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Bacterial Pathogenicity Regulation by RNA-binding Antiterminators

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Bacterial Pathogenicity Regulation by RNA-binding Antiterminators

Antiterminators are essential components of bacterial transcriptional regulation, allowing the control of gene expression in response to fluctuating environmental conditions. RNA-binding antiterminators are particularly important regulatory proteins that play a significant role in preventing transcription termination by binding to specific RNA sequences. These RNA-binding antiterminators have been extensively studied for their roles in regulating various metabolic pathways. However, their role in modulating the physiology of pathogens requires further investigations. This review focuses on these RNA-binding proteins in both Gram-positive and Gram-negative bacteria, particularly on their structures, mechanism of action, and target genes. Additionally, the involvement of the antitermination mechanisms in bacterial pathogenicity will be discussed. This knowledge is crucial for understanding the regulatory mechanisms that govern bacterial pathogenicity, opening up exciting prospects for future research, and potentially new alternative strategies to fight against infectious diseases.

Keywords: antiterminator, expression regulation, bacterial pathogen, virulence, biofilm, metabolism

Introduction

RNA polymerase (RNAP) is a multi-component enzyme that performs transcription in all living organisms. After the initial recognition of a gene promoter region, sigma factor-assisted RNAP transcribes the first 10 to 15 nucleotides before the sigma factor is released to start elongation. RNAP then transcribes DNA until termination signals are encountered, inducing rapid and irreversible dissociation of the nascent RNA transcript from RNAP. Transcription termination is caused by two main processes, one of which is based on the helicase Rho protein that dislodges the RNAP (Rho-dependent termination), while the other one depends on the RNA sequence itself (intrinsic termination), and refers to the Rho-independent termination ¹. In the latter, RNAP stops at a DNA sequence characterized by a GC rich hairpin followed by a poly-T track, resulting in its dissociation from the DNA template without the assistance of auxiliary factors ².

In bacteria, most transcription termination is performed *via* intrinsic RNA loops located either at the end of genes thus preventing transcription of the downstream genes ¹, or in the upstream regulatory leader regions (or untranslated regions [UTR]) to adjust gene expression in response to metabolic and/or environmental signals ³. Since the formation of RNA loops is mainly a passive process in bacteria, transcription antitermination is considered as an important regulatory mechanism to allow RNAP to bypass or prevent hairpins from folding, and consequently to control gene expression ⁴.

Bacteria have several pathways that allow RNAP to override a terminator, most of which affect the formation of hairpins. The first passive mechanism discovered in bacteria refers to the action of stalled ribosomes and is known as termination attenuation. This is the case of the *Escherichia coli trp* operon during tryptophan starvation, in which the ribosome stalls within the leader peptide, allowing the formation of an antiterminator loop, leading RNAP to proceed into the Trp biosynthesis genes ⁵. Uncharged tRNA can also be sensed independently

of the ribosome, through interactions between RNA and the leader encoding an antiterminator⁶. Another antitermination mechanism involves small molecules and mRNA leader riboswitches that undergo structural rearrangements in response to ion concentration variations, or upon binding to small compounds as purine, flavin mononucleotide, or ppGpp for instance^{7,8}. Some intrinsic terminators are located upstream of the open reading frames, within 5' leader regions, where they participate in signal-responsive regulatory mechanisms. Antiterminators with RNA-binding domain can interact with these regions, thereby controlling downstream gene expression⁹.

Among the RNA-binding antiterminators, proteins with an ANTAR (AmiR and NasR Transcription Antitermination Regulators) domain bind RNA hexaloop motifs within the nascent RNA chain to prevent termination^{10,11}. This domain has been detected within various response regulators of Two-Component Systems (TCS), and binds RNA instead of DNA, unlike the majority of TCS output domains¹². ANTAR domains are modular and include several types of sensors, or receiving domains, such as cGMP-related phosphodiesterases, Adenylyl cyclases, FhlA (GAF), and Per-Arnt-Sim (PAS) domains^{10,13}. Consequently, proteins with ANTAR domains have been suggested to be involved in the regulation of numerous bacterial metabolic and regulatory processes¹³⁻¹⁵.

Another RNA-binding domain encountered in bacteria is the CAT (Co-AntiTerminator) domain found in antiterminators like BglG or SacY in *E. coli* and *Bacillus subtilis*, respectively^{16,17}. The CAT domain binds to specific RNA motifs known as Ribonucleotidic AntiTerminator (RAT) sequences. By binding to these RAT sequences, CAT domains prevent the formation of terminator structures in nascent mRNA, allowing transcription to continue¹⁸⁻²⁰. In many antitermination proteins, the CAT domain is followed by regulatory domains, such as phosphorylable PhosphoTransferase System (PTS) Regulatory Domains (PRDs). These regulatory domains play a crucial role in modulating CAT domain activity. Most of the

antiterminators of the BglG/SacY family are involved in metabolism, and possibly also in virulence, noticeably in the case of opportunistic bacteria ²¹.

This review gathers the up-to-date knowledge on RNA-binding antiterminators from both Gram-positive and Gram-negative bacteria at the levels of structure, mode of action, regulation, and identified targets. In addition, their functions in cellular processes are highlighted, with a special focus on their involvement in bacterial pathogenicity in terms of infection, virulence, and biofilm formation.

RNA-binding antiterminators in Gram-negative bacteria

The BglG antiterminator in Escherichia coli

In *E. coli*, the *bgl* operon is composed of three genes: *bglG*, *bglF* and *bglB*, encoding functions needed to regulate the uptake and degradation of β -glucosides. These polysaccharides are composed of disaccharide units linked by covalent β -glycosidic bonds ^{22,23}. The BglG protein is a transcriptional antiterminator, BglF a β -glucoside-transport PTS enzyme EIIBCA, and BglB a phospho- β -glucosidase that hydrolyzes the phosphorylated carbohydrates ^{16,22,23}.

BglG structure

The BglG antiterminator family is a group of regulatory proteins with an inducer-dependent phosphorylation activity, first identified in *E. coli* ^{18,24,25}. They are composed of an RNA-binding domain, corresponding to the so-called CAT domain, and two PRDs, named PRD1 and PRD2. BglG exists in monomeric and dimeric forms, the latter being initiated by PRD2 to create the RNA-binding domain ^{26,27}. The BglG protein of *E. coli* has not been

crystallized, unlike its counterpart SacY in *B. subtilis*, which is described in the section corresponding to Gram-positive bacterial antiterminators.

BglG antitermination mechanism

In the absence of β -glucoside, *bgl* operon transcription is initiated by the RNAP. Still, it stops due to the formation of a transcription terminator located in the untranslated region (5'UTR) of the *bglGFB* operon (Figure 1). Phosphorylation of BglG is mediated by BglF, which is also involved in sugar phosphorylation. BglF, as a PTS transporter, has two phosphorylation sites, *i.e.* His-547, phosphorylated by HPr (Histidine-containing Phosphocarrier protein), and Cys-24, from which the phosphoryl group is transferred to the sugar²⁸. When BglG is phosphorylated by BglF, it remains inactive allowing the formation of the Rho-independent transcription terminator^{16,22}. In the presence of the inducer sugar, BglF transports the carbon source and transfers its phosphate group to β -glucosides, resulting in the dephosphorylation and dimerization of BglG, and the formation of an active RNA-binding domain^{24,29-31}. When BglG is active, it binds to the RAT palindromic RNA sequence, found in the *bgl* operon leader region. This 30-nucleotides RNA sequence can adopt a stem-loop structure that partly overlaps the intrinsic terminator²⁶. BglG bound to the RAT sequence stabilizes its secondary structure in nascent transcripts, prevents terminator formation, and allows the RNAP to progress through the downstream encoding gene sequence^{16,18,25}.

HPr phosphorylation was suggested to be required for BglG activation³¹, but direct phosphorylation has never been described. Görke and Rak have proposed that the BglG phosphorylation sites by BglF and HPr are different. One of the two PRDs was suggested to be the target of the BglF-negative-regulation, while the second could be that of HPr-related positive-regulation³¹. Two different hypotheses have been depicted concerning the amino acids involved in regulation by phosphorylation/dephosphorylation. Both hypotheses showed that

BglG is phosphorylated on a PRD2-histidine residue (position 208) ^{28,32,33}. According to the first hypothesis, the His-208 is phosphorylated by BglF ^{32,34}. On the contrary, according to the second study, HPr phosphorylates PRD2-His-208, and BglF, PRD1-His-101 ³³. Görke suggests a phosphorylation transfer, during which, HPr transiently phosphorylates PRD1, and the phosphoryl group is then transferred to His-208 of the second monomer to stabilize the preformed dimer, in a *trans* intramolecular reaction ^{33,35}.

Other levels of regulation

HPr-mediated phosphorylation of BglG is considered as a shortcut route to control the flux of other PTS sugars and their coordinated utilization ³¹. The *E. coli bgl* operon is also subject to Carbon Catabolic Repression (CCR) *via* the CRP protein (cAMP Receptor Protein, also known as CAP for Catabolite Activator Protein) and the glucose-specific enzyme EIIA^{Glc}. When EIIA^{Glc} is phosphorylated, it activates adenylate cyclase, leading to the synthesis of cAMP. In high concentrations of cAMP, the cAMP/CRP complex forms and binds to the promoters of catabolic genes to induce their expression. Thus, the CRP-cAMP complex facilitates the expression of the *bgl* operon in the absence of glucose and the presence of β -glucosides. The EIIA^{Glc} protein stimulates the phosphorylation of BglG by BglF in the presence of glucose to cause a drastic decrease of the operon transcription. Glucose also interferes with BglG activity, modulating BglF phosphorylation and thus its activity ^{30,36}.

To maintain BglG inactive in the presence of glucose or the absence of β -glucosides, the antiterminator is phosphorylated and sequestered at the membrane by BglF. The presence of β -glucosides in the growth medium results in rapid BglG dephosphorylation ³², which enables its release by BglF before being attracted to the cell pole *via* an interaction with EI and HPr ^{37,38}. BglG is finally phosphorylated by HPr on His-208 and released from the pole into the cytoplasm ³⁵. A PRD1/PRD2 interaction also takes place when BglG is in a monomeric form,

to limit dimer formation, and acts as a reservoir. This interaction is favored by BglF, whereas the presence of β -glucosides supports the dimeric form^{27,39}. Interestingly, it was shown that if BglG is artificially anchored to the inner membrane, it remains active without migration to the transcription site⁴⁰.

Dole *et al.* have shown that operon expression can be mediated by other players, notably the H-NS protein, which is more specifically involved in repression^{41,42}. H-NS is an important pleiotropic regulator in the bacterial physiology adaptation to external signals. This protein accentuates *bgl* operon repression when cellular transcription rates are low. The H-NS binds to DNA upstream of the promoter and represses transcription initiation. This protein is also able to bind to the coding region of the first gene, approximately 600-700 bp downstream of the transcription initiation site, where it induces Rho-dependent repression and thus transcription termination. The presence of two binding sites also suggests the possible formation of a DNA loop supported by the H-NS proteins. This loop either prevents RNAP from binding to the promoter region or traps this enzyme within the loop, preventing transcription of mature *bgl* mRNA⁴³.

Cellular processes involving BglG

As a regulator, BglG regulates its expression and the BglF transporter but has also a large impact on bacterial gene expression. For example, Gordon *et al.* searched for RATs-like motifs on the genomes of *E. coli* K12 or uropathogenic *E. coli*, and identified that most of the putative RATs do not overlap terminators. Their results suggest that the ability of RAT sequences has been explored during evolution to mediate activities, rather than antitermination⁴⁴. For example, BglG remains bound to RNA even after performing its antiterminator role. Thus, BglG protects and enhances the stability of the 5'UTR of the *bgl* mRNA, by blocking accessibility to ribonucleases. Moreover, the 5'UTR of *bgl* operon in the presence of BglG

adopts an alternative secondary structure that protects itself from endonucleases⁴⁵. In *E. coli*, the regulator also binds to the RNAP and more specifically to the β' -subunit, to facilitate subsequent transcriptional steps, without acting as an antiterminator⁴⁶.

A proteomic study in *E. coli* revealed that BglG regulates at least 12 target genes⁴⁷. Among them, BglG influences the *gcvA* gene expression by destabilizing the mRNA and preventing its translation. This gene encodes a regulator of a non-coding RNA called *gcvB*, which plays a role in the expression of the oligopeptide transporter OppA. Through this intermediary, BglG is thought to have an indirect impact on the stationary growth phase. BglG is also involved in the expression of the *gadE* gene, encoding a major regulator of pH homeostasis, and Lipopolysaccharide (LPS) biosynthesis. GadE regulates the expression of the *ridA* and *lrp* genes, which encode a deaminase and a leucine-sensitive regulatory protein, respectively. BglG binds RAT sequences upstream of the *gcvA* and *gadE* genes, without involving a terminator. Thus, BglG has a pleiotropic role, with a strong impact on cell physiology, independently of its antiterminator function and in the absence of the inducing sugar^{48,49}.

In 1998, Ayub Khan *et al.* identified the genes involved in *E. coli* pathogenesis during sepsis to define its adaptive capacity. Among these genes, the *bgl* operon is exclusively induced *in vivo*. These results raise questions about the regulation of this phenotypically silent operon and the importance of these genes during infection, also found in other pathogens. Thus, a complete operon *bglGFB* is identified in *Shigella sonnei*, while bacteria like *Salmonella* spp., *Proteus mirabilis*, *Enterobacter aerogenes*, and *P. aeruginosa*, possess only the *bglG* homolog⁵⁰. A more recent publication showed that BglG is involved in *Listeria monocytogenes* virulence. Indeed, *bglG* mutation severely impairs bacterial invasion and reduces virulence in mice. The authors suggest that *bglG* is involved in the sensing and consumption of carbohydrates from the host cell during infection, thus facilitating the pathogenesis of *L.*

*monocytogenes*⁵¹. Consequently, BglG is a well-studied regulator with a pleiotropic role not limited to metabolism or transport, but also membrane homeostasis and virulence.

The AmiR antiterminator in Pseudomonas aeruginosa

AmiR is an antiterminator expressed in all *Pseudomonas aeruginosa* strains and belongs to the *ami* operon. This operon was first described and characterized by Clarke's team⁵². The products of this operon enable *P. aeruginosa* to metabolize a couple of aliphatic amides to use them as carbon and nitrogen sources *via* the tricarboxylic acid cycle (Krebs cycle)⁵³.

The *ami* operon is composed of four to six genes depending on the *P. aeruginosa* strain. In PAO1 strain, the operon consists of five genes, *i.e.* *amiS*, *amiR*, *amiC*, *amiB* and *amiE* (annotated PA3362 to PA3366, respectively) (www.pseudomonas.com) (Figure 2A and 2B). By contrast, the PA14 strain displays only *amiR*, *amiC*, *amiB*-like, and *amiE* (annotated PA14_20560 to PA14_20590, respectively) (www.pseudomonas.com), lacking *amiS* (Figure 2B). Note that in both strains, the *ami* operon starts with a 'leader' sequence, sometimes referred to as *amiL* or *amiE* leader (PA3366.1, Figure 2B). This non-coding RNA enables the binding of ribosomes and contains a binding site for Hfq chaperone⁵⁴. The *amiE* gene encodes an aliphatic amidase, which hydrolyses aliphatic amides allowing *P. aeruginosa* to recover carbon and nitrogen sources. Therefore, the first characterized function of the *P. aeruginosa* *ami* operon was to convert short-chain aliphatic amides into their corresponding organic acids, an activity supported by AmiE amidase. The *amiR* and *amiC* genes encode AmiR and AmiC, which are the positive and negative regulators of the *ami* operon, respectively⁵⁵. The *amiB* and *amiS* genes are poorly described in the literature. AmiB could be a chaperone that, when coupled with AmiS, could be involved in an ABC-type transport system⁵⁶.

AmiR structure

The AmiR protein is constituted by a dimeric structure due to the binding of a monomeric protein of 196 amino acids in length (Figure 2C, PDB 1QO0) ⁵⁷. The monomer is composed of a N-terminal globular domain, a central coiled-coil region and a C-terminal helical domain. The presence of an ANTAR domain in AmiR is predicted in the region from the amino acid 135 to the amino acid 190. It has been shown that the AmiC–AmiR complex crystallizes with an AmiR dimer and two AmiC monomers in the asymmetric unit (Figure 2C) ⁵⁷. This crystal structure allowed the identification of the AmiC-dependant regulation mechanism of AmiR by sequestration rather than phosphorylation.

AmiR antitermination mechanism

Activation of the *ami* operon is induced by the presence of amides such as acetamide, propionamide or lactamide. Conversely, its expression is repressed by formamide and butyramide, considered to be antagonists of the AmiC sensor of the *ami* operon ^{53,58}. Recently, other natural compounds have been identified as AmiC agonists. It has been demonstrated that natriuretic peptides can bind AmiC with differential affinity. Using the microscale thermophoresis approach, the C-type Natriuretic Peptide (CNP) ⁵⁹ and the human Atrial Natriuretic Peptide (hANP) ⁶⁰ are shown to bind purified AmiC with a quite similar affinity (K_D of 2 μ M for CNP and K_D of 5 μ M for hANP). In addition, CNP strongly activates transcription of the whole *ami* operon, suggesting that the complex CNP-AmiC would release AmiR regulator allowing its antiterminator activity ⁵⁹.

It was shown that AmiR activates *ami* operon expression. Over-expressing AmiR leads to increased amidase activity, and constitutive expression of AmiR leads to AmiE constitutive expression ⁶¹. Then, it was discovered that the operon leader sequence harbors a Rho-independent transcription termination loop ⁶². Deletion of this loop resulted in AmiE

constitutive expression and led to the hypothesis that AmiR acts as a transcriptional antiterminator, an hypothesis that was supported by the sequence similarities between the binding sites of BglG and AmiR ⁶³.

AmiC and AmiR control together expression of the *ami* operon resulting into AmiE expression ^{61,62}. Without a substrate like acetamide, AmiC sequesters AmiR, thereby inhibiting its activity as an antiterminator of transcription (Figure 2C). To release AmiR and enable its activity, an agonist must bind to AmiC to remove its anchoring to AmiR ^{55,58,64}. This is precisely what happens in the presence of acetamide, which binds to AmiC, thus releasing AmiR, and allowing expression of the *ami* operon ^{57,65}.

Under inducing conditions leading to the release of an important amount of free AmiR, *amiE* is the most abundant transcript of the *ami* operon ⁶⁵. Indeed, AmiR interacts with the nascent mRNA upstream of the terminator to remove the loops that inhibit prematurely the full transcription of *ami* genes. Since *amiE* is the first gene behind the first loop, this gene is highly transcribed after the removal of the first loop (named T1), whereas the second gene *amiB* is less transcribed, and *amiC* is very poorly transcribed. This control of *amiC* expression would avoid limiting AmiR activity ⁶⁵. Therefore, the AmiR target is a mRNA leader sequence of the amidase operon, and it is suggested that AmiR binding to this sequence prevents specific hairpin loop structure, allowing RNAP activity ¹⁴. Finally, Norman *et al.* found that the stoichiometry of the interactants AmiR dimer and RNA was 152:1, corresponding to a K_D value of 1.1 nM ⁶⁴.

Cellular processes involving AmiR

Since these pioneering studies, little news has been reported in the literature about the *ami* operon, the AmiR regulator, and the final AmiE enzyme, until the discovery 10 years ago that a family of human hormones, the natriuretic peptides, are capable of binding directly the AmiC sensor. It has been demonstrated that the binding of natriuretic peptides to AmiC and the

release of AmiR strongly modified the ability of *P. aeruginosa* to form biofilm^{59,66}, and triggered mature biofilm dispersion, suggesting that the antiterminator AmiR, in addition to its metabolic activity, could be involved in *P. aeruginosa* biofilm regulation^{60,67}. The putative role of AmiR in the anti-biofilm activities of these compounds thus has been investigated, and the presence of the AmiC-AmiR pair was shown essential to the effect of natriuretic peptides on biofilm^{60,67}. More interestingly, it has been demonstrated that over-expression of AmiR strongly impairs the ability of *P. aeruginosa* to form a biofilm⁶⁰.

This hypothesis of the role of AmiR in *P. aeruginosa* biofilm regulation is reinforced by the observation that the *amiE* mRNA synthesis is enhanced by 18.3- or 34.4-fold in biofilm state versus planktonic or dispersed state, respectively⁶⁸. In addition, it appears that AmiE abundance is regulated during biofilm formation since it is weakly expressed in planktonic conditions, while strongly increased in a 48h-old biofilm. In addition, AmiE abundance is increased in 96h-old biofilm compared to 48h-old ones⁶⁹. Finally, a second study confirmed that AmiE concentration is strongly enhanced when bacteria are grown in a biofilm state as compared to a planktonic state⁷⁰. More surprisingly and therefore interestingly, it appears that AmiE is the protein with the highest increased expression among all the proteins (8.4-fold) identified in *P. aeruginosa* biofilm grown for 96h compared to planktonic cells.

Near to biofilm activities modified by AmiR and the products of the *ami* operon, a direct impact of *P. aeruginosa* pathogenesis could be suspected. Indeed, over-production of AmiE provokes a total loss of *P. aeruginosa* virulence in a model of mouse acute infection⁷¹. The involvement of AmiR and members of the *ami* operon in the regulation of various physiological processes such as biofilms strongly suggests that AmiR has more molecular targets than initially identified^{59,60,71}.

Altogether these data suggest that an antiterminator could have many different functions depending on the physiological state of the bacteria. The data concerning the *ami* operon in

general, and AmiR, in particular, pave the way to a new vision of AmiR as a putative regulator of *P. aeruginosa* biofilm.

The NasR antiterminator in Klebsiella oxytoca

In *Klebsiella oxytoca* (*pneumoniae*) M5a1, a bacterium belonging to the family *Enterobacteriaceae*, transcription antitermination serves as a regulatory mechanism to modulate nitrogen metabolism. This microorganism can use nitrate and nitrite as sole nitrogen sources during aerobic growth. The genes involved in nitrate and nitrite assimilation are organized in an operon, *nasFEDCBA* (Figure 3A), hereafter referred to as the *nasF* operon⁷². This non-catabolic operon shares regulatory similarities with catabolic operons and encodes components essential for nitrate assimilation. Specifically, *nasF*, *nasE*, and *nasD* genes encode a nitrate and nitrite uptake system⁷³. Nitrate is enzymatically converted through nitrite to ammonium via assimilatory nitrate reductase, encoded by *nasC* and *nasA*, and assimilatory nitrite reductase, encoded by *nasB* (Figure 3A). Upstream of *nasF* lies *nasR*, encoding an antiterminator protein⁷⁴. In the presence of nitrate or nitrite, NasR triggers the expression of the *nasF* operon, likely by facilitating transcription readthrough at a terminator within the *nasF* operon leader⁷⁵.

The expression of the *nasF* operon in *K. oxytoca* M5a1 is under two levels of regulation. The first control, Ntr control, operates during ammonium-limited growth, where phosphorylated NtrC protein activates transcription from upstream binding sites. Promoters regulated by NtrC activation necessitate σ^N (or σ^{54}) encoded by the *rpoN* gene, a specialized form of sigma factor directing RNAP to bind to -12/-24 promoters (Figure 3A)^{75,76}. The second control of nitrate and nitrite assimilation is governed by the key regulator NasR (Figure 3A). Genetic analyses showed that *nasF* operon expression in response to nitrate/nitrite occurs

specifically through a transcriptional antitermination mechanism, in which NasR interacts with the *nasF* leader region^{74,75}.

NasR structure

In the absence of added nitrate or nitrite, NasR forms dimers both in the crystal and in solution states⁷⁷. The NasR protein structure exhibits an organization featuring two separate domains connected by an elongated linker (Figure 3B). Each subunit within this dimer comprises two distinct all-helical domains: an amino-terminal NIT (Nitrate- and nitrite-sensing) domain responsible for sensing nitrate/nitrite levels⁷⁸ and a carboxyl-terminal ANTAR (AmiR and NasR Transcription Antitermination Regulator) signaling domain (Figure 3B).

NIT domain. The NIT domain found in the NasR protein which spans residues 9 to 287, encompassing helices H1 to H8 (as illustrated in Figure 3B), has been detected in various receptor components of signal transduction pathways in a wide range of bacterial species^{77,78}. The NIT domain comprises two closely resembling four-helix bundles (Figure 3B), each bearing structural similarity to periplasmic input domains found in transmembrane receptors like Tar and NarX. How these two bundles associate within the NIT domain closely mirrors the homodimeric structures formed by periplasmic input domains. Notably, within the NIT domain, the amino-terminal helix H1' extends from the packed bundle and interacts with the adjacent monomer.

Linker region. The linker region consists of three components: a disordered peptide (residues 288 to 299), a small helical peptide labeled as H9' (residues 302 to 312), and a lengthy helix denoted as H9, which packs tightly against the helical bundle domain⁷⁷.

ANTAR domain. The ANTAR domains play crucial roles in modulating various bacterial processes, either through TCS or *via* direct interaction with protein sensor domains^{10,15}. In *K. oxytoca* M5a1, the ANTAR domain of NasR protein, which spans residues 333 to 393, shares

similarities with ANTAR domains identified in transcription antiterminators such as AmiR of *P. aeruginosa* and Rv1626 of *Mycobacterium tuberculosis*. The ANTAR domain (Figure 3B) comprises a triangular configuration of a three-helix bundle (designated as helices H10 to H12), similar to the arrangement observed in the ANTAR domains of AmiR and Rv1626^{57,79}. Notably, the first helix (H10) of the ANTAR domain is significantly longer than the other two and is oriented perpendicular to helix H9 originating from the linker (Figure 3B)⁷⁷. The ANTAR signaling domain of NasR targets a regulatory element known as an "antiterminator" secondary structure located within the leader region of the *nasF operon* mRNA transcript^{77,80}.

NasR antitermination mechanism

In *Klebsiella oxytoca* M5a1, the transcription antitermination regulatory mechanism of NasR has been extensively studied^{75,80,81}. The NasR-mediated antitermination occurs following its interaction with *nasF* leader RNA. NasR target is a two-hairpin RNA motif in the *nasF* leader region. Specifically, each hairpin structure is capped with a terminal hexanucleotide loop that contains A and G residues at the first and fourth positions, which are essential for NasR binding. In a recent study, the molecular mechanism by which NasR couples its ligand signal to RNA-binding activity has been proposed⁸¹. In the absence of the nitrate signal, the NasR antiterminator forms a constitutive dimer incapable of binding RNA by adopting an autoinhibited conformation (Figure 3C, left). However, in the presence of nitrate, the autoinhibitory conformation is disrupted and NasR adopts an alternate configuration by releasing the key RNA recognition residues of the ANTAR domain to bind the hairpin RNA structures contributing to the antitermination (Figure 3C, right). The binding of nitrate to the NIT domain might transmit structural transitions into the ANTAR domain to adopt an alternative dimer configuration which allows the release of its RNA binding surface⁸¹.

Cellular processes involving NasR

Nitrogen is a crucial nutrient for growth and is readily available to bacteria in diverse environments in the form of ammonium, nitrate, and nitrite. When ammonium is scarce, many bacterial species, including *K. oxytoca* M5a1, which possess an assimilatory nitrate/nitrite reductase pathway, can use nitrate or nitrite as the sole nitrogen source for growth and to maximize their fitness in the host environment ⁸². During infection, species of the genus *Klebsiella* can use a plethora of virulence factors for survival and immune evasion ⁸³. Especially, cytotoxins, capsular polysaccharide (K antigen), LPS (containing O antigen), fimbriae, outer membrane proteins, and determinants for iron acquisition and nitrogen source utilization are common virulence factors in *K. pneumoniae* and *K. oxytoca* ^{83,84}. In addition, some studies reported that clinical isolates of *K. oxytoca* collected from patients with antibiotic-associated hemorrhagic colitis (AAHC) ⁸⁵ and colorectal cancer ⁸⁶ were able to produce moderate biofilms which prevent the efficient infection eradication.

The relationship between nitrogen metabolism and bacterial pathogenicity is multifaceted. Bacterial pathogens often encounter varying nitrogen environments during infection, and their ability to adapt and utilize nitrogen sources can significantly impact their virulence and survival within the host. Changes in nitrogen availability within the host can impact the expression of host defense mechanisms and the ability of bacteria to evade immune responses. Moreover, nitrogen metabolism can also influence biofilm formation, a critical aspect of bacterial pathogenesis. Overall, nitrogen metabolism plays a pivotal role in shaping bacterial pathogenicity by influencing nutrient acquisition, virulence factor production, host-pathogen interactions, and biofilm formation ⁸². The relationship between bacterial pathogenicity and nitrogen metabolism is intricate. It is difficult to delineate the boundaries between the two, however, it is apparent that they are interconnected. Thereby, we can speculate that the transcription antitermination regulatory mechanism of NasR involved in nitrogen

metabolism may affect directly or indirectly the pathogenicity of *K. oxytoca* M5a1 enabling its persistence within the host environment, evasion from immune responses, and resistance to antimicrobial treatments. Therefore, understanding the molecular regulatory mechanisms that governs this complex relationship is essential for developing strategies to combat infectious diseases.

RNA-binding antiterminators in Gram-positive bacteria

The SacT/SacY/LicT antiterminators in Bacillus subtilis

Although the *bglG* system was first described in *E. coli*, such systems are highly conserved in bacteria and also present in Gram-positive bacteria, like SacT, SacY and LicT in *B. subtilis*^{16,87,88}, with similar physiological functions.

SacT/SacY/LicT structure

These antiterminators have two functional domains: the CAT domain involved in RNA binding, and the regulatory PRD domains.

CAT domain. The CAT domain of SacT/SacY/LicT (with RNA-binding and antitermination function) is an N-terminal domain of 55 amino acids. This domain shares a high level of sequence similarity with BglG. Each monomer is composed of a four-stranded antiparallel β -sheet, with tight turns connecting strand 1 to strand 2 and strand 2 to strand 3, and a long loop connecting strand 3 and strand 4 (Figure 4A)⁸⁹. Dimers are stabilized by the orthogonal stacking of the two β -sheets, which forms a β -barrel closed on one side by four main-chain hydrogen bonds between the two β 4-strands of the two monomers. On the other side, each barrel is closed by a symmetrical hydrogen bond. While one side of the β strands forms the dimer

interface, the other face is in contact with a hydrophobic cluster of residues from the long loop between the third and the fourth β -strand, forming the hydrophobic core of the monomer^{26,89}. *PRD*. The regulatory region consists of two structurally similar domains corresponding to PRD1 and PRD2⁹⁰. Each domain forms a compact bundle comprising five helices ($\alpha 1$ to $\alpha 5$). The PRD module contains a core that consists of two pairs of antiparallel helices. The first pair includes the antiparallel helices $\alpha 1$ and $\alpha 4$, while the second pair contains $\alpha 2$ and $\alpha 5$. The third helix ($\alpha 3$) is oriented perpendicularly to $\alpha 5$ at the periphery of the bundle (Figure 4B). In its dimerized form, phosphorylation sites of PRD2 are inaccessible to enzymatic partners. The PRD undergoes significant rearrangements between the inactive and the active forms, notably the PRD2 which is highly mobile in the inactive state, and locked upon activation by phosphorylation⁹¹. Thus, in the inactive state, the PRD2 undertakes a wide movement resulting in a dimer opening which exposes the phosphorylation sites. This movement provokes additional structural rearrangements of the PRD1 interface and the CAT-PRD1 linker.

SacT/SacY/LicT antitermination mechanism

SacT is a transcriptional antiterminator involved in the control of the *sacPA* operon (Figure 5), encoding a sucrose-transporting PTS enzyme EII^{Suc} and a sucrose-6-phosphate hydrolase, respectively^{87,92}. SacT of *B. subtilis* is homologous to BglG and SacA to BglF⁹². Several studies showed that the regulation mechanism of *sacPA* by SacT is identical to that of *bglGFB* by BglG (Figure 1)^{25,88,93}. While the work of Tortosa *et al.* raises the possibility that SacT also requires phosphorylation by HPr to be active, Arnaud *et al.* suggest the possibility of antitermination activity with non-phosphorylated forms of SacT^{19,93,94}. Two hypotheses were proposed to explain these discrepancies: (i) the importance of the dimerization in activation of BglG-like antiterminators, which is influenced by phosphorylation of the transporter and/or HPr enzyme and (ii) both phosphorylated and dephosphorylated forms are able to promote

antitermination, although protein phosphorylation may enhance interaction with the RAT sequence and/or facilitate antitermination activity. These two possibilities are not mutually exclusive, since active dimers can form independently of phosphorylation and enable antitermination in the presence of high protein concentration ¹⁹.

SacT is not the only antiterminator involved in sucrose utilization since the presence of this carbon source also induces the expression of *sacXY* operon (Figure 5). SacY encodes an antiterminator, which regulates its own expression and the expression of *sacB* ^{87,95,96}. SacX is a sucrose specific EIIBC^{Suc} PTS transporter, and SacB a levan-sucrase involved in sucrose degradation ^{87,94,95,97}. A palindrome enabling the formation of a Rho-independent terminator has been identified upstream of the *sacB* gene and, when this palindrome is mutated, constitutive expression of the locus is observed ⁹⁸. Identification of this regulatory site established that the regulatory pattern of *sacB* is similar to that of *sacP* and *bglG* ^{17,25,99–101}. Surprisingly, the study of the RAT sequence of the *sacXY* operon revealed the presence of an imperfect palindrome, corresponding to a long imperfect hairpin structure. In the presence of sucrose, activated antiterminators stabilize the structure by binding to the RAT sequence and thus prevent the formation of the hairpin, whose function remains to be established. Its function as a Rho-independent terminator was suggested by Tortosa and Le Coq, based on its structure and the absence of a noteworthy phenotype when the *rho* gene is deleted. However, the existence of a second *rho* gene or the intervention of an endonuclease cannot be ruled out ^{96,102,103}.

Unlike SacT, SacY activity is not positively regulated by HPr ^{93,104}. Conversely, SacY is negatively regulated by HPr thanks to phosphorylation at His-99, which is dependent on the formation of the SacX/SacY complex ^{17,94}. Indeed, in the absence of sucrose, SacX is phosphorylated by the PTS general enzymes *via* an unknown EIIA. Phosphorylated SacX then traps SacY, allowing its efficient phosphorylation on His-99 by HPr. Thus, in the absence of

sucrose, SacX exerts double negative control over antitermination activity, by trapping SacY and increasing its phosphorylation rate. In the presence of sucrose, SacX is dephosphorylated, preventing its interaction with SacY, which in turn is also dephosphorylated^{94,105}.

A third antiterminator named LicT was identified in *B. subtilis*, which is involved in the regulation of β -glucan utilization, such as lichenan or salicin, and which regulates the expression of the *licTS* (Figure 5) and *bglPH* operons¹⁰⁶. The *licS*, *bglP* and *bglH* genes encode an extracellular β -glucanase, a β -glucoside-specific EII transporter, and a phospho- β -glucosidase, respectively^{20,106,107}. LicT exhibits about 40% of sequence identity with its *E. coli* homolog BglG or other antiterminators of the BglG/SacY family^{107,108}. LicT activity is positively regulated by HPr^{20,109}. Thus the activated form of LicT is dephosphorylated at the His-100 of PRD1, which is the target of BglP, and phosphorylated at the His-207 and His-269 of PRD2 by HPr^{89,105,110}. As with BglG, the LicT phosphorylation by PTS components induces dynamic subcellular localization: in the presence of an inducer such as salicin, LicT displays a subpolar localization, whereas in its absence, it is uniformly distributed throughout the cell¹¹¹.

The last antiterminator identified in *B. subtilis* is GlcT, which regulates the expression of the *ptsGHI* operon (Figure 5) and is induced in the presence of glucose. The *ptsH* and *ptsI* genes encode the general EI and HPr PTS enzymes, respectively, and *ptsG* the EIICBA^{Glc} transporter¹¹². The system is also activated by sucrose and salicin which are transported by the glucose permease. However, although GlcT is a member of the BglG/SacY family of transcriptional antiterminators, the RAT sequence of the *ptsG* gene is not similar to the consensual RATs¹¹³. HPr-dependent phosphorylation has a slight stimulatory effect on GlcT activity, probably due to the reduced efficiency of the phosphorylation, whereas EII^{Glc}-dependent phosphorylation inactivates the protein, as expected¹¹⁴.

Recently, the antiterminator NagY was identified in *Enterococcus faecalis*, presenting 36% identity with SacY from *B. subtilis*²¹ (Figure 5). The *nagY* gene forms an operon with

nagE, encoding a N-acetylglucosamine specific EIICBA PTS transporter ¹¹⁵. The search of RAT sequence allowed the identification of an imperfect inverted repeat suggesting a regulation similar to that of SacY ^{21,25,105}.

Other levels of regulation

In *B. subtilis*, CCR involves the pleiotropic repressor CcpA and an ATP-dependent phosphorylation by the HPr protein. In the presence of a glycolytically metabolized carbon source, HPr is phosphorylated at Ser-46 by an ATP-dependent kinase. P-Ser-HPr then interacts with the transcriptional regulator CcpA, and the complex binds to specific operator sites called *cre* sequences (catabolite-responsive element) to inhibit the expression of CCR-sensitive operons. Antiterminator targets can be directly subjected to CCR, such as the *sacPA* and *bglPH* operons. While expression of the *sacPA* operon is strongly decreased in the presence of glucose, the *bglPH* operon has a *cre* site ^{93,107}. It was shown for the LicT-regulated *bglPH* operon that mutants deleted for *cre bglPH* and the RAT/terminator sequence are completely independent of CCR, whereas *cre bglPH* mutations caused only partial relief ²⁰. This suggests a complementary regulation of LicT and CCR. A second CCR-linked mechanism is indirectly mediated by P-Ser-HPr, which cannot phosphorylate (and consequently activate) SacT or LicT ^{20,93,109,116}. In agreement with this concept, Lindner *et al.* have shown that a mutant insensible to HPr phosphorylation is no longer susceptible to CCR ¹¹⁷.

It was also shown that *sacPA* and *sacB* expression is dependent on sucrose concentration. Indeed, the *sacPA* operon is induced at low sucrose concentration, and when no preferential carbon source is available. At the same time, the *sacB* gene is induced by high sucrose concentration, in the presence or absence of glucose. Then the levansucrase SacB can break sucrose producing: (i) glucose, which can be transported and metabolized, and (ii) levan,

which can provide long-term energy storage ⁹⁷. These results show that *B. subtilis* has two alternate systems for utilizing sucrose ^{19,25,104}.

In addition to sucrose induction, expression of *sacXY* and *sacB* is affected by the DegS-DegU TCS (formerly *sacU*), a regulatory system with pleiotropic regulatory role ^{87,96}. The DegU regulatory protein is a positive regulator, allowing a high level of *sacB* transcription, in the presence of sucrose ^{96,98,118}. The signal transmitted by DegU and the presence of sucrose could represent the conditions under which levan synthesis by levansucrase is useful for the cell^{96,104}.

Cellular processes involving SacT/SacY/LicT-like antiterminators

Gordon et al. searched for RATs-like motifs on *B. subtilis* genome and identified 11 putative RAT sequences, in addition to those known for the antiterminators operons. Similarly to *E. coli*, most of the putative RATs identified do not overlap with a terminator, suggesting that these elements do not play a role in antitermination ⁴⁴. Cross-regulation has also been demonstrated in *B. subtilis*, due to the high degree of similarity between the SacY and SacT systems and common putative targets. For example, SacY is able to regulate the expression of the SacT-regulated *sacPA* operon ^{19,93}. Consequently, these regulators have a large impact on cell metabolism.

Given the central role of metabolism during colonization and the extent of cross-talk between cellular metabolites across pathways, antitermination mechanisms are widely prevalent in pathogens. Human pathogenic Gram-positive bacteria are supposed to use β -glucosides derived from human tissues, such as extracellular matrix or glycosaminoglycans, on the respiratory tract or colon. This degradation has two advantages: firstly, it facilitates the spread of bacteria by degrading the host extracellular matrix, and secondly, it provides carbon and energy sources. A LicT homolog has been identified in *Streptococcus pyogenes* as involved

in bloodstream survival and soft tissue infection. Indeed, the β -glucoside PTS transporter and the β -glucosidase enzyme can impact the regulation of virulence-related genes that control biofilm formation, hemolysis, or the progression of localized ulcerative lesions during subcutaneous infections in mice ¹¹⁹. The same observations were described in *Streptococcus gordonii* where β -glucosides metabolism has a central role in *in vitro* adhesion, biofilm formation, growth, and *in vivo* colonization ¹²⁰.

This hypothesis was also proposed by Soussan *et al.* who studied the degradation by *E. faecalis* of hyaluronic acid, a glycosaminoglycan component of the host extracellular matrix and mucus, composed of N-acetylglucosamine and glucuronic acid. *E. faecalis* is an opportunistic pathogen with metabolic characteristics that allow it to colonize its host ²¹. Under infection conditions, *E. faecalis* degrades the hyaluronic acid thanks to the NagY-regulated HylA hyaluronidase, allowing the cell to recover N-acetylglucosamine from its environment. HylA was also shown to be involved in biofilm formation and pathogenicity in the *Galleria mellonella* model ²¹, and urinary tract infection ¹²¹. NagY homologous antiterminator was then proposed to be a link between metabolism and the opportunistic properties of *E. faecalis*. Consequently, knowledge of the NagY regulon may open up interesting prospects for a better understanding of the colonization mechanism of the *E. faecalis* pathobiont.

The HutP antiterminator in Bacillus subtilis

L-histidine is an amino acid that can be utilized by *B. subtilis* as a source of energy, carbon, and nitrogen for growth, although its degradation is tightly regulated considering its expensive synthesis in the cell ¹²². The *B. subtilis hutPHUIGM* operon, henceforth referred to as the *hutP* operon, encodes enzymes for the transport and catabolism of L-histidine, as well as an RNA-binding protein positively regulating its expression ^{123–125}. The *hutM* gene encodes a

histidine transporter, whereas *hutHUIG* genes are involved in histidine utilization. The pathway of histidine catabolism is highly conserved among bacteria, although two different routes can be distinguished. In *B. subtilis*, histidine is deaminated by HutH histidinase to yield urocanate, which is further hydrated by HutU urocanase to give imidazolone propionate; ring cleavage of the latter is accomplished by HutI hydrolase to obtain formiminoglutamate, that is finally hydrolyzed to formamide and glutamate by HutG^{122,123,126}. The regulatory elements consist of a *hutP* gene, that encodes a protein involved in antitermination of the whole *hutPHUIGM* transcript, and a transcription terminator, located between *hutP* and *hutH*, whose mRNA hairpin HutP binds¹²⁷⁻¹²⁹.

HutP structure

The HutP (Histidine utilization positive regulatory Protein) from *B. subtilis* forms a homohexamer complex resembling a flattened cylinder, with three individual dimers related by three-fold symmetry¹³⁰. According to the elucidated crystal structure of HutP, each monomer consists of four antiparallel β -strands forming a β -sheet, with two α -helices on the front side and two more at the back, and five loop regions (L) (Figure 6A). A crystal structure of the HutP hexamer revealed that it recognizes and binds a single-stranded RNA molecule on each side of the cylinder in a triangular conformation (Figure 6B). However, previous binding of both L-histidine and Mg^{2+} ion to each HutP monomer is required to activate the RNA-binding activity of the hexamer¹³¹. The L-histidine and Mg^{2+} ligands are associated with each other and buried inside the HutP cylinder at the interface between two HutP monomers, whereas the RNA molecules are bound to both outer surfaces. Therefore, the structure of the HutP quaternary complex contains a protein homohexamer with six L-histidines, six Mg^{2+} ions, and two bound single-stranded RNA molecules^{130,132} (Figure 6B).

HutP antitermination mechanism

Unlike *B. subtilis*, the *hut* genes of Gram-negative organism (*i.e.* enteric bacteria and several species of *Pseudomonas*) do not lie in a single operon¹²². Moreover, a *hutT* gene, encoding a histidine/urocanate transporter instead of the abovementioned histidine transporter (HutM), and a Hut-specific repressor HutC, rather than the HutP antiterminator, have been identified in Gram-negative bacteria. Therefore, regulation of the *hutP* operon in enteric bacteria and pseudomonads is quite different from that observed in *B. subtilis*, with HutC-mediated repression in the former and HutP-mediated antitermination in the latter^{122,127–129}.

Hut enzymes in *B. subtilis* are induced by L-histidine, not urocanate as in enteric bacteria and pseudomonads^{122,133}, and repressed by rapidly metabolized carbon sources such as glucose and the presence of amino acids^{133,134}. L-histidine-mediated induction of the *hutP* operon in *B. subtilis* is regulated by an antitermination mechanism¹²². Carbon catabolite repression is mediated by CcpA protein, binding to the *cre* site located in the middle of the *hutP* gene^{122,135}, whilst transcription repression in response to amino acid availability is controlled by CodY protein, acting at *hutO_A* site, placed just downstream from the *hutP* operon promoter (Figure 6C)^{128,136}.

Expression regulation of the histidine utilization system in *B. subtilis* by antitermination mechanisms has been previously reviewed^{122,125,137–139}. In the presence of the inductor, six molecules of L-histidine bind to inactive HutP hexamer, which undergoes a conformational change. Then, six Mg²⁺ ions also bind to the HutP-L-histidine structure, triggering the active conformation of the HutP-L-histidine-Mg²⁺ complex for the recognition of RNA^{130,140–142}. When activated by the coordination of L-histidine and Mg²⁺ ions, HutP binds specifically to the target sequence within the terminator RNA, placed in the *hutP-hutH* intercistronic region (Figure 6C), and initiates the destabilization process that impairs the terminator hairpin structure. In particular, the hexameric activated HutP interacts with two clusters (denoted as

site I and II) within the target RNA, each one consisting of three NAG (Nucleobase-Adenine-Guanine) motifs separated by two to four unconstrained spacer nucleotides, with a GC-rich linker region of 20 bp between binding sites I and II (Figure 6D)^{125,143–145}. Each HutP monomer recognizes one NAG triplet of the terminator RNA, so that the six NAG regions are bound by the six protein subunits, three motifs (one cluster) on each surface of the hexamer, with the 20-bp linker separating each cluster^{130,132}. Therefore, in conditions of excess L-histidine, the activated HutP hexamer firstly accesses to NAG-rich site I within the nascent *hut* transcript by using one of its two RNA-binding surfaces, and then binds to site II utilizing the opposite RNA-binding side to mediate the conformational changes of the terminator RNA stem, thus preventing premature termination of transcription and allowing readthrough of the terminator¹⁴⁵.

In addition to the antitermination regulation of the Hut enzymes, HutP can also activate the promoter of the *hutP* operon of *B. subtilis*. However, the mechanism by which HutP activates transcription from the *hutP* promoter remains unknown¹²².

RNA-binding antiterminators in Mycobacterium tuberculosis and Enterococcus faecalis

ANTAR antiterminators bind RNAs with a conserved dual stem-loop structure found in 5'UTRs of specific mRNAs. As described for Gram-negative bacteria, this bond stabilizes a transcription antiterminator, thus allowing transcription. In some species of the phyla *Bacillota* and *Pseudomonadota*, it was observed that histidine kinases phosphorylate the ANTAR response regulator protein, and then activates ANTAR for RNA binding.

An extensively studied ANTAR antiterminator involved in the switch from commensal living to pathogenesis is the EutV protein in *E. faecalis*. This bacterium can use ethanolamine as a nutrient source which provides a selective advantage in the intestinal tract and contributes

to its pathogenic potential. The complex regulatory model of the *eut* ethanolamine utilization operon involves a cobalamin-dependent riboswitch and the TCS EutW-EutV¹⁴⁶. Ethanolamine acts as the signal ligand that induces autophosphorylation of the histidine kinase EutW, which in turn phosphorylates the response regulator EutV, following the consensual model of TCS, except that the C-terminal domain of EutV contains an ANTAR domain like the AmiR protein in *P. aeruginosa*. Thus, the response regulator binds to the *eut* 5'UTR, on an RNA sequence upstream from a Rho-independent terminator. The antitermination of transcription occurs thanks to the prevention of the terminator hairpin formation. Termination at a co-enzyme B12-dependent riboswitch in the presence of cobalamin also increases the efficiency of both transcription initiation and antitermination by EutV^{146,147}. A model of protein/RNA interaction was developed following a successful structural characterization¹¹. This ethanolamine catabolic enzyme regulation is also described in pathogens like *Listeria* and *Clostridium*¹³.

A large number of ANTAR proteins was also identified in the mycobacterial genome, with significant overrepresentation compared to *Pseudomonas* or *Clostridium*¹⁴⁸ and a similar structure to ANTAR antiterminator proteins¹⁴⁹. ANTAR RNA target sequences often overlap with the ribosome binding site or the translation start site of the regulated genes, triggering translation inhibition. In mycobacteria, the Rv1626 ANTAR protein is constitutively active and the control levels occur on its activating histidine kinase Rv3220c. It was shown that ANTAR antiterminator targets are genes involved in lipid and redox pathways, with an important impact on virulence for *M. tuberculosis* for example¹⁴⁸. The link between ANTAR proteins and lipid catabolism was also found in *M. smegmatis*, *M. vaccae* and *M. vanbalenii*, but also in *E. coli* and *Rhodococcus* spp. Both pathogenic and saprophytic mycobacteria utilize host/environment lipids for growth and survival, and resistance to oxygen and nitrogen species give advantages to survive inside hosts. This underlines the important role of ANTAR antiterminators regulation in actinobacteria.

Conclusion

Antitermination regulation is an important strategy used by bacteria to adapt to their environment. More specifically, pathogens need to optimize their nutrient acquisition and metabolism, involving RNA-binding antiterminators described in this review, with an impact on virulence traits. Moreover, the interaction dynamics between antiterminators and RNA represents excellent targets for the development of alternative and innovative drugs, as it can be targeted through direct and allosteric inhibition of enzymatic activity or inhibition of protein-protein or protein-nucleic acid interactions. Antiterminators were also suggested to be interesting targets for possible vaccine candidates ¹⁵⁰, due to their large impact on bacterial physiology. Therefore, understanding the molecular regulatory mechanisms that govern the complex relationship between transcription antitermination and pathogenicity is essential for developing strategies to combat infectious diseases, opening up interesting perspectives.

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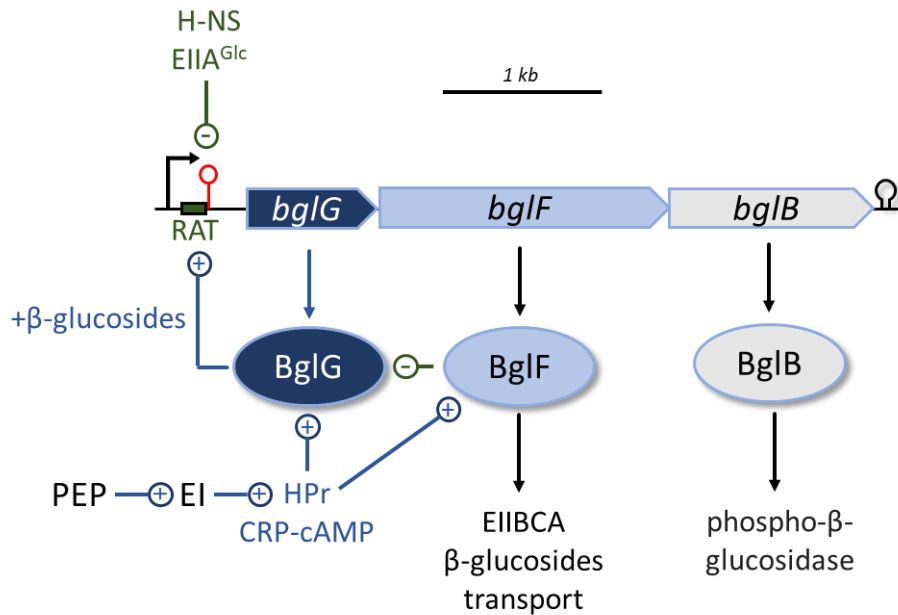


Figure 1. BglG antitermination mechanism in *E. coli*. BglG activity is dependent on its phosphorylation steps, involving HPr from the phosphotransferase system (PTS), and the CRP-cAMP complex. The first step of the phosphorylation cascade catalyzed by the PTS is the autophosphorylation of Enzyme I (EI) with phosphoenolpyruvate (PEP). The phosphate is then transferred to HPr which phosphorylates BglF (EIIBCA). In the absence of β -glucoside, BglG is inactivated by BglF. The *bgl* operon transcription is initiated by RNAP but stops at the terminator hairpin (in red). In the presence of β -glucoside, BglF phosphorylates the carbohydrate during its uptake, and BglG is activated. After dimerization, BglG binds the Ribonucleic Antiterminator sequence (RAT), promoting antitermination, and induction of the *bgl* operon. H-NS and EIIA^{Glc} also provides regulation on the *bgl* operon.

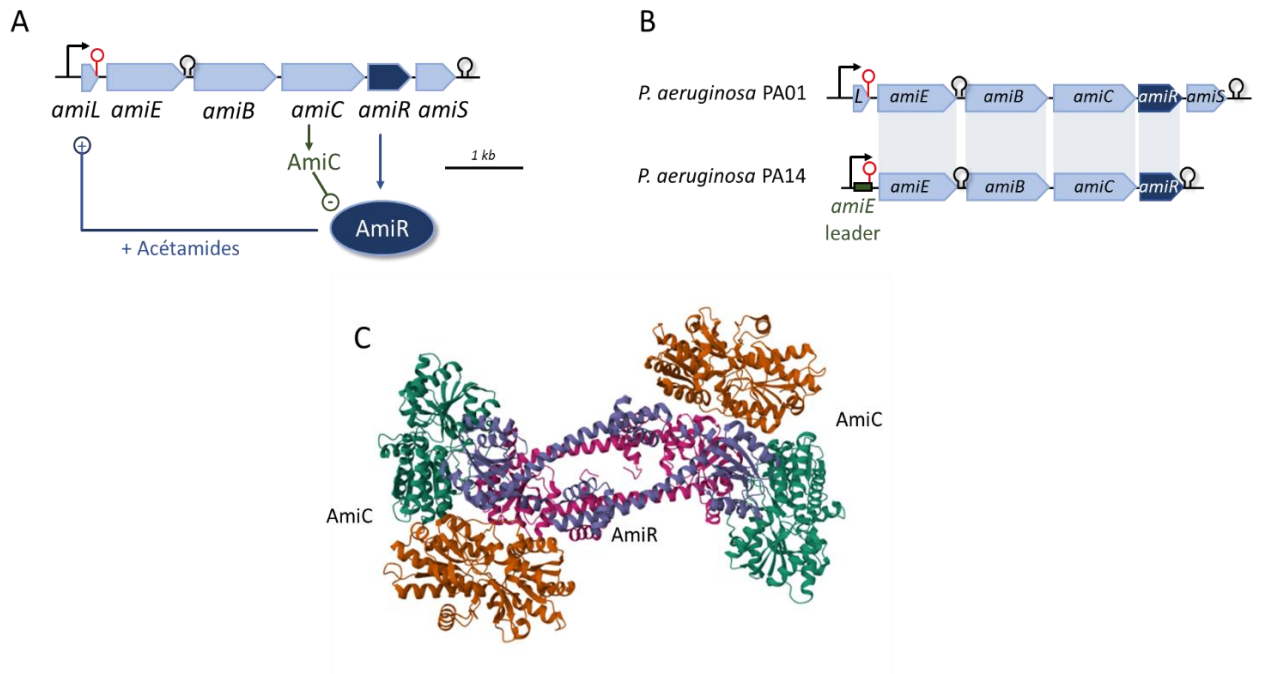


Figure 2. (A) AmiR mechanism in *P. aeruginosa* PAO1 strain. Antitermination occurs in the red hairpin (B) Operon structure in *P. aeruginosa* PAO1 and PA14 strain, adapted from www.pseudomonas.com. Antiterminator binding sequences are represented by red hairpins (C) Amidase sensor of the amidase operon of *P. aeruginosa* (AmiC) complexed with the AmiR antiterminator (PDB 1QO0). AmiR monomers are displayed in pink and purple, and AmiC proteins in green and orange.

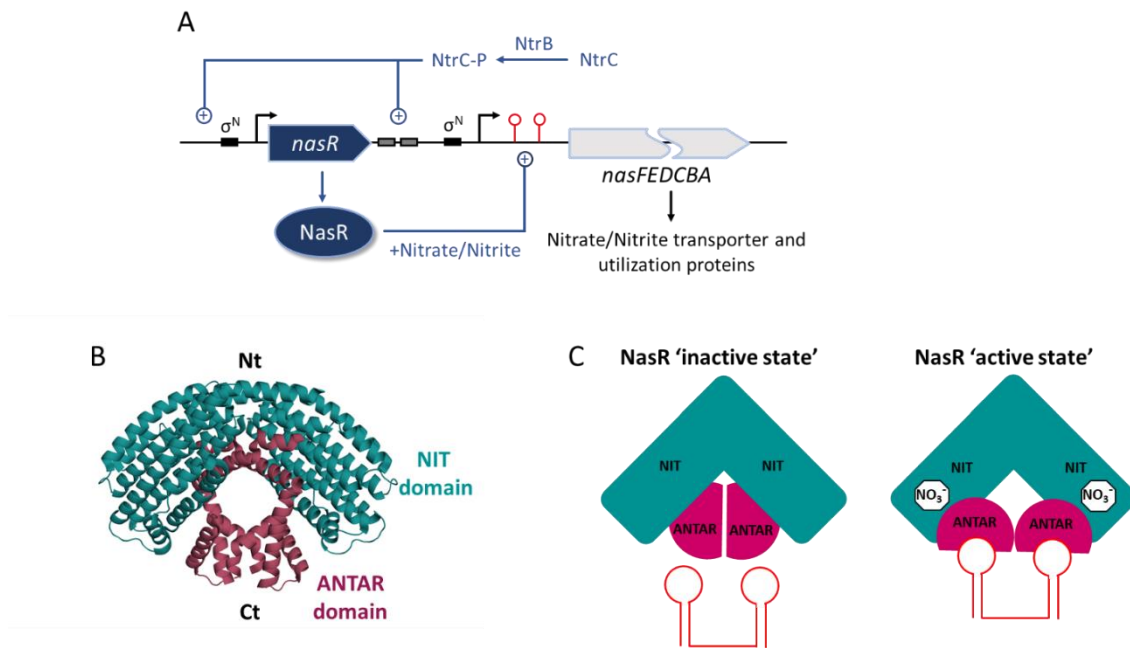


Figure 3. The antiterminator NasR in *Klebsiella oxytoca* M5a1. (A) Genomic context of *nasR* gene and *nasF* operon regulation, antiterminator binding sequences are represented by red hairpins; (B) Structure of NasR transcription antiterminator (PDB 4AKK) (Nt: N-terminal, Ct: C-terminal); (C) Model of molecular mechanism of NasR antitermination.

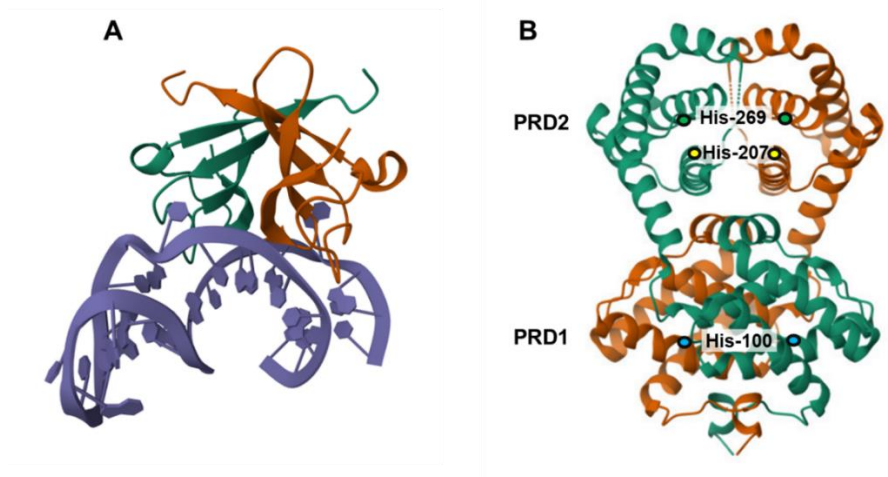


Figure 4. Structure of the SacT/SacY/LicT domains. (A) Dimer of the RNA-binding domain in complex with its hairpin RNA target (PDB 1L1C) and (B) PRD with the phosphorylatable histidines (position in LicT) (PDB 1H99). The two monomers are represented in orange and green, respectively, and the double-strand RNA is displayed in purple.

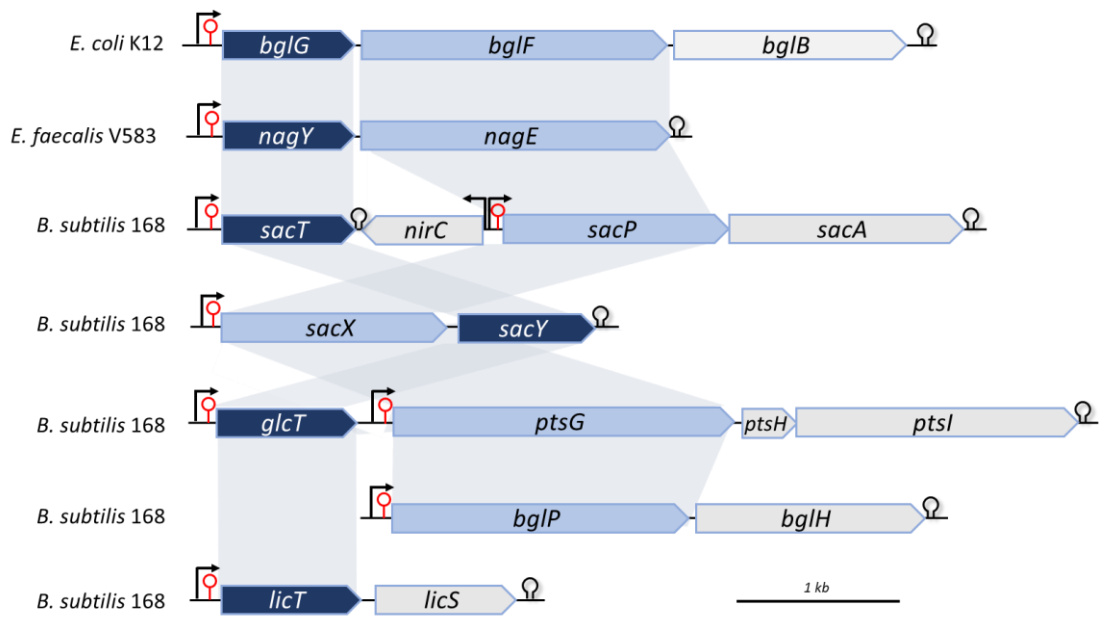


Figure 5. Operonic structure encoding BglG in *E. coli*, SacT/SacY/GlcT/LicT antiterminators in *B. subtilis*, and NagY antiterminator in *E. faecalis*. Genes encoding antiterminators and carbohydrate transporters are represented in dark and light blue, respectively. Antiterminators binding sequences are displayed in red.

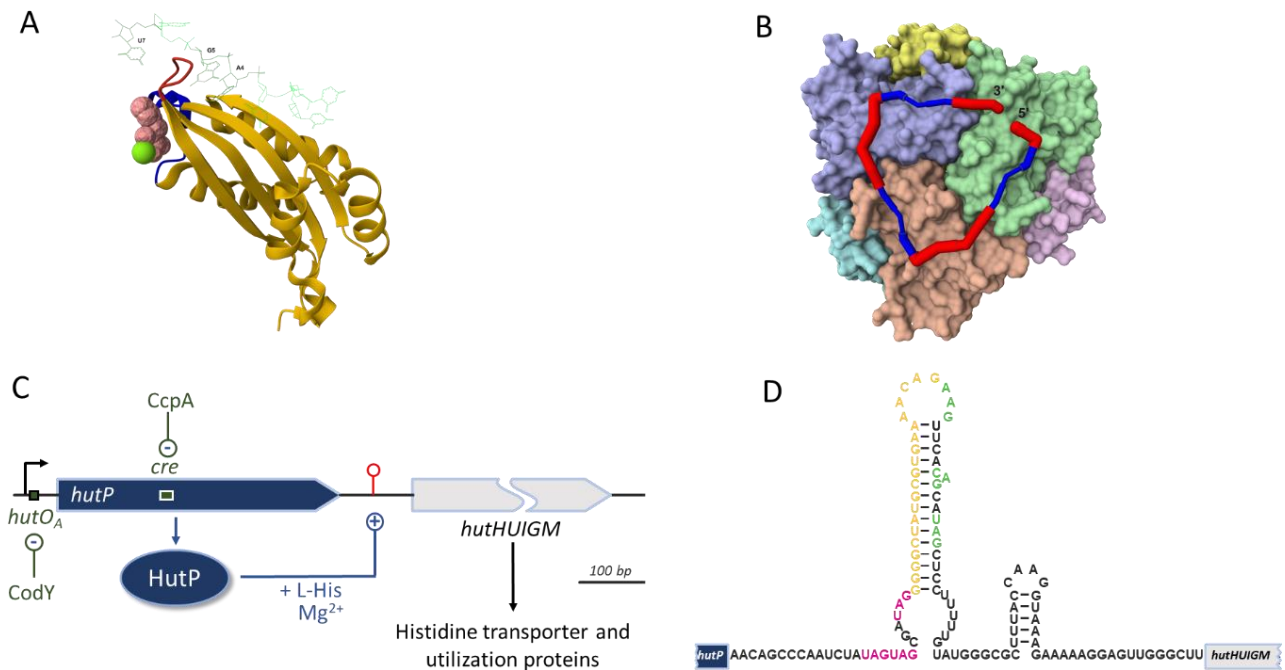


Figure 6. (A) Detail of one HutP protein monomer (PDB 3BOY) interacting with the RNA, histidine, and Mg^{2+} . The HutP protein is displayed in yellow, with protein loops suggested to be involved in the structural communication between the ligand-binding pocket and the RNA in red and blue. The RNA is shown in green, with protein-bound nucleotides highlighted in dark green and labeled. The surface of the histidine ligand is shown in pink and the Mg^{2+} ion as a green sphere. (B) Top view of the structure of the HutP quaternary complex (PDB 3BOY). Each monomeric protein unit is represented with a different color, the mRNA segment is represented as a red tube, and the blue residues highlight the three specific NAG binding sites (Nucleobase-Adenine-Guanine). The ligand-binding pockets and the second bound RNA are not visible in this view. (C) Schematic representation of the *hutPHUIGM* operon structure and regulation. Binding sites for regulatory proteins are indicated by green boxes and the antiterminator binding sequence by a red hairpin. (D) HutP-target terminator RNA structure. NAG recognition motifs of sites I and II are highlighted in pink and green, respectively, and the 20-bp linker region between the two HutP binding sites is shown in orange.