Isoform-specific phosphorylation of human linker histone H1.4 in mitosis by the kinase Aurora B

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Summary

The linker histone H1 plays an essential role in maintaining and establishing higher-order chromatin structure. As with core histones, histone H1 is also extensively covalently modified. We showed previously that phosphorylation of S27 in human histone H1.4 (H1.4S27-*P*), prevents binding of heterochromatin protein 1 (HP1) family members (officially known as chromobox protein homologs) to the neighboring dimethylated K26. Here, we present the first functional characterization of H1.4S27-*P* in vivo and in vitro. We show that H1.4S27 phosphorylation is cell-cycle-regulated and its levels peak on metaphase chromosomes. We identify further Aurora B as the kinase phosphorylating H1.4S27. We demonstrate that histone H1.4 is the only somatic linker histone variant targeted by Aurora B and that Aurora B exclusively phosphorylates S27. Adjacent K26 dimethylation can regulate Aurora B activity towards S27, uncovering a crosstalk between these modifications. Finally, our fluorescence recovery after photobleaching (FRAP) analysis on histone H1.4 mutants suggests a role of S27 phosphorylation in the regulation of histone H1.4 mobility and chromatin binding in mitosis.

Key words: Aurora B, Histone H1, Histone modification, Phosphorylation

Introduction

Together with the nucleosomal core particle, the linker histone H1 is part of the basic repeat unit of chromatin, the nucleosome. It binds to the linker DNA and is essential for establishment and maintenance of higher-order chromatin structures (Thoma et al., 1979). Histone H1 comprises a short N-terminal tail, a globular domain and a long C-terminal tail, which adopts specific secondary structures upon binding to DNA (Roque et al., 2008). Of the 11 human H1 isoforms, H1.4 belongs to the ubiquitously expressed somatic H1 variants and in many cells it is expressed at high levels (Izzo et al., 2008).

Phosphorylation of H1 has been recognized as its most prominent modification (e.g. Balhorn et al., 1972). Bulk H1 phosphorylation was found to be strongly cell-cycle-regulated, peaking in M phase and occurring predominantly on (S/T)PxK consensus motifs in the C-terminal tail, which are recognized by cyclin-dependent kinases (CDKs). In the past years, however, a number of mass spectrometrical studies have demonstrated that linker histones are also subject to many more modifications, including methylation, acetylation and ADP-ribosylation (Garcia et al., 2004; Wisniewski et al., 2007). This raises the question of whether a 'linker histone code' exists and modifications of the H1 histones contribute to the regulation of chromatin dynamics and transcriptional activity similar to core histone modifications. In fact, methylation of H1.4K26 (Garcia et al., 2004; Lu et al., 2009) seems to display 'coding properties', as it is able to recruit heterochromatin protein 1 (HP1) family members (officially known as chromobox protein homologs) (Daujat et al., 2005). H1.4K26 methylation is catalyzed by the histone lysine methyl transferases (HKMTs) Ezh2 and G9a (Kuzmichev et al., 2004; Trojer et al., 2008; Weiss et al., 2010). Interestingly, the neighboring S27 is phosphorylated and both modifications had been found to coexist (Garcia et al., 2004). We have recently shown that H1.4S27 phosphorylation prevents binding of the HP1 chromo domain to H1.4K26-Me_2 (where Me represents a methyl group) (Daujat et al., 2005). Interestingly, this creates a similar 'methylation–phosphorylation switch' outcome to that observed with HP1 binding to H3K9-Me_2 or H3K9-Me_3 (Fischle et al., 2005; Hirota et al., 2005). The kinase phosphorylating H1.4S27 has, however, remained elusive, especially as this residue is not within a consensus motif for CDKs.

In this study, we functionally characterize H1.4S27-*P* using a newly raised specific antibody. We show that H1.4S27-*P* is a novel mitotic mark that is set by the kinase Aurora B. We find that adjacent K26 methylation can regulate the Aurora B activity towards S27. Furthermore, fluorescence recovery after photobleaching (FRAP) analysis demonstrates that H1.4S27 mutations alter the mobility of H1.4. Our data is therefore the first report of a modification site in the N-terminal tail of histone H1 regulating mobility and binding to condensed mitotic chromatin.

Results and Discussion

Characterization of a H1.4 S27-*P*-specific antibody

Histone H1.4 can be phosphorylated at S27 in the N-terminus (H1.4S27) (supplementary material Fig. S1A) (Garcia et al., 2004). In order to gain insight into the function of this H1.4 phosphorylation, we raised a novel H1.4S27-*P*-specific antibody. This antibody specifically recognized the immunizing peptide but not the unmodified peptide (supplementary material Fig. S1B,C). We confirmed these results by peptide competition experiments on native H1 (Fig. 1A). Dephosphorylation of native H1, with λ-phosphatase (λ-PPase) resulted in a loss of signal, demonstrating

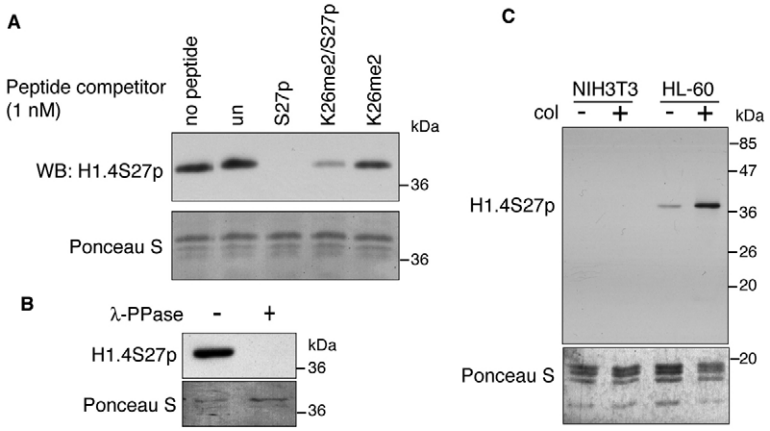


Fig. 1. Characterization of the anti-H1.4S27-*P* antibody. (**A**) Western blot (WB) and peptide competition. HCl-extracted histones from HL-60 cells were probed with the anti-H1.4S27-*P* antibody in the presence of competitor peptides, as indicated, testifying for the specificity of the antibody. un, unmodified peptide; S27p, H1.4S27-P peptide; K26me2/S27p, H1.4K26-Me_2 and S27-P double modified peptide, K26me2, H1.4K26-Me_2 peptide. (**B**) Treatment of linker histones with (+) λ-PPase abolishes recognition by the anti-H1.4S27-*P* antibody. (**C**) Western blot analysis of hyperphosphorylated histones from colcemid (col)-treated mouse NIH3T3 (+) and human HL-60 cells. Note that the anti-H1.4S27-*P* antibody is specific for human H1.4 containing the unique ARKS site and does not cross-react with H3 and no cross-reaction with H3 was detected.

that the antibody is specific for phosphorylated H1.4S27 (Fig. 1B). S27 is within an ARKS motif in histone H1. In contrast to human H1.4, murine H1 contains no ARKS motif. Indeed, hyperphosphorylated linker histones isolated from colcemide-treated murine NIH3T3 cells were not recognized by our H1.4S27-*P* antibody, demonstrating its specificity (Fig. 1C). Importantly, the antibody was highly specific for linker histone H1.4 and showed no cross-reactivity with histone H3, despite the similar ARKS sequences in the H3 tail, or non-histone proteins in whole-cell lysate (Fig. 1C and supplementary material Fig. S1D).

H1.4S27-*P* is a novel mitotic histone mark

C-terminal histone H1 phosphorylation is mediated by CDKs, whereas less is known about phosphorylation of the N-terminus (Roque et al., 2008; Sarg, 2006). We first investigated whether H1.4S27 phosphorylation levels are regulated in a cell-cycle-

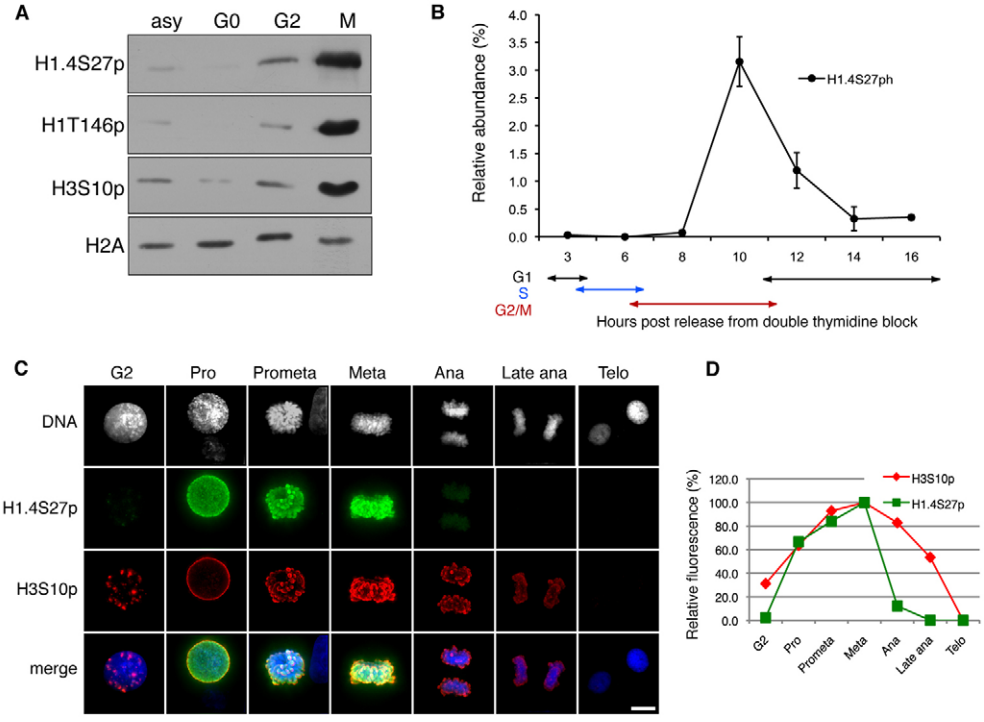


Fig. 2. H1.4S27 phosphorylation is a novel mitotic mark. (**A**) Western blot analysis of histones from MCF-7 cells, either asynchronously grown (asy), arrested in G0, blocked in G2 or in mitosis (M). Phosphorylation of H1.4S27 is strongly elevated in mitotic cells, in a comparable manner to that of H3S10-*P* or H1T146-*P*. (**B**) Mass spectrometrical analysis of H1.4S27 phosphorylation in HeLaS3 cells released from a double thymidine block at different time points (hours). The relative abundance of H1.4S27-*P* is shown. Results are means±s.d. for three replicates (with the exception of the 10-hour timepoint, which is for two replicates). Approximations of cell cycle stages are based on the Watson Pragmatic extrapolation of flow cytometry data. (**C**) Double immunofluorescence labeling of H1.4S27-*P* (green) and H3S10-*P* (red) in MCF-7 cells. H1.4S27 phosphorylation is present at high levels on chromosomes from prophase to metaphase, but staining decreases sharply in anaphase. H3S10-*P* is already visible in characteristic foci in G2 and persists until decondensation of chromatin. DNA was stained with DAPI (blue). Scale bar: 10 µm. (**D**) Graph depicting relative fluorescence intensity levels of H1.4S27-*P* and H3S10-*P* staining in individual pictures during mitotic progression.

dependent manner. We blocked MCF-7 cells in distinct cell cycle phases and analyzed H1.4S27 phosphorylation together with H3S10-*P* and H1T146-*P*, two well-described cell-cycle-regulated histone markers by immunoblotting. Cell cycle stages were verified by FACS analysis (supplementary material Fig. S2A). Asynchronously growing cells showed a low level of H1.4S27 phosphorylation (Fig. 2A). Although a slight increase in H1.4S27-*P* levels could be detected in cells in G2 phase, phosphorylation clearly peaked in cells arrested in mitosis. When cells were arrested in G0 by serum starvation, H1.4S27-*P* levels were reduced. H3S10-*P*, as well as the CDK1-dependent H1T146-*P* followed a similar pattern. We confirmed this dynamics of H1.4S27 phosphorylation over the cell cycle by HPLC-MS analysis of H1.4S27-*P* levels in HeLaS3 cells (Fig. 2B) and in HEK-293 cells by immunoblotting (supplementary material Fig. S2B). Co-immunofluorescence of MCF-7 cells with anti-H1.4S27-*P* and anti-H3S10-*P* antibodies resulted in strong labeling of mitotic chromosomes but with a different kinetics for the two phosphorylations (Fig. 2C,D). Whereas H3S10-*P* was already detectable in characteristic foci at G2 phase (Hendzel et al., 1997), H1.4S27-*P* could not be detected before prophase. Additionally, there was a sharp reduction in H1.4S27-*P* levels during the transition from metaphase to anaphase, and the staining was reduced to undetectable levels during anaphase progression. A control staining with an anti-H1 antibody confirms that H1 remains bound to chromatin during anaphase (supplementary material Fig. S2C). In contrast to H1.4S27-*P*, H3S10-*P* persisted until chromatin finally undergoes telophase, albeit with decreased levels.

Aurora B is a mitotic H1.4S27 kinase

Our next aim was to identify the kinase responsible for mitotic H1.4S27 phosphorylation. To this end we performed an in silico protein motif scan using GPS2.0 software (Xue et al., 2008) (Fig. 3A). Aurora B was among kinases with the highest score. We therefore chose this kinase, as well as the closely related Aurora A, as candidates to test in the subsequent assays. Although CDK1 was not predicted to be a putative H1.4S27 kinases, we decided to include it in our analyses as it has been shown that CDKs can phosphorylate serine and threonine residues in non-CDK consensus sites (Swank et al., 1997). We found that both recombinant Aurora A and Aurora B could phosphorylate H1.4 at S27 in vitro, whereas a CDK1–cyclinB1 complex could not (Fig. 3B). We confirmed the activity of CDK1 in this assay by immunodetection of phosphorylated H1T146, a CDK-dependent target. To investigate whether Aurora kinases can also phosphorylate H1.4S27 in cells, we treated HL-60 cells with the Aurora-kinase-specific inhibitor ZM447439 or with the CDK1-kinase-specific inhibitor RO-3306, as a control. To raise general phosphorylation levels, cells were simultaneously treated with the phosphatase inhibitor calyculin A (CalA). As expected, treatment with CalA elevated the levels of all analyzed histone phosphorylations (H1.4S27-*P*, H1T146-*P* and H3S10-*P*) (Fig. 3C, lane 2). However, simultaneous treatment with increasing amounts of the Aurora inhibitor impeded the phosphorylation of H1.4S27, as well as of H3S10, but not the CDK-dependent phosphorylation of H1T146 (Fig. 3C, lanes 3–6). On the other hand, treatment with RO-3306 did not affect H1.4S27 levels, whereas H1T146 phosphorylation was strongly impaired (Fig. 3C, lanes 7 and 8). To rule out non-specific effects of the kinase inhibitors, and to distinguish the Aurora kinase family members, we performed knockdown experiments. We used small interfering RNAs (siRNAs) targeting specifically Aurora A or Aurora B (Yang et al., 2005) in MCF-7 cells. These siRNAs efficiently knocked down their corresponding targets (Fig. 3D) and H3S10-*P* levels decreased upon depletion of Aurora B, as expected

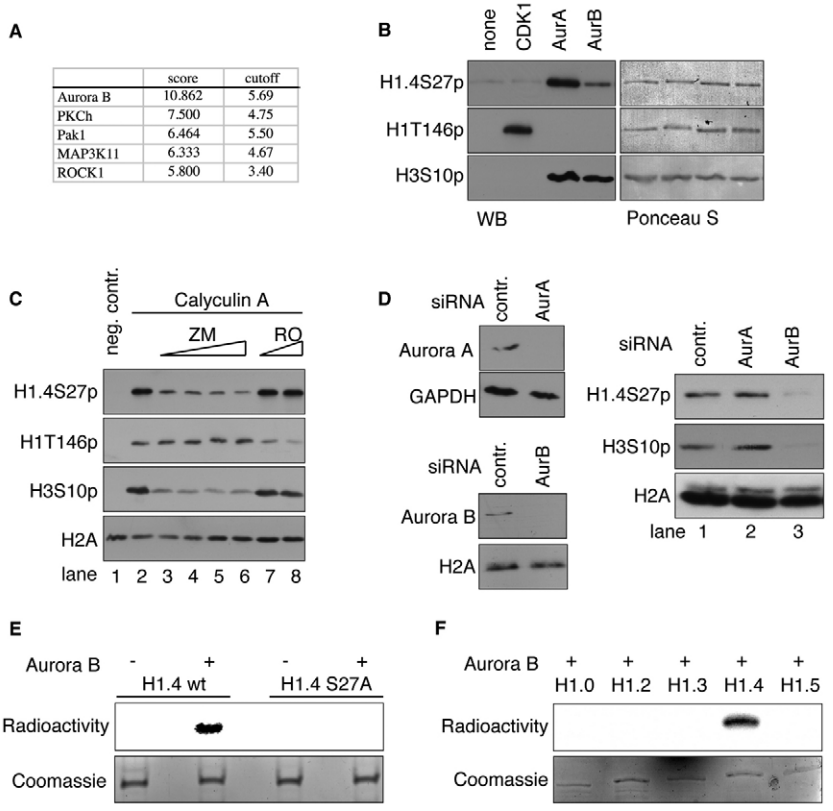
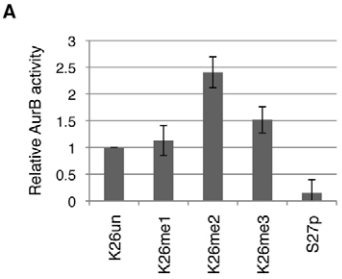
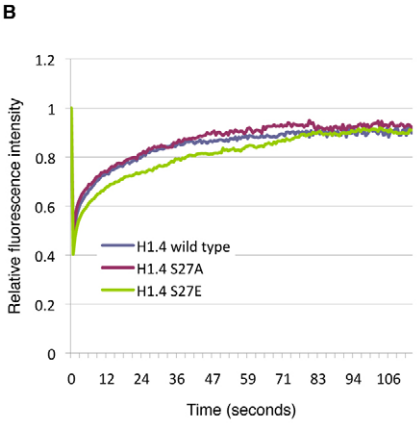


Fig. 3. Aurora B is a mitotic H1.4S27 kinase. (**A**) Table of kinases predicted to target H1.4S27 by GPS2.0 software in an in silico protein motif scan. (**B**) Aurora A and Aurora B (AurA and AurB) but not CDK1–CycB phosphorylate H1.4S27 as determined by an in vitro kinase assay followed by SDS-PAGE and subsequent western blot analysis with phosphorylation-site-specific antibodies. (**C**) Inhibition of Aurora kinase results in reduced H1.4S27-*P* levels. HL60 cells were treated with 50 nM CalA in order to raise histone phosphorylation levels. The effect of ZM447439 (ZM, 0.2 µM–2 µM), an inhibitor of Aurora kinases, or RO-3306 (RO, 4.5 and 9 µM), an inhibitor of CDK1, was tested through western blotting for the indicated epitopes. As a control for the CDK inhibitor, H1T146-*P* levels were also monitored. (**D**) Depletion of Aurora B but not Aurora A reduces H1.4S27 phosphorylation levels. MCF-7 cells were transfected with siRNA targeting Aurora A or Aurora B. Western blot analysis of MCF-7 cell lysates (upper left-hand panels) or solubilized chromatin fraction (lower left-hand panels) transfected with siRNAs targeting specifically Aurora A or Aurora B. Protein levels of both kinases are efficiently reduced. Histone phosphorylations were analyzed by SDS-PAGE and immunoblotting (right-hand panel). contr., control siRNA. (**E**) The H1.4S27A mutation abolishes the incorporation of radioactivity in an in vitro kinase assay with γ-[^{32}P]ATP. (**F**) Aurora B preferentially phosphorylates H1.4 among the main human somatic linker histone variants as determined by an in vitro phosphorylation assay using recombinant H1 variants.





	t_{80} (seconds)	s.d.
H1.4 wt	23.86	+/- 5.57
H1.4 S27A	22.69	+/- 6.04
H1.4 S27E	38.57	+/- 8.27

Fig. 4. Functional analysis of H1.4S27 phosphorylation. (**A**) In vitro kinase assays using H1.4 peptides as substrates. Aurora B activity towards S27 is enhanced on the H1.4K26 dimethylated peptide, compared with the unmodified (un) and mono- (me1) or tri-methylated (me3) peptides. (**B**) The GFP–H1.4S27A mutant has slower FRAP recovery kinetics. FRAP analysis on mitotic cells expressing GFP–H1.4wt, S27A or S27E. Left-hand panel: a circular spot with the diameter of 60 pixels was bleached and the fluorescence recovery was measured every 0.59 seconds over 3 minutes. A total of 20–30 selected cells were used for quantification. The recovery curve for GFP–H1.4wt is in blue, GFP–H1.4S27A in red and for GFP–H1.4S27E in green. Right-hand panel: calculated t_{80} recovery times and standard deviations.

(Crosio et al., 2002). Importantly, knockdown of Aurora B, but not of Aurora A, led to a decrease in H1.4S27 phosphorylation levels (Fig. 3D). Taken together, these results clearly show that the kinase Aurora B catalyzes H1.4S27 phosphorylation. This is also the first demonstration that Aurora B can phosphorylate a member of the linker histone family.

Next we addressed whether S27 is the only site in H1.4 that is phosphorylated by Aurora B. To examine this we performed kinase assays using γ-[^{32}P]ATP with recombinant Aurora B on wild-type H1.4 or H1.4 in which S27 was mutated to an alanine residue. The S27A mutation totally abolished H1 phosphorylation, suggesting that S27 is indeed the only phosphorylation site targeted by Aurora B in H1.4 (Fig. 3E). Furthermore, among all the five somatic H1 variants tested, Aurora B had a strong preference for H1.4 (Fig. 3F). Taken together, our results show that Aurora B phosphorylates specifically H1.4S27 during mitosis, with a different kinetics than to that of S10 on H3. The later onset of H1.4S27 phosphorylation compared with that of H3S10-*P* might be due to different targeting of Aurora B or different susceptibility to counteracting phosphatases, as proposed for H3S28 compared with H3S10 (Goto et al., 2002). Alternatively, the accessibilities of the H1.4 N-terminal tail compared with the H3 tail might be different at distinct sites. In line with this, Baatout and Derradji have proposed a model where the N-terminal tail of H1 (or parts of it) is released from chromatin at a certain time, thus potentially regulating the access to distinct kinases, for example, after onset of mitosis (Baatout and Derradji, 2006).

Functional relevance of S27 phosphorylation

The adjacent residue to H1.4S27, K26 can be methylated and acetylated (Garcia et al., 2004; Vaquero et al., 2004). Indeed, H1.4S27-*P* is known to inhibit binding of HP1 to H1.4K26-Me$_2$ (Daujat et al., 2005) in accordance with the methylation–phosphorylation switch model proposed by Fischle et al. (Fischle et al., 2003). Methylation of K26 is in fact the most abundant linker histone methylation (Lu et al., 2009) and is linked to transcriptional repression (Kuzmichev et al., 2004). To assess whether different states of K26 methylation can influence phosphorylation of H1.4S27 by Aurora B, we performed kinase assays on H1.4 peptides that were either unmodified or methylated at K26. Whereas monomethylation of K26 seemed not to influence Aurora B activity towards S27 (Fig. 4A), Aurora B activity was slightly elevated with the H1.4K26-Me$_3$ peptide and was more than twofold higher with the H1.4K26-Me$_2$ peptide (Fig. 4A), suggesting a crosstalk between these modifications. Interestingly, and in contrast to this result, Rea et al. demonstrated a reduced kinase activity of Aurora B towards H3S10 when the adjacent H3K9 was dimethylated (Rea et al., 2000). However, the quite different sequences following the serine residues in the different histone types might be responsible for the different outcomes. As H1.4K26 methylation is associated with heterochromatin and HP1 can bind this mark, we speculate that the enhanced Aurora B activity towards di- or tri-methylated H1.4K26 provides an additional mechanism to dissociate HP1 from mitotic chromatin.

To gain further insights into the functional role of H1.4S27 phosphorylation, we next determined the impact of S27 mutations on histone H1 mobility. We established stable HEK-293 cell lines expressing GFP-tagged wild-type H1.4 (H1.4wt), H1.4S27A (a non-phosphorylatable mutant) or H1.4S27E (a mutant mimicking constitutive phosphorylation). Using FRAP, we analyzed the mobility of these GFP fusion molecules during mitosis (Fig. 4B). Whereas GFP–H1.4wt and GFP–S27A showed very similar recovery kinetics after bleaching of the mitotic chromosomes, the GFP–H1.4S27E mutant displayed a slower recovery [time taken to recover 80% of the fluorescence (t_{80}): GFP–H1.4wt, 23.86 seconds; GFP–H1.4S27A, 22.69 seconds; GFP–H1.4S27E, 38.57 seconds, Fig. 4B]. This suggests that S27 phosphorylation influences the

binding of H1.4 to mitotic chromatin and that the fully phosphorylated form binds mitotic chromatin with higher affinity than the unphosphorylated form. The low abundance (~3%) of S27 phosphorylation (see Fig. 2B) could explain the similar recovery kinetics of H1.4wt and the S27A mutant.

These data are in line with previous reports suggesting that the N-terminal tail can be important for linker histone affinity to chromatin, as demonstrated by experiments with N-terminally truncated H1. Thus, although the C-terminal tail of linker histones is considered to determine the binding of H1 to chromatin to a large extent (Hendzel et al., 2004), the N-terminal tail can also contribute to H1 binding. In the case of the H1 C-terminus, it has been proposed that its partial phosphorylation leads to a reduced aggregation of DNA, but that full phosphorylation results in a high capacity to aggregate DNA (Roque et al., 2008). On the basis of this model, we speculate that similar mechanisms also apply to the N-terminal tail of H1 where H1.4S27 phosphorylation might induce structural changes that increase H1.4 binding in the context of mitotic chromatin.

Materials and Methods

Antibodies and peptides

The specific polyclonal antibody against H1.4S27-*P* was raised in collaboration with Sigma–Aldrich. Antibodies against H1T146-*P* and the monoclonal anti-H3S10-*P* antibody were purchased from Abcam. Anti-H2A antibody (acidic patch) was from Millipore. Antibodies against Aurora A and Aurora B were from BD Transduction. Anti-GAPDH antibody was from Ambion. H1.4 peptides corresponding to amino acids 22–32 of human H1.4 were from GeneCust, Biosyntan and Clonestar.

Plasmids

Human H1.4 was cloned as a C-terminally His_6-tagged protein into a pET-30z vector for bacterial expression. For eukaryotic expression, human H1.4 with a N-terminal GFP fusion was cloned into pcDNA3. Mutation of S27 was performed by PCR mutagenesis.

Cell culture, synchronization and mass spectroscopy analysis

To arrest HL-60 and NIH3T3 cells in mitosis, cells were treated with 500 ng/ml colcemide (Alexis) for 18 hours. To arrest MCF-7 cells in G0 phase, cells were serum-starved for 3 days in medium containing 5% stripped serum. Accumulation in G2 phase was achieved by treatment with 9 μM RO-3306 (Alexis) and enrichment in M phase by treatment with 300 ng/ml nocodazole (Calbiochem) for 16 hours. HeLaS3 cells (2×10^5 –5×10^5 cells per ml) were maintained as previously described (Zee et al., 2010), and were arrested at the G1–S transition by first blocking with 2 mM thymidine (ACROS) for 19 hours, allowing a recovery for 10 hours, followed by a second thymidine block and release into new medium. Cell cycle states were analyzed by propidium iodide staining and flow cytometry as described previously (Darzynkiewicz et al., 2001). Histones were extracted and derivatized with propionic anhydride and analyzed as described previously (Weiss et al., 2010). Relative abundances for the phosphorylated and all detectable modified forms of the H1.4 peptide (amino acids 26–33) were determined from integration of the area under the chromatographic peaks of the respective peptide. All spectra were manually verified.

Extraction of native histones and λ-PPase treatment

For extraction of native histones, cells were lysed in PBS, 0.5% Triton X-100 and phosphatase inhibitors (1 mM Na_3VO_4, 10 mM NaF and 10 mM 2-glycerophosphate). The pellet was extracted in 0.2 M HCl for 4 hours at 4°C. Dephosphorylation was carried out using λ-PPase (New England Biolabs) according to the manufacturer's instructions.

Inhibition of kinases, siRNA treatment and preparation of lysates

HL-60 cells were incubated with ZM447439 (Biomol) (0.2 μM to 2 μM) or RO-3306 (4.5 μM and 9 μM) and simultaneously with 50 nM calyculin A (Cell Signaling Technology) for 30 minutes for inhibition of kinases and phosphatases. siRNA duplexes targeting Aurora A and Aurora B were obtained from Dharmacon and allStars negative control siRNA from Qiagen. MCF-7 cells were transfected with 50 nM siRNA using Lipofectamine 2000 (Invitrogen) according to the reverse transfection protocol. Cells were harvested at 64 hours post transfection.

Immunoblotting and peptide competition

Proteins were resolved by electrophoresis using SDS-PAGE (17.5% gels) and blotted onto nitrocellulose. Immunodetected proteins were visualized with the ECL system (Millipore). For peptide competition experiments, primary antibody was preincubated with the indicated peptides (1 nM) for 30 minutes at 4°C.

Immunofluorescence

Immunofluorescence was performed as described previously (Daujat et al., 2009). Images were taken with a Deltavision RT microscope and data analyzed using softWoRxSuite software (Applied Precision).

Expression and purification of recombinant H1.4

C-terminally His_6-tagged recombinant histone H1.4 was expressed in *E. coli* and extracted from inclusion bodies with 0.83 M perchloric acid, precipitated with trichloracetic acid and resuspended in sodium phosphate buffer pH 8. Further purification was achieved using nickel-affinity gels (Sigma).

Kinase assays

Recombinant GST-tagged kinases were obtained from ProQinase and Cell Signaling, and H1 variants from Calbiochem. Protein or peptide substrates were incubated at 30°C in 60 mM HEPES pH 7.5, 30 mM $MgCl_2$, 30 mM $MnCl_2$, 12 mM DTT, 5% glycerol and phosphatase inhibitors with 200 ng kinase and 100 μM or 10 μM ATP with or without γ-[^{32}P]ATP for Aurora kinases or CDK1, respectively. Samples were analyzed by western blotting or detection of radioactivity using a phosphoimager (Fuji).

Establishment of stable GFP–H1.4 cell lines and FRAP

HEK-293 cells were transfected with pcDNA3 plasmids containing GFP–H1.4 constructs using ExGen500 (Fermentas). GFP–H1.4 expression levels were <5% of endogenous H1.4. Cells were selected and maintained with G418 (750 μg/ml). The experiments were performed on a Zeiss LSM 510 confocal microscope as described previously (Dundr et al., 2002). Mitotic cells with comparable GFP fluorescence in metaphase and early anaphase were selected. For quantification, the total fluorescent intensities of a circular region of interest in the bleached area of mitotic chromosomes and in the total mitotic chromosome area were monitored using Zeiss LSM software. Background fluorescence (BG) was measured in a random field outside the cells. The relative fluorescence intensity double-normalized to the pre-bleach value was calculated at each time point (t) as: $I_{rel}=(T_o\text{–BG}) \times (I_{(t)}\text{-BG})/(T_{(t)}\text{–BG}) \times (I_o\text{–BG})$, where T_o is the average intensity of the entire mitotic chromosome area during pre-bleach and I_o is the average intensity of the region of interest during the pre-bleach. For quantification 20–30 cells per specific construct were used.

Bioinformatics

We used GPS2.0 software (Xue et al., 2008) to predict putative kinases able to phosphorylate H1.4S27.

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Supplementary material available online at
http://jcs.biologists.org/cgi/content/full/124/10/1623/DC1

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