

Review

Control of foreign DNA: emerging roles of xenogeneic silencers

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Bacteria continuously acquire foreign DNA through horizontal gene transfer, yet its successful integration depends on regulatory mechanisms that balance genome protection with evolutionary innovation. Xenogeneic silencers are central to this process: they preferentially bind AT-rich DNA, a common feature of many horizontally acquired genetic elements, and repress its transcription. Recent studies, however, reveal a much broader regulatory repertoire. Beyond transcriptional repression, these proteins contribute to chromosome organization by forming higher-order nucleoprotein complexes and phase-separated condensates that shape bacterial nucleoid architecture. Furthermore, they play roles in regulating bacteriophage infection cycles, including mechanisms by which phages hijack host silencing activities for their own benefit. Their extensive regulatory reach, spanning virulence genes, biofilm formation, specialized metabolite production, and mobile genetic elements (MGEs), underscores their central role in connecting environmental signals, including fluctuations in the second messenger c-di-GMP, with gene expression, and genome organization. The diversification of xenogeneic silencers across bacterial chromosomes, plasmids, phages, and other MGEs highlights their evolutionary significance. Together, these recent findings position xenogeneic silencers as dynamic regulatory modules that shape the fate of foreign DNA across the horizontal gene transfer network.

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Introduction

Bacteria are constantly exposed to foreign DNA. Horizontal gene transfer (HGT), mediated by bacteriophage infection, conjugative and transposable elements, and natural competence, drives the acquisition of new genetic material [1,2]. Such incoming DNA often differs in nucleotide composition from the host genome, providing a basis for its recognition [3,4].

While HGT can be a major driver of bacterial evolution by enabling the acquisition of new genetic traits [5,6], it also poses significant challenges for the host cell. Newly acquired genes may interfere with existing regulatory networks or impose metabolic burden if expressed in an uncontrolled manner [7]. Bacteria must therefore balance the evolutionary advantages of gene acquisition with the potential fitness costs of unregulated foreign DNA expression.

One key mechanism to manage this challenge is the use of xenogeneic silencer proteins (XS) [4]. XS proteins are nucleoid-associated proteins (NAPs) that preferentially bind AT-rich DNA, a common feature of horizontally acquired genetic elements, and repress their transcription. Through the formation of higher-order oligomeric assemblies, DNA bridges, and/or long-range looping structures, these proteins contribute to DNA compaction and genome organization. Deletion of XSs often results in extensive pleiotropic effects [8–10], making functional characterization challenging while also highlighting their broad regulatory and structural roles.

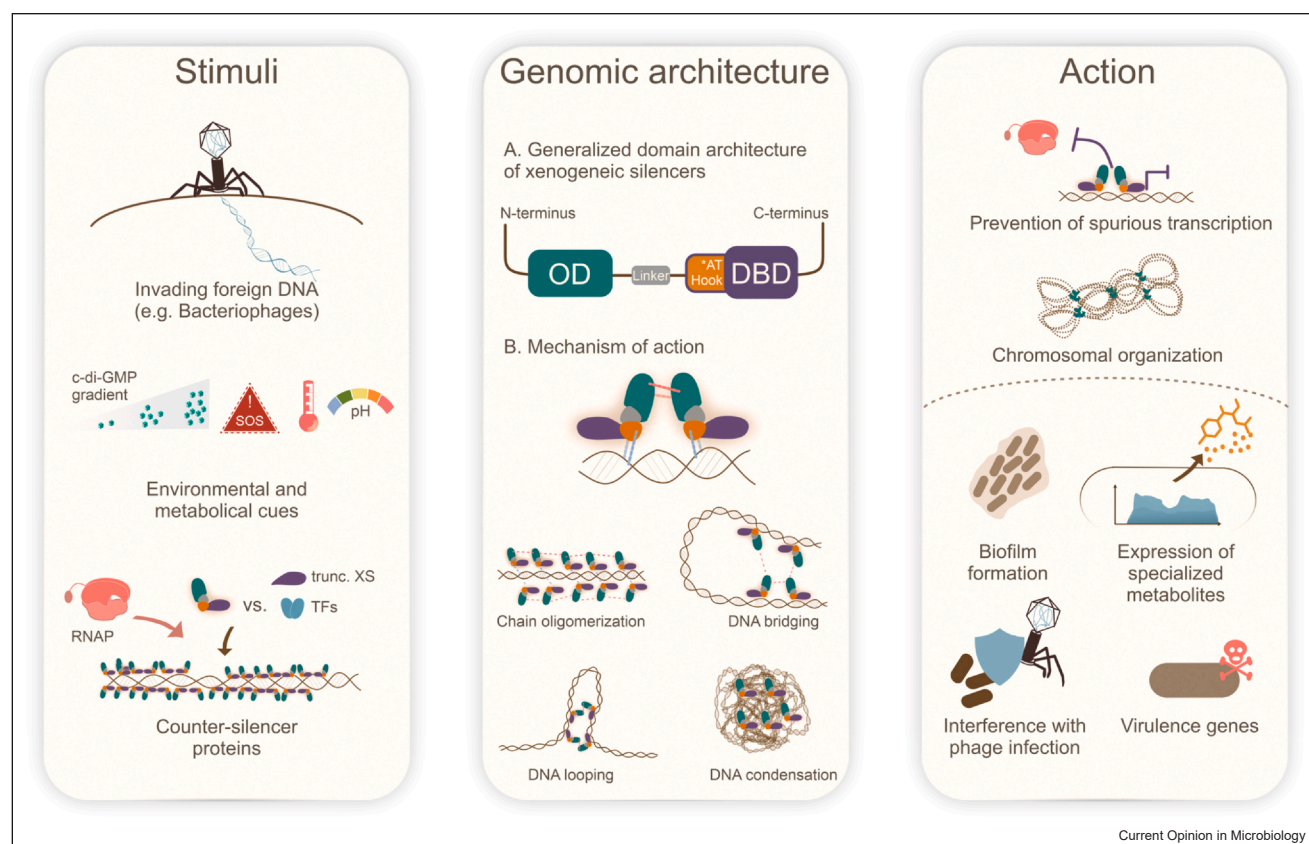
XSs have been identified across diverse bacterial lineages, yet they share little sequence homology. Known examples of proteins exemplifying the aforementioned definition include H-NS and MucR in Proteobacteria [11,12], Lsr2 in Actinomycetota [13], Rok proteins in Firmicutes [14], and the MvaT/MvaU proteins in *Pseudomonas* [15]. Despite their sequence diversity, these proteins (typically <15 kDa in size) share a common domain organization, comprising an N-terminal oligomerization domain (OD) and a C-terminal DNA-binding domain (DBD), and both domains are essential for gene-silencing function. In addition, the Lsr2 and H-NS families also encode an AT-hook-like 'Q/RGR' motif (Q for H-NS, R for Lsr2 and the related Bv3F family [16,17]) within their DBDs.

Although XSs were initially characterized as transcriptional repressors of foreign chromosomal DNA, recent studies have revealed a broader functional repertoire. These proteins modulate core gene regulatory networks, influencing

processes as diverse as virulence gene expression [18], biofilm formation [19], and specialized metabolite production [20]. Beyond individual gene targets, recent work shows that they also shape large-scale chromosome organization [21] and interact with MGEs, including the regulation of (pro-) phage activity [9]. Importantly, homologs of XSs are not restricted to bacterial chromosomes but are also encoded by plasmids, conjugative elements, and bacteriophages [22]. The regulatory roles of these phage- and plasmid-encoded variants remain largely unexplored and may represent an important, yet mainly overlooked, dimension of phage-host and MGE-host interactions.

In this review, we discuss recent advances in understanding the distribution, function, and evolutionary origins of XSs across chromosomes and MGEs, highlighting their critical roles in genome stability, phage-host interactions, and the fate of horizontally acquired DNA. [Figure 1](#).

Figure 1



Overview of the regulatory landscape of xenogeneic silencing. Xenogeneic silencing is modulated by a diverse range of stimuli (including second messengers, temperature, and phage-encoded factors) that influence chromosomal organization and the expression of the targeted gene cluster (left). Although xenogeneic silencers share a common domain architecture, comprising an N-terminal oligomerization domain (OD), a linker region, and a C-terminal DNA-binding domain (DBD), the higher-order nucleoprotein complexes and chromatin organization they form differ between silencer families. In the Lsr2 and H-NS families, the DBD contains an AT-hook-like motif, marked in orange. The modulation of xenogeneic silencing has broad physiological consequences, impacting processes as diverse as biofilm formation, virulence gene expression, defense against invading DNA, and specialized metabolite production (right).

Counter-silencing enables regulatory assimilation of foreign DNA

A central question emerging from this expanded view is how bacteria preserve the benefits of silencing while allowing selected horizontally acquired genes to become functionally integrated. This balance is achieved through counter-silencing mechanisms that relieve repression of XS-controlled loci in a global or locus-specific manner. Thus, xenogeneic silencing is not simply an ON/OFF barrier to foreign gene expression, but a reversible state that can enable regulatory assimilation [23]. Work in *Salmonella* first illustrated this principle. Here, the two-component regulator PhoP activates horizontally acquired genes that are otherwise silenced by H-NS. Whereas PhoP-dependent activation of ancestral genes requires a conserved promoter architecture, H-NS-bound horizontally acquired promoters can be activated across a broader range of promoter configurations [24]. This supports the notion that xenogeneic silencing buffers newly acquired loci while permitting more flexible routes for their regulatory assimilation.

Counter-silencing can occur through global or locus-specific mechanisms. Truncated XS variants lacking the DBD [9], or proteins with incompatible oligomerization interfaces, can disrupt nucleoprotein complex formation and de-repress silenced targets. Such strategies have been used to activate specialized metabolism in *Streptomyces* [20] or induce MGEs [9]. However, genome-wide counter-silencing can be detrimental, as activation of silenced prophage or MGE genes may trigger toxicity and cell death [9].

More targeted counter-silencing relies on local disruption, displacement, or bypass of repressive nucleoprotein complexes. Transcription factors can remodel XS-DNA complexes and facilitate RNAP recruitment, as shown for VirB-mediated relief of H-NS repression at virulence promoters in *Shigella flexneri* [25]. Regulators can also compete directly with silencers for overlapping DNA-binding sites, exemplified by LuxR counteracting H-NS repression of quorum-sensing genes in *Vibrio harveyi* [25]. In addition, transcription itself can promote counter-silencing: elongating RNAP and associated changes in DNA topology can destabilize H-NS or Lsr2 nucleoprotein complexes, reinforcing promoter accessibility and transcriptional progression [26].

Synthetic approaches have further defined the mechanistic rules of counter-silencing. In *Corynebacterium glutamicum*, the Lsr2-like XS protein CgpS preferentially binds AT-rich regions marked by local GC drops near transcription start sites and nucleates at sites enriched in A/T-step motifs. Systematic insertion of transcription factor operator sites into CgpS-silenced promoters showed that regulator binding can efficiently relieve repression, with the strongest activation when the

operator overlaps the region of maximal CgpS occupancy, consistent with disruption of the nucleoprotein complex at or near its nucleation site [27]. This principle was further implemented in synthetic regulatory circuits, demonstrating that XS-based repression can be converted into inducible, reversible gene control [28]. More recently, CRISPR/dCas-based strategies extended this concept by converting programmable DNA-binding proteins into targeted antagonists of Lsr2-mediated repression, enabling selective activation of otherwise silent gene clusters [29]. Thus, counter-silencing ranges from broad disruption of silencer assemblies to precise, programmable remodeling of individual XS-regulated loci.

Regulation of xenogeneic silencing via second messengers

Xenogeneic silencing influences a wide range of biological processes, including motility [30], biofilm formation [31], cell cycle progression [32], and virulence [33]. Recent studies have further revealed that silencer activity can be regulated by second messengers, with XS proteins identified as direct receptors for the cyclic dinucleotide c-di-GMP.

The first reported example of second messenger-mediated regulation of XSs demonstrated that c-di-GMP modulates the DNA-binding activity of H-NS in *Salmonella*. C-di-GMP binds to H-NS with a 1:1 stoichiometry, and this interaction disrupts H-NS binding to target promoters. Notably, this effect appears specific to c-di-GMP, as similar interactions were not observed for other cyclic messengers such as c-di-AMP or cGMP. The identified binding residues are located near the AT-hook motif of H-NS, supporting a mechanism in which c-di-GMP interferes with H-NS-DNA interactions [34]. However, conflicting results leave the proposed mechanism of c-di-GMP and H-NS jointly regulating T6SS expression in *Salmonella* partially unresolved [35].

While the interaction between c-di-GMP and H-NS remains debated, studies in Actinomycetota point that Lsr2 can be an interaction partner of c-di-GMP signaling. In *Mycobacterium bovis*, elevated levels of this second messenger correlate with increased production of keto-mycolic acids [36]. As proposed for H-NS, c-di-GMP binds Lsr2 with a 1:1 stoichiometry; however, this interaction enhances rather than inhibits its regulatory activity. Mechanistically, c-di-GMP binding promotes Lsr2-dependent activation of *hadD*, encoding a (3R)-hydroxyacyl-ACP dehydratase of the fatty acid synthase type II (FAS-II) system. In this context, Lsr2 functions as a transcriptional activator rather than a silencer [37]. While this remains the only well-characterized example in Actinomycetota to date, in *Streptomyces venezuelae*, which also encodes a divergent Lsr2 homolog, LsrL, an analogous enhancement of gene expression dependent

on Lsr2 (rather than silencing) has been detected [20]. Given that c-di-GMP regulates developmental progression in *Streptomyces* through interactions with regulators such as the global developmental regulator BldD [38], it is plausible, although not yet experimentally tested, that analogous c-di-GMP–Lsr2 regulatory mechanisms may exist in this genus. Evidence that c-di-GMP-mediated regulation of DNA-binding proteins extends beyond Actinomycetota is provided by CdbA from *Myxococcus xanthus*, a c-di-GMP receptor that coordinates nucleoid organization with chromosome segregation, suggesting that second-messenger control of chromatin-associated proteins may represent a conserved regulatory principle across bacterial lineages [39].

By contrast, the MucR silencer in the α -proteobacterium *Sinorhizobium fredii* represents a distinct mechanism. Here, c-di-GMP binding does not affect DNA association but disrupts higher-order bridging required for silencing, thereby enabling transcription independently of additional regulators [40].

Together, these studies indicate that second messenger signaling can modulate XS activity in diverse and context-dependent ways; however, further physiological, biochemical, and structural analyses across different XS families and bacterial lineages will be required to determine whether common regulatory principles or family-specific mechanisms emerge.

From host chromosomes to MGEs: diversification of XSs

XSs are widely distributed across bacterial chromosomes, phages, plasmids, and ICEs [3,9,41–44]. While host-encoded XSs, such as H-NS and Lsr2, are considered as global ‘core’ regulators, the roles of MGE-encoded variants remain poorly understood. Phylogenetic analyses separate host-encoded XSs from MGE-encoded homologs, even within the same host (Figure 2a,b) [44–46]. Furthermore, their presence in distinct genetic regions that lack sequence and functional similarity (Figure 2c), suggests that XSs have diversified beyond canonical host chromosome silencing.

While some MGE-encoded Lsr2 proteins (e.g. CgpS of CGP3 [44]) have an anti-MGE function, the host-encoded Lsr2 of *M. smegmatis* is required for efficient infection cycles of phages [46]. Moreover, although plasmid-encoded H-NS homologs, for example, of R27 of *Salmonella enterica* [45], can complement some *hns* deletion phenotypes, their primary role appears more nuanced. By specifically downregulating plasmid-borne genes to minimize the fitness costs, it is suggested that these homologs enable a seamless integration of the plasmids into the host regulatory network [43,47].

In actinobacterial phages, Lsr2 proteins have probably diverse functions, as they are found in both temperate and virulent phages [42,48], and their genes reside within nonconserved genetic neighborhoods [22]. Notably, some Lsr2 actinophages are phage-plasmids, widespread temperate phages that replicate as plasmids during lysogeny [49]. In certain phage-plasmids, *lsr2* is encoded next to partitioning and replication modules [22] (e.g. CRB1 in Figure 2C), suggesting an involvement in the plasmid life cycle. However, since not all phage-plasmids, even from the same Cluster, encode Lsr2 (e.g. RedRock [50]), the protein likely serves an accessory role.

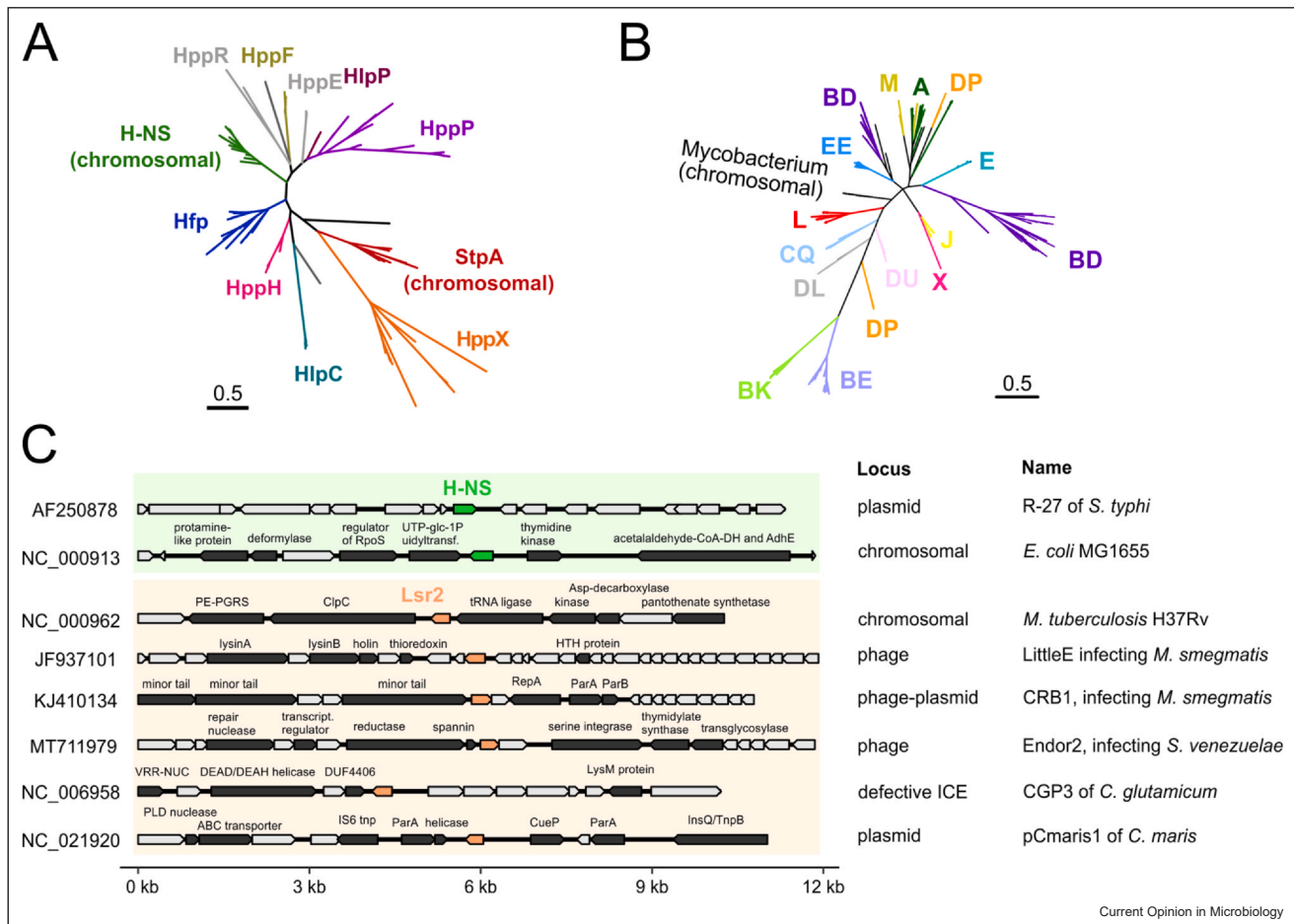
Multiple XS genes are often encoded by MGEs or bacterial chromosomes. These paralogs are typically located at distant genomic loci and have complementary or safeguard functions. For instance, enterobacterial H-NS and StpA are partially redundant, form heterodimers, and can compensate for each other [51]. Similarly, most *Streptomyces* species have Lsr2 and a second copy, LsrL. Next to the main functions (global silencers, chromosomal organizer), Lsr2 serves as a gatekeeper for biosynthetic gene clusters [20]. LsrL functions in tandem with Lsr2, fine-tuning, reinforcing, and modulating broader silencing programs while contributing to the organization of higher-order chromosome structures [21]. Additional LsrL-specific functions remain to be discovered. In *M. smegmatis*, Lsr2 paralogs exhibit different DNA binding specificities [52]. While exact roles have not been investigated, the diversity of the Lsr2 variants suggests distinct, versatile functions.

Lastly, whether XSs originated from MGEs or from their bacterial hosts remains a matter of debate. Their high prevalence, context-specific functions, and frequent paralogy suggest recurrent exchanges and rapid specialization during bacteria-MGE co-evolution. This dynamic is underscored by their active deployment in the host–pathogen arms race. For instance, some pathogenic *E. coli* strains have distinct genomic regions that encode Ler, an H-NS homolog, or H-NST, a truncated H-NS variant lacking the DBD. When expressed, both proteins act as counter-silencers that alleviate native H-NS repression, effectively reshaping the host’s regulatory output [53].

Bacteriophages as targets and modulators of xenogeneic silencing

Bacteriophages, the viruses that infect bacteria [54,55], exploit the host cellular machinery to promote the production of phage progeny, often culminating in host cell lysis [56]. In response, bacteria have multiple regulatory strategies to limit phage replication [57]. Consequently, successful phage infection frequently requires interactions with host regulatory networks, either by hijacking

Figure 2



Diversification of Xs across host chromosomes, MGEs, and phages. **(a)** Phylogenetic tree of chromosomal and plasmid-encoded H-NS homologs [47] (adapted and simplified). H-NS and StpA are host-encoded, whereas Hpp and Hlp are plasmid-encoded. **(b)** Phylogenetic tree comparing host-encoded (*Mycobacterium*) Lsr2 proteins to phage-encoded homologs, color-coded and labeled by cluster [46] (adapted and simplified). **(c)** Genetic regions of plasmid-, phage-, and host-encoded XS proteins (H-NS in green, and Lsr2 in orange). Gene annotations were derived from corresponding GenBank files. Known functions are indicated for black genes, while unknown functions in gray.

host regulators or by encoding factors that modulate host transcription [58,59].

Early evidence came from bacteriophage T7, whose early protein Gp5.5 directly interacts with *E. coli* H-NS and relieves H-NS-mediated repression, allowing transcription by both host and phage RNA polymerases [60]. Similarly, *Pseudomonas* phage LUZ24 encodes Mip (MvaT-interfering protein), a small protein that interacts with the H-NS-like silencer MvaT [61]. Given the GC-content difference between LUZ24 and its host, Mip was proposed to act as an antidefense factor that protects phage DNA from silencing. Mechanistically, Mip does not block MvaT-DNA binding, but prevents higher-order bridging required for repression [62]. However, whether Mip is essential for the LUZ24 infection cycle remains unresolved. Beyond antagonizing host silencers,

some phages encode silencer homologs themselves. For example, EPV1, a phage infecting *Candidatus Accumulibacter phosphatis*, encodes an H-NS homolog that may cooperate with the host protein to silence host genes, potentially including defense systems [63].

Within Actinomycetota, the XS Lsr2 is broadly distributed, and several phages infecting *Mycobacterium*, *Streptomyces*, and *Gordonia* species encode Lsr2-like proteins [3]. The mycobacterium-infecting phage Fionnbharth appears to rely on host Lsr2 for successful infection, implying a role in organizing the phage genome during the infectious cycle [46]. However, this conclusion is based solely on microscopy data and *lsr2* deletion phenotypes, and does not account for potential indirect effects arising from altered transcription of Lsr2-regulated genes. In contrast, in *C. glutamicum*, the Lsr2-

like protein CgpS encoded within the defective ICE CGP3 provides an additional defense layer by silencing the incoming DNA of AT-rich phages [44]. The functional roles of phage-encoded Lsr2 homologs remain largely uncharacterized, though they have been proposed to potentially interfere with host silencing proteins as well [48].

Novel interactions between phage proteins and XSs continue to be uncovered. The *E. coli* phage T4 protein MotB was initially characterized as a counter-silencer of H-NS [64], but more recent work suggests that MotB may instead act as a DNA-compacting protein that remodels nucleoid architecture and thereby indirectly affects H-NS-mediated silencing [65].

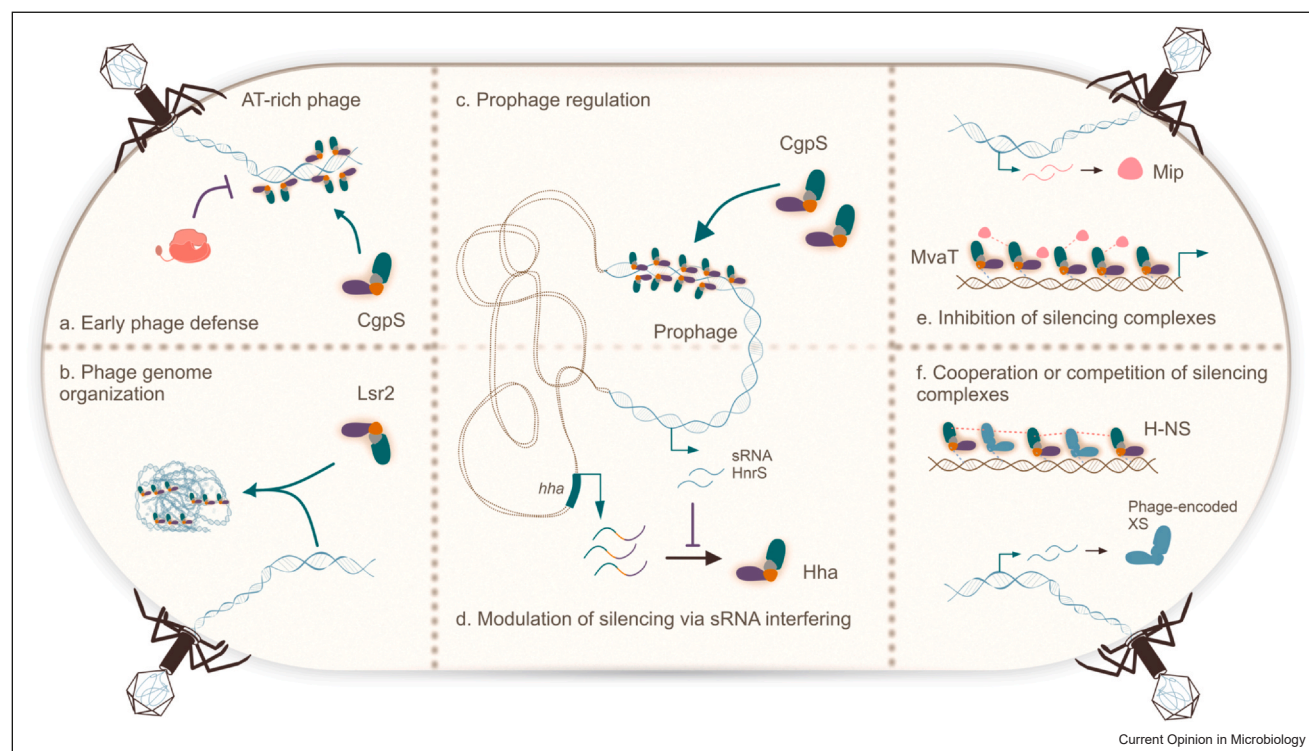
Prophage-encoded regulatory elements add another layer to xenogeneic silencing dynamics. Prophages, the dormant form of phages integrated into bacterial genomes, can encode factors that modulate host regulatory networks [66]. In pathogenic *E. coli*, the prophage-encoded small RNA HnrS represses translation of the H-

NS paralog Hha by pairing with its ribosome-binding site. This derepression promotes expression of virulence genes, ultimately enhancing host fitness and supporting persistence of the temperate phage [67]. Together, these examples show that bacteriophages and prophages have repeatedly evolved strategies to manipulate xenogeneic silencing, either by counteracting host silencers, encoding silencer homologs, or rewiring silencer-associated regulatory circuits (Figure 3). This mechanistic breadth highlights the functional diversity of xenogeneic silencing systems and their central role at the interface between bacterial genome defense and phage infection.

Chromosomal organization by xenogeneic silencers

XSs have also been orthologically compared to eukaryotic histone proteins, given their shared role in compacting and organizing chromosomal DNA, with some proposals suggesting a common evolutionary origin [17,68]. Recent work has implicated XSs as contributors to nucleoid architecture across multiple phyla. Despite sharing a conserved DBD with a preference for AT-rich

Figure 3



Interactions between bacteriophages and XSs. **(a)** The ICE-encoded Lsr2-like silencer CgpS can defend against infection by AT-rich phages by binding to incoming phage DNA, thereby repressing phage gene expression. **(b)** In *Mycobacterium smegmatis*, the xenogeneic silencer Lsr2 is required for productive infection and successful phage propagation by certain mycobacteriophages. **(c)** CgpS additionally silences cryptic prophage regions within the bacterial chromosome. **(d)** A prophage-encoded sRNA can bind the mRNA of the H-NS paralog Hha and inhibit its translation. **(e)** The *Pseudomonas* phage-encoded Mip protein interferes with the formation of higher-order xenogeneic silencing complexes, leading to reactivation of gene expression. **(f)** Phage-encoded xenogeneic silencer (XS) proteins can either cooperate with or compete against preexisting chromosomal silencing complexes, thereby modulating silencing activity.

sequences, these proteins display notable structural diversity in their influence on chromatin architecture. H-NS in Enterobacteriaceae can form both linear and bridged nucleoprotein assemblies, whereas MvaT in *P. aeruginosa* and Lsr2 in Actinomycetota adopt predominantly filamentous bridged structures [69], reflecting again a functional diversification across bacterial lineages.

Characterizing the oligomeric assemblies formed by XSs has proven challenging due to their lack of fixed stoichiometry [70]. Recent work on H-NS shows that chromosome organization critically depends on higher-order oligomerization rather than DNA binding or dimerization alone. The Y61E mutant, which abolishes this higher-order assembly, consequently eliminates the capacity to condense DNA and form liquid-like phase-separated condensates [69]. These condensates can involve up to 17 different proteins, including the co-repressor Hha. Notably, temperature emerges as a key physiological cue capable of naturally dissolving H-NS:DNA condensates, linking nucleoid organization to environmental sensing [71].

A similarly complex organizational role has recently been described for MucR, one of the most recently proposed H-NS-like silencers. Using chromosome conformation capture (Hi-C) in *Brucella*, an organism that harbors two chromosomes, DNA contacts within and between chromosomes were examined. Deletion of *mucR* revealed a marked increase in both short- and long-range DNA contacts, demonstrating that MucR actively restricts inter-regional chromosome interactions. Among the most striking outcomes of its loss were reduced chromosome alignment and a significant increase in interchromosomal contacts, pointing to a role for MucR in maintaining the spatial segregation of the two chromosomes [72].

In Actinomycetota, Lsr2 can form long-range chromosomal loops and protein-DNA co-condensates, as shown in *M. tuberculosis* [73]. This capacity appears to arise from the overall binding energy landscape rather than recognition of specific sequence motifs, suggesting a mode of global chromosome organization distinct from simple targeting of AT-rich DNA. Beyond *M. tuberculosis*, Lsr2 has also been shown to remodel chromosomal topology at both local and global scales in *Streptomyces venezuelae*, acting in concert with its paralog LsrL. As observed for H-NS, this organizational activity could be further modulated through interplay with other NAPs, underscoring a recurring theme of cooperative nucleoid structuring across phylogenetically distant silencer families [21].

Chromatinization dynamics, while a relatively recent topic in bacteria, have also been described for viruses. Human cytomegalovirus, for example, undergoes rapid

chromatinization of its genome upon nuclear entry, influencing gene expression across different phases of infection [74]. Kaposi's sarcoma-associated herpesvirus is circularized into an extrachromosomal episome that relies on cellular histone proteins for its stability [75]. Adenoviruses encode the core protein VII, which has sequence similarity to histone H3 [76], and eukaryotic histone H3.3 has also been shown to deposit onto incoming viral genomes, silencing their expression [77]. Since bacteriophages encode their own XSs, and recent research has begun to uncover interactions between host- and phage-encoded silencers [44,46], it remains a compelling open question whether these proteins also participate in the chromatinization of bacteriophage genomes or other MGEs.

Conclusion & future perspectives

XSs are emerging as multifunctional regulators at the intersection of genome defense, regulatory innovation, and chromosome architecture. Beyond their classical role in repressing AT-rich foreign DNA, recent work shows that these proteins can be dynamically modulated by counter-silencing, second messengers, and MGEs, while also contributing to large-scale nucleoid organization. Their widespread occurrence on chromosomes, plasmids, ICEs, and bacteriophages [22,46] suggests that XS proteins are not static host barriers, but mobile regulatory modules exchanged and specialized during bacteria-MGE co-evolution.

Future studies will provide mechanistic insights into the function of MGE-encoded XS homologs and their roles during plasmid maintenance, lysogeny, and phage infection. This also includes the interaction between different MGEs, like prophages, plasmids, ICEs, and co-infecting phages [42], where XS proteins may mediate competition, cooperation, or exclusion by silencing, counter-silencing, or structuring foreign DNA [44]. Integrating comparative genomics, single-cell infection models, chromosome-conformation approaches, and structural biology will be essential to define how XS proteins operate along a continuum from local transcriptional regulation to global genome organization [78]. Understanding this continuum may enable targeted control of silent gene clusters, virulence genes, mobile element activity, and phage susceptibility.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could

have appeared to influence the work reported in this paper.

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- of outstanding interest

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