The DNA static curvature has a role in the regulation of the *ompS1* porin gene in *Salmonella enterica* serovar Typhi

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The DNA static curvature has been described to play a key role as a regulatory element in the transcription process of several bacterial genes. Here, the role of DNA curvature in the expression of the *ompS1* porin gene in *Salmonella enterica* serovar Typhi is described. The web server MUTACURVE was used to predict mutations that diminished or restored the extent of DNA curvature in the 5′ regulatory region of *ompS1*. Using these predictions, curvature was diminished by site-directed mutagenesis of only two residues, and curvature was restored by further mutagenesis of the same two residues. Lowering the extent of DNA curvature resulted in an increase in *ompS1* expression and in the diminution of the affinity of the silencer proteins H-NS and StpA for the *ompS1* 5′ regulatory region. These mutations were in a region shown not to contain the H-NS nucleation site, consistent with the notion that the effect on expression was due to changes in DNA structural topology.

INTRODUCTION

*Salmonella enterica* serovar Typhi (S. Typhi) is the aetiological agent of typhoid fever in humans (Pang et al., 1998). In our laboratory we have identified the S. Typhi *ompS1* gene that encodes the OmpS1 quiescent porin belonging to the OmpC/OmpF superfamily (Fernández-Mora et al., 1995). OmpS1 has been reported to have a role in swarming motility, biofilm formation and virulence in mice (Toguchi et al., 2000; Mireles et al., 2001; Rodriguez-Mora et al., 2006).

Expression of *ompS1* is dependent on two overlapping promoters, P1 and P2. The P1 promoter is dependent on the OmpR response regulator. The P2 promoter does not require OmpR for activation, being active only in the absence of OmpR (Oropeza et al., 1999; Flores-Valdez et al., 2003; De la Cruz et al., 2007). Another key element in the transcriptional regulation of *ompS1* is the global regulatory protein H-NS, a nucleoid protein of 137 amino acids (15 kDa) that negatively regulates its expression (Flores-Valdez et al., 2003; De la Cruz et al., 2007). In *Salmonella*, H-NS has been proposed to selectively silence horizontally acquired genes by targeting sequences with a GC content lower than the resident genome, regulating around 12% of its genes (Lucchini et al., 2006; Navarre et al., 2006). StpA, an H-NS parologue, was found to repress *ompS1* in an *hns* background; and LeuO, a LysR-type regulator, positively regulates *ompS1* expression by antagonizing H-NS and StpA (De la Cruz et al., 2007). LeuO has been implicated in several functions, such as stress resistance, virulence and biofilm formation (VanBogelen et al., 1996; Fang et al., 2000; Majumder et al., 2001; Tenor et al., 2004; Lawley et al., 2006; Moorthy & Watnick, 2005; Rodríguez-Morales et al., 2006). Recently, our group has described several genes regulated by LeuO in S. Typhi (Hernández-Lucas et al., 2008).

In bacterial genomes, the recognition of their binding targets by regulatory proteins is commonly considered to be sequence-dependent, although DNA curvature plays a well-characterized role in many transcriptional regulation mechanisms in prokaryotes ( Jáuregui et al., 2003; Olives-Mazaleta et al., 2006). For example, static DNA curvature has been shown to activate transcription, facilitating the binding of RNA polymerase to promoters, or favouring the interaction of activator proteins ( Pérez-Martín et al., 1994; Gourse et al., 2000). Curved DNA regions have also been found to repress transcription initiation, where DNA curvature generally plays an indirect role, being the target for the binding of specific silencer proteins or by stabilizing or enhancing a preexisting DNA loop, thus effectively blocking transcription of downstream regions (Olives-Mazaleta et al., 2006). In particular, such would be the case for the *ompF* porin gene (Mizuno, 1987).

Previously ( Jáuregui et al., 2003; Olives-Mazaleta et al., 2006), we performed computer analyses to study the prevalence of DNA static curvature in the regulatory
regions of *Escherichia coli* and established that most of the global transcription factors (ArcA, CRP, FIS, FNR, Lrp, IHF and H-NS), as well as some specific regulators, have a tendency to regulate operons with curved DNA sequences in their upstream regions.

Here we present a topological analysis of the *ompS1* 5’ upstream regulatory region and the identification of a static curvature that plays an important role in the binding of H-NS and StpA, the silencer proteins of *ompS1*.

### METHODS

**Computational design of site-directed mutagenesis.** In order to modify the extent of DNA static curvature in the regulatory region of *ompS1*, we used our web server MUTACURVE (http://www.ibt.unam.mx/biocomputo/dna_curvature.html). This server first evaluates the amplitude of the intrinsic DNA curvature of every nucleotide in a given sequence using the algorithm of Goodsell & Dickerson (1994), with the contribution matrices for rotational and spatial displacements reported by Satchwell et al. (1986). Secondly, centered at the maximum curvature value, the server evaluates the effect of every double point mutation in a window of 31 nucleotides (three helix turns) to select those changes that would produce a significant reduction in the intrinsic DNA curvature of the fragment. Finally, the server generates the curvature profiles of the original and mutated sequences for their comparative analysis.

**Site-directed mutagenesis.** Site-directed mutageneses were performed using complementary oligonucleotides that contained the mutations predicted by MUTACURVE (Table 2). The plasmid pRO310-wt was used as template to generate pRO310-mt and pRO310-re by inverse PCR (Table 1). The expected mutations were verified by nucleotide sequencing.

**Analysis of static curvature.** We amplified several DNA fragments using a series of oligonucleotides that encompass the *ompS1* regulatory region (Table 2). The PCR fragments were separated by 6% PAGE at 4 °C (polyacrylamide gels in 0.5× Tris/borate/EDTA buffer at 4 °C, 70 V, without buffer recirculation), a condition favouring slower migration of curved DNA sequences (Falconi et al., 1993; Flores-Valdez et al., 2003; Olivares-Zavaleta et al., 2006). Gels were stained with ethidium bromide and photographed in an Alpha Imager system (Alpha Innotech).

### RESULTS

**Diminished and restored ompS1 DNA curvature**

Previously, our group, working with three DNA fragments of the 5’ upstream regulatory region of the *S. Typhi ompS1* porin gene, described the presence of intrinsic DNA curvature peaking around −230 and −149 bp upstream of the transcriptional initiation site at promoter P1 (Flores-Valdez et al., 2003). MUTACURVE (Olivares-Zavaleta et al., 2006), a software developed to determine the topographic profile of a particular DNA region in *silico*, was used to further define regions with the highest degree of curvature; one of them was found between −151 and −135 bp upstream of the P1 transcriptional start point (Fig. 1A). This region was of interest for further research in this work, because it is located in the vicinity of the binding sites of two main transcription factors, LeuO and H-NS, which regulate *ompS1* expression (De la Cruz et al., 2007). In addition, our previous studies showed that removal of the region from −310 to −153 had a modest effect on derepression as compared to removal of the region between −153 and −117 (Oropeza et al., 1999), which includes the curved region studied here. The advantage of the software

### Table 1. Bacterial strains and plasmids

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Genotype and/or relevant markers</th>
<th>Reference</th>
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<tbody>
<tr>
<td><strong>E. coli</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MC4100</td>
<td>F’ araD139 Δ(argF-lac)U169 rpsL150 relA1 30C1 ptsF25 rbsR</td>
<td>Casadaban (1976)</td>
</tr>
<tr>
<td><strong>S. Typhi</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IMSS-1</td>
<td><em>S. enterica</em> serovar Typhi 9.12, d, Vi serotype; Mexican reference clinical strain</td>
<td>Puente et al. (1987)</td>
</tr>
<tr>
<td>STYhns99</td>
<td>IMSS-1 hns99::Km’ mariner</td>
<td>Flores-Valdez et al. (2003)</td>
</tr>
<tr>
<td><strong>Plasmids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pRO310-wt</td>
<td>pMC1871-derived plasmid, containing a translational fusion of the <em>ompS1</em> 5’ regulatory region, up to 31–27 bp upstream of the transcriptional start point, to the lacZ reporter gene</td>
<td>Oropeza et al. (1999)</td>
</tr>
<tr>
<td>pRO310-mt</td>
<td>pRO310-derived plasmid carrying mutations A151G and G135T, lowering curvature</td>
<td>This work</td>
</tr>
<tr>
<td>pRO310-re</td>
<td>pRO310-derived plasmid carrying mutations C151G and A135T, restoring curvature</td>
<td>This work</td>
</tr>
<tr>
<td>pFMTrc12</td>
<td>pTrc99A modified with replication origin p15A1; Ap’</td>
<td>De la Cruz et al. (2007)</td>
</tr>
<tr>
<td>pFMTrc12</td>
<td>pFMTrc12 carrying the S. Typhi leuO gene fused to His n under tac promoter</td>
<td>De la Cruz et al. (2007)</td>
</tr>
</tbody>
</table>
The mutations that affected the DNA curvature in silico (Fig. 1A, B). were introduced into the wild-type ompS1 et al. previously by EMSA and DNase footprinting analysis (De la Cruz et al., 2003; Flores-Valdez et al., 2003). Several groups have reported that DNA static curvature at the ompS1 regulatory region produced different effects on expression, depending on whether they did or did not change the extent of DNA curvature.

We have reported previously that H-NS silences ompS1 expression (Flores-Valdez et al., 2003; De la Cruz et al., 2007). Thus, the effect of the mutations affecting the curvature was evaluated in an hns background (STYhns99) (Fig. 2B). The pRO310-mt fusion increased ompS1 expression 40% with respect to the wild-type (Fig. 2B). Hence, the removal of the intrinsic curvature at −151 to −135 also derepressed ompS1 expression in the hns background, although in a smaller proportion than in the wild-type background, where a fivefold effect was observed (Fig. 2A). This smaller effect could be due to the lowering of the affinity of the binding of StpA in an hns background, as shown in Fig. 5D.

Osmoregulation and thermoregulation of ompS1 is not affected by changes in DNA curvature

The expression of ompS1 is not osmoregulated in a wild-type background but is negatively regulated at high osmolarity in an hns background (Oropeza et al., 1999; Flores-Valdez et al., 2003). Several groups have reported that DNA static curvature is involved in the thermoregulation and osmoregulation of certain genes (Ramani et al., 1992; Kaji et al., 2003; Prosseda et al., 2004). The expression of the pRO310-mt fusion was therefore analysed in a wild-type and hns background at low and high osmolarity and at 37 °C and 30 °C (Fig. 3). As can be observed, the removal of the DNA curvature did not have a major effect on expression in these growth conditions: although a slight derepression was observed at high osmolarity in the hns background, no effect of temperature was seen (Fig. 3).

LeuO and DNA curvature at the ompS1 regulatory region

LeuO is an antagonist of H-NS and of its paralogue protein StpA, allowing ompS1 expression; furthermore, the expres-

Table 2. Oligonucleotide primers used

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5’–3’)</th>
<th>Alignment</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>310b-1</td>
<td>TAGCCCTTTATCATTTATTTATC</td>
<td>−303 Fwd</td>
<td>De la Cruz et al. (2007)</td>
</tr>
<tr>
<td>310b-2</td>
<td>ATGATTTAGTGGTTTGGTTGGA</td>
<td>−153 Rev</td>
<td>De la Cruz et al. (2007)</td>
</tr>
<tr>
<td>310b-3</td>
<td>CAAAAGCATCAAATACATAATAAAAA</td>
<td>−226 Fwd</td>
<td>De la Cruz et al. (2007)</td>
</tr>
<tr>
<td>310b-4</td>
<td>TGGTTCTATTGGTTTGGTTATAC</td>
<td>−76 Rev</td>
<td>De la Cruz et al. (2007)</td>
</tr>
<tr>
<td>310b-7</td>
<td>5’ATTCTTATGGTCTATATGCTTTGAT TAT</td>
<td>−114 Fwd</td>
<td>De la Cruz et al. (2007)</td>
</tr>
<tr>
<td>310b-8</td>
<td>ATATGTAGGCACTTACAAAAAC</td>
<td>+27 Rev</td>
<td>De la Cruz et al. (2007)</td>
</tr>
<tr>
<td>310-mt-5’</td>
<td>CACATACTCTATTAAAAATGATA ATGATAGACTATATATTC</td>
<td>−164 Fwd</td>
<td>This work</td>
</tr>
<tr>
<td>310-mt-3’</td>
<td>GAATATATAGTTATGTATGTTTT TATTATGTTATGTTG</td>
<td>−164 Rev</td>
<td>This work</td>
</tr>
<tr>
<td>310-re5’</td>
<td>CACATACTCTATTAAAAATGATA ATGATAGACTATATATTC</td>
<td>−164 Fwd</td>
<td>This work</td>
</tr>
<tr>
<td>310-re3’</td>
<td>GAATATATAGTTATGTATGTTTT TATTATGTTATGTTG</td>
<td>−164 Rev</td>
<td>This work</td>
</tr>
</tbody>
</table>

used was that it allowed the evaluation of the effect on DNA curvature of every double point mutation that encompassed the region from −226 to −76 (fragment Fb-wt) (Fig. 1A, B).

The mutations that affected the DNA curvature in silico were introduced into the wild-type ompS1 5’ upstream regulatory region near the H-NS binding site as defined previously by EMSA and DNase footprinting analysis (De la Cruz et al., 2007). First, a double mutation was introduced to diminish the extent of DNA curvature (pRO310-mt). We then used this mutated DNA as substrate for a second PCR-mediated mutagenesis to introduce the required changes, different from the wild-type sequence, which would restore the original extent of DNA curvature (pRO310-re) (Table 1). The effects of these mutations on DNA bending were evaluated by the PCR amplification of these regions (Fb-wt, Fb-mt and Fb-re) and their corresponding analysis by PAGE at 4 °C (Falconi et al., 1993; Flores-Valdez et al., 2003; Olivares-Zavaleta et al., 2006). The mutations had the expected effect on the electrophoretic migration as predicted in silico (Fig. 1A, C): the Fb-mt fragment migrated faster, consistent with an abolished or diminished curvature, while the Fb-re fragment co-migrated with Fb-wt, suggesting a restored curvature.

DNA curvature is required for the negative regulation of ompS1 expression

In order to evaluate the effect of DNA curvature on the expression of ompS1, the ompS1-lacZ reporter activity of the plasmids pRO310-wt, pRO310-mt and pRO310-re was evaluated in S. Typhi. Interestingly, the pRO310-mt fusion, carrying the ompS1 regulatory region with diminished curvature, was derepressed fivefold relative to the wild-type fusion (pRO310-wt), whereas the positive control, pRO310-re, carrying two point mutations in the same position as the ones introduced into pRO310-mt but restoring the curvature of the ompS1 regulatory region, rendered the same low expression level as the pRO310 wild-type (Figs 1 and 2A). Thus, point mutations located in precisely the same position in the ompS1 5’ upstream region produced different effects on expression, depending on whether they did or did not change the extent of DNA curvature.
Fig. 1. In silico design of point mutations decreasing the DNA static curvature in the ompS1 5’ regulatory region and their effect on electrophoretic migration. (A) The DNA curvature value of each nucleotide at the ompS1 5’ upstream regulatory sequence was calculated and used to plot its curvature profile (solid line, wt). Centred at the nucleotide with the greatest curvature value, a window of 31 bp was used to look for the double nucleotide change that would produce the most significant reduction in intrinsic DNA curvature. This change was introduced into the original sequence and a new curvature profile was plotted (dotted line labelled mt). From these plots, one of the highest curvature regions was localized −151 to −135 bp upstream of the P1 transcription start site (indicated as +1). The thick bar on the x-axis represents the binding site for LeuO and H-NS, as detailed in Fig. 5A. Below the plot, the three DNA fragments (Fa, Fb and Fc) used for electrophoresis experiments are represented, with their coordinates. (B) Nucleotide sequences of the wild-type (wt), the mutant (mt) abolishing the curvature, and the mutant (re) restoring the curvature, at the −151 to −135 region, contained in the respective Fb fragments. The curvature values of the sequence are shown on the right and the relevant or mutagenized nucleotides are indicated in bold with their position upstream of the P1 transcriptional start site. (C) Electrophoretic migration of Fa, Fb and Fc wild-type fragments, and Fb-mt and Fb-re mutant fragments, at room temperature and at 4 °C. Lanes: M, 100 bp ladder DNA markers; 1, Fa-wt (−304 to −154); 2, Fb-wt (−226 to −76); 3, Fc-wt (−124 to +27); 4, Fb-mt (A151C, G135A); 5, Fb-re (C151G, A135T). The intrinsic bending of each fragment quantified by the k-value (Prosseda et al., 2004) is shown at the bottom of the 4 °C gel.
sion of ompS1 is dependent on the concentration of LeuO (De la Cruz et al., 2007). The maximum level of ompS1 expression was obtained when LeuO was induced at 100 μM IPTG from pFMTrcLeuO-50, the same as that attained in a double hns stpA background (De la Cruz et al., 2007). Hence, the effect of curvature on LeuO-mediated regulation of ompS1 was examined by evaluating the expression of the pRO310-wt, pRO310-mt and pRO310-re fusions in the presence of cloned and expressed LeuO at different concentrations of IPTG (Fig. 4). Interestingly, the pRO310-mt fusion reached the maximum level when LeuO was induced at 50 μM IPTG, contrasting with pRO310-wt and pRO310-re, which reached the maximum level at 100 μM IPTG (Fig. 4). Furthermore, at 20 and 25 μM IPTG the expression was higher in the -mt construct than in the -wt and -re plasmids, at the same IPTG concentration. The observation that the degree of LeuO induction required to obtain the highest levels of ompS1-lacZ expression in strains carrying the plasmids with the curved ompS1 regulatory region (plasmids pRO310-wt and pRO310-re) was almost double that required for those carrying the non-curved ompS1 regulatory region (plasmid pRO310-mt) is consistent with the notion that diminution of the curvature enhanced the LeuO antagonistic effect on H-NS.

Effect of curvature on H-NS, StpA and LeuO binding

The effect of diminishing the DNA curvature of the ompS1 regulatory region on the affinity of H-NS, StpA and LeuO was explored by EMSA. The binding of these proteins was analysed at 4 °C using amplified fragments of the ompS1 regulatory region containing different lengths towards the 5’ terminus. They encompassed from +27 to either −310 (F1), −226 (F2), −164 (F3) or −114 (F4) (Fig. 5A). The corresponding F1-mt, F2-mt and F3-mt fragments contained the point mutations that diminished curvature (Fig. 1). In the presence of H-NS, the wild-type F1 fragment, which contains the whole regulatory region, shifted at 90 nM H-NS (Fig. 5B). In contrast, F1-mt shifted at 175 nM H-NS (Fig. 5B). Moreover, the shorter F2 and F3 fragments, which contain less of the 5’ upstream regulatory sequences, shifted at higher H-NS concentrations than F1: at 270 and 350 nM (Fig. 5B). The corresponding F2-mt and F3-mt fragments fully shifted at 350 and 435 nM H-NS (Fig. 5B). These data show that H-NS binding is favoured by the degree of curvature and illustrate the existence of further H-NS-binding sites upstream of the DNA curvature centre.

As a comparison, the F2 fragment was analysed by EMSA with StpA and LeuO (Fig. 5D, E). F2 shifted at 900 nM and F2-mt at 1100 nM StpA; both fragments shifted at 300 nM LeuO. We have previously described an H-NS nucleation site in the 5’ regulatory region of ompS1 within the LeuO (II) binding box (Fig. 6A). Mutations in this nucleation site cause derepression of ompS1 expression in the absence of LeuO and a reduction in the H-NS binding affinity, and this site showed the highest affinity to H-NS by DNase footprinting analysis (De la Cruz et al., 2007). Moreover, this site shows homology to the H-NS nucleation site for the proU gene (AATATATCGA) (Bouffartigues et al., 2007). In contrast, mutations throughout the LeuO (I) binding box (Fig. 6A) did not have an effect on ompS1 expression in the absence of LeuO, nor did they render an altered H-NS binding (De la Cruz et al., 2007). These observations are in agreement with the experiment shown in Fig. 6B, where 50-mer double-stranded oligonucleotides encompassing the LeuO (II) or the LeuO (I) regions showed differential binding to H-NS. The LeuO (II) fragment was bound by H-NS and the LeuO (I) fragment did not bind (Fig. 6B). Most importantly, the LeuO (I) fragment contains the −151 and −135 residues of the curved region that were mutated to render either a lowering (mt) or a restoration (re) of the curvature (Fig. 1b). Thus, the curved region studied did not encompass the H-NS nucleation site.

These data are in accord with a model where the derepression observed with the pRO310-mt fusion (Fig. 2) is due to the lowering of the affinity of these two silencing proteins by the change in DNA curvature of the ompS1 regulatory region, and with the notion that the effect of the introduced mutations is indeed on the DNA curvature and not on the alteration of the binding of H-NS to its nucleation site.
**Fig. 3.** Effect of *ompS1* intrinsic curvature upon growth under different osmolarity and temperature conditions. $\beta$-Galactosidase activity of the *lacZ* fusions pRO310-wt, -mt and -re was determined in *S. Typhi* wild-type and STYhns99 at low (NB) and high (NB + 0.3 M NaCl) osmolarity (A), and at 30 and 37 °C (B).

**Fig. 4.** Abolition of *ompS1* curvature favours derepression by LeuO: $\beta$-galactosidase activity of the *lacZ* fusions pRO310-wt, -mt and -re, in the presence of cloned LeuO on plasmid pFMTrcLeuO-50 induced with increasing concentrations of IPTG. The pFMTrc12 vector plasmid was included as control.
DISCUSSION

Transcription initiation is one of the most regulated steps of gene expression in bacteria. This process is mainly carried out by regulatory proteins that specifically recognize their DNA targets in a sequence-dependent fashion based on the interactions of the regulatory protein with the nucleotides. In addition, it has been demonstrated that primary DNA sequence is not the only recognized feature in the genome for the transcription regulatory process: the DNA topology has been shown to have a relevant role in

Fig. 5. Effect of DNA curvature on the binding of H-NS, StpA and LeuO to ompS1. (A) Schematic representation of the 5’ upstream regulatory region of ompS1, depicting the P1 and P2 promoters, and the binding sites for OmpR, H-NS and LeuO. The F1, F2, F3 and F4 fragments used in panels B, C, D and E are shown. (B–E) EMSAs for the binding of H-NS, StpA and LeuO to the F (wild-type) and the F-mt mutant fragment at different protein concentrations.
in order to restore the curvature (re; Fig. 1B). It is worth mentioning that the computer design of our mutagenesis protocol provided the minimal number of changes in the DNA regulatory region. In addition, as our second internal control, we demonstrated by EMSAs, that the H-NS nucleation site is not present on the DNA regulatory region used in our point mutation analysis (LeuO (I) binding box; Fig. 6). Thus, the observations reported here are consistent with the concept that the effect of these nucleotide changes on the regulation of ompS1 expression is due to the lowering of the DNA curvature in the region.

Using the wild-type and the aforementioned non-curved and curved mutagenized ompS1 regulatory regions, we obtained in vivo and in vitro evidence that supports the role of a curved DNA sequence in the repression of ompS1 expression. First, our theoretically predicted effects of the point mutation to diminish (mt) and restore (re) the intrinsic curvature of the ompS1 regulatory region were verified by PAGE mobility analysis at 4°C (Fig. 1). Secondly, our EMSA experiments demonstrated that the affinity of H-NS for the regulatory region is diminished following point mutagenesis that lowers the curvature and that there are H-NS binding sites upstream of the H-NS nucleation centre (Fig. 5). In further support of this notion, fragment F4 (−114 to +27), which contains only the H-NS nucleation site and no further upstream sequences, including the curved region, did not shift with H-NS. Similarly, StpA binding was reduced for the non-curved ompS1 regulatory region (Fig. 5D) and, interestingly, LeuO affinity was not affected by the extent of curvature (Fig. 5E).

Finally, there was a fivefold increase in the ompS1 activity as assayed with a lacZ reporter fusion, upon removal of curvature in plasmid pRO310-mt (Fig. 2). This upregulation effect was not observed in our internal control of strains carrying plasmid pRO310-re with restored DNA curvature (Fig. 2).
All the results show that the curved region located at −151 to −135 in the ompS1 regulatory region participates in the repression of ompS1 transcription initiation. We previously accounted for the repression of ompS1 expression by the formation of an H-NS nucleofilament (De la Cruz et al., 2007). The new findings reported here now support a DNA-curvature-dependent bridging model that would account for full repression of ompS1 expression (Fig. 7A), where the role of the curvature would be to facilitate the formation of DNA–H-NS–DNA bridges between downstream and upstream sites. Chromatin organization by loop domain formation conducive to DNA bridging has been discussed previously in detail (Dame et al., 2005, 2006; Noom et al., 2007; Dorman & Kane, 2009). In the F-mt fragments, where the curvature has been lowered by two point mutations, H-NS would still be binding, albeit at lower affinity, and could still cause some repression of expression by the formation of a nucleofilament-type structure (Figs 5B, C and 7B). Moreover, the enhancement of LeuO activation upon removal of DNA curvature (Fig. 4) is consistent with our previous model of regulation for the ompS1 porin gene in which LeuO acts as an antirepressor of H-NS and StpA (De la Cruz et al., 2007), and where changes in DNA structural topology also affect repression. H-NS has been proposed to selectively silence a great number of horizontally acquired genes (Lucchini et al., 2006; Navarre et al., 2006) and also to act as a modulator of environmentally regulated gene expression (Atlung & Ingmer, 1997). H-NS modulates other important biological processes, such as DNA recombination, DNA replication and organization of the bacterial chromosome (Pérez-Martín et al., 1994; Jáuregui et al., 2003).

ACKNOWLEDGEMENTS

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REFERENCES


Fig. 7. DNA curvature and the binding of H-NS to the ompS1 regulatory region. (A) Current model, which involves the formation of a DNA-curvature-mediated bridge structure that participates in the repression of ompS1 expression in the wild-type. (B) Diminution of the DNA curvature in the mt mutant would favour the formation of an H-NS nucleofilament, where H-NS binds to DNA with lower affinity.


