Published in final edited form as:

Biochem Biophys Res Commun. 2016 July 22; 476(2): 57–62. doi:10.1016/j.bbrc.2016.05.063.

Sequence-directed nucleosome-depletion is sufficient to activate transcription from a yeast core promoter *in vivo*

Yuichi Ichikawa^{a,b,1}, Nobuyuki Morohashi^{c,1}, Nobuyuki Tomita^c, Aaron P. Mitchell^b, Hitoshi Kurumizaka^a, and Mitsuhiro Shimizu^{c,*}

^aGraduate School of Advanced Science and Engineering/RISE, Waseda University, 2-2 Wakamatsu-cho, Shinjuku-ku, Tokyo 162-8640, Japan

^bDepartment of Biological Sciences, Carnegie Mellon University, 4400 Fifth Avenue, Pittsburgh, PA 15213, U.S.A

^cProgram in Chemistry and Life Science, School of Science and Engineering, Department of Chemistry, Graduate School of Science and Engineering, Meisei University, 2-1-1 Hodokubo, Hino, Tokyo 191-8506, Japan

Abstract

Nucleosome-depleted regions (NDRs) (also called nucleosome-free regions or NFRs) are often found in the promoter regions of many yeast genes, and are formed by multiple mechanisms, including the binding of activators and enhancers, the actions of chromatin remodeling complexes, and the specific DNA sequences themselves. However, it remains unclear whether NDR formation *per se* is essential for transcriptional activation. Here, we examined the relationship between nucleosome organization and gene expression using a defined yeast reporter system, consisting of the *CYCI* minimal core promoter and the *lacZ* gene. We introduced simple repeated sequences that should be either incorporated in nucleosomes or excluded from nucleosomes in the site upstream of the TATA boxes. The (CTG)₁₂, (GAA)₁₂ and (TGTAGG)₆ inserts were incorporated into a positioned nucleosome in the core promoter region, and did not affect the reporter gene expression. In contrast, the insertion of (CGG)₁₂, (TTAGGG)₆, (A)₃₄ or (CG)₈ induced *lacZ* expression by 10–20 fold. Nucleosome mapping analyses revealed that the inserts that induced the reporter gene expression prevented nucleosome formation, and created an NDR upstream of the TATA boxes. Thus, our results demonstrated that NDR formation dictated by DNA sequences is sufficient for transcriptional activation from the core promoter *in vivo*.

*Corresponding author: Mitsuhiro Shimizu, shimizum@chem.meisei-u.ac.jp, Phone: +81-42-591-7483 Fax: +81-42-591-7419.

¹Equal contribution.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflict of interest

None declared.

Keywords

DNA structure; nucleosome positioning; nucleosome depleted-region; transcription; chromatin; yeast

1. Introduction

In eukaryotic genomes, DNA is packaged into arrays of nucleosomes, where protein binding to DNA is sterically occluded. Thus, the nucleosome is considered to act as a barrier to transcription, and the positioning of nucleosomes is critical for regulating DNA activities [1–3]. In general, positioned nucleosomes flank the promoter, and the degree of nucleosome positioning gradually decreases toward the end of the coding region in eukaryotic genomes. Another common feature of the chromatin architecture is that the nucleosome density is especially low in the promoter and terminator regions, where nucleosome-depleted regions (NDRs, also called nucleosome-free regions, NFRs) are thus formed [1–3]. In fact, NDRs are found in most promoters in yeast as well as other eukaryotes [4–9].

Activator or enhancer binding competes with nucleosome formation and creates NDRs in the regions upstream of promoters [10–14]. Chromatin remodeling factors are also known to create NDRs after nucleosome deposition [14–20]. In addition to these protein factors, the properties of the DNA sequence are recognized as being important for forming NDRs [4,9,21–24]. These multiple factors are mutually involved in NDR formation.

DNA sequences have a preferred rotational setting on the histone octamers, which suggested the idea of a genomic code for nucleosome positioning [25]. Although other factors besides the sequence preference contribute to the nucleosome organization in cells, the intrinsic DNA sequence plays a central role in determining the organization of nucleosomes *in vivo*, because similar nucleosome organization has been detected in chromatin reconstituted *in vitro* without other protein factors [4]. However, some studies have argued against this model, and concluded that the intrinsic histone-DNA interactions are not the major determinant of the *in vivo* nucleosome positions [26,27]. Even though DNA sequences have minor effects on the preferential formation of positioned nucleosomes, it is critically important to consider the chromatin architectures of certain DNA sequences that intrinsically disfavor or block nucleosome formation.

(A)_n•(T)_n tracts (also called poly dA•poly dT) reportedly form an unusual conformation that disrupts nucleosome positioning in yeast cells [22,28,29]. Similarly, (A)_n tracts and their flanking DNA regions are relatively depleted of nucleosomes in the yeast genome [9]. Actually, promoters containing (A)_n tracts, such as the yeast *HIS3* promoter and the *Candida glabrata* *AMT1* promoter, reportedly stimulate transcription [30,31]. Consistently, nucleosome positioning by the yeast $\alpha 2$ repressor in the promoter region is disrupted by the insertion of (A)_n, causing derepression of the α -cell-specific gene *BARI* [32]. (CG)_n also disfavors nucleosome formation [32], and (CG)₉ stimulates gene activity by forming Z-DNA in yeast cells [33]. Recently, we showed that the human telomeric repeated sequence (TTAGGG)_n acts as a nucleosome-disfavoring sequence, whereas its sequence-isomer (TGTAGG)_n is incorporated into nucleosomes *in vivo* [34]. Thus, the contribution of the

intrinsic physical properties of DNA to nucleosome organization, including NDR formation, is an important issue to understand chromatin structure and dynamics in the genome.

Herein, we examined the relationship between NDR formation and transcriptional activity using a defined yeast reporter system, consisting of the minimal core promoter of *CYCI* fused to the *lacZ* gene. To this end, we introduced simple repeated sequences that are either incorporated into the nucleosome or excluded from the nucleosome, in the site upstream of the TATA boxes in the *CYCI* core promoter. Our results clearly showed that the sequence-directed NDR formation remarkably induced the activation of basal transcription from the core promoter.

2. Materials and methods

The pLG 312S SS plasmid is a derivative of the μ m plasmid, and harbors a *CYCI-lacZ* reporter gene that lacks the *CYCI* UASs (Upstream Activating Sequences) [35–38]. The inserted DNA fragments were prepared from pairs of chemically synthesized oligonucleotides, and were ligated into the *XhoI* site of the plasmid pLG 312S SS. The (CTG)₁₂, (GAA)₁₂ and (CGG)₁₂ inserts have *KpnI* sites at both ends. All of the constructed plasmids were verified by DNA sequencing. The pLG 12S SS plasmid and its derivatives were transformed into the *S. cerevisiae* strain AMP105 [*MATa ura3 leu2 lys2 ho::LYS2*], which is a derivative of SK-1 [35,36]. Standard culture conditions and media were used, and the yeast transformation and the β -galactosidase assay were performed according to the previously reported procedures [35]. Preparation of yeast nuclei, digestion with micrococcal nuclease (MNase) and subsequent indirect end-labeling mapping of the MNase cleavage sites were described previously [29,34].

3. Results

3.1. Experimental design

The pLG 312S SS plasmid contains the downstream core promoter region of the *CYCI* gene (–274 to +3), from which UAS1 and UAS2 were deleted, fused to the *E. coli lacZ* gene [35–38]. This core promoter contains five TATA or TATA-like boxes at –178 to –170 (TATA1), –123 to –115 (TATA2), –93 to –86 (TATA3), –79 to –71 (TATA4), and –56 to –49 (TATA5) (see Fig. 1A) [37,39–41]. When this UAS-deleted *CYCI-lacZ* plasmid is introduced in the yeast cells, it assembles an array of nucleosomes and forms a yeast minichromosome. We previously showed that nucleosomes are positioned in the UAS-deleted *CYCI* core promoter region in the pLG 312S SS minichromosome *in vivo* [35]. Since NDRs are formed by the binding of activators or enhancers [10–14,15,20], it is difficult to assess the NDR function for transcription activation without the effects of activators. Thus, this system is likely to be appropriate to examine the relationship between the nucleosome organization and the transcriptional activation from the core promoter *in vivo*. Based on our previous results, as well as *in vitro* studies by others [22,28,29,30–34, 42, 43], we designed (CGG)₁₂, (TTAGGG)₆, (A)₃₄ and (CG)₈ as nucleosome-excluding sequences, and (CTG)₁₂ and (TGTAGG)₆ as nucleosome-incorporating sequences. We were unsure about the character of (GAA)₁₂, and thus tested it together with the other sequences. We introduced these simple repeat sequences into the *XhoI* site, which is located just

upstream of the TATA boxes (Fig. 1A). Then, we examined their effects on transcriptional activities and nucleosome organization *in vivo*.

3.2. Effects of inserted sequences on reporter gene expression

We monitored the *CYCI-lacZ* expression as the β -galactosidase activity in Miller units, in the yeast cells harboring pLG 312S SS and its derivatives with inserts (Fig. 1B). The *lacZ* expression was very low (0.8 Miller units) from the UAS-deleted *CYCI* promoter in pLG 312S SS (no insert). The insertion of (CTG)₁₂, (GAA)₁₂ or (TGTAGG)₆ (sequence isomer of human telomeric repeat) did not affect the *lacZ* expression (1.0 Miller units). In contrast, the insertion of (CGG)₁₂, (TTAGGG)₆, (A)₃₄, or (CG)₈ caused the induction of *lacZ* expression (11.5, 18.8, 19.4 and 9.5 Miller units for (CGG)₁₂, (TTAGGG)₆, (A)₃₄, or (CG)₈, respectively, Fig.1B) by 10–20 fold, as compared to pLG 312S SS (no insert). Previously, we showed that the insertion of (A)_n or (CG)_n into the *BAR1* promoter disrupted the positioned nucleosomes, leading to the partial activation of *BAR1* in the repressed *MAT α* cells [32]. In other studies, human telomeric repeat (TTAGGG)₆ disfavored nucleosome assembly and altered nucleosome positioning in the yeast minichromosome, in contrast to its sequence isomer (TGTAGG)₆ [34]. Thus, the results shown in Fig. 1 suggest that the (CGG)₁₂, (TTAGGG)₆, (A)₃₄, and (CG)₈ sequences altered the nucleosome organization in the *CYCI* core promoter to activate transcription.

3.3. Effect of the insertion on the chromatin structure of the core promoter region

To examine the effects of the inserted sequences on the nucleosome organization, we analyzed the chromatin structure of the *CYCI* core promoter region by limited digestion with MNase. The MNase cleavage sites in isolated nuclei (regarded as chromatin samples, lanes labeled with C) and in naked DNA (lanes labeled with D) as a control are shown in Fig. 2. The locations of the nucleosomes were determined by the comparison of the MNase cleavage sites between the chromatin and naked DNA samples (the main cleavage sites in the naked DNA are marked with *a – *h in Fig. 2). For the chromatin structure of the pLG 312S SS minichromosome (no insert), the sites marked with *a, *b, *c, and *f were protected in the chromatin samples (lanes 1 and 2), but were not protected in the naked DNA (lane 3), indicating the formation of the positioned nucleosomes I, II, and III in the core promoter region. In addition, chromatin sample-specific cleavage sites were detectable, and assigned to the linker DNA regions. These results are consistent with our previous study [35].

The insertion of (CTG)₁₂, (GAA)₁₂ or (TGTAGG)₆, which did not affect the *CYCI-lacZ* expression as shown in Fig. 1B, changed the MNase cleavage pattern in the region around nucleosomes II and III, but not around the nucleosome I region (Fig. 2, lanes 4–12). The MNase cleavage sites marked with *b and *d were cleaved in both the chromatin and naked DNA samples, but the site marked with *c was protected in the chromatin samples, indicating that nucleosome II is positioned between bands *b and *d in these constructs. The region between bands *b and *d was approximately 140 bp, which is narrower than the region protected by nucleosome II (about 165 bp) in the pLG 312S SS minichromosome (no insert). These results imply that nucleosome II is more tightly packed by the incorporated (CTG)₁₂, (GAA)₁₂, and (TGTAGG)₆ sequences. For these constructs,

nucleosome III is positioned redundantly in two frames: one is at the same position as that in the vector (no insert), and the other is positioned upstream from the band marked with *b, just adjacent to nucleosome II.

In contrast, the nucleosome architecture in the core promoter region was remarkably changed by the insertion of (CGG)₁₂, (TTAGGG)₆, (A)₃₄, or (CG)₈, which caused the induction of *lacZ* expression by 10–20 fold (Fig. 1B). As shown in Fig. 2 (lanes 13–24), the most pronounced region was around the inserts: the site marked with *c, which was located upstream of these inserts, was extensively cleaved in the chromatin samples, and the intensity was much stronger than that in naked DNA, indicating that the insert is exposed in the promoter region. The site marked with *b was completely protected in these chromatin samples, as compared to that in the naked DNA samples. Thus, these inserts act as nucleosome-disfavoring sequences and form an approximately 100 bp NDR (between bands *c and *d), just upstream of the TATA boxes in the core promoter, and the position of nucleosome II changed to the region between bands *a and *c, which was designated as nucleosome II'.

Regarding nucleosome I in the constructs with activated promoters containing the (CGG)₁₂, (TTAGGG)₆, (A)₃₄, or (CG)₈ insert, the band labeled *e in the chromatin samples was partially protected as compared to their naked DNA samples. This protection was not detectable in the chromatin samples of the repressed promoters, such as pLG 312S SS and its derivatives containing the (CTG)₁₂, (GAA)₁₂, or (TGTAGG)₆ insert. The intensity of the band *d also became stronger than that of *e in the chromatin samples of the activated promoters, as compared to the chromatin samples of the repressed promoters. These results indicated that, due to the NDR formation, nucleosome I shifted from the non-redundant position to redundant positions between bands *d and *h (about 170–190 bp), which were wider than those in the repressed promoters.

As summarized in Fig. 3, the nucleosome organization in the core promoter activated by the inserts (bottom panel) is different from that in the repressed promoters (top and middle panels): the inserts are incorporated in the positioned nucleosome II in the repressed promoters, whereas the inserts are exposed in the NDR in the activated promoters. The NDR formation upstream of the TATA boxes leads to the destabilization of the nucleosome I positioning.

4. Discussion

Prior studies revealed that nucleosome loss activates the transcription of several genes, including the *CYCI* core promoter in *S. cerevisiae* [44]. We also previously showed that nucleosomes are positioned on the *CYCI* core promoter in the repressed state [35]. In the present study, we demonstrated the relationship between the reporter gene expression and the nucleosome organization dictated by DNA sequences in the core promoter. The (CTG)₁₂, (GAA)₁₂, and (TGTAGG)₆ inserts were incorporated in a positioned nucleosome in the promoter region, and they did not affect the reporter gene expression. In contrast, the insertion of (CGG)₁₂, (TTAGGG)₆, (A)₃₄, or (CG)₈ altered the nucleosome architecture in the promoter and caused the induction of the reporter gene expression. The most pronounced

chromatin structural feature commonly found in the activated promoters is the NDR formation by the insertion of (CGG)₁₂, (TTAGGG)₆, (A)₃₄, or (CG)₈, leading to the destabilization of the positioning of nucleosome I (Fig. 3). The insertions we analyzed are diverse, yet they share the common feature of the nucleosome-disfavoring property. Thus, in the absence of UASs, which are binding sites for activators, the activity of this core promoter is mainly governed by nucleosome formation and depletion.

Regarding NDR formation, one possible mechanism is the intrinsic nucleosome-disfavoring property of the inserts, since the relatively longer poly dA•poly dT, (CGG)_n, (CG)_n, and (TTAGGG)_n sequences are resistant to nucleosome formation *in vivo* and *in vitro* [22,28,29,32–34,42]. The other possible mechanism is the action of the RSC (Remodel the Structure of Chromatin) chromatin remodeling complex through the inserts, since the subunits of RSC bind to G/C motifs as well as short A tracts [14–16]. Based on the recent report by Kubic *et al.* [15], our results can be interpreted as the intrinsic nucleosome-disfavoring property of the DNA. They showed that an NDR greater than 240 bp in length is formed by the action of RSC through two short sequence motifs, (A)₇ and CGSC (S=G or C) [15]. In contrast, in the present study, the length of the NDR formed by the insertion of (CGG)₁₂, (TTAGGG)₆, (A)₃₄, or (CG)₈ in the *CYCI* core promoter is about 100 bp, which is much shorter than the typical RSC-mediated NDR. In addition, the RSC action through these sequence motifs alone is not sufficient to determine the nucleosome location, and sequence-specific general regulatory factors, such as transcription factors, play a key role in the formation and destabilization of fragile nucleosomes [15]. We know of no *trans*-acting factors that are involved in transcriptional regulation of the *CYCI* core promoter in the present experiments. Thus, we consider that the longer nucleosome-disfavoring sequences, which we employed here, act as determinants of NDR formation, although our results do not rule out the possibility of the actions of nucleosome remodeling factors, such as RSC.

To activate transcription from the *CYCI* core promoter, TBP binding to the TATA box is an initial and critical step, and it is severely restricted by the incorporation of the sequence into a nucleosome [45]. Here, the NDR formation by the nucleosome-disfavoring inserts causes nucleosome I to shift from the non-redundant position to redundant positions. This destabilization would facilitate the access of TBP to the TATA boxes, as the regions near the entry-exit sites of the nucleosome are highly dynamic and accessible [46–48]. Our results emphasize that NDR formation *per se* is essential to activate the *CYCI* core promoter, and support the idea that NDR formation upstream of TATA boxes and transcriptional start sites in the core promoter is spatially important for loading the components of the pre-initiation complex [24].

Acknowledgments

This work was supported by JSPS KAKENHI Grant Numbers 26430186 (to MS), 25250023 (to HK) and NIH R01 AI070272 (to APM). HK was also supported by the Waseda Research Institute for Science and Engineering. YI was supported by the Early Bird Program of Waseda University. We thank Masashi Fujita for his participation in the early stage of this work, and Hirotsada Mikai for technical assistance with the *lacZ* assay in his undergraduate studies. We thank Professors Yoichi Shibusawa and Akio Yanagida, Tokyo University of Pharmacy and Life Science, for their helpful support. MS thanks Professors Mamoru Sato and Yoshifumi Nishimura, Yokohama City University, and Professor Yoshizumi Ishino, Kyushu University, for their encouragement and support.

Abbreviations

NDR	nucleosome-depleted region
UAS	upstream activating sequencing
MNase	micrococcal nuclease
RSC	remodel the structure of chromatin

References

1. Rando OJ, Winston F. Chromatin and transcription in yeast. *Genetics*. 2012; 190:351–387. [PubMed: 22345607]
2. Cairns BR. The logic of chromatin architecture and remodelling at promoters. *Nature*. 2009; 461:193–198. [PubMed: 19741699]
3. Li B, Carey M, Workman JL. The role of chromatin during transcription. *Cell*. 2007; 128:707–719. [PubMed: 17320508]
4. Kaplan N, Moore IK, Fondufe-Mittendorf Y, Gossett AJ, Tillo D, Field Y, LeProust EM, Hughes TR, Lieb JD, Widom J, Segal E. The DNA-encoded nucleosome organization of a eukaryotic genome. *Nature*. 2009; 458:362–366. [PubMed: 19092803]
5. Schones DE, Cui K, Cuddapah S, Roh T-Y, Barski A, Wang Z, Wei G, Zhao K. Dynamic regulation of nucleosome positioning in the human genome. *Cell*. 2008; 132:887–898. [PubMed: 18329373]
6. Mavrich TN, Jiang C, Ioshikhes IP, Li X, Venters BJ, Zanton SJ, Tomsho LP, Qi J, Glaser RL, Schuster SC, Gilmour DS, Albert I, Pugh BF. Nucleosome organization in the *Drosophila* genome. *Nature*. 2008; 453:358–362. [PubMed: 18408708]
7. Mavrich TN, Ioshikhes IP, Venters BJ, Jiang C, Tomsho LP, Qi J, Schuster SC, Albert I, Pugh BF. A barrier nucleosome model for statistical positioning of nucleosomes throughout the yeast genome. *Genome Res*. 2008; 18:1073–1083. [PubMed: 18550805]
8. Lee W, Tillo D, Bray N, Morse RH, Davis RW, Hughes TR, Nislow C. A high-resolution atlas of nucleosome occupancy in yeast. *Nat. Genet*. 2007; 39:1235–1244. [PubMed: 17873876]
9. Yuan G-C, Liu Y-J, Dion MF, Slack MD, Wu LF, Altschuler SJ, Rando OJ. Genome-scale identification of nucleosome positions in *S. cerevisiae*. *Science*. 2005; 309:626–630. [PubMed: 15961632]
10. Struhl K, Segal E. Determinants of nucleosome positioning. *Nat. Struct. Mol. Biol*. 2013; 20:267–273. [PubMed: 23463311]
11. You JS, Kelly TK, De Carvalho DD, Taberlay PC, Liang G, Jones PA. OCT4 establishes and maintains nucleosome-depleted regions that provide additional layers of epigenetic regulation of its target genes. *Proc. Natl. Acad. Sci. U.S.A.* 2011; 35:14497–14502. [PubMed: 21844352]
12. Ganapathi M, Palumbo MJ, Ansari SA, He Q, Tsui K, Nislow C, Morse RH. Extensive role of the general regulatory factors, Abf1 and Rap1, in determining genome-wide chromatin structure in budding yeast. *Nucleic Acids Res*. 2011; 39:2032–2044. [PubMed: 21081559]
13. Bai L, Ondracka A, Cross FR. Multiple Sequence-Specific Factors Generate the Nucleosome-Depleted Region on *CLN2* Promoter. *Mol. Cell*. 2011; 42:465–476. [PubMed: 21596311]
14. Badis G, Chan ET, van Bakel H, Pena-Castillo L, Tillo D, Tsui K, Carlson CD, Gossett AJ, Hasinoff MJ, Warren CL, Gebbia M, Talukder S, Yang A, Mnaimneh S, Terterov D, Coburn D, Li Yeo A, Yeo ZX, Clarke ND, Lieb JD, Ansari AZ, Nislow C, Hughes TR. A library of yeast transcription factor motifs reveals a widespread function for Rsc3 in targeting nucleosome exclusion at promoters. *Mol. Cell*. 2008; 32:878–887. [PubMed: 19111667]
15. 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*. 2015; 60:422–434. [PubMed: 26545077]
16. Lorch Y, Maier-Davis B, Kornberg RD. Role of DNA sequence in chromatin remodeling and the formation of nucleosome-free regions. *Genes Dev*. 2014; 28:2492–2497. [PubMed: 25403179]

17. Ganguli D, Chereji RV, Iben JR, Cole HA, Clark DJ. RSC-dependent constructive and destructive interference between opposing arrays of phased nucleosomes in yeast. *Genome Res.* 2014; 24:1637–1649. [PubMed: 25015381]
18. Yen K, Vinayachandran V, Pugh BF. SWR-C and INO80 Chromatin remodelers recognize nucleosome-free regions near +1 nucleosomes. *Cell.* 2013; 154:1246–1256. [PubMed: 24034248]
19. Tolkunov D, Zawadzki KA, Singer C, Elfving N, Morozov AV, Broach JR. Chromatin remodelers clear nucleosomes from intrinsically unfavorable sites to establish nucleosome-depleted regions at promoters. *Mol. Biol. Cell.* 2011; 22:2106–2118. [PubMed: 21508315]
20. Hartley PD, Madhani HD. Mechanisms that specify promoter nucleosome location and identity. *Cell.* 2009; 137:445–458. [PubMed: 19410542]
21. Müller F, Tora L. Chromatin and DNA sequences in defining promoters for transcription initiation. *Biochim. Biophys. Acta- Gene Regulatory Mechanisms.* 2014; 1839:118–128.
22. Segal E, Widom J. Poly(dA:dT) tracts: major determinants of nucleosome organization. *Curr. Opin. Struct. Biol.* 2009; 19:65–71. [PubMed: 19208466]
23. Sekinger EA, Moqtaderi Z, Struhl K. Intrinsic histone-DNA interactions and low nucleosome density are important for preferential accessibility of promoter regions in yeast. *Mol. Cell.* 2005; 18:735–748. [PubMed: 15949447]
24. Venters BJ, Pugh BF. A canonical promoter organization of the transcription machinery and its regulators in the *Saccharomyces* genome. *Genome Res.* 2008; 19:360–371. [PubMed: 19124666]
25. Segal E, Fondufe-Mittendorf Y, Chen L, Thåström A, Field Y, Moore IK, Wang J-PZ, Widom J. A genomic code for nucleosome positioning. *Nature.* 2006; 442:772–778. [PubMed: 16862119]
26. Stein A, Takasuka TE, Collings CK. Are nucleosome positions *in vivo* primarily determined by histone-DNA sequence preferences? *Nucleic Acids Res.* 2010:709–719. [PubMed: 19934265]
27. Zhang Y, Moqtaderi Z, Rattner BP, Euskirchen G, Snyder M, Kadonaga JT, Liu XS, Struhl K. Intrinsic histone-DNA interactions are not the major determinant of nucleosome positions *in vivo*. *Nat. Struct. Mol. Biol.* 2009; 16:847–852. [PubMed: 19620965]
28. Suter B, Schnappauf G, Thoma F. Poly(dA•dT) sequences exist as rigid DNA structures in nucleosome-free yeast promoters *in vivo*. *Nucleic Acids Res.* 2000; 28:4083–4089. [PubMed: 11058103]
29. 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.* 2000; 19:3358–3365. [PubMed: 10880448]
30. Iyer V, Struhl K. Poly(dA:dT), a ubiquitous promoter element that stimulates transcription via its intrinsic DNA structure. *EMBO J.* 1995; 14:2570–2579. [PubMed: 7781610]
31. Zhu Z, Thiele DJ. A specialized nucleosome modulates transcription factor access to a *C. glabrata* metal responsive promoter. *Cell.* 1996; 87:459–470. [PubMed: 8898199]
32. Morohashi N, Yamamoto Y, Kuwana S, Morita W, Shindo H, Mitchell AP, Shimizu M. Effect of sequence-directed nucleosome disruption on cell-type-specific repression by $\alpha 2$ /Mcm1 in the yeast genome. *Eukaryotic Cell.* 2006; 5:1925–1933. [PubMed: 16980406]
33. Wong B, Chen S, Kwon J-A, Rich A. Characterization of Z-DNA as a nucleosome-boundary element in yeast *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. U.S.A.* 2007; 104:2229–2234. [PubMed: 17284586]
34. Ichikawa Y, Morohashi N, Nishimura Y, Kurumizaka H, Shimizu M. Telomeric repeats act as nucleosome-disfavouring sequences *in vivo*. *Nucleic Acids Res.* 2014; 42:1541–1552. [PubMed: 24174540]
35. Tanase J, Morohashi N, Fujita M, Nishikawa J-i, Shimizu M, Ohyama T. Highly efficient chromatin transcription induced by superhelically curved DNA segments: The underlying mechanism revealed by a yeast system. *Biochemistry.* 2010; 49:2351–2358. [PubMed: 20166733]
36. Bowdish KS, Yuan HE, Mitchell AP. Positive control of yeast meiotic genes by the negative regulator UME6. *Mol. Cell. Biol.* 1995; 15:2955–2961. [PubMed: 7760793]
37. Hahn S, Hoar ET, Guarente L. Each of three ‘TATA elements’ specifies a subset of the transcription initiation sites at the *CYC-1* promoter of *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. U.S.A.* 1985; 82:8562–8566. [PubMed: 3001709]

38. Guarente L, Mason T. Heme regulates transcription of the *CYC1* gene of *S. cerevisiae* via an upstream activation site. *Cell*. 1983; 32:1279–1286. [PubMed: 6301690]
39. Chen J, Ding M, Pederson DS. Binding of TFIID to the *CYC1* TATA boxes in yeast occurs independently of upstream activating sequences. *Proc. Natl. Acad. Sci. U.S.A.* 1994; 91:11909–11913. [PubMed: 7991556]
40. Li WZ, Sherman F. Two types of TATA elements for the *CYC1* gene of the yeast *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 1991; 11:666–676. [PubMed: 1846668]
41. Hahn S, Buratowski S, Sharp PA, Guarente L. Yeast TATA-binding protein TFIID binds to TATA elements with both consensus and nonconsensus DNA sequences. *Proc. Natl. Acad. Sci. U.S.A.* 1989; 86:5718–5722. [PubMed: 2569738]
42. Wang YH, Griffith JD. The [(G/C)₃NN]_n motif: a common DNA repeat that excludes nucleosomes. *Proc. Natl. Acad. Sci. U.S.A.* 1996; 93:8863–8867. [PubMed: 8799118]
43. Godde JS, Wolffe AP. Nucleosome assembly on CTG triplet repeats. *J. Biol. Chem.* 1996; 271:15222–15229. [PubMed: 8663027]
44. Han M, Grunstein M. Nucleosome loss activates yeast downstream promoters *in vivo*. *Cell*. 1988; 55:1137–1145. [PubMed: 2849508]
45. Imbalzano AN, Kwon H, Green MR, Kingston RE. Facilitated binding of TATA-binding protein to nucleosomal DNA. *Nature*. 1994; 370:481–485. [PubMed: 8047170]
46. Kujirai T, Horikoshi N, Sato K, Maehara K, Machida S, Osakabe A, Kimura H, Ohkawa Y, Kurumizaka H. Structure and function of human histone H3.Y nucleosome. *Nucleic Acids Res.* 2016 in press.
47. Poirier MG, Bussiek M, Langowski J, Widom J. Spontaneous access to DNA target sites in folded chromatin fibers. *J. Mol. Biol.* 2008; 379:772–786. [PubMed: 18485363]
48. Hieb AR, Gansen A, Böhm V, Langowski J. The conformational state of the nucleosome entry–exit site modulates TATA box-specific TBP binding. *Nucleic Acids Res.* 2014; 42:7561–7576. [PubMed: 24829456]

Highlights

- The relationship between nucleosome organization and gene expression was examined.
- Nucleosome-depleted region (NDR) was created by intrinsic DNA properties.
- Sequence-directed NDR formation is sufficient to activate a core promoter in yeast.

Fig. 1.

A. Experimental design in this study. Reporter constructs of pLG 312S SS and its derivatives. +1, translation start site of the *lacZ* gene; xh, *XhoI* site; *UAS-pCYC1*, the *CYC1* core promoter region; and thick bars numbered 1, 2, 3, 4 and 5, TATA1 (–178 to –170), TATA2 (–123 to –115), TATA3 (93 to –86), TATA4 (–79 to –71) and TATA5 (–56 to –49), respectively. DNA inserts, indicated by a gray box, were cloned into the *XhoI* site, which is located –247 bp upstream from the translation start site. **B. Effects of DNA inserts on the reporter gene expression.** *LacZ* expression is shown in Miller units of β-

galactosidase. The average values of triplicate determinations are shown with the standard deviation values.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig. 2. Indirect end-label mapping of MNase cleavage sites in pLG 312S SS and its derivatives
 The samples were digested with *Stu*I and *Cla*I (corresponding to the region from -698 to +498 of pLG 312S SS), and resolved by electrophoresis on a 1.5% agarose gel. The samples were transferred to a nylon membrane by Southern blotting. The MNase cleavage sites were detected by indirect-end labeling, using the DNA fragment (corresponding to the region from -698 to -902 of pLG 312S SS) as a probe. The reporter construct and the nucleosome positions are illustrated on the left side of each gel. The locations of the DNA inserts are indicated by gray boxes. Lanes labeled with 'C' indicate MNase digestion of

isolated nuclei (C samples) at two nuclease concentrations, and lanes labeled with 'D' indicate digestion of the naked DNA (D samples), as a control. The characteristic MNase cleavage sites in the D samples are indicated by asterisks. The activity of the *CYCI* core promoter is shown by 'repressed' or 'activated' in the top of gels, according to the results of Fig. 1B.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

No insert (vector)

(CTG)₁₂, (GAA)₁₂, (TGTAGG)₆

(CGG)₁₂, (TTAGGG)₆, A₃₄, (CG)₈

Fig. 3. Summary of nucleosome mapping in pLG 312S SS and its derivatives
The designations are the same as those in Fig. 1. Gray ellipses indicate the locations of nucleosomes in the *CYC1* core promoter.