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Centromere-specific acetylation of histone H4 in barley detected through three-dimensional microscopy

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Abstract

Histone acetylation affects chromatin conformation and transcriptional activity. However, the structural role of histone acetylation at specific chromosomal regions, such as the centromere, is poorly understood. In this study, histone H4 acetylation and its localization in barley interphase nuclei are revealed by three-dimensional microscopy. The centromeres form a ring-like allocation near the nuclear membrane in barley. Immunofluorescence studies on non-fixed, interphase nuclei treatment revealed ring-like distribution of the highly acetylated histone H4, located near the nuclear membrane at one pole of the nucleus. This fluorescent structure was similar to the centromere cluster and referred to as hyperacetylated region (HAR). The distribution pattern of the acetylated histone H4 was similar to each of the K5, K8, K12 and K16 lysine residues, although H4 acetylated at K5, K8 and K12 residues was found in almost all nuclei, whereas H4 acetylated at K16 was weakly observed in only half of the nuclei. Each HAR consists of two strongly acetylated cores and a halo-like, less acetylated surrounding area. Fluorescence signals from centromere-specific repetitive sequences of barley, detected through three-dimensional fluorescence *in situ* hybridization (3D-FISH), co-localized with the HAR corresponding to the K5 residue acetylation, but the signals did not completely overlap each other. These findings indicate that histone acetylation specifically occurring at the centromeres likely have certain structural roles for the centromere.

Introduction

Histone acetylation is a post-translational modification which occurs in nucleosomal histones at the N-terminal lysine residues of histone H4, such as K5, K8, K12 and K16. Acetylation affects both the interaction between DNA and the nucleosomal core and among nucleosomes themselves (Luger *et al.*, 1997). Highly acetylated nucleosomes inhibit full condensation of chromatin to the 30nm fiber (Annunziato *et al.*, 1988; Iwano *et al.*, 1997) and remain in an extended conformation (Gracia-Ramirez *et al.*, 1995), indicating that histone acetylation exerts a conformational effect on the chromatin fiber. Such conformational changes in chromatin fiber affect accessibility of DNA targeted factors, such as DNase I and transcriptional factors.

The discovery that histone acetyltransferases (HATs) are the components of some transcription factors (Brownell *et al.*, 1996) supports the existence of a close association between histone acetylation and transcriptional activity. DNA-binding repressors, transcriptional co-repressors and methylated DNA-binding proteins are associated with histone deacetylase complexes (reviewed by Struhl, 1998; Jones *et al.*, 1998; Nan *et al.*, 1998; Ng and Bird, 2000).

Antibodies against histone isoforms acetylated at specific lysine residues (Turner and Fellows, 1989; White *et al.*, 1999) have been established and used to investigate the pattern of lysine residue-specific acetylation. Because amino acid sequence of N-terminus tail of histone H4 is highly conserved in eukaryotes, immunocytological methods have been used across biological species, i.e., mitotic metaphase chromosomes of mammals (Jeppesen and Turner, 1993) and plants (Houben *et al.*, 1997; Belyaev *et al.*, 1997; Belyaev *et al.*, 1998; Wako *et al.*, 2002), polytene chromosomes of *Drosophila* (Turner *et al.*, 1992) and lampbrush chromosomes of amphibians (Sommerville *et al.*, 1993). These results clearly indicate the existence of a lysine-specific acetylation pattern of the chromosomes.

However, the acetylation pattern in interphase nuclei, in which transcription and replication occur, are poorly understood and, moreover, the microscopic preparations are usually treated with fixatives. The interphase nucleus has a globular structure, and a simple, two-dimensional microscopic observation cannot discriminate the events between the nuclear surface and the interior. For a better discrimination of spatial events inside the nuclei, we used two different types of three-dimensional microscopy: the laser confocal microscopy and the deconvolution method. In the laser confocal microscope, the blur originating from fluorescence outside the focal plane is removed by a physical method, placing a confocal pinhole just in front of the detector. The deconvolution method removes fluorescence blur from the digitally captured image set by a mathematical method (Agard *et al.*, 1989). Optical sections are obtained and a three-dimensional view is reconstructed. The deconvolution system has a higher resolution than confocal microscopy, but takes longer for the 3D-image reconstruction (Wako *et al.*, 1998).

In this report we present the three-dimensional localization of the highly acetylated regions and centromeric repeats in barley interphase nuclei and discuss the biological significance of histone acetylation in detail based on the results.

Materials and methods

Plant materials and sample preparation

Barley, *Hordeum vulgare* L. cv. Minorimugi ($2n = 14$), was used for preparing interphase nuclei and chromosome samples. Seeds were germinated at 25 °C in the dark. The root tips were macerated using an enzymatic cocktail and then spread on glass slides by the tapping method (Wako *et al.*, 1998). Root tips fixed with 4% paraformaldehyde were used for following fluorescence *in situ* hybridization (FISH). Non-fixed root tips were used for double detection of histone acetylation by immunostaining and centromeric repeat by FISH. The slides were stored at −80 °C before use.

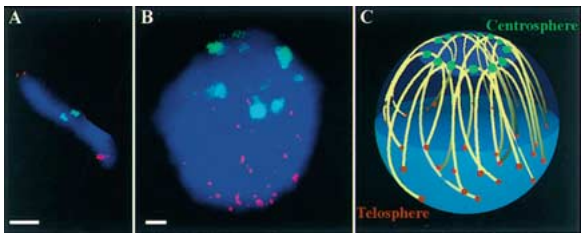

Figure 1. Multi-color FISH (McFISH) results using probes for the centromeric (green) and telomeric (red) repeats on chromosomes and nuclei from barley. A. Metaphase chromosome. B. Interphase nucleus. C. Spatial distribution model of barley chromosomes in an interphase nucleus. Bar = 2 μm.

Immunostaining and fluorescence in situ *hybridization (FISH)*

The indirect immunofluorescence method for the detection of acetylated histone H4 was described previously (Wako *et al.*, 1998). Rabbit polyclonal antisera against histone H4 at defined lysine residues were kindly supplied from Prof. Bryan M. Turner at the University of Birmingham Medical School, UK. These antisera were established and confirmed their specificities against each acetylated lysine residue (Turner *et al.*, 1989; White *et al.*, 1999). The antisera used in this study were: R41 (for AcH4K5, acetylated H4 at lysine 5), R232 (AcH4K8), R101 (AcH4K12), and R14 (AcH4K16). Specificity of these antisera was confirmed with acetyl-histone peptides (Upstate Biotechnology, USA) as the blocking reagents. After immunostaining, the samples were fixed in 4% paraformaldehyde (Houben *et al.*, 1995). Briefly, the slides were washed with 1× PBS for 5 min, treated with 1 mg/ml of RNase for 1 h. The slides were washed 3 times with 2× SSC for 3 min each, then treated with 0.01 M HCl for 2 min, and 5 μg/ml pepsin in 0.01 M HCl for 20 min. Slides were rinsed in distilled water for 2 min, twice in 2× SSC for 3 min, 1× PBS with 50 mM $MgCl_2$ for 5 min, 1% formaldehyde in 1× PBS with 50 mM $MgCl_2$ for 10 min, 1× PBS for 5 min and 2× SSC for 5 min. Dehydration with 70%, 90% and 100%

ethanol series was performed before air-drying. The biotin-labeled centromeric probe, *CEREBA* (Courtesy of Prof. I. Schubert and Dr G. Presting, Institut für Pflanzengenetik und Kulturpflanzenforschung; Presting *et al.*, 1998) and digoxigenin-labeled telomere probes, $(TTTAGGG)_n$ (Richards and Ausubel, 1998), were hybridized on the slides, followed by washing and fluorescence detection the following day. The detection procedure was described previously (Ohmido and Fukui, 1997; Ohmido *et al.*, 2001).

Three-dimensional construction of nuclear images

Three-dimensional nuclei from FISH images with the telomere and the centromeric repetitive sequences were obtained by confocal microscopy (LSM410, Zeiss, Germany). Fluorescent signals of histone acetylation and/or centromeric repetitive sequence were captured and deconvolved three-dimensionally under a deconvolution microscopic system (DeltaVision, Applied Precision, USA). Then stereogram and polygonized images were delivered from deconvolved image sets with a program of DeltaVision system.

Results

Distribution of centromeres and telomeres in barley interphase nuclei

The position of the telomeric repeats (Richards and Ausubel, 1998) and the centromeric repetitive sequence *CEREBA*, with a unique 809 bp unit (Presting *et al.*, 1998), on a metaphase chromosome is shown in Figure 1A. The centromeric signal clearly shows a typical doublet pattern, indicating the two sister chromatids of the chromosome after the S phase. The centromeric fluorescent signals on each chromatid are elongated along the chromatid axis, and have a constriction at the center of the signal. This may indicate that the centromeric sequence includes many copies of *CEREBA*, and that the *CEREBA* itself is distributed in and/or around the kinetochore.

The three-dimensional orientation of the barley centromeres and telomeres in interphase nuclei was also examined (Figure 1B). The centromeres show a cluster localization in a limited region near the nuclear membrane, forming ring-like distribution. The numbers of detected centromere signals is usually below the chromosome number of barley, 14, suggesting that fusion or co-localization of the centromeres might occur. The telomere sequences spread in a more divergent manner over the hemisphere, which is on the opposite side of the nucleus. A model of the spatial distribution of barley chromosomes in interphase nuclei is drawn in Figure 1C. Within a globular nucleus, the hemisphere where the centromeres are distributed is referred to as the 'centrosphere', whereas the hemisphere where telomere sequences are distributed is referred to as the 'telosphere'. The centromeres are positioned at limited region in the nucleus, then each chromosomal arm extends toward the telosphere of the nucleus. The chromosomal arms in barley have different lengths, which may be one reason why the telomere sequences do not cluster in a limited region, but are rather widely spread within the telosphere.

Intact interphase nucleus has a ring-shaped formation of hyperacetylated regions

To reveal the detailed location of the hyperacetylated regions (HARs) in interphase nuclei, non-fixed barley cells were examined by using the deconvolution system. In unfixed interphase nuclei, acetylated histone H4 at lysine 5 residue (AcH4K5) showed strong signals near one side of the nuclear membrane, where they cluster in ring-like formation (Figure 2A) as previously reported (Wako *et al.*, 1998). Neither the HARs nor other AcH4K5 signals were observed when the acetyl-histone H4 peptide at lysine 5 (K5) was used as a blocking reagent for immunostaining. Addition of acetyl-histone peptides at lysine 8, 12 and 16 (K8, K12 and K16) in the course of immunostaining experiment with the antibody against AcH4K5 did not prevent the immunostaining of AcH4K5 (data not shown). The maximum number of HARs was 14, equal to the chromosome number of barley, although the average number was less than 14 as in the case of centromeres. Microscopic examination of 100 interphase nuclei showed that 97 nuclei carried more than six HARs, and the average number was 8.9. This ring-like formation was clearly detected only in non-fixed material, and was barely visible on fixed preparations. Even in the case of K5, which usually showed the brightest fluorescent spots and the most visible ring formation among the four lysine residues, the use of fixed material led to only occasional and faint signals and even the disappearance of the ring formation. Fluorescence was observed only at the telosphere in the case of K16 in the fixed material (Wako *et al.*, in press).

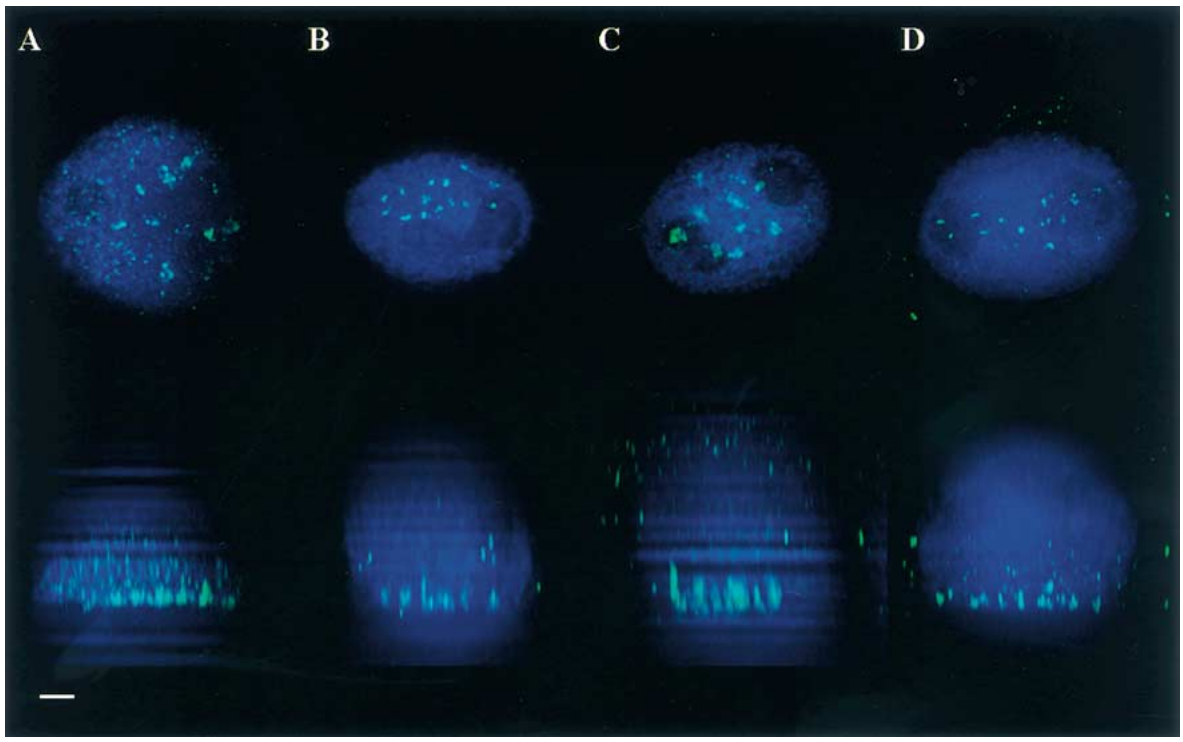

Figure 2. The distribution of hyperacetylated histone H4 in an unfixed barley interphase nuclei. All figures are projection images captured using the deconvolution system. Acetylated histone H4 is shown in green and nuclear DNA is stained blue with DAPI. The upper and the lower panels show a reconstruction of the polar view and the side view of the nuclei, respectively. A. Acetylation at lysine 5. B. Acetylation at lysine 8. C. Acetylation at lysine 12. D. Acetylation at lysine 16. Bar = 2 μm.

Fluorescent detection of histones hyperacetylated at lysine 8, 12 and 16 (K8, K12 and K16) resulted in the similar ring-like formations near the nuclear membrane (Figure 2B, 2C and 2D, respectively). Acetylhistone H4 peptides blocked staining completely only when the acetylated lysine residue of the peptides was the same to the recognition residues of the corresponding antibodies (data not shown). Although these were similar to the AcH4K5 pattern in number and nuclear position, the fluorescent intensity of K16 was low and the ring formation was observed only half the number of nuclei examined. Each HAR was about 1 μm in diameter and contained two strongly acetylated cores, 0.1–0.2 μm in diameter. The two cores were surrounded by a lower fluorescence halo. The acetylation level of the halo seemed intermediate, between the strongly-fluorescent cores and the low-level signal of other nuclear regions. The number of the fluorescent dots within the strongly acetylated cores did not vary along with the progression of the cell cycle (G_1, S and G_2 phases).

Close association between the centromeres and the hyperacetylated regions

Simultaneous detection of the acetylated histone H4 and the centromere sequence was achieved, by combining indirect immunofluorescence staining (with an antibody against AcH4K5) with three-dimensional fluorescence *in situ* hybridization (3D-FISH) with probes targeting the centromere repeats. The HARs of AcH4K5 (Figure 3A, green) and the centromere repeats (Figure 3A, red) were co-localized in interphase nuclei, where they seem to partially overlap each other. These overlapping regions are represented by yellow color in Figure 3A instead of red and green colors.

The two signal areas, of the acetylated spots and of the centromeric hybridization, were extracted from the original images by assigning a threshold to the gray value of the digital images. Then the extracted area was polygonized and colored by the computer software on the deconvolution system. In Figure 3B, the strongly acetylated cores of the HARs are depicted in solid light green, whereas the signals of the

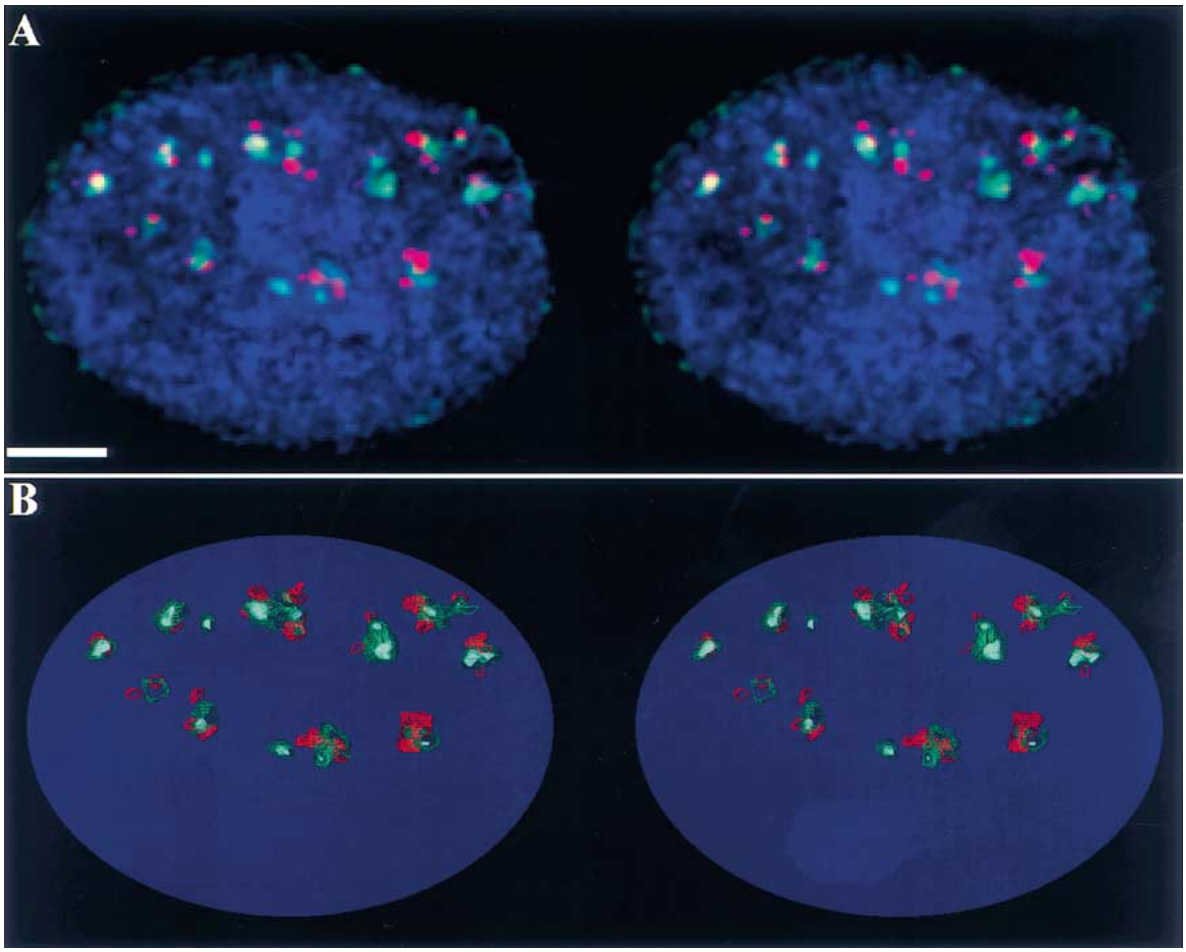

Figure 3. Co-localization of the hyperacetylated regions (HARs) of AcH4K5 and centromeric repeats. A. Stereographic representation of centromeres (red) and acetylated histone H4 (green) captured by the deconvolution system. The nucleus is counterstained with DAPI (blue). B. Polygonized stereogram. The HARs are depicted in solid light green and wire-framed green according to signal intensity. The light green color shows the strongly acetylated cores and the green color the less acetylated halo of the HARs. The red wire-framed color corresponds to the centromeric repeats. Bar = 2 μm.

less acetylated halo and the centromeric repeats are wire-framed green and red, respectively. The polygonized image shows that the HARs and the centromeric repeats share the same compartment in the nucleus, however, they do not completely overlap. Especially, the centromeric signals (Figure 3B, red) were not overlapped to the strongly acetylated cores (Figure 3B, light green). Figure 4 depicts a superimposed stereogram of a HAR and the corresponding centromeric repeats. The strongly acetylated cores are depicted solid light green, the less acetylated halo is wire-framed green, and the centromeric repeats are wire-framed red. The centromeric signals spread beyond the less acetylated halo of the HARs and they overlap each other only at their outer peripheral area. This suggests that the centromeric repeats occupy mostly the two independent domains outside of the HAR, and the two strongly acetylated cores also exist inner domains of the centromeric repeats.

Discussion

Possible function of a ring like formation of centromere cluster

Polarized orientation of the centromeres and telomeres in the interphase nucleus after cell division is referred to as Rabl orientation (reviewed by Comings, 1980), but its mechanism and function are not yet understood. The Rabl structure has been investigated in mammals

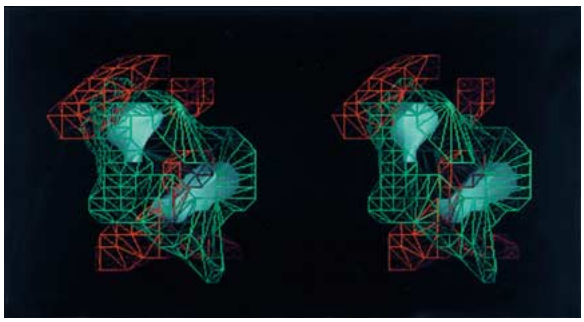

Figure 4. Close, but not identical association of the centromeric repeats and the hyperacetylated region (HAR) of AcH4K5. Enlarged image of a polygonized HAR. Colors solid light green, wire-framed green and red indicate the strongly acetylated cores, the acetylated halo and the centromeric repeats, respectively.

(Moroi *et al.*, 1981; Cremer *et al.*, 1982), fission yeast (Funabiki *et al.*, 1993) and plants (Noguchi and Fukui, 1995; Aragón-Alcaide *et al.*, 1996; Dong and Jiang, 1998) with either direct approaches such as FISH with centromere and/or telomere sequences or indirect detection systems. Previous reports showed that barley centromeres cluster at one pole of the interphase nucleus. Chromomycin A_3, which specifically stains barley centromeres, was used to stain and reveal the three-dimensional clustering of centromeres (Noguchi and Fukui, 1995). FISH with cereal-specific centromere and telomere DNA probes also demonstrated the Rabl orientation two-dimensionally. However, the Rabl model is not universal in some plant species (Dong and Jiang, 1998). A cell cycle dependent, non-Rabl orientation was also reported in mouse lymphocyte cells (Vourc'h *et al.*, 1993) in human nuclei (Cremer *et al.*, 1995) and in plants (Dong and Jiang, 1998).

The three-dimensional distribution of barley centromeres and telomeres described in our study reflects the typical Rabl orientation. The relationship between genome size and the Rabl structure was previously discussed (Dong and Jiang, 1998). It is known that large genomes include numerous non-transcribed sequences, such as heterochromatic repeats. Barley chromosomes carry large centromeric heterochromatins (Fukui and Kakeda, 1990) and the coding regions are limited to the terminal regions of the chromosomes (Künzel *et al.*, 2000). Thus coding regions on each chromosome are divided into the most separated regions on the chromosomes. When the Rabl structure is formed in the interphase nucleus, the coding regions come together within the telosphere, whereas the non-transcribed centromeric heterochromatin stays in the centrosphere. As a result, compartmentalization of the telomeric coding region and the centromeric heterochromatin is attained within a globular nucleus. The Rabl structure 'concentrates' the coding regions within a limited nuclear space and probably contributes to the efficient allocation and recycling of the transcriptional machinery in the nucleus, especially for species with a large genome such as barley, with 4873 Mb (Arumuganathan and Earle, 1991).

Hyperacetylation at the centrosphere

Our three-dimensional analysis shows that the *CEREBA* centromeric repeats closely associated with the hyperacetylated regions (HARs), and they both formed similar ring formation in interphase nuclei. This result indicates that the HARs were localized at the centromeres in wider sense. However, the centromeric repeats and the HARs were not completely overlapped. The centromeric repeats tended to occupy two independent clusters near the individual HARs and partially overlapped to the less acetylated halo in the HARs, although the repetitive sequence was not observed in the strongly acetylated cores in the HARs. It means that the acetylated cores do not contain the centromeric repeats and the most of the centromeric repeats do not involve the strongly acetylated histone H4. Barley centromeric repetitive sequence *CEREBA*, that is homologous to cereal centromeric repetitive sequence *pSau3A9* (Jiang et al., 1996), is part of a *Ty3 gypsy* retrotransposon-like sequence (Presting et al. 1998). Another *gypsy*-type retrotransposon sequence RIRE7 was identified as a component of the pericentromeric heterochromatin of rice (Kumekawa *et al.*, 2001). Therefore the retrotransposon-like centromeric repetitive sequences may not have a significant contribution to the centromere function, although they locate on the centromeres in a wide sense. Our result showed that the HARs, especially the cores, are slightly apart from the centromeric repeats. There is evidence that indicates functional centromere locates apart from the centromeric repeat. A homolog of barley centromeric repeat, *pSau3A9* and plant CENP-C homolog in maize were closely associated with each other but did not show complete co-localization (Dawe et al., 1999). This finding supports functional centromere is apart from the *gypsy*-type retrotransposon sequence. It is likely that the acetylated cores of the HARs have some functional role for the centromeres.

The HARs are observed only in the nuclei prepared without fixation, and these nuclei have a hazy shape, indicating certain levels of degradation of the nuclear structure. In nuclei fixed with paraformaldehyde, the outline of the nuclei is sharp, but the HARs become hazy or completely disappear especially in case of K16, although strong fluorescent signals appear at the telosphere even after fixation (Wako *et al.*, 2002). This suggests that fixation affects nuclear structure, especially at the membrane level, and thus disrupts the HARs but the appearance of telomeric signals. Therefore, the HARs are probably in close association with the nuclear membrane. Hyperacetylation may contribute to the attachment of the centromeres to the nuclear membrane or nuclear lamina, and thus to the spatial compartmentalization of the nucleus. Alternatively, centromeric attachment to the nuclear membrane and/or nuclear lamina may induce acetylation of histone H4. In support for this idea, Hendzel *et al.* (1994) reported that the nuclear matrix has histone acetyltransferase activity.

Similar ring-formed hyperacetylated regions were reported in a stage-dependent manner with *Vicia faba* (Jasencakova *et al.*, 2000) and barley (Jasencakova *et al.*, 2001) interphase nuclei. However, almost all nuclei have the ring-formed hyperacetylated regions of K5 as shown by the present results and the fact indicates that this structure is not replication timing dependent. The stage-dependent (mid S-phase to G2-phase) and infrequent (maximum 56.9% at late S-phase) fluorescent signals appearance at K5 in barley reported by Jasencakova *et al.* (2001) may be due to the fixation. On the other hand, in the hyperacetylated ring formation of AcH4K16 which is observed about half the number of nuclei may be mitotic stage-dependent.

Interphase nuclei can be classified in before or after replication using FISH. Before DNA replication FISH signals usually appear as single dots, whereas double dots are visible after replication in G_2 nuclei and metaphase chromosomes (Selig *et al.*, 1992). Similar observations were made with the centromere-specific human proteins CENP-A and CENP-C (Sugimoto *et al.*, 2000) and the maize CENP-C protein (Dawe *et al.*, 1999). In our observations, each HAR has two strongly acetylated cores. If the structure of the cores were G_1 specific, some nuclei would have HARs with four cores. If G_2 specific, some nuclei would have HARs with one core. However, no such nuclei were identified. Therefore, this phase-independent structure, with two strongly acetylated cores of the HAR is probably not related to the CENP proteins.

Role of acetylation of individual lysine residues

Our experiments revealed centromere clustering near the nuclear membrane and centromeric H4 histone hyperacetylation at all four acetylatable lysine residues. Acetylation at K5, K8 and K12 was observed in almost all nuclei, but acetylation at K16 was detected only in about half of the nuclei counted. Barley chromosomes have centromeric heterochromatins that are transcriptionally inactive and more condensed than euchromatin area (Fukui and Kakeda, 1990). We previously described high-level acetylation of K5 at the centrosphere of fixed interphase nuclei, and discussed K5 would play a role for avoiding over-condensation of the heterochromatin at the centrosphere in interphase nuclei (Wako *et al.*, 2002). Similarly, K5 acetylation at the centromeres would constitutively play a role for avoiding over-condensation of the centromeres. Acetylation at K8 and K12 were also observed in almost all nuclei. They might play some roles for centromere function together with K5. On the other hand, K16 acetylation at the centromeres was much weaker than that of K5 and was observed only half of nuclei. Acetylation of K16 was linked to transcriptional activity (Jeppesen and Turner, 1993; Kuo *et al.*, 1998) and centromeric regions are known as transcriptionally inactive, because of heterochromatin accumulation (Künzel *et al.*, 2000). Our previous observation revealed that K16 acetylation at the telomeric hemisphere and we have discussed that correlation between K16 acetylation and transcriptional activity (Wako *et al.*, 2002). In this study, the nuclei that showed ring-formed HARs of K16 at the centromere also showed strong telomeric acetylation. This suggests that K16 acetylation at the centromere may be subsidiary and that may not have functional and/or structural role at the centromeres and that, perhaps, the other three lysines could have a structural role, contributing to centromeric clustering in interphase nuclei.

All these results strongly suggest that histone H4 acetylation at the centromeres in the centrosphere contributes to both the function and the structure of the centromeres in a lysine-specific manner.

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References

Agard, D.A., Hiraoka, Y., Shaw, P. and Sedat, J.W. 1989. Fluorescence microscopy in three dimensions. Meth. Cell Biol. 30: 353–377.

Annunziato, A.T., Frabo, L.Y., Seale, R.L. and Woodcock, C.L.F. 1988 Treatment with sodium butyrate inhibits the complete condensation of interphase chromatin. Chromosoma 96: 132–138.

Aragón-Alcaide, L., Miller, T., Schwarzacher, T., Reader, S. and Moore, G. 1996. A cereal centromeric sequence. Chromosoma 105: 261–268.

Arumuganathan, K. and Earle, E.D. 1991. Nuclear DNA content of some important plant species. Plant Mol. Biol. Rep. 9: 208–218.

Belyaev, N.D., Houben, A., Baranczewski, P. and Schubert, I. 1997. Histone H4 acetylation in plant heterochromatin is altered during the cell cycle. Chromosoma 106: 193–197.

Belyaev, N.D., Houben, A., Baranczewski, P. and Schubert, I. 1998. The acetylation patterns of histone H3 and H4 along *Vicia faba* chromosomes are different. Chromosome Res. 6: 59–63.

Brownell, J.E. and Allis, C.D. 1996. Special HATs for special occasions: linking histone acetylation to chromatin assembly and gene activation. Curr. Opin. Genet. Dev. 6: 176–184.

Comings, D.E. 1980 Arrangement of chromatin in the nucleus. Hum. Genet. 53: 131–143.

Cremer, T., Cremer, C., Baumann, H., Luedtke, E.K., Sperling, K., Teuber, V. and Zorn, C. 1982. Rabl's model of the interphase chromosome arrangement tested in Chinese hamster cells by premature chromosome condensation and laser-UV-microbeam experiments. Hum. Genet. 60: 46–56.

Cremer, T., Dietzel, S., Eils, R., Lichter, P. and Cremer, C. 1995. Chromosome territories, nuclear matrix filaments and interchromatin channels: a topological view on nuclear architecture and function. In: P.E. Brandham and M.D. Bennett (Eds.) Kew Chromosome Conference IV, Kew Royal Botanic Gardens, pp. 63–81.

Dawe, R.K., Reed, L.M., Yu, H., Muszynski, M.G. and Hiatt, E.N. 1999. A maize homolog of mammalian CENPC is a constitutive component of the inner kinetochore. Plant Cell 11: 1227–1238.

Dong, F. and Jiang, J. 1998. Non-Rabl patterns of centromere and telomere distribution in the interphase nuclei of plant cells. Chromosome Res. 6: 551–558.

Fukui, K. and Kakeda, K. 1990 Quantitative karyotyping of barley chromosomes by image analysis methods. Genome 33: 450–458.

Fukui, K. 1996. Plant Chromosomes: Laboratory Methods. CRC Press, Boca Raton, FL, p. 1.

Funabiki, H., Hagan, I., Uzawa, S. and Yanagida, M. 1993. Cell cycle-dependent specific positioning and clustering of centromeres and telomeres in fission yeast. J. Cell Biol. 121: 961–976.

Garcia-Ramirez, M., Rocchini, C. and Ausio, J. 1995. Modulation of chromatin folding by histone acetylation. J. Biol. Chem. 270: 17923–17928.

Hendzel, M.J., Sun, J., Chen, H.Y., Rattner, J.B. and Davie, J.R. 1994. Histone acetyltransferase is associated with the nuclear matrix. J. Biol. Chem. 269: 22894–22901.

Houben, A., Guttenbach, M. and Schubert, I. 1995. Nucleolus-like bodies in field bean cells. Biol. Zentralbl. 114: 339–345.

Houben, A., Belyaev, N.D., Leach, C.R. and Timmis, J.N. 1997. Differences of histone H4 acetylation and replication timing between A and B chromosomes *Brachycome dichromosomatica*. Chromosome Res. 5: 233–237.

Iwano, M., Fukui, K., Takaichi, S. and Isogai, A. 1997. Globular and fibrous structure in barley chromosomes revealed by high-resolution scanning microscopy. Chromosome Res. 5: 341–349.

Jasencakova, Z., Meister, A., Walter, J., Turner, B.M. and Schubert, I. 2000. Histone H4 acetylation of euchromatin and heterochromatin is cell cycle dependent and correlated with replication rather than with transcription. Plant Cell 12: 2087–2100.

Jasencakova, Z., Meister, A. and Schubert, I. 2001. Chromatin organization and its relation to replication and histone acetylation during the cell cycle in barley. Chromosoma 110: 83–92.

Jeppesen, P. and Turner, B.M. 1993. The inactive X chromosome in female mammals is distinguished by a lack of histone H4 acetylation, a cytogenetic marker for gene expression. Cell 74: 281–289.

Jiang, J., Nasuda, S., Dong, F., Scherrer, C.W., Woo, S., Wing, R.A., Gill, B.S. and Ward, D.C. 1996. A conserved repetitive DNA element located in the centromeres of cereal chromosomes. Proc. Natl. Acad. Sci. USA 93: 14210–14213.

Jones, P.L., Veenstra, G.J.C., Wade, P.A., Vermaak, D., Kass, S.U., Landsberger, N., Stroubouli, J. and Wolffe, A.P. 1998. Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription. Nature Genet. 19: 187–191.

Kumekawa, N., Ohmido, N., Fukui, K., Otsubo, E. and Otsubo, H. 2001. A new *gypsy*-type retrotransposon RIRE7: preferential insertion into the tandem repeat sequence TrsD in the pericentromeric heterochromatin regions of rice chromosomes. Mol. Gen. Genet. 265: 480–488.

Künzel, G., Korzun, L. and Meister, A. 2000. Cytologically integrated physical restriction fragment length polymorphism maps for the barley genome based on translocation breakpoints. Genetics 154: 397–412.

Kuo, M.H., Brownell, J.E., Sobel, R.E., Ranalli, T.A., Cook, R.G., Edmonson, D.G., Roth, S.Y. and Allis, C.D. 1996. Transcription-linked acetylation by Gcn5p of histone H3 and H4 at specific lysines. Nature 383: 269–272.

Luger, K., Mäder, A.W., Richmond, R.K., Sargent, D.F. and Richmond, T.J. 1997. Crystal structure of the nucleosome core particle at 2.8Å resolution. Nature 389: 251–260.

Moroi, Y., Hartman, A.L., Nakane, P.K., Tan, E.M. 1981. Distribution of kinetochore (centromere) antigen in mammalian cell nuclei. J. Cell Biol. 90: 245–259.

Nan, X., Ng, H., Johnson, C.A., Laherty, C.D., Turner, B.M., Eisenman, R.N. and Bird, A. 1998. Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. Nature 393: 386–388.

Ng, H.H. and Bird, A. 2000. Histone deacetylases: silencers for hire. Trends Biol. Sci. 25: 121–126.

Noguchi, J. and Fukui, K. 1995. Chromatin arrangements in intact interphase nuclei examined by laser confocal microscopy. J. Plant Res. 108: 209–216.

Ohmido, N. and Fukui, K. 1997. Visual verification of close disposition between a rice A genome-specific DNA sequence (TrsA) and the telomere sequence. Plant Mol. Biol. 35: 963–968.

Ohmido, N., Kijima, K., Ashikawa, I., de Jong, J.H. and Fukui, K. 2001 Visualization of the terminal structure of rice chromosomes

6 and 12 using multicolor FISH to chromosomes and extended DNA fibers. Plant Mol. Biol. 47: 413–421.

Presting, G., Malysheva, L., Fuchs, J. and Schubert, I. 1998. A *TY3/GYPSY* retrotransposon-like sequence localizes to the centromeric regions of cereal chromosomes. Plant J. 16: 721–728.

Richards, E.J. and Ausubel, F.M. 1988. Isolation of a higher eukaryotic telomere from *Arabidopsis thaliana*. Cell 53: 127–136.

Selig, S., Okumura, K., Ward, D.C. and Cedar, H. 1992. Delineation of DNA replication time zones by fluorescence *in situ* hybridization. EMBO J. 11: 1217–1225.

Sommerville, J., Baird, J. and Turner, B.M. 1993. Histone H4 acetylation and transcription in amphibian chromatin. J. Cell Biol. 120: 277–290.

Struhl, K. 1998. Histone acetylation and transcriptional regulatory mechanisms. Genes Dev. 12: 599–606.

Sugimoto, K., Fukuda, R. and Himeno, M. Centromere/kinetochore localization of human centromere protein A (CENP-A) exogenously expressed as a fusion to green fluorescent protein. Cell Struct. Funct. 25: 253–261.

Turner, B.M. and Fellows, G. 1989. Specific antibodies reveal ordered and cell-cycle-related use of histone-H4 acetylation sites in mammalian cells. Eur. J. Biochem. 179: 131–139.

Turner, B.M., Birley, A.J. and Lavender, J. 1992. Histone H4 isoforms acetylated at specific lysine residues define individual chromosomes and chromatin domains in *Drosophila* polytene nuclei. Cell 69: 375–384.

Vourc'h, C., Taruscio, D., Boyle, A.L. and Ward, D.C. 1993. Cell cycle-dependent distribution of telomeres, centromeres, and chromosome-specific subsatellite domains in the interphase nucleus of mouse lymphocytes. Exp. Cell Res. 205: 142–151.

Wako, T., Fukuda, M., Furushima-Shimogawara, R., Belyaev, N.D., Turner, B.M. and Fukui, K. 1998. Comparative analysis of topographic distribution of acetylated histone H4 by using confocal microscopy and a deconvolution system. Anal. Chim. Acta 365: 9–17.

Wako, T., Fukuda, M., Furushima-Shimogawara, R., Belyaev, N.D. and Fukui, K. Cell cycle dependent and lysine residue-specific dynamic changes of histone H4 acetylation in barley. Plant Mol. Biol. 46: 645–653.

White, D.A., Belyaev, N.D. and Turner, B.M. 1999. Preparation of site-specific antibodies to acetylated histones. Methods 19: 417–421.