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H-NS gives invading DNA the silent treatment

Mary Kay H Pflum

Though uptake of beneficial foreign DNA confers fitness advantages to bacteria, the mechanisms protecting bacteria from harmful foreign DNA have been unclear. A new study suggests that the H-NS protein transcriptionally silences invading DNA by recognizing its low G-C content, thereby protecting cell viability during bacterial evolution.

Bacterial pathogens are a serious human health concern, particularly given the emergence of antibiotic-resistant strains¹. Insight into the evolutionary events that lead to antibiotic resistance is likely to uncover new strategies for controlling drug-resistant strains. One mechanism of bacterial evolution involves the uptake of foreign DNA through horizontal gene transfer². Although it is well acknowledged that horizontal gene transfer has the potential to enhance bacterial fitness, the ways in which bacteria have evaded the possible negative influences of foreign DNA remain unclear. In a recent issue of *Science*, Navarre *et al.*³ shed light on one possible mechanism used by bacteria to maximize the benefits of horizontal gene transfer.

Nucleoid-associated proteins regulate gene expression in bacteria. In various pathogenic bacteria, including *Salmonella enterica* serovar *typhimurium* (*S. typhimurium*), the leading cause of human gastroenteritis⁴, the histone-like nucleoid structuring (H-NS) protein governs expression of many genes, including those required for host infection⁵. H-NS has functional similarities to histones and acts as a transcriptional repressor by altering DNA structure and supercoiling⁶. H-NS is known to recognize DNA by its structure rather than its sequence. In particular, H-NS binds with high affinity to intrinsically curved DNA⁷, suggesting that H-NS associates with DNA and represses gene expression promiscuously throughout the genome.

Navarre *et al.*³ explored the extent of H-NS regulation using complementary DNA microarray analysis to monitor the changes in gene

expression in *S. typhimurium* lacking the gene encoding H-NS (*hns*). Of the 4,529 open reading frames (ORFs) assessed in the study, roughly 13% of transcripts were influenced in the *hns* mutant strain compared with the wild-type strain. When the authors compared the ORFs influenced by *hns* mutation with genes likely to have been horizontally acquired (as determined by comparison to related genomes⁴), they observed a striking correlation. Namely, transcription of foreign DNA was often repressed by H-NS, but expression of genes common to related pathogenic bacteria was not. Whereas H-NS activity was previously linked to the efficiency of horizontal gene transfer and regulation of horizontally acquired genes^{5,8}, the cDNA microarray results highlight the global influence of H-NS on silencing foreign DNA.

The average G-C content of bacterial genomes varies from 25% to 75%, with Gram-positive bacteria having either low (25–42%) or high (67–75%) C-G content compared with Gram-negative bacteria (50–60%, ref. 9). Not surprisingly, foreign DNA is therefore often distinguished from the native bacterial genome by its unusual G-C content^{2,4}. With this understanding in mind, Navarre *et al.*³ determined the G-C content of the ORFs influenced by *hns* mutation in *S. typhimurium*. They found that genes repressed by H-NS had 46.8% G-C content (the genome average is 52.2%), suggesting that H-NS preferentially recognizes DNA with a reduced G-C content. To confirm these results, the authors recombined a gene from *Helicobacter pylori* that maintains 39.7% G-C content into the *S. typhimurium* genome; using chromatin immunoprecipitation with quantitative PCR, they observed significant interactions between H-NS and the foreign gene. In total, the results indicate that H-NS prefers interaction with foreign DNA

sequences because of its relatively low G-C content.

The correlation between H-NS recognition and G-C content is perhaps not surprising, given that foreign DNA in *S. typhimurium* is A-T rich⁴, A-T-rich DNA sequences are often curved¹⁰ and H-NS interacts preferentially with curved DNA⁷. The model that emerges posits that DNA sequences having low G-C content contain regions of curvature, which attracts H-NS and results in transcriptional repression (Fig. 1). It would be interesting to correlate the curvature of the foreign DNA sequences in *S. typhimurium* with H-NS binding and transcriptional regulation to confirm this structural model of recognition. However, the evidence suggests that recognition of curved A-T-rich DNA by H-NS allows bacteria to distinguish foreign from native genes. Discriminating 'self' from 'nonself' by G-C content is reminiscent of the restriction and modification (R-M) system in bacteria¹¹ in which unmethylated foreign DNA is selectively digested by restriction enzymes in the presence of methylated native DNA, preventing genomic incorporation of the foreign DNA. Taken together, the R-M system and H-NS repression may combine to provide two levels of defense against foreign-DNA invasion; the R-M system reduces the occurrence of horizontal gene transfer while H-NS represses the genetic instability caused by foreign DNA.

Though the study by Navarre *et al.* explains the protective influence of H-NS in bacterial evolution, the ways in which genes conferring a fitness advantage are selectively activated, or derepressed, remain unclear. To address this issue, the authors propose that "antisilencing" agents are evolutionarily developed to activate expression of beneficial genes. Antisilencing agents may be protein factors that competitively displace H-NS binding (Fig. 1c), as observed

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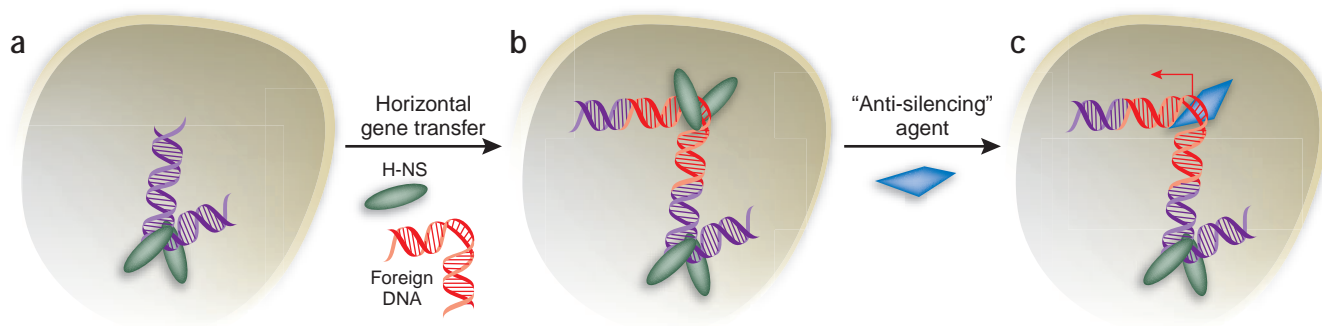


Figure 1 A model for the regulation of foreign DNA in bacteria. (a) Prior to horizontal gene transfer, H-NS proteins (green ovals) interact with curved regions of genomic DNA to repress gene transcription. (b) Once incorporated in the genome, foreign DNA (red), having relatively low G-C content and therefore also having regions of curvature, attracts H-NS, leading to repression of transcription. H-NS repression neutralizes the possible harmful effects of unregulated foreign gene expression on cell viability. (c) Antisilencing agents, such as competitive DNA-binding proteins (blue diamond), derepress H-NS-regulated transcription, resulting in the selective expression of genes (red arrow) that provide a fitness advantage to the cell.

with virulence regulators³. Because DNA methylation alters transcriptional repression of H-NS¹², another possibility is that DNA methylation acts as an antisilencing event. Altogether, H-NS and antisilencing events may cooperate to selectively regulate expression of foreign genes to maximize bacterial fitness. Exploring the cellular events that result in selective derepression of beneficial foreign genes will be an exciting area of future work.

Considering the threat of antibiotic resistance¹, it is important to discuss the ways an understanding of bacterial evolution might provide new strategies for limiting the spread of antibiotic resistance. H-NS attenuates both the conjugal transfer of DNA harboring

multidrug-resistant genes⁸ and the expression of multidrug efflux transporters¹³, which suggests that antisilencing mechanisms could facilitate antibiotic resistance. Therefore, drugs targeting the antisilencers of H-NS might counteract the spread and maintenance of antibiotic resistance. Alternatively, because *hns* mutant strains of *S. typhimurium* are non-viable³, another interesting possibility is the development of new antibiotics that inhibit H-NS activity. Determining the proteins involved in bacterial fitness may reveal unanticipated drug targets and open the door to future drug development.

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Greasing the gears of potassium channels

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Enzymatic conversion of sphingomyelin to ceramide-1-phosphate in the external leaflet of the cellular membrane has now been shown to markedly facilitate opening of classical voltage-activated potassium channels. This discovery raises the possibility that lipids may have more prominent roles in the gating mechanism of these important ion channels than was previously appreciated.

Signaling across cell membranes has attracted the interest of scientists for generations. At some times the lipid components of the membrane have been the center of attention, whereas at

others the proteins molecules embedded in the lipid have generated the most excitement. Today there is a growing appreciation for the importance of the interface where membrane proteins and surrounding lipid molecules meet. The phosphoinositide family of lipids within the intracellular leaflet of the bilayer are a good example of lipids that interact with membrane proteins and have prominent roles in signaling mechanisms throughout biology¹. There are increasing reports of relatively abundant lipids that copurify with membrane proteins, are resolved in their

crystal structures and appear to interact specifically with their protein partners^{2,3}, as observed with the KcsA potassium channel⁴. The anionic phosphatidylglycerol molecules bound to KcsA seem to be required for channel function^{4,5}, reminiscent of the cardiolipin requirement^{4,5} of many proteins involved in cellular bioenergetics^{2,3}. In a recent report by Ramu *et al.*⁶, the importance of specific lipid-protein interactions is highlighted by the discovery that two classical voltage-activated potassium (Kv) channels are exquisitely sensitive to extracellular sphingomyelinase,

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