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Integrated circuits: how transcriptional silencing and counter-silencing facilitate bacterial evolution

W Ryan Will[1], William W Navarre[3] and Ferric C Fang[1,2]

Horizontal gene transfer is a major contributor to bacterial evolution and diversity. For a bacterial cell to utilize newly-acquired traits such as virulence and antibiotic resistance, new genes must be integrated into the existing regulatory circuitry to allow appropriate expression. Xenogeneic silencing of horizontally-acquired genes by H-NS or other nucleoid-associated proteins avoids adventitious expression and can be relieved by other DNA-binding counter-silencing proteins in an environmentally-responsive and physiologically-responsive manner. Biochemical and genetic analyses have recently demonstrated that counter-silencing can occur at a variety of promoter architectures, in contrast to classical transcriptional activation. Disruption of H-NS nucleoprotein filaments by DNA bending is a suggested mechanism by which silencing can be relieved. This review discusses recent advances in our understanding of the mechanisms and importance of xenogeneic silencing and counter-silencing in the successful integration of horizontally-acquired genes into regulatory networks.

Addresses
[1] Department of Laboratory Medicine, University of Washington School of Medicine, Seattle, WA 98195, USA
[2] Department of Microbiology, University of Washington School of Medicine, Seattle, WA 98195, USA
[3] Department of Molecular Genetics, University of Toronto, Toronto, Ontario M5S 1A8, Canada

Corresponding author: Fang, Ferric C (fcfang@u.washington.edu)

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Introduction

A brief comparison of the genomes of a bacterial pathogen and its close non-pathogenic relatives will typically reveal that these strains differ primarily by the presence of horizontally-acquired virulence-associated genomic islets and islands. The model enteric pathogen *Salmonella enterica* serovar Typhimurium is a well-studied example, possessing five separate *Salmonella* pathogenicity islands (SPIs) that are not present in its close relative *Escherichia coli* [1]. This demonstrates the ability of bacteria to evolve in quantum leaps made possible by horizontal gene transfer [2]. Horizontal gene transfer can provide a bacterial cell with new traits or phenotypes in a single genetic event, rather than via the gradual accumulation of beneficial point mutations over time, allowing recipient cells to rapidly take advantage of and colonize new environmental niches. This has made horizontal gene transfer a primary driver of bacterial evolution [3].

To produce an adaptive advantage, newly-acquired genes must be integrated into existing regulatory circuits so that they are expressed in an environmentally and physiologically appropriate manner. If a new gene is too highly expressed, it risks imposing a significant fitness cost upon the host and placing it at a competitive disadvantage. Conversely, if a new gene is only weakly expressed, it may not provide the host with a selective advantage and hence will not be maintained. Thus, the acquisition or evolution of an appropriate regulatory circuit represents an evolutionary threshold. This review focuses on the mechanisms used by *S.* Typhimurium to integrate new genes into existing transcriptional regulatory networks. Recent findings to suggest the existence of analogous mechanisms in distantly related bacterial species will also be discussed.

Global repression by xenogeneic silencing

Horizontally-acquired DNA in bacteria is readily distinguished from ancestral DNA on the basis of its sequence characteristics, in particular, AT-content higher than that of the ancestral genome [3,4]. Findings over the past decade have revealed that many species of bacteria possess DNA-binding proteins that recognize and silence the expression of AT-rich DNA. First described in *S.* Typhimurium [5•,6•], 'xenogeneic silencing' of AT-rich DNA by nucleoid-associated proteins [5•,7] effectively allows a cell to discriminate between 'self' and 'non-self' sequences, repressing foreign gene expression to avoid potential fitness costs. This suggests that horizontally-acquired DNA that is relatively AT-rich is more likely to be retained by recipient cells because it is recognized by xenogeneic silencing proteins and thus better tolerated. Xenogeneic silencing proteins fall into at least three different classes based on structural similarity: the H-NS-like proteins of *Salmonella* and other species of proteobacteria [8,9], the MvaT-like proteins of *Pseudomonas* spp. [10,11], and the Lsr2-like proteins of the Actinomycetes [12–14]. These classes of proteins

share an ability to selectively bind AT-rich DNA and prevent gene expression by forming higher order oligomers that further polymerize to comprise extensive nucleoprotein structures.

A remarkable feature common to all three classes of silencing proteins is their ability to target AT-rich DNA without strict sequence specificity. Studies of the DNA-binding domains of H-NS and Lsr2 have determined that both proteins recognize structural features unique to the minor groove of AT-rich DNA [15••], which is typically narrower and deeper than that of GC-rich DNA [16]. AT-rich DNA sequences vary considerably in their affinity for H-NS, and several studies have shown that a critical determinant for high-affinity binding by H-NS and Lsr2 is the 'TpA step', a thymine base immediately followed by an adenine, which distorts the shape of the minor groove more than other dinucleotide steps and imparts a high degree of flexibility to DNA. Both H-NS and Lsr2 utilize a 'prokaryotic AT-hook' motif (Q/RGR) that inserts into the minor groove and forms extensive interactions along the groove floor [15••]. The flexibility and distortions provided by TpA steps facilitate the insertion of the AT-hook motif [15••,17]. It remains unclear how MvaT-like proteins, which lack an AT-hook motif, selectively bind AT-rich DNA.

Multiple studies have shown that the higher-order structure of the nucleoprotein complex plays a critical role in silencing, and that DNA binding alone is insufficient to block gene expression at most loci [18–20]. Structural and mutational studies of H-NS reveal that the molecule contains two separate dimerization domains. Oligomers consist of chains of H-NS molecules linked 'head-to-head/tail-to-tail' [21••]. Two dimerization interfaces have also been experimentally observed in MvaT and Lsr2 [22,23], suggesting that these molecules oligomerize in a similar chain-like fashion. The manner in which xenogeneic silencing protein chains interact with DNA has recently been a subject of controversy. Atomic force microscopy studies of H-NS [24,25], MvaT [26] and Lsr2 [20] have demonstrated that these molecules can bridge adjacent DNA duplexes, suggesting that silencing may occur when RNA polymerase is trapped in the loops formed between such bridges [25,27,28]. However other studies have shown that the bridging effect is strongly dependent on the concentration of intracellular magnesium [29,30•]. At ~1 mM magnesium concentrations thought to more closely approximate the intracellular environment [31], all three classes of xenogeneic silencing proteins form a stiffened filament when bound to DNA [19,30•,32]. Furthermore, mutational analyses have demonstrated that mutant H-NS proteins that are incapable of forming stiffened filaments *in vitro* are also incapable of silencing gene expression *in vivo*, suggesting that stiffening is the mode of DNA binding responsible for transcriptional repression [33].

Transcriptional activation is dependent on a conserved promoter architecture

As H-NS is constitutively associated with the bacterial chromosome [34], xenogeneic silencing renders most horizontally-acquired genes transcriptionally inactive by default. This places H-NS in a central role within the regulatory network of many virulence genes. Expression requires the regulated relief of H-NS-mediated repression, otherwise known as counter-silencing [7,35]. The regulation of bacterial gene expression in response to environmental or physiological cues is often achieved by two-component systems, exemplified by the PhoPQ system, which is essential for *Salmonella* virulence [36,37]. The PhoPQ response regulator, PhoP, is a prototypical transcriptional activator, regulating gene expression in response to low extracellular Mg^{2+} [38], acidic pH [39], and cationic antimicrobial peptides [40]. Recent studies have demonstrated that PhoP acts via different mechanisms at ancestral and horizontally-acquired promoters [41••]. Bioinformatic analysis of the PhoP regulon has demonstrated that horizontally-acquired genes exhibit variable promoter architectures, with PhoP-binding sites at a variety of positions and orientations relative to the transcription start site [42•,43] (Figure 1). In contrast, ancestral genes exhibit a conserved promoter architecture, with a single PhoP binding site overlapping the −35 box. *In vitro* reconstitution of these regulatory circuits using supercoiled templates has revealed that PhoP is only capable of activating promoters with ancestral architectures, at which it presumably interacts directly with the RNA polymerase (RNAP) holoenzyme [41••]. PhoP is unable to up-regulate promoters exhibiting alternative architecture unless they are silenced by H-NS, indicating that PhoP acts at these promoters by counter-silencing rather than by classical activation. However, H-NS-mediated silencing does not prevent activation, as horizontally-acquired promoters possessing an ancestral promoter architecture, such as *orgB*, are capable of being activated by PhoP.

Diversity in promoter architecture [44] suggests flexibility in the interaction of PhoP with horizontally-acquired promoters, whereas the interaction of PhoP with ancestral promoters appears to be highly constrained. Structural analyses of a related OmpR-family regulator, PhoB, suggests that a response regulator must be precisely positioned at the −35 box for interaction with RNAP holoenzyme and transcription activation to occur. The interaction results in remodeling of the linker between domains 3 and 4 of the σ-subunit, which allows passage of the nascent transcript through the RNA exit channel to result in promoter escape [45•]. Such activation by RNAP remodeling is inconceivable at many horizontally-acquired promoter architectures, at which the PhoP binding site is situated at a variable distance and orientation upstream of the −35 box. It is unknown whether this mechanism applies to other response