Such questions of the physiological basis for spatial distribution in natural biofilms are being explored in other ecosystems (Boetius *et al.*, 2000), and the paradigm system of dental plaque presents many such opportunities.

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H-NS and genomic bridge building: lessons from the human pathogen *Salmonella* Typhi

The H-NS protein has emerged as one of the leading causes of transcriptional repression in Gram-negative bacteria. In a paper published in this issue, De la Cruz and colleagues shed new light on the role of DNA curvature in the repressive mechanism, using a porin gene promoter from the human pathogen *Salmonella* Typhi as their experimental system (De la Cruz *et al.*, 2009). Their data help to deepen our understanding of the importance of local DNA structure in facilitating the interaction of H-NS with its target sites in DNA and in establishing an effective nucleoprotein complex for the repression of transcription.

DNA curvature was described as being an important feature of H-NS binding sites many years ago (Yamada *et al.*, 1990) but the reason for its importance remained obscure until recently. This is in spite of the considerable effort that has been expended in analysing H-NS interaction with regions of curvature, using both naturally occurring and artificial sequences with intrinsic curvature (Jordi *et al.*, 1997; Rimsky *et al.*, 2001). A requirement for A+T-rich DNA has also been noted, which is interesting in the light of the facts that (1) A+T-richness is a common feature of bacterial promoters, (2) an appropriate spacing of A+T-rich patches in B-DNA can impose curvature and (3) curved DNA is often found close to promoters (Barbic *et al.*, 2003; Jauregui *et al.*, 2003; Lang *et al.*, 2007). The coincidence of these features seems to produce an ideal platform upon which H-NS can repress the very large number of promoters that it is known to regulate. Central to the repression mechanism is the creation of DNA–H-NS–DNA bridges that impede transcription initiation (Dame *et al.*, 2005; Dorman & Kane, 2009).

De la Cruz *et al.* (2009) have examined the role of DNA static curvature in the promoter region of the *ompS1* porin gene in *S.* Typhi. Bends were predicted *in silico* and detected by an electrophoretic technique in which the position of the bend centre relative to the ends of a DNA fragment results in a temperature-dependent alteration in the migration of the DNA molecule through a polyacrylamide gel. Impressively, the authors have been able to remove the bend by making just two changes to the base composition of the DNA, at positions −135 and −151 upstream of the *ompS1* transcription start site (+1). The presence of these base substitutions derepresses transcription of the promoter by about sevenfold. H-NS is known to bind to the *ompS1* regulatory sequences, as is the paralogous protein StpA (De la Cruz *et al.*, 2007). The previously determined binding sites extend from the region of intrinsic curvature to the transcription start site. In this new study additional binding sites for H-NS are identified upstream of the curve, raising the possibility that H-NS may build bridges between the DNA sequences located upstream and downstream of the curve, resulting in the creation of a nucleoprotein complex that represses the *ompS1* promoter. This is an intuitively appealing scenario that is consistent with earlier repression models that involve DNA–H-NS–DNA bridges (Prosseda *et al.*, 2004). Interestingly, the suppression of DNA curvature in the *ompS1* upstream regulatory region does not result in full derepression of the promoter; this requires inactivation of the *hns* gene as well. If the *hns* gene is inactivated but the curve is left intact (or is restored following mutation) a lower level of derepression results. This hints at the presence in the cell of another curve-dependent repressor of *ompS1* transcription. StpA is an attractive candidate, not least because it shares so many properties with H-NS and is known to regulate *ompS1* transcription (De la Cruz *et al.*, 2007).

The observation that H-NS continues to exert a negative effect on *ompS1* transcription in the absence of the curve suggests that H-NS can still interact with the regulatory region DNA in the absence of bridging across the curve; electrophoretic mobility shift data support this. Of course these data do not rule out the possibility that H-NS may engage in highly localized bridging within each of the 'arms' of the regulatory region as well as more conventional binding and nucleation.

The *ompS1* promoter is also under the control of other regulators, and the DNA–H-NS–DNA bridging model provides a useful framework in which to consider their contributions at a mechanistic level. Prominent among these are the OmpR and LeuO DNA-binding proteins (De la Cruz *et al.*, 2007; Flores-Valdez *et al.*, 2003; Oropeza *et al.*, 1999). Both are pleiotropic regulators of transcription. OmpR belongs to the response regulator protein family, most of whose members are transcription regulators that are subject to control by phosphorylation by histidine protein kinase sensor proteins (Kenney, 2002). LeuO is a member of the LysR family of

DNA-binding proteins (Chen & Wu, 2005; Hernández-Lucas *et al.*, 2008). LeuO is an antagonist of H-NS repression and can act by blocking oligomerization of H-NS along the DNA molecule; it can also form bridges that introduce loops into DNA (Chen & Wu, 2005; Stoebel *et al.*, 2008). Both OmpR and LeuO act positively at the *ompS1* promoter (De la Cruz *et al.*, 2007). The data presented by De la Cruz *et al.* (2009) are consistent with a regulatory mechanism in which alternative DNA interactions associated with LeuO and OmpR, such as DNA bending (OmpR) or the formation of alternative bridged structures (LeuO), diminish the ability of H-NS to maintain the repressive nucleoprotein complex through DNA bridging. When the intrinsic curve upstream of the *ompS1* gene is removed by site-directed mutagenesis, the LeuO protein is found to be an even more effective anti-repressor, presumably because its opponent, H-NS, is interacting less strongly with the DNA due to its impeded ability to establish a bridged structure. These findings will assist future investigations of H-NS-repressed promoters in bacteria by helping investigators to make informed predictions of the types of regulatory features they may encounter (i.e. regions of intrinsically curved DNA and H-NS binding sites) and their likely locations relative to the promoter.

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