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Cytosine Methylation Enhances Z-DNA Formation In Vivo

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The influence of cytosine methylation on the supercoil-stabilized B-Z equilibrium in *Escherichia coli* was analyzed by two independent assays. Both the M · *Eco*RI inhibition assay and the linking-number assay have been used previously to establish that dC-dG segments of sufficient lengths can exist as left-handed helices in vivo. A series of dC-dG plasmid inserts with Z-form potential, ranging in length from 14 to 74 base pairs, was investigated. Complete methylation of cytosine at all *Hha*I sites, including the inserts, was obtained by coexpression of the *Hha*I methyltransferase (M · *Hha*I) in cells also carrying a dC-dG-containing plasmid. Both assays showed that for all lengths of dC-dG inserts, the relative amounts of B and Z helices were shifted to more Z-DNA in the presence of M · *Hha*I than in the absence of M · *Hha*I. These results indicate that cytosine methylation enhances the formation of Z-DNA helices at the superhelix density present in *E. coli*. The B-Z equilibrium, in combination with site-specific base methylation, may constitute a concerted mechanism for the modulation of DNA topology and DNA-protein interactions.

DNA structural microheterogeneity is believed to play a fundamental role in genetic regulatory mechanisms (16, 20). Left-handed DNA (Z-DNA) is a structural alternative to right-handed DNA (B-DNA) that has been well characterized in vitro (14, 17, 21). Recently, it was demonstrated that Z-DNA can exist in living *Escherichia coli* cells and that a given sequence can coexist in the Z form and the B form in the same cell (7, 8, 15, 22, 23). The in vivo B-Z equilibrium is influenced by active biological processes (like transcription, replication, supercoil and toroid formations, and DNA-protein interactions).

There is evidence that cytosine methylation may have an important signaling function in eucaryotic gene expression (4, 11). The hypomethylation of gene regulatory regions is often connected to an expressed state of those genes, whereas silent genes are generally overmethylated. In vitro, cytosine methylation also facilitates the B-to-Z transition in synthetic linear DNA polymers (1, 2, 5) and in certain sequences in supercoiled recombinant plasmids (10, 25).

In previous studies, we employed two independent assays for structural analyses under in vivo conditions. The first assay was based on the observation that a recognition sequence (GAATTC) is not methylated by the corresponding bacterial methyltransferase (M · *Eco*RI) when this site is near or inside a left-handed helix, whereas other *Eco*RI sites in the same plasmid molecule were completely methylated (8, 23). The presence of a Z structure around the unmethylated sites was confirmed by a variety of chemical, enzymatic, and topological approaches (14, 16, 17, 21, 24).

Lack of methylation by a temperature-sensitive M · *Eco*RI of *Eco*RI sites in suitable plasmid constructs, as detected by *Eco*RI restriction digests of purified DNAs, revealed the presence of a left-handed helix inside *E. coli* cells.

The second assay was based on the well-characterized topological changes in plasmids that have undergone a B-to-Z DNA structural transition with helix unwinding (23, 25). Z-DNA formation in circular plasmids causes the relaxation of negative supercoils. The amount of relaxation indicates the type of structural change, since approximately two supercoils are lost for each helix turn of B-DNA undergoing the transition (14, 25).

On this basis, the formation of Z-DNA inside growing bacterial cells could be analyzed by a linking-number assay. The B-to-Z transition inside *E. coli* cells results in the relaxation of a number of supercoils, in proportion to the length of the Z-region involved. This process causes DNA gyrase to decrease the linking number until the original superhelix density is reestablished. Analysis of purified native DNA on gels containing the intercalator chloroquine revealed the distribution of linking numbers generated in vivo. Thus, the appearance of a second population of topoisomers with more-negative linking numbers or the complete shift of a topoisomer population relative to a control plasmid indicated the formation of Z-DNA in vivo (7, 23).

The non-B conformations detected by a combination of both in vivo assays were indeed left-handed Z-DNA, as revealed by the close agreement (i) between the extents of inhibition of methylation by M · *Eco*RI in *E. coli* and the capacities of the inserted sequences to form Z helices in vitro and (ii) between the extents of relaxation measured by the in vivo linking-number assay and the results of in vitro two-dimensional gel electrophoresis (14, 23, 25).

Herein, we have analyzed the influence of cytosine methylation on the supercoil-stabilized B-Z equilibrium in vivo. The M · *Eco*RI inhibition assay and the linking-number assay were used for DNA structural analyses inside living *E. coli* both in the presence and in the absence of a constitutively expressed M · *Hha*I. Cytosine methylation had a stabilizing effect on supercoil-induced Z-DNA formation in vivo that was similar to its enhancing effect in vitro.

MATERIALS AND METHODS

Plasmids. The constructions and sequence features of the plasmids used were described previously for pRW1567, pRW1557, pRW1563, pRW1561, pRW1554, pRW478, pRW1556, pRW1558, pRW1559, and pRW1564 (23) and pRW1602 (8). pRW1560 is pBR322 with an 8-base-pair (bp) *Bgl*II linker cloned into the filled-in *Eco*RI site (23). pRW1610 was constructed by inserting a 1,476-bp *Hin*dIII

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fragment containing the M · *Hha*I gene (3) into the *Hin*dIII site of pLV59 (13, 18), thus inactivating the *Eco*RI restriction enzyme gene. The M · *Hha*I gene was constitutively expressed from its own promoter.

All cotransformants containing either pRW1602 or pRW1610 (both pACYC184 derivatives) in addition to one of the Z-DNA-containing pBR322 derivatives were grown in *E. coli* HB101 with double selection with ampicillin and chloramphenicol. Plasmids were isolated by the phenol-lysozyme method and purified by two CsCl gradient centrifugations as described previously (8, 23).

Chemicals and enzymes. Chloramphenicol and chloroquine phosphate were obtained from Sigma Chemical Co. Restriction and DNA-modifying enzymes were from New England BioLabs, Inc., and Boehringer Mannheim.

Kinetics of in vivo methylation by M · *Eco*RI. Experiments to determine the time courses of in vivo methylation of *Eco*RI sites were performed as described previously (23). At both the nonpermissive (42°C) and the permissive (22°C) temperatures, all *Hha*I sites were fully methylated in vivo by the M · *Hha*I expressed from pRW1610, as determined by restriction digests with *Hha*I or with *Pst*I plus *Bss*HII of purified plasmids (19, 24).

Linking-number assays. Plasmid DNAs for the linking-number assays were prepared as described previously (23). After growth at 42°C to an optical density at 550 nm of 1.0, the cultures were shifted to 22°C and grown for an additional 6 h to maintain the same growth conditions as for the M · *Eco*RI inhibition assays. The distributions of linking numbers in the purified native DNAs were analyzed on 0.8% agarose gels run at 60 V for 48 h in 1× TBE buffer (89 mM Tris-borate, 2 mM EDTA [pH 8.3]) containing 4 μm chloroquine in the gel, buffer, and loading samples. After several 30-min washes in water to dilute out the chloroquine, the gels were stained with ethidium bromide and photographed under UV illumination with Polaroid type 55 negative film. The negatives were traced with a model 620 videodensitometer (Bio-Rad Laboratories).

RESULTS

Rationale. Two different approaches were employed to study the influence of cytosine methylation on the B-Z equilibrium in living *E. coli*. The M · *Eco*RI inhibition assay and linking-number assay enabled us previously to detect the presence of Z-DNA and to estimate the relative amounts of Z-DNA versus B-DNA in recombinant plasmid inserts in vivo.

In the present study, both assays were performed in *E. coli* HB101 with a variety of pBR322 derivatives containing dC-dG inserts of various lengths (14 to 74 bp). *Eco*RI recognition sites were located either inside the dC-dG segment or at the junction between insert and vector DNA (Fig. 1). A temperature-sensitive M · *Eco*RI activity was introduced into *E. coli* HB101 with the pACYC184 derivative pRW1602 or pRW1610 (Fig. 1), both of which express an active M · *Eco*RI protein at 30°C or lower (8, 23). In addition, pRW1610 also contained the gene for M · *Hha*I which is constitutively expressed from its own promoter. M · *Hha*I fully methylated all *Hha*I sites [d(GCGC)] on plasmids in vivo, even at the nonpermissive temperature for M · *Eco*RI (42°C).

Inhibition of methylation by M · *Eco*RI in the absence and presence of M · *Hha*I. *E. coli* HB101 was cotransformed with either pRW1602 or pRW1610 and each of the dC-dG containing plasmids listed in Fig. 1. The quantitation of Z-DNA

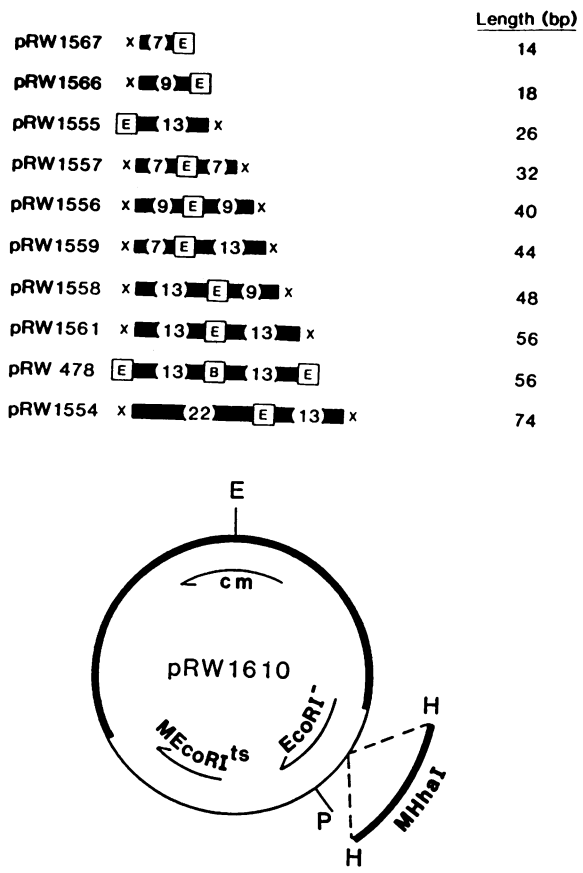

FIG. 1. Plasmid inserts used in this study. The numbers in black boxes indicate the numbers of dC-dG dinucleotide repeat units. When E or B sites are internal to a dC-dG region, 4 bp (AATT or GATC, respectively) are added to the total length. All inserts were cloned in the *Eco*RI site of pBR322 or the *Bgl*II site of pRW1560. The inserts are not drawn to scale. Sites: E, *Eco*RI; B, *Bam*HI; X, *Xho*II; H, *Hin*dIII; P, *Pvu*II. A map of pRW1610 carrying both the gene for a temperature-sensitive *Eco*RI methylase (M*Eco*RI^{ts}) and the gene for the *Hha*I methylase (M*Hha*I) inserted into the *Eco*RI restriction enzyme gene (EcoRI$^-$) is also shown. cm, Chloramphenicol resistance gene.

formation in vivo with M · *Eco*RI as a structural probe was performed as described previously (23). After growth of the cotransformants at the nonpermissive temperature (42°C) to an optical density at 550 nm of 1.0, the cultures were shifted to the permissive temperature (22°C) and grown for various periods of time. The plasmids were purified and analyzed in vitro by digestion with *Pst*I and *Eco*RI to quantitate the extent of methylation in vivo at *Eco*RI sites in the inserts, which reflects the amount of Z-DNA.

The time courses of in vivo M · *Eco*RI methylation for inserts of various lengths in seven plasmids grown in the presence of M · *Hha*I were determined (Fig. 2). Also, pRW1610, which contains one isolated *Eco*RI site surrounded by random-sequence DNA, was included as a control for the kinetics of methylation of an unperturbed *Eco*RI site. For each plasmid, the percentage of methylation (relative to the total amount of plasmid) was plotted as a function of time at the permissive temperature. The rate of in vivo M · *Eco*RI methylation at the target sites decreased substantially as the length of the insert increased. The control plasmid pRW1610 was fully methylated at the *Eco*RI

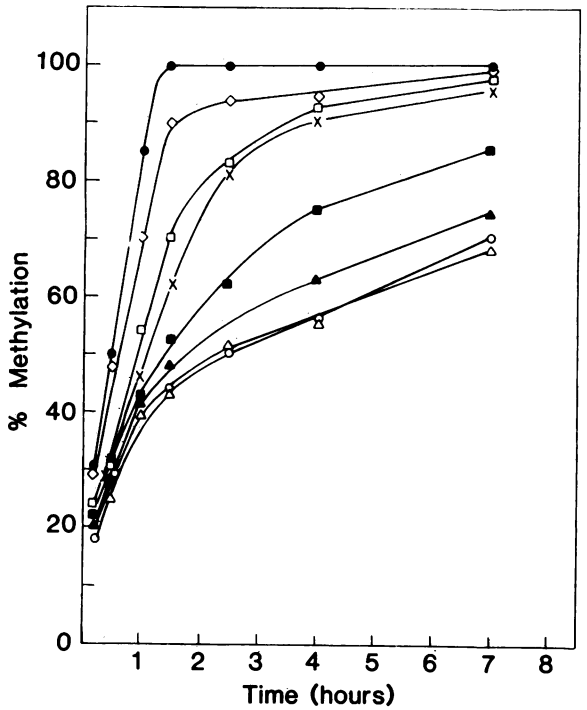

FIG. 2. Kinetics of in vivo methylation by M · *Eco*RI (the quantitation of in vivo methylation at *Eco*RI sites is described in Materials and Methods). The percentage of methylated plasmid (relative to the total amount of plasmid DNA) was plotted as a function of growth time at the permissive temperature (22°C). *E. coli* HB101 cells were transformed with pRW1610 alone (●) or cotransformed with pRW1610 and pRW1567 (◇), pRW1556 (□), pRW1559 (×), pRW1558 (■), pRW1554 (▲), pRW1561 (○), or pRW478 (△). Each point is the average of three to five determinations, with deviations of 6 to 7% between individual measurements.

sites after approximately 1 h, whereas plasmids with a 56- or 74-bp dC-dG insert were methylated to only approximately 60 to 70%, even after 6 to 7 h. A qualitatively similar behavior was observed recently when pRW1602 instead of pRW1610 was used as the M · *Eco*RI-carrying plasmid (23).

To evaluate the contribution of cytosine methylation to the kinetic behavior of the plasmids examined in Fig. 2, we compared the time courses obtained for pRW1610 cotransformants (i.e., with the M · *Hha*I expressed in the same cell) with those obtained for pRW1602 cotransformants (i.e., in the absence of M · *Hha*I). The extent of methylation (relative to that of the control pRW1602 or pRW1610, which was taken as 100%) after 1 h of growth at the permissive temperature was plotted as a function of the lengths of the Z forming inserts (Fig. 3). The curve for the pRW1610 cotransformants was generally shifted to the left by approximately 10 to 15 bp relative to the curve for the pRW1602 cotransformants. This indicates that the presence of M · *Hha*I in the cell has an inhibitory effect on methylation by M · *Eco*RI for all of the insert lengths examined. Also, the plateau that was reached in both cases when insert lengths were 56 or more bp was reached at a significantly lower level of relative methylation (45%) in the presence of M · *Hha*I than in the absence of M · *Hha*I (60%). We interpret this as a reflection of a longer lifetime for the Z conformation, especially for the longer inserts, in which the cytosines in the dC-dG regions were methylated by M · *Hha*I. Thus, cytosine methylation has a stabilizing effect on supercoil-induced Z-DNA formation in vivo (Fig. 3) similar to that observed in vitro (10, 25).

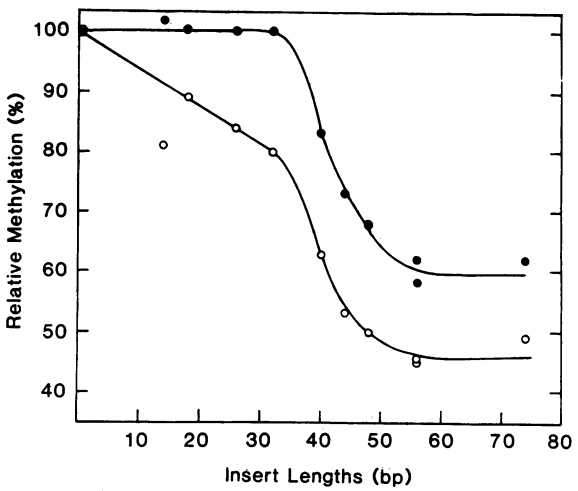

FIG. 3. Extent of in vivo methylation by M · *Eco*RI as a function of the lengths of the dC-dG tracts in the plasmid inserts. The percentage of methylation was calculated from the data in Fig. 2 by defining the value for pRW1610 as 100%. The values for the 60-min points were used. Symbols: ○, cotransformants with pRW1610; ●, cotransformants with pRW1602 (the latter data were taken from reference 23).

Effects of M · *Hha*I expression on in vivo linking-number distributions. A linking-number assay was previously employed to demonstrate the formation of Z-DNA helices in supercoiled plasmids in *E. coli* (7, 23). The topoisomer distributions of pRW1602 and pRW1610 cotransformants, grown in *E. coli* HB101 in the same way as for the M · *Eco*RI inhibition studies, were analyzed after separation on agarose gels containing chloroquine and densitometric tracing of the negative-gel photographs. The native topoisomer distributions of various pairs of cotransformants were resolved on agarose gels containing chloroquine (Fig. 4). In each lane, the lowest topoisomer family, belonging to the pBR322 derivative with dC-dG insert, shows significant variations, depending on the length of the insert and the presence or absence of M · *Hha*I. However, the positions of the pRW1602 and pRW1610 topoisomer families, which served as internal control molecules (Fig. 4A, uppermost family in each lane), are the same among the different pairs. These topoisomer distributions were not influenced by the pBR322 derivatives cotransformed in the same host cell. Also, the topoisomer distributions of single transformants obtained with pRW1602, pRW1610, or pRW1560 were not influenced after cotransformation with a second plasmid. This indicates that a change of linking numbers in vivo was correlated with the presence of the dC-dG inserts and was not a general consequence of two plasmids residing in the same host cell.

Tracings of the native linking-number distributions were obtained for the control plasmid pRW1560 without a dC-dG insert and for three Z-DNA-containing plasmids with various lengths of dC-dG inserts (Fig. 4B).

pRW1560 formed only one linking-number distribution that was not significantly effected by M · *Hha*I. For pRW1567, with a 14-bp dC-dG insert, one population of topoisomers was observed in the absence of M · *Hha*I, in agreement with previous results (23). When cotransformed with pRW1610, however, the center of the distribution was shifted to the right by about three linking numbers, i.e., to more-negative values. This is in close agreement with the approximately 2.5 turns of relaxation expected for a 14-bp

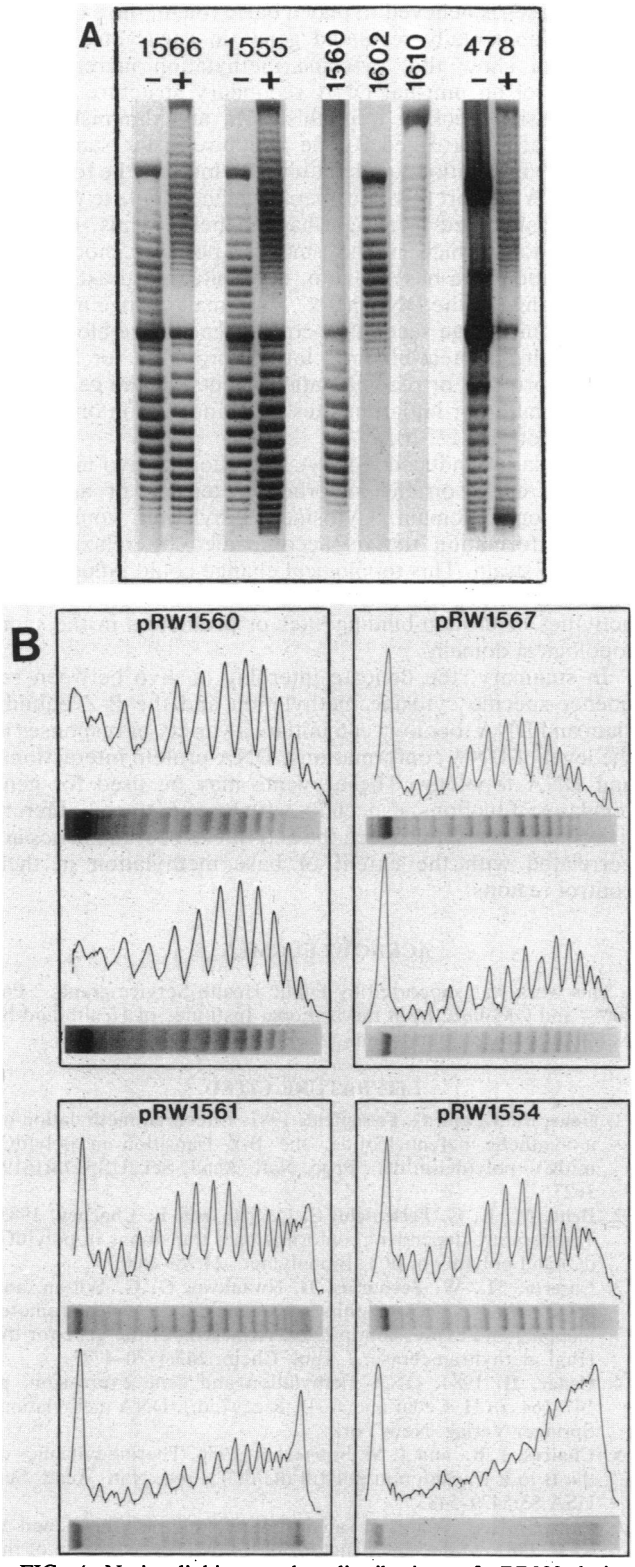

FIG. 4. Native linking-number distributions of pBR322 derivatives grown in the presence or absence of M · *Hha*I. (A) Topoisomer distributions in agarose-chloroquine gels obtained for pRW1566, pRW1555, and pRW478 containing $(dC\text{-}dG)_9$, $(dC\text{-}dG)_{13}$, and $(dC\text{-}dG)_{13}B(dC\text{-}dG)_{13}$ inserts, respectively. In lanes 1560, 1602, and 1610, control plasmid pRW1560 and plasmids pRW1602 and pRW1610 alone, respectively, are included for comparison. Gels for cotransformants with pRW1602 (no M · *Hha*I) (−) and for cotransformants with pRW1610 (with M · *Hha*I) (+) are shown. (B) Densitometric tracings of selected cotransformants. pRW1560 is the control plasmid without a dC-dG insert. pRW1567 contains $(dC\text{-}dG)_7$, pRW1561 contains $(dC\text{-}dG)_{13}E(dC\text{-}dG)_{13}$, and pRW1554 contains $(dC\text{-}dG)_{22}E(dC\text{-}dG)_{13}$ as insert. Each of these plasmids was cotransformed with either pRW1602 (top tracing and gel in each box) or pRW1610 (bottom tracing and gel in each box). The topoisomer distribution generated inside *E. coli* was analyzed on agarose-chloroquine gels, and then densitometric tracings of the negative-gel photographs were performed. Migration was from left to right. The intense peaks on the left end of each tracing correspond to the nicked plasmid. The topoisomers of pRW1602 or pRW1610 do not appear on this gel region since they are much longer than the pBR322 derivatives shown here.

Z-DNA insert. We believe that in the bottom tracing for pRW1567, a Z-DNA population, shifted by about 2.5 to 3 supercoil turns, is superimposed on the B-DNA population of the upper tracing (Fig. 4B). This shows that in the presence of M · *Hha*I, a 14-bp dC-dG insert at least partially forms a left-handed helix in vivo, whereas without M · *Hha*I, no such behavior was detected by this method.

For pRW1561 and pRW1554 (containing 56- and 74-bp inserts, respectively), bimodal topoisomer distributions were observed even in the absence of M · *Hha*I, confirming previous results (23). However, from the lower tracings in the boxes for pRW1561 and pRW1554, it is obvious that the presence of M · *Hha*I in the same cell significantly increases the intensities of the topoisomers with more-negative linking numbers (which were not resolved into individual bands under these gel conditions) (Fig. 4B). These bands represent molecules in which a B-to-Z transition had occurred inside the cell. On the other hand, the bands corresponding to the topoisomer families in the centers of the top tracings, representing molecules without a left-handed helix structure in vivo, have strongly decreased in intensity (Fig. 4B). The linking-number assay was also performed with several other plasmids (pRW1557 and pRW1563 [Fig. 1]; Fig. 4A) after cotransformation with pRW1602 or pRW1610. Although quantitative differences were observed because of the different lengths of the inserts, the in vivo behavior of these plasmids with respect to the absence or presence of M · *Hha*I was qualitatively similar to the behavior of the plasmids described above (data not shown).

These changes in the relative intensities of B- and Z-type topoisomer populations, caused by the expression of M · *Hha*I in *E. coli*, are in complete agreement with the results obtained with the M · *Eco*RI inhibition experiments described above. Both types of study indicate that cytosine methylation enhances the formation of left-handed helix structures in supercoiled plasmids in vivo.

Effects of chloramphenicol amplification. Several of the cotransformants with pRW1610 were grown at the permissive temperature for 12 to 16 h after the addition of chloramphenicol to inhibit protein synthesis. We wished to investigate whether protein depletion after cell amplification would effect the B-Z equilibrium in the presence of the M · *Hha*I gene in vivo. However, it was found that after amplification, the plasmids were partially susceptible to cleavage by *Hha*I, indicating that M · *Hha*I was no longer able to completely methylate all the *Hha*I sites in the plasmids. Therefore, this approach to measuring the in vivo effects of cytosine methylation was not applicable to this growth condition. In previous experiments which used cotransformants with pRW1602 only, no significant influence

of protein synthesis inhibition on the in vivo B-Z equilibrium was observed (23).

DISCUSSION

The effects of cytosine methylation on the supercoil-driven formation of Z-DNA in vivo were determined by two different methods. Both the M · *Eco*RI inhibition assay and the linking-number assay were used previously to establish that dC-dG regions of sufficient length can exist in left-handed helices in growing *E. coli* (8, 23).

We believe that the percentage of inhibition of methylation at *Eco*RI sites in our plasmid constructs by the temperature-sensitive M · *Eco*RI reflects the relative stabilities of the B form and Z form of the inserts inside living cells. Thus, the amount of M · *Eco*RI inhibition was used to quantitate the amount of Z form for each length of potential Z region. By cotransformation with pRW1610, which contained the genes for both M · *Eco*RI and M · *Hha*I, complete methylation of cytosines in the multiple *Hha*I sites in the dC-dG inserts was obtained.

Longer dC-dG segments containing an *Eco*RI site were methylated by M · *Eco*RI at a slower in vivo rate than were shorter segments (Fig. 2). This result qualitatively confirms data obtained previously in the absence of M · *Hha*I (23). In the presence of a constitutively expressed M · *Hha*I, cytosine methylation decreased the amount of M · *Eco*RI methylation for each length of Z-DNA insert. Whereas without M · *Hha*I a significant inhibition of M · *Eco*RI was observed only with inserts of at least approximately 40 bp in length, a measurable amount of M · *Eco*RI inhibition was observed even for the shortest insert (14 bp of dC-dG) when M · *Hha*I was also present in the cell. Thus, cytosine methylation stabilizes the formation of a Z helix even in inserts that are predominantly in a right-handed form when the dC-dG regions are not methylated.

The analysis of the plasmid linking-number distributions generated in vivo confirmed this observation. For short inserts (14, 18, and 26 bp of dC-dG), we observed a pattern in which a subpopulation of topoisomers, generated by a B-to-Z transition in vivo, was superimposed on the normal distribution of topoisomers that had not contained Z-DNA in *E. coli*.

For longer inserts (56 and 74 bp of dC-dG), a Z-helix structure is already stabilized in the absence of M · *Hha*I (23), as reflected by the bimodal topoisomer distributions in the top tracing for each plasmid (Fig. 4B). In the presence of M · *Hha*I, the population with lower linking numbers (representing molecules that had contained Z-DNA in the cell) for both plasmids greatly increased in intensity relative to the unperturbed B-form population centered at higher linking numbers. These results are in complete agreement with those of the M · *Eco*RI inhibition studies described above. They also indicate that expression of M · *Hha*I (i.e., the methylation of cytosines in the dC-dG regions) stabilizes the formation of Z-DNA inside living cells and causes a significant shift in the proportion of B-form and Z-form structures in the inserts towards more Z-DNA than B-DNA.

It is obvious that for a plasmid not containing any insert with Z-form potential (pRW1560), expression of M · *Hha*I leads to a change in the linking-number distribution of only approximately 0.5 supercoil. This shift is probably caused by the alteration of the helix repeat parameters in the multiple *Hha*I sites [d(GCGC)] in the plasmid, caused by methylation of the cytosines (6, 25).

Cytosine methylation by sequence-specific DNA methyltransferases is believed to play a basic role in the expression of developmentally regulated genes in eucaryotes (4, 11). Our data show that cytosine methylation increases the stability of an unusual DNA secondary structure in vivo, thus possibly locking it in this form and diminishing the amount of unperturbed B-type structure in the same DNA segment. In addition, methylation also induces the formation of Z-DNA in short DNA segments which otherwise would be stably maintained in a right-handed helix. Thus, cytosine methylation, which is the smallest possible biochemical modification of a base in vivo, may initiate a drastic structural change in the DNA helix. This small change may then lead to quite large secondary consequences like blocking of site-specific protein-binding, target formation for Z-DNA-binding proteins or recombination events, or the generation of termination or initiation sites for transcription or replication or both (9, 12, 20).

Methylation-induced Z-DNA formation in vivo may have a global effect on the superhelical tension of an entire chromosomal domain. Cytosine methylation would favor Z-DNA formation that is accompanied by relaxation of torsional strain. This topological change could influence the stabilities of supercoil-dependent DNA structures and the activities of protein-binding sites or promoters in the same topological domain.

In summary, the delicate interplay in vivo between sequence-specific cytosine methylation and the B-Z equilibrium and DNA topology can initiate a variety of responses at the level of DNA conformations, DNA-protein interactions, and DNA topology. These events may be used for gene regulatory functions, especially with respect to cell differentiation processes, for which the activities of certain genes are correlated with the extent of base methylation in their control regions.

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LITERATURE CITED

1. **Behe, M. J., and G. Felsenfeld.** 1981. Effects of methylation on a synthetic polynucleotide: the B-Z transition in poly(dG-m^{5}dC) · poly(dG-m^{5}dC). Proc. Natl. Acad. Sci. USA **78**:1619–1623.
2. **Behe, M. J., G. Felsenfeld, S. C. Szu, and E. Charney.** 1985. Temperature-dependent conformational transitions in poly(dG-dC) and poly(dG-m^{5}dC). Biopolymers **24**:289–300.
3. **Caserta, M., W. Zacharias, D. Nwankwo, G. G. Wilson, and R. D. Wells.** 1987. Cloning, sequencing, in vivo promoter mapping and expression in Escherichia coli of the gene for the HhaI methyltransferase. J. Biol. Chem. **262**:4770–4777.
4. **Cedar, H.** 1984. DNA methylation and gene expression, p. 147–164. *In* H. Cedar and A. D. Riggs (ed.), DNA methylation. Springer-Verlag, New York.
5. **Chaires, J. B., and J. M. Sturtevant.** 1986. Thermodynamics of the B to Z transition in poly(m^{5}dG-dC). Proc. Natl. Acad. Sci. USA **83**:5479–5483.
6. **Fujii, S., H.-J. Wang, G. van der Marel, J. H. van Boom, and A. Rich.** 1982. Molecular structure of (m^{5}dC-dG)$_3$: the role of the methyl group on 5-methyl cytosine in stabilizing Z-DNA. Nucleic Acids Res. **10**:7879–7892.
7. **Haniford, D. B., and D. E. Pulleyblank.** 1983. The in vivo occurrence of Z-DNA. J. Biomol. Struct. Dyn. **1**:593–609.
8. **Jaworski, A., W.-T. Hsieh, J. A. Blaho, J. E. Larson, and R. D. Wells.** 1987. Left-handed DNA in vivo. Science **238**:773–777.
9. **Jove, R., D. E. Sperber, and J. L. Manley.** 1984. Transcription of methylated eucaryotic viral genes in a soluble in vitro system.

Nucleic Acids Res. **12**:4715–4730.

10. **Klysik, J., S. M. Stirdivant, C. K. Singleton, W. Zacharias, and R. D. Wells.** 1983. Effects of 5-cytosine methylation on the B-Z transition in DNA restriction fragments and recombinant plasmids. J. Mol. Biol. **168**:51–71.
11. **Marinus, M. G.** 1984. Methylation of procaryotic DNA, p. 81–109. *In* H. Cedar and A. D. Riggs (ed.), DNA methylation. Springer-Verlag, New York.
12. **Nickol, J., M. J. Behe, and G. Felsenfeld.** 1982. Effect of the B-Z transition in poly(dG-m^5dC) · poly(dG-m^5dC) on nucleosome formation. Proc. Natl. Acad. Sci. USA **79**:1771–1775.
13. **O'Connor, C. D., and G. O. Humphreys.** 1982. Expression of the EcoRI restriction-modification system and the construction of positive-selection cloning vectors. Gene **20**:219–225.
14. **Peck, L. J., and J. C. Wang.** 1983. Energetics of B-to-Z transition in DNA. Proc. Natl. Acad. Sci. USA **80**:6206–6210.
15. **Rahmouni, A., and R. D. Wells.** 1989. Stabilization of Z-DNA in vivo by localized supercoiling. Science **246**:358–363.
16. **Rich, A., A. Nordheim, and A. H.-J. Wang.** 1984. The chemistry and biology of left-handed Z-DNA. Annu. Rev. Biochem. **53**:791–846.
17. **Singleton, C. K., J. Klysik, S. M. Stirdivant, and R. D. Wells.** 1982. Left-handed Z-DNA is induced by supercoiling under physiological conditions. Nature (London) **299**:312–316.
18. **Smith, H. R., G. O. Humphreys, G. A. Willshaw, and E. S. Anderson.** 1976. Characterization of plasmids coding the restriction endonuclease EcoRI. Mol. Gen. Genet. **143**:319–325.
19. **Vardimon, L., and A. Rich.** 1984. In Z-DNA the sequence G-C-G-C is neither methylated by HhaI methyltransferase nor cleaved by HhaI restriction endonuclease. Proc. Natl. Acad. Sci. USA **81**:3268–3272.
20. **Wang, R. Y.-H., H.-Y. Zhang, R. Khan, Y. Zou, L.-H. Huang, and M. Ehrlich.** 1986. Methylated DNA-binding protein from human placenta recognizes specific methylated sites on several procaryotic DNAs. Nucleic Acids Res. **14**:9843–9860.
21. **Wells, R. D.** 1988. Unusual DNA structures. J. Biol. Chem. **263**:1095–1098.
22. **Wittig, B., T. Dorbic, and A. Rich.** 1989. The level of Z-DNA in metabolically active, permeabilized mammalian cell nuclei is regulated by torsional strain. J. Cell Biol. **108**:755–764.
23. **Zacharias, W., A. Jaworski, J. E. Larson, and R. D. Wells.** 1988. The B-Z DNA equilibrium in vivo is perturbed by biological processes. Proc. Natl. Acad. Sci. USA **85**:7069–7073.
24. **Zacharias, W., J. E. Larson, M. W. Kilpatrick, and R. D. Wells.** 1984. HhaI methylase and restriction endonuclease as probes for B to Z DNA conformational changes in d(GCGC) sequences. Nucleic Acids Res. **12**:7677–7692.
25. **Zacharias, W., T. R. O'Connor, and J. E. Larson.** 1988. Methylation of cytosine in the 5-position alters the structural and energetic properties of the supercoil-induced Z-helix and of B-Z junctions. Biochemistry **27**:2970–2978.