Cell cycle behavior of human HP1 subtypes: distinct molecular domains of HP1 are required for their centromeric localization during interphase and metaphase

Tomohiro Hayakawa[1,2], Tokuko Haraguchi[1,2], Hiroshi Masumoto[3] and Yasushi Hiraoka[1,2,*]

[1]CREST Research Project, Kansai Advanced Research Center, Communications Research Laboratory, 588-2 Iwaoka, Iwaoka-cho, Nishi-ku, Kobe 651-2492, Japan
[2]Department of Biology, Graduate School of Science, Osaka University,1-1 Machikaneyama, Osaka 560-0043, Japan
[3]Division of Biological Science, Graduate School of Science, Nagoya University, Chikusa-ku, Nagoya 464-8602, Japan
*Author for correspondence (e-mail: yasushi@crl.go.jp)

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Summary

Heterochromatin protein 1 (HP1) plays an important role in heterochromatin formation. Three subtypes of HP1, namely HP1α, β, and γ, have been identified in humans. In this study, using yellow fluorescent protein (YFP) fusion constructs, we examined the intracellular localization of human HP1 subtypes during the cell cycle. During interphase, all three HP1 subtypes were localized to centromeric heterochromatin and to promyelocytic leukemia (PML) nuclear bodies. Different preferences, however, were observed among the subtypes: during interphase HP1β localized most preferentially to centromeric heterochromatin, whereas HP1α and γ were more preferentially localized to PML nuclear bodies. During metaphase, only HP1α, was localized to the centromere. We thus determined which molecular domains of HP1 were necessary for their intracellular localization. Our results showed that the C-terminal fragment (amino acid residues 101-180) of HP1α was necessary for localization to the metaphase centromere and the N-terminal fragment (amino acid residues 1-76) of HP1β was necessary for localization to the interphase centromere. Interestingly, simultaneous observations of residues 101-180 of HP1α and residues 1-76 of HP1β in living HeLa cells revealed that during late prophase, the HP1β fragment dissociated from centromeric regions and the HP1α fragment accumulated in centromeric regions. These results indicate that different specific regions of human HP1α and HP1β mediate localization to metaphase and interphase centromeric regions resulting in association of different subtypes of HP1 with the centromere at different times during the cell cycle.

Key words: Heterochromatin, HP1, PML, Centromere, CENP-B

Introduction

Eukaryotic chromosomes consist of cytologically defined domains of euchromatin and heterochromatin. Euchromatin contains transcriptionally potent genes which are subject to transcriptional activation or inactivation. In contrast, heterochromatin contains transcriptionally silent genes. Constitutive heterochromatin also has specialized chromosome structures such as centromeres and telomeres.

Although the molecular details that define euchromatin and heterochromatin are not fully understood, it is now clear that heterochromatin protein 1 (HP1) is a major constituent of heterochromatin and plays a key role in its formation and maintenance. HP1 is highly conserved from yeasts to humans (Eissenberg and Elgin, 2000). HP1 was first identified as a gene product of an allele of *Su(var)2-5* in *Drosophila melanogaster* (James and Elgin, 1986; Eissenberg et al., 1990). In *Drosophila*, HP1 is localized to pericentric and telomeric heterochromatin. When mutated HP1 was expressed in *Drosophila*, abnormal condensation and segregation of chromosomes was observed (Kellum and Alberts, 1995). Involvement of HP1 in heterochromatin formation is now known to occur through its interaction with SUV39H1, the histone methyltransferase that methylates histone H3 at lysine 9. HP1 binds to histone H3, which has been methylated at lysine 9 by SUV39HI, and in turn it recruits SUV39H1 to the DNA, which further propagates methylation along the chromatin (Jenuwein, 2001). This relationship between HP1 and SUV39H1 is conserved in their *Schizosaccharomyces pombe* homologues Swi6 and Clr4 (Bannister et al., 2001), suggesting evolutionary conservation of this mechanism of heterochromatin formation.

In humans, three subtypes of HP1 have been identified, HP1α, HP1β, and HP1γ (Singh et al., 1991; Saunders et al., 1993; Ye and Worman, 1996). They share significant similarities in their amino acid sequences (see Fig. 7). HP1 consists of two highly conserved regions, the N-terminal chromo domain (CD) (Paro and Hogness, 1991) and the structurally related C-terminal chromo shadow domain (CSD) (Aasland and Stewart, 1995; Cowieson et al., 2000). These two domains are connected by a less well conserved hinge region

(IVR) (see Fig. 7). The CSD is a unique motif found only in the HP1 family; the CD motif, however, is also found in Su(var)3-9 family proteins and Polycomb (Pc) family proteins (Jacobs et al., 2001).

HP1 seems to have pleiotropic functions in the genetic activities of chromosomes. It has been shown that mammalian HP1 homologues have a number of interacting partners such as the CAF-1 subunit p150 (Murzina et al., 1999), SP100 (Lehming et al., 1998; Seeler et al., 1998), TIF1-β (KAP-1) (Le Douarin et al., 1996; Ryan et al., 1999), Ku70 (Song et al., 2001), lamin B receptor (Ye and Worman, 1996; Ye et al., 1997), SUV39H1 (Aagaard et al., 1999), Ki-67 (Kametaka et al., 2002; Scholzen et al., 2002), histone H1-like protein (Nielsen et al., 2001), methylated histone H3 (Bannister et al., 2001; Lachner et al., 2001) and INCENP (Ainsztein et al., 1998). These proteins have a wide variety of functions involved in chromatin assembly, transcriptional regulation, telomere maintenance, and nuclear membrane formation. Many of the HP1 interacting partners listed above bind to the CSD of HP1, but interaction of lysine 9-methylated histone H3 with the CD region (Lachner et al., 2001) and INCENP with the IVR region (Ainsztein et al., 1998) have also been documented.

HP1 subtypes have shown heterogeneous patterns of intracellular localization in accordance with the presence of a wide variety of interacting molecules. Localization of HP1 subtypes during the cell cycle have been extensively studied in human and mouse cells (Wreggett et al., 1994; Horsley et al., 1996; Furuta et al., 1997; Minc et al., 1999; Yamada et al., 1999; Minc et al., 2000; Sugimoto et al., 2001). In interphase nuclei, HP1 subtypes are localized in heterochromatin (Wreggett et al., 1994; Minc et al., 1999; Minc et al., 2000) in accordance with their interaction with SUV39H1 (Aagaard et al., 1999), and localized at the PML nuclear bodies in interphase nuclei (Everett et al., 1999) in accordance with their interaction with SP100, a component of the PML nuclear body (Lehming et al., 1998; Seeler et al., 1998); HP1 subtypes are also localized at the periphery of nucleoli (Minc et al., 1999). In metaphase, HP1α is localized at the centromeric region (Minc et al., 1999; Yamada et al., 1999; Minc et al., 2001), as is its interacter INCENP (Ainsztein et al., 1998). Furthermore, live cell imaging of GFP-fused HP1α throughout the cell cycle in human cells demonstrated that HP1α is colocalized with DsRed-fused CENP-B at the centromere during metaphase (Sugimoto et al., 2001). Compared with the reproducible results reported for HP1α, localization of HP1β and γ at the metaphase centromere has been debatable, depending on cell types and antibodies used (Wreggett et al., 1994; Minc et al., 1999; Saffery et al., 1999; Minc et al., 2000; Minc et al., 2001).

To determine molecular domains of HP1 subtypes required for their intracellular localization, we examined human HP1 subtypes and various truncation constructs in living HeLa cells, and determined the molecular domains responsible for their localization to centromeres and PML nuclear bodies. Here we report that HP1β associates with interphase centromeres but is replaced at the centromere by HP1α as the cell enters metaphase. The N-terminal region of HP1β, including the CD, is responsible for localization to the interphase centromere and the C-terminal region of HP1α including the CSD and a portion of the IVR, is responsible for localization to the metaphase centromere. Simultaneous observations of the N-terminal fragment of HP1β and the C-terminal fragment of HP1α in living cells revealed that during late prophase the HP1β fragment dissociated from the centromere and as the cells entered metaphase the HP1α fragment accumulated at the centromere. Thus, localization of human HP1β and HP1α to centromeric heterochromatin during different phases of the cell cycle is mediated by distinct regions of these proteins. These results suggest specific roles for HP1β and HP1α at the interphase and metaphase centromere, respectively.

Materials and Methods

Cell lines, antibodies and reagents

HeLa cells were obtained from the Riken Cell Bank. Cells were grown at 37°C in Dulbecco's Modified Eagle Medium (DMEM) (Invitrogen) containing 10% calf serum (Invitrogen). Human anti-centromere antisera was derived from a patient with scleroderma (Masumoto et al., 1989). Anti-PML monoclonal antibody was purchased from Santa Cruz Biotech (Santa Cruz, CA, USA). Species-specific, fluorescent secondary antibodies were purchased from Jackson Immunoresearch Laboratories (West Groove, PA, USA). Hoechst 33342 and 4,6-diamidino-2-phenylindole (DAPI) were purchased from Calbiochem (La Jolla, CA, USA). An anti-fluorescence fading reagent, 1,4-diazobicyclo-(2,2,2)-octane (DABCO), was purchased from Sigma-Aldrich (St. Louis, MO, USA).

YFP and CFP fusion constructs

The open reading frames (ORF) of human HP1α (GenBank accession number: NM_012117.1), HP1β (GenBank accession no.: NM_006807.2) and HP1γ (GenBank accession no.: AB030905.1) were cloned by RT-PCR using mRNA isolated from HeLa cells as the PCR template. Total RNA was isolated from HeLa cells using TORIZOL (Invitrogen) according to the manufacturer's instructions. Complementary DNAs were amplified with *Pyrobest* DNA polymerase (TaKaRa, Ohtsu, Japan) using specific primers for the ORF of each gene; the *Eco*RI linker was added to the 5′ primer, and a stop codon plus the *Bam*HI linker was added to the 3′ primers. Synthesized cDNAs were digested with *Eco*RI and *Bam*HI, then cloned into the pEYFP-C1, pECFP-C1 or pDsRed2-C1 vector (Clontech, Palo Alto, CA, USA). Truncated constructs of human HP1 were produced by PCR-based amplification using its ORF as the template. All clones were checked for their nucleotide sequence using ABI310 or ABI377 (Applied Biosystems Inc., Foster City, CA, USA).

The plasmids pGFP-CB1-160c and pCFP-CB1-160c, containing the N-terminal fragment of human CENP-B fused with GFP and CFP, respectively, were generated as follows: The N-terminal fragment of CENP-B (–2 to 481 bp) was excised from pETCBN-160 (Kitagawa et al., 1995) with *Nco*I (the *Nco*I digestion product was blunt-ended using Klenow) and *Bam*HI. A *Bam*HI- *Hin*dIII adaptor was prepared by annealing oligonucleotides 5′-GATCCATGGCTAGCATGACTG-GTGGACAGCAAATGGGTCGGTAGTCGACA-3′ and 5′-AGC-TTGTCGACTACCGACCCATTTGCTGTCCACCAGTCATGCTAG-CCATG-3′. The CENP-B fragment with the adaptor was cloned into *Sal*I (the *Sal*I digestion product was blunt-ended using Klenow)-*Hin*dIII-cut pMTID13S (Stuart et al., 1984) to create pMTCB1-160c. Then, to create the plasmid pGFP-CB1-160c, the N-terminal fragment of CENP-B was excised from pMTCB1-160c with *Xba*I and *Hin*dIII (the *Hin*dIII digestion product was blunt-ended using Klenow) and introduced into *Xba*I-*Bcl*I-digested and blunt-ended pGFP-C1 (Clontech). To create the plasmid pCFP-CB1-160c, the N-terminal fragment of CENP-B was excised from the plasmid pGFP-CB1-160c with *Sal*I and *Bam*HI, and introduced into *Sal*I-*Bam*H-digested pECFP-C1 (Clonetech).

Transfection

Transfection reactions were carried out with LipofectAmine PLUS (Invitrogen). HeLa cells cultured in a 35 mm glass-bottom culture dish (MatTek, Ashland, MA, USA) were transfected with 250 ng of plasmid DNA, and incubated at 37°C for 1.5 hours. For co-transfection with two or three kinds of plasmid DNA, 125 ng or 85 ng DNA of each plasmid were applied, respectively. Transfected cells were incubated in fresh DMEM medium containing 10% calf serum for 1 or 2 days before observation.

Indirect immunofluorescent staining

Indirect immunofluorescent staining was carried out essentially as described previously (Haraguchi et al., 2000). Cells cultured in a 35 mm glass-bottom dish were fixed with a mixture of 3.7% formaldehyde and 0.2% glutaraldehyde in DMEM culture medium for 20-30 minutes at room temperature. Fixed cells were then treated twice with 0.1% sodium borohydrate in phosphate-buffered saline (PBS) for 15 minutes at room temperature. The cells were then permeabilized with 0.1% Triton X-100 in PBS for 5 minutes, followed by incubation in 1% bovine serum albumin in PBS at room temperature for 1 hour. Anti-centromere human antisera and anti-PML mouse monoclonal antibody were then added at a dilution of 1:100 and 1:200, respectively, and incubated for 3 hours at room temperature. After the first antibody reaction, cells were washed five times with PBS for 10 minutes, and stained for 3 hours at room temperature with the relevant fluorescent secondary antibodies: Cy3-conjugated goat anti-mouse IgG or Cy5-conjugated goat anti-human IgG, both at a dilution of 1:200. The stained cells were washed five times with PBS for 10 minutes and then taken sequentially with 20%, 40%, 60% and 80% glycerol in PBS containing 0.5 μg/ml DAPI and 2.5% DABCO. Finally, cells were mounted in 90% glycerol for microscopic observation.

Live cell observation

observation of live cells was carried out as previously described (Haraguchi et al., 1997; Haraguchi et al., 1999). Briefly, cells cultured in a 35 mm glass-bottom dish were stained with 100 ng/ml Hoechst 33342 for 30 minutes and then washed twice with DMEM containing 10% calf serum. The Hoechst 33342-stained cells were then cultured in phenol red-free DMEM supplemented with 10% fetal bovine serum (Invitrogen), antibiotics (50 unit/ml penicillin and 50 unit/ml streptomycin; purchased from Invitrogen), and 20 mM Hepes pH 7.4. These cells were incubated in a CO_2 incubator for at least 30 minutes and then placed on a microscopic stage in a temperature-controlled room. Mineral oil was layered onto the culture medium to avoid evaporation during microscopic observation. After 30 minutes of culture on the microscope stage, observation was carried out using a DeltaVision microscope system (Applied Precision, Issaquah, WA, USA) kept at 37°C in a temperature-controlled room (Haraguchi et al., 1999). The oil-immersion objective lens UApo40x/NA1.35 (Olympus Opitcal, Tokyo, Japan) was used for the observation.

Non-fixed chromosome spread

HeLa cells cultured in a 60 mm culture dish were transfected with 750 ng DNA for 1.5 hours using LipofectAmine PLUS. The transfected cells were then cultured in DMEM supplemented with 10% calf serum at 37°C for 24 hours. Thymidine was added to the culture at a final concentration of 2.5 mM to synchronize the cell cycle. After incubation in the thymidine-containing medium for 18 hours, the cells were washed twice with DMEM and cultured in DMEM supplemented with 10% calf serum at 37°C for 8 hours to allow cells to proceed into mitosis. Mitotic cells were enriched by collecting cells that detached from the dish by gentle pipetting. Cells with no mitotic enrichment were collected using a rubber scraper. For hypotonic treatment, the collected cells were washed once with 75 mM KCl that had been pre-warmed to 37°C, and incubated for 20 minutes at 37°C. Mitotic cells were collected by pipetting, resuspended in 5 ml of 75 mM KCl and kept on ice. Then an equal volume of ice-cold buffer A (15 mM Hepes pH 7.4, 80 mM KCl, 20 mM NaCl, 0.5 mM EGTA, 2 mM EDTA, 0.5 mM spermidine, 0.2 mM spermine and 0.1% 2-mercaptoethanol) (Hiraoka et al., 1990) containing 0.1% Triton X-100 (buffer A-T) was added to the cells while on ice. Cells were centrifuged, washed and then resuspended with buffer A-T at 4°C. The cells were placed on a glass slide, DAPI added for DNA counterstaining, and the cells were covered with a coverslip. Chromosome spreads were prepared by gently tapping the coverslip.

Fluorescence microscopy

Fluorescence microscope images were obtained on a Peltier-cooled CCD using a DeltaVision microscope system. Details of the microscope system have been described previously (Haraguchi et al., 1997; Haraguchi et al., 1999). For high-resolution analysis of fixed specimens, the oil-immersion objective lens PlanApo60x/NA1.4 (Olympus Optical) was used. Three-dimensional optical section images were taken at 0.5 μm focus intervals and computationally processed by an iterative deconvolution method (Agard et al., 1989).

Results

Intracellular localization of YFP-fused HP1 subtypes

We examined the localization of YFP-fusion constructs of human HP1 subtypes in HeLa cells. HeLa cells transiently expressing YFP-fused HP1 were fixed and observed under a fluorescence microscope. In interphase nuclei, YFP-fused HP1 showed two different staining patterns: one, a series of bright regularly shaped spots; and the other, less bright irregularly shaped areas with some diffuse staining of the nucleoplasm. HP1β was predominantly found in the irregularly shaped bodies associated with centromeric regions (Fig. 1A, arrowhead), and HP1α and γ were found also in the round bright spots of the PML nuclear bodies (Fig. 1A, arrow). During metaphase, HP1 subtypes diffused throughout the cytoplasm, and were excluded from the chromosomal region (Fig. 1B). HP1α, however, did show spot staining on the metaphase chromosomes (Fig. 1B,b); HP1β and γ did not (Fig. 1B,e,h). Immunofluorescence staining confirmed that the spot-like staining pattern of HP1α on metaphase chromosomes was at the centromere (Fig. 1C).

Localization of YFP-fused HP1 subtypes was also examined on spread preparations of metaphase chromosomes. When metaphase chromosome spreads were prepared from cell populations enriched with mitotic cells HP1α was clearly present at the centromere (Fig. 2a) whereas HP1β and γ showed faint or no localization at the centromere (Fig. 2c and e, respectively). In contrast, when chromosome spreads were prepared from whole cell populations with no enrichment, HP1β showed a somewhat increased tendency to be associated with the centromere (Fig. 2d) although its localization was much fainter than that of HP1α. Quantitative data is summarized in Table 1. The apparent centromere localization of HP1β was the result of interphase cells being present in the preparations whereas localization of HP1α and γ was not significantly affected by the presence of interface cells. This would suggest that relocalization of HP1β occurs during the cell cycle and may explain the discrepancies in the reported localization of HP1β at the metaphase centromere (see Discussion).

Taken together, these results show that YFP-fused HP1 subtypes change their intracellular localization from interphase to metaphase. All HP1 subtypes associate with interphase centromeres, but only HP1α shows strong association with metaphase centromeres. These intracellular localization patterns of YFP-fused HP1 are consistent with the previous observations obtained using antibodies or GFP-fusion constructs (Minc et al., 1999; Sugimoto et al., 2001) (see

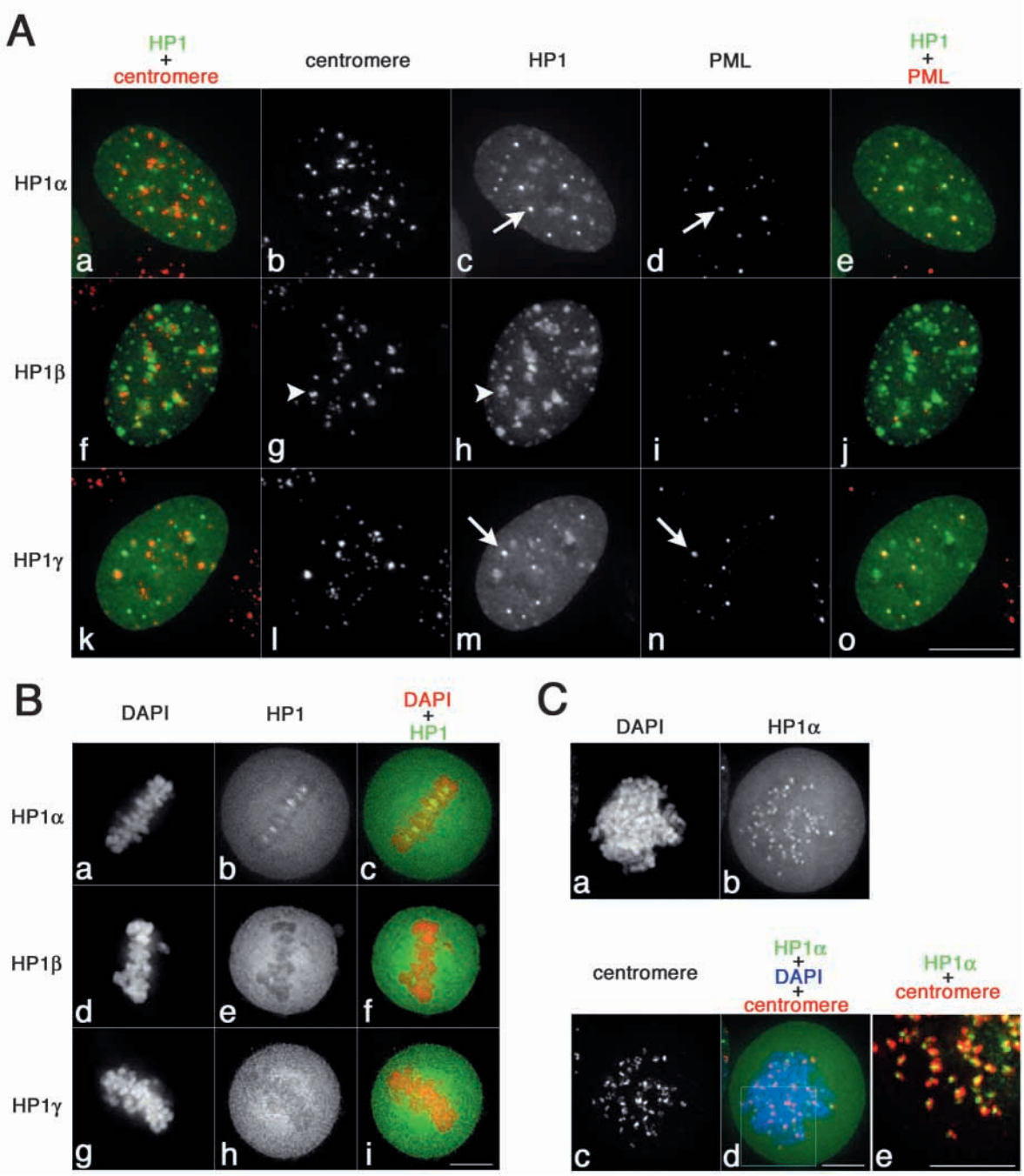

Fig. 1. Intracellular localization of human HP1 subtypes. (A) Localization of human HP1 subtypes in interphase nuclei. HeLa cells transiently expressing YFP-HP1α (a-e), YFP-HP1β (f-j), or YFP-HP1γ (k-o) were fixed, and centromeres and PML nuclear bodies were viewed by indirect immunofluorescent staining. (b,g,l) centromeres, (c,h,m) YFP-HP1 and (d,i,n) PML nuclear bodies in the same cells. Merged images in a, f and k, of HP1 (green) and of centromeres (red) and in e, j and o for HP1 (green) and the PML nuclear bodies (red). Bar, 10 μm. (B) Metaphase localization of HP1 subtypes. HeLa cells transiently expressing YFP-HP1α (a-c), YFP-HP1β (d-f), or YFP-HP1γ (g-i) were fixed and stained with DAPI (a, d and g). (c, f, and i) Merged images of HP1 (green) and DAPI (red). Bar, 10 μm. (C) HeLa cells transiently expressing YFP-HP1α were arrested at mitosis by treatment with 650 nM nocodazole for 8 hours. Centromeres were stained with human antisera that recognizes CENP-A, CENP-B, and CENP-C proteins (Masumoto et al., 1989). (a) DAPI, (b) YFP-HP1α and (c) centromeres. (d) A merged image of DAPI (blue), YFP-HP1α (green) and centromeres (red). (e) An enlarged image of the boxed area in d showing YFP-HP1α (green) and centromeres (red). Scale bar, 10 μm.

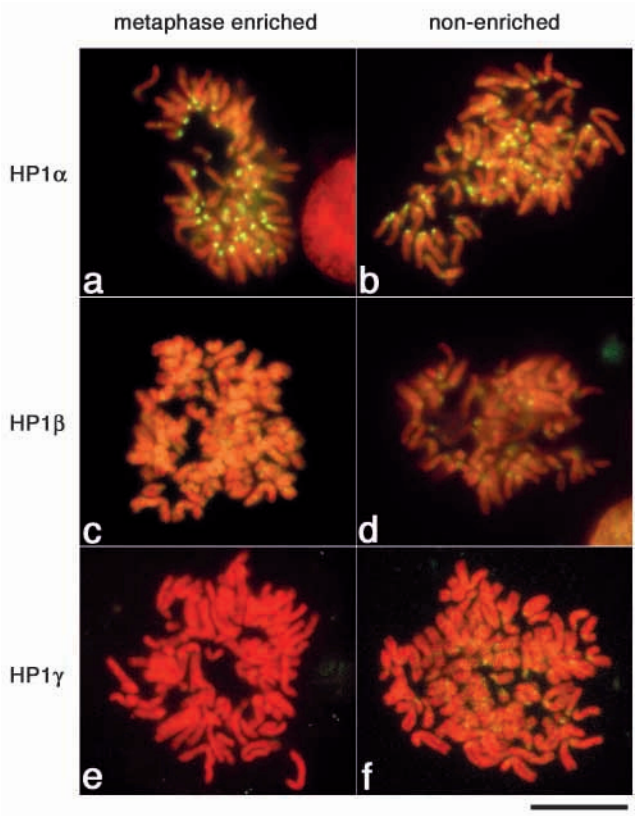

Fig. 2. Localization of HP1 subtypes in metaphase chromosome spreads. HP1α (a,b), HP1β (c,d) and HP1γ (e,f) are shown in green on metaphase chromosomes (red). Metaphase chromosome spreads were prepared from cells collected with (a,c,e) or without (b,d,f) enrichment of mitotic cells (see Materials and Methods). Mitotic cells were about 22-24% of the total cell population in enriched preparation and about 6-9% in the non-enriched prerapation. Scale bar, 10 μm.

Introduction). Thus, we determined molecular domains required for the intracellular localization using truncation constructs of the YFP-fusion HP1 subtypes in HeLa cells.

Molecular domains in HP1 necessary for their intracellular localization

As HP1α was uniquely localized to the metaphase centromere, we determined the necessary molecular domains of HP1α required for this localization. To this end, we constructed a series of truncated HP1α peptides tagged with YFP (Fig. 3A). First, we divided HP1α into three domains, namely the N-terminal region, consisting of residues 1-75, which contains the CD; the C-terminal region consisting of residues 121-191, which contains the CSD; and the IVR region, consisting of residues 76-120. However, none of these domains was found to be localized to the metaphase centromere (data not shown).

We then examined residues 1-170 containing the CD and the IVR and residues 76-191 containing the IVR and the CSD. We found that the latter peptide was localized to the metaphase centromere (Fig. 3A; image data not shown). Further experiments using truncations of HP1α determined that the peptide consisting of residues 101-180 was localized to the metaphase centromere, whereas the peptide consisting of residues 111-180 was not (Fig. 3B), indicating that residues 101-180 of HP1α is the minimum region necessary for localization of HP1α to the metaphase centromere. Interestingly, residues 98-176 of HP1β and residues 83-170 of HP1γ, which correspond to residues 101-180 of HP1α, were also localized to the metaphase centromere (Fig. 4B, Fig. 5B) in spite of the fact that neither full-length HP1β nor HP1γ was localized to the metaphase centromere (Fig. 1B), suggesting that this region of HP1 has a fundamental role in the localization to the metaphase centromere.

HP1 peptides that were localized to the centromere at metaphase were not localized to the centromere in interphase (HP1α 101-180, compare Fig. 3C with Fig. 3B; HP1β 98-176, compare Fig. 4C with B; HP1γ 83-170, compare Fig. 5C with B). Instead they localized exclusively to PML nuclear bodies (data not shown). The shorter fragments containing only the CSD rgion were also present (HP1α 121-191 in Fig. 3D; HP1β 117–185 in Fig. 4D; HP1γ 111-173 in Fig. 5D). Thus, the CSD portion of HP1 is sufficient for localization to PML nuclear bodies in interphase, whereas the additional IVR residues are required for localization to the metaphase centromere.

As the residues of HP1 that were localized to the metaphase centromere were not localized to the interphase centromere, we examined the molecular domains of HP1 necessary for localization to the centromere within the interphase nucleus. Our results showed that in HP1α and HP1β the N-terminal fragments containing the CD were localized to the interphase centromere, but this fragment in HP1γ was poorly localized (HP1α 1-75 in Fig. 3C; HP1β 1-76 in Fig. 4C; HP1γ 1-75 in Fig. 5C). These results indicate that the domains of HP1 required for centromeric localization during interphase are different from those required for centromeric localization during metaphase.

Simultaneous observations of HP1α and HP1β in living cells

Of the three HP1 subtypes HP1β was localized to the interphase centromere most strongly, whereas HP1α was the only subtype localized to the metaphase centromere. In order to examine the switching of HP1α and HP1β during the cell cycle, we attempted to observe HP1α and HP1β simultaneously in living cells. To avoid possible interaction

Table 1. Localization of HP1 at the metaphase centromere

Subtype	Localization +	Localization +/–	Localization –	Total no. of cells examined
HP1α				
Metaphase enriched	38	2	3	43
Non-enriched	33	6	2	41
HP1β				
Metaphase enriched	4	14	21	39
Non-enriched	25	16	1	42
HP1γ				
Metaphase enriched	1	24	17	42
Non-enriched	2	29	13	44

Numbers are from two independent preparations for each data set. Examples of localization assigned for positive (+) are given in Fig. 2a, b and d; those for ambiguous (+/–) are in Fig. 2c and f; those for negative (–) are in Fig. 2e.

between HP1α and HP1β through their CSD (Le Douarin et al., 1996; Ye et al., 1997), we used the N-terminal fragment of HP1β (residues 1-76) and the near C-terminal fragment of HP1α (residues 101-180); these fragments do not interact with each other. Fig. 6 shows an example of HeLa cells expressing the residues 1-76 of HP1β fused with YFP, the residues 101-180 of HP1α fused with DsRed2, and the N-terminal fragment of CENP-B fused with CFP. Between interphase and prophase, the HP1α fragment was not localized to the centromere, but instead localized to PML nuclear bodies (Fig. 6, frames at

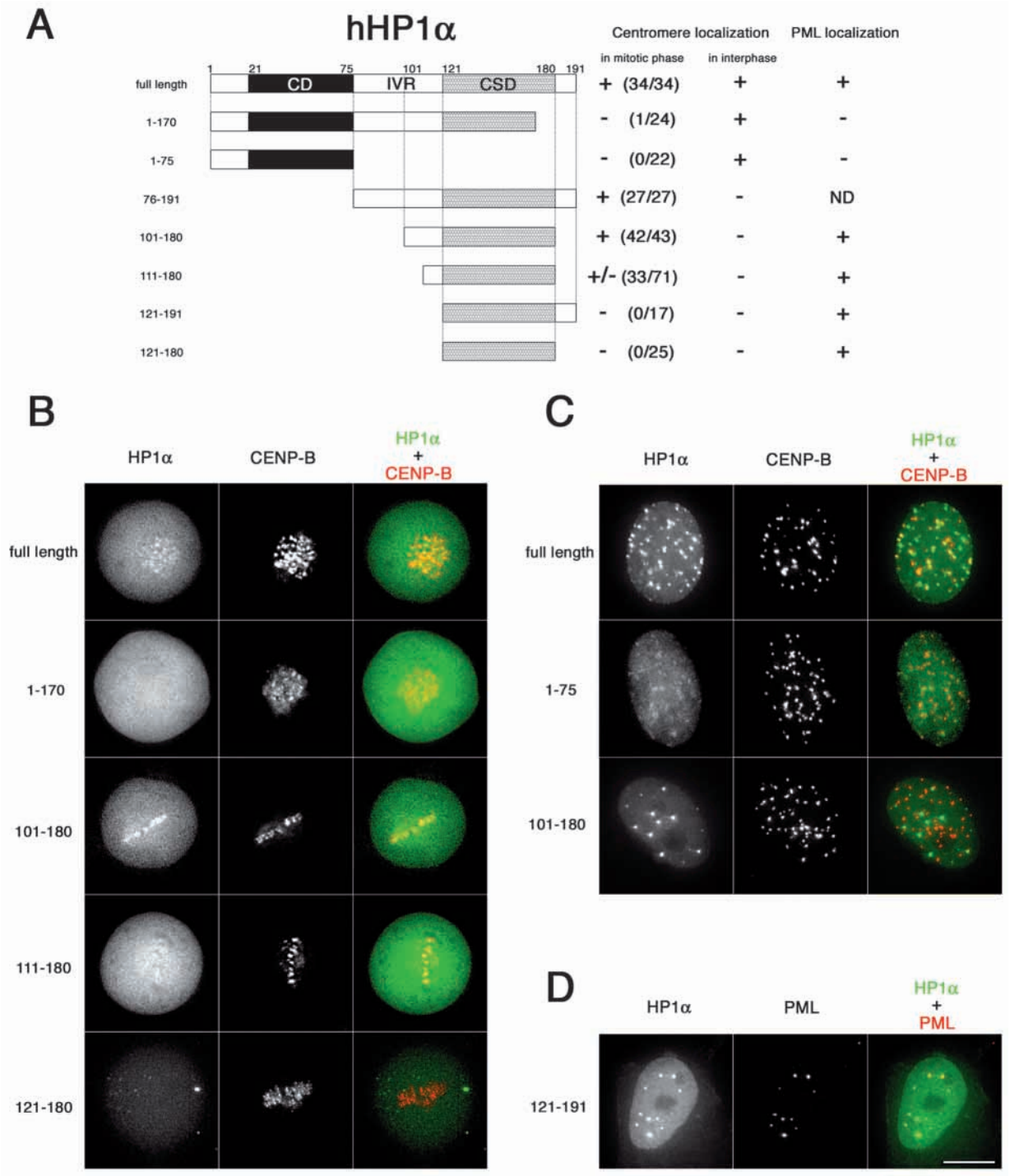

Fig. 3. Domains of HP1α required for its localization. HeLa cells transiently expressing HP1α fragments fused with YFP and the CENP-B N-terminal region fused with CFP. (A) Diagram of YFP-HP1α truncation constructs and their intracellular localization: +, localized; –, not localized; +/–, ambiguous; and ND, not determined. Quantitative data were obtained from three independent experiments. (B) YFP-HP1α fragment localization during metaphase: YFP-HP1α fragment (left column), CFP-CENPB-N-terminal fragment (middle column), and merged images (right column). (C) Localization of HP1α fragments in the interphase nucleus. (D) Localization of HP1α and PML nuclear bodies. Numbers on the left of each row refer to the amino acid numbers of the fragments. Scale bar, 10 μm.

0:00-1:44). However, the HP1β fragment did localize to the centromere, stained with CENP-B-CFP, during this period (Fig. 6, frames at 0:00-1:02). In late prophase, the HP1β fragment dissociated from the centromere (Fig. 6, frames at 1:44-2:01), and, in the same cell, the HP1α fragment accumulated at the centromere just prior to metaphase (Fig. 6, frame at 2:29). During anaphase, the HP1α fragment dissociated from the centromere (Fig. 6, frame at 2:34) and the HP1β fragment began to accumulate first on the chromosome (Fig. 6, frame at 2:45), and then on the centromere (Fig. 6, frame at 2:51). Accumulation of the HP1α fragment at the midbody was also observed during cytokinesis (Fig. 6, frames at 2:45 and 2:51). Full-length HP1α also showed accumulation at the midbody (data not shown). By the next interphase, the

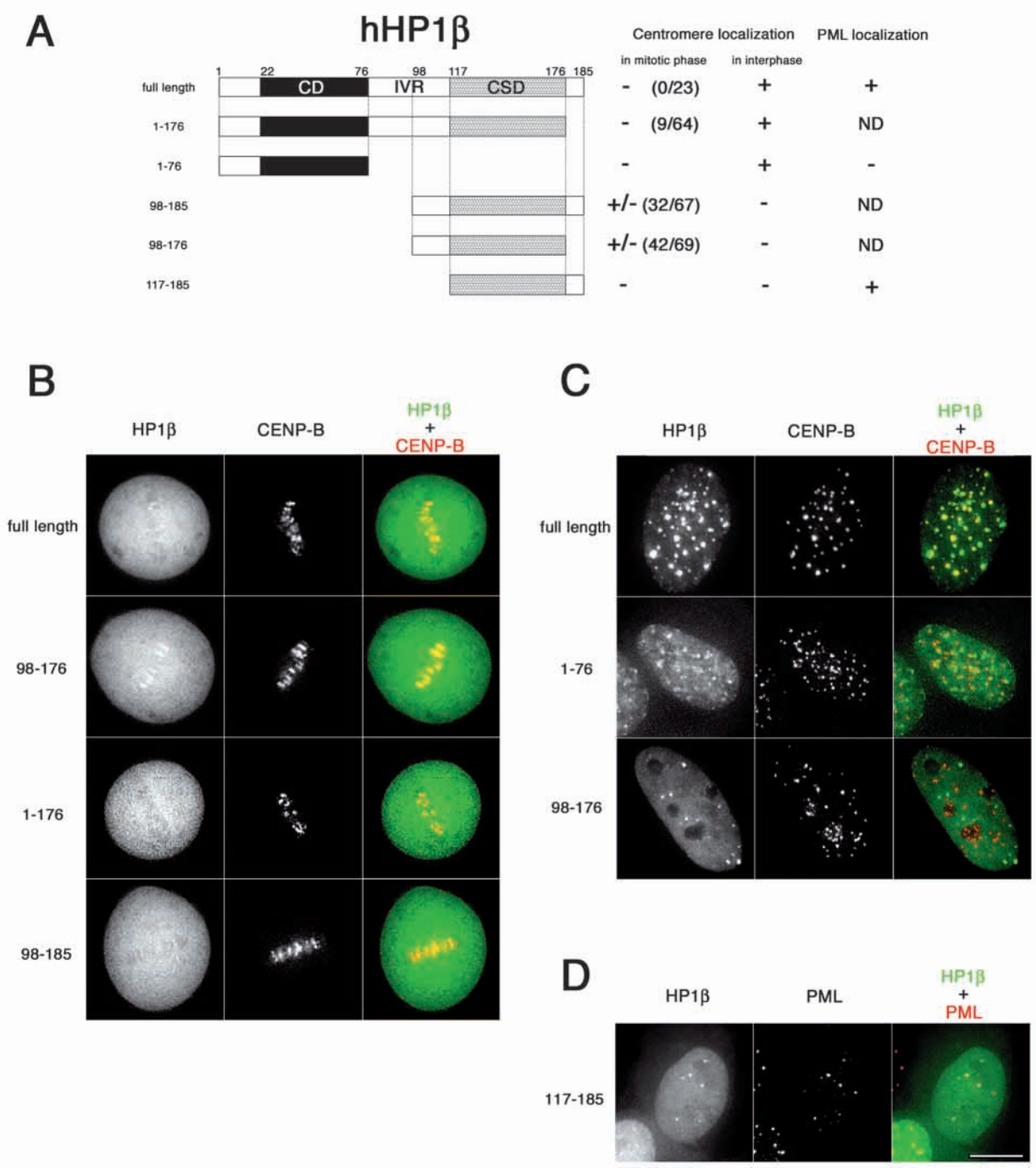

Fig. 4. Domains of HP1β required for its localization. HeLa cells transiently expressing HP1β fragments fused with YFP and the CENP-B N-terminal region fused with CFP. (A) Diagram of YFP- HP1β truncation constructs and their intracellular localization: +, localized; –, not localized; +/–, ambiguous; ND, not determined. Quantitative data were obtained from three independent experiments. (B) YFP-HP1β fragment localization during metaphase: YFP-HP1β fragment (left column), CFP-CENPB-N-terminal fragment (middle column), and merged images (right column). (C) Localization of HP1β fragments in the interphase nucleus. (D) Localization of HP1β and PML nuclear bodies. Numbers on the left of each row refer to the amino acid numbers of the fragments. Scale bar, 10 μm.

HP1α fragment reformed foci with PML nuclear bodies and the HP1β fragment relocalized to centromeric regions (an example for later interphase is shown in Fig. 6, frame at 16:09). These results demonstrate that as a result of the interaction of different domains of human HP1 proteins with interphase and metaphase centromeric regions, HP1β is localized to the centromere during interphase and is replaced by HP1α during metaphase (summarized in Fig. 7; see Discussion).

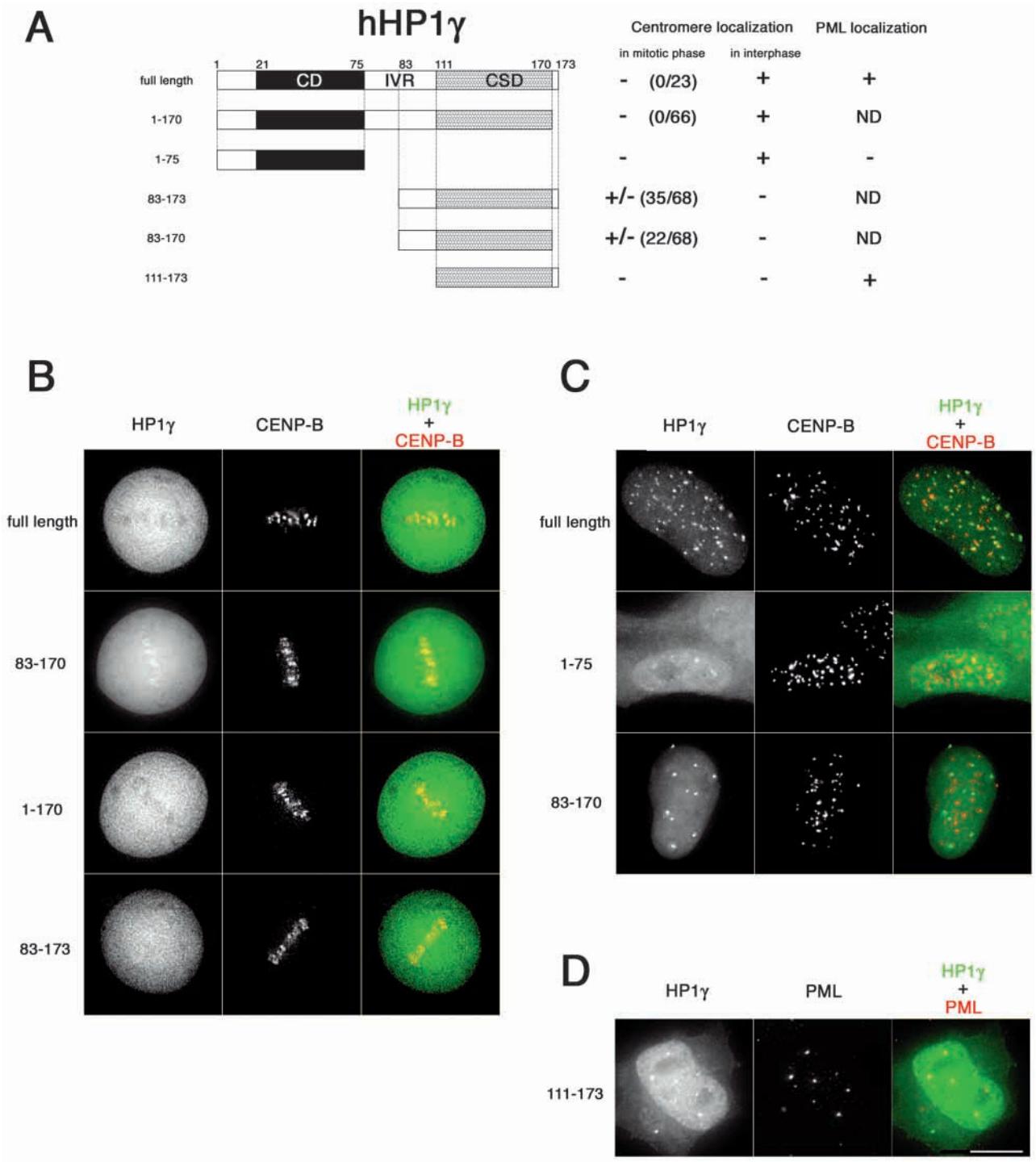

Fig. 5. Domains of HP1γ required for its localization. HeLa cells transiently expressing HP1γ fragments fused with YFP and the CENP-B N-terminal region fused with CFP. (A) Diagram of YFP- HP1γ truncation constructs and their intracellular localization: +, localized; –, not localized; +/–, ambiguous; and ND, not determined. Quantitative data were obtained from three independent experiments. (B) YFP- HP1γ fragment localization during metaphase: YFP- HP1γ fragment (left column), CFP-CENPB-N-terminal fragment (middle column), and merged images (right column). (C) Localization of HP1γ fragments in the interphase nucleus. (D) Localization of HP1γ and PML nuclear bodies. Scale bar, 10 μm.

Discussion

In this study, we determined the molecular domains of human HP1 that are required for the intracellular localization of its subtypes. While localization of full-length HP1 is heterogeneous over several different intracellular structures, truncated domains of HP1 showed distinct localization to specific structures (summarized in Fig. 7). Within the interphase nucleus, the CD region of HP1 localizes to the

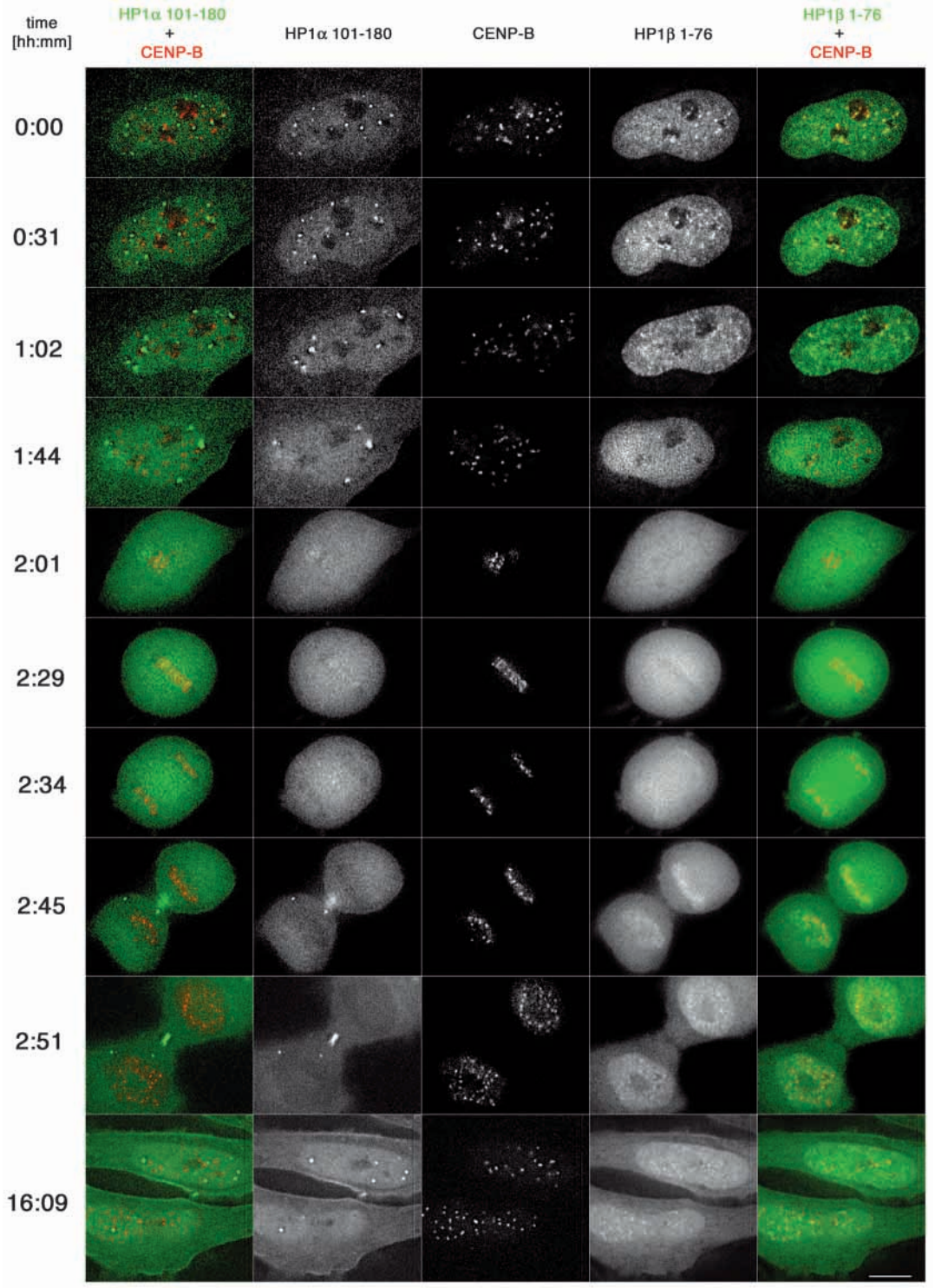

Fig. 6. Simultaneous observation of HP1α C-terminal fragment and HP1β N-terminal fragment. Living HeLa cells transiently expressing a DsRed2-fused HP1α fragment that localized to the mitotic centromere, the N-terminal fragment of HP1β fused with YFP, and the N-terminal fragment of CENP-B fused with CFP. From left to right, merged images of the HP1α fragment (green) and CENP-B (magenta), the HP1α fragment, CENP-B, the HP1β fragment, and merged images of the HP1β fragment (green) and CENP-B (magenta). Numbers on the left indicate time in hours and minutes. Scale bar, 10 μm.

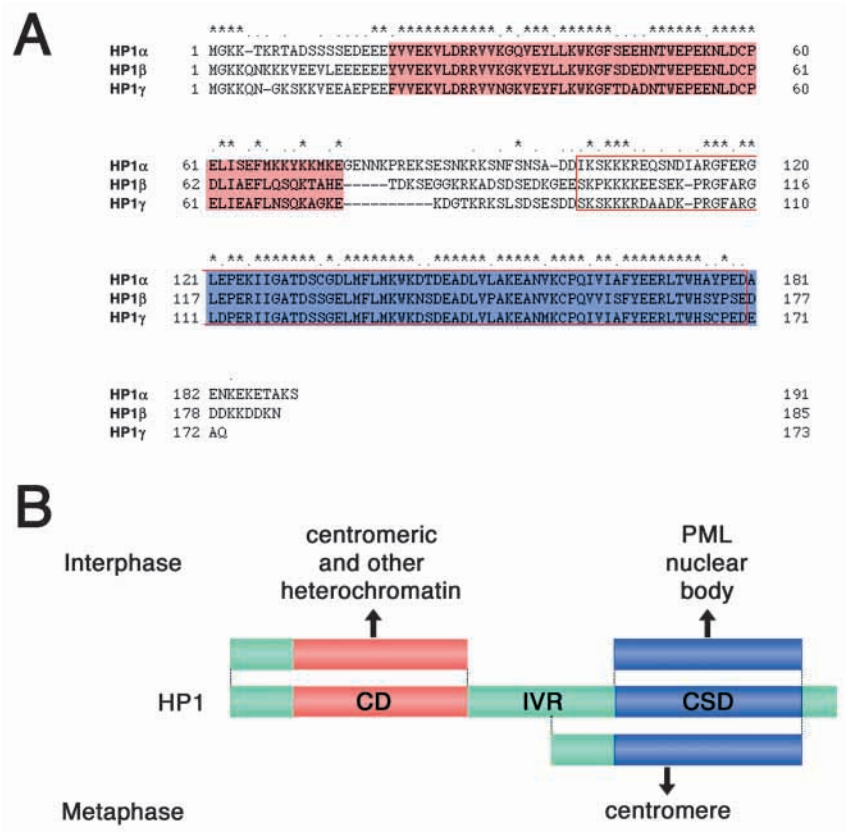

Fig. 7. Human HP1 molecular domains. (A) Amino acid sequence of human HP1 subtypes α, β, and γ. Asterisks indicate amino acids conserved among the three subtypes and dots indicate amino acids conserved among two of the subtypes. The red-shading is the chromo domain (CD), and the blue shading is the chromo shadow domain (CSD). These domains are connected by the IVR. The domain that mediates centromere localization during metaphase is indicated by a red outline. (B) In interphase, the CD motif (red) is required for localization to centromeric heterochromatin and other heterochromatin, and the CSD motif (blue) is required for localization to PML nuclear bodies. However, during metaphase, centromeric localization of HP1 requires the region containing a portion of the IVR and the CSD motif.

centromere and other heterochromatin, but does not associate with PML nuclear bodies, whilst the CSD region localizes exclusively to PML nuclear bodies. Interestingly, in metaphase, centromere localization requires the CSD but not the CD. Heterogeneous localization of HP1 reflects the presence of its various interacting molecules, and the distinct localization of different domains reflects domain-specific binding partners. We speculate that centromeric localization of HP1 probably involves distinct binding partners during interphase and metaphase.

HP1 proteins in the interphase nucleus

In interphase, all three human HP1 subtypes localize to centromeric regions and other heterochromatin regions to varying extents. Of the subtypes, HP1β most preferentially localized to heterochromatin. We determined that the N-terminal fragment containing the CD was the molecular domain of HP1 required for localization to interphase centromeric heterochromatin. Localization of the CD region of HP1 to heterochromatin probably reflects the fact that the CD of HP1 binds to lysine 9 methylated histone H3 (Bannister et al., 2001; Lachner et al., 2001) while a CSD interacting partner, SUV39H1, methylates lysine 9 of histone H3 (Rea et al., 2000). It is now hypothesized that the CD of HP1 recognizes modified histones, the ‘histone code’, and recruits a variety of proteins through interaction with the CSD to establish chromatin structure (Jenuwein and Allis, 2001).

Our results also showed that all three human HP1 subtypes were localized to the PML nuclear bodies, as previously reported (Everett et al., 1999). HP1α and γ were preferentially localized to PML nuclear bodies. The PML nuclear body is an intranuclear structure that contains a number of proteins, such as PML, SP100, ISG20, PIC1/SUMO1, LYSP100, PLZF, INT6, CBP, RB1, RFP and ribosomal protein P (Lamond and Earnshaw, 1998; Seeler and Dejean, 1999). It has been shown that HP1 interacts with SP100, one of the major components of the PML nuclear body (Lehming et al., 1998; Seeler et al., 1998; Seeler and Dejean, 1999). In this study, we determined that the CSD is the molecular domain of HP1 required for localization to PML nuclear bodies. This is consistent with the fact that the CSD portion of HP1 interacts with SP100 (Lehming et al., 1998; Seeler et al., 1998).

HP1 proteins in the metaphase centromere

In metaphase, HP1α localized to the centromere whereas HP1β and HP1γ did not. This result is consistent with previous observations obtained from antibodies or GFP fusion constructs (Sugimoto et al., 2001). However, it has also been reported that HP1β localizes to the centromere of some, but not all, metaphase chromosomes (Saffery et al., 1999) and that HP1γ is localized to the metaphase centromere (Minc et al., 2000). Furuta et al. (Furuta et al., 1997) demonstrated that centromere localization of HP1β is observed at metaphase, but is most prominent at anaphase, suggesting cell cycle regulations. We also occasionally observed some faint centromere staining for HP1β or HP1γ in metaphase chromosome spreads that also contained some interphase cells (Fig. 2; Table 1), suggesting that their localization may be

affected by unknown factors from interphase cells. Since our experiments of HP1 truncations showed that all HP1 subtypes are potentially capable of binding to the metaphase centromere, centromere localization of HP1 is probably regulated during mitosis by protein modification or interaction with other proteins. Also their localization may depend on heterochromatin structures, which vary in different cell types.

We defined the domain of HP1α that is required for its localization to the metaphase centromere. This domain consists of a portion of the IVR and the CSD region, as opposed to the CD which is required for localization to the interphase centromere (Fig. 7). Replacement of an HP1β peptide containing the CD region with an HP1α peptide containing the IVR/CSD region at the centromere during mitosis was clearly demonstrated by observation of live cells (see Fig. 6). Interestingly, the corresponding IVR/CSD domain of HP1β and HP1γ was also localized to the metaphase centromere whereas full-length HP1β or HP1γ was not. These results show that HP1β and HP1γ potentially have the capability of localizing to the metaphase centromere, but the neighboring domains somehow interfere with metaphase centromere localization. These results also suggest that their localization at metaphase centromere is regulated by small variations of amino acid sequences or modifications such as phosphorylation. Putative phosphorylation sites regulated during the cell cycle have been reported (Minc et al., 1999) although there is no direct evidence that phosphorylation is involved in HP1 localization.

Centromeres are likely to be reorganized during progression through mitosis. The human centromere/kinetochore consists of many structural units (Clarke et al., 1999): the sister chromatids cohesion element (INCENP and Rad21), centromeric chromatin element (CENP-B), inner kinetochore element (CENP-A, CENP-C), outer kinetochore element (CENP-F), and fibrous corona (CENP-E, dynein, dynactin). Additionally, the spindle checkpoint apparatus, including Mad, Bub and other proteins, is localized on the outer kinetochore element. It should be noted that a portion of the IVR, in addition to the CSD, of HP1 is required for its localization to the metaphase centromere. INCENP is known to interact with HP1 through the IVR (residues 64-111) (Ainsztein et al., 1998). Our results show that neither the IVR nor the CSD alone is sufficient for localization to the metaphase centromere. Thus, INCENP is not sufficient for localizing HP1 to the metaphase centromere, and other protein molecules interacting with the CSD appear to be required, in combination with INCENP or other unknown IVR interactive molecules, for this effect.

In accordance with the specific localization of HP1α to the metaphase centromere, some cellular events are known to be specifically associated with HP1α. In highly invasive, metastatic breast carcinoma cell lines, HP1α expression is decreased at both mRNA and protein levels, whereas expression of HP1β or HP1γ is unaffected (Kirschmann et al., 2000). Conversely, the protein level of HP1α increases under anti-IgM induced apoptotic conditions in a human Burkitt lymphoma cell line (Brockstedt et al., 1998). HP1α interacts with INCENP (Ainsztein et al., 1998), and INCENP interacts with Aurora B kinase (Adams et al., 2001). Deletion of Scc1/Rad21 in DT40 cells eliminates the localization of INCENP to the inner centromeric domain (Sonoda et al., 2001). Furthermore, deletion of Swi6 (*S. pombe* HP1) causes loss of sister centromeric cohesion (Bernard et al., 2001). These results would suggest that the HP1α localized to the mitotic centromeric heterochromatin may contribute to the stability of sister chromatid cohesion or activation of the kinetochore checkpoint. Reduced HP1α at the metaphase centromere may also be a cause of chromosome instability in cancer cells.

HP1 subtypes interact with a variety of molecules. This generates a heterogeneous pattern of intracellular localization and dynamic relocalization during the cell cycle. Molecular dissection of their domains provides a simpler view of their localization through interaction with specific binding partners.

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References

Aagaard, L., Laible, G., Selenko, P., Schmid, M., Dorn, R., Schotta, G., Kuhfittig, S., Wolf, A., Lebersorger, A., Singh, P. B. et al. (1999). Functional mammalian homologues of the Drosophila PEV-modifier Su(var)3-9 encode centromere-associated proteins which complex with the heterochromatin component M31. *EMBO J.* **18**, 1923-1938.

Aasland, R. and Stewart, A. F. (1995). The chromo shadow domain, a second chromo domain in heterochromatin-binding protein 1, HP1. *Nucleic Acids Res* **23**, 3168-3174.

Adams, R. R., Eckley, D. M., Vagnarelli, P., Wheatley, S. P., Gerloff, D. L., Mackay, A. M., Svingen, P. A., Kaufmann, S. H. and Earnshaw, W. C. (2001). Human INCENP colocalizes with the Aurora-B/AIRK2 kinase on chromosomes and is overexpressed in tumour cells. *Chromosoma* **110**, 65-74.

Agard, D. A., Hiraoka, Y., Shaw, P. and Sedat, J. W. (1989). Fluorescence microscopy in three dimensions. *Methods Cell Biol.* **30**, 353-377.

Ainsztein, A. M., Kandels-Lewis, S. E., Mackay, A. M. and Earnshaw, W. C. (1998). INCENP centromere and spindle targeting: identification of essential conserved motifs and involvement of heterochromatin protein HP1. *J. Cell Biol.* **143**, 1763-1774.

Bannister, A. J., Zegerman, P., Partridge, J. F., Miska, E. A., Thomas, J. O., Allshire, R. C. and Kouzarides, T. (2001). Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. *Nature* **410**, 120-124.

Bernard, P., Maure, J. F., Partridge, J. F., Genier, S., Javerzat, J. P. and Allshire, R. C. (2001). Requirement of heterochromatin for cohesion at centromeres. *Science* **294**, 2539-2542.

Brockstedt, E., Rickers, A., Kostka, S., Laubersheimer, A., Dorken, B., Wittmann-Liebold, B., Bommert, K. and Otto, A. (1998). Identification of apoptosis-associated proteins in a human Burkitt lymphoma cell line. Cleavage of heterogeneous nuclear ribonucleoprotein A1 by caspase 3. *J. Biol. Chem.* **273**, 28057-28064.

Clarke, A. S., Lowell, J. E., Jacobson, S. J. and Pillus, L. (1999). Esa1p is an essential histone acetyltransferase required for cell cycle progression. *Mol. Cell Biol.* **19**, 2515-2526.

Cowieson, N. P., Partridge, J. F., Allshire, R. C. and McLaughlin, P. J. (2000). Dimerisation of a chromo shadow domain and distinctions from the chromodomain as revealed by structural analysis. *Curr. Biol.* **10**, 517-525.

Eissenberg, J. C., James, T. C., Foster-Hartnett, D. M., Hartnett, T., Ngan, V. and Elgin, S. C. (1990). Mutation in a heterochromatin-specific chromosomal protein is associated with suppression of position-effect variegation in Drosophila melanogaster. *Proc. Natl. Acad. Sci. USA* **87**, 9923-9927.

Eissenberg, J. C. and Elgin, S. C. (2000). The HP1 protein family: getting a grip on chromatin. *Curr. Opin. Genet. Dev.* **10**, 204-210.

Everett, R. D., Earnshaw, W. C., Pluta, A. F., Sternsdorf, T., Ainsztein, A. M., Carmena, M., Ruchaud, S., Hsu, W. L. and Orr, A. (1999). A

dynamic connection between centromeres and ND10 proteins. *J. Cell Sci.* **112**, 3443-3454.

Furuta, K., Chan, E. K. L., Kiyosawa, K., Reimer, G., Luderschmidt, C. and Tan, E. M. (1997). Heterochromatin protein HP1Hsbeta (p25beta) and its localization with centromeres in mitosis. *Chromosoma* **106**, 11-19.

Haraguchi, T., Kaneda, T. and Hiraoka, Y. (1997). Dynamics of chromosomes and microtubules visualized by multiple-wavelength fluorescence imaging in living mammalian cells: effects of mitotic inhibitors on cell cycle progression. *Genes Cells* **2**, 369-380.

Haraguchi, T., Ding, D. Q., Yamamoto, A., Kaneda, T., Koujin, T. and Hiraoka, Y. (1999). Multiple-color Fluorescene Imaging of Chromosomes and Microtubules in Living Cells. *Cell Struct. Funct.* **24**, 291-298.

Haraguchi, T., Koujin, T., Hayakawa, T., Kaneda, T., Tsutsumi, C., Imamoto, N., Akazawa, C., Sukegawa, J., Yoneda, Y. and Hiraoka, Y. (2000). Live fluorescence imaging reveals early recruitment of emerin, LBR, RanBP2, and Nup153 to reforming functional nuclear envelopes. *J. Cell Sci.* **113**, 779-794.

Hiraoka, Y., Agard, D. A. and Sedat, J. W. (1990). Temporal and spatial coordination of chromosome movement, spindle formation, and nuclear envelope breakdown during prometaphase in Drosophila melanogaster embryos. *J. Cell Biol.* **111**, 2815-2828.

Horsley, D., Hutchings, A., Butcher, G. W. and Singh, P. B. (1996). M32, a murine homologue of Drosophila heterochromatin protein 1 (HP1), localises to euchromatin within interphase nuclei and is largely excluded from constitutive heterochromatin. *Cytogenet Cell Genet.* **73**, 308-311.

Jacobs, S. A., Taverna, S. D., Zhang, Y., Briggs, S. D., Li, J., Eissenberg, J. C., Allis, C. D. and Khorasanizadeh, S. (2001). Specificity of the HP1 chromo domain for the methylated N-terminus of histone H3. *EMBO J.* **20**, 5232-5241.

James, T. C. and Elgin, S. C. (1986). Identification of a nonhistone chromosomal protein associated with heterochromatin in Drosophila melanogaster and its gene. *Mol. Cell Biol.* **6**, 3862-3872.

Jenuwein, T. and Allis, C. D. (2001). Translating the histone code. *Science* **293**, 1074-1080.

Jenuwein, T. (2001). Re-SET-ting heterochromatin by histone methyltransferases. *Trends Cell Biol.* **11**, 266-273.

Kametaka, A., Takagi, M., Hayakawa, T., Haraguchi, T., Hiraoka, Y. and Yoneda, Y. (2002). Interaction of the chromatin compaction-inducing domain (LR domain) of Ki-67 antigen with HP1 proteins. *Genes Cells* **7**, 1231-1242.

Kellum, R. and Alberts, B. M. (1995). Heterochromatin protein 1 is required for correct chromosome segregation in Drosophila embryos. *J. Cell Sci.* **108**, 1419-1431.

Kirschmann, D. A., Lininger, R. A., Gardner, L. M., Seftor, E. A., Odero, V. A., Ainsztein, A. M., Earnshaw, W. C., Wallrath, L. L. and Hendrix, M. J. (2000). Down-regulation of HP1Hsalpha expression is associated with the metastatic phenotype in breast cancer. *Cancer Res.* **60**, 3359-3363.

Kitagawa, K., Masumoto, H., Ikeda, M. and Okazaki, T. (1995). Analysis of protein-DNA and protein-protein interactions of centromere protein B (CENP-B) and properties of the DNA-CENP-B complex in the cell cycle. *Mol. Cell Biol.* **15**, 1602-1612.

Lachner, M., O'Carroll, D., Rea, S., Mechtler, K. and Jenuwein, T. (2001). Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. *Nature* **410**, 116-120.

Lamond, A. I. and Earnshaw, W. C. (1998). Structure and function in the nucleus. *Science* **280**, 547-553.

Le Douarin, B., Nielsen, A. L., Garnier, J. M., Ichinose, H., Jeanmougin, F., Losson, R. and Chambon, P. (1996). A possible involvement of TIF1 alpha and TIF1 beta in the epigenetic control of transcription by nuclear receptors. *EMBO J.* **15**, 6701-6715.

Lehming, N., Le Saux, A., Schuller, J. and Ptashne, M. (1998). Chromatin components as part of a putative transcriptional repressing complex. *Proc. Natl. Acad. Sci. USA* **95**, 7322-7326.

Masumoto, H., Masukata, H., Muro, Y., Nozaki, N. and Okazaki, T. (1989). A human centromere antigen (CENP-B) interacts with a short specific sequence in alphoid DNA, a human centromeric satellite. *J. Cell Biol.* **109**, 1963-1973.

Minc, E., Allory, Y., Worman, H. J., Courvalin, J. C. and Buendia, B. (1999). Localization and phosphorylation of HP1 proteins during the cell cycle in mammalian cells. *Chromosoma* **108**, 220-234.

Minc, E., Courvalin, J. C. and Buendia, B. (2000). HP1gamma associates with euchromatin and heterochromatin in mammalian nuclei and chromosomes. *Cytogenet. Cell Genet.* **90**, 279-284.

Minc, E., Allory, Y., Courvalin, J. C. and Buendia, B. (2001). Immunolocalization of HP1 proteins in metaphasic mammalian chromosomes. *Methods Cell Sci.* **23**, 171-174.

Murzina, N., Verreault, A., Laue, E. and Stillman, B. (1999). Heterochromatin dynamics in mouse cells: interaction between chromatin assembly factor 1 and HP1 proteins. *Mol. Cell* **4**, 529-540.

Nielsen, A. L., Oulad-Abdelghani, M., Ortiz, J. A., Remboutsika, E., Chambon, P. and Losson, R. (2001). Heterochromatin formation in mammalian cells: interaction between histones and HP1 proteins. *Mol. Cell* **7**, 729-739.

Paro, R. and Hogness, D. S. (1991). The Polycomb protein shares a homologous domain with a heterochromatin-associated protein of Drosophila. *Proc. Natl. Acad. Sci. USA* **88**, 263-267.

Rea, S., Eisenhaber, F., O'Carroll, D., Strahl, B. D., Sun, Z. W., Schmid, M., Opravil, S., Mechtler, K., Ponting, C. P., Allis, C. D. et al., (2000). Regulation of chromatin structure by site-specific histone H3 methyltransferases. *Nature* **406**, 593-599.

Ryan, R. F., Schultz, D. C., Ayyanathan, K., Singh, P. B., Friedman, J. R., Fredericks, W. J. and Rauscher, F. J., 3rd (1999). KAP-1 corepressor protein interacts and colocalizes with heterochromatic and euchromatic HP1 proteins: a potential role for Kruppel-associated box-zinc finger proteins in heterochromatin-mediated gene silencing. *Mol. Cell Biol.* **19**, 4366-4378.

Saffery, R., Earle, E., Irvine, D. V., Kalitsis, P. and Choo, K. H. (1999). Conservation of centromere protein in vertebrates. *Chromosome Res.* **7**, 261-265.

Saunders, W. S., Chue, C., Goebl, M., Craig, C., Clark, R. F., Powers, J. A., Eissenberg, J. C., Elgin, S. C., Rothfield, N. F. and Earnshaw, W. C. (1993). Molecular cloning of a human homologue of *Drosophila* heterochromatin protein HP1 using anti-centromere autoantibodies with anti-chromo specificity. *J. Cell Sci.* **104**, 573-582.

Scholzen, T., Endl, E., Wohlenberg, C., van Der Sar, S., Cowell, I. G., Gerdes, J. and Singh, P. B. (2002). The Ki-67 protein interacts with members of the heterochromatin protein 1 (HP1) family: a potential role in the regulation of higher-order chromatin structure. *J. Pathol.* **196**, 135-144.

Seeler, J. S., Marchio, A., Sitterlin, D., Transy, C. and Dejean, A. (1998). Interaction of SP100 with HP1 proteins: a link between the promyelocytic leukemia-associated nuclear bodies and the chromatin compartment. *Proc. Natl. Acad. Sci. USA* **95**, 7316-7321.

Seeler, J. S. and Dejean, A. (1999). The PML nuclear bodies: actors or extras? *Curr. Opin. Genet. Dev.* **9**, 362-367.

Singh, P. B., Miller, J. R., Pearce, J., Kothary, R., Burton, R. D., Paro, R., James, T. C. and Gaunt, S. J. (1991). A sequence motif found in a Drosophila heterochromatin protein is conserved in animals and plants. *Nucleic Acids Res.* **19**, 789-794.

Song, K., Jung, Y., Jung, D. and Lee, I. (2001). Human Ku70 interacts with heterochromatin protein 1alpha. *J. Biol. Chem.* **276**, 8321-8327.

Sonoda, E., Matsusaka, T., Morrison, C., Vagnarelli, P., Hoshi, O., Ushiki, T., Nojima, K., Fukagawa, T., Waizenegger, I. C., Peters, J. M. et al., (2001). Scc1/Rad21/Mcd1 is required for sister chromatid cohesion and kinetochore function in vertebrate cells. *Dev. Cell* **1**, 759-770.

Stuart, G. W., Searle, P. F., Chen, H. Y., Brinster, R. L. and Palmiter, R. D. (1984). A 12-base-pair DNA motif that is repeated several times in metallothionein gene promoters confers metal regulation to a heterologous gene. *Proc. Natl. Acad. Sci. USA* **81**, 7318-7322.

Sugimoto, K., Tasaka, H. and Dotsu, M. (2001). Molecular behavior in living mitotic cells of human centromere heterochromatin protein HPLalpha ectopically expressed as a fusion to red fluorescent protein. *Cell Struct. Funct.* **26**, 705-718.

Wreggett, K. A., Hill, F., James, P. S., Hutchings, A., Butcher, G. W. and Singh, P. B. (1994). A mammalian homologue of Drosophila heterochromatin protein 1 (HP1) is a component of constitutive heterochromatin. *Cytogenet. Cell Genet.* **66**, 99-103.

Yamada, T., Fukuda, R., Himeno, M. and Sugimoto, K. (1999). Functional domain structure of human heterochromatin protein HP1(Hsalpha): involvement of internal DNA-binding and C-terminal self-association domains in the formation of discrete dots in interphase nuclei [In Process Citation]. *J. Biochem. (Tokyo)* **125**, 832-837.

Ye, Q. and Worman, H. J. (1996). Interaction between an integral protein of the nuclear envelope inner membrane and human chromodomain proteins homologous to Drosophila HP1. *J. Biol. Chem.* **271**, 14653-14656.

Ye, Q., Callebaut, I., Pezhman, A., Courvalin, J. C. and Worman, H. J. (1997). Domain-specific interactions of human HP1-type chromodomain proteins and inner nuclear membrane protein LBR. *J. Biol. Chem.* **272**, 14983-14989.