Note

The Yeast Minichromosome System Consisting of Highly Positioned Nucleosomes *in Vivo*

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In eukaryotic genomes, the nucleosome is the structural and functional unit, and its position and dynamics are important for gene expression control and epigenetic regulation. Epigenetics is an important mechanism in development and homeostasis, and aberrant epigenetics is a common feature in cancer. Although understanding the mechanistic basis that determines nucleosome positioning *in vivo* is important for elucidating chromatin function and epigenetic regulation, a suitable experimental system to examine such mechanisms is still being developed. Herein, we examined nucleosome organization in yeast minichromosomes, using a parallel mapping method we previously developed that involve site-directed chemical cleavage and micrococcal nuclease digestion. This parallel mapping is capable of revealing the differences in the occupancy and the stability of individual nucleosomes in the minichromosome. Based on the previously characterized minichromosome, we engineered a set of new minichromosomes, aimed at strengthening the positioning of the nucleosomes. The site-directed chemical mapping method demonstrated that the nucleosome positioning in the newly designed yeast minichromosome system was significantly more stable. This system will be useful for elucidating the determinants of nucleosome organization, such as DNA sequences and/or nucleosome binding proteins, and for determining the relationships between nucleosome dynamics and epigenetic regulation, which are targets for therapeutic agents.

Key words chromatin; nucleosome positioning; yeast minichromosome; site-directed chemical mapping; enzymatic mapping

INTRODUCTION

The nucleosome is the structural and functional unit of eukaryotic chromatin. In the canonical nucleosome, 146 bp of DNA are wrapped around the histone octamer, which is composed of two molecules each of histones H2A, H2B, H3, and H4. Each nucleosome is separated by 10 to 60 bp of linker DNA, which varies among species.¹⁾ It is widely accepted that nucleosome positioning and its dynamics are involved in regulating not only gene expression, but also the epigenetic status of the cell.^{2–6)} Histone modifications, chromatin remodeling, DNA structures, and transcription influence the nucleosome positioning on genomic DNA.^{7–9)} In addition, recent studies have revealed the existence of non-canonical nucleosomes *in vivo* and *in vitro*.^{10–15)} Although the mechanisms of their formation and biological consequences remain to be elucidated *in vivo*, the presence of non-canonical nucleosomes highlights the importance of nucleosome organization in chromatin function and epigenetic regulation.

Epigenetics is an important mechanism in development and homeostasis, and its disorders are closely related to human diseases, including cancer.¹⁶⁾ Many agents targeting epigenetic regulation, such as DNA methylation, histone modifications, and chromatin remodelers, are currently being developed and studied in clinical trials for cancer treatment.¹⁷⁾ To examine the detailed actions of therapeutic agents on the nucleosome, an assay system, in which the nucleosomes are stably positioned on the DNA, would be useful. However, a universal experimental system has not been developed yet, although

a yeast minichromosome system may serve as a valuable tool. In fact, the yeast TRPIARS1 minichromosome¹⁸⁾ and its derivatives, including the TALS minichromosome,¹⁹⁾ have been utilized to examine the effects of DNA sequences on nucleosome formation and the binding of transcription factors to nucleosomal DNA.^{20–28)} Recently, we established a parallel mapping method that utilizes site-directed hydroxyl radical cleavage and micrococcal nuclease (MNase) digestion to characterize individual nucleosomes in more detail.²⁹⁾ We found that the occupancy and stability of each nucleosome in the minichromosome were different. In the present study, we improved the stability of the nucleosome positioning in the yeast minichromosome by manipulating its DNA sequence.

MATERIALS AND METHODS

Plasmids and Yeast Strains The TALS plasmid was constructed by Roth *et al.*¹⁹⁾ TALS was inserted into the *Hind*III site of a pBR322 derivative to manipulate plasmid construction in *Escherichia coli*, to form the TALS-pBR322ΔRI plasmid.²²⁾ To eliminate the pUC19 DNA sequence from TALS, the 358 bp region between the two *Eco*RI sites was replaced by the 378 bp fragment containing the upstream sequence of *STE6* (–457 to –83, the translation start site of the gene is defined as +1), forming spTALS1-pBR322ΔRI (spTALS is designated from superior-positioning-TALS). The spTALS2-pBR322ΔRI plasmid was constructed by inverse PCR, using spTALS1-pBR322ΔRI as the template and primers attached to the *STE6* upstream sequences at

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the 5'-end, to extend the *STE6* region from -83 to -66 . The spTALS3-pBR322 Δ RI plasmid was constructed by replacing the region of spTALS1 with the upstream region of TRP1ARS1, to extend the promoter region of *TRP1* to -170 . The spTALS4-pBR322 Δ RI plasmid was constructed by inverse PCR, using spTALS3 as the template and primers attached to the *STE6* upstream sequences at the 5'-end, to extend the *STE6* region from -83 to -66 . All constructs were verified by DNA sequencing. The sequences of spTALS1–4 were deposited in the DDBJ (The accession numbers for spTALS1, spTALS2, spTALS3 and spTALS4 are LC424430, LC424431, LC424432 and LC424433, respectively). The pBR322 Δ RI portion was eliminated by a digestion with *Hind*III, and each portion of TALS and spTALS1–4 was self-ligated and transformed into the *Saccharomyces cerevisiae* strain MHS3006 [*MATa ura3-52 trp1 Δ 63 leu2 Δ 1 hhf1::S47C hhf2::KanMX*], as described.²⁹⁾

Analyses of Nucleosome Positions by Chemical and Enzymatic Cleavages Yeast nuclei were isolated from the harvested cells harboring the TALS or spTALS1–4 minichromosomes, as described.³⁰⁾ The histone H4 S47C site-directed chemical cleavage and MNase digestion of the isolated nuclei, the analysis of their cleavage sites by indirect end-labeling, and the visualization of the indirect end-labeling results with a Typhoon FLA7000 biomolecular imager (GE Healthcare UK Ltd., England) were performed as described.²⁹⁾

RESULTS AND DISCUSSION

Parallel Mapping of Nucleosomes in TALS Minichromosome As shown in Fig. 1, the TALS plasmid, constructed by Roth *et al.*,¹⁹⁾ consists of TRP1ARS1 (1453 bp) and the 358 bp insert, which contains the *STE6* upstream sequences (-244 to -118) and an *E. coli*-derived DNA fragment that includes the *lac* promoter from the pUC19 plasmid. The backbone of the TALS plasmid is the TRP1ARS1 minicircular DNA (1453 bp) derived from *Saccharomyces cerevisiae* chromosome IV, which contains the *TRP1* gene (selectable marker, encoding the phosphoribosylanthranilate isomerase, which catalyzes the third step in tryptophan biosynthesis) with its 103 bp promoter region and the ARS1 (Autonomously Replicating Sequence 1) region.¹⁸⁾ The *STE6* is an *a*-cell-specific gene, which is expressed and repressed in *MATa* and *MAT α* cells, respectively.

Previous MNase mapping studies of the TALS minichromosome showed that nucleosomes are positioned adjacent to the $\alpha 2$ operator in *a*-cell-specific genes,^{19,30)} which is bound by the $\alpha 2$ /Mcm1 repressor, together with the Tup1-Ssn6 corepressors and the Isw2 chromatin remodeling complex.^{23,31–33)} We recently established a parallel mapping method,²⁹⁾ as described in Fig. 2A. In this method, MNase preferentially digests the linker DNA regions between nucleosomes, and prefers A + T-rich over G + C-rich sequences.⁸⁾ In the chemical mapping, hydroxyl radicals generated near the S47C residue of histone H4 attack the DNA backbone near the dyad axis of the nucleosome.^{34,35)} Thus, the combination of MNase and chemical mappings allows us to determine the nucleosome positions more precisely.²⁹⁾

We examined the nucleosome organization in the TALS minichromosome by the parallel mapping method, as shown in Fig. 2B. Since the MNase-cleaved sites are interpreted as linker DNA and nucleosome-free regions, whereas the re-

Fig. 1. Maps of the Plasmids TALS and spTALS1–4

The backbone of the plasmid is the TRP1ARS1 minicircular DNA (1453 bp) derived from *S. cerevisiae* chromosome IV (Black circle).¹⁸⁾ TALS contains a 358 bp insert between the two EcoRI sites in TRP1ARS1, which includes a 127 bp fragment from the *STE6* promoter region (-244 to -118 , the translation start site of the gene is defined as +1) as indicated by STE6pr (middle gray), as well as the pUC19 sequence (light gray) containing the *lac* operator and the CAP site.¹⁹⁾ Position numbers begin at the EcoRI site (1) and continue clockwise. spTALS1 contains the 378 bp insert between the EcoRI sites including the *STE6* upstream sequence (-457 to -83). spTALS2 contains the 383 bp insert including the *STE6* upstream sequence (-457 to -66). spTALS3 contains the 378 bp insert including the *STE6* upstream sequence (-390 to -83) and the *TRP1* upstream sequence (-170 to -104) connected to the *TRP1* promoter (-103 to -1) and the *TRP1* gene (curved arrow with bold line). spTALS4 contains the 383 bp insert including the *STE6* upstream sequence (-390 to -66) and the *TRP1* upstream sequence (-170 to -104) connected to the promoter (-103 to -1) of the *TRP1* gene. Locations of the α2 operator, the lac operator, the CAP site, and the A + T-rich sequence are indicated by boxes. ARS is an autonomously replicating sequence. Restriction endonuclease sites are also indicated, and have the following position numbers: EcoRI, 1 and 1453; EcoRV, 387; HindIII, 615; StuI, 829; NheI, 1037 and 1623.

gions protected from MNase digestion with approximately 140–150 bp are assumed to contain nucleosomes, the positions of the nucleosomes were assigned by comparing the MNase-cleaved sites between the chromatin and naked DNA samples (compare lanes 3 and 4 with lane 2 in Fig. 2B). In contrast, the hydroxyl radical-cleaved sites were observed in the middle of the regions that were protected from MNase digestion (lanes 5–9 in Fig. 2B). The positions of nucleosomes I to IV in the TALS minichromosome nucleosomes can be more precisely assigned between the -72 bp and $+72$ bp locations from the chemically cleaved sites, as shown in Fig. 2B, in good agreement with previous reports.^{19,20,22,23,28,30)}

It is noteworthy that the tentatively assigned nucleosome between nucleosomes V and VI in the previous MNase mapping experiments (white dotted ellipse between lanes 4 and 5 in Fig. 2B) was absent in the H4 S47C-site directed chemical mapping experiments. There were no chemically cleaved sites for the tentative nucleosome (lanes 6–9 in Fig. 2B), demonstrating that a nucleosome was not present in this region. The DNA region of the tentative nucleosome was not cleaved in

Fig. 2. A. Scheme of the Parallel Mapping Using MNase or Site-Directed Chemical Cleavages *in Vivo*

Chromatin in nuclei isolated from yeast cells were treated with MNase or H₄ S47C-mediated hydroxyl radicals, which cleaved the linker DNA or the nucleosome dyads, respectively. MNase has a preference for A + T-rich sequences, whereas hydroxyl radicals do not.

B. Indirect End-Labeling Mapping of the Sites Cleaved with MNase or Site-Directed Hydroxyl Radicals in the TALS and spTALS1 Minichromosomes

The samples were digested with *EcoRV*, resolved by electrophoresis on a 1.2% agarose gel, and transferred to a nylon membrane for Southern blotting. The cleavage sites were mapped by indirect end-labeling from the *EcoRV* site in the clockwise direction, using the *EcoRV*-*HindIII* probe.²⁹⁾ In principle, nucleosomes near the *EcoRV* cleavage site (nucleosomes VII and VIII) are not detectable in the indirect end-labeling method. MN, MNase-digested samples; CHM, site-directed chemically cleaved samples; D, naked DNA digested with MNase; C, chromatin in nuclei digested with MNase. The nucleosomes (gray ellipses) are shown between the MNase and chemical mapping gels in each mapping experiment, and their positions are assigned by the chemically cleaved sites. The designation of nucleosomes I to V is derived from the previous reports,¹⁹⁾ and nucleosomes IV to IX are designated as such for consistency. The tentative nucleosome in TALS reported by Roth *et al.*¹⁹⁾ is shown by the white dotted ellipse. M, DNA markers, with sizes indicated on the left side of the gel. Dots indicated chemically cleaved sites corresponding to nucleosome VI at two positions that were mutually exclusive in spTALS1. Asterisk between lanes 17 and 18 indicated the A + T-rich region in spTALS1.

both the naked DNA and chromatin samples (lanes 2–4 in Fig. 2B), thus leading to the ambiguous assignment of this nucleosome. In addition, the faint bands below the band derived from nucleosome I (lanes 6–9 in Fig. 2B) were assigned as minor fractions of a redundant nucleosome near nucleosome I. These results indicate that the site-directed chemical mapping is a more sensitive method than the MNase mapping.

More importantly, we found that the occupancy of nucleosome IV was relatively lower than those of nucleosomes I to III in the TALS minichromosome, as judged from the band intensities of the chemical cleavages (lanes 5–9 in Fig. 2B). This finding was unexpected, since previous MNase and DNase I mappings showed that nucleosome IV is precisely positioned adjacent to the $\alpha 2$ operator.³⁰⁾ It should be noted that enzymatic mappings provide information about the positions of nucleosomes, but not about their occupancy. Thus, an advantage of the H₄ S47C-directed chemical method is that the occupancy of the nucleosome can be directly judged from the intensity of the cleaved DNA band. The dyad positions of nucleosomes III and IV in TALS were determined as position numbers 1301 and 1458, respectively, from the chemically cleaved sites, based on a standard curve constructed by the migrations of the DNA size markers (lane 10 in Fig. 2B). Assuming that the nucleosomal DNA is 146 bp, this result indicated that the linker DNA length between the nucleosomes was 10 bp (subtracted 146 from 156), which is shorter than the average linker DNA length of 18 bp observed in *S. cerevisiae* chromatin.⁹⁾ This may imply that the formation of nucleosome

III interferes with that of nucleosome IV. In other words, the space between the edge of nucleosome III and the $\alpha 2$ operator may not be sufficient for the stable formation of nucleosome IV.

Parallel Mapping of Nucleosomes in spTALS1 Minichromosome To improve the stability of nucleosome IV, we constructed spTALS1 by engineering the sequences and lengths of the initial 358 bp insert in TALS as follows. First, we eliminated the portion of the pUC19-derived DNA, which was present in TALS, by substituting it with the yeast *STE6* upstream sequences (–457 to –83). The rationale behind this substitution is that nucleosomes strongly prefer yeast DNA over *E. coli* DNA, in general.⁷⁾ Second, we extended the length of the insert from 358 to 378 bp; *i.e.*, the region between the *EcoRI* site (1453) and the $\alpha 2$ operator in spTALS1 was extended by 20 bp over that of TALS (hatched boxes in Fig. 1). The rationale for this design is the fact that the linker DNA between nucleosomes III and IV in the original TALS minichromosome was shorter than the average linker DNA length of *S. cerevisiae* (*ca.* 18 bp).⁹⁾ In spTALS1, the site-directed chemically cleaved bands became stronger and more discrete for not only nucleosome IV, but also nucleosomes I and II (compare lanes 11–15 with lanes 5–10 in Fig. 2B). The regions corresponding to nucleosomes I to V were protected from MNase digestion (lanes 16–18 in Fig. 2B). However, it should be noted that the chemically cleaved sites derived from nucleosome III were fainter, as compared to those from nucleosomes I, II, and IV in spTALS1. Thus, the spTALS1 minichromosome was not

Fig. 3. Indirect End-Labeling Mapping of the Site-Directed Chemically Cleaved Sites in the TALS and spTALS1–4 Minichromosomes

The samples were digested with *EcoRV* (A) or *NheI* (B), resolved by electrophoresis on a 1.2% agarose gel, and transferred to a nylon membrane for Southern blotting. The cleavage sites were detected by indirect end-labeling. The *EcoRV*-*HindIII* probe was used for mapping from the *EcoRV* site in the clockwise direction (A), and the *NheI*-*Stul* probe was used for mapping from the *NheI* site in the counter-clockwise direction (B).²⁹⁾ The nucleosomes (gray ellipses) are shown on the right side of the gel. In principle, nucleosomes near the *EcoRV* cleavage site (nucleosomes VII and VIII), shown in Fig. 3A, are not detectable in the indirect end-labeling method. Similarly, nucleosomes near the *NheI* cleavage site (nucleosomes I and II), shown in Fig. 3B, are also not detectable. M, DNA markers, with sizes indicated on the left side of the gel, along with their position numbers (pos. #) in spTALS4. Dots indicated chemically cleaved sites corresponding to nucleosome VI at two positions that were mutually exclusive in spTALS1 and -2.

satisfactory for our purpose to stabilize the positioning in the array of nucleosomes I to IV.

Chemical Mapping of Nucleosomes in spTALS2–4 Minichromosomes To improve the nucleosome positioning in spTALS1, we extended the length of the insert from 378 to 393 bp for spTALS2; *i.e.*, the region between the *EcoRI* site (1453) and the $\alpha 2$ operator was extended, by 15 bp over that of spTALS1 and 35 bp over that of TALS (hatched boxes in Fig. 1). We then examined the nucleosome organization in spTALS2, together with TALS and spTALS1 (Fig. 3), using the H4 S47C site-directed chemical mapping method. The band derived from nucleosome III in spTALS2 became more discrete, as compared to that in spTALS1 (lanes 10, 12, 16, and 18 in Fig. 3A). The formation of nucleosomes I, II, and IV was similar among spTALS1 and -2. This result indicated that nucleosome III was stably formed at a non-redundant position in spTALS2, whereas it was formed at redundant positions in spTALS1.

We noticed that nucleosome VI was formed at two positions that were mutually exclusive in spTALS1 and -2 (indicated by dots between lanes 9 and 10, between 15 and 16 in Fig. 3A and between lanes 27 and 28, between 33 and 34 in Fig. 3B, also see lane 11 in Fig. 2B), although nucleosome VI was formed primarily at one position in the TALS minichromosomes. It should be noted that 45 bp of the A + T-rich region in *STE6* (position numbers 1764 to 1808 and 1779 to 1823 for spTALS1 and -2, respectively, also see Fig. 1) were cleaved between nucleosomes V and VI by MNase in both the chromatin and naked DNA (asterisk between lanes 17 and 18 in Fig. 2B), indicating that this region tended to be on the outside of the nucleosomes. Thus, to stabilize the positioning of nucleosome VI in spTALS1 and -2, the A + T-rich region in the *STE6* pro-

moter was eliminated by the substitution with the upstream sequence of the *TRP1* promoter region (*TRP1*pr-up, -170 to -104), forming spTALS3 and -4, respectively. As a result, the position of nucleosome VI in spTALS3 and -4 was restored to the one major position, as in the TALS minichromosome (lanes 11, 12, 17, and 18 in Fig. 3A, lanes 29, 30, 35, and 36 in Fig. 3B). Thus, regarding the nucleosome organization in the *TRP1* region, spTALS3 and -4 appeared to be similar to TALS. The site-directed chemical mapping showed that the organization of the array of nucleosomes I to IV in spTALS3 and -4 was quite similar to those in spTALS1 and -2, respectively (lanes 11–12, 15–18 in Fig. 3A). Thus, the elimination of the *E. coli* DNA fragment and the increase in the length of the insert between the *EcoRI* sites successfully improved the positioning of nucleosomes I to IV.

We also examined the organization of nucleosomes V to IX around the *TRP1* region by indirect end-labeling of the chemical cleavages from the *NheI* site in the counter-clockwise direction, as shown in Fig. 3B. As shown in Fig. 1, since there were two *NheI* sites (position numbers 1037 and 1623) in TALS, the parental band appeared about 1200 bp in length and nucleosomes III to V in TALS were not detectable by indirect end-labeling from the *NheI* site in the counter-clockwise direction (lanes 20, 26, and 32 in Fig. 3B). As expected from our previous results,²⁹⁾ the chemical cleavage signals of nucleosomes V to IX appeared to be relatively low, as compared to that of nucleosome IV within the same lanes (Fig. 3B). This result supports the idea that nucleosomes V to IX in the promoter and the coding region of the *TRP1* gene are not stably formed, and instead are dynamically altered, possibly due to the transcription.²⁹⁾

Analyses of the Dyad Positions of Nucleosomes and the

Table 1. The Dyad Positions of Nucleosomes I to IX Determined by Chemical Cleavage Sites through the H4 S47C Site

	The dyad position								
	I	II	III	IV	V	VI	VII	VIII	IX
TALS	946	1131	1301	1458	1685	126	286 / 344	469	623
spTALS1	939	1127	1322	1481	1690	52 / 170	348	465 / 506	624
spTALS2	948	1130	1328	1497	1712	43 / 159	343	462 / 497	617
spTALS3	947	1134	1329	1481	1741	99	283 / 342	459 / 509	620
spTALS4	943	1129	1325	1492	1748	102	289 / 344	465 / 503	618

Fig. 4. Summary of Nucleosome Mapping in TALS and spTALS1-4 Minichromosomes

Gray ellipses indicate the locations of nucleosomes. The dyad positions of the nucleosomes were determined by indirect end-labeling of chemically cleaved sites (Fig. 3). The designations are the same as those in Fig. 1.

Linker DNA Lengths Based on the results shown in Fig. 3, the chemically cleaved sites (the center of each band) corresponding to the dyad positions of the nucleosomes in the minichromosomes were determined (Table 1 and Fig. 4), using the standard curve constructed by the migrations of the DNA size markers, which were prepared by PCR using the naked TALS DNA as the template. Within the resolution of agarose gel electrophoresis, the dyad positions of nucleosomes I (943 ± 4 –5) and II (1130 ± 3 –4) were nearly identical between the original TALS and spTALS1-4 minichromosomes. These results support the idea that the ARS region acts as a boundary that is a critical determinant of nucleosome positioning. The dyad position of nucleosome III was similar among spTALS1-4 (1325 ± 3 –4), and differed from that in TALS (1301). Although the position of nucleosome IV seemed to differ among the TALS and spTALS1-4 minichromosomes (position numbers 1458–1497), the distances between the dyad of nucleosome IV and the $\alpha 2$ operator were quite similar in all of the constructs (74 ± 2 –3), consistent with the idea that $\alpha 2$ /Mcm1,^{19,30} together with the Ssn6-Tup1 co-repressors and the Isw2 chromatin remodeling complex, positions nucleosomes in an active manner.^{23,31–33}

We also examined the lengths of the linker DNA, which can be determined by subtracting 146 bp (the length of nucleosomal DNA) from the distance between two adjacent nucleosomes. As seen in Fig. 4, the length of the linker DNA between nucleosomes I and II was nearly the same, 38 ± 3 bp, in TALS and spTALS1-4. The linker DNA length between nucleosomes II and III was increased in spTALS1-4 (49 ± 1 –2 bp), as compared with that in TALS (23 bp). The linkers between nucleosomes III and IV were increased to 22 and 20 bps in spTALS2 and -4, respectively, but not in TALS

and spTALS1 and -4 (10, 12, and 5 bps, respectively). Thus, the increase in the linker DNA length between nucleosomes II and III was most likely caused by the extension of 20 bp between the *EcoRI* site (1453) and the $\alpha 2$ operator in the insert (hatched boxes in Fig. 1), and the increase in the linker between nucleosomes III and IV was due to the additional 15 bp extension (total 35 bp extension). Based on these results, we interpreted the stable formation of nucleosome IV in spTALS1-4 (lanes 9–12 and 15–18 in Fig. 3A) to be attributable to the increase in the linker DNA between nucleosomes II and III. While nucleosome III was formed at several redundant positions in spTALS1 and -3 (lanes 9, 11, 15, and 17 in Fig. 3A), it formed at a non-redundant position in spTALS2 and -4 (lanes 10, 12, 16, and 18 in Fig. 3A). This implied that the increase in the linker DNA length between nucleosomes III and IV in spTALS2 and -4 causes the positioning of the nucleosome III. Thus, the appropriate linker DNA length is important for nucleosome positioning.

Conclusions and Perspectives In summary, we improved the yeast minichromosome system by manipulating the DNA sequences and the lengths of the linker DNA, to form highly positioned nucleosomes *in vivo*. We conclude that spTALS2 and -4 are superior to TALS, and spTALS1 and -3, in terms of the stability of nucleosome positioning. Nucleosomes I to IV were highly positioned, whereas nucleosomes V to IX were relatively labile due to the transcription of the *TRP1* gene. This *in vivo* system will be useful for not only analyses of the structural features and dynamics of nucleosomes, but also examinations of factors that affect epigenetic regulators, such as histone acetyltransferases or deacetylases, lysine methyltransferases or histone demethylases, and bromo-domain ligands.

Aberrant epigenetics are commonly observed in cancer, and chemopreventive approaches for targeting histone modification enzymes, chromatin remodelers, and transcription factors are currently underway in the field.^{16,17} The presently developed yeast minichromosomes can be utilized to examine the effects of these agents on nucleosome alterations, which are likely to be associated with changes in the activities of histone modification enzymes and chromatin remodelers. For example, an inhibitor of a certain epigenetic regulator can be added to yeast cultures, and its effect on nucleosome alterations in the minichromosome can be detected using our system. In addition, the effects of drug actions can be examined using strains with a mutation in an epigenetic regulator gene, together with the wild-type strain. Thus, the minichromosome system consisting of highly positioned nucleosomes will be a useful tool for assessing the detailed actions of epigenetic therapeutic agents, and for unraveling the relationships between aberrant epigenetics and nucleosome dynamics *in vivo*.

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Conflict of Interest The authors declare no conflict of interest.

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