A Small Bacterial RNA Regulates a Putative ABC Transporter*

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A small noncoding bacterial ribonucleic acid of 62–64 nucleotides, RydC, was identified in the genomes of *Escherichia coli*, *Salmonella*, and *Shigella*. In vivo, RydC binds to the RNA-binding protein Hfq, and it is unstable when Hfq is absent. Mobility assays reveal that complex formation between RydC and Hfq is specific, with an apparent binding constant of ~300 nM. Sequence alignments combined with structural probing demonstrate that RydC folds as a pseudoknot. Hfq binds the loops crossing the deep and shallow grooves of the pseudoknotted RNA and reorganizes its overall conformation. An interaction with a polycysteinic mRNA, *yejABEF*, which encodes a putative ABC transporter, was detected by affinity purification of immobilized RNA-Hfq complexes. In vivo, the *yejABEF* operon is expressed on minimal medium. Remarkably, its expression is reduced when RydC is absent, and the operon is degraded when RydC expression is stimulated. This observation correlates with the growth defects associated with a stimulation of its expression *in vitro*, generating a thermosensitive phenotype that affects growth on minimal media supplemented with glycerol, maltose, or ribose. We conclude that RydC regulates the *yejABEF*-encoded ABC permease at the mRNA level. This small RNA may contribute to optimal adaptation of some Enterobacteria to environmental conditions.

A number of 40–400-nt² RNAs that generally do not encode proteins or function as transfer or ribosomal RNAs have been characterized in *Escherichia coli*. Because of their small sizes, they have been referred to as small (s) or noncoding (nc) RNAs. Initially, a dozen sRNAs were identified in *E. coli* on the basis of their high abundance or by serendipity. In the last few years, computational, microarray and cloning-based screens have led to the identification of around 50 additional sRNAs in *E. coli* (for recent reviews, see Refs. 1 and 2). These sRNAs act mainly by pairing with other RNAs, are part of RNA-protein complexes, or adopt the structures of other nucleic acids (3). Bacterial sRNAs base pairing with target mRNAs can have various regulatory fates: sRNAs can repress or activate translation by blocking or promoting ribosome binding to mRNAs (3). They can also destabilize or stabilize mRNAs by increasing or decreasing accessibility to RNases. Base pairing between some sRNAs and their RNA targets requires the participation of the Hfq protein, a homolog of the Sm and Sm-like eukaryotic proteins involved in mRNA splicing. Hfq binds AU-rich sequences and forms a homohexameric ring (4). It has been proposed that Hfq acts as an RNA chaperone to promote base pairing interactions between Hfq-binding sRNAs and their targets. It can protect many sRNAs and also mRNAs against RNase E digestion (5). The mechanisms by which Hfq facilitates interactions between sRNAs and their targets are, however, poorly understood.

In this report, the functional and structural identification of a novel sRNA that folds as a pseudoknot and binds Hfq *in vitro* and *in vivo* is described. The RNA was detected in the enterobacteriaceae family, in 21 sequenced strains from the three genera *Escherichia*, *Salmonella*, and *Shigella*. Its existence was independently detected by microarrays of Hfq-immunoprecipitated *E. coli* RNAs and Northern analysis (6), and it was termed RydC. We have identified by affinity chromatography an mRNA target of RydC that specifies a predicted ABC transport system. In *vivo*, RydC regulates the expression of the ABC permease at the mRNA level. When the expression of RydC is stimulated *in vitro*, the mRNA encoding the transporter gets degraded, and growth defects are observed on minimal medium with glyceral, maltose, or ribose as the carbon sources.

**EXPERIMENTAL PROCEDURES**

**rydC Gene Disruption and Overexpression**—The chromosomal *rydC* gene was deleted by targeted gene substitution using a combination of two described protocols. The cat gene flanked by FLP recognition target flanks was amplified by PCR using P1 (5′-CTGCGATGATGCCGGTAAAGCTTCTCTGGAAAGGACCA-3′) and P2 (5′-GATTAAAAAAATAGCCGATGTGAAGGGAGTTATGATGAAGCTGC-C TTC-3′) and P3 (5′-GATTAAAAAAATAGCCGATGTGAAGGGAGTTATGATGAAGCTGC-C TTC-3′), as described previously (7). Strain KY330 was transformed with the PCR product as described previously (8). Homologous recombination between the PCR product and the chromosome leads to chloramphenicol-resistant clones in which the chromosomal *rydC* gene is replaced by the pKD3-encoded *cat* gene, as confirmed by PCR using P3 (5′-CGGATCTCCGCGATGGGCAAAAGG-3′) and P4 (5′-CGGATCTCCGCGATGGGCAAAAGG-3′), and the construct was then introduced into MG1655Z1 (9) by P1 sir-mediated transduction, resulting in strain PhB3079. To overexpress RydC, the *rydC* gene was PCR-amplified using P5 (5′-CGGATCTCCGCGATGGGCAAAAGG-3′) and P6 (5′-CGGATCTCCGCGATGGGCAAAAGG-3′). The resulting fragment was digested by BamHI and EcoRI and cloned into BamHI and EcoRI restricted pUC18, resulting in strain PhB3203. Constructions were verified on an ABI310 automatic DNA sequencer (Applied Biosystems).

**RNA Isolation and Northern Blots**— *E. coli* strains were grown in either Luria-Bertani (LB) or minimal media (M9) and harvested at the indicated A₆₀₀. The cell pellets were resuspended in Trizol (Invitrogen). Total RNA extraction was performed as suggested by the manufacturer. Total RNAs were isolated either by Trizol reagent (Invitrogen) or by acid-phenol extraction, in which the cell pellet was dissolved in 0.2 M sodium acetate, 10 mM EDTA, 1% SDS (pH 5.0), with a volume of water-saturated phenol pre-heated at 65 °C. Incubation was performed...
for 10 min at 65 °C and 0.5 volume of CHCl3:isoamylic alcohol (24:1).

The sample was incubated for 10 additional minutes at 65 °C. Total RNAs were precipitated overnight at 4 °C. For the blots performed on agarose gels, the transfer of the RNAs was achieved in 0.5 mM MgCl2. The incubation was for 1 h at 37 °C, and then 5 units of avian myeloblastosis virus reverse transcriptase (Qbiogene) were added to 1% agarose gel in 2.2 M Tris-borate EDTA supplemented with 0.5% glycerol at 4 °C. The samples were supplemented with 10% glycerol (final concentration) and loaded on a native 4% polyacrylamide gel containing 5% glycerol. The electrophoresis was performed in 0.5× Tris-borate EDTA supplemented with 0.5% glycerol at 4 °C for 4 h (100 V). The results were analyzed by autoradiography or directly by autoradiography.

Isolation of RNAs That Bind to a RydC-Hfq Complex—Total RNAs were extracted at A260 = 0.4. Both the 18S and the 23S rRNAs were removed (Single Place Magnetic Stand; Ambion). RNAs (10 µg) were loaded on both 16S and 23S rRNAs, corresponding to roughly 30 µg of total RNAs, were 3′-end-labeled (as described for RydC, see above) and added to the untagged 3′-P hymN mRNA. The samples were then divided into 10% glycerol, and 0.1% Triton X-100. Protein concentration was determined using the Bradford assay, and the protein was stored at 4 °C.

The transcripts were denatured in 50 mM Heps (pH 6.9), 50 mM NaCl, 5 mM KCl, and 1 mM MgCl2 for 3 min at 85 °C, followed by refolding for 10 min at 30 °C and chilled on ice. 0.2 to 1 pmoI of labeled RNA was incubated with a 1000–1500 molar excess of carrier yeast tRNA and 2 units of RNase T1 (final volume, 5 µl) and incubated in a similar buffer, except for the concentration of imidazole (300 mM). The protein was pure, as shown on a 12% SDS-PAGE (the monomeric and multimeric forms of Hfq were visible, even after boiling the sample in SDS buffer), heated for 15 min at 80 °C, and centrifuged for 10 min at 13,000 g at room temperature, and the supernatant was concentrated on a Skd Amicon in 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 50 mM KCl, 5% glycerol, and 0.1% Triton X-100. Protein concentration was determined using the Bradford assay, and the protein was stored at 4 °C.

in vivo, the 3′-end-labeled primers P7 (5′-GATGCACTGGATTTCACTGGG-3′) was used for hybridization. To monitor RydC expression in vivo in a wild-type strain, ΔRydC, and pUC-RydC strain, primer P8 (5′-ACGGACGCTTTGAGAATACGGG-3′) was used for primer extension. To monitor expression of the putative ABC transporter, primer P9 (5′-TATGCACTGGATTTCACTGGG-3′) was used for hybridization.
with either 10−4 or 5.10−5 unit of RNase V1 or 0.5 unit to 5 units of nuclease S1 supplemented with 1 mM ZnCl2. There was either a 400- or a 1200-fold molar excess of yeast tRNA when probing the conformation of RydC in complex with Hfq.

RESULTS

Identification, Expression, and End Mapping of a Novel sRNA—Based on sequence conservations between phylogenetically related species, a computer approach was developed (11) to identify novel sRNAs expressed from the intergenic regions of the E. coli genome. All those previously characterized (1) were identified, plus new ones. Among them, −10 and −35 promoter sequence signals flanked at their 3'-sides by a 50−60-nt-long "GC-rich" region ending by a putative "Rho-independent" terminator could be predicted (Fig. 1A). We selected one of these putative sRNA-encoding genes that was identified in 21 sequenced bacterial genomes, all from the family of the enterobacteriaceae (6 E. coli, 11 Salmonella, and 4 Shigella sequences), for additional studies. A sequence alignment of those that possess sufficient sequence variations is presented in Fig. 1A. While our work was in progress, the presented putative E. coli sRNA was isolated by co-immunoprecipitation with Hfq, identified using microarray and Northern blot analysis, and named RydC (6), the name by which we will refer to it. The RydC-encoding gene, rydC, is located at 32 min on the E. coli genetic map between cybB and ydcA encoding cytochrome b561 and a hypothetical protein of 5.9 kDa, respectively, both located on the complementary DNA strand (Fig. 1B).

Based on the alignment of seven sequences with high nucleotide identity, RydC is proposed to fold as a pseudoknot, as confirmed experimentally using structural probes (see below). Two RNA helices H1 (7 bp) and H2 (9 bp) are predicted. H2 is part of a Rho-independent terminator. Divergent rydC sequences possess three compensatory mutations to maintain the pairings within RNA helix H2. Predicted helices H1 and H2 are
entangled and connected by three nt stretches L1, L2, and L3. Based on the known genes, L1 has 6–7 “pyrimidine-rich” nt, L2 has a single conserved Cys residue, and L3 has 7–8 nt.

Primer extension analysis on total cellular RNA demonstrates that the predicted promoter is functional \textit{in vivo}, and assessment of the affinity and specificity of the interaction. The detection of RydC expression by Northern hybridization using a labeled strand-specific probe during bacterial growth in LB medium in cells that do not express Hfq because of chromosomal gene disruption (HfqΔ) compared with a control strain (Hfq wt). B. Native gel retardation assay of purified labeled RydC with increasing amounts of purified His-tagged Hfq. C. The interaction between Hfq and RydC is specific. Native gel retardation assay of labeled RydC and Hfq in the presence of increasing amounts of either unlabeled total \textit{E. coli} tRNA or unlabeled RydC.

\textbf{RydC Forms a Complex with Hfq—}Because RydC interacts with Hfq \textit{in vivo} (6), we hypothesized that its quantity could be Hfq-dependent. RydC expression was compared by Northern blots between a strain deficient for Hfq (Δhfq) and its parental strain. In contrast to the parental strain, RydC could not be detected in the derivative that does not express Hfq (Fig. 2A). It suggests that in the absence of Hfq, RydC is unstable and rapidly degraded by RNases. Stability of sRNAs can be reduced in the absence of Hfq that protects them against RNase activity, including RNase E (1, 5). Alternatively, Hfq might be required for the transcription of RydC. The binding of Hfq to RydC was tested \textit{in vitro} by gel-shift assays using increasing amounts of purified Hfq and constant amounts of labeled synthetic RydC. Binding assays were performed at various salt concentrations, constant pH (pH 7.5–8.0), and a large excess of bulk tRNA from yeast to reduce aspecific binding (from a 30 to a 1500 molar excess relative to RydC). The apparent binding constant between RydC and Hfq varies from 120 to 500 nM, depending on the amount of carrier tRNA (the lowest association constant corresponds to the lowest molar excess of competitor RNA). Retardation assays with increasing amounts of purified Hfq indicate that the RNA-protein complex is detected \textit{in vitro} at physiological pH and salt concentrations (Fig. 2B shows a typical assay). The binding between RydC and Hfq is specific because a 4000-fold molar excess of total tRNA displaces only a minor fraction of RydC from a preformed RydC-Hfq complex, whereas a 50-fold excess of cold RydC competed labeled RydC out of the complex (Fig. 2C).

\textbf{Structural Analysis of RydC and Its Interaction with Hfq—}Sequence variation is too low (the sequences aligned have a high sequence identity) to fully establish the secondary structure of RydC by a phylogenetic analysis. Therefore, its conformation was analyzed further by structural probes in solution, an approach that was instrumental in establishing the secondary structures for many RNAs (12). A RydC transcript was end-labeled, and its solution conformation was probed by enzymes. RNase V1 cleaves double-stranded RNA or stacked nucleotides, whereas nuclease S1 cleaves single-stranded RNA. The reactivity toward these probes was monitored for each nucleotide of a 64-nt-long synthetic RNA, in the absence and presence of the protein Hfq. Four independent experiments were performed on RydC alone, and four additional ones were performed on the Hfq-RydC complex (Fig. 3, A–C, is representative). These data are summarized on secondary structure models that they, together with the phylogenetic analysis, support (Fig. 3, D and E).

Double-stranded-specific cuts from C15 to U18 and at the predicted G12-C41 pair and the absence of nuclease S1 cleavages at G12-U18 and A35-C41 suggest that helix H1 forms in solution (Figs. 1A and 3D). RNase V1 cuts at U29-U31, G54, and G57 and the absence of S1 cleavages between C25-C33 and G49-G57 support the existence of a 9-bp helix H2 (Figs. 1A and 3D). S1 cleavages at A19-U23 and U44-U47 are consistent with loops L1 and L3 being mostly single-stranded in solution. According to the sequences, the nt content of loop L1 varies from six to seven, and that of L3 varies from seven to eight. L1 and L3 cross the deep and shallow grooves of the RNA structure that folds as a pseudoknot (13). A conserved unpaired Cys residue is in between helices H1 and H2 that forms L2. L2 is not cut by S1, probably because it is not accessible for cleavage. The strong S1 cleavages between U58 and U63 suggest that the “uridine-rich” 3′-end of RydC is unpaired. Nucleotides C5 and G6 are cleaved by both single-stranded- and double-stranded-specific probes, suggesting that the 5′-end of RydC breathes in solution, as for other bacterial RNAs (14). Alternatively, the nt segment 4CGAU8 can pair transiently with 39UGCG43 (Fig. 3D, boxed nt), accounting for the V1 cuts at nt C5 and G6, forming an extended helix interrupted by an internal bulge at nt G9-C14. There are no detectable degradation sites within the sequence of RydC.

The interaction between RydC and Hfq was also monitored by structural probes (Fig. 3, B, C, and E). In the presence of Hfq, nuclease S1 cleavages disappear at U21-U24 in loop L1 and at U44-U47 in loop L3. These regions are accessible “uridine-rich” sequences. Because Hfq binds “uridine-rich” sequences close to structured domains in target RNAs (15), it suggests that the protein binds L1 and L3, flanked by H1 and H2. Protection of L3 by Hfq is less obvious than for L1. L3 may only be a secondary binding site, a fraction of the RNA does not bind Hfq at L3 or Hfq does not bind L3 and the reactivity change is induced by an indirect structural effect. The stoichiometry of the binding of Hfq to RydC is not known and might
be higher than 1:1 because the protein folds as a hexamer in solution. The pattern of some RNase V1 cuts at stems H1 and H2 also varies: V1 cuts at C15-C16 in H1 and at U29-U31 in H2 are weaker in the presence of Hfq, probably because Hfq stacks onto each other. In the presence of Hfq, G39-U40 gets cleaved by V1, and a stronger V1 cut is observed at C41 (Fig. 3). Hydrolysis ladders (lanes L) indicate the position of each guanosine residue within the RNA sequence. D, experimentally supported secondary structure of RydC. H1 and H2 are the helices; L1 and L3 are the loops crossing the major and minor grooves of the RNA structure, respectively; and L2 connects H2 to H1. Arrowheads show the V1 cleavages, stars show the S1 cuts. Black symbols represent strong cleavage, gray symbols represent moderate cleavage, and white symbols represent weak cleavage. E, enzymatic cleavages that are reduced (−) or enhanced (+) upon protein binding are indicated. Upon Hfq binding, five alternate pairings between nt located within the two boxes are proposed.

Affinity Purification of an mRNA Fragment That Binds a Preformed RydC-Hfq Complex—Bacterial sRNAs that interact with Hfq regulate gene expression at the transcriptional and/or translational level via mRNA-sRNA pairings (3). Affinity chromatography was used to pull putative target RNA(s) out of a preformed RydC-Hfq complex from cellular extracts. Complex was formed between purified His-tagged Hfq and synthetic RydC at a 25-fold:1-fold molar ratio; at that ratio, >95% of Hfq interacts with RydC (Fig. 2B and “Experimental Procedures”). The RNAs that are retained by the column (the RydC-Hfq complexes were preloaded onto a nickel resin) were eluted, ligated at both ends with RNA adapters, amplified by reverse transcription-PCR, cloned, and sequenced. A 350-nt fragment (part of its sequence is 5'-AACGCTTGCAGG1ATCGCAGCACATAAACCCTGT-3') had a perfect match with nt 192–228 (nt 2272390–2272426) from the coding region of the 1095-nt-long yejB mRNA, a putative membrane permease (364 amino acids at position 2272190–2272293 in E. coli K12 MG1655, extracted from the colihi data base at genolist.pasteur.fr/Colibri/). According to the size of the PCR fragment, it also contains 30–40 nt upstream of the AUG initiation codon, which correspond to the 3'-end of the coding region of yejA mRNA, the putative periplasmic binding protein from the transport system. yejA and yejB belong to a predicted 5532-nt operon that encodes two additional genes: yejE is the second half of the permease, and yejF is the ATP binding component of the putative transport system. When blasting the selected sequence against all the sequenced bacterial genomes, only those from Shigella and E. coli have a nearly perfect sequence identity with yejA and yejB. Remarkably, those bacteria also encode...
RydC, whereas the others do not. It suggests a functional link between the selected sequence and rydC.

The mRNA Target Binds Hfq—Hfq binds some mRNAs with affinity and specificity (16, 17). A purified synthetic 129-nt-long RNA (yejAB), corresponding to the nucleotide sequence of the yejA-yejB junction (91 nt from yejA and 38 nt from yejB) within the polycistronic mRNA yejABEF, was produced by in vitro transcription. Initial work was performed with a 350-nt-long RNA that was shortened to minimize nonspecific binding in the gel-shift assays. Native gel retardation assays were performed between labeled yejAB and increasing amounts of purified Hfq, in the presence of an excess of total tRNA or unlabeled yejAB. RydC does not bind yejAB in the absence of Hfq, even at a 100-fold molar excess.

whereas a 500-fold excess of cold yejAB prevents complex formation (Fig. 4B). Two conformations of yejAB probably coexist in solution because two bands are detected on a native gel. The one that migrates faster is predominant, and both conformers bind Hfq (Fig. 4). Alternatively, one could be a degradation product. In the presence of a 100-fold molar excess of cold RydC, there is no detectable complex formation between RydC and labeled yejAB (Fig. 4C).

RydC Regulates the Expression of the yejABEF mRNA Operon—A direct correlation between the expression levels of RydC and its target mRNA was detected in vivo. Northern blot experiments demonstrate that the mRNA corresponding to yejABEF is expressed in wild-type cells grown in minimal media (Fig. 5). The expression of the mRNA is maximal during mid-log and stationary phases. As estimated from 200-nt to 10-kb RNA markers, the mRNA has ~5200 nt (±15% variation), in agreement with its predicted size of 5532 nt based on the genomic sequence. Remarkably, in a strain deficient for RydC (ΔrydC; Fig. 6B), a lower amount of mRNA is detected up to mid-log phase, compared with wild-type. Also, the mRNA operon is degraded in ΔrydC at A_{600} = 0.2, but not in wild-type cells. Fig. 5 is a representative of results that have been reproduced three times with different RNA extractions. In a strain that overexpresses RydC (rydC^{++}; its expression profile during cell growth is shown in Fig. 6B) the mRNA operon is degraded, whereas the overall cellular RNAs are not (Fig. 5, bottom panel). A DNA probe specific for 16S rRNA was used as an internal control of loading identical amounts of total RNA per lane and also as a ~1500-nt RNA size marker. In rich medium (LB), the three bacterial strains do not express the yejABEF mRNA (data not shown). Interestingly, the well-characterized ABC oligopeptide transporter in E. coli, oligopeptide permease (opp), has an expression that is also repressed in rich medium (LB) but is identifiable in minimum medium (18).

Growth Defects Associated with Enhancing RydC Expression—The expression profile of RydC during cell growth was monitored by Northern blots with a labeled DNA oligonucleotide complementary to 16S ribosomal RNA and ethidium bromide staining of the total RNAs extracted from each strain loaded onto an 8% PAGE (8 M urea).

![Fig. 4. In vitro binding assays between yejAB and Hfq. A, native gel retardation assay of a labeled synthetic mRNA fragment (two conformations, gray and black arrows) corresponding to the nt sequence at the junction between yejA and yejB with increasing amounts of purified Hfq (molar ratios of 10–1000 Hfq per mRNA). B, the interaction between Hfq and yejAB is specific. In vitro competition assays with an excess of either total tRNA or unlabeled yejAB. C, RydC does not bind yejAB in the absence of Hfq, even at a 100-fold molar excess.](http://www.jbc.org/)

![Fig. 5. The expression of the yej mRNA operon is regulated by RydC at the mRNA level In vitro. Northern blots with a labeled DNA complementary to yejA in the overproducing strain (RydC^{++}) and the deletion strain (RydCΔ), compared with wild-type levels (wt) in minimum media. Cells were harvested at A_{600} = 0.2, 0.4, and 0.8. The estimated size of the operon is 5000–6000 nt (predicted size, 5532 nt). Bottom panels, Northern blots probed with a labeled DNA oligonucleotide complementary to 16S ribosomal RNA and ethidium bromide staining of the total RNAs extracted from each strain loaded onto a 8% PAGE (8 M urea).](http://www.jbc.org/)
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FIG. 6. Growth defects associated with enhancing RydC expression. A, detection of RydC expression levels by Northern hybridization using strand-specific probes during bacterial growth of K12 C25113 cells in LB medium. Northern blots with labeled DNA complementary to either RydC (bottom panel) or transfer-messenger RNA, which was used as a control (top panel). B, RydC expression in vivo is either prevented because of chromosomal gene disruption (RydC Δ) or increased from a multicopy plasmid that encodes RydC expressed from its natural promoter sequence (RydC ++). C, a multicopy plasmid containing rydC confers thermosensitive growth on certain media. Strain MG1655Z1 containing either pUC18 or pUC-RydC grown overnight in LB medium at 30 °C was pelleted and resuspended in M63. Serial dilutions (10-fold, from left to right) were spotted (5 μl) on M9 plates supplemented with either glucose, glycerol, maltose, or ribose at 30 °C, 37 °C, or 42 °C, as indicated.

DISCUSSION

A small, noncoding, ribonucleic acid, RydC, was identified in three Enterobacteria by a systematic search for novel small bacterial RNAs. In E. coli cells, the expression of RydC during growth was monitored, and its 5′- and 3′-ends were mapped, leading to a size of 62–64 nt. RydC co-immunoprecipitates with Hfq (6), and we show here that the protein is required for its stability and activity in vivo. In vitro, RydC binds Hfq with high affinity and specificity, and the apparent binding constant of complex formation between the RNA and the protein is compatible with its presence in vivo. In solution, RydC folds as an RNA pseudoknot, and its conformation is modified upon binding of Hfq. Probing suggests that the protein binds the two connecting single-stranded loops within the pseudoknot, induces a rearrangement of the pairing in one helix, and stacks the two helices, probably to set RydC for optimal pairing with its target mRNA.

All the sRNAs that bind and require Hfq for activity act by pairing to target mRNA(s), perturbing their stability and/or translation. Hfq increases RNA unfolding or local target mRNA concentration. Affinity chromatography could detect a fragment of a cellular message that binds to immobilized RydC-Hfq complexes. It corresponds to a fragment of an operon encompassing four genes, yejA, yejB, yejE, and yejF. In E. coli cells, we have detected expression of the ~5.5-kb yejABEF polycistronic mRNA in minimal media. We have monitored the phylogenetic distribution of this sRNA-mediated regulation to assess its functional importance in Enterobacteria. In the genome of Salmonella paratyphi A, we could not identify RydC, and yejF is missing from the yejABEF operon, suggesting that the putative transport system is not functional in that species and that its associated sRNA is also missing. In the genome of the closely related species Salmonella paratyphi B, however, we have identified both RydC and yejABEF genes. Based on the apparent binding affinities, the RydC-Hfq complex forms and then binds to yejABEF mRNA. The binding of Hfq to RydC is probably required to fold the RNA in an active conformation and to facilitate short and perhaps noncontiguous pairing between the trans-encoded RydC and its target mRNA. Transient ternary complex formation between RydC, the mRNA, and Hfq could facilitate recognition between the two RNAs. Hfq binds and affects the stability of several bacterial mRNAs (19). Because Hfq binds both the target message and RydC, one Hfq hexamer could bind RydC and yejABEF simultaneously to increase local RNA concentration. Alternatively, one Hfq hexamer could bind RydC, a second hexamer could bind yejABEF, and the two hexamers could be brought together via interactions between their hydrophobic backs, as suggested previously (3).

The targeted operon encodes a putative ABC permease, but RydC might also regulate other targets encoded at separate locations of the chromosome. In Gram-negative bacteria, the basic units of the importers consist of an auxiliary periplasmic binding protein (the transporters that possess a periplasmic component are involved in cellular import), two membrane-associated domains, and two ATP-binding domains. Based on sequence similarity, yejABEF probably encodes a permease (20), in which yejA encodes a periplasmic binding protein, yejB and yejE encode the two membrane proteins, and yejF is the ATP-binding protein. In E. coli, the major peptide transport systems are the dipeptide permease, the oligopeptide permease, and the murein tripeptide transport from cell wall turnover (21). The expression of the operons encoding oligopeptide permease and dipeptide permease is regulated by a small RNA, gevB, by an unknown mechanism (22).

The regulation of the yejABEF operon by RydC is achieved at the mRNA level. Putative pairings between RydC and yejABEF mRNA have been identified but require direct experimental support. Impairing RydC expression reduces the amount of the target mRNA without triggering mRNA degradation and alters the temporal expression of the operon. Therefore, endogenous expression of RydC in wild-type cells positively affects the accumulation of its target RNA, as recently described for an-
other sRNA in E. coli (23). No growth defects are observed in the absence of RydC, probably because there is still some intact yeABEF mRNA in the cells that can be translated into functional permeases. When RydC expression is stimulated, however, it leads to yejABEF mRNA degradation. High amounts of RydC destabilize the cellular message, probably by enhancing ribonuclease access and turnover, as for other bacterial sRNAs (3). The absence of the mRNA encoding the permease might trigger the thermosensitive phenotype that affects growth on minimal medium supplemented with specific carbohydrates, especially if the transporter is involved in sugar import. When monitoring the expression of yejABEF mRNA at identical absorbances (at stationary phase) in wild-type cells grown in minimal media supplemented with either glucose or ribose, those grown on ribose express higher amounts of the mRNA operon than those grown on glucose. This preliminary result suggests that the growth defect observed on ribose as the carbon source when RydC expression is stimulated is due, at least in part, to the degradation of the mRNA yejABEF. It also suggests that ribose is imported by the yejABEF-encoded permease. Alternatively, although Hfq is abundant in E. coli cells, the induction of RydC may compete with the binding of other sRNAs for the protein and impact their regulatory functions. In summary, the amount of RydC per cell is precisely set to allow the optimal expression of the yejABEF mRNA operon when the nutrients are scarce.

In some Enterobacteria, the expression of two abundant outer membrane porins is also regulated by two sRNA genes (24). Enterobacteria colonize the digestive tract of humans and animals and are attached to the epithelial cells within the digestive tract where the absorption of nutrients takes place. They are facing variations in nutrients concentrations, and a tight regulation of nutrient uptake may be required for growth and survival. 10^{13} bacteria representing 400 species live in the digestive tract. The Bacteroides are the dominant flora, and the Enterobacter are only subdominant and located exclusively in the colon. Glucose absorption takes place in the stomach. Enterobacteria may require specific nutrient uptake mechanisms to capture sugars different from glucose to survive in a competitive environment.

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