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Positioned nucleosomes inhibit Dam methylation *in vivo*

(chromatin/Dam methyltransferase/α2 operator/*Saccharomyces cerevisiae*)

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ABSTRACT **_Escherichia coli_ Dam DNA methyltransferase can methylate genomic GATC sites when expressed in _Saccharomyces cerevisiae_. Others have observed changes in the level of methylation at specific sites and suggested that these changes are related to transcriptional state or chromosomal context. To test directly the influence of nucleosome location on the ability of Dam methyltransferase to modify GATC sites in chromatin, we analyzed minichromosomes containing precisely positioned nucleosomes in _dam_-expressing yeast strains. Levels of methylation at individual GATC sites were rigorously quantified by an oligonucleotide-probing procedure. Within the linker and adjacent 21 bp of nucleosome-associated DNA, GATC sites were highly methylated, whereas methylation was severely inhibited by histone–DNA contacts nearer to the nucleosomal pseudodyad. Other DNA–protein complexes also interfere with Dam methylation. These data are consistent with a model in which nucleosomes exert a repressive influence on the biological functions of DNA by restricting access of trans-acting factors to DNA.**

Analysis of eukaryotic chromatin structure has relied largely on the isolation of organelles or subcellular fractions from cells, followed by analysis with either enzymatic or chemical reagents. These techniques require the disruption of intact cells. Recently, several laboratories have expressed the *Escherichia coli* Dam methyltransferase, which methylates GATC sites at the N^6 position of adenine, in yeast to obtain *in vivo* information about chromatin structure (1–3). Although wild-type *Saccharomyces cerevisiae* contains undetectable levels of N^6-methyladenine (<0.05%) (4, 5), the only reported phenotype of *dam* gene expression in yeast is a minimal effect on the rate of mitotic recombination (6). The level of methylation at specific sites depends on the transcriptional state and chromosomal location of a gene; transcriptionally repressed loci are refractory to Dam methylation, whereas active genes exhibit increased levels of *in vivo* modification (1, 2). These observations have been attributed to "closed" or "open" chromatin conformations, respectively. However, as this terminology suggests, it is unclear exactly what changes in chromatin structure are responsible for modulating modification by Dam methyltransferase.

As a first step toward defining the structural features of chromatin that prevent Dam methylation *in vivo*, we asked whether specifically positioned nucleosomes, as opposed to higher order chromatin conformation, can limit modification by the methyltransferase. We introduced plasmids (see Fig. 1) containing GATC reporter sites into *dam*-expressing yeast strains. These plasmids, which also contain a yeast α2 operator sequence, are packaged into precisely positioned nucleosomes in haploid yeast α cells but not in haploid a cells (7–9). These defined minichromosomes enabled us to quantify rigorously the *in vivo* levels of methylation at specific GATC reporter sites in a nucleosome that is positioned both translationally and rotationally. We demonstrate that GATC sites in the central 84 bp of DNA in a positioned nucleosome are highly refractory to modification by the methyltransferase. These data indicate that sequence-specific modification can be hindered by positioned nucleosomes and thereby support the hypothesis that the repressive influence of nucleosomes on the biological functions of DNA may be exerted by restricting access of sequence-specific binding proteins to DNA.

MATERIALS AND METHODS

Yeast Strains and Reporter Plasmids. The strains used were UCC1023 (*MATa ura3-52 lys2-801:dam⁺:LYS2 ade2-101 trp1-Δ1 his3-Δ200 leu2-Δ1*) (2) and the isogenic *MATα* strain, MKY2. MKY2 was created by transforming (10) UCC1023 with a plasmid expressing the HO endonuclease (kindly provided by A. J. S. Klar), curing this plasmid by sporulation, and isolating the α-cell variant by tetrad dissection. These strains constitutively express the *dam* gene from a single copy integrated at the *LYS2* locus, where it is transcribed from a fortuitous bacterial sequence.

TALS-2 and TALS-3 were derived from the parent plasmid, TALS (7), to create (11) six and two new GATC sites for potential Dam methylation, respectively (see Fig. 1 *B* and *C*). Each GATC site was inserted to correspond to a site of DNase I cleavage within the exposed minor groove on the surface of nucleosome IV (8). TALS-3.5 was made by cleaving TALS-3 at its unique *Hga* I site and filling in the 5-bp overhang with the Klenow fragment of *E. coli* DNA polymerase I. The relevant regions for each modified plasmid were verified by DNA sequencing (12).

Isolation and Analysis of Chromatin. Chromatin from two independent yeast clones was prepared according to Fedor *et al.* (13) in which 0.3 ml of supernatant (obtained from 40 ml of yeast cells) from a spheroplast lysate containing minichromosomes was incubated at 37°C with micrococcal nuclease for 10 min at concentrations varying from 0 to 60 units/ml. Digestion by micrococcal nuclease was stopped, and the DNA was purified as previously detailed (7). For the "naked" DNA controls, DNA from spheroplast lysates was first deproteinized before treatment with 0–3 units of micrococcal nuclease per ml and subsequent purification. All samples were cleaned over G-50 spin columns before analysis by indirect end labeling.

Sites of micrococcal nuclease cleavage were mapped by indirect end labeling (14, 15). Briefly, purified DNA was digested to completion with *Eco*RV, electrophoresed on 1.3% agarose gels, transferred to Duralon nylon membranes (Stratagene), and hybridized (16) to a 199-bp *Eco*RV–*Xba* I fragment [385–186 map units (mu); see Fig. 1*A*] that was labeled with ^{32}P by random priming (17). Isolated chromatin was cleaved in a dose-dependent manner by micrococcal nuclease, and control chromatin samples incubated without

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Abbreviation: mu, map units.

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the enzyme were intact, indicating the absence of contaminating endogenous nucleases (data not shown).

Restriction Endonuclease Digestions. Total genomic DNA was rapidly isolated (18) from 10–20 ml of early- to midlogarithmic phase cultures (absorbance at 600 nm of 1.0–1.6) grown in Trp$^-$ selective media (synthetic defined medium plus 0.5% Casamino acids). One-third of the genomic DNA purified from each culture was first digested with *Eco*RI (TALS-2) or *Hin*dIII (TALS-3 and TALS-3.5) to linearize the plasmids. Subsequently, samples were treated with either no secondary enzyme, *Dpn* I, *Dpn* II, or *Sau*3A1 (20 units per approximately 0.25–0.8 μg of DNA for 4–5 hr). As a control to monitor the extent of secondary enzyme digestion, 50 ng each of pBluescript KS+ (Stratagene) and pBR322 isolated from *dam*$^+$ (XL1-Blue; Stratagene) and *dam*$^-$ (GM2163; New England Biolabs) *E. coli* strains, respectively, were added to the genomic DNA prior to *Eco*RI digestion. Although *Dpn* I cleavage is reported to be specific for dimethylated GATC sites (19, 20), our extensive *Dpn* I digestion conditions also yielded cleavage of hemimethylated and unmethylated substrates. These activities were present in all lots of *Dpn* I (New England Biolabs) utilized, albeit at varying levels. Under our conditions, cutting by *Dpn* II was exclusive to unmethylated GATC sites (data not shown) (20).

Primer Extension. One-tenth of each digested genomic DNA sample was then cleaved with *Rsa* I and used for primer extension as in Shimizu *et al.* (8) except that the primer was hybridized at 58°C and extended at 72°C for only 1 min. The radiolabeled primer-extension products were resolved on a denaturing 6% polyacrylamide/50% (wt/vol) urea gel. The primer used for extension corresponds to the bottom strand of the α2 operator, TGCCATGTAATTACCTAATAGGGAAATTTACACG.

Slot Blot Hybridization. The remaining fraction of each digested genomic DNA sample was denatured and applied to GeneScreen*Plus* membranes (New England Nuclear) for slot blot analysis. Oligonucleotide probes for bottom strand cleavage at each GATC site are (*i*) 854 (*ARS1*), CAAGCATAAAAGATCTAAACATAAAA; 955, GCAGGGGGTTGATCTTTACCATTTC; 1369, ATCAATGCGAGATCCGTTTAACCG; 1383, CGTTTAACCGGATCCTAGTGCACT; 1395, CCCTAGTGCGGATCCCCCACGTTC; 1405, CTTACCCCACGATCGGTCCACTG; 1416, TTCGGTCCACGATCTGCCGGACGC; 1423, ACTGTGTGCCGATCATGCTCCTTC; 1445, CTTCACTATTTGATCATGTGGA; 1454, TCATGTGGATCGAGCTCGA; and 1464, TCGAGCTCGATCGAAAATGCT for TALS-derived plasmids; (*ii*) an equimolar mixture of GGAAGGGCGATCGGTGCGGG and AGAACTAGTGGATCCCCCGGGCT for pBluescript KS+; and (*iii*) an equimolar mixture of CCGTCCTGTGGATCCTCTACGCCG and GATGGGGAAGATCGGGCTCGCCA for pBR322. Oligonucleotide probes for top strand cleavage are the complementary sequence. Oligonucleotides were end labeled with ^{32}P to a specific activity of 1–2 × 10^8 cpm/μg and hybridized (16) at 10°C below t_m as calculated (21) for 4–16 hr. After hybridization, the membranes were washed twice at room temperature for 5 min followed by one wash at 10°C below t_m for 2–3 min with 2× SSC (0.3 M NaCl/0.03 M sodium citrate, pH 7.0). The slot blot data were authenticated by primer extension as described above, which yielded identical values of cleavage for several analyzed samples.

RESULTS AND DISCUSSION

To assess how a nucleosome that is precisely positioned both translationally and rotationally would affect *in vivo* modification of DNA by Dam methyltransferase, we introduced a series of TALS-derived plasmids (Fig. 1) into *dam*-expressing strains of *S. cerevisiae*. Each TALS variant contains a number of reporter GATC sites that were introduced at precise locations relative to the edge of nucleosome IV.

To demonstrate that the new TALS-derived constructs maintain strict positioning of nucleosome IV in α cells, we prepared spheroplast lysates that contain minichromosomes (13), digested them with micrococcal nuclease, and mapped the nuclease cleavage sites by indirect end labeling (Fig. 2) (14, 15). In this technique, sites cleaved by micrococcal nuclease in chromatin and in naked DNA are mapped relative to a common restriction endonuclease cleavage site. Nucleosome positions are inferred from regions of at least 140 bp (146 bp of DNA is associated with a nucleosome) that are protected from micrococcal nuclease digestion in chromatin

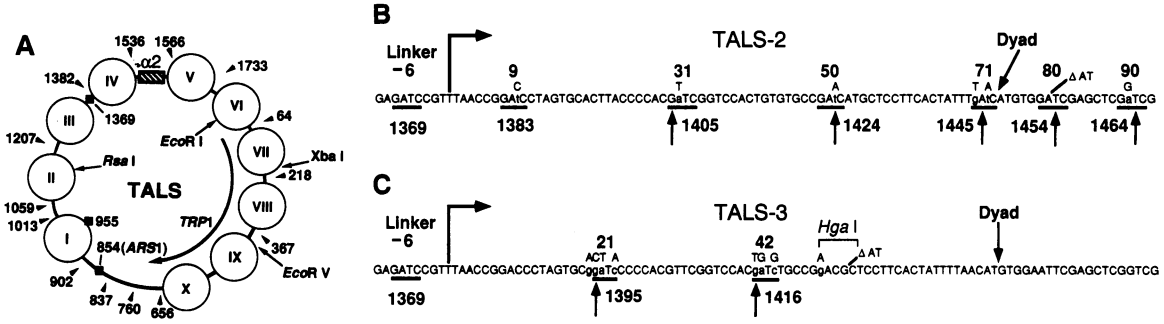

FIG. 1. Chromatin structure of the TALS plasmids in α cells. (*A*) Nucleosome positions as determined by indirect end labeling are shown (7). The locations of micrococcal nuclease cut sites (arrowheads) in chromatin are given in mu, where position 1 is at the *Eco*RI site within nucleosome VI. The α2 operator (α2) is indicated by the hatched box. The locations of GATC sites (except those in nucleosome IV; see *B* and *C*), which are potential targets of the Dam methyltransferase, are marked by solid boxes; the sites at 854 (within the autonomously replicating sequences, *ARS1*) and 1369 mu (internucleosomal linker) are naturally occurring and thus present in all TALS-derived plasmids, whereas that at 955 mu was introduced by changing C 956 and T 957 to T and C, respectively, near the pseudodyad of nucleosome I by PCR mutagenesis (11) to create TALS-955. The selective marker, *TRP1*, is indicated by the curved arrow. (*B* and *C*) Partial nucleotide sequences of the nucleosome IV region in TALS-2 and TALS-3, respectively. Only the top DNA strand is shown. TALS-2 and TALS-3 were derived from the parent plasmid, TALS, to create (11) six and two new GATC sites for potential Dam methylation, respectively (underlined). Bases within TALS-2 or TALS-3 that represent changes from TALS are indicated in lowercase. The ΔAT indicates a dinucleotide deletion that, in the case of TALS-2, destroys the *Eco*RI site within nucleosome IV of TALS. As determined by indirect end labeling, the positions of nucleosomes I–VII in the three TALS-derived constructs are indistinguishable from those in the parent TALS plasmid (Fig. 2). Each inserted GATC site corresponds to a site of DNase I cleavage (indicated by arrows below the sequence) within the exposed minor groove on the surface of the nucleosome (edge demarcated by bent arrow) (8). The numbers above and below the sequence refer to the number of bases from the upstream edge of the nucleosome IV and the map coordinates, respectively, that the adenine within each GATC site occupies in α cells.

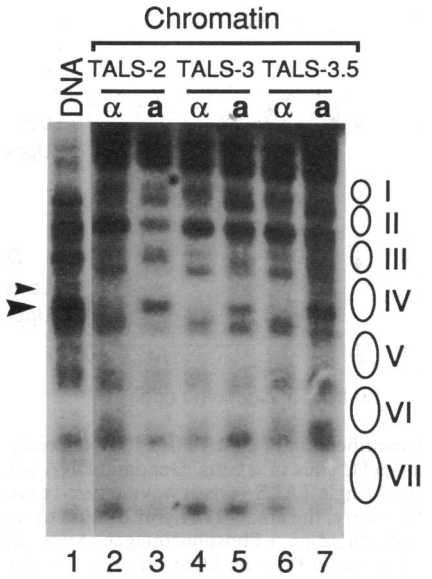

FIG. 2. Chromatin structure of the TALS plasmids in α and **a** cells. Micrococcal nuclease cleavage sites in chromatin (lanes 2–7) and TALS-2 DNA (lane 1) were mapped relative to the unique *Eco*RV site (385 mu) (see Fig. 1*A*). Samples were treated with micrococcal nuclease at 3 units/ml (lane 1) or 60 units/ml (lanes 2–7) at 37°C for 10 min. Locations of nucleosomes I–VII are indicated as ellipses at the right. In particular, the arrowheads to the left of the gel indicate two micrococcal nuclease cleavage sites present in naked DNA that are protected by nucleosome IV in chromatin isolated from α-cell (lanes 2, 4, and 6) but not in **a**-cell (lanes 3, 5, and 7) chromatin.

(Fig. 2, lanes 2–7) but not in naked DNA (Fig. 2, lane 1). In Fig. 2, for all three TALS-derived minichromosomes, it is clear that two sites cleaved by micrococcal nuclease in naked DNA (bands marked on the left by arrowheads) are protected by nucleosome IV, which is precisely positioned in α cells (Fig. 2, lanes 2, 4, and 6) but not in **a** cells (Fig. 2, lanes 3, 5, and 7). For these new DNA sequences, and in other cases where completely foreign sequences are located next to the α2 operator (22–24), a nucleosome remains precisely positioned in α cells.

We assessed the extent of *in vivo* methylation at GATC sites by restriction endonuclease cleavage followed by primer extension (Fig. 3). In α cells, where nucleosome IV is precisely positioned on the TALS-2 DNA, *Dpn* I, which primarily (see *Materials and Methods*) cuts dimethylated substrates, cleaved relatively few molecules at five GATC sites located at map positions 1405–1464 or 31–90 bp interior to the nucleosome (Fig. 3, lane 8). Thus sites more than 30 bp away from the linker appear refractory to Dam methyltransferase activity. The lack of cleavage at these sites was not due to partial activity of *Dpn* I since control DNA in the same reaction was cut to completion (see *Materials and Methods*; see Fig. 4). Moreover, at 1464 mu, the large amount of cleavage by *Dpn* II (which cuts only unmethylated sites) in the same sample demonstrates the near quantitative absence of both dimethylation and hemimethylation (Fig. 3, lane 6). In contrast, most of the TALS-2 DNA in α cells was digested by *Dpn* I at 1383 mu, indicating a high degree of methylation (Fig. 3, lane 8). The extensive *in vivo* methylation of this site near the edge of nucleosome IV shows that the lack of methylation at the pseudodyad is not due to insufficient levels of methyltransferase activity.

In contrast to the above data, *Dpn* I cleaved most of the TALS-2 molecules at 1464 mu in DNA isolated from **a** cells (Fig. 3, lane 7). This high degree of methylation is consistent with the lack of a stably positioned nucleosome adjacent to the α2 operator (7–9). This result also rules out the possibility

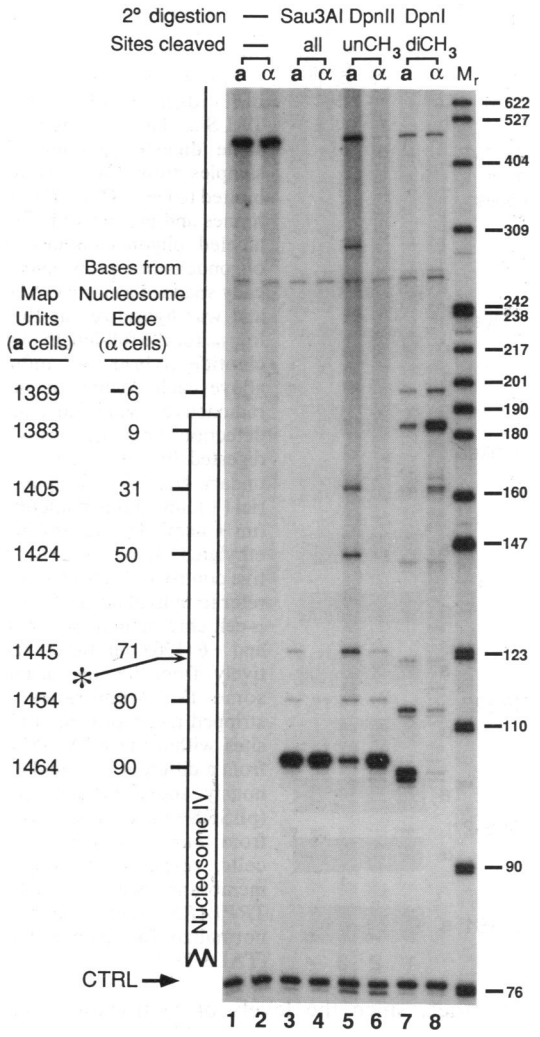

FIG. 3. Primer extension analysis of Dam methylation in the TALS-2 plasmid. Total genomic DNA from *dam*-expressing **a** (UCC1023) and α (MKY2) cells harboring TALS-2 was treated as indicated (see *Materials and Methods*): no secondary enzyme (—); *Dpn* I, which primarily (see *Materials and Methods*) digests GATC sites methylated on both strands (dimethylated; $diCH_3$); *Dpn* II, which cleaves unmethylated ($unCH_3$) sites; or *Sau*3A1, which digests sites irrespective of their methylation state (all) (19, 20, 25). In the absence of secondary enzyme cleavage, primer extension (8) from the α2 operator primer creates the 467-base product at the top of the gel due to runoff at the cleaved *Rsa* I site (1097 mu) (lanes 1 and 2). The lower molecular weight bands are generated by runoff at secondarily cleaved GATC sites (labeled on the left to correspond to Fig. 1) within nucleosome IV (pseudodyad marked by asterisk). Digestion with *Sau*3A1 (lanes 3 and 4) demonstrates the result that is expected if the first GATC site encountered by the primer (at 1464 mu) is quantitatively cleaved. The offset of the extension ladders by 2 bases is due to the different overhangs created by the various enzymes (*Dpn* I versus *Dpn* II and *Sau*3A1). To control for sample loading and primer extension efficiency, a second primer (MS6) (8) was included as an internal control to produce a 76-base product (arrow labeled as CTRL) due to runoff at a unique *Eco*RI site (1 mu). The inclusion of primer MS6 did not yield different results from those obtained with the α2 operator primer alone. The molecular weight ladder (M_r) is pBR322 digested with *Msp* I.

that the lack of *Dpn* I digestion at 1464 mu in the DNA isolated from α cells was due to site preference in naked DNA for either Dam methyltransferase or *Dpn* I endonuclease activity. Taken together, the above results demonstrate that the *in vivo* methylation of certain GATC sites within this region of TALS-2 correlates with the presence (in α cells) or absence (in **a** cells) of a translationally positioned nucleo-

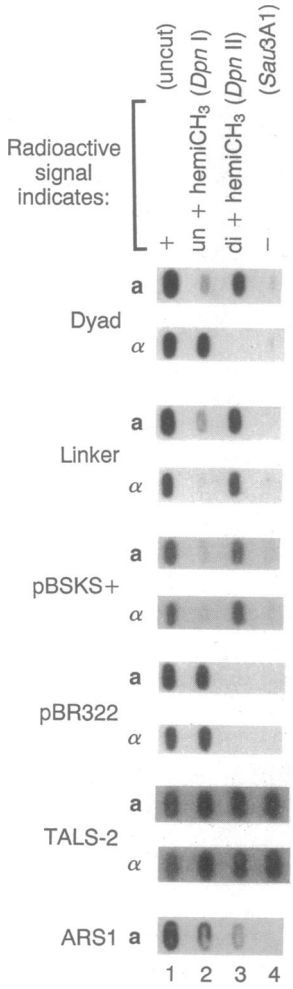

FIG. 4. Slot blot analysis of individual GATC sites in TALS-2. The remainder of the same digested genomic DNA samples from Fig. 3 were slot blotted to GeneScreen*Plus* membranes and probed with ^{32}P end-labeled oligonucleotides. Each oligonucleotide probe symmetrically spanned a single GATC site and was hybridized at 10°C below t_m for the full-length oligonucleotide hybrid. As indicated above each column of slots, a radioactive signal indicates the detection of material that was not digested by the respective secondary enzyme: all input material (+); un- and hemimethylated (un + hemiCH_3); di- and hemimethylated (di + hemiCH_3); negative control (−). Dyad and linker refer to individual GATC sites in α-cell chromatin at 80 (1454 mu) and −6 (1369 mu) bases, respectively, from the edge of nucleosome IV. Membranes were stripped and reprobed at GATC sites within the *ARS1* (854 mu, from a different blot) and exogenously added pBluescriptKS+ (pBSKS+) and pBR322 isolated from *dam*$^+$ and *dam*$^-$ *E. coli* cells, respectively. Last, the membranes were probed with *TRP1ARS1* (26) sequences to normalize for sample loading (TALS-2).

some. Further, since the levels of methylation near the pseudodyad are inversely related to the presence of a translationally positioned nucleosome, it is likely that positioned nucleosomes restrict the access of the Dam methyltransferase to its specific DNA-binding site. However, it is formally possible that histone–DNA contacts in the nucleosome interfere with the catalytic methylation of GATC sites.

To rigorously quantify the degree of *in vivo* methylation at each GATC site independent of both neighboring GATC sites and convenient restriction sites, we developed an oligonucleotide-probing procedure (Fig. 4). In this approach, a radiolabeled oligonucleotide probe spanning each GATC site hybridizes only to uncleaved sites to produce an autoradiographic signal. Treatment of each sample with *Sau*3A1 (Fig. 4, lane 4), which is insensitive to methylation (25), abolishes most of the signal (compare to untreated samples Fig. 4, lane 1; except for the loading control marked TALS-2).

A GATC site near the pseudodyad (1454 mu) of nucleosome IV (Fig. 4, Dyad) was quantitatively cleaved by *Dpn* II in DNA isolated from α cells. Thus, this site was unmethylated in virtually all of the minichromosomes *in vivo*, demonstrating the complete inhibition of methyltransferase DNA modification by the histone–DNA contacts in this region. The absence of dimethylation at this site was corroborated by the lack of *Dpn* I cutting (lane 2). In contrast, the same GATC site was strongly methylated in a cells, consistent with the absence of positioned nucleosome IV.

The linker region (1369 mu) between nucleosomes III and IV (Fig. 4, Linker) exhibited the converse of the dyad results: In α cells, ≈100% of the molecules were cleaved at this site by *Dpn* I, whereas only a small fraction was cleaved by *Dpn* II, indicating that most of the minichromosomes were methylated *in vivo* in the linker region. Thus, the absence of histone–DNA contacts within the internucleosomal linker permits access and modification by Dam methyltransferase.

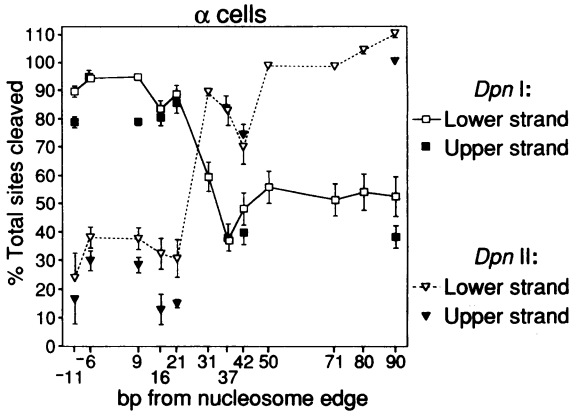

FIG. 5. Quantification of methylation levels of each DNA strand at individual GATC sites in α cells. Genomic DNA isolated from α cells harboring a single TALS variant was analyzed exactly as in Fig. 4. To assure linearity, the amount of hybridization to each treatment sample was quantified on a PhosphorImager. After subtracting the membrane background from an identical area that did not contain sample, the specific signal in each sample (uncut, *Dpn* I-treated, or *Dpn* II-treated) was obtained by subtracting the *Sau*3A1 signal, which represents nonspecific oligonucleotide binding (≤10% for every data point) to genomic DNA. Data points are reported as the mean ± SD (minimum of three independent experiments utilizing two different α-cell transformants for each construct).

To more precisely delimit the region of the nucleosome that is accessible to the methyltransferase, we constructed TALS-3 (Fig. 1*C*). In DNA isolated from α cells, the GATC site introduced at 21 bp (1395 mu) from the edge of nucleosome IV was as highly methylated as were more linker-proximal sites in TALS-2 (Fig. 5). In contrast, sites beyond 30 bp from the edge of nucleosome IV were relatively refractory to methylation. Furthermore, the sites at 50–90 bp from the nucleosome's edge were not appreciably dimethylated or hemimethylated, as evidenced by quantitative *Dpn* II cleavage. The significant amount of *Dpn* I cutting observed in Fig. 5 is probably due to cleavage of unmethylated GATC sites by the particular lot of enzyme that was used (see *Materials and Methods*). This is further supported by the fact that the cumulative cleavage at each GATC site by both enzymes exceeds 100%. Although we observed some strand variation in the level of endonuclease cleavage, the regions of differential methyltransferase accessibility in the nucleosome remained evident. Cleavage of DNA isolated from a cells for GATC sites from 1369 to 1464 mu indicated that, with some variability, all sites were accessible to the methyltransferase, consistent with the lack of precisely located nucleosomes (total sites cleaved: *Dpn* I, 78.6% ± 5.6%, range = 64.5–85.9%; *Dpn* II, 33.3% ± 17.2%, range = 11.6–67.2%).†

To investigate the effect of rotational positioning on methyltransferase accessibility, we shifted the GATC sites in TALS-3 closer to the linker by 5 bp, to create TALS-3.5 (Fig. 1 legend; *Materials and Methods*). In α cells, the A-6 position of each GATC in nucleosome IV on TALS-3.5 is in the major groove facing the solution and projecting away from the histone octamer surface, whereas in TALS-3, the A-6 position projects toward the octamer. In contrast to the pronounced effect of translational positioning on methyltransferase accessibility, altering rotational positioning of GATC sites did not affect their methylation levels [Fig. 5, compare

†Mean ± SD for all data points indicated in Fig. 5 from a minimum of three independent experiments with two different a-cell transformants.

points at 16 and 37 bp (TALS-3.5), respectively, to 21 and 42 bp (TALS-3) from the nucleosome edge.]

To assay a nucleosome that is positioned by an α2-independent mechanism, we constructed TALS-955 (11) by introducing a GATC site near the pseudodyad of nucleosome I in TALS (7–9) (see Fig. 1). Similar to nucleosome IV, in α cells, *Dpn* II cleaved 83.8% ± 2.6% (range = 79.2–88.7% assaying both DNA strands)‡ of the DNA molecules at this site. This suggests that the inaccessibility of the central region of nucleosome-associated DNA to Dam methyltransferase is a general property of positioned nucleosomes.

At the autonomously replicating sequence (*ARS1*; 854 mu) of the three TALS-derived constructs, *Dpn* I cleaved only 53.6% ± 6.9% (range = 42.8–68.8%)‡ of the GATC sites in α cells and only 42.5% ± 6.0% (range = 33.4–53.5%)‡ in **a** cells even though this region is hypersensitive to micrococcal nuclease (26, 27). This is in contrast to the ≈80% *Dpn* I cleavage observed for GATC sites from 1369 to 1464 mu on the same **a**-cell blots (see above). Presumably, the GATC site at *ARS1* is protected from methylation by the recently identified origin recognition complex (28, 29). This significant protection from methylation, even in logarithmic-phase growing cells, suggests that components of the origin recognition complex remain stably bound at the *ARS1* throughout both the cell cycle and the duration of the experiment. In other *in vivo* experiments, DNA-bound GAL4/GAL80 also inhibited Dam methylation at an adjacent GATC site (R. H. Morse, personal communication).

Our results indicate that at least the central 84 bp of DNA bound by the histone octamer are refractory to methyltransferase activity. Histone–DNA contacts near the nucleosomal pseudodyad appear sufficient to prevent the methyltransferase from binding and/or modifying GATC sites. More generally, these data indicate that sequence-specific DNA-binding proteins can potentially be excluded from their recognition sequences near the nucleosomal pseudodyad and support the hypothesis that nucleosomes might suppress the biological functions of DNA by restricting access of trans-acting factors to DNA (22, 30–32).

In contrast, GATC sites in the linker and adjacent 21 bp of DNA in nucleosome IV of the TALS variants are highly methylated by Dam methyltransferase *in vivo*. This is consistent with other studies that indicate an increased deformability of sequences at the edge of, or between, core particles (33–36). While our data do not address the influences of more highly organized chromatin structure(s) on the accessibility of specific sequences to Dam methyltransferase, other studies indicate that transcriptionally silent domains in yeast are refractory to methylation *in vivo* (1–3). Thus, as yet undefined chromatin structures can modify the accessibility of specific GATC sites. We also found that, in addition to the histone octamer ($M_r \approx 100{,}000$), the origin recognition complex ($M_r \approx 250{,}000$) was able to significantly inhibit methylation at the *ARS1* element. Further studies are needed to determine whether other trans-acting complexes (e.g., GAL4/GAL80) in addition to nucleosomes and the *ARS* origin recognition complex can also interfere with the enzyme's ability to access and/or methylate DNA.

Our finding that certain sites remain accessible to Dam methyltransferase even in the context of a highly organized region of chromatin stresses the need for caution in interpreting accessibility of sites in less well-characterized domains; methylation of a given site should not be equated with the absence of a nucleosome, since our data indicate that methylation can occur at the termini of stably positioned nucleosomes. Used in conjunction with existing methods for studying chromatin, *in vivo* modification by the Dam methyltransferase provides a valuable, noninvasive methodology.

‡Mean ± SD for a minimum of three independent experiments with two different yeast cell transformants.

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