>532.3221</td>
<td>-0.0093</td>
<td>SLVSK</td>
<td>86-90</td>
</tr>
<tr>
<td>H1.2, 3, 4</td>
<td>844.5997</td>
<td>844.5018</td>
<td>0.0979</td>
<td>SGVSLAALK</td>
<td>55-63</td>
</tr>
<tr>
<td>H1.1, 2, 3, 4</td>
<td>1105.5247</td>
<td>1106.5608</td>
<td>-1.036</td>
<td>ALAAAGYDVEK</td>
<td>65-75</td>
</tr>
<tr>
<td>H1.1, 2, 3, 4</td>
<td>1106.5107</td>
<td>1106.5608</td>
<td>-0.05</td>
<td>ALAAAGYDVEK</td>
<td>65-75</td>
</tr>
<tr>
<td>H1.1, 2, 3, 4</td>
<td>1106.7597</td>
<td>1106.5608</td>
<td>0.199</td>
<td>ALAAAGYDVEK</td>
<td>65-75</td>
</tr>
<tr>
<td>H1.1, 2, 3, 4</td>
<td>1107.7027</td>
<td>1106.5608</td>
<td>1.142</td>
<td>ALAAAGYDVEK</td>
<td>65-75</td>
</tr>
<tr>
<td>H1.1, 2, 3, 4</td>
<td>1235.6297</td>
<td>1234.6557</td>
<td>0.974</td>
<td>KALAAAGYDVEK</td>
<td>64-75</td>
</tr>
<tr>
<td>H1.1, 2, 3, 4</td>
<td>1324.5847</td>
<td>1325.7554</td>
<td>-1.1707</td>
<td>KASGPPVSELITK</td>
<td>34-46</td>
</tr>
<tr>
<td>H1.1, 2, 3, 4</td>
<td>1326.7687</td>
<td>1325.7554</td>
<td>1.0133</td>
<td>KASGPPVSELITK</td>
<td>34-46</td>
</tr>
<tr>
<td>H1.1, 2, 3, 4</td>
<td>1577.8697</td>
<td>1577.7797</td>
<td>0.09</td>
<td>ALAAAGYDVEKNNSR</td>
<td>65-79</td>
</tr>
<tr>
<td>H1.1, 2, 3, 4, 5</td>
<td>745.4667</td>
<td>745.4334</td>
<td>0.0333</td>
<td>GTLVQTK</td>
<td>91-97</td>
</tr>
</tbody>
</table>

Measured peptide mass (in Da), a theoretical mass, difference between measured and theoretical mass (Δ), and corresponding peptide sequence are shown. The numbers of amino acids corresponding to given peptide of histone H1.2 (Start-End) are shown.
<table>
<thead>
<tr>
<th>Histone H1</th>
<th>Measured</th>
<th>Theoretical</th>
<th>$\Delta$</th>
<th>Peptide sequence</th>
<th>Start-End</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1.2</td>
<td>1014.1907</td>
<td>1013.6233</td>
<td>0.5674</td>
<td>KPAAATVTKK</td>
<td>160-169</td>
</tr>
<tr>
<td>H1.4</td>
<td>912.4767</td>
<td>911.5552</td>
<td>0.9215</td>
<td>KPAAAAGAKKK</td>
<td>160-169</td>
</tr>
<tr>
<td>H1.5</td>
<td>982.7347</td>
<td>982.5923</td>
<td>0.1424</td>
<td>AKKPAAAAGAKK</td>
<td>158-168</td>
</tr>
<tr>
<td>H1.5</td>
<td>487.6657</td>
<td>487.3118</td>
<td>0.3539</td>
<td>AAKAK</td>
<td>215-219</td>
</tr>
<tr>
<td>H1.5</td>
<td>967.8177</td>
<td>967.5814</td>
<td>0.2363</td>
<td>AKKPAGATPK</td>
<td>131-140</td>
</tr>
<tr>
<td>H1.5</td>
<td>1013.7037</td>
<td>1013.6233</td>
<td>0.0804</td>
<td>NGLSLAALKK</td>
<td>58-67</td>
</tr>
<tr>
<td>H1.5</td>
<td>1211.8937</td>
<td>1211.6761</td>
<td>0.2176</td>
<td>ATGPPVSELITK</td>
<td>38-49</td>
</tr>
<tr>
<td>H1.5</td>
<td>1563.8517</td>
<td>1563.7641</td>
<td>0.0876</td>
<td>ALAAGGYDVEKNNSR</td>
<td>68-82</td>
</tr>
<tr>
<td>H1X</td>
<td>458.4697</td>
<td>459.2805</td>
<td>-0.8108</td>
<td>GGKAK</td>
<td>183-187</td>
</tr>
<tr>
<td>H1X</td>
<td>702.7817</td>
<td>702.4024</td>
<td>0.3793</td>
<td>KTAAGGK</td>
<td>188-195</td>
</tr>
<tr>
<td>H1X</td>
<td>723.4947</td>
<td>723.3803</td>
<td>0.1144</td>
<td>IYTEAK</td>
<td>70-75</td>
</tr>
<tr>
<td>H1X</td>
<td>924.2297</td>
<td>924.5756</td>
<td>-0.3459</td>
<td>KAAKPSVPK</td>
<td>199-207</td>
</tr>
<tr>
<td>H1X</td>
<td>1206.9547</td>
<td>1206.6608</td>
<td>0.2939</td>
<td>YSQLVGETIR</td>
<td>48-57</td>
</tr>
<tr>
<td>H1X</td>
<td>1330.1967</td>
<td>1330.6993</td>
<td>-0.5026</td>
<td>GAPAAATAPAPTAHK</td>
<td>129-143</td>
</tr>
<tr>
<td>H1X</td>
<td>1330.6117</td>
<td>1330.6993</td>
<td>-0.0876</td>
<td>GAPAAATAPAPTAHK</td>
<td>129-143</td>
</tr>
<tr>
<td>H1X</td>
<td>1341.7497</td>
<td>1340.7664</td>
<td>0.9834</td>
<td>ALVQNDTLQQVK</td>
<td>95-106</td>
</tr>
<tr>
<td>H1X</td>
<td>1874.4787</td>
<td>1873.9343</td>
<td>0.5445</td>
<td>SVELEEALPVTTAEGMAK</td>
<td>2-19</td>
</tr>
</tbody>
</table>

Measured peptide mass (in Da), a theoretical mass, difference between measured and theoretical mass ($\Delta$), and corresponding peptide sequence are shown. The numbers of amino acids corresponding to given peptide of each histone H1 (Start-End) are shown.