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. 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. Histone modifications, chromatin remodeling, DNA structures, and transcription influence the nucleosome positioning on genomic DNA. In addition, recent studies have revealed the existence of non-canonical nucleosomes in vivo and in vitro. 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. 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. 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 minichromosome, and its derivatives, including the TALS minichromosome, have been utilized to examine the effects of DNA sequences on nucleosome formation and the binding of transcription factors to nucleosomal DNA. 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. 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. TALS was inserted into the HindIII site of a pBR322 derivative to manipulate plasmid construction in Escherichia coli, to form the TALS-pBR322ARI plasmid. To eliminate the pUC19 DNA sequence from TALS, the 358 bp region between the two EcoRI 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-pBR322ARI (spTALS is designated from superior-positioning-TALS). The spTALS2-pBR322ARI plasmid was constructed by inverse PCR, using spTALS1-pBR322ARI 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-pBR322ΔAR1 plasmid was constructed by replacing the region of spTALS1 with the upstream region of TRPIARS1, to extend the promoter region of TRPI to −170. The spTALS4-pBR322ΔAR1 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 pBR322ΔAR1 portion was eliminated by a digestion with HindIII, and each portion of TALS and spTALS1–4 was self-ligated and transformed into the Saccharomyces cerevisiae strain MHS3006 [MATα ura3-52 trp1Δ63 leu2Δ1 hhf1::S47C hhf2::KanMX], as described.

**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.

**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 TRPIARS1 (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 TRPIARS1 minicircular DNA (1453 bp) derived from Saccharomyces cerevisiae chromosome IV, which contains the TRPI 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 α-cell-specific gene, which is expressed and repressed in MATα and MATα cells, respectively.

Previous MNase mapping studies of the TALS minichromosome showed that nucleosomes are positioned adjacent to the α2 operator in an α-cell-specific genes,19,30) which is bound by the α2/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.3) 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 regions 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
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 α2 operator. 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 146bp, this result indicated that the linker DNA length between the nucleosomes was 10bp (subtracted 146 from 156), which is shorter than the average linker DNA length of 18bp observed in S. cerevisiae chromatin. 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 α2 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 358bp 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 378bp; i.e., the region between the EcoRI site (1453) and the α2 operator in spTALS1 was extended by 20bp 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. 18bp).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...
In principle, nucleosomes near the RV cleavage site (nucleosomes VII and VIII), shown in Fig. 3A, are not detectable in the indirect end-labeling method. Similarly, EcoI exclusive in spTALS1 and -2.

The samples were digested with EcoRV (A) or Nhel (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 Nhel-Stul probe was used for mapping from the Nhel site in the counter-clockwise direction (B). 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 Nhel cleavage site (nucleosomes I and II), shown in Fig. 3B, are also not detectable.

The 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.

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 the STE6 (position numbers 1764 to 1808 and 1779 to 1823 for spTALS1, 1200 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 also examined the organization of nucleosomes V to IX in spTALS2, which is the parental band appeared about 1200 bp in length and nucleosomes III to V in spTALS2 were not detectable by indirect end-labeling from the EcoRI sites successfully improved the positioning of nucleosome 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 α2 operator was extended, by 15 bp over that of spTALS1 and 35 bp over that of TALS (lanes 11, 12, 17, and 18 in Fig. 3A). Thus, the elimination of the EcoRI site successfully eliminated the upstream sequence of the TRPI promoter region (TRPIpr-up, −170 to −104), forming spTALS3 and -4, respectively. As a result, the position of nucleosome V 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 TRPI region, spTALS3 and -4 appeared to be similar to TALS.

We also examined the organization of nucleosomes V to IX around the TRPI region by indirect end-labeling of the chemical cleavages from the Nhel site in the counter-clockwise direction, as shown in Fig. 3B. As shown in Fig. 1, since there were two Nhel 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 Nhel site in the counter-clockwise direction (lanes 20, 26, and 32 in Fig. 3B). As expected from our previous results, 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 TRPI gene are not stably formed, and instead are dynamically altered, possibly due to the transcription.

Analyses of the Dyad Positions of Nucleosomes and the
The dyad positions of 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, thechemically 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 α2 operator were quite similar in all of the constructs (74 ± 2–3), consistent with the idea that α2/Mcm1 together with the Ssn6-Tup1 co-repressors and the Isw2 chromatin remodeling complex, positions nucleosomes in an active manner.  

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 II and IV were increased to 22 and 20 bp 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 α2 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. 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.

REFERENCES

1) van Holde KE. Chromatin. Springer New York, New York, NY (1989).
2) Almouzni G, Cedar H. Maintenance of epigenetic information. Cold Spring Harb. Perspect. Biol., 8, a019372 (2016).
3) Becker PB, Workman JL. Nucleosome remodeling and epigenetics. Cold Spring Harb. Perspect. Biol., 5, a017905 (2013).
4) Lai WKM, Pugh BF. Understanding nucleosome dynamics and their links to gene expression and DNA replication. Nat. Rev. Mol. Cell Biol., 18, 548–562 (2017).
5) Rando OJ, Winston F. Chromatin and transcription in yeast. Genet., 190, 351–387 (2012).
6) Turner BM. The adjustable nucleosome: an epigenetic signaling module. Trends Genet., 28, 436–444 (2012).
7) Stryh K, Segal E. Determinants of nucleosome positioning. Nat. Struct. Mol. Biol., 20, 267–273 (2013).
8) Chereji RV, Clark DJ. Major determinants of nucleosome positioning. Biophys. J., 114, 2279–2289 (2018).
9) Jansen A, Verstrepen KJ. Nucleosome positioning in Saccharomyces cerevisiae. Microbiol. Mol. Biol. Rev., 75, 301–320 (2011).
10) Koyama M, Kurumizaka H. Structural diversity of the nucleosome. J. Biochem., 163, 85–95 (2018).
11) Kato D, Osakabe A, Arimura Y, Mizukami Y, Horikoshi N, Saikusa K, Akashi S, Nishimura Y, Park SY, Szymkowiak L, Ohkawa Y, Matsumoto A, Kono H, Inoue R, Sugiyama M, Kurihara H, Kurumizaka H, Akashi S, Nishimura Y, Park SY, Nogami J, Maehara K, Ohkawa Y, Matsumoto A, Kono H, Inoue R, Sugiyama M, Kurumizaka H, Crystal structure of the overlapping dinucleosome composed of hexasome and octasome. EMBO J., 2160 (1997).
12) Ocampo J, Cui F, Zhuhrin VB, Clark DJ. The proto-chromatosome: a fundamental subunit of chromatin? Nucleus, 7, 382–387 (2016).
13) Kubik S, Bruzzone MJ, Jacquet P, Falcone J-L, Rougemont J, Shore D. Nucleosome stability distinguishes two different promoter types at all protein-coding genes in yeast. Mol. Cell, 60, 422–434 (2015).
14) Ramachandran S, Zentenni GE, Henikoff S. Asymmetric nucleosomes flank promoters in the budding yeast genome. Genome Res., 25, 381–390 (2015).
15) Rhee HS, Bataille AR, Zhang L, Pugh BF. Subnucleosomal structures and nucleosome asymmetry across a genome. Cell, 159, 1377–1388 (2014).
16) Zoghbi HY, Beaudet AL. Epigenetics and human disease. Cold Spring Harb. Perspect. Biol., 8, a019497 (2016).
17) Ahuja N, Sharma AR, Baylin SB. Epigenetic therapeutics: a new weapon in the war against cancer. Annu. Rev. Med., 67, 73–89 (2016).
18) Thomas F, Bergman LW, Simpson RT. Nuclease digestion of circular TRPLARS1 chromatin reveals positioned nucleosomes separated by nuclease-sensitive regions. J. Mol. Biol., 177, 715–733 (1984).
19) Roth SY, Dean A, Simpson RT. Yeast a2 repressor positions nucleosomes in TRPLARS1 chromatin. Mol. Cell. Biol., 10, 2247–2260 (1990).
20) Roth SY, Shimizu M, Johnson L, Grunstein M, Simpson RT. Stable nucleosome positioning and complete repression by the yeast a2 repressor are disrupted by amino-terminal mutations in histone H4. Genes Dev., 6, 415–425 (1992).
21) Balasubramanian B, Morse RH. Binding of Gal4p and bicoid to nucleosomal sites in yeast in the absence of replication. Mol. Cell. Biol., 19, 2977–2985 (1999).
22) Shimizu M, Mori T, Sakurai T, Shindo H. Destabilization of nucleosomes by an unusual DNA conformation adopted by poly(dA)-poly(dT) tracts in vivo. EMBO J., 19, 3358–3365 (2000).
23) Morohashi N, Nakajima K, Kurihara D, Mukai Y, Mitchell AP, Shimizu M. A nucleosome positioned by a2/Mcm1 prevents Hap1 activator binding in vivo. Biochem. Biophys. Res. Commun., 364, 583–588 (2007).
24) Suter B, Livingstone-Zatchej M, Thoma F. Chromatin structure modulates DNA repair by photolyase in vivo. EMBO J., 16, 2150–2160 (1997).
25) Slon CE, Leblanc BP, Alfieri JA, Clark DJ. Remodeling of yeast CUP1 chromatin involves activator-dependent repositioning of nucleosomes over the entire gene and flanking sequences. Mol. Cell. Biol., 21, 534–547 (2001).
26) Fink M, Imholz D, Thoma F. Contribution of the siren 129 of histone H2A to chromatin structure. Mol. Cell. Biol., 27, 3589–3600 (2007).
27) Fink M, Thompson JS, Thoma F. Contributions of histone H3 nucleosome core surface mutations to chromatin structures, silencing and DNA repair. PloS ONE, 6, e26210 (2011).
28) Ichikawa Y, Morohashi N, Nishimura Y, Kurumizaka H, Shimizu M. Telomeric repeats act as nucleosome-disfavoring sequences in vivo. Nucleic Acids Res., 42, 1541–1552 (2014).
29) Furse T, Katsunuma K, Morohoshi K, Mukai Y, Ichikawa Y, Kurumizaka H, Yanagida A, Ubara T, Kato H, Shimizu M. Parallel mapping reveals with site-directed hydroxyl radicals and micrococcal nuclease mapping reveals structural features of positioned nucleosomes in vivo. PLOS ONE, 12, e0186974 (2017).
30) Shimizu M, Roth SY, Szent-Gyorgyi C, Simpson RT. Nucleosomes are positioned with base pair precision adjacent to the a2 operator in Saccharomyces cerevisiae. EMBO J., 10, 3033–3041 (1991).
31) Watson AD, Edmondson DG, Bone JR, Mukai Y, Yu Y, Du W, Stillman DJ, Roth SY. Ssn6-Tup1 interacts with class I histone deacetylases required for repression. Genes Dev., 14, 2737–2744 (2000).
32) Zhang Z, Reese JC. Ssn6-Tup1 requires the ISW2 complex to position nucleosomes in Saccharomyces cerevisiae. EMBO J., 18, 2246–2257 (2004).
33) Fazzio TG, Geiβl ME, Tsukiyama T. Two distinct mechanisms of chromatin interaction by the Isw2 chromatin remodeling complex in vivo. Mol. Cell. Biol., 25, 9165–9174 (2005).
34) Henikoff S, Ramachandran S, Krassovsky K, Bryson TD, Codomo CA, Brogaard K, Widom J, Wang J-P, Henikoff SG. The budding yeast Centromere DNA Element II wraps a stable Cse4 hemisome in either orientation in vivo. eLife, 3, e01861 (2014).
35) Brogaard K, Xi L, Wang J-P, Widom J. A map of nucleosome positions in yeast at base-pair resolution. Nature, 486, 496–501 (2012).