Histone H1 phosphorylation affects chromatin condensation and function, but little is known about how specific phosphorylations impact the function of H1 variants in higher eukaryotes. In this study, we show that specific sites in H1.2 and H1.4 of human cells are phosphorylated only during mitosis or during both mitosis and interphase. Antibodies generated to individual H1.2/H1.4 interphase phosphorylations reveal that they are distributed throughout nuclei and enriched in nucleoli. Moreover, interphase phosphorylated H1.4 is enriched at active 45S preribosomal RNA gene promoters and is rapidly induced at steroid hormone response elements by hormone treatment. Our results imply that site-specific interphase H1 phosphorylation facilitates transcription by RNA polymerases I and II and has an unanticipated function in ribosome biogenesis and control of cell growth. Differences in the numbers, structure, and locations of interphase phosphorylation sites may contribute to the functional diversity of H1 variants.

Introduction

Nonallelic variants of histone H1 in metazoans share a common tripartite structure, with a conserved globular domain flanked by a short N-terminal domain and a longer C-terminal domain (CTD). FRAP analyses of cells expressing H1-GFP fusions have revealed that H1 variants bind chromatin dynamically in vivo and that both the globular domain and CTD contribute to chromatin binding (Lever et al., 2000; Misteli et al., 2000; Hendzel et al., 2004; Brown et al., 2006). H1-binding dynamics affect the chromatin access of high mobility group proteins, MeCP2 (methyl-CpG–binding protein), upstream-binding factor (UBF), the glucocorticoid receptor, and other regulators by modulating H1-mediated chromatin folding and by enabling factors to compete with H1 for chromatin-binding sites (Zlatanova et al., 2000; Phair et al., 2004; Bustin et al., 2005).

CTD interactions with linker DNA are important for higher order folding of chromatin (Allan et al., 1980, 1986; Bednar et al., 1998; Carruthers et al., 1998; Lu et al., 2009). S/TPXK/R Cdk substrate motifs that are repeated in the CTD contribute to its DNA binding (Suzuki, 1989; Vila et al., 2001; Roque et al., 2005), and phosphorylation at these motifs affects CTD–DNA interactions (Roque et al., 2008). These motifs are phosphorylated to varying degrees in H1 prepared from asynchronous or mitosis-arrested mammalian cells (Garcia et al., 2004; Sarg et al., 2006; Wisniewski et al., 2007), but how this affects chromatin processes is unclear. Analyses of synchronized cells suggest that H1 phosphorylation increases progressively during interphase before peaking transiently during mitosis (Gurley et al., 1975; Ajiro et al., 1981a,b), but few details are known about the site specificity of phosphorylation during interphase and mitosis because phosphorylation sites were not identified in these early analyses. Site-specific phosphorylation of an H1 variant during H1-mediated chromatin condensation and function, but little is known about histone H1 phosphorylation is associated with transcription by RNA polymerases I and II.

Histone H1 phosphorylation affects chromatin condensation and function, but little is known about how specific phosphorylations impact the function of H1 variants in higher eukaryotes. In this study, we show that specific sites in H1.2 and H1.4 of human cells are phosphorylated only during mitosis or during both mitosis and interphase. Antibodies generated to individual H1.2/H1.4 interphase phosphorylations reveal that they are distributed throughout nuclei and enriched in nucleoli. Moreover, interphase phosphorylated H1.4 is enriched at active 45S preribosomal RNA gene promoters and is rapidly induced at steroid hormone response elements by hormone treatment. Our results imply that site-specific interphase H1 phosphorylation facilitates transcription by RNA polymerases I and II and has an unanticipated function in ribosome biogenesis and control of cell growth. Differences in the numbers, structure, and locations of interphase phosphorylation sites may contribute to the functional diversity of H1 variants.

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consistent with previous analyses of H1 phosphorylation stoichiometry in synchronized cells (Ajiro et al., 1981a,b; Gurley et al., 1995; Talasz et al., 1996).

H1 is phosphorylated at a specific subset of sites during interphase

We identified interphase phosphorylation sites in HIC fractions from mid–S phase cells because H1 phosphorylation was clearly increasing at this time, but the chance of contamination by hyperphosphorylated H1 from mitotic cells was less than at later time points. SDS gel electrophoresis confirmed that the five major HIC peaks for mid–S phase samples were essentially homogenous fractions of H1.2 or H1.4 (Fig. S1 A). TDMS analyses of these fractions provided striking evidence that interphase H1 phosphorylation is site specific. H1.2 contains four Cdk substrate motifs, but MS/MS analysis of the 1p-H1.2 peak localized phosphorylation exclusively to S173 in both allelic variants. Similarly, although H1.4 has five Cdk substrate motifs,
**Table I. H1 phosphorylation during interphase and mitosis in HeLa S3 cells**

| HIC peak | Molecular mass | Δm | Variant | Modifications |
|----------|----------------|-----|---------|---------------|
|          | Measured       | Predicted |         |               |
|          | D              | D               |         |               |

**Interphase**

| 1        | 21,491.6      | 21,332.8     | +160    | H1.4          | pS172, pS187 |
| 2        | 21,412.3      | 21,332.8     | +80     | H1.4          | pS187        |
| 3        | 21,333.1      | 21,332.8     | 0       | H1.4          | None         |
| 4        | 20,912.1      | 20,832.6     | +80     | H1.4 [A142]   | pS173        |
| 5        | 2,0941.7      | 20,832.6     | +110    | H1.4 [T142]   | None         |
| 5        | 20,862.2      | 20,832.6     | +30     | H1.2 [T142]   | None         |

**Mitosis**

| 6pH1.4   | 21,813.9      | 21,332.8     | +480    | H1.4          | pT18, pS27, pT146, pT154, pS172, pS187 |
| 4pH1.2   | 21,153.3      | 20,832.6     | +320    | H1.2 [A142]   | pT31, pT146, pT154, pS173 |
| 4pH1.2   | 21,182.9      | 20,832.6     | +350    | H1.2 [T142]   | pT31, pT146, pT154, pS173 |

HIC peaks are labeled as in Fig. 1 B. Molecular masses are reported as neutral monoisotopic species. Predicted values were found using NCBI Protein database accession no. NP_005312 (H1.4) and NP_005310 (H1.2), assuming the loss of Met1 during protein maturation in vivo and residues 2–5 during electrospray ionization. Peak identifications are based on MS/MS sequencing of multiple electron capture dissociation fragment ions. The A142 and T142 allelic variants of H1.2 were detected initially by MS and confirmed by genotyping as described in Fig. S1. Phosphorylated residues were identified by MS/MS sequencing of electron capture dissociation fragment ions. Analyses of the H1.2 [T142] forms indicated that phosphorylated residues were identical to those determined for the H1.2 [A142] forms.

Phosphorylation localized exclusively to S187 in the 1p-H1.4 peak and to S172 plus S187 in the 2p-H1.4 peak (Table I, Fig. S1 B, and Fig. S2 F).

Analyses of the 4p-H1.2 and 6p-H1.4 peaks from colchicine-treated cells (Fig. 1 B) revealed uniform phosphorylation at the four and five Cdk substrate motifs present in H1.2 and H1.4, respectively (Table I and Fig. S2 F). An additional site, S27, in an RKX motif that is unique to H1.4 was phosphorylated concurrently. We refer to T31, T146, and T154 of H1.2 and T18, S27, T146, and T154 of H1.4 as M sites because they appear to be phosphorylated exclusively during mitosis. In contrast, we refer to H1.2-S173, H1.4-S172, and H1.4-S187 as I sites because they can be phosphorylated during mitosis and interphase.

Our results suggest that H1.2-S173 and H1.4-S172 and -S187 are the sole sites of interphase phosphorylation in these proteins in human cells. Notably, monophosphorylation at H1.4-S172 was not detected, implying that interphase H1.4 phosphorylation/dephosphorylation occurs hierarchically or that other mechanisms prevent the accumulation of detectable H1.4-S172 monophosphorylation. Our findings also suggest that interphase H1 kinases preferentially phosphorylate Ser-containing Cdk substrate motifs, which is consistent with evidence that three such sites in H1.5 are phosphorylated during mitosis (Talasz et al., 2009). With the exception of H1.4-S27, all of the M sites identified in this study are Thr-containing Cdk substrate motifs. Identification of H1.4-S27 as an M site is noteworthy because phosphorylation at this residue during mitosis may affect acetylation or methylation at H1.4-K26 or the interaction of factors that recognize these modifications (Kuzmichev et al., 2004; Vaquero et al., 2004; Daujat et al., 2005; Trojer et al., 2007). We did not detect either modification at H1.4-K26 using TDMS, suggesting that they affect <1% of total H1.4 in HeLa S3 cells (Pesavento et al., 2008).

pS173-H1.2 and pS187-H1.4 are enriched in nucleoli
To investigate the roles of specific H1 phosphorylations, we generated antisera against phosphopeptides containing the pS173-H1.2 and pS187-H1.4 I sites and the pS27-H1.4 and pT154-H1.4 M sites (Fig. S2 A). We also generated antiserum against recombinant human H1.4 that recognizes H1.4 regardless of its phosphorylation state to use as a control. The specificity of these antisera was validated using Western blotting (Fig. S2, B–E), ELISA assays (not depicted), and immunocytochemistry (Fig. S3).

The pT154 antiserum stained the chromatins of mitotic HeLa cells intensely but did not stain interphase nuclei (Fig. 2 A). Similar results were observed for the pS27 and pT146 antiserum (unpublished data), confirming that these sites are phosphorylated exclusively in mitosis. In contrast, the pS173 and pS187 antiserum stained chromatin in both interphase and mitotic cells. The pS173 antiserum stained mitotic chromatins less intensely than either the pT154 or the pS187 antiserum. Because these residues are expected to be phosphorylated to similar degrees during mitosis, this suggests that the pS173 epitope may be less accessible in mitotic chromosomes. Differences were also observed for the staining of interphase cells by the pS173 and pS187 antiserum (Fig. 2 A). Most interphase cells displayed stippled nuclear staining and clusters of punctate nucleolar staining for pS173. Many interphase cells showed similar staining for pS187, but others displayed speckled staining similar to that of mouse 10T1/2 cells stained by antiserum to phosphorylated *Tetrahymena thermophila* H1. The latter antiserum preferentially recognizes phosphorylated mouse H1.5, and it has been suggested that the speckled staining represents the localization of transcriptionally active chromatin near sites of RNA splicing (Chadee et al., 1997). Other evidence suggests that speckled staining with this same antibody occurs primarily during G1 phase in human cells (Lu et al., 1994), but we have
not investigated whether this is the case for pS187 staining. In contrast, stippled staining distributed throughout interphase nuclei was observed for the α-H1.4 antisera, with weaker staining associated with nucleoli in some cases. Because this antisera recognizes H1.4 regardless of its phosphorylation state (Fig. S2 D), the stippled pattern may reflect a nonuniform distribution of H1.4, as suggested previously for two H1 variants (Parseghian et al., 1994) or differences in the accessibility of H1.4 at different loci to the antisera.

Fibrillarin colocalized extensively with clustered punctate pS173 and pS187 staining, but not with α-H1.4 staining, confirming that these H1.2/H1.4 phosphorylations are enriched in nucleoli (Fig. 2 B). Moreover, punctate pS187 staining colocalized with centers of bromo-UTP (BrUTP) incorporation foci when cells were pulse labeled with BrUTP to detect nascent 45S pre–ribosomal RNA (rRNA) transcripts (Fig. 2 C; Koberna et al., 2002; Olson and Dundr, 2005). Similar results were obtained for pS173 staining (unpublished data), suggesting that interphase phosphorylated H1.2/H1.4 are associated with transcribing rDNA (45S preribosomal RNA genes) and may facilitate RNA pol I transcription.

pS187-H1.4 is preferentially associated with active rDNA promoters

Mammalian cells contain several hundred rDNA repeats. The transcriptional activity of individual repeats is regulated by mechanisms including histone modification to match cellular demand for ribosomal biogenesis (Lawrence and Pikaard, 2004; Moss et al., 2007; McStay and Grummt, 2008). To investigate whether interphase H1 phosphorylation contributes to this regulation, we used chromatin immunoprecipitation (ChIP) to compare the association of pS187-H1.4 with rDNA promoters before and after selective inhibition of RNA pol I transcription with actinomycin D (ActD; Jordan et al., 1996; Olson and Dundr, 2005). The levels of 45S pre–rRNA were assessed using RT-PCR to provide a semiquantitative measure of rDNA transcription (Huang et al., 2008; Murayama et al., 2008). A brief ActD treatment that markedly reduced the levels of nascent 45S pre–rRNA significantly reduced the promoter association of pS187-H1.4 compared with untreated cells (Fig. 3, A and B). Although treatments that impair RNA pol II transcription are associated with global H1 dephosphorylation (Chadee et al., 1997), immunoblots revealed that the limited ActD treatment used had little effect on the global levels of pS187-H1.4 and total H1.4 (Fig. 3 C). Moreover, ChIP with the α-H1.4 antisera revealed that ActD treatment actually enhanced the level of total H1.4 at the promoter (Fig. 3 A). Collectively, the data suggest that pS187-H1.4 is enriched at active rDNA promoters and that this association is dynamically regulated.

The ultrastructural elements of nucleoli in higher eukaryotes, the fibrillar center (FC), the dense fibrillar component (DFC), and the granular component, are thought to reflect vectorial organization of major steps in ribosome biogenesis: pre–rRNA transcription, pre–rRNA processing, and ribosome assembly on mature rRNA (Olson and Dundr, 2005; Hernandez-Verdun, 2006). This organization is affected when rDNA transcription is inhibited by ActD (Jordan et al., 1996; Olson and Dundr, 2005), so we investigated how ActD affects pS187-H1.4 colocalization with markers for these nucleolar compartments. Fibrillarin localizes primarily to the DFC in punctate staining that rings FCs in untreated cells but becomes concentrated in large foci at the nucleolar periphery when the FC and DFC dissociate from each other in ActD-treated cells (Olson and Dundr, 2005). Although pS187-H1.4 and fibrillarin colocalized extensively in untreated cells, ActD caused a characteristic change in this relationship (Fig. 3 D). Both proteins formed similar numbers of foci near the nucleolar periphery, but the pS187-H1.4 foci were typically smaller and shifted relative to the fibrillarin foci.

Similarly, pS187-H1.4 colocalized extensively with UBF in untreated cells (Fig. 3 D). ActD caused the characteristic punctate UBF staining to coalesce into a few large granules at the nucleolar periphery, which is in agreement with previous work (Zatsepina et al., 1993). However, in contrast to the fibrillarin...
with UBF and RNA pol I before and after ActD treatment reported in this study (Fig. 3) suggest the possibility that H1 kinases are recruited to transcriptionally active/competent rDNA. S187 phosphorylation could promote rDNA decondensation and transcription by enhancing H1.4 dissociation and facilitating UBF binding.

Induced enrichment of pS187-H1.4 at hormone response elements

Data from approaches that do not account for H1 phosphorylation suggest that H1 represses transcription by RNA pol II (Laybourn and Kadonaga, 1991; Cheung et al., 2002; Lee et al., 2004; Kim et al., 2008). In contrast, ChIP analyses using antisera to phosphorylated T. thermophila H1 suggest that H1 phosphorylation is required for glucocorticoid-dependent transcription from the murine mammary tumor virus (MMTV) promoter in mammary cells (Lee and Archer, 1998; Bhattacharjee et al., 2001). This led us to investigate whether pS187-H1.4 is involved in transcription by RNA pol II.

We used ChIP to compare the association of pS187-H1.4 with the multicopy MMTV long terminal repeat glucocorticoid response element (GRE [MMTV-GRE]) in murine 3134 mammary tumor cells before and after hormone stimulation. Dexamethasone rapidly induced pS187-H1.4 association with the MMTV-GRE, increasing the level of pS187-H1.4 at this locus by approximately threefold in 60 min (Fig. 4 A). Rapid, hormone-induced enrichment of pS187-H1.4 was also observed at the

![Graph showing the levels of pS187-H1.4 and total H1.4 at the 45S pre-rRNA promoter in untreated and 50 ng/ml ActD-treated HeLa cells (3 h). The data are expressed as fold change relative to a parallel ChIP without primary antibody from untreated cells.](Image)

![Graph showing the abundance of 45S pre-rRNA transcript in untreated and ActD-treated cells assayed by RT-PCR and normalized to β-actin expression.](Image)

![Image of confocal images of control and ActD-treated HeLa cells costained with antibodies to pS187-H1.4 and fibrillarin, UBF, or the large subunit of RNA polymerase I (RPA 194).](Image)
Chromatin, respectively, our findings imply that phosphorylation of Cdk sites enhances or diminishes H1-GFP dissociation from that mutations mimicking phosphorylation or dephosphorylation for other regulatory factors.

By nuclear hormone receptors by enhancing chromatin access H1.4-S187 phosphorylation promotes transcriptional activation at the pS2-ERE and sites recognized by AP-1 or by other nuclear hormone receptors.

Analyses of glucocorticoid receptor–regulated transcription at the MMTV promoter in different systems suggest that H1 is depleted immediately after hormone stimulation (Bresnick et al., 1992; Belikov et al., 2007) but reassociates with refractory promoters after prolonged hormone treatment (Lee and Archer, 1998), which is consistent with the notion that H1 generally acts as a repressor. In contrast, overexpression of H1c or H1° enhanced basal and hormone-stimulated transcription of stably integrated MMTV-LTR reporter genes in murine 3T3 cells and prevented their repression during prolonged hormone stimulation (Gunjan and Brown, 1999). Our data are consistent with the proposal that H1 affects MMTV promoter chromatin architecture to facilitate the binding of liganded nuclear hormone receptors, their synergism with transcription factors such as NF1 and AP-1, and the recruitment or activation of kinases that phosphorylate and facilitate H1 displacement after hormone stimulation (Sancho et al., 2008; Talasz et al., 2009).

Evidence for H1 variant–specific effects on transcription and repression in different systems suggests that interphase phosphorylation enhances H1 accessibility to factors that regulate transcription and other processes. Our evidence that the association of pS187-H1.4 with specific loci is dynamic implies that H1 kinases and phosphatases are recruited to these loci in a targeted fashion, although little is known about the mechanisms involved. Kinases that mediate interphase H1 phosphorylation in vivo have not been directly identified, but several lines of evidence implicate Cdk2 (Herrera et al., 1996; Bhattacharjee et al., 2001; Contreras et al., 2003). Less is known about whether specific phosphatases regulate interphase H1 phosphorylation. Although our data support the general model that interphase phosphorylation enhances H1 dissociation from chromatin, variation in the numbers, locations, and structures of I sites among H1 variants suggests that interphase phosphorylation may affect H1 chromatin binding in a variant-specific fashion. Such differences may underlie recent evidence for H1 variant–specific effects on transcription and replication (Sancho et al., 2008; Talasz et al., 2009).

Materials and methods

Cell culture

HeLa S3 cells were grown in suspension in Joklik’s modified minimal essential medium supplemented with 10% newborn calf serum (NCS) and synchronized using the double-thymidine block procedure as described previously (Pesavento et al., 2008). 1 µM colchicine was added to growing asynchronous cells for 18 h to enrich for mitotic cells. For the experiments shown in Fig. 3, adherent HeLa cells were grown in DME supplemented with 10% FBS.

Cells were treated with 0.05 µg/ml ActD for 3 h to selectively inhibit RNA pol I transcription. 3134 cells were maintained in DME supplemented with 10% FBS, sodium pyruvate, nonessential amino acids, and 2 mM glutamine as described previously (John et al., 2009). Cells were transferred to DME supplemented with 10% charcoal dextran–treated FBS for 48 h before treatment with 100 nM dexamethasone. MCF-7 cells were maintained in DME supplemented with 5% NCS as described previously (Schultz-Norton et al., 2007). Cells were transferred to phenol red–free DME containing 5% charcoal dextran–treated NCS for 72–96 h before treatment with 10 nM estradiol.

Histone preparation, chromatography, and MS

Crude H1 was prepared by 5% perchloric acid fractionation of crude histones (Pesavento et al., 2008). Recombinant human H1.4 was expressed in Escherichia coli BL21 cells from a pET3d vector using standard procedures.
Reverse phase HPLC used a column (4.6 mm ID x 250 mm; Vydac C18) with a multistep linear gradient from buffer A (0.1% vol/vol TFA in 5% vol/vol CH3CN) to buffer B (0.094% TFA in 95% CH3CN). HIC used a column (4.6 mm ID x 100 mm; PolyPROPYL A; PolyLC Inc.) with a multistep linear gradient from buffer A (2.5 M [NH4]2SO4 and 50 mM ethylene-diamine, pH 7.0) to buffer B (1.0 M [NH4]2SO4 and 50 mM ethylenediamine, pH 7.0). Hydrophilic interaction chromatography used a column (4.6 mm ID x 200 mm; PolyCAT A; PolyLC Inc.) with a multistep linear gradient from buffer A (70% CH3CN and 15 mM triethylamine/H2PO4, pH 3.0) to buffer B (70% CH3CN, 0.68 M NaClO4, and 15 mM triethylamine/H2PO4, pH 3.0).

MS data were acquired on a custom B.5-t quadrupole Fourier transform ion cyclotron resonance mass spectrometer with an electrospray ionization source operated in positive-ion mode as described previously (Pesonen et al., 2005). Desalted HIC fractions or crude H1 were dissolved in 50% methanol + 1% formic acid for infusion into the mass spectrometer. Masses are reported as neutral, monoisotopic species. Figs. 1 and S1 show the spectrum of the most abundant charge state.

Immunochemical methods

Phosphorylated and nonphosphorylated peptides were custom synthesized. Rabbits were immunized with phosphopeptides coupled to keyhole limpet hemocyanin (Thermo Fisher Scientific) using standard procedures. Recombinant human H1.4 was complexed with yeast tRNA before rabbit immunization as described previously (Stollar and Ward, 1970). Antibodies to pT146-H1.4 and fibrillarin were obtained from Abcam. Antibodies to UBF and RNA polymerase II were obtained from Santa Cruz Biotechnology, Inc. Antibodies to α-tubulin (clone DM1A) and bromodeoxyuridine (clone BRD.3) were obtained from Sigma-Aldrich and Neomarkers, respectively.

Microscopy and BrUTP labeling of cells

Hela cells grown on glass coverslips were fixed with 4% (wt/vol) paraformaldehyde in PBS for 10 min at room temperature, permeabilized with 0.2% Triton X-100 in PBS for 15 min, and immunostained with primary antisera using standard procedures. Staining was visualized with FITC-conjugated donkey anti–rabbit antibody (1:200; Jackson Immunoresearch Laboratories, Inc.) and Cy3-conjugated anti–mouse antibody (1:800; Jackson Immunoresearch Laboratories, Inc.). Nuclei were counterstained with TO-PRO-3 (Invitrogen), and coverslips were mounted with Vectashield media (Vector Laboratories). Images were captured at room temperature with a confocal microscope and software (LSM 510; Carl Zeiss, Inc.) using a Plan Apochromat 63× 1.4 NA oil immersion objective lens and processed with ImageJ (National Institutes of Health) and Photoshop (Adobe). For peptide competition, primary antibodies were preincubated with peptides at 2x final concentration for 2 h at room temperature and diluted to a final peptide concentration of 1.0 µg/ml before use.

FuGENE 6 was diluted 1:10 in Hepes-buffered saline (25 mM Hepes, 150 mM NaCl, 0.68 M NaClO4, and 15 mM triethylamine/H3PO4, pH 7.0) and incubated at room temperature for 5 min. BrUTP was added to achieve 5 mM BrUTP (70% CH3CN, 0.68 M NaClO4, and 15 mM triethylamine/H3PO4, pH 3.0).

ChIP

Cells were cross-linked by adding formaldehyde directly to cultures at room temperature for 5 min. BrUTP was added to achieve 5 mM BrUTP (70% CH3CN, 0.68 M NaClO4, and 15 mM triethylamine/H3PO4, pH 3.0).

ChIP products were quantitated by real-time PCR using SYBR Green master mix (Applied Biosystems) and the following primers: rRNA promoter (forward), 5′-GGTGCTGCAGATGGTGCGCTT3′; [reverse] 5′-TTCGCCAGTCGCAGGAAAGAAGA3′; MMTV-GRE (forward), 5′-TCTTCACTCCAGAGGGGACATG3′; and [reverse] 5′-CTTCTCTATCACAGGTCACTG3′ and [reverse] 5′-GGTATCGTGCAGCCTGTC3′ and [reverse] 5′-CCTCCCGGCGGAAATAC3′.

ChIP to monitor the association of ERα and pS187-H1.4 with the pS2ERE included minor modifications as described previously (Schultz-Norton et al., 2007). The ERα antibody (sc-8002) for ChIP was obtained from Santa Cruz Biotechnology, Inc.

Online supplemental material

Fig. S1 shows SDS gel and TDMs analyses of H1 fractions with different levels of phosphorylation prepared from mid–S phase HeLa cells using HIC. Fig. S2 shows the specificity of antisera for individual H1 phosphorylation sites. H1 antisera to total H1.4 in immunobLOTS, and the phosphorylation sites identified in this study relative to an alignment of the human H1.1–H1.5 protein sequences. Fig. S3 shows the specificity of the pS173-H1.2 and pS187-H1.4 antisera using peptide competition in immunofluorescence microscopy. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.201001148/DC1.

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