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SHORT COMMUNICATION

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# Truncated HP1 lacking a functional chromodomain induces heterochromatinization upon in vivo targeting

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**Abstract** Packaging of the eukaryotic genome into higher order chromatin structures is tightly related to gene expression. Pericentromeric heterochromatin is typified by accumulations of heterochromatin protein 1 (HP1), methylation of histone H3 at lysine 9 (MeH3K9) and global histone deacetylation. HP1 interacts with chromatin by binding to MeH3K9 through the chromodomain (CD). HP1 dimerizes with itself and binds a variety of proteins through its chromoshadow domain. We have analyzed at the single cell level whether HP1 lacking its functional CD is able to induce heterochromatinization in vivo. We used a lac-operator array-based system in mammalian cells to target EGFP-lac repressor tagged truncated HP1α and HP1β to a lac operator containing gene-amplified chromosome region in living cells. After targeting truncated HP1α or HP1β we observe enhanced tri-MeH3K9 and recruitment of endogenous HP1α and HP1β to the chromosome region. We show that CD-less HP1α can induce chromatin condensation, whereas the effect of truncated HP1β is less pronounced. Our results demonstrate that after lac repressor-mediated targeting, HP1α and HP1β without a functional CD are able to induce heterochromatinization.

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## Introduction

In the eukaryotic nucleus, various levels of chromatin packaging can be discerned (reviewed in Horn and Peterson 2002; Verschure et al. 2005). The condensation state of chromatin is a key aspect of epigenetic gene control. Different chromatin condensation states are related to the presence or absence of proteins involved in the regulation of gene expression (reviewed in Lachner and Jenuwein 2002). Pericentromeric heterochromatin, for instance, is typified by the accumulations of heterochromatin protein 1 (HP1), methylation of histone H3 at lysine 9 (MeH3K9) and global histone deacetylation (reviewed in Dillon 2004; Maison and Almouzni 2004). The molecular mechanisms underlying the structural and functional aspects of heterochromatin are still unresolved.

In mammals, three isoforms of HP1 have been identified, HP1α, HP1β and HP1γ, showing different subnuclear distributions. HP1α is primarily found in pericentromeric heterochromatin. HP1β and to a much lesser extent HP1γ are also present in pericentromeric heterochromatin (Horsley et al. 1996; Minc et al. 1999; Cowell et al. 2002; Li et al. 2003; Wreggett et al. 1994). HP1 proteins have emerged as key structural and regulatory components for the assembly of compact chromatin (Maison and Almouzni 2004). HP1 consists of a chromodomain (CD) at the amino terminus and a chromoshadow domain (CSD) at the carboxy terminus, separated by a hinge domain (HD). The CD recognizes MeH3K9, mediating binding to chromatin (Bannister et al. 2001; Jacobs et al. 2001; Lachner et al. 2001; Cowell et al. 2002; Jacobs and Khorasanizadeh 2002; Nielsen et al. 2002). The CSD interacts with a consensus peptide that is present in a number of proteins, including the CSD of HP1 itself, thereby targeting proteins to

heterochromatin and forming HP1 dimers (Aasland and Stewart 1995; Smothers and Henikoff 2001; Thiru et al. 2004; Lechner et al. 2005). The conserved CD and CSD are essential for the function of HP1. The HD of HP1 is less well characterized; a few studies indicate that it plays a direct role in HP1 function (Li et al. 2002; Muchardt et al. 2002; Meehan et al. 2003; Badugu et al. 2005).

Recently, we showed that targeting of the intact HP1α and HP1β is sufficient to induce large-scale chromatin compaction, enhanced tri-MeH3K9 and recruitment of proteins involved in heterochromatinization (Verschure et al. 2005). In the present study, we investigate whether HP1 that lacks a functional CD is able to induce heterochromatinization. Our results demonstrate that when HP1α or HP1β without a functional CD is targeted to chromatin, it is able to induce heterochromatinization.

## Materials and methods

### Construction of plasmids

A plasmid expressing the enhanced green fluorescent protein (EGFP)-dimer lac repressor-simian virus 40 nuclear localization signal (NLS) fusion protein under control of the F9-1 promoter, called p3'SS-EGFP-dimer lac repressor (Tumbar et al.1999) was used as the basis for these studies. This plasmid was modified as described previously to generate EGFP-lacR-AscI-NLS (NYE4) (Nye et al. 2002).

HP1α and HP1β correspond to full sequences of the human *HP1*. Both human *HP1*α and *HP1*β only have minor amino acid difference from the Chinese hamster *HP1*α and *HP1*β (for *HP1*β one amino acid difference in the hinge domain and for *HP1*α one amino acid difference in the CD, two in the HD and two outside of the domains). HP1α-Δ(2–39) and HP1β-Δ(2–40) have deletions of amino acids 2–39 and 2–40 in HP1α and HP1β, respectively. These proteins lack a large part of the CD, including V21 (HP1α) and V23 (HP1β), which are required for MeH3K9 binding (Platero et al. 1995; Bannister et al. 2001; Lachner et al. 2001). Full length HP1 and HP1 as well as HP1α-Δ(2–39) and HP1β-Δ(2–40) fragments were PCR-amplified using primers that contain *Asc*I sites in frame. The PCR products were digested with *Asc*I and ligated into the *Asc*I-digested EGFP-lacR-*Asc*I-NLS (NYE4) vector to create full length HP1α and HP1β as well as HP1α-Δ(2–39) and HP1β-Δ(2–40) C-terminal in frame fusions with the EGFP-lacR-*Asc*I-NLS (NYE4) vector. All regions of constructs that had undergone PCR were sequenced to ensure fidelity.

### Cell culture and transfection assay

We used the CHO-derived cell line RRE_B1, which contains large amplified genomic domains (several tens of Megabase pairs) consisting of arrays of lac operator binding sites, in an extended, often fibrillar conformation, suggesting a euchromatin-like structure (Robinett et al. 1996; Verschure et al. 2005). The in situ appearance of the RRE_B1 cell line as visualized with the light microscope is described in detail in Verschure et al. (2005). Cells were cultured in F12 Ham's media without hypoxanthine or thymidine supplemented by 10% dialyzed fetal bovine serum (HyClone Labs, Logan, UT, USA) containing 10% dialyzed fetal bovine serum and 10 μM methotrexate at 37°C in a 5% $CO_2$ atmosphere.

Transient transfections were performed with FuGENE6 reagent (Roche, Indianapolis, IN, USA) according to the manufacturer's instructions, using 500 ng of DNA and 6 μl FuGENE6 reagent per milliliter of culture medium using cells growing on coverslips. Fresh medium was added 24 h after transfection. After 48 h, cells were rinsed with phosphate buffered saline (PBS) and used for immunofluorescent labeling.

### Immunofluorescent labeling

Immunofluorescent labeling was performed as described previously (Verschure et al. 2002). Briefly, cells were fixed for 10 min at 4°C in 2% (w/v) formaldehyde in PBS. After fixation, cells were permeabilized with 0.5% (w/v) Triton-X 100 in PBS for 5 min and incubated in PBS containing 100 mM glycine for 10 min. Subsequently, cells were incubated for 1 h at 37 °C with the primary antibodies diluted in PBG: PBS containing 0.5% (w/v) bovine serum albumin (BSA) and 0.1% (w/v) gelatin (Sigma, St Louis MO, USA). The following primary antibodies were used: rabbit anti-tri-MeH3K9 (Nakayama et al. 2001), mouse anti-HP1α and anti-HP1β (Euromedex; Mundolsheim France) (Nielsen et al. 2001). After several washes with PBS, cells were incubated with the appropriate secondary antibodies, using Cy3-conjugated-donkey anti-rabbit antibody or Cy3-conjugated-donkey anti-mouse antibody (Jackson, West Grove, PA, USA). Secondary antibodies were diluted in PBG. Incubations were performed for 1 h at room temperature. Cells were then rinsed with PBS at room temperature and DNA staining was performed with 0.4 μg/ml DAPI 33258 (Sigma) in PBS. Slides were mounted in Vectashield (Brunschwig, Burlingame, CA, USA). Slides were stored at 4°C and analyzed within 24 h. As a control, the primary antibody was omitted.

### Confocal scanning laser microscopy

All the experiments were performed at least twice in duplicate. Images were recorded using a Zeiss LSM 510 (Zeiss, Jena, Germany) confocal laser scanning microscope, equipped with a 100×/1.23 NA oil immersion objective. We used an argon laser at 488 nm in combination with a helium neon laser at 543 nm to excite

the green and red fluorochromes simultaneously. Emitted fluorescence was detected with a 505–530 nm bandpass filter for the green signal and a 560 nm longpass filter for the red signal. Pairs of images were collected simultaneously in the 'green' and 'red' channels. Three dimensional (3D) images were scanned as 512×512×32 voxel images (sampling rate, 49 nm lateral and 208 nm axial).

### Image analysis

For a semi-quantitative analysis of the spatial relationship between the relative spatial distributions of components in dually labeled cells, line scans were made. The signal intensities of the two labels are plotted along a line through the nucleus. The line scan method provides a rapid and straightforward way to determine to what extent two components colocalize.

To quantitatively analyze changes in large-scale chromatin structure, we applied 3D image analysis tools as described in a previous study (Verschure et al. 2005). Briefly, 3D images of the amplified chromosome region were acquired and attributes of the 3D structure were determined. A region of interest, i.e. the EGFP labeled chromosome region, is defined. The images were Gaussian filtered to reduce noise with a size of 20 pixels (sigma = 250 nm). For each set we record the intensity gradient over the chromatin region. The distribution of the intensity gradient is plotted in a box-plot. The second and third quartiles of the observed values are within the box, the median value is shown by the thick horizontal line, the vertical small lines show the first and fourth quartiles of the observed values. We used the Wilcoxon nonparametric test to calculate the *P*-value giving the probability that two populations are different from each other.

## Results

### Experimental approach

To investigate the ability of HP1 lacking a functional CD to induce heterochromatinization, we used the CHO cell line RRE_B1. This cell line contains a large amplified chromatin region consisting of arrays of lac operator binding sites (Robinett et al. 1996). When visualized in vivo by binding of EGFP tagged lac repressor (lacR), the amplified region occupies a large nuclear area and displays an open chromatin structure, suggesting an euchromatin-like state (Robinett et al. 1996; Verschure et al. 2005). These cells constitute a useful system to investigate euchromatin–heterochromatin transitions. In a previous study, we have shown that targeting of full length HP1α or HP1β to the lac operator arrays induced chromatin compaction, enhanced tri-MeH3K9 and recruitment of endogenous HP1 and the histone methyltransferase SETDB1 (Verschure et al. 2005). These results demonstrated that HP1 binding is sufficient to trigger heterochromatinization.

DNA constructs were made such that the code for truncated human HP1α and HP1β were lacking the C-terminal part of their CD tagged with EGFP-lacR: EGFP-lacR-HP1α-Δ(2–39) and EGFP-lacR-HP1β-Δ(2–40). Two controls were used: (1) similar constructs that code for full length wild-type HP1α and HP1β instead of truncated protein and (2) a construct coding for EGFP-lacR. Proteins coded by all constructs bind to the amplified chromatin region via their lacR domain. As expected, after transient transfection in cells not containing lacR-binding sites, CD-less HP1 does not accumulate in pericentromeric heterochromatin, since it lacks the binding site for MeH3K9 (data not shown). In contrast, EGFP-lacR full length HP1 does accumulate in pericentromeric domains (Verschure et al. 2005). These findings agree with the notion that the CD is responsible for localization of HP1α and HP1β in pericentromeric heterochromatin through MeH3K9 binding (Bannister et al. 2001; Jacobs et al 2001; Lachner et al 2001; Nakayama et al 2001; Nielsen et al. 2002; Cheutin et al. 2003).

To analyze whether targeting of truncated HP1 results in changes in the chromatin structure we measured three independent parameters: (1) tri-MeH3K9 using immunofluorescent labeling with an anti-tri-MeH3K9-specific antibody, (2) accumulation of endogenous HP1α or HP1β and (3) changes in in vivo 3D chromatin structure of the EGFP-marked amplified chromatin domain, imaged by confocal microscopy followed by quantitative image analysis.

### Histone H3 lysine 9 methylation

MeH3K9 creates a binding site for the CD of HP1, whereas the CSD has been shown to interact with a variety of proteins, including HP1 itself (Aasland and Stewart 1995; Bannister et al. 2001; Jacobs et al. 2001; Lachner et al. 2001; Smothers and Henikoff 2001; Cowell et al. 2002; Jacobs and Khorasanizadeh 2002; Nielsen et al. 2002; Thiru et al. 2004; Lechner et al. 2005). Recently, we showed that lacR-mediated targeting of full length HP1α or HP1β results in strong heterochromatinization, including an increase of tri-MeH3K9 and recruitment of HP1 and histone methyltransferase (Verschure et al. 2005). Figure 1 shows that in cells transfected with EGFP-LacR-HP1α-Δ(2–39) or EGFP-LacR-HP1β-Δ (2–40) the level of tri-MeH3K9 is considerably increased in the amplified chromatin domain. In addition, tri-MeH3K9 is present diffusely throughout the nucleus. Line scans confirm that enhanced methylation levels colocalize with the binding of CD-less HP1 to the chromatin array. This phenomenon is observed in all the cells transfected with either full length (Verschure et al. 2005) or truncated HP1α or HP1β (Fig. 1). In control cells transfected with EGFP-lacR, the methylation level is not changed

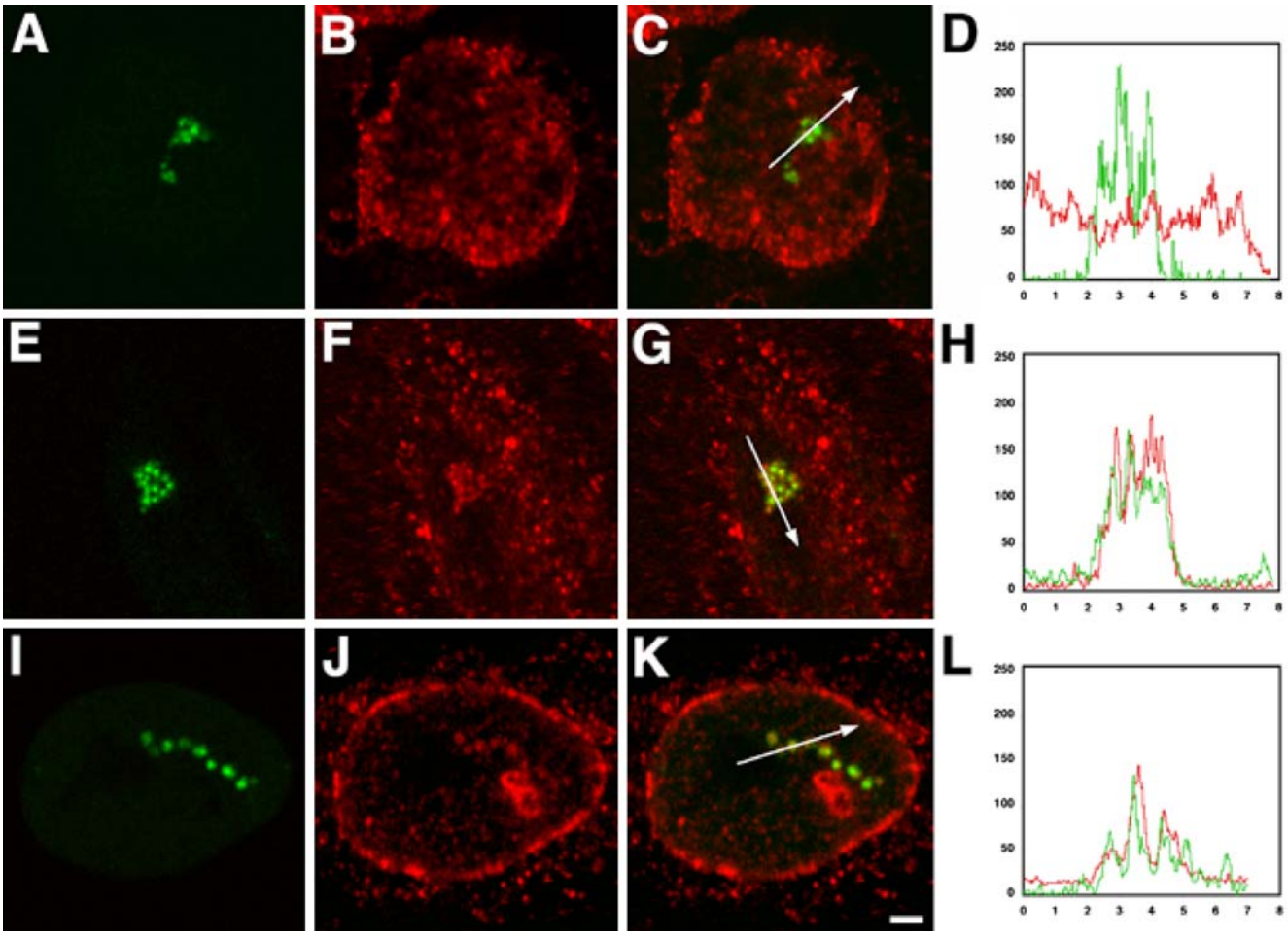


**Fig. 1** Enhanced tri-MeH3K9 at the amplified chromosome region RRE_B1 cells transfected with EGFP-lacR (as a contol) (**a**–**d**), EGFP-lacR-HP1α-Δ(2–39) (**e**–**h**) or EGFP-lacR-HP1β-Δ (2–40) (**i**–**l**), were immunofluorescently labeled with an antibody against tri-MeH3K9 after 48 h. The *green signal* shows the EGFP-tagged chromosomal array and the *red signal* marks the distribution of the immunolabeled tri-MeH3K9. Three dimensional images were recorded. Images shown are individual mid-nuclear optical sections. Figure **d**, **h** and **l** show *line scans* through the nuclei shown in **a**–**c**, **e**–**g** and **i**–**k**, respectively. The position of the line scans is shown by the *white arrow*. Nuclei in **a**–**c**, **e**–**h** and **i**–**l** are the same scale; *bar* represents 2 μm

(Fig. 1a–d). These observations show that CD-less HP1 is able to recruit directly or indirectly histone methyl transferase, similar to wild-type HP1.

## Recruitment of endogenous HP1

LacR-mediated targeting of wild-type HP1α or HP1β has been shown to result in local accumulation of endogenous HP1α or HP1β at the chromatin array. Here we analyze whether targeting of HP1 without a functional CD has the same effect. Cells transfected with EGFP-LacR-HP1α-Δ(2–39) were fluorescently labeled with an antibody against HP1β, and cells transfected with EGFP-LacR-HP1β-Δ(2–40) with an antibody specific for HP1α. Figure 2 shows that both the truncated proteins are able to recruit a considerable amount of endogenous HP1 to the amplified chromatin domains. Line scans confirm that endogenous HP1 accumulates at the sites where the CD-less HP1 protein is bound (Fig. 2). In control cells transfected with EGFP-lacR the distribution of endogenous HP1 is not changed (Fig. 2a–d). These results indicate that targeting of HP1 without the CD domain is able to recruit endogenous HP1.

## Large-scale chromatin structure

In a recent study, we showed that lacR-mediated binding of wild-type HP1 induces considerable chromatin compaction (Verschure et al. 2005). Figure 3 shows that also HP1 without a functional CD is able to induce a similar condensation of chromatin, as can be visualized by confocal microscopy of the EGFP-tagged amplified chromatin domain. In many cells targeting of truncated HP1 induces condensation of the amplified chromatin regions in a cluster or chain of highly condensed spherical chromatin domains. There is a significant variability in the appearance of the chromatin domains

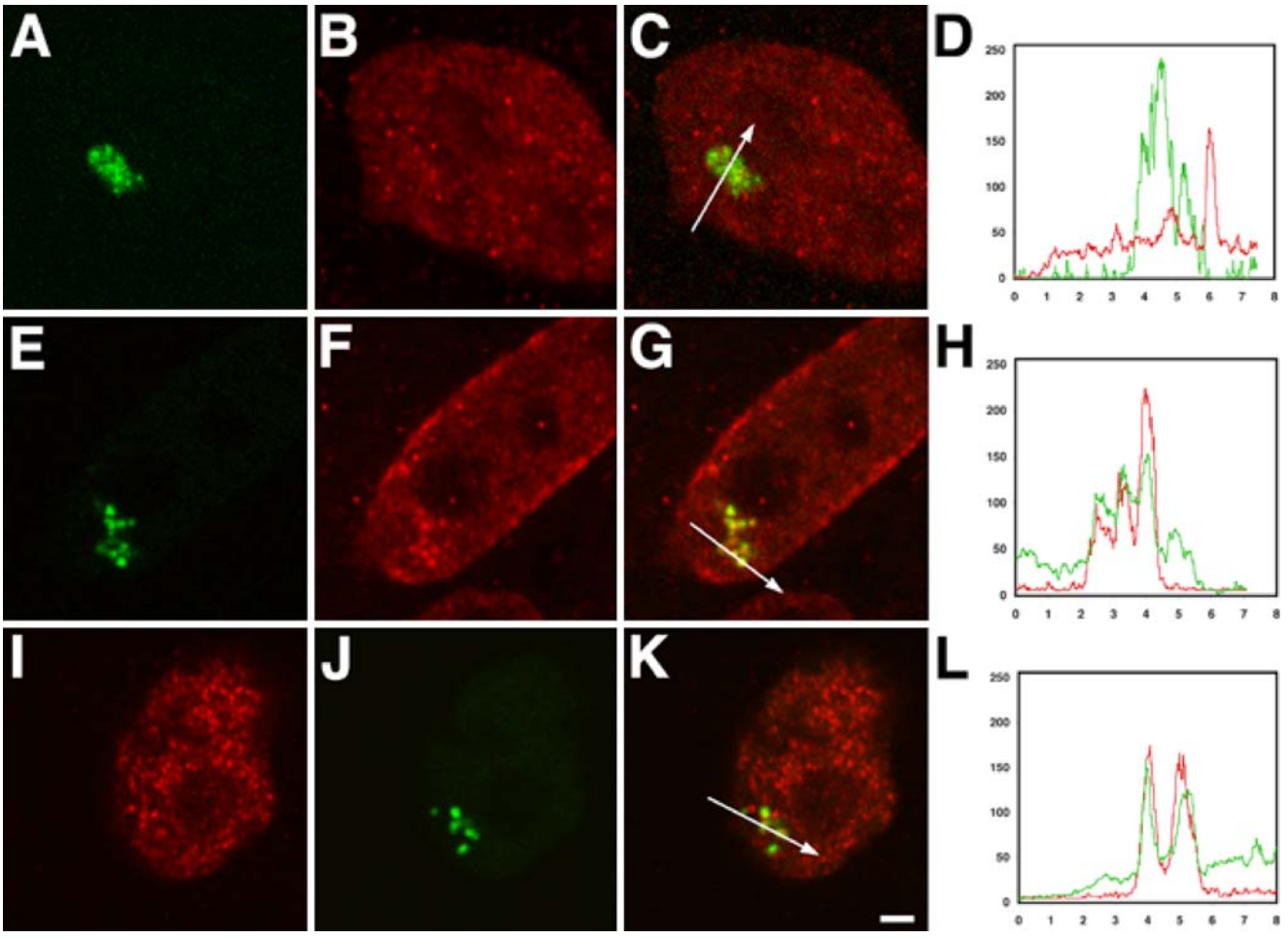


**Fig. 2** Recruitment of endogenous HP1 at the amplified chromosome region RRE_B1 cells transfected with EGFP-lacR (**a**–**d**), EGFP-lacR-HP1β-Δ(2–39) (**e**–**h**) or EGFP-lacR-HP1α-Δ(2–40) (**i**–**l**) were after 48 h immunofluorescently labeled with an antibody against HP1α (**a**–**d**) and (**e**–**h**) or against HP1β (**i**–**l**). Three dimensional images were recorded. Images shown are individual mid-nuclear optical sections. The *green signal* shows the EGFP-lacR tagged chromosomal array and the *red signal* marks the distribution of the immunolabeled endogenous HP1 proteins. Figure **d**, **h** and **l** show line scans through the nuclei shown in (**a**–**c**), (**e**–**g**) and (**i**–**k**), respectively. The position of the line scans is shown by the *white arrow*. Nuclei in (**a**–**c**), (**e**–**g**) and (**i**–**l**) are the same scale; *bar* represents 2 μm

in a population of otherwise identical cells, for full length HP1 (Verschure et al. 2005), for CD-less HP1 and for control cells (Fig. 3). Therefore, we carried out a quantitative analysis of the change in compaction of the chromatin structure of the array. As a measure for the degree of compaction we used a parameter that quantifies the discreteness of the individual subdomains in the amplified chromatin domain (Verschure et al. 2005). In control cells transfected with EGFP-lacR, such subdomains are relatively poorly defined (Fig. 3a). In contrast, after HP1-induced chromatin condensation (either wild-type or truncated HP1), the individual domains are well-defined (Fig. 3b–e). As a quantitative parameter we measured the gradient of the EGFP signal over such chromatin subdomains. The analysis is carried out on 3D images using a novel quantitative image analysis software package as is described in greater detail by Verschure et al. (2005). For each experiment several tens of cells were analyzed (*n*-values are shown in Table 1).

Results are summarized in Fig. 4 and Table 1. Figure 4 shows the median value (thick horizontal line) of the observed values, the second and third quartiles (open box) and the first and fourth quartiles of the detected values (thin vertical lines). The results confirm the large cell-to-cell variation observed earlier (Verschure et al. 2005). Statistical analysis (Wilcoxon test, Table 1) shows that CD-less HP1α induces significant chromatin condensation, similar to wild-type HP1α and HP1. Although condensed chromatin is seen in a number of cells transfected with EGFP-LacR-HP1β-Δ(2–40), for this parameter the difference with the control cell population (transfected with lacR-GFP) is statistically not significant. This suggests that CD-less HP1α is more effective in chromatin condensation than CD-less HP1α. In contrast, both mutated proteins seem equally effective in inducing enhanced tri-MeH3K9 and in recruiting endogenous HP1 according to light microscopical evaluation (Figs. 1, 2) Unexpectedly, our re-

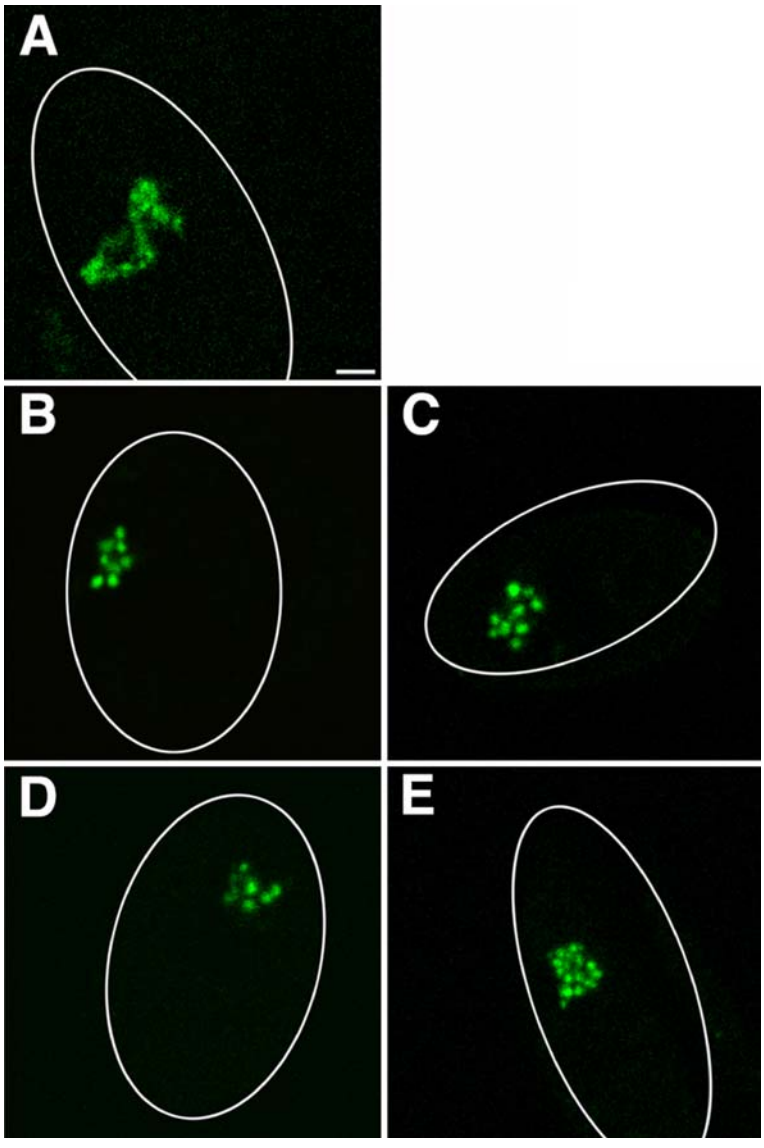


**Fig. 3** Local compaction of the chromatin structure of the amplified chromosome region RRE_B1 cells were transfected with EGFP-lacR-HP1α, EGFP-lacR-HP1β, EGFP-LacR-HP1α-Δ (2–39) or EGFP-LacR-HP1β-Δ (2–40) fusion constructs or with EGFP-lacR as a control. Three dimensional images were collected 48 h after transfection. Images shown represent individual mid-nuclear optical sections illustrating the EGFP signal of the amplified chromosome region. The chromosomal array in a control nucleus transfected with EGFP-lacR (**a**) occurs as an extended fibrillar structure. After HP1α (**b**) or HP1β (**c**) targeting, the amplified chromosome region is compacted occurring as a cluster of distinct spherical subdomains. After targeting of HP1α-Δ (2–39) (**d**) or HP1β-Δ(2–40) (**e**) similar compaction of the large-scale chromatin structure was observed. The approximate position of the nuclear envelope is shown by the *white line*. Nuclei in **a**–**e** are the same scale; **bar** represents 0.9 μm

**Table 1** Statistical evaluation of the structural analysis.

| Populations analyzed | Number of cells | *P*-value | Significant |
|---|---|---|---|
| Control–wild-type HP1α | 57–27 | $P < 0.0001$ | Yes |
| Control–wild-type HP1β | 57–53 | $P = 0.001$ | Yes |
| Control–CD-less HP1α | 57–27 | $P < 0.0001$ | Yes |
| Control–CD-less HP1β | 57–29 | $P = 0.29$ | No |
| wild type HP1α–wild type HP1β | 27–53 | $P = 0.0008$ | Yes |

Statistical evaluation of the differences in chromatin structure after targeting EGFP-lacR tagged constructs; differences between control cells transfected with EGFP-lacR and cells transfected with EGFP-lacR-HP1α, EGFP-lacR-HP1β, EGFP-LacR-HP1α-Δ (2–39), or EGFP-LacR-HP1β-Δ (2–40) (rows 1 through 4), and between EGFP-lacR-HP1α and EGFP-lacR-HP1β (row 5). Shown are the *P*-values determined in a Wilcoxon statistical test, indicating the probabilities that two populations are different (column 1), the number of cells that were analyzed (column 2) and the interpretation in term of significance of the difference, choosing a cut-off value of $P = 0.05$ (column 3).

sults also show that the effect of chromatin condensation after targeting full length HP1α is more pronounced than the effect of full length HP1β. Apparently, both wild-type HP1α and CD-less HP1α are more effective than their HP1β counterparts.

## Discussion

Heterochromatin is a specialized chromatin state that is typically condensed, late replicating, and incompatible

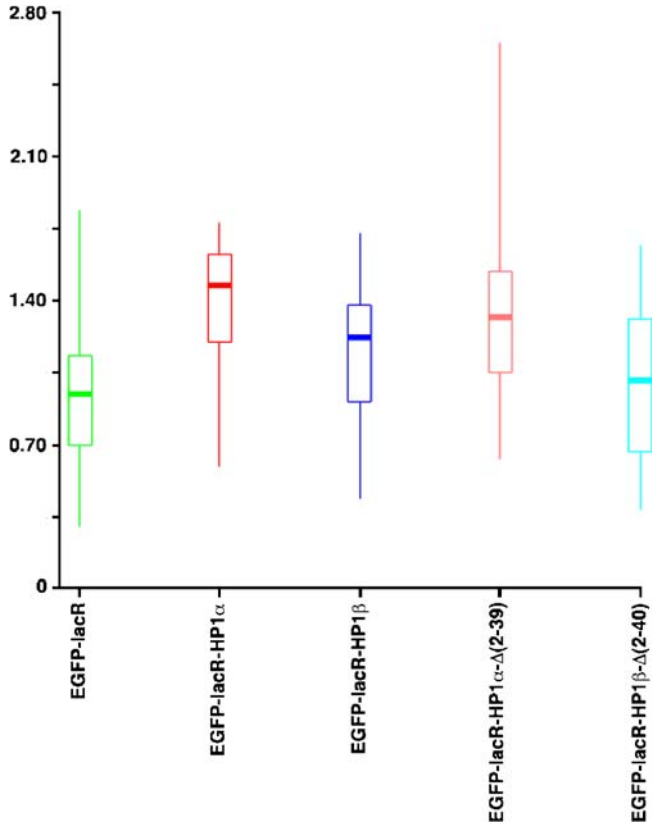


**Fig. 4** Quantitative analysis of the change in chromatin structure. Three dimensional images of RRE_B1 cells transfected with EGFP-lacR-HP1α, EGFP-lacR-HP1β, EGFP-LacR-HP1α-Δ (2–39), EGFP-LacR-HP1β-Δ (2–40) or EGFP-lacR as a control were acquired 48 h after transfection. We measured the gradient of the intensity of the EGFP signal over the amplified chromosome region. The results are shown as a box-plot of the measured values for cells transfected with EGFP-lacR (*green*) EGFP-lacR-HP1α (*dark red*), EGFP-lacR-HP1β (*dark blue*), EGFP-LacR-HP1α-Δ (2–39) (*light red*) EGFP-LacR-HP1β-Δ (2–40) (*light blue*). The second and third quartiles of the observed values are within the box (*open box*), the median value is shown by the thick *horizontal line*, the vertical *small lines* show the first and fourth quartiles of the observed values

with gene activity. The HP1 protein family is shown to be involved in the formation of heterochromatin. The observation that HP1 specifically binds to MeH3K9 via its chromodomain (CD), directly links these proteins to epigenetic gene control mechanisms (reviewed in Lachner and Jenuwein 2002). In the present study, we analyze in vivo and at the single cell level whether HP1 that lacks a functional CD can induce heterochromatinization. We use three parameters to assess euchromatin-heterochromatin transitions: (1) enhanced tri-MeH3K9, (2) recruitment of endogenous HP1α and HP1β and (3) condensation of chromatin. Results show that CD-less HP1α and HP1β are able to cause tri-MeH3K9 and recruitment of endogenous HP1α and HP1β. Targeting of truncated HP1α induces chromatin compaction, similar to what is observed after targeting full length HP1α and HP1β (Verschure et al. 2005). The effect of truncated HP1β is less pronounced. Our data demonstrate that both CD-less HP1α and CD-less HP1β are able to induce heterochromatinization, CD-less HP1α being more effective than CD-less HP1β.

## Comparison of HP1α and HP1β

In the present study, we compared the effect of targeting HP1α with that of HP1β (both wild type and CD-less HP1 proteins) on heterochromatinization of an amplified chromatin region. Results show that the effect of chromatin condensation after targeting full length HP1α is more pronounced than that of full length HP1β. Similarly, CD-less HP1α is more effective in inducing chromatin compaction than truncated HP1β. In contrast, both proteins are equally effective in inducing enhanced tri-MeH3K9 and in recruiting endogenous HP1. The cause of this difference between HP1α and HP1β in their ability to induce chromatin compaction is unclear. The amino acid sequence of both proteins differs only marginally (Minc et al. 1999). In the present study, we used human HP1 in Chinese hamster cells, but there is only a minor amino acid difference between the human HP1α and HP1β and Chinese hamster HP1α and HP1β (see Materials and methods). Therefore, we do not believe that the difference in effect between HP1α and HP1β is related to less efficient binding of the human HP1 in Chinese hamster cells. The difference in effect between HP1α and HP1β is probably related to the fact that HP1α and HP1β have distinct nuclear and mitotic distributions and differ in cell cycle-related phosphorylation (Minc et al. 1999). HP1α is phosphorylated throughout the cell cycle, whereas HP1β remains unphosphorylated. HP1α and HP1β are predominantly located in the centric heterochromatin. A third member of the HP1 family, HP1γ is found in euchromatin and to a much lesser extent in heterochromatin. In a recent study, using a dominant negative approach by overexpressing truncated HP1 lacking a functional CD, we show that endogenous HP1α or HP1β are competed out of mouse chromocenters without changing their condensed chromatin structure (Mateos et al. unpublished). Together, these observations indicate that full length HP1α and HP1β as well as CD-less HP1α and HP1β are sufficient to induce heterochromatinization, but are not necessarily required to maintain the condensed state.

## Three HP1 domains

The CD of HP1 recognizes MeH3K9 and is essential for the association of HP1 with heterochromatin. HP1 can form homodimers and bind a variety of other proteins through its CSD (Aasland and Stewart 1995; Bannister et al. 2001; Jacobs et al. 2001; Lechner et al. 2005; Smothers and Henikoff 2001; Cowell et al. 2002; Jacobs and Khorasanizadeh 2002; Nielsen et al. 2002; Thiru et al. 2004; Lachner et al. 2001). It is known from in vitro studies that HP1 also binds to DNA and linker

histones through its HD (Meehan et al. 2003). Also, the HD is known to be involved in targeting HP1 to heterochromatin through an RNA binding activity (Muchardt et al. 2002).

It is generally believed that the interaction of HP1 with MeH3K9 facilitates the formation of heterochromatin and represses gene activity (Lachner et al. 2001). Our results show that when HP1 without a functional CD is targeted to chromatin, here via the lac repressor–lac operator interaction, its ability to induce heterochromatin is maintained. This indicates that the CSD and HD are sufficient to recruit and bind the protein factors required for the heterochromatinization process. Interestingly in this context, Stewart et al. (2005) recently showed that H3K9 methylation and HP1 can also act independently. Probably, incorporation of HP1 into heterochromatin is a multistep process, involving interactions with histone methyltransferases, binding to MeH3K9 and possibly stabilization by RNA components (Muchardt et al. 2002; Maison and Almouzni 2004). Our data suggest that the complex process of heterochromatin formation does not require the CD domain if HP1 is bound to chromatin in a CD-independent manner.

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