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Gene silencing via protein-mediated subcellular localization of DNA

(membrane patches of DNA-binding proteins/chromatin sequestration/gene repression in *Escherichia coli*/position effects in eukaryotes/SopB protein)

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ABSTRACT **We previously reported that overexpression of SopB, an *Escherichia coli* F plasmid-encoded partition protein, led to silencing of genes linked to, but well-separated from, a cluster of SopB-binding sites termed *sopC*. We show here that in this SopB-mediated repression of *sopC*-linked genes, all but the N-terminal 82 amino acids of SopB can be replaced by the DNA-binding domain of a sequence-specific DNA-binding protein, provided that the *sopC* locus is also replaced by the recognition sequence of the DNA-binding domain. These results, together with our previous finding that the N-terminal fragment of SopB is responsible for its polar localization in cells, suggest a mechanism of gene silencing: patches of closely packed DNA-binding domains are formed if a sequence-specific DNA-binding protein is localized to specific cellular sites; such a patch can capture a DNA carrying the recognition site of the DNA-binding domain and sequestrate genes adjacent to the recognition site through nonspecific binding of DNA. The generalization of this model to gene silencing in eukaryotes is discussed.**

Transcriptional silencing of long sections of eukaryotic chromosomes is well known (1). There have been few hints of this phenomenon, however, in prokaryotes. In a previous study of the F plasmid partition protein SopB, we reported that overexpression of SopB led to the inactivation of genes that are linked to, but well separated from, a cluster of SopB-binding sites termed *sopC* (2). Interestingly, this inactivation or silencing of *sopC*-linked genes can extend at least over a distance of 10 kbp from the *sopC* site, whether the *sopC* locus is plasmid borne or chromosomally located (2). Genes within this silenced region appear to be inaccessible to cellular proteins, as evidenced by the monitoring of the sites of modification by *dam* methylase and of the sites of cleavage by DNA gyrase (2).

Two plausible mechanisms were proposed to account for these observations (2). In the first, binding of SopB to *sopC* is postulated to nucleate the formation of a nucleoprotein filament, within which the DNA is inaccessible to transcription. In the second, binding of SopB to *sopC* is postulated to sequestrate the DNA to a subcellular region inaccessible to cellular enzymes, including RNA polymerase. Recently, a study of a P1 plasmid partition protein ParB, a homologue of SopB, has provided evidence in favor of the nucleoprotein-filament mechanism (3). Similar to the silencing of genes mediated by SopB, overexpression of ParB was found to silence genes linked to *parS*, a cluster of ParB-binding sites. When cells were treated with formaldehyde to form protein–DNA crosslinks under conditions in which the *parS*-linked genes were silenced, ParB was found to crosslink not only to the region containing parS, but also to adjacent regions spanning a length of about 11 kbp (3). Furthermore, when tandem sites for the binding of a protein RepA were placed between *parS* and a reporter gene, ParB-mediated silencing of the gene was no longer observed on induction of RepA, suggesting that the binding of RepA to the intervening sites might serve as a roadblock to the extension of a nucleoprotein filament nucleated at *parS* (3).

Although these recent findings with ParB (3) provide new support of the nucleoprotein-filament model proposed earlier (2), in the meantime we have been entertaining alternative interpretations. There were two subtle hints from our earlier studies that favored the DNA sequestration model. First, SopB-mediated gene silencing is known to be abolished by the deletion of the N-terminal 71 amino acids of the protein (4). This particular N-terminal region of SopB can apparently mediate the polar localization of the protein in *Escherichia coli* cells (5). Visualization of SopB and its fragments fused to the green fluorescence protein (GFP) in *E. coli* cells indicates that intact SopB is localized to the "quarter-cell" positions near the cell poles, and deletion of the N-terminal 71 amino acids abolishes this localization (5). N-terminal SopB fragments as short as 82 aa also show a polar localization, though not confined to the quarter-cell positions (5). These observations suggest that SopB-mediated gene silencing and the polar localization of the protein might be related (5). Second, although binding of purified SopB to the *sopC* cluster of sites occurs readily, the binding of the protein to DNA does not spread outside the cluster even at very high concentrations of the protein, and the formation of an extended nucleoprotein filament is not seen (4).

In the present communication, we report our study of fusion proteins in which fragments of SopB are linked to the DNA-binding domain of the yeast *GAL4* gene product (6), or a multiple zinc-finger protein engineered for binding to a specific DNA sequence (7). We found that such fusion proteins containing an N-terminal segment of SopB as short as 82 aa could silence genes linked to their respective sequence-specific DNA-binding sites. These results are not readily explained by the nucleoprotein-filament model. We propose instead that gene silencing can be effected by the formation of a cellular patch of DNA-binding domains through their localization to specific cellular sites: a DNA carrying the recognition site of a DNA-binding protein can first bind to the patch through interactions between the recognition site and a DNA-binding domain located in the patch; genes adjacent to the recognition site are then silenced through nonspecific binding to the closely packed DNA-binding domains within the same patch.

MATERIALS AND METHODS

Materials. A plasmid bearing a cluster of yeast Gal4 protein-binding sites (pRY171) and rabbit antibodies against yeast

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Abbreviations: GFP, green fluorescence protein; IPTG, isopropyl β-D-thiogalactopyranoside.

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Gal4 protein were kindly provided by M. Ptashne, Memorial Sloan–Kettering Cancer Center (New York, NY), and pGEX-6P-3–268//NRE was kindly provided by C. Pabo, Massachusetts Institute of Technology (Cambridge, MA). The plasmid for the expression of intact SopB protein, ptacSopB, was previously constructed in this laboratory (2). *E. coli* K-12 strain DH5α and various chemical and biochemical reagents were purchased from commercial suppliers, and oligodeoxynucleotide primers were either prepared by the use of an automated nucleic acid synthesizer (PE Biosystems Model 394) or purchased from commercial sources.

Construction of Plasmids. For the inducible expression of the SopB(1–323)Gal4 fusion protein, the 441-bp region of pGBT9 (CLONTECH) encoding the 147- aa DNA-binding domain of yeast Gal4 protein (6) was amplified by the PCR, by using a pair of primers 5′-CTCCCCGCGGTC(ATGAAGCTACTGTCTTCTATC)-3′ and 5′-AACTGCAGTTA-(GAATTCCGGCGATACAGTCAA)-3′. The primer sequences shown in parentheses are complementary to those at the ends of the DNA segment being amplified, and the underlined hexameric sequences are the cleavage sites for *Sac*II and *Pst*I, which were introduced to facilitate the construction of the fusion protein expression plasmid. The use of this pair of PCR primers also added a stop codon after codon 147 of *GAL4* and immediately before the *Pst*I site. The PCR product was digested with *Sac*II and *Pst*I and purified by agarose gel electrophoresis; replacing the *Sac*II-*Pst*I segment in pSopB-GFP (4) by the purified PCR-amplification product then yielded pSopB(1–323)Gal4. The fusion protein expression plasmids pSopB(1–82)Gal4 and pSopB(72–323)Gal4 were similarly constructed by using the appropriate *Sac*II-*Pst*I segments from pSopB(1–82)GFP and pSopB(72–323)GFP (4) as the backbones. For expression of SopB-Zif fusion proteins, the GAL4 coding region was replaced by a 651-bp segment encoding a multiple zinc finger DNA-binding domain Zif268//NRE (7), which was amplified from pGEX-6P-3–268//NRE (7) by the use of a pair of primers 5′-CTCCCCGCGGTC(GAACGCCCATATGCTTGCCCT)-3′ and 5′-AACTGCAGTCAGAC(GTCCTTCTGTCTTA-AGTG)-3′. The underlined hexameric sequences in the pair of primers are restriction sites added for cloning purposes. The placement of the *Aat*II site GACGTC also added one extra codon to the end of the Zif coding region.

The lacZ reporter plasmid carrying *sopC* was derived from pASLS4 (2) as follows. The *lacZ* gene was obtained from pBAD-Myc-His-LacZ (Invitrogen) by first digesting the plasmid with *Nco*I and repairing the 5′-overhangs generated with the Klenow fragment of *E. coli* DNA polymerase I. The product was then digested with *Bgl*II, and the 3,165-bp *lacZ* fragment was gel isolated and used to replace the segment between the *Eco*RV and *Bam*HI sites of pASLS4 (2). In the final product, the *lacZ* reporter gene is placed under the control of Ptet, the promoter expressing the tetracycline-resistance marker of pBR322, with the *sopC* segment located about 1 kbp upstream of the promoter. The control lacZ reporter plasmid without *sopC* was similarly constructed, by using pASLS3 (2) instead of pASLS4 in the last step. This control plasmid also served as the backbone in the construction of lacZ reporter plasmids carrying $(UAS)_4$, a cluster of four binding sites of yeast Gal4 protein, or $(N//Z)_8$, a cluster of eight sites for the binding of Zif268//NRE (7). Sites that are recognized by sequence-specific DNA-binding proteins were readily introduced into the *Xho*I site, located about 1 kbp upstream of Ptet, of the pASLS3-derived lacZ reporter plasmid. A 240-bp segment carrying $(UAS)_4$ was amplified from pRY171 (8) by the use of a pair of oligodeoxynucleotide primers 5′-ACGCCGCTCGAG(TGGAACTTTCAGTAAT-ACGCTT)-3′ and 5′-ACGCCGCTCGAG(TTCTGGGGC-CAGGTTACTGCCA)-3′, each of which contained a *Xho*I recognition sequence (underlined). The PCR product was digested with *Xho*I, purified by agarose gel electrophoresis, and inserted into the *Xho*I site of the control lacZ reporter plasmid to give pSKK$(UAS)_4$. To obtain a fragment containing $(N//Z)_8$, a pair of oligonucleotides 5′-TCGAGAAGGGTT-CAGTGCGTGGGCGCGCCAAGGGTTCAGTGCGTGG-GCG-3′ and 5′-TCGACGCCCACGCACTGAACCCTTGG-CGCGCCCACGCACTGAACCCTTC-3′ were synthesized, annealed, and ligated. These were then digested with both *Xho*I and *Sal*I to generate fragments containing tandem repeats of Zif268//NRE-binding sites, and one that contains eight binding sites was used to obtain pSKK$(N//Z)_8$.

Other Methods. β-Galactosidase assay was carried out according to standard procedures (9). Briefly, *E. coli* DH5α cells harboring a pair of compatible fusion protein expression plasmid and lacZ reporter plasmid were grown overnight in Luria broth containing ampicillin (100 μg/ml) and chloramphenicol (15 μg/ml). The overnight stock was diluted 100-fold into culture tubes containing Luria broth without antibiotics, grown to early-log phase, and induced with various concentrations of isopropyl β-D-thiogalactopyranoside (IPTG). β-Galactosidase activity in each culture was assayed 2 hr after induction or at the specified times described. To determine the copy numbers of the pair of plasmids pSopB(1–323)Gal4 and the lacZ reporter plasmid bearing $(UAS)_4$ during the induction periods, cells harboring the plasmids were grown to early-log phase as described, and IPTG was added to a final concentration of 1 mM. One-milliliter aliquots were removed at 30-min intervals, chilled in ice, and cells from each aliquot were pelleted and used for plasmid isolation by the use of a Qiagen (Chatsworth, CA) plasmid preparation kit. To monitor the recovery of plasmids in each preparation, 100 ng of pBluescript (Stratagene) was added to each cell suspension before lysis. Plasmids were linearized by digestion with *Sca*I restriction endonuclease, and the linearized plasmids were separated by electrophoresis in a 0.6% agarose gel slab. DNA bands were stained with ethidium, destained by soaking in the electrophoresis buffer without ethidium, and photographed over a UV illuminator. The same set of aliquots was also used in quantitating the level of expression of SopB(1–323)Gal4 at different times after the addition of IPTG. The apparent optical density of the aliquots at 590 nm was measured, and samples, each containing 1 OD unit of cells, were lysed for separation of the cellular proteins by gel electrophoresis, as described by Schägger and Jagow (10). A 25-cm-long 9.8% acrylamide–0.3% bis-acrylamide gel was run at 140 V for 16 hr. Duplicate sets of samples were analyzed, one set for staining with Coomassie blue and the other for transferring the protein bands to a nylon membrane for immunochemical identification of SopB(1–323)Gal4 by using rabbit antibodies against yeast Gal4 protein.

RESULTS

Design of Fusion Protein and lacZ Reporter Expression Plasmids. Two sets of expression plasmids were constructed. In one, codons for full length SopB or its fragments were joined to those encoding the DNA-binding domain of a sequence-specific DNA-binding protein. In the other, a *lacZ* reporter gene was placed under the control of a constitutive promoter, and a cluster of sites for the binding of the sequence-specific DNA-binding protein was placed about 1 kbp upstream of this promoter. Because a pair of a fusion protein expression plasmid and a *lacZ* reporter plasmid must be compatible if the effect of the fusion protein on *lacZ* expression in the same cell is to be examined, all fusion protein expression plasmids contained the replication origin of ColE1 plasmid and all reporter gene constructs, that of p15A.

The relevant regions of a typical pair of fusion protein and reporter gene expression plasmids are depicted in Fig. 1. In the SopB(1–323)Gal4 plasmid (*Upper*), the 323 codons of intact

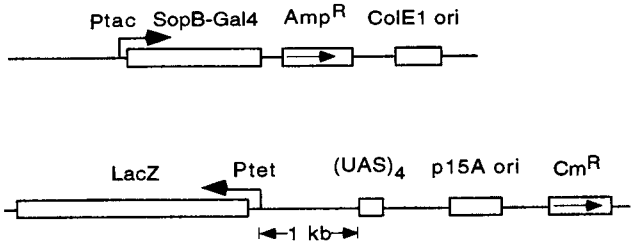

FIG. 1. Relevant regions of the pBR322 derivative expressing SopB(1–323)Gal4 from the IPTG-inducible tac promoter (*Upper*) and a lacZ reporter plasmid with a p15A replication origin and a cluster of four Gal4-binding sites $(UAS)_4$ about 1 kbp upstream of the Ptet promoter expressing β-galactosidase (*Lower*).

SopB are placed under the control of an inducible promoter Ptac, and 147 codons for the DNA-binding domain of yeast Gal4 protein are added to the end of the SopB ORF. In the lacZ reporter plasmid carrying $(UAS)_4$, a cluster of four yeast Gal4 protein-binding sites, the β-galactosidase ORF is placed under the control of a constitutive promoter Ptet, the promoter for the tetracycline-resistance marker in pBR322, about 1 kbp downstream of $(UAS)_4$ (*Lower*). For other members of the fusion protein plasmids, the SopB(1–323)Gal4 region was replaced by the various ORFs of the other fusion proteins; for congeners of the lacZ reporter construct shown in Fig. 1, the $(UAS)_4$ cassette depicted was replaced by the appropriate recognition sites of the DNA-binding domains of the fusion proteins.

Effect of Fusion Protein Expression on β-Galactosidase Level. Fig. 2 depicts the effect of expressing SopB(1–323)Gal4 on the level of β-galactosidase in cells harboring the pair of plasmids depicted in Fig. 1. Induction of fusion protein expression by the addition of IPTG resulted in a reduction of the β-galactosidase level (Fig. 2*A*, open symbols). If the lacZ reporter plasmid does not contain the sequence $(UAS)_4$, however, no reduction in β-galactosidase level was observed (Fig. 2*A*, filled symbols). Similarly, no effect on β-galactosidase level was seen if the 147-aa Gal4 DNA-binding domain was expressed instead of the fusion protein (data not shown).

In the above experiments, β-galactosidase activity was measured 2 hr after induction of fusion protein expression. To ensure that the reduction in β-galactosidase activity was not caused by reduced copy number of the reporter plasmid during this time period, plasmid DNA was isolated from cells at various times, treated with appropriate restriction enzymes to linearize both the fusion-protein expression plasmid and the lacZ reporter plasmid, and analyzed by agarose gel electrophoresis (Fig. 2*B*). The amounts of both plasmids recovered from a constant volume of culture increased with time, but the copy numbers of the plasmids per cell were not significantly altered. Quantitation of the DNA bands in Fig. 2*B* shows that the copy numbers of the ColE1-based 6-kbp fusion protein expression plasmid and the 8-kbp p15A-based lacZ reporter plasmid were around 55 and 13 per cell, respectively, throughout the 2-hr period. During this time period, an increase in the amount of the fusion protein in cells (Fig. 2*C*) was accompanied by a decrease in the level of β-galactosidase (Fig. 2*D*). The cellular concentration of SopB(1–323)Gal4 protein 2 hr after the addition of IPTG to 1 mM was estimated to be about 6×10^4 molecules per cell from the amount of the protein band in the gel photograph shown in Fig. 2*C* (band marked by an arrow; identity of the protein was confirmed by blotting with antibodies specific to Gal4).

The above results show that the SopB-mediated silencing of *sopC*-linked genes does not depend on interaction between SopB protein and *sopC* DNA, its natural substrate, as fusion of SopB to the Gal4 DNA-binding domain yields a protein capable of silencing a reporter gene linked to a cluster of

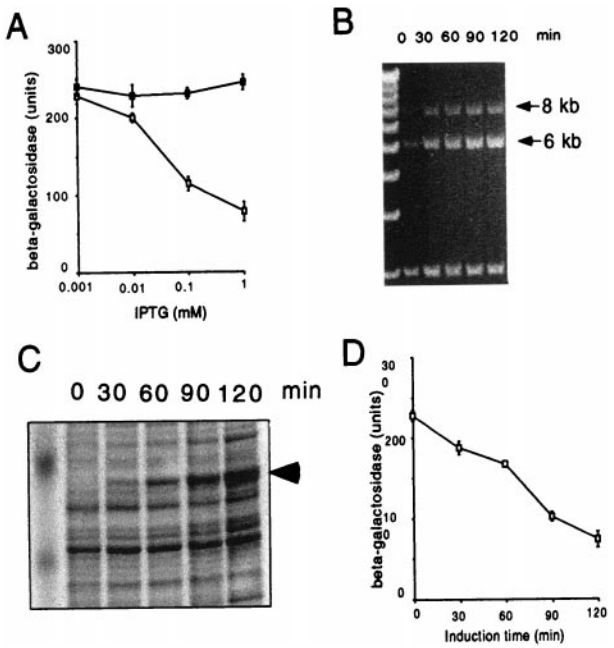

FIG. 2. Repression of a lacZ reporter gene by a fusion protein SopB(1–323)Gal4. (*A*) The levels of β-galactosidase 2 hr after the addition of the indicated amounts of IPTG to cells harboring a pair of plasmids depicted in Fig. 1 (open squares) or to cells harboring the same pair of plasmids, except that the lacZ reporter plasmid carries no Gal4-binding site (closed squares). The levels of β-galactosidase in cells not exposed to IPTG were not significantly different from those induced with 0.001 mM IPTG. (*B–D*) Measurements of the plasmid copy numbers (*B*), the concentration of SopB(1–323)Gal4 fusion protein (*C*), and β-galactosidase level (*D*) at various times after the addition of IPTG to 1 mM. Aliquots (1 ml each) of cells harboring the fusion protein expression plasmid and the lacZ reporter plasmid depicted in Fig. 1 were sampled at the indicated times. The fastest migrating band in each sample run in (*B*) was a linearized 3-kbp plasmid, which was added to the cell suspensions before lysis to monitor the recovery of plasmid DNA.

Gal4-binding sites. Strikingly, when all but the N-terminal 82 amino acids of SopB were deleted from SopB(1–323)Gal4 to give SopB(1–82)Gal4, silencing of $(UAS)_4$-linked lacZ reporter was again seen (Fig. 3*A*). Furthermore, when the Gal4 DNA-binding domain in SopB(1–82)Gal4 was replaced by a poly-zinc finger protein 268//NRE (7), the resulting fusion protein SopB(1–82)Zif was found to repress β-galactosidase expression from a reporter plasmid carrying a cluster of eight Zif268//NRE-binding sites denoted $(N//Z)_8$ (Fig. 3*B*).

Results obtained with the pairs of plasmids described above as well as additional pairs are summarized in Table 1. In all cases, expression of a chimeric protein in which a sequence-

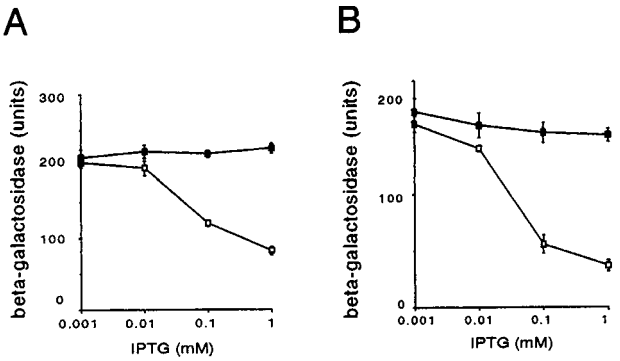

FIG. 3. Repression of a lacZ reporter gene by the fusion proteins SopB(1–82)Gal4 and SopB(1–82)Zif.

Table 1. Summary of protein-mediated gene silencing

Protein	DNA recognition site	Repression of *lacZ*
SopB(1–323)	*sop*C	Yes
SopB(1–323)	None	No
SopB(1–323)	$(UAS)_4$	No
SopB(1–323)	$(N//Z)_8$	No
SopB(1–323)Gal4	None	No
SopB(1–323)Gal4	$(UAS)_4$	Yes
SopB(1–323)Gal4	$(N//Z)_8$	No
SopB(1–82)Gal4	None	No
SopB(1–82)Gal4	$(UAS)_4$	Yes
SopB(72–323)Gal4	$(N//Z)_8$	No
Gal4	$(UAS)_4$	No
SopB(1–323)Zif	None	No
SopB(1–323)Zif	$(UAS)_4$	No
SopB(1–323)Zif	$(N//Z)_8$	Yes
SopB(1–82)Zif	None	No
SopB(1–82)Zif	$(UAS)_4$	No
SopB(1–82)Zif	$(N//Z)_8$	Yes
SopB(72–323)Zif	None	No
SopB(72–323)Zif	$(UAS)_4$	No
SopB(72–323)Zif	$(N//Z)_8$	No
Zif	$(N//Z)_8$	No

specific DNA-binding domain is fused to the C terminus of SopB, or the SopB N-terminal fragment spanning amino acids 1 to 82, leads to the repression of the lacZ reporter gene if, and only if, a cluster of recognition sites of the DNA-binding domain is present. SopB lacking the first 71 amino acids does not show this gene-silencing effect when fused to the same DNA-binding domains, and similarly the expression of Gal4 or Zif itself has no effect on the expression of lacZ whether the reporter gene is linked to a cluster of its recognition sites.

DISCUSSION

Our results indicate that abutting the N-terminal 82-aa residues of SopB to any sequence-specific DNA-binding domain yields a protein capable of repressing genes linked to the recognition site of the particular DNA-binding domain. The three DNA-binding proteins examined, SopB, Gal4, and Zif268//NRE, are not known to share common structural features in their interactions with their respective DNA-binding sites. As a consequence, the SopB N-terminal 82-aa fragment fused to each of these proteins is expected to assume a different spatial position relative to the DNA-binding surface of the fusion protein. It is therefore difficult to envision, structurally, how these fusion proteins could all form a nucleoprotein filament with the differently positioned SopB fragment serving as the common glue. Our earlier observation that SopB readily saturates its binding sites within *sopC* but does not form a nucleoprotein filament even at a very high concentration (5) also argues against the presence of a sticky SopB segment capable of acting as an amorphous cement.

The nucleoprotein filament model also provides no explanation of the observation that the same N-terminal region of SopB necessary for SopB-mediated gene silencing is involved in the polar localization of the protein. Although by itself this correlation could be coincidental and therefore discounted, it invites a reexamination of the various models proposed for gene silencing by SopB/ParB (2–5) when viewed in combination with the shortcomings of the nucleoprotein filament model described earlier. We believe that the results reported both here and previously can be better interpreted in terms of gene silencing through sequestration of a DNA to a subcellular location.

According to this gene sequestration model, interaction between a DNA-binding protein and a component specifically localized to a subcellular site or sites leads to the formation of a patch or patches of closely packed DNA-binding proteins. In the case of the SopB fusion proteins reported here, the N-terminal fragment of SopB fused to a DNA-binding domain serves the role of a homing factor, which, presumably through interaction with a specifically localized membrane component, targets the DNA-binding domain linked to it to the cell poles. Patches of the fusion proteins are thus formed at the cell poles, with the DNA-binding domains exposed to the cytoplasmic side. For a DNA segment carrying a cluster of recognition sites of the DNA-binding domains in such a patch, these recognition sites would first bind to the patch through sequence-specific DNA–protein interactions. DNA segments adjacent to the bound cluster of recognition sites would then bind, through nonspecific interactions, to nearby DNA-binding domains in the same patch. In this model, the transcription machinery is either excluded from the particular subcellular location, perhaps because of its preferential localization to other cellular regions, or is incapable of accessing the DNA because of the fixation of the DNA to multiple points within the patch of DNA-binding domains.

Could this gene sequestration model explain the recent results with the ParB protein (3)? Formally, the spreading of a protein along a DNA to form a nucleoprotein filament is very similar to the anchoring of multiple points along a DNA to a patch of DNA-binding domains: both involve initiation through the binding of a protein to a specific DNA sequence, and both involve subsequent formation of DNA–protein complexes through less specific DNA–protein interactions. Spreading of a protein along a DNA from a nucleated site is helped by protein–protein interactions between the closely positioned proteins along the DNA contour; anchoring of DNA segments adjacent to an already anchored segment is facilitated by the closely spaced DNA-binding domains within such a patch. Thus we believe that the gene sequestration model can adequately account for all available data.

A number of proteins that interact with DNA, including the chromosomally encoded SopB homologs of *Bacillus subtilis*, *Pseudomonas putida*, and *Caulobacter crescentus*, and bacterial DNA topoisomerase IV A-subunit and MinD protein, have recently been shown to have a polar localization (11–15). In *B. subtilis*, localization of a number of replication proteins to a midcell location has also been reported (16). Thus silencing of genes through protein-mediated localization of a DNA to cell poles, and perhaps to other particular subcellular locations as well, may play significant roles in chromosome partition as well as other chromosomal transactions in various bacteria. It should be pointed out, however, that the gene sequestration model of gene silencing proposed here requires the formation of a patch of closely packed DNA-binding domains but is not limited to specific localization of such a patch to cell poles.

It is likely that sequence-specific and -nonspecific interactions between a long DNA segment and a patch of DNA-binding proteins may represent a mode of gene regulation much more general than the special cases discussed here. Perinuclear localization of inactive regions of chromosomes in eukaryotes ranging from yeasts to mammals, for example, is well known (17–20). In the repression of a reporter gene flanked by the silencer elements of yeast mating loci, repression of the reporter gene occurs if such a cassette is placed close to, but not if it is placed far from, telomeres that appear to localize to a limited number of discrete perinuclear sites (reviewed in ref. 21). Interestingly, overexpression of Gal4 DNA-binding domain fused to integral membrane proteins was recently reported to facilitate transcriptional silencing of a modified yeast mating locus in which the silencer elements had been replaced by the Gal4-binding sites (22). Although the authors did not consider it likely that membrane localization of their fusion proteins was participating directly in gene silencing and instead interpreted their observation in terms of

a higher perinuclear concentration of the limiting SIR proteins known to be required in mating locus silencing, the similarity between their observation in yeast and the results reported here on protein-mediated silencing of genes in *E. coli* is intriguing.

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