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Highly Efficient Chromatin Transcription Induced by Superhelically Curved DNA Segments: The Underlying Mechanism Revealed by a Yeast System[†]

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ABSTRACT: Superhelically curved DNA structures can strongly activate transcription in mammalian cells. However, the mechanism underlying the activation has not been clarified. We investigated this mechanism in yeast cells, using 108, 180, and 252 bp synthetic curved DNA segments. Even in the presence of nucleosomes, these DNAs activated transcription from a UAS-deleted *CYC1* promoter that is silenced in the presence of nucleosomes. The fold-activations of transcription by these segments, relative to the transcription on the control that lacked such segments, were 51.4, 63.4, and 56.4, respectively. The superhelically curved DNA structures favored nucleosome formation. However, the translational positions of the nucleosomes were dynamic. The high mobility of the nucleosomes on the superhelically curved DNA structures seemed to influence the mobility of the nucleosomes formed on the promoter and eventually enhanced the access to the center region of one TATA sequence. Functioning as a dock for the histone core and allowing nucleosome sliding seem to be the mechanisms underlying the transcriptional activation by superhelically curved DNA structures in chromatin. The present study provides important clues for designing and constructing artificial chromatin modulators, as a tool for chromatin engineering.

DNA with a curved helix axis, referred to as bent DNA or curved DNA, is found in DNA from viruses to humans. This type of DNA occurs frequently in biologically important regions, such as origins of DNA replication, control regions of transcription, and recombination loci in cellular and viral genomes. The biological significance and the essential roles of these curved DNA regions seem to be well understood because of the extensive studies performed in the last century (*1*−*3*).

In eukaryotic transcription, naturally occurring curved DNA structures are likely to function in several ways. These include acting as a structural (conformational) signal for transcription factor binding; juxtaposing the basal transcription machinery with effector domains on upstream-bound factors; regulating transcription in association with transcription-factor-induced bending of DNA; and appropriately organizing the local chromatin structure for transcription initiation (*4*). Among these functions, the last one seems to be useful for developing technology, chromatin engineering, which uses synthetic curved DNAs for improved expression of endogenous genes or highly efficient expression of transgenes.

The first possibility of chromatin engineering was found with a synthetic left-handedly curved 36 bp DNA (subsequently named T4) in 2003. This T4 DNA increased the accessibility of the TATA box of the reporter promoter in chromatin, in a transient transfection assay system using COS-7 cells, and activated transcription by about 10-fold (*5*). Furthermore, when a T4-containing reporter was delivered into mouse liver by a hydrodynamics-based injection, T4 also activated transcription of the reporter gene (*6*). Its longer homologues activated transcription further in the transient expression system (*7*). For example, the herpes simplex virus (HSV[1]) thymidine kinase (*tk*) promoter was activated by a 288 bp superhelically curved DNA segment (named T32) by ca.140-fold. Furthermore, in HeLa genomic chromatin, the *tk* promoter was activated by ca. 90-fold at maximum by a similar 180 bp segment (named T20) (*7*). The chicken β-actin promoter, a well-known strong promoter, was even activated by this structure in mouse genomic chromatin in both ES cells and the hepatocytes differentiated from the ES cells (to be submitted elsewhere).

At the very first step of chromatin engineering, many issues must be clarified, i.e., the effective shape of the synthetic curved DNA structures, the type of chromatin they can form, the effects they generate, the mechanism employed to regulate gene expression, and so on. As described above, the superhelically curved DNA segments are definitely effective. However, their mechanism of transcriptional activation has not been clarified, except for the mechanism of the short segment T4 (*7*). This is mainly due to the experimental difficulty in using mammalian cells for fine

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[1]Abbreviations: ChIP, chromatin immunoprecipitation; *CYC1*, iso-1-cytochrome *c* gene; HSV, herpes simplex virus; *lacZ*, β-galactosidase gene; MNase, micrococcal nuclease; TBP, TATA binding protein; *tk*, thymidine kinase gene; UAS, upstream activating sequence; *URA3*, orotidine-5′-phosphate decarboxylase gene.

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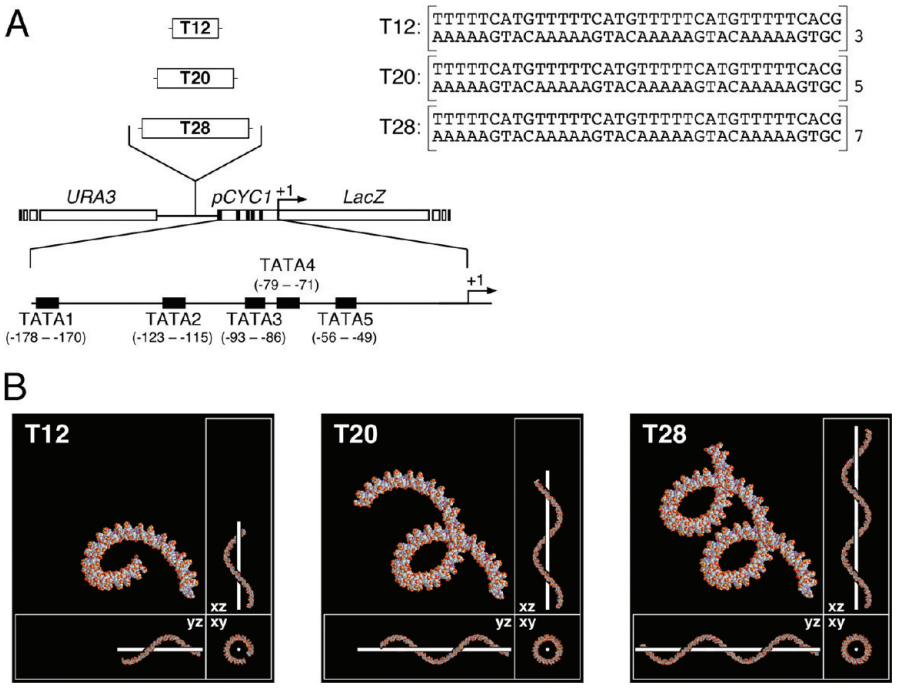

FIGURE 1: Reporter constructs used in this study and the architecture of superhelically curved DNAs. (A) Reporter constructs. *pCYC1*, +1, and TATA indicate the *CYC1* promoter, translation start site, and TATA box, respectively. The *CYC1* promoter contains five TATA sequences, and they were distinguished by the numbering according to the report by Li and Sherman (*16*). The nucleotide sequence of T20 is slightly different from that in the previous report (*7*): the sequence started with 5′-TCAGTTTTT and ended with TTTTT-3′ in the previous T20, but the corresponding sequences are TTTTT and TTTTTCACG-3′ in the present T20. However, since they have the same three-dimensional architecture, the same name was used for convenience. (B) Three-dimensional architectures of T12, T20, and T28. They were drawn as reported previously (*7*). The white line indicates the superhelical axis.

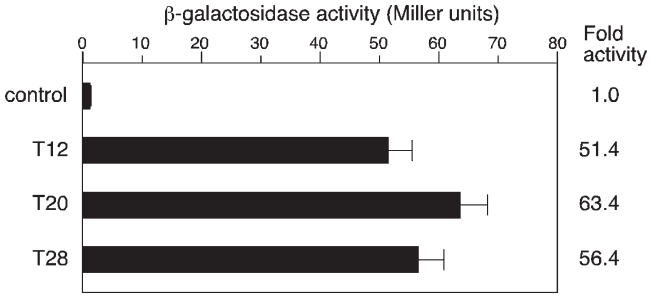

FIGURE 2: Effect of superhelically curved DNA on transcription. The promoter activity was determined in an β-galactosidase assay. Values shown are means ± SD (n = 3).

analyses of chromatin architectures. The principal cause is the size of mammalian genomes. They are very large and thus require a large amount of DNA to obtain a sufficient molarity for the analyses, which reduces the resolution. Another problem is the lack of convenient episomal systems for analyses of chromatin structure. Since it is also established that yeast gene promoters often contain a superhelical DNA curvature for the regulation of transcription (*8*), in the present study, we analyzed the activation mechanism by T20 and its homologues, using the yeast minichromosome system.

EXPERIMENTAL PROCEDURES

Plasmids and Strains. A fragment containing the T20 segment was prepared by PCR from the plasmid pLHC20/*loxP*/TLN-6 as a template (*7*), using the set of primers, 5′-GCGGTACCCTGGAGCGTCAGTCAGTTTTTCATG-3′ and 5′-GCGGTACCCTCGAGGTTATGATATCGGTGAAAAA-3′. The PCR fragment was digested with *Asp*718 and inserted into the *Kpn*I (*Asp*718) site of pKB112, the multicopy *CYC1-lacZ* plasmid (*9*). During plasmid screening, those harboring a T12 or T28 segment at the same site were also obtained. The plasmids were digested with *Xho*I to delete the UAS region from the *CYC1* promoter, to construct pTM6-U11-5 (T12), pTM44-U4-5 (T20), and pTM67-U8-15 (T28). All of the plasmids thus obtained were verified by DNA sequencing. The plasmid pLGΔ312ΔSS (*10*), in which the UAS region was deleted from the *CYC1-lacZ* gene, was used as a control plasmid. These plasmids were introduced into *Saccharomyces cerevisiae* strain AMP105 [*MATa ho::LYS2 ura3 lys2 leu2::hisG*].

β-Galactosidase Assay. The β-galactosidase assay was performed with early exponential phase cells grown in SC-Ura medium. The assay was carried out according to the standard protocol (*11*) with a minor modification: Y-PER (Pierce Chemical, Rockford, IL, USA) was used to prepare permeabilized yeast cells, instead of chloroform. The assay results were the average of three independent cultures of each strain.

MNase Digestion-Based Analysis of Chromatin Structure. Yeast cells harboring plasmids were cultured in SC-Ura medium until the OD_{600} reached ~1.0. Nuclei were isolated, and micrococcal nuclease (MNase) digestion was performed as described previously (*12*). The cleavage sites for MNase were analyzed by indirect end-labeling, as described previously (*13*). The samples were digested with *Stu*I, which cut at +442 (the translation start site of *URA3* gene is +1) of the *URA3* gene in the plasmids. The products were separated on a 1.5% agarose gel prepared in 1 × TBE buffer, transferred onto a Hybond-XL membrane (GE-Healthcare, Little Chalfont,UK), and detected by a radioactively labeled probe (201 bp) corresponding to the region from +442 to +642 in the *URA3* gene. The nucleosome repeat assay was performed as described (*13*). The MNase

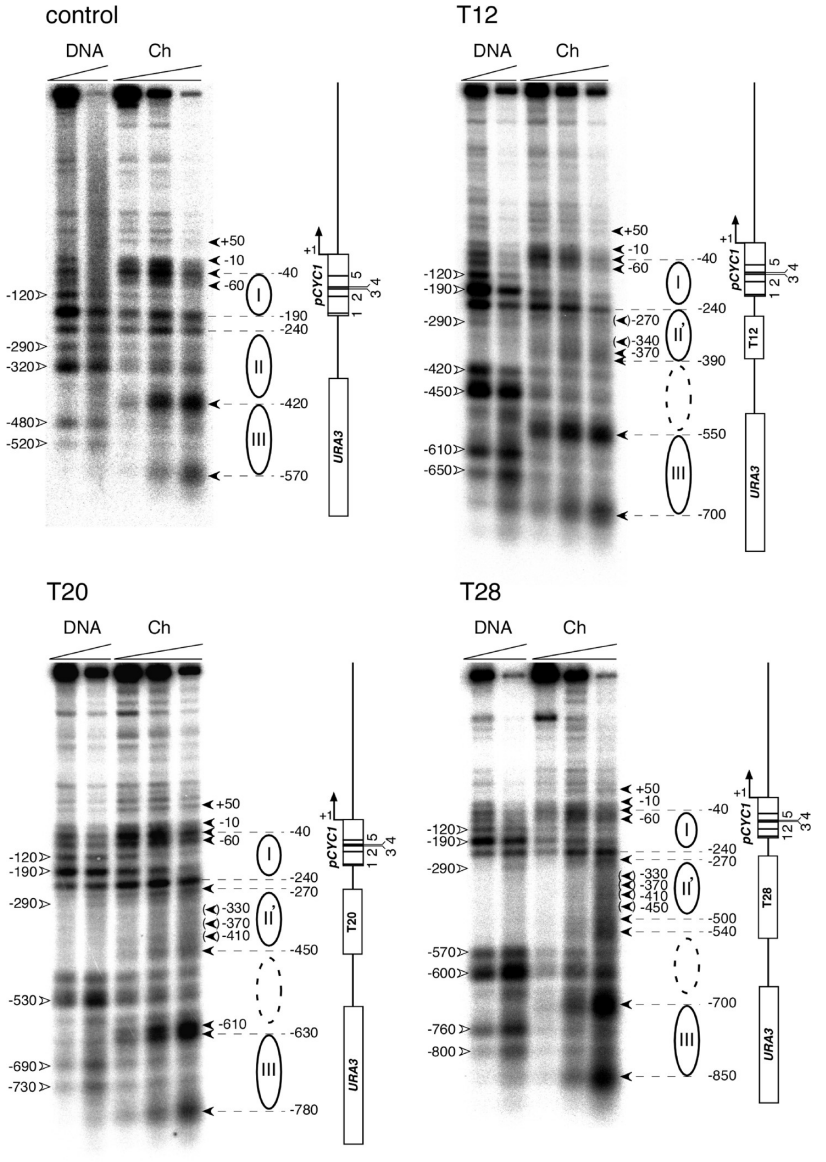

FIGURE 3: Analysis of translational positions of nucleosomes on the upstream region of the reporter gene. The upstream structure of the translation start site is illustrated on the right side of each gel. The lines with a number in the *CYC1* promoter (*pCYC1*) indicate the five TATA sequences. In each set of data, the lanes labeled Ch indicate the MNase digestion of isolated nuclei (chromatin) at three nuclease levels, and the lanes labeled DNA indicate the MNase digestion of the naked DNA at two nuclease levels, as a control. Arrowheads indicate the following sites: white, fully or partially protected in chromatin; black, highly digested or specifically digested in chromatin; black in parentheses, digested more in chromatin than in naked DNA, but the digestion itself was slight. Position numbers are relative to the translation start site (+1). The ellipses with I, II, II′, or III indicate putative nucleosomes, as deduced from the band patterns. Dashed-lined ellipses: possible additional positions.

digested samples were separated on a 1.3% agarose gel, transferred onto the Hybond-XL membrane, and detected by radioactively labeled probes corresponding to the region of the *CYC1* promoter, the T12, T20, or T28 segments, and the *URA3* region. The probe for the promoter was prepared by using primers 5′-ATG GCC AGG CAA CTT TAG T-3′ and 5′-GCT ACA AAG GAC CTA ATG TAT AAG GAA-3′. The primer sets used to prepare the other probes are described in the ChIP Assay section.

DNase I Footprinting Assay. Aliquots (200 μL) of the nuclei suspension were incubated at 37 °C for 1 min. Then, 0.01 U, 0.1 U, or 1 U of DNase I was added, and digestion was performed at 37 °C for 2 min. Naked DNA was digested with 0.0001 U, 0.002 U, or 0.003 U of DNase I at 37 °C for 2 min. After digestion, the products were purified and dissolved in water. Subsequently, the DNase I cleavage sites were detected by a PCR-based primer extension, using a [5′-^{32}P]-labeled primer 5′-AGT GAG ACG GGC AAC AGC-3′ (to detect the cleavage sites in the promoter region) or 5′-CAC ATG CAT GCC ATA TGA T-3′ (to detect the cleavage sites in the upstream region of the promoter) under the following conditions: 95 °C for 5 min and 35 cycles at 95 °C for 30 s, 63 °C for 15 s, and 72 °C for 2 min. All samples were purified and resolved in 6% polyacrylamide-7 M urea gels.

ChIP Assay. The ChIP assay was performed according to Kuo and Allis (*14*), with slight modifications. To obtain the chromatin fragment, the cell lysate was digested with 20 U of MNase at 37 °C for 20 min. After the cell debris was removed, 60 μL of the lysate was used for immunoprecipitation and subsequent DNA purification, which was performed in triplicate with a OneDay ChIP Kit (Diagenode, Liège, Belgium) according to the manufacturer's instructions, using antibodies against histone H3 (ab1791; Abcam, Cambridge, UK). Using 2 μL of

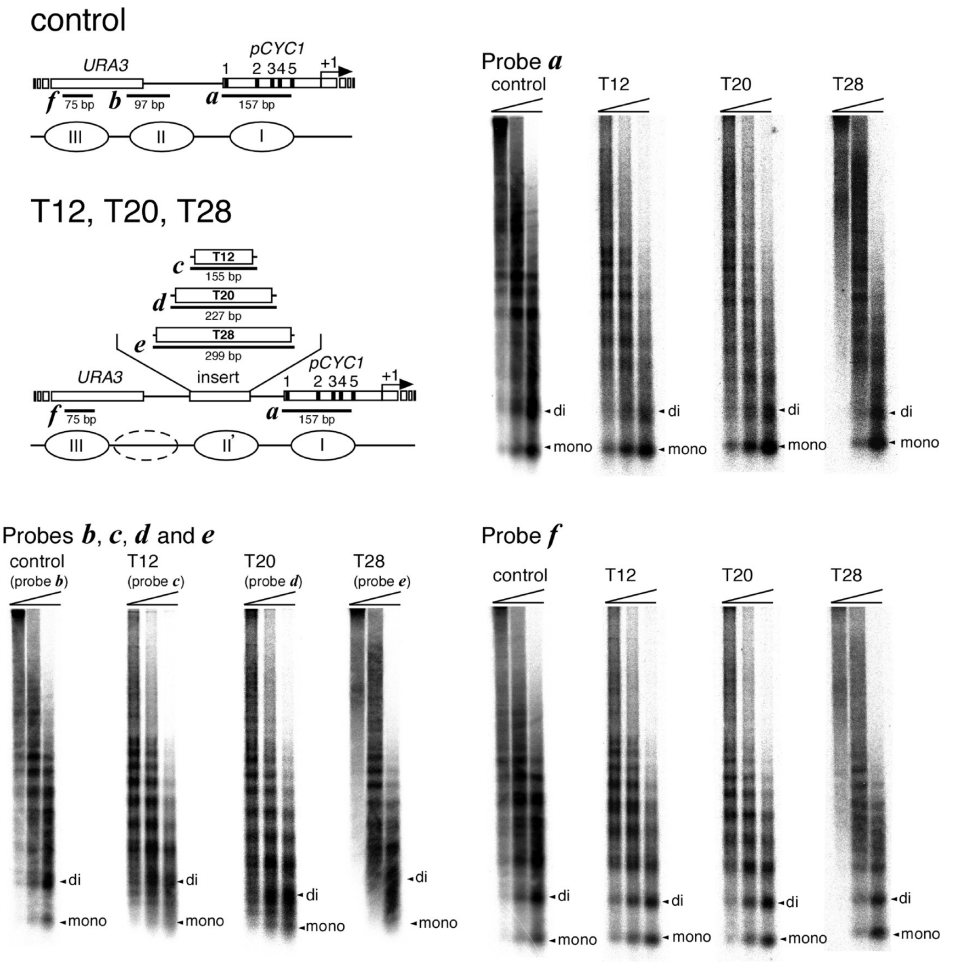

FIGURE 4: Southern blot analysis of DNA fragments obtained from MNase digests of nuclei. The digestion products were hybridized with probes obtained from the promoter (probe ***a***), the curved DNA regions (probes ***c***, ***d***, and ***e***), the *URA3* region (probe ***f***), or the region between the promoter and the *URA3* region in the control construct (probe ***b***). The positions and lengths of these probes are illustrated. Black boxes in the *CYC1* promoter indicate the TATA sequences 1 to 5. Nucleosomes are shown as ellipses with Roman numerals. The MNase digestion of isolated nuclei (chromatin) was performed at three nuclease levels. mono and di indicate mono- and dinucleosomes, respectively.

the DNA samples thus obtained, we performed quantification of fragments containing a part of the nucleosome I, II, or II′ DNA by SYBR Green quantitative real-time PCR with the StepOne Plus real-time PCR system and StepOne software v. 2.1. Quantification of fragments containing part of the nucleosome III DNA was carried out simultaneously, as an internal control. The primer sets were as follows: for the nucleosome I region, 5′-GTG TGC GAC GAC ACT GAT-3′ and 5′-AGA GAA AAG AAG AAA ACA AGA GTT-3′; for the nucleosome II region, 5′-GGC TGG GAA GCA TAT TTG AG-3′ and 5′-TTG AAG CTC TAA TTT GTG AGT TTA GT-3′; for the nucleosome II′ region, 5′-TGG TAC CCT GGA GAG TAG TCA G-3′ and 5′-CGG ATC TGC TCG AGG TTA TG-3′; for the nucleosome III region, 5′-AGA ACC GTG GAT GAT GTG GT-3′ and 5′-CCT TCC CTT TGC AAA TAG TCC-3′. The relative sample enrichment was calculated using the formula: $2^{-(\text{Ct target region} - \text{Ct nucleosome III region})}$

RESULTS

Effect of Superhelically Curved DNA on Transcription in Yeast. The *CYC1-lacZ* plasmids were constructed by Guarente and Mason (*10*) and have been utilized to study gene expression in *S. cerevisiae* (*9*, *15*−*17*). In the wild-type *CYC1* promoter, activators Hap1 and Hap2/3/4/5 bind to UAS1 and UAS2, respectively, to activate transcription (*18*, *19*). When the UAS region is deleted from the *CYC1* promoter in a *CYC1-lacZ* plasmid, the remaining downstream promoter is activated by nucleosome depletion (*15*). This implies that nucleosome occupancy is a major determinant of promoter activity in this system. Thus, we chose it to examine mechanisms of transcriptional activation by superhelically curved DNA structures for we assumed that the activation would be mediated by chromatin alteration. The superhelically curved DNA segments T12, T20, and T28 were introduced into the UAS-deleted *CYC1* promoter (Figure 1), and their effects on transcription were examined by measuring the β-galactosidase activity. As shown in Figure 2, all of the curved DNA segments activated transcription, and the observed fold activations relative to the control were 51.4 (T12), 63.4 (T20), and 56.4 (T28), respectively. Copy numbers of the reporter constructs in yeast cells were almost the same and about 20 copies/cell (analyzed by quantitative real-time PCR; data not shown). Thus, superhelically curved DNAs can activate transcription not only in mammalian cells but also in yeast cells. Furthermore, the extents of activation by these segments were comparable to that induced by the nucleosome-free effect described above (94-fold activation was reported in ref *15*) and those observed in mammalian cells (*7*).

Positioning of Nucleosomes on and around the Promoter. To determine how T12, T20, and T28 activated transcription, at first, the potential sequence-specific localization (translational positioning) of the nucleosomes on the region containing a

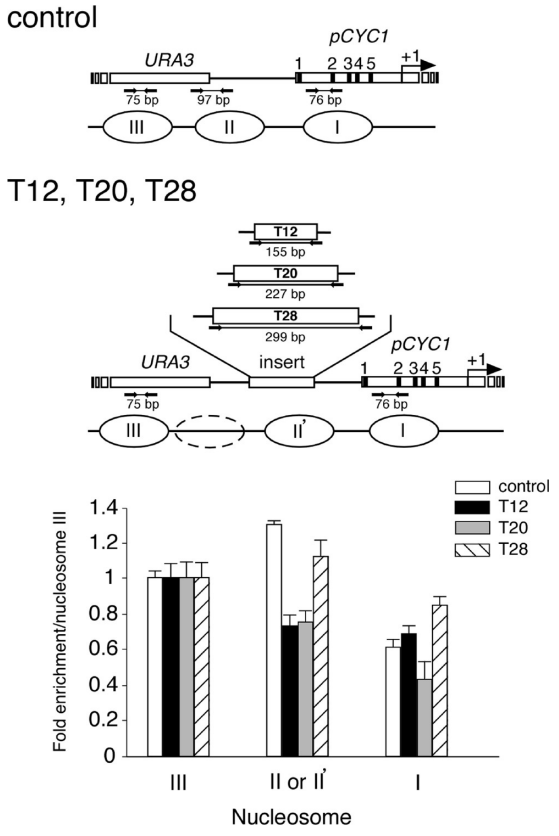

FIGURE 5: Relative populations of nucleosomes I, II, II′, and III, as determined by a ChIP assay. The assay was performed using the antibodies against histone H3. In the schematic drawing, the amplified regions and their locations are indicated with lines, with arrows indicating PCR primers. In the histogram, the fold enrichment of each nucleosome relative to nucleosome III is indicated with different bars: white, control chromatin; black, T12 chromatin; gray, T20 chromatin; striped, T28 chromatin (means ± SD of three determinations).

curved DNA and the *CYC1* promoter was investigated by an indirect end-labeling analysis. Plasmid-containing nuclei were isolated and were subjected to digestion with micrococcal nuclease (MNase), which cuts the linker regions between nucleosomes, and subsequently to analysis (Figure 3). The bands shown with white arrowheads indicate the sites that were digested by the enzyme in the naked state but were fully or partially protected in chromatin. On the other hand, the bands shown with black arrowheads indicate the sites that were newly or highly digested in chromatin. On the basis of these signal profiles and the distances between the black arrowheads, the nucleosome positions were deduced.

Regarding the chromatin formed on the control construct (control chromatin), three nucleosomes, referred to as I, II, and III, were suggested to be present as indicated in Figure 3: the latter two were located upstream of the TATA1 sequence and the first one was on the promoter. Nucleosomes I and III also seemed to lie on the corresponding regions in the curved DNA harboring each construct. In the control chromatin, nucleosome I was deduced to be located between −40 and −190 relative to the translation start site, and the distance between these positions was 150 bp, while in each chromatin formed on the curved DNA-containing construct (referred to below as T12, T20, or T28 chromatin, or together as Tn chromatin), it was presumably located between −40 and −240, and the distance between them was 200 bp. Therefore, nucleosome I seemed to cover a wider region in the Tn chromatin. The presence of a nucleosome (referred to as nucleosome II′) on the T12, T20, or T28 region was also speculated. In the locus of nucleosome II′, we detected several sites that were slightly but obviously digested in the chromatin, as indicated with black arrowheads in parentheses. They seemed to indicate the edges of nucleosomes with different translational positions. Furthermore, another nucleosome might be located between nucleosomes II′ and III, as indicated by the dashed ellipse in Figure 3.

Confirmation of the Nucleosome Presence. To determine whether nucleosomes I, II, II′, and III were actually formed on the regions indicated in Figure 3, the chromatin digests with MNase were subjected to a Southern blot analysis, using probes prepared from the relevant regions. As shown in Figure 4, so-called nucleosomal ladder patterns were detected by all of the probes, which supported the presence of the putative nucleosomes indicated in Figure 3. However, the curved DNA probes *c*, *d*, and *e* hardly detected DNA fragments of mononucleosome size but detected fragments with sizes between mono- and dinucleosomal DNAs, which appeared as smeared bands between these two positions. This pattern was not observed when the other probes were used. These smeared patterns could be a consequence of the difficulty in digesting the curved DNA regions, as shown in Figure 3. In addition, it is possible that nucleosome II′ had multiple translational positionings, as described in the previous section. In such a case, the outside regions of each curved DNA could be protected from MNase digestion by the positioned histone cores. These two factors could generate DNA fragments longer than that of mononucleosomal DNA.

The relative populations of the nucleosome-forming constructs were analyzed by a ChIP assay. The immunoprecipitation was performed using the antibody against histone H3. The population of nucleosome III was used as a reference for comparison (Figure 5). The relative populations of nucleosome I to nucleosome III were 61% for the control construct, 69% for the T12-harboring construct (T12 construct), 43% for the T20 construct, and 85% for the T28 construct. Thus, the populations of nucleosome I were nearly the same for the control and T12 constructs and higher for the T28 construct, while the promoter activities of the T12 and T28 constructs were much higher (more than 50-fold) than that of the control construct. Obviously, the transcription data in Figure 2 could not be explained in terms of the extent of histone deposition. Furthermore, these data showed that transcription was highly activated, even from the nucleosome-harboring promoter.

The promoter proximal nucleosomes were nucleosomes II and II′. Regarding these, the control construct and the T28 construct had comparable populations, which were higher than those for the T12 and T20 constructs. However, T28 activated the promoter by more than 50-fold. Therefore, the presence of nucleosomes II and II′ themselves was not implicated in determining the transcription level.

Accessibility of DNA in Chromatin. We subjected the same nuclei fraction to DNaseI digestion, to investigate the accessibility of the DNA in chromatin in order to understand how curved DNAs could activate transcription. The control construct and the curved DNA-containing constructs share the same sequence, except for the curved DNA region (Figure 1). In the region with the same sequence, a notable difference was only detected within the TATA3 sequence (Figure 6). In the control construct, A_{-92} and A_{-91} were highly accessible in the naked DNA, and their accessibility was maintained even in chromatin.

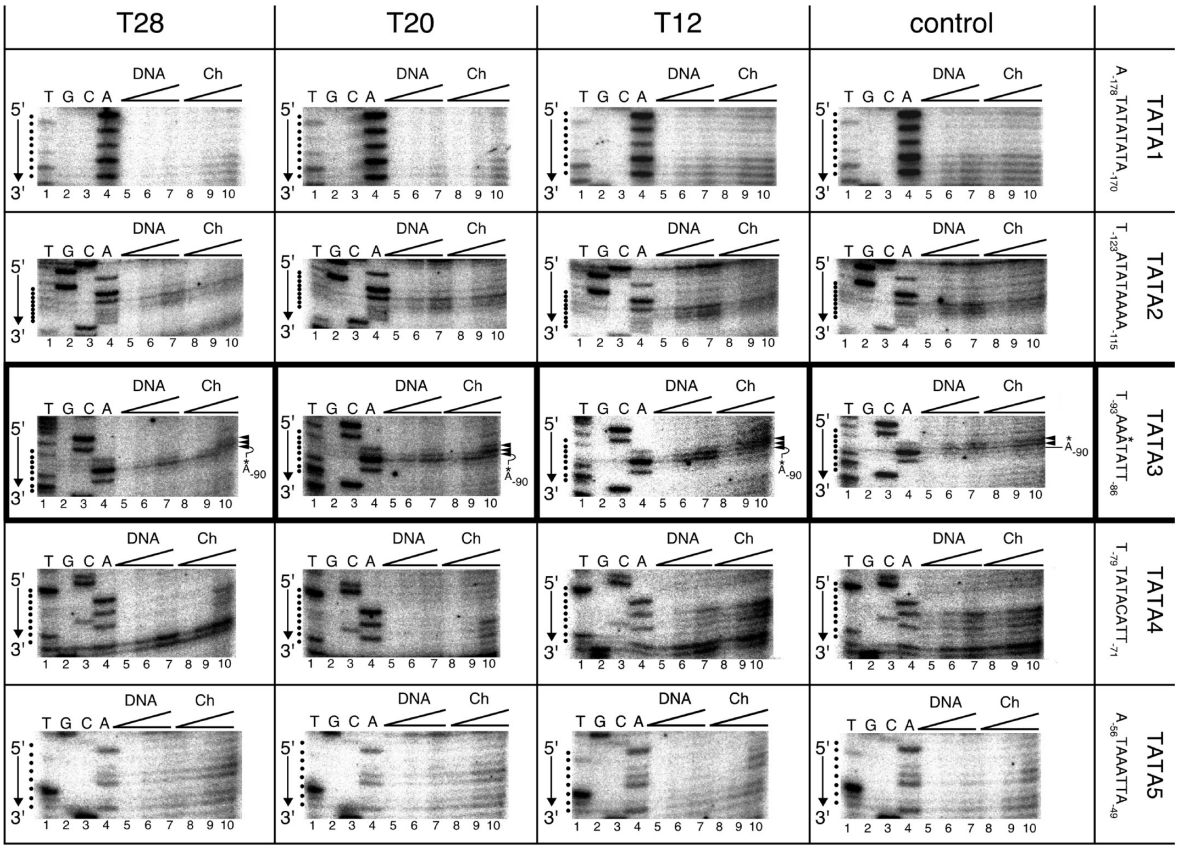

FIGURE 6: Accessibility of DNA in chromatin. Nuclei harboring each construct were digested with DNase I, and the cleavage sites were analyzed as described in the Experimental Procedures. DNA and Ch indicate DNase I cleavage sites in naked DNA and chromatin, respectively. The dots on the left-hand side of each gel indicate the positions of TATA-comprising nucleotides. Highly digested nucleotides within the TATA3 sequence in the chromatin are indicated with black arrowheads, and the position of A_{-90} is also indicated.

However, the sites with high accessibility were shifted by one nucleotide toward the center of the TATA3 sequence in the curved DNA-containing constructs in the naked states. As a result, A_{-91} and A_{-90} became highly accessible.

The accessibility of these two sites was maintained in the T20 chromatin. In addition, as in the case of the control chromatin, A_{-92} was also accessible in this chromatin. Although the cleavage signal at A_{-90} was less marked in the T12 chromatin and the T28 chromatin, as compared to that in the T20 chromatin, the signal definitely existed (see lane 9 in each panel). In conclusion, the exposed sequence was shifted toward the center of the TATA3 sequence in the curved DNA-containing constructs in the naked DNA, and this exposure was maintained even in chromatin. Since the distance between −240 and −90 is 150 bp (Figure 3), position −90 seemed to be the downstream end of a certain population of nucleosome I.

Regarding the curved DNA region, interestingly, both the positions and intensities of the cleavage signals were almost the same between naked DNA and chromatin in each Tn chromatin. Clear 10 bp ladder cleavage patterns with a rung spacing of 9 bp were observed, indicating that the rotational setting of the DNA was maintained even in the chromatin (Supporting Information).

DISCUSSION

Using a yeast minichromosome system, the present study investigated why superhelically curved DNA segments can activate transcription in chromatin. Although they did not cause any big change in the nucleosome occupancy in the promoter, they enhanced the accessibility of the TATA3 sequence. The activation mechanism can be explained in terms of increased mobility of nucleosomes promoted by the curved DNA structures.

Mechanism of Transcriptional Activation by Superhelically Curved DNA Segments. Chromatin analyses confirmed that the major populations of the reporter constructs formed nucleosomes on the *CYC1* promoter, the curved DNA region, and the *URA3* region. Regarding the promoter region, there were two clear differences between the control chromatin and the Tn chromatin. One was that nucleosome I was located between −40 and −190 in the control chromatin, while it was located between −40 and −240 in the Tn chromatin (Figure 3). The other was that the center region of the TATA3 sequence was more exposed in the Tn chromatin than in the control chromatin (Figure 6). Previous reports described that the TATA1 and TATA2 sequences were responsible for transcription from the *CYC1* promoter (*16*, *17*). However, considering that the extent of exposure of these sequences in the Tn chromatin was almost the same as that in the control chromatin (Figure 6) and that the transcription from the *CYC1* promoter is repressed in the presence of nucleosomes (*15*), the TATA3 sequence was presumably responsible for the transcription of the reporter gene in the present case.

If the upstream edge of nucleosome I was −240 in the Tn chromatin, then the downstream edge should have been located around −90, which is the center region of the TATA3 sequence. A certain population of chromatin seemed to adopt this positioning since −190 was considerably protected against the digestion (Figure 3). However, considering that the locus of nucleosome I (the protected region) spanned from −40 to −240, different translational positions were also presumably adopted by the

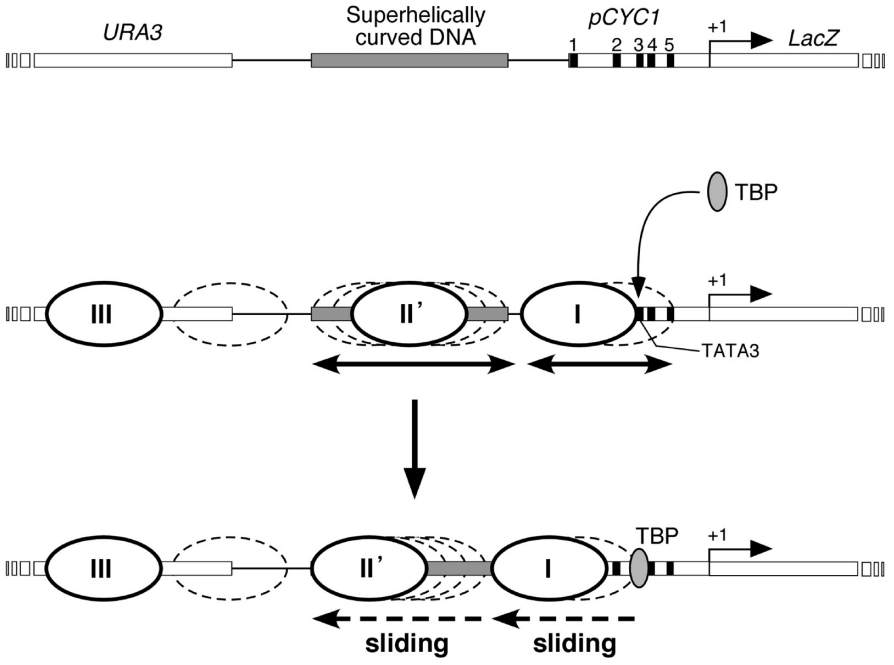

FIGURE 7: Putative mechanism of transcriptional activation in chromatin induced by superhelically curved DNA structures. The high mobility of nucleosome II′ on the curved DNA region generates room to accept transiently and largely sliding nucleosome I that is caused by TBP binding, which exposes the promoter and activates transcription.

other populations. The increased mobility of nucleosome I in the Tn chromatin seems to have generated the wide signal for the translational positioning. Under this condition, the TATA3-exposing population of nucleosome I was generated, and it was presumably implicated in the transcriptional activation in the Tn chromatin. A quite similar result was obtained in the system using a short, left handedly curved DNA segment, T4. When T4 (36 bp) was linked to the HSV *tk* promoter at a specific rotational phase and distance, it attracted the histone core, and the TATA box was thus left in the linker DNA with its minor groove facing outward, which led to the activation of transcription (*5*). In the present case, presumably, the long Tn segments increased the mobility of nucleosome II′, which enabled nucleosome I to fluctuate and expose the TATA3 sequence as described below.

Nucleosome II′ seemed to have multiple translational positions, while in contrast, the position of nucleosome II was almost fixed (Figures 3 and 4). This difference was obviously caused by the underlying DNA, and the superhelically curved DNAs were certainly implicated in the multiple positioning of nucleosome II′. It is known that curved DNA structures favor nucleosome formation (*20*−*24*). Therefore, the wide-ranging DNA curvature is likely to have the ability to slide the histone core. This hypothesis is supported by the report that nucleosome sliding is a general phenomenon that is dependent on the underlying DNA sequence (*25*). Furthermore, nucleosome sliding is considered as an important way of regulating access to DNA sites that are near nucleosome borders (*26*). The data shown in Figures 3 and 4 strongly suggest that the sliding of nucleosome II′ indeed occurred in the curved DNA region. The high mobility of the histone core seems to have increased the mobility of nucleosome II′. Furthermore, it must also be noted that TBP binding to DNA can induce nucleosome sliding (*27*). In conclusion, we can envision a scenario in which the high mobility of nucleosome II′ enabled nucleosome I to fluctuate. This fluctuation enhanced the chance of TBP binding to the TATA3 sequence, which then induced further sliding of these nucleosomes transiently, and the resulting open structure highly facilitated transcription initiation (Figure 7). Although the dynamic features of these events could not be detected in our static analyses, it is quite possible that a superhelically curved DNA segment could function as an acceptor of the sliding histone core and assist with the dynamic process of transcription.

The rotational orientation and the distance of a curved DNA segment relative to the promoter are important parameters for transcriptional activation (*5*, *7*). However, these were not optimized in the present study. Therefore, further positional refinement of each segment might have produced even greater transcriptional activation.

SUPPORTING INFORMATION AVAILABLE

Whole data for the DNase I footprinting assay. This material is available free of charge via the Internet at http://pubs.acs.org.

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