Human Centromere Protein B Induces Translational Positioning of Nucleosomes on α-Satellite Sequences*

Received for publication, September 1, 2005. Published, JBC Papers in Press, September 23, 2005, DOI 10.1074/jbc.M509666200

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THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 280, NO. 50, pp. 41609 –41618, December 16, 2005
Printed in the U.S.A.

The human centromere proteins A (CENP-A) and B (CENP-B) are the fundamental centromere components of chromosomes. CENP-A is the centromere-specific histone H3 variant, and CENP-B specifically binds a 17-base pair sequence (the CENP-B box), which appears within every other α-satellite DNA repeat. In the present study, we demonstrated centromere-specific nucleosome formation in vitro with recombinant proteins, including histones H2A, H2B, H4, CENP-A, and the DNA-binding domain of CENP-B. The CENP-A nucleosome wraps 147 base pairs of the α-satellite sequence within its nucleosome core particle, like the canonical H3 nucleosome. Surprisingly, CENP-B binds to nucleosomal DNA when the CENP-B box is wrapped within the nucleosome core particle and induces translational positioning of the nucleosome without affecting its rotational setting. This CENP-B-induced translational positioning only occurs when the CENP-B box sequence is settled in the proper rotational setting with respect to the histone octamer surface. Therefore, CENP-B may be a determinant for translational positioning of the centromere-specific nucleosomes through its binding to the nucleosomal CENP-B box.

The centromere is a chromosomal locus that plays an essential role in chromosome segregation at mitosis and meiosis. These specific loci are located in the primary constriction of each chromosome and are organized into a specialized chromatin structure composed of α-satellite DNA repeats and their associated proteins (reviewed in Refs. 1–4). The human centromere proteins A, B, and C (CENP-A,4 CENP-B, and CENP-C, respectively) are such centromere-specific DNA-binding proteins (5–11). CENP-A and CENP-C do not show any sequence specificity in DNA binding; however, in contrast, CENP-B specifically binds a 17-base pair sequence (the CENP-B box), which appears in every other α-satellite repeat (171 base pairs) in human centromeres (12–14).

CENP-A shares sequence similarity with histone H3 and binds to α-satellite DNA in human centromeres in vivo (7, 8, 15–18). In vitro experiments have shown that CENP-A can be incorporated into nucleosomes instead of histone H3 (19–21). Interspersed CENP-A-containing nucleosomes (CENP-A nucleosomes) and histone H3-containing nucleosomes (H3 nucleosomes) in the centromere have been observed in stretched chromatin fibers (22). These findings indicate that CENP-A is the centromere-specific histone H3 variant.

CENP-A is also required for centromere function. Removal of CENP-A causes chromosome missegregation (23–27), suggesting that the CENP-A nucleosomes recruit other centromere and kinetochore components. Consistent with this idea, the depletion of CENP-A causes significant dispersions of CENP-B and CENP-C, which are fundamental components of the centromere, in mouse cells (28). A biochemical study also showed that CENP-A, CENP-B, and CENP-C cooperatively constitute functional centromeres in mammalian cells (29, 30).

CENP-B is a dimeric protein composed of 80-kDa subunits (6) and contains DNA-binding and dimerization domains at its N terminus and C terminus, respectively (31, 32). CENP-B specifically binds the CENP-B box within α-satellite DNA (12, 33–35). We previously determined the crystal structures of the CENP-B N-terminal DNA-binding domain (amino acid residues 1–129) complexed with the CENP-B box DNA and the C-terminal dimerization domain (amino acid residues 540–599). The C-terminal dimerization domain consists of two α-helices, which are folded into an antiparallel configuration, and forms a dimer with a symmetrical, antiparallel, four-helix bundle structure (36). On the other hand, the N-terminal DNA-binding domain forms two helix-turn-helix motifs, which are bound to adjacent major grooves of the CENP-B box DNA (37).

The existence of the CENP-B box sequence within the α-satellite sequence is required for the formation of a functional centromere in vivo (38, 39). However, CENP-B null mice appeared to be normal (40–42), probably due to the existence of functional homologues of CENP-B (43, 44). A nucleosome reconstitution experiment with canonical histones and CENP-B suggested that CENP-B has the potential to modulate nucleosome formation in the vicinity of the CENP-B box (45).

Therefore, CENP-B may function as a trans-acting factor that regulates the formation of centromere-specific chromatin on the α-satellite DNA repeat at the nucleosome assembly level.

* This work was supported by the RIKEN Structural Genomics/Proteomics Initiative, the National Project on Protein Structural and Functional Analyses, and grants-in-aid from the Japanese Society for the Promotion of Science and the Ministry of Education, Sports, Culture, Science, and Technology, Japan. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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¶ The abbreviations used are: CENP-A, -B, and -C, centromere protein A, B, and C, respectively; NTA, nitritotriacetic acid; MNase, micrococcal nuclease.
In the present study, we reconstructed the centromere-specific nucleosome containing histones H2A, H2B, H4, and CENP-A and studied the role of CENP-B binding in nucleosome formation on α-satellite DNA in vitro.

EXPERIMENTAL PROCEDURES

Recombinant Proteins—The recombinant human histones H2A, H2B, H3, H4, and CENP-A were purified as described previously (20). The DNA fragments encoding histones H2A, H2B, H3, and CENP-A were inserted into the pHEC vector (BioLeaders) (46), and that encoding histone H4 was inserted into the PET15b vector (Novagen). The codons of the histone H4 and CENP-A genes were optimized for expression in Escherichia coli cells (20). These recombinant proteins were expressed as N-terminal hexahistidine (His<sub>6</sub>)-tagged proteins. The His<sub>6</sub>-tagged histones and CENP-A were recovered in the insoluble fraction. The pellets were dissolved in 100 mM Tris-HCl buffer (pH 8.0), containing 2M NaCl, 5 mM dithiothreitol, 1 mM EDTA, 1m M phenylmethylsulfonyl fluoride, and 5% glycerol. After the NaCl concentration was reduced from 2 to 0.6M by adding 10 mM MgCl<sub>2</sub>, the His<sub>6</sub>-tagged histones and CENP-A were recovered in the insoluble fraction. The pellets were dissolved in 20 mM Tris-HCl buffer (pH 8.0), containing 500 mM NaCl, 5% glycerol, and 6 mM urea. The recombinant histones and CENP-A were purified by nickel-nitrioltriacetic acid (Ni<sup>2+</sup>-NTA)-agarose (Qiagen) chromatography under denaturing conditions. The H2A/H2B dimer, the H3/H4 tetramer, and the CENP-A/H4 tetramer were reconstituted by dialysis against 20 mM Tris-HCl buffer (pH 8.0), containing 2 mM NaCl and 0.1 mg/ml bovine serum albumin. The mixtures were first dialyzed against 10 mM Tris-HCl buffer (pH 8.0), containing 1 mM EDTA, 10 mM 2-mercaptoethanol, and 0.1 M NaCl for 16 h at 4 °C.

When the nucleosomes were reconstituted in the presence of CENP-B-(1–129), CENP-B-(1–129) (3.5 µg) was mixed with the H2A/H2B dimer (6 µg) and the H3/H4 tetramer or the CENP-A/H4 tetramer (6 µg), along with the α-satellite DNA fragment (10 µg), at 4 °C in the presence of 6 mM urea, 2 M NaCl, and 0.1 mg/ml bovine serum albumin. The mixtures were first dialyzed against 10 mM Tris-HCl buffer (pH 8.0), containing 1 mM EDTA (or 0.1 mM EDTA), 1 mM 2-mercaptoethanol, and 0.1 M NaCl for 16 h at 4 °C.

The nucleosomes reconstituted with the recombinant histones and CENP-A were analyzed by nondenaturing 6% polyacrylamide gel electrophoresis in 0.5× TBE buffer (45 mM Tris base, 45 mM boric acid, and 1.25 mM EDTA). The gel (20 × 20 × 0.1 cm) was run at 10 V/cm for 90 min. Bands were visualized by autoradiography and were quantitated with a BAS2500 image analyzer (Fuji).

DNAse I Footprinting—The α-satellite DNA was labeled by T4 polynucleotide kinase (New England Biolabs) in the presence of [γ-<sup>32</sup>P]ATP at the NheI end (Fig. 1B). The reconstituted nucleosomes containing the <sup>32</sup>P-labeled α-satellite DNA were dialyzed against 10 mM Tris-HCl buffer (pH 8.0), containing 0.1 mM EDTA, 1 mM 2-mercaptoethanol, and 0.1 M NaCl, for 16 h at 4 °C. After the dialysis, the Mg<sup>2+</sup> concentration was adjusted to 3 mM, concomitantly with the addition of DNAse I. Naked DNA and the CENP-B-(1–129)-DNA complex were treated with 0.2 units of DNAse I (Takara, Japan)/µg of DNA. Nucleosomes were treated with 0.5 units of DNAse I/µg of DNA. DNAse I reactions were carried out at room temperature for 2 min and were terminated by the addition of EDTA to 25 mM. For the footprinting by the gel-purified method, the nucleosomes and the CENP-B-(1–129)-nucleosome complexes were separated by nondenaturing 6% polyacrylamide gel electrophoresis in 0.5× TBE buffer (45 mM Tris base, 45 mM boric acid, and 1.25 mM EDTA), and the DNA fragments were isolated from the gel (47). DNA fragments from these complexes were isolated and analyzed by denaturing 8% polyacrylamide gel electrophoresis.

Micrococcal Nuclease (MNase) Protection and Translational Positioning Assays—Reconstituted nucleosomes, containing 10 µg of DNA, were dialyzed against 10 mM Tris-HCl buffer (pH 8.0), containing 0.1 mM EDTA, 1 mM 2-mercaptoethanol, and 0.1 M NaCl, for 16 h at 4 °C. After the dialysis, the Ca<sup>2+</sup> concentration was adjusted to 1 mM, concomitantly with the addition of MNase. Naked DNA was digested with 2.5 and 1.25 units/ml MNase (Worthington) for 5 min at 22 °C. The nucleosomes were digested with 1.5, 0.75, and 0.25 units/ml MNase, and the reactions were terminated by the addition of EDTA to 25 mM. Then the DNA fragments were extracted with phenol/chloroform and were precipitated by ethanol. The purified DNA fragments were resuspended in 100 µl of TE buffer, and a 10-µl aliquot was used for the labeling reaction by T4 polynucleotide kinase in the presence of...
[γ-32P]ATP. Then the 32P-labeled DNA fragments were analyzed by nondenaturing 8% polyacrylamide gel electrophoresis in 0.5 × TBE buffer. Lane 1, the 186-base pair α-satellite DNA fragment. Lanes 2 and 3, the H3 nucleosomes and the CENP-A nucleosomes, respectively. D, the MNase assay. The reconstituted nucleosomes were treated with MNase, and the resulting DNA fragments were analyzed by nondenaturing 6% PAGE. Lanes 1, 4, 8, 9, and 13, molecular mass markers (MspI digests of pBR322 for lanes 1, 4, and 9; 100-base pair ladders for lanes 8 and 13). Lanes 2 and 3 indicate control experiments with the naked 186-base pair α-satellite DNA fragment. Lanes 5–7 indicate the experiments with the H3 nucleosomes, and lanes 10–12 indicate the experiments with the CENP-A nucleosomes. The amounts of MNase used in these experiments were 2.5 units/ml (lane 2), 1.25 units/ml (lane 3), 5 units/ml (lanes 5 and 10), 7.5 units/ml (lanes 6 and 11), and 2.5 units/ml (lanes 7 and 12). CP, the DNA fragments incorporated into the nucleosome core particle.

RESULTS

CENP-A Is Properly Incorporated into the Nucleosome Core Particle with the Human α-Satellite Sequence—The nucleosome core particle is composed of a histone octamer (containing two H2A/H2B dimers and an H3/H4 tetramer) and a 147-base pair DNA fragment, which is tightly

FIGURE 1. Human CENP-A forms nucleosomes. A, schematic representation of the nucleosome reconstitution assay by the salt dialysis method. B, the human α-satellite DNA fragment used in the present study. The end of the DNA fragment close to the CENP-B box is defined as the proximal edge, and the other DNA end is defined as the distal edge. The numbers correspond to the bases from the proximal edge of the 186-base pair DNA fragment. The blue box indicates the CENP-B box sequence, which is located from base pair 44 to 60. The arrows indicate the sites for the HindIII, NheI, EcoRI, and DraIII restriction enzymes. C, nucleosomes were reconstituted with the recombinant human histones and CENP-A by the salt dialysis method and were analyzed by nondenaturing 6% polyacrylamide gel electrophoresis in 0.5 × TBE buffer. Lane 1, the 186-base pair α-satellite DNA fragment. Lanes 2 and 3, the H3 nucleosomes and the CENP-A nucleosomes, respectively. D, the MNase assay. The reconstituted nucleosomes were treated with MNase, and the resulting DNA fragments were analyzed by nondenaturing 6% PAGE. Lanes 1, 4, 8, 9, and 13, molecular mass markers (MspI digests of pBR322 for lanes 1, 4, and 9; 100-base pair ladders for lanes 8 and 13). Lanes 2 and 3 indicate control experiments with the naked 186-base pair α-satellite DNA fragment. Lanes 5–7 indicate the experiments with the H3 nucleosomes, and lanes 10–12 indicate the experiments with the CENP-A nucleosomes. The amounts of MNase used in these experiments were 2.5 units/ml (lane 2), 1.25 units/ml (lane 3), 5 units/ml (lanes 5 and 10), 7.5 units/ml (lanes 6 and 11), and 2.5 units/ml (lanes 7 and 12). CP, the DNA fragments incorporated into the nucleosome core particle.
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wrapped around the histone octamer (48, 49). This 147-base pair fragment is protected from MNase digestion, and therefore, it can be detected after MNase digestion if the α-satellite DNA is properly wrapped around the histone octamer (MNase assay). To test whether CENP-A properly forms nucleosomes, we reconstituted the CENP-A nucleosome with recombinant histones H2A, H2B, H4, and CENP-A in the presence of a 186-base pair human α-satellite DNA fragment (Fig. 1, A–C).

As shown in Fig. 1D, when the CENP-A nucleosomes, formed with the 186-base pair human α-satellite DNA fragment, were digested with MNase, a 147-base pair DNA fragment was detected by nondenaturing polyacrylamide gel electrophoresis (lanes 10 and 11). As controls, a 147-base pair DNA fragment was also detected when H3 nucleosomes were digested with MNase (Fig. 1D, lanes 5 and 6), but it was not obvious when the naked DNA fragment was used as a template for this assay (Fig. 1D, lanes 2 and 3). Therefore, these results indicate that the CENP-A nucleosome wraps 147 base pairs of the α-satellite sequence within its nucleosome core particle, like the canonical H3 nucleosome.

CENP-B Can Bind to Nucleosomal DNA—Next, we performed the nucleosome reconstitution assay in the presence of the DNA-binding domain of human CENP-B (CENP-B-(1–129)) (32, 37, 50). CENP-B specifically binds the CENP-B box (a 17-base pair DNA sequence) with this DNA-binding domain. The 186-base pair α-satellite DNA fragment used in this experiment contains a CENP-B box, and it is located in the region from base pair 44 to 60 (Fig. 1B). Since the nucleosome core particle contained 147 base pairs of DNA (Fig. 1D), the CENP-B box sequence could be located within the nucleosome core particle, even if the nucleosome was formed on an edge of this α-satellite DNA fragment (Fig. 1B).

As shown in Fig. 2A, CENP-B-(1–129) bound to the H3 and CENP-A nucleosomes. The nucleosome–CENP-B-(1–129) complexes migrated more slowly than the nucleosomes without CENP-B-(1–129) on a non-denaturing 6% polyacrylamide gel (Fig. 2A). A footprint of CENP-B-(1–129) binding to the CENP-B box sequence was detected in both the H3 and CENP-A nucleosomes by DNase I probing (Fig. 2B, lanes 5 and 7, respectively). However, the CENP-B footprint of the nucleosomal DNA was very weak as compared with that of the naked DNA (Fig. 2B, lanes 3, 5, and 7). To ensure that the histones and CENP-B-(1–129) did not dissociate during the DNase I treatment and to confirm that the footprint did not result from two distinct complexes with or without CENP-B-(1–129), gel-purified the CENP-B-(1–129)–nucleosome complexes and the nucleosomes after DNase I treatment, but the intensity of the CENP-B footprint did not result from two distinct complexes with or without CENP-B-(1–129), we gel-purified the CENP-B-(1–129)–nucleosome complex and confirmed that the alphatranslational positioning of the nucleosome, suggesting that the translational positioning of nucleosomes is induced by CENP-B binding on α-satellite DNA. CENP-B binding to the nucleosomal CENP-B box sequence may restrict the translational positioning of the histone octamer along the DNA fragment, like linker histones (52).

Design of the Mutant α-Satellite DNA—To test whether the location of the CENP-B box sequence in the α-satellite DNA affects CENP-B binding to nucleosomal DNA, we constructed 12 mutant α-satellite DNAs (Fig. 4A). These mutant α-satellite DNAs were named −CB, −20, −10, −9, −6, −3, +3, +6, +9, +10, and +20. In the −CB α-satellite DNA, the 17-base pair CENP-B box was replaced by the sequence (5′-TAT CGT CTG CAG CGC CA-3′), which is inactive for CENP-B binding. In the −20, −10, −9, −6, and −3 α-satellite DNAs, the CENP-B box was moved 20, 10, 9, 6, and 3 base pairs, respectively, toward the proximal edge of the α-satellite DNA fragment. On the other hand, in the +3, +6, +9, +10, and +20 α-satellite DNAs, the CENP-B box was moved 3, 6, 9, 10, and 20 base pairs, respectively, toward the distal edge of the α-satellite DNA fragment. In the dyad α-satellite DNA, the CENP-B box was moved to a location close to the dyad axis of the nucleosome. These mutant α-satellite DNA fragments (192 base pairs) were amplified by polymerase chain reaction and were purified by agarose gel electrophoresis. As shown in Fig. 4B, CENP-B-(1–129) bound to all of these mutant α-satellite DNAs, except for the −CB α-satellite DNA. The CENP-B-(1–129)–DNA complexes showed different mobilities on a non-denaturing 6% polyacrylamide gel (Fig. 4B), probably due to the intrinsic curvature of the α-satellite sequence and the local DNA kinks (about 60°) induced by the CENP-B binding (37, 53).
CENP-B Binds to CENP-A Nucleosomes Containing Mutant α-Satellite DNAs—We tested CENP-B binding to the centromere-specific nucleosome, the CENP-A nucleosome, containing these mutant α-satellite DNAs. To do so, the CENP-A nucleosomes were reconstituted with the mutant α-satellite DNAs (192 base pairs, Fig. 4A) in the presence or absence of CENP-B-(1–129), and the resulting nucleosomes

FIGURE 2. CENP-B-(1–129) binds to nucleosomal DNA. A, nucleosomes were reconstituted with or without CENP-B-(1–129) in the presence of a 186-base pair α-satellite DNA fragment. The reconstituted nucleosomes were analyzed by 6% PAGE. B, DNase I footprinting. Aliquots containing 10 μg of naked DNA and nucleosomal DNA were treated with DNase I for 2 min. The DNA fragments were analyzed by denaturing 8% PAGE. Lane 1 indicates molecular mass markers (10-bp ladder marker). Dots indicate the 10-base pair periodicity of the DNase I-hypersensitive sites. The small arrows indicate the bands with densities that were weakened by CENP-B-(1–129) binding. C, DNase I footprinting with the gel-purified nucleosomes and the nucleosome-CENP-B-(1–129) complexes.
were analyzed by gel mobility shift assays. Surprisingly, CENP-B-(1–129) bound to all of the mutant \( \alpha \)-satellite nucleosomes containing the CENP-B box sequence, as judged by the retarded mobilities of the CENP-B-(1–129)-nucleosome complexes (Fig. 5A). CENP-B-(1–129) bound neither naked nor nucleosomal \( \alpha \),satellite DNA (Figs. 4B and 5A, lanes 1 and 3, respectively), indicating that CENP-B-(1–129) binding to these mutant \( \alpha \)-satellite nucleosomal DNAs depends on the presence of the CENP-B box sequence.

In order to confirm the CENP-B-(1–129) binding to the mutant \( \alpha \)-satellite nucleosomes, we performed a Ni\(^{2+}\)-NTA pull-down assay (Fig. 6A). In this assay, the mutant \( \alpha \)-satellite nucleosomes containing His\(_6\)-tagged histones and CENP-A were reconstituted in the presence of CENP-B-(1–129). CENP-B-(1–129) bound to the nucleosomal CENP-B box DNA was co-precipitated with His\(_6\)-tagged histones and CENP-A by the Ni\(^{2+}\)-NTA-agarose beads (Qiagen) and was detected on an SDS-16% polyacrylamide gel stained with Coomassie Brilliant Blue as a band just above the histone H4 band. CENP-B-(1–129) was successfully co-precipitated with the histones in the presence of the wild-type \( \alpha \)-satellite DNA (Fig. 6C, lane 2), but was not co-precipitated in the presence of the –CB \( \alpha \)-satellite DNA (Fig. 6B, lane 2). These results indicate that CENP-B-(1–129) bound to the CENP-B box DNA but not to histones and nonspecific DNA. No proteins were detected in control experiments without His\(_6\)-tagged histones (Fig. 6B and C), indicating that the CENP-B-(1–129) bound to the nucleosome-free CENP-B box DNA was not precipitated.

Consistent with the gel shift analysis, the CENP-B-(1–129) binding was detected with the –20, –10, –9, –6, –3, +3, +9, and +10 nucleosomes (Fig. 6B, lanes 4, 6, 8, 10, and 12) and C (lanes 4, 8, and 10). However, the CENP-B-(1–129) binding could not be detected with the +6, +20, and dyad nucleosomes (Fig. 6C, lanes 6, 12, and 14), although their interactions with CENP-B-(1–129) were detected in the gel shift analysis (Fig. 5A, lanes 19, 25, and 27). We repeated this entire experiment twice and confirmed that the results were consistent. Therefore, CENP-B may bind to the nucleosomes containing the +6, +20, and dyad mutant \( \alpha \)-satellite, but its affinity for them could be very low.
The Rotational Setting of the CENP-B Box Sequence Is Important for the Translational Positioning Induced by CENP-B—Next, we tested whether CENP-B induces the translational positioning of these CENP-A nucleosomes containing the mutant α-satellite DNA, like the case of the wild-type α-satellite DNA. As shown in Fig. 5B, multiple translational positions of the CENP-A nucleosomes containing the mutant α-satellite DNAs were observed without CENP-B-(1–129) (lanes 2, 5, 7, 10, 12, 15, 17, 19, 22, 24, 27, and 29), although two major translational positions were observed with the wild-type α-satellite DNA (Fig. 3B). Surprisingly, upon CENP-B-(1–129) binding, single translational positioning (position A) of the CENP-A nucleosome was induced in the −20, −10, +9, and +10 nucleosomes (Fig. 5B, lanes 6, 8, 23, and 25, respectively). Therefore, the mobility of the histone octamer may be restricted in the −20, −10, +9, and +10 nucleosomes. In contrast, like the −CB nucleosome, the induction of translational positioning by CENP-B-(1–129) binding was not observed in the −9, −6, −3, +3, +6, +20, and dyad nucleosomes (Fig. 5B), although these nucleosomes have the ability to bind CENP-B-(1–129) (Fig. 5A).

The −20 nucleosome may contain the CENP-B box in a linker region, not in the nucleosome core particle, if the nucleosome was located at the distal edge of the DNA (position A). However, part of the CENP-B box of the −10 nucleosome could be located within the nucleosome core particle, if the nucleosome formed at position A. Furthermore, the entire CENP-B box could be located within the nucleosome core parti-
cle in the +9 and +10 nucleosomes (Fig. 4A). Since the average pitch of nucleosomal DNA is about 10 base pairs/turn (48, 54), the CENP-B box sequences in the +9, +10, and +20 nucleosomes may have similar rotational settings to that of the wild-type α-satellite DNA with respect to the histone octamer surface. Therefore, the restriction of the nucleosome mobility by CENP-B may occur only when the CENP-B box is located with the proper rotational setting, with respect to the surface of the histone octamer within the nucleosome. CENP-B may be a determinant for the translational positioning of centromere-specific nucleosomes by its functional binding to the nucleosomal CENP-B box sequence.

**DISCUSSION**

In the present study, we showed that CENP-B has the ability to bind to the CENP-B box sequence located within the nucleosome core particle. This suggests that CENP-B binds to nucleosomal DNA without seriously disrupting the contacts between the histone octamer and DNA. Actually, CENP-B binding did not disrupt the nucleosome. We tested two types of nucleosomes: a centromere-specific CENP-A nucleosome containing histones H2A, H2B, H4, and CENP-A and a canonical H3 nucleosome containing histones H2A, H2B, H3, and H4. Genetic analyses with CENP-A-depleted mouse cells revealed that the absence of CENP-A caused significant dispersion of CENP-B (28), suggesting that CENP-B binding to the centromere may depend on the presence of CENP-A. However, our *in vitro* analyses did not show any preference for CENP-B binding to the CENP-A nucleosome rather than the H3 nucleosome. Additional factor(s) or an unrevealed mechanism may be involved in the functional links between CENP-A and CENP-B.

According to the crystal structure of CENP-B-(1–129) complexed with CENP-B box DNA, CENP-B-(1–129) directly contacts three essential sites (sites 1–3) within the 17-base pair CENP-B box sequence (37). All of these direct interactions between CENP-B-(1–129) and DNA are formed on only one side of the DNA helix and induce local DNA kinks of about 60° (Fig. 7A). Therefore, we constructed a docking model between the nucleosome core particle and the CENP-B-(1–129) structures (Fig. 7B). In this model, we supposed that the nucleosome is formed at position A, because CENP-B binding induced preferential nucleosome formation at position A. As shown in Fig. 7B, CENP-B-(1–129) could bind to the nucleosomal DNA without serious steric hindrance in this model. This model structure does not contain the DNA kinks induced by CENP-B binding. No significant difference in MNase susceptibility was observed between the nucleosome and the nucleosome-CENP-B-(1–129) complex, indicating that the nucleosome core particle somehow accommodates the CENP-B-induced
DNA kinks and maintains nucleosome stability upon CENP-B binding. Further structural studies of the nucleosome-CENP-B complex are required to understand the functional accommodation of CENP-B within the nucleosome core particle.

The CENP-B box sequence reportedly functions as a cis-element for nucleosome assembly on α-satellite DNA (45). Consistent with this observation, in the present study, we directly showed that CENP-B binding to the nucleosomal α-satellite DNA induced translational positioning of the nucleosome core particles. Nucleosome reconstitution experiments with the mutant α-satellite DNAs showed that inappropriate localization of the CENP-B box within the nucleosomal α-satellite DNA still allowed CENP-B binding to the nucleosomal CENP-B box sequence but did not induce translational positioning of the nucleosome core particle. These facts indicate that the proper rotational setting of the CENP-B box within the nucleosome is essential for the CENP-B-induced translational positioning, which may function in the centromere-specific chromatin formation. The DNase I footprinting experiment showed that the α-satellite DNA sequence was rotationally phased in both the H3 and CENP-A nucleosomes, probably due to its intrinsic curvature (55–57). This intrinsic rotational setting ability of the α-satellite sequence is important to determine the CENP-B box rotational setting, which regulates the CENP-B-induced translational positioning of the nucleosome. Therefore, the rotational setting and translational positioning of the centromere-specific nucleosomes are tightly linked in the presence of CENP-B and may be important for the formation of a functional centromere-specific chromatin structure.

**FIGURE 6.** The Ni²⁺-NTA-agarose pull-down assay for the CENP-B-(1–129) binding to the mutant nucleosomal α-satellite DNA. A, a schematic representation of the Ni²⁺-NTA-agarose pull-down assay with His₆-tagged histones (H2A, H2B, CENP-A, and H4), CENP-B-(1–129), and the wild-type or mutant α-satellite DNAs (192 bp). B and C, proteins precipitated with Ni²⁺-NTA-agarose beads as detected by SDS-10% PAGE.

**FIGURE 7.** Model for the CENP-B-nucleosome interaction. A, the crystal structure of CENP-B-(1–129) complexed with CENP-B box DNA (37). Right, electrostatic surface potential of CENP-B-(1–129) in the complex with the CENP-B box DNA. CENP-B-(1–129) makes direct contacts with DNA in the basic patch (blue area) on one side of the DNA helix. B, docking model between the nucleosome core particle and CENP-B-(1–129) structures. The structural coordinates of the nucleosome core particle and CENP-B-(1–129) were obtained from the RCSB Protein Data Bank (1AOI (54) and 1HLV (37), respectively). In this model, the translational position of the nucleosome is position A.
Nucleosome Positioning Induced by CENP-B

It has been reported that demethylation of centromeric satellite DNA sequences, accomplished by growing cells in the presence of a DNA methyltransferase inhibitor, resulted in the redistribution of CENP-B (58). Intriguingly, the CENP-B box sequence contains two CpG dinucleotides within its essential sites for CENP-B binding (37). We previously found that CENP-B preferentially binds to an unmethylated CENP-B box, and the DNA-binding ability of CENP-B is reduced nearly to the level of nonspecific binding by CpG methylation at both sites (50). In the present study, we showed that CENP-B binding to the nucleosomal CENP-B box induces the translational positioning of nucleosomes on the α-satellite sequence. Therefore, CpG methylation at the CENP-B box may be an important regulator of centromere-specific chromatin formation, through the CENP-B-induced nucleosome positioning on the α-satellite DNA repeats.

Acknowledgments—We thank N. Nakatanii and E. Takeuchi (Waseda University) for technical assistance.

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