Ménage à trois

Post-transcriptional control of the key enzyme for cell envelope synthesis by a base-pairing small RNA, an RNase adaptor protein, and a small RNA mimic

Yvonne Göpel, Muna A Khan, and Boris Görke*

Max F. Perutz Laboratories; Department of Microbiology; Immunobiology and Genetics; Center of Molecular Biology; University of Vienna, Vienna, Austria

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In Escherichia coli, small RNAs GlmY and GlmZ feedback control synthesis of glucosamine-6-phosphate (GlcN6P) synthase GlmS, a key enzyme required for synthesis of the cell envelope. Both small RNAs are highly similar, but only GlmZ is able to activate the glmS mRNA by base-pairing. Abundance of GlmZ is controlled at the level of decay by RNase adaptor protein RapZ. RapZ binds and targets GlmZ to degradation by RNase E via protein–protein interaction. GlmY activates glmS indirectly by protecting GlmZ from degradation. Upon GlcN6P depletion, GlmY accumulates and sequesters RapZ in an RNA mimicry mechanism, thus acting as an anti-adaptor. As a result, this regulatory circuit adjusts synthesis of GlmS to the level of its enzymatic product, thereby mediating GlcN6P homeostasis. The interplay of RNase adaptor proteins and anti-adaptors provides an elegant means how globally acting RNases can be re-programmed to cleave a specific transcript in response to a cognate stimulus.

Introduction

Small transcript-encoded RNAs present an eminent class of ribo-regulatory molecules found in all three domains of life. In bacteria, sRNAs engage in regulatory circuits in virtually all physiological processes. Nutrient intake, metabolism, and metabolism fluxes are intricately controlled by small RNAs, as exemplified by sRNA Spot42 participating in the regulation of substrate transport and carbon catabolite repression, sRNA GlmZ, mediating glucosamine-6-phosphate (GlcN6P) homeostasis, and the dual-function sRNA SgrS counteracting sugar phosphate stress. Many sRNAs regulate multiple transcripts by imperfect base-pairing, thereby altering translation efficiency and/or stability of the paired RNA. In Gram-negative bacteria, most base-pairing sRNAs require the Sm-like RNA chaperon Hfq for functionality. Hfq stabilizes sRNAs and stimulates formation of cognate sRNA/mRNA duplexes. In contrast, few sRNAs act by protein-binding to alter the activity of their cognate binding partners. Due to their pivotal regulatory roles, the abundance of small RNAs must be firmly controlled. Often, sRNA genes are elaborately regulated at the level of transcription. Many global regulators, such as alternative sigma factors and two-component systems (TCS), expand and invert their regulatory repertoires by integrating sRNAs into their regulons. Regulatory proteins and sRNAs often form various network motifs, resulting in feedback- and feed-forward loops or more elaborate regulatory circuits to coordinate complex physiological responses and social behavior. As opposed to biogenesis, sRNA decay and how it might be regulated is barely understood. Endoribonucleases RNase E and RNase III and the 3′→5′ exoribonuclease PNPase are key factors responsible for sRNA degradation in Gram-negative bacteria. RNase E often promotes the coupled degradation of sRNAs paired to their target transcripts. However, RNase E may also cleave unpaired sRNAs, either initiating their further decay or leading to variants with distinct regulatory properties. Research on the GlmY/GlmZ system in E. coli has revealed novel principles in how sRNA activities can be controlled at the level of transcription as well as decay. The homologous sRNAs GlmY and GlmZ feedback regulate expression of the key enzyme for cell wall biosynthesis, glucosamine-6-phosphate synthase (GlmS), in a hierarchical manner. In contrast, other homologous small RNAs act redundantly and/or additively on their targets. GlmZ activates translation of the glmS mRNA by base-pairing. Abundance of GlmZ is governed at the level of degradation catalyzed by RNase E. GlmZ is not recognized by its decay machinery. Rather, degradation of GlmZ requires the specific RNase adaptor protein RapZ, which binds GlmZ and targets it to decay by a mechanism involving physical interaction with RNase E. This process can be specifically countered by anti-adaptor GlmY, which functions as a decoy sRNA and sequesters RapZ when GlcN6P is limiting. The GlmY/GlmZ regulatory cascade therefore features a unique mechanism to specifically control sRNA decay in response to physiological cues by employing a dedicated RNase adaptor protein.
GlmS is the key enzyme in the hexosamine biosynthesis pathway, which generates precursors for synthesis of important structural macromolecules in bacteria and eukaryotes (Fig. 1A). The product of this pathway is UDP-N-acetyl D-glucosamine (UDP-GlcNAc), an essential building block of peptidoglycan in bacteria. GlmS catalyzes synthesis of GlcN6P, which provides the first and rate-limiting step in biosynthesis of lipopolysaccharides of the outer membrane. GlmS is also essential, unless amino sugars are available in the environment. These sugars can be taken up and converted to GlcN6P, thereby bypassing the need for GlmS. GlcN6P can also be recycled from degradation of peptidoglycan. Degradation by enzyme NagB allows utilization of GlcN6P as nitrogen and carbon source.

In order to achieve metabolite homeostasis in the hexosamine pathway, activity of GlmS must be tightly controlled. Although very different in Gram-positive and Gram-negative bacteria, post-transcriptional mechanisms of glmsS regulation perform the same physiological task: they mediate feedback inhibition of GlmS synthesis by its product GlcN6P, thereby regulating flux through the hexosamine pathway. Gram-positive bacteria use a metabolite responsive ribozyme to adjust GlmS enzymatic activity to the requirements of the cell. In contrast, Gram-negative bacteria also possess powerful mechanisms allowing them to overcome GlmS inhibition by instant and drastic overproduction of this enzyme. This response acts at the post-transcriptional level and involves sophisticated mechanisms of riboregulation.

**The Central Role of Enzyme GlmS for Synthesis of Bacterial Cell Envelope Precursors**

GlmS is the key enzyme in the hexosamine biosynthesis pathway, which generates precursors for synthesis of important structural macromolecules in bacteria and eukaryotes (Fig. 1A). The product of this pathway is UDP-N-acetyl D-glucosamine (UDP-GlcNAc), an essential building block of peptidoglycan in bacteria. GlmS converts fructose-6-phosphate (Fru6P) into GlcN6P, which provides the first and rate-limiting step in biosynthesis of lipopolysaccharides of the outer membrane. GlmS catalyzes synthesis of GlcN6P, which is the key reaction. If available, various amino sugars can be taken up and converted to GlcN6P, bypassing the need for GlmS. GlcN6P can also be recycled from degradation of peptidoglycan. Degradation by enzyme NagB allows utilization of GlcN6P as nitrogen and carbon source.

The Gram-Negative Silver Bullet: sRNA GlmZ Mediates Intra-Operonic Regulation of glmsS

In the Gram-negative bacterium *E. coli* and related species, the genes encoding GlmU and GlmS are present in one operon (Fig. 1B). The two genes are separated by an intercistronic region of 161 nt (Fig. 2A), but a ribozyme is not detectable. Transcription initiation at the glmUS promoter is modulated 3-fold by the transcriptional regulator NagC in response to external amino sugars (Fig. 1B). Since GlmS is dispensable in the presence of exogenous amino sugars, this modulation
of transcription frequency cannot account for the required differential expression of both enzymes.

This paradox was solved when sRNA GlmZ was found to mediate differential regulation within the glmUS operon. GlmZ (formerly RyiA or SraJ) is encoded in an intergenic region opposite to the adjacent genes aslA and hemY (Fig. 4A).34,35 As a prerequisite for regulation, the glmUS co-transcript undergoes rapid and seemingly unregulated cleavage by RNase E at the glmU stop codon, generating monocistronic glmU and glmS mRNAs (Figs. 1B and 2A).28,36 The resulting glmU mRNA lacks a stop codon and is rapidly degraded, indicating that protein GlmU is synthesized from the primary co-transcript.28,36,37 The glmS mRNA is also unstable, unless it becomes activated by base-pairing with GlmZ (Fig. 1B).28,38 Thus, GlmZ selectively activates a downstream cistron within an operon. In silico analysis predicted interaction of GlmZ with nucleotides located between positions -41 and -19 upstream of the glmS start codon (Fig. 1B).28 Indeed, activation of glmS was abolished by mutations in this region and could be rescued through compensatory base mutations in GlmZ (Fig. 2A).38 The base-pairing site in GlmZ is composed of 15 nt located in the single-stranded region between stem loops 2 and 3 (Fig. 2B).

**GlcN6P-Regulated Decay of GlmZ Triggers Feedback Control of GmS Synthesis**

Primarily, GlmZ activates translation of glmS through an anti-antisense mechanism similar to the few other base-pairing sRNAs known to stimulate translation.39 Base-pairing with GlmZ disrupts an inhibitory stem-loop structure that sequesters the Shine-Dalgarno sequence (SD) of glmS (Fig. 2A).28,38 However, at least in a fraction of glmS mRNAs the SD might be accessible. That is, the basal level of glmS translation in absence of GlmZ is sufficient to allow growth under standard laboratory conditions. In addition, base-pairing with GlmZ also stabilizes the glmS mRNA,40 possibly resulting from protection against nucleolytic attack by increased translation.40 As expected for a base-pairing sRNA, Hfq is essential for activation of glmS by GlmZ.28,38 GlmZ strongly associates with Hfq in vivo and in vitro.22,35,41 This interaction also contributes to stability of GlmZ as observed for many other base-pairing sRNAs.6,22 Consistently, Hfq also binds with high affinity to the 5' UTR of glmS.51 Two tripartite ARN repeats (i.e., [ARN] 3 motifs, where R denotes a purine and N any nucleotide) are detectable in this region (Fig. 2A). (ARN) motifs are believed to mediate binding to the distal surface of Hfq.51
the (ARN) 3-2 motif is essential for regulation of glmS by GlmZ. In conclusion, Hfq facilitates base-pairing of GlmZ and glmS similar to many other sRNA/target RNA interactions. Small RNA GlmZ is an exceptional case as its activity is controlled at the level of decay rather than expression. There are two versions of GlmZ: the primary GlmZ transcript, which is 207 nt long, and a shorter variant of ~151 nt resulting from processing. Cleavage removes the base-pairing nucleotides generating a species that is unable to activate glmS (Fig. 2B).

Intriguingly, processing of GlmZ is not a constant process, but is modulated by GlcN6P. Upon GlcN6P limitation, the processed variant of GlmY accumulates and sequesters RapZ by an RNA mimicry mechanism. As a result, GlmZ cannot be cleaved by RNase E. Consequently, unprocessed GlmZ accumulates and base-pairs with the glmS mRNA in an Hfq-dependent manner. Base-pairing disrupts the inhibitory stem loop occluding the SD, thereby allowing translation of glmS, which concomitantly stabilizes the transcript. The newly synthesized GlmS replenishes GlcN6P.

Figure 3. Maintenance of GlcN6P homeostasis by the regulatory GlmY/GlmZ/RapZ circuit. Under ample GlcN6P supply, sRNA GlmY is present in low amounts. Therefore, adapter protein RapZ recruits the homologous sRNA GlmZ for cleavage by RNase E in a process that involves physical interaction of both proteins. Processed GlmZ lacks complementarity to glmS and is unable to activate glmS expression. Consequently, the glmS SD is not accessible to ribosomes, leading to low translation rates and rapid degradation of the mRNA. In addition, high GlcN6P concentrations trigger conversion of preexisting GlmS dimers to enzymatically inactive hexamers, providing feedback regulation at the protein level. Upon GlcN6P limitation, the processed variant of GlmY accumulates and sequesters RapZ by an RNA mimicry mechanism. As a result, GlmZ cannot be cleaved by RNase E. Consequently, unprocessed GlmZ accumulates and base-pairs with the glmS mRNA in an Hfq-dependent manner. Base-pairing disrupts the inhibitory stem loop occluding the SD, thereby allowing translation of glmS, which concomitantly stabilizes the transcript. The newly synthesized GlmS replenishes GlcN6P.

The diagram illustrates the regulation of glmS by GlmZ in E. coli. Under GlcN6P abundance, the sRNA GlmY is present in low amounts, allowing RapZ to recruit GlmZ for cleavage by RNase E. However, when GlcN6P levels decrease, GlmZ accumulates and basepairs with glmS mRNA, activating its translation and stabilizing the transcript. The newly synthesized GlmS replenishes GlcN6P, maintaining homeostasis.
full-length GlmZ accumulates and activates synthesis of GlmS, which replenishes GlcN6P. Thus, GlcN6P homeostasis is established at the level of GlmZ decay.

It Takes Two to Tango: RapZ is an Adaptor Protein Targeting GlmZ to Cleavage by RNase E

A search for the corresponding RNase catalyzing cleavage of GlmZ in vivo indicated involvement of RNase E. Surprisingly, in a pure in vitro system RNase E alone is insufficient to cleave GlmZ, indicating requirement for an additional factor. It was fortuitously observed that mutants lacking protein RapZ (formerly YhbJ) accumulate enormous amounts of GlmS.\textsuperscript{28} Subsequent studies established that RapZ exerts its effect on GlmS synthesis via sRNA GlmZ. Indeed, in \textit{rapz} mutants, processing of GlmZ is abolished resulting in chronic activation of \textit{glmS} expression.\textsuperscript{28,38} Vice versa, overproduction of RapZ increases GlmZ cleavage rates beyond wild-type levels suggesting that RapZ is a limiting factor for processing. However, RapZ is not a ribonuclease as it lacks nucleolytic activity. In fact, cleavage of GlmZ requires the simultaneous presence of both proteins, RNase E and RapZ. In vitro, RapZ triggers correct processing of GlmZ by RNase E in a concentration-dependent manner.\textsuperscript{22}

In \textit{E. coli}, RapZ is encoded in the \textit{rpoN} (Sigma 54) operon. Although located in different genetic contexts, homologs of RapZ are present in a wide range of bacteria indicating an important function.\textsuperscript{22,45,46} Apart from a Walker A/Walker B motif,\textsuperscript{45} RapZ does not exhibit any extended homology to other proteins. However, a C-terminal RNA binding domain was predicted for RapZ in Enterobacteriaceae.\textsuperscript{22} Notably, occurrence of this domain coincides with the presence of GlmZ (and GlmY; see below), suggesting a functional connection. Indeed, RapZ specifically binds GlmZ in vivo and in vitro with high affinity and this interaction is a prerequisite for proper processing of the sRNA.\textsuperscript{22} Intriguingly, processing of GlmZ by the concerted action of RapZ and RNase E also involves physical interaction between these proteins.\textsuperscript{22,47} Initial experiments suggest that RapZ forms a homotrimer and might associate with RNase E in a 3:1 stoichiometry. In bacteria that do not possess sRNAs GlmZ (and GlmY), the roles of RapZ homologs remain elusive. However, at least for the \textit{Bacillus subtilis} homolog, a function in regulation of late competence genes has been described.\textsuperscript{45}

In conclusion, GlmZ can meet two fates: at limiting GlcN6P concentrations GlmZ remains unprocessed and binds Hfq to activate \textit{glmS} through base-pairing. In contrast, GlmZ is preferably bound by RapZ, and consequently, degraded at high GlcN6P concentrations (Fig. 3). Hence, RapZ acts as an adaptor protein targeting GlmZ for cleavage by RNase E.
specifically directing cleavage of a sRNA by a globally acting RNase.

**Reprogramming RNase E Activity by Association with Accessory Proteins**

RNase E consists of an N-terminal catalytic domain and an unstructured C-terminal scaffolding domain, which binds RNA. RNase E is also an interaction site for RNA helicase RhlB, the glycolytic enzyme enolase, and PNPase. The resulting complex, designated RNA degradosome, is required for degradation of bulk RNA. However, RNase E can associate with additional proteins leading to formation of alternative degradosomes that may serve specialized functions. For instance, helicase RhlB can be replaced by other helicases under specific conditions such as cold shock. Additional association of the degradosome with ribosomal protein L4 may selectively inhibit degradation of stress-related transcripts. Proteins RraA and RslB are able to change the activity and/or composition of the degradosome upon binding, rerouting cleavage activity. Hfq replaces helicase RhlB in the degradosome, thereby recruiting RNase E for degradation of sRNA-targeted transcripts. Although the various canonical and alternative degradosome components impact RNA decay by different mechanisms, they have two features in common: they simultaneously influence a multitude of transcripts and they all bind to the scaffolding domain of RNase E. This raises the possibility that the role of the catalytic domain as potential hub for interacting proteins has been underappreciated. Targeting the N terminus could provide a means for direct regulation of the nucleolytic activity of RNase E. At least for a sRNA, such a direct mode has recently been demonstrated: sRNA MicE allosterically activates RNase E through interaction with its 5′-monophosphate to trigger cleavage of its target mRNA ompD. The discovery of RapZ implies that more adaptors exist, which could confer substrate specificity to general ribonucleases such as RNase E and RNase III. This might extend beyond the central stem loop structures. Thus, GlmY lacks complementarity to the glmUs mRNA (Fig. 2C). Nonetheless, GlmY mediates coordinated expression within the glmUS operon similar to GlmS. Multiple sequence alignments show that homology does not extend beyond the central stem loop structures. Thus, GlmY lacks complementarity to the glmS mRNA (Fig. 2C). Nonetheless, GlmY mediates coordinated expression within the glmUS operon similar to GlmS. Subsequent studies revealed that GlmY and GlmZ operate in a hierarchical manner to jointly attain synthesis of GlmS to the cellular GlcN6P concentration. Essentially, GlmY controls GlmS levels indirectly by antagonizing processing of GlmZ. Limiting GlcN6P concentrations induce accumulation of the processed form of GlmY by a yet unknown mechanism, ultimately leading to activation of glmS. In conclusion, GlmY and GlmZ represent a unique mechanism employed by homologous sRNAs (Fig. 3). Regulation of gene expression by redundant or additive action of homologous sRNAs is widespread in bacteria. In contrast, a hierarchical mode of action has so far only been observed for GlmY and GlmZ.

Unlike GlmZ, GlmY is not bound by Hfq with high affinity and does not require Hfq for stability, indicating a protein-binding rather than base-pairing function. Indeed, RapZ binds GlmY with a slightly higher affinity as compared with GlmZ. In a ligand-fishing experiment using RapZ as bait, GlmY and GlmZ were highly enriched and collectively accounted for 80% of the co-purifying RNA, emphasizing that RapZ is highly specific for both sRNAs. RapZ interacts with the sRNAs’ central stem loop, which is a structure shared by both molecules. Consequently, GlmY and GlmZ compete for binding to RapZ. When GlmY accumulates in the cell as a consequence of GlcN6P deprivation, it sequesters RapZ and precludes GlmZ from binding. As a result, RNase E cannot be recruited to cleave GlmZ and glmS is activated (Fig. 3). This regulation could even be reconstituted in vitro: Presence of GlmY strongly inhibits processing of GlmZ by the concerted action of RNase E and RapZ. Thus, GlmY is the first example for a sRNA that regulates another sRNA through molecular mimicry.

**RNA Mimicry—A Hot Topic in Post-Transcriptional Regulation**

As exemplified by the role of GlmY as mimic for GlmZ, RNA mimicry becomes an increasingly recognized mechanism governing RNA activity through titration. A paradigm is provided by the carbon storage regulatory Csr system in γ-Proteobacteria. Protein CsrA regulates translation and/or stability of target RNAs by direct binding. The cognate sRNAs CsrB and CsrC antagonize CsrA. Both sRNAs are enriched in GGA-motifs that function as CsrA-recognition sequences and are therefore capable of sequestering multiple CsrA proteins. Further, CsrA can even be counteracted through sequestration by an mRNA, as demonstrated for regulation of fimbral gene expression in Salmonella. Another example found in the chitinoclastic carbon metabolism highlights the importance of decoy RNAs for regulation of interaction between RNA molecules themselves. Presence of substrate induces the mph operon

**The Homologous Decoy sRNA GlmY Indirectly Activates glmS by Sequestration of Adaptor RapZ**

RapZ targets GlmZ to cleavage by RNase E. Yet, how is this process controlled by GlcN6P? The homologous sRNA GlmY acts as a molecular mimic for GlmZ (Fig. 2B and C). When GlcN6P is limiting, GlmY accumulates and sequesters RapZ. As a consequence, GlmZ remains unprocessed and associates with Hfq to activate synthesis of GlmS (Fig. 3). GlmY (formerly SspF or Tck1) is a 184 nt long sRNA that undergoes rapid and apparently unregulated processing by a yet unknown enzyme at its 3′-end. The resulting 148 nt variant represents the molecule responsible for regulation in vivo. Strikingly, GlmY and GlmZ are highly similar in structure and sequence (Fig. 2B and C). Both sRNAs are conserved in Enterobacteriaceae. Multiple sequence alignments show that homology does not extend beyond the central stem loop structures. Thus, GlmY lacks complementarity to the glmS mRNA (Fig. 2C). Nonetheless, GlmY mediates coordinated expression within the glmUS operon similar to GlmS.
required for utilization of chitosugars. Synthesis of the separately
encoded chitosaccharide-specific outer membrane protein
ChiP is repressed by sRNA ChiX. ChiX also base-pairs with the

\( \text{chb} \) mRNA. Interestingly, this interaction functions as an RNA
trape that relieves \( \text{chb} \) from repression by ChiX.

These findings may just be scratches at the surface: for
eukaryotes, evidence is accumulating that transcripts may cross-
regulate one another via competition for shared microRNAs.89
Similarly, bacterial RNAs may communicate with each other by
trapping sRNA regulators, or acting as sponges for global RNA-

binding proteins, such as CsrA or Hfq.7,61,64 Using this mechanism,
untranslated regions of RNAs may also communicate with other
transcripts as opposed to solely controlling stability and expression
of the cognate RNA molecule. In sum, competition between
RNAs for binding of shared regulators emerges as a widespread
mechanism adopted for post-transcriptional regulation in all
living organisms.

**Polyadenylation Impacts on glmS Expression by Targeting GlmY Stability**

GlmY was also the first sRNA reported to influence gene
expression dependent on the poly(A) status of its 3′-end. Initially,
it was observed that absence of poly(A) polymerase 1 (PAP I)
causes accumulation of GlmS, reminiscent of the phenotype of a

\( \text{tap2} \) mutant.90 Generally, polyadenylation by PAP I facilitates
the degradation of transcripts in bacteria.91 However, rather than
being directly targeted by PAP I, the glmS transcript is indirectly
controlled via polyadenylation of GlmY.90,92 Absence of PAP I leads
to stabilization of GlmS, and consequently, of GlmZ and glmS.
That is, the processed form of GlmS requires polyadenylation
at its 3′-end for efficient decay. The poly(A) tail presumably
provides a retooling for regulation by PNPase to overcome the extensive
stem loop structure in GlmY.92,93 Similarly, sRNAs MicA and
StrA are polyadenylated by PAP I to facilitate their degradation
by PNPase.92,94 Finally, stability of antisense RNAs maintaining
plasmid copy numbers are controlled by PAP I-dependent
polyadenylation.94 Thus, it is possible that many of the effects
exerted by PAP I on gene expression and bacterial physiology are
the indirect consequence of differentially polyadenylated sRNA regulators.95

**Exceptional Promoter Architectures Control Expression of glmY and glmZ**

GlmY was the first sDNA shown to be controlled by sigma
factor \( \sigma^54 \) in *Enterobacteriaceae*.

**Response regulator GlrR activates transcription initiation at the \( \sigma^54 \) promoter directing expression of \( \text{glmY} \).**
The cognate TCS GlrK/GlrR (formerly YbhK/YbhA) is encoded downstream of \( \text{glmY} \) and transcribed from an independent promoter (Fig. 4A). Sensor histidine
kinase GlrK activates GlrR by phosphorylation. Phosphorylated
GlrK binds to three conserved TGGCGN_GACA motifs located
more than 100 bp upstream of the \( \text{glmY} \) promoter (Fig. 4B).95,96

Multiple binding sites may facilitate formation of GlrR hexamers.
Generally, activator proteins assemble into hexamers to catalyze
open complex formation at \( \sigma^54 \) promoters.97 In addition,
binding of integration host factor IIF to two distinct sites may
facilitate GlrR–RNA polymerase contacts through RNA looping
(Fig. 4B).98 Gene \( \text{glmZ} \) encoding an outer membrane protein,
co-localizes with the genes encoding GlrK and GlrR, suggesting
a functional connection (Fig. 4A).

Surprisingly, expression of \( \text{glmY} \) is not abolished in mutants
lacking \( \sigma^54 \). This is explained by an overlapping \( \sigma^54 \) promoter
(Fig. 4B), an arrangement that is also observed in other
*Enterobacteriaceae*.99–101 Intriguingly, both promoters start
transcription at the same nucleotide, thus preventing the
generation of GlmY species with altered 5′-ends,90 which may lead
to functionally different variants as observed for sRNAs IstR1 and IstR2.102
Although such an overlapping \( \sigma^54/\sigma^57 \) promoter
architecture was never observed before, more recent studies
indicate that it may also apply to other genes.95 The \( \sigma^54 \) promoter
only marginally contributes to \( \text{glmY} \) transcription, suggesting
that its activity could be increased under specific conditions.95

The TCS GlrK/GlrR and small RNAs GlmY and GlmZ are
highly conserved among *Enterobacteriaceae* and their occurrence
strictly coincides.90 Strikingly, in most species, \( \text{glmZ} \) is also
transcribed from \( \sigma^54 \) promoters controlled by GlrK and GlrR.
Again, overlapping \( \sigma^54/\sigma^57 \) promoters are present upstream of
\( \text{glmZ} \) in a subset of species. In contrast, in *Escherichia* species,
\( \text{glmZ} \) is transcribed exclusively from an apparently unregulated
\( \sigma^57 \) promoter (Fig. 4A). Hence, \( \text{glmY} \) and \( \text{glmZ} \) compose a regulon
controlled by GlrK/GlrR and \( \sigma^54 \) in most *Enterobacteriaceae*.
However, in a subset of species including *E. coli*, this regulon is
apparently in evolutionary transition to a \( \sigma^57 \)-dependent system
for reasons that remain elusive.95

**A Second Function for GlmY and GlmZ in Interaction with Host Cells?**

The TCS GlrK/GlrR also plays a role in virulence of various
entero bacteriaal pathogens. In *Salmonella*, GlrK is required
for an undisturbed expression of virulence genes and glmK
mutants are impaired in invasion of epithelial cells, survival
within macrophages, and in vivo colonization of liver and
spleen in mice.96 In *Yersinia pseudotuberculosis*, glmR mutants
are significantly less virulent than the wild-type as assessed in
a mouse model.103 In enterohemorrhagic *E. coli* (EHEC),
the orthologs of GlrK/GlrR are named QseE/QseF for quorum-
sensing regulators E and F. Together, with the second TCS QseB/
QseC, QseE/QseF controls virulence functions presumably in response to autoinducer 1 (AI-1), which is a quorum-sensing
signal produced by the intestinal microbiota, and to host signals
epinephrine/norepinephrine. Thus, these TCSs may function
in inter-kingdom signaling and virulence regulation during
host colonization.103–106 In TCSs coordinate expression of epdFU,
which encodes an effector protein translocated to host cells,
and genes located within the locus of enterocyte effacement
(LEE). The LEE genes are required for adhesion of EHEC to

www.landesbioscience.com  RNA Biology 439
epithelial cells and for effacement of the colonic epithelium, which includes actin rearrangement within host cells. However, as direct regulation of virulence gene expression by QseF could not be demonstrated, GlmY is a likely candidate linking TCS QseE/QseF to pathogenicity. Indeed, attachment of EHEC to host cells and remodeling of the host cytoskeleton by bacterial effector proteins was recently shown to rely on GlmY and GlmZ, providing the first example for sRNA-mediated virulence gene expression in EHEC. The sRNAs promote expression of qseE/F and derepression of core-gene and LEE5 loci by so far unknown mechanisms. These antagonistic regulatory effects on the expression of virulence genes seem to be confounding. However, GlmY and GlmZ may contribute to proper timing, precise modulation, and rapid adaptation of virulence gene expression during host infection. Interestingly, response regulator QseB was shown to modulate glmY expression in EHEC 2-fold. QseB apparently binds to the glmY promoter in vitro and a corresponding binding site has also been suggested in E. coli K-12 (Fig. 4B). Hence, both TCSs may employ GlmY and GlmZ for regulation of virulence functions. In conclusion, GlmY and GlmZ provide a further example for core-genome encoded sRNAs that were coopted for regulation of horizontally acquired genes within pathogenicity islands.

Open Questions and Perspectives

The enterobacterial GlmYZ system represents a novel mechanism in sRNA-based regulation of unusual complexity: In response to a specific stimulus the regulatory output of a base-pairing RNA (GlmZ) is determined by its programmed decay, which involves an adaptor protein (RapZ) for the degrading Rnas, and a decay sRNA (GlmY) that functions as an anti-adaptor (Fig. 3). So far, regulation of sRNAs has mainly been studied at the level of biogenesis revealing sophisticated and extensive control of transcription (e.g., Fig. 4). In contrast, the regulatory potential of programmed degradation of sRNAs has long been neglected. Interestingly, degradation of sRNAs CarB and CarC by Rnas E also relies on an additional protein designated CarD. The discovery of RapZ and CarD opens the intriguing possibility that selective targeting of sRNAs to degradation by dedicated adaptor proteins might provide a ubiquitous mechanism to control sRNAs. Switching sRNA activities by regulated decay may allow cells to adapt instantly to changing physiological conditions. RapZ physically interacts with the N-terminal catalytic domain of RNase E. Hence, RapZ could serve as a co-factor to activate RNase E allosterically. Alternatively, RapZ could deliver GlmZ to membrane-bound RNase E increasing its local concentration or remodel the structure of GlmZ to a substrate that is recognized by RNase E. In conclusion, GlmYZ may represent a model system for similar mechanisms of programmed decay of sRNA regulators, not only in bacteria but perhaps even in eukaryotes.

Another elusive question concerns the mechanism of GlmS sensing by the GlmYZ cascade. GlmY accumulates upon GlmS depletion and counteracts processing of GlmZ (Fig. 3). In a glmY mutant, GlmS has no effect on GlmZ, emphasizing that GlmY is essential for perception of the metabolite. However, the TCS Glk/Glr, which controls glmY transcription, does not sense GlmS, and consequently, activity of the dual glmY promoter is not affected by GlmNP. Therefore, GlmNP acts post-transcriptionally. Does GlmYP facilitate decay of GlmY or does it act by preventing its association with RapZ?

The unusually complex GlmY/GlmZ sRNA circuit provides a potential hub for interconnection with additional processes and regulatory pathways in the cell. Recent findings suggest that sRNAs GlmY and GlmZ have been recruited for regulation of virulence functions in EHEC and perhaps in other pathogens. How GlmY and GlmZ cooperate in fine-tuning of virulence gene expression and whether RapZ also plays a role in this process remains elusive. GlmY might serve additional regulatory functions even in non-pathogenic Enterobacteriacae as it strongly accumulates at the onset of stationary phase, when GlmS synthesis is dispensable. The crucial role of GlmS attracts much interest to target this enzyme for antimicrobial chemotherapy. However, inhibitors of GlmS enzymatic activity are only marginally effective against Enterobacteriaceae. Activation of the GlmY/GlmZ cascade triggers overproduction of GlmS, which overcomes inhibition. Consequently, co-administration of compounds that prevent activation of GlmY/GlmZ is expected to potentiate the antimicrobial activity of GlmS inhibitors. The recently discovered involvement in bacterial virulence even in non-pathogenic Enterobacteriacae as it strongly accumulates at the onset of stationary phase, when GlmS synthesis is dispensable. The crucial role of GlmS attracts much interest to target this enzyme for antimicrobial chemotherapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Storz G, Vogel J. Small RNAs in bacteria: expanding frontiers. Mol Cell 2011; 43:880-88; PMID:21925377; http://dx.doi.org/10.1016/j.molcel.2011.08.022
2. De Lay J, Schier AJ, Grossmann S. Bacterial small RNA-based negative regulation: 11H1 and its consequences. J Biol Chem 2013; 288:7994-8005; PMID:23202507; http://dx.doi.org/10.1074/jbc.R112.441386.
3. Bond CL, Storz G. The base-pairing RNA spot 42 participates in a multistep feedback loop to help enact catabolite repression in Escherichia coli. Mol Cell 2011; 43:286-97; PMID:21531216; http://dx.doi.org/10.1016/j.molcel.2010.12.027
4. Gersch R, Vogel J. Noncoding RNA control of the making and breaking of sugars. Genes Dev 2008; 22:2194-219; PMID:18981470; http://dx.doi.org/10.1101/gad.1717808
5. Belovskyy M, Vandevoorde CK. Regulation of bacterial metabolism by small RNAs using diverse mechanisms. Annu Rev Genet 2011; 45:209-32; PMID:21062835; http://dx.doi.org/10.1146/annurev-genet-112210-155445
6. Vogel J, Leser U, Schirmer and its contribution of RNA. Nat Rev Microbiol 2011; 9:578-89; PMID:21706622; http://dx.doi.org/10.1038/nrmicro2815
transcriptional regulation on a global scale: form and function of CarR in Bacillus subtilis. Mol Microbiol 2010; 34:866-82; PMID:20662934

mediated regulation at the level of transcript stability. FEMS Microbiol Rev 2010; 34:866-82; PMID:20662934

their roles in global regulatory networks. FEMS Microbiol Rev 2010; 34:866-82; PMID:20662934

controlled destruction of the target mRNA by the degradosome. Q Rev Biophys 2012; 45:105-45; PMID:2282857; http://dx.doi.org/10.1017/S0033583511000144

Ferré-D’Amaré AR. The seed region of a small RNA drives two separated NagC binding sites. EMBO J 1995; 14:251-61; PMID:7424111; http://dx.doi.org/10.1002/jem.250140305

and preliminary X-ray diffraction analysis of YhbJ. J Mol Microbiol Biotechnol 2010; 20:143-51; PMID:19717263; http://dx.doi.org/10.1159/000259884

RNA processing and decay. Nat Rev Microbiol 2013; 11:45-57; PMID:23241849; http://dx.doi.org/10.1038/nrmicro3454

J Bacteriol 1999; 181:47-54; PMID:9864311

Beisel CL, Storz G. Base pairing small RNAs and regulators to control gene expression. Curr Opin Microbiol 2009; 12:674-80; PMID:19588496; http://dx.doi.org/10.1016/j.copmic.2009.08.009

Mandin P, Guillier M. Expanding control in bacteria: eukaryote-like mechanisms, and structures of metabolite-responsive ribozymes. Nature 2011; 478:403-9; PMID:21839169; http://dx.doi.org/10.1038/nature10327

The assembly and distribution in vivo of a small RNA. Nucleic Acids Res 2013; 41:45-57; PMID:23241849; http://dx.doi.org/10.1038/nrmicro3454

RNA Biology
...that alter the composition of the degradosome. Mol Microbiol 2006; 61:394-406; PMID:16771842; CJ, Cohen SN, Georgiou G. Differential modulation of comparative genomics. Curr Biol 2001; 11:1369-73; PMID:11553332; http://dx.doi.org/10.1016/j.cell.2001.05.022. Hénon P, Temple S, Liang J, Larson J, Vakulskas CA, Yakhnin AV, Baker CS, Yakhnin H, Sterzenbach T, Nguyen KT, Nuccio SP, Winter MG, Vogel J, Bartels V, Tang TH, Churakov G, Slagter-Górna MW, Pietras Z, Tsai YC, Callaghan AJ, Hernández H, Robinson CV, Luisi BF. The regulatory VR, Lin-Chao S. Regulation of ribonuclease E activity by perfectly overlapping sigma 54- and sigma 70-promoters. Nucleic Acids Res 2010; 38:1273-83; PMID:20757522; http://dx.doi.org/10.1016/j.cell.2001.07.040. Mcvey K, Gottman S. Competitive among Hfq-binding small RNAs in Escherichia coli. Mol Microbiol 2011; 82:1545-52; PMID:21240176; http://dx.doi.org/10.1093/jb microbes/bmr149. Benezet I, Bouchez V, Tulli TL, Chenier G, Huguenot C, Bélanger M. Evolution of the RNA degradosome. RNA 2012; 18:955-62; PMID:22081195; http://dx.doi.org/10.1261/rna.049803. Guad J, Jukl Z, Zhou N, Qiu Z, Zhou X, Antonova ME, Cys M, Colos M, Georgiou G. Differential modulation of the E. coli mRNA degradome by inhibitory proteins that alter the composition of the degradosome. Mol Microbiol 2013; 87:851-66; PMID:23305111; http://dx.doi.org/10.1111/j.1365-2958.2013.07454.x. Daigle A, Fritschi A, Hommel M, Achenbach M. Phenotypic analysis of Salmonella enterica serovar Typhi-Mueller-80. Infect Immun 2012; 80:4344-53; PMID:22144490; http://dx.doi.org/10.1128/MMBR.00006-12. Pöggeler S. A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? Cell 2011; 146:353-8; PMID:21980993; http://dx.doi.org/10.1016/j.cell.2011.07.014. Đorđević B, Mužek M, Vračár I, Česte G, Babić M, Kovačević J, Arraiano CM. PNPase is a key player in the degradation of small RNAs that are not associated with Hfq. RNA 2012; 18:814-23; PMID:22251294; http://dx.doi.org/10.1261/rna.029413.111. Vogel SC, Phifer V, Stomba A, Bier J, Vogel S, Arraiano CM. Characterization of the role of chaperones in Salmonella small RNA regulation. Nucleic Acids Res 2007; 35:6435-43; PMID:17629293; http://dx.doi.org/10.1093/nar/gkm1425. Berezin I, Babitzke P. CsrA activates transcription by sRNAs in Escherichia coli. Mol Microbiol 2004; 54:1422-30; PMID:15554979; http://dx.doi.org/10.1093/nar/gkm916. Dersch P, Görke B. Common and divergent features of microbe-induced rearrangement of host cell actin. Proc Natl Acad Sci U S A 2009; 106:864-9; PMID:19144914; http://dx.doi.org/10.1073/pnas.0810205106. Faivre-Bonnin N, Valentin-Mistelbauer M, Knaus M, Florin P, Bossi L. Caught at its own game: regulatory small RNA mediated by an inducible transcrip-...