RIBOSWITCHES

Sequestration of a two-component response regulator by a riboswitch-regulated noncoding RNA

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Riboswitches are ligand-binding elements contained within the 5'-untranslated regions of bacterial transcripts, which generally regulate expression of downstream open reading frames. Here, we show that in Listeria monocytogenes, a riboswitch that binds vitamin B12 controls expression of a noncoding regulatory RNA, Rli55. Rli55, in turn, controls expression of the eut genes, whose products enable ethanolamine utilization and require B12 as a cofactor. Defects in ethanolamine utilization, or in its regulation by Rli55, significantly attenuate Listeria virulence in mice. Rli55 functions by sequestering the two-component response regulator EvuT by means of a EvuT-binding site contained within the RNA. Thus, Rli55 is a riboswitch-regulated member of the small group of regulatory RNAs that function by sequestering a protein and reveals a distinctive mechanism of signal integration in bacterial gene regulation.

Ethanamine is an abundant molecule in the vertebrate intestine (1, 2), and genes of the ethanolamine utilization pathway (eut) are widely conserved in pathogenic bacteria (3–5). This includes the Gram-positive intracellular human pathogen Listeria monocytogenes, in which eut expression has been shown to be up-regulated in the intestine during infection of mice (6), which suggests that ethanolamine is important for Listeria pathogenesis. In Enterococcus faecalis, eut expression is activated in response to ethanolamine by a two-component response regulator, EvuTW (7, 8). In Salmonella enterica, ethanolamine utilization requires vitamin B12 as a cofactor (9), and we noted the presence of a B12-binding riboswitch located upstream of the first gene in the eut locus of L. monocytogenes (Fig. 1A) (10), which suggested that eut expression might also be regulated in response to B12 availability.

To investigate a role for B12, we examined expression of the eut locus in response to B12 and ethanolamine by RNA sequencing (RNA-seq) (Fig. 1B) and quantitative reverse transcription polymerase chain reaction (qRT-PCR) (fig. S1). We observed expression of the eutFW genes under all conditions, albeit at low levels, which suggested that the cell maintains a pool of EvuTW to sense and respond to ethanolamine. In contrast, higher-level expression of eutFW and expression of other eut genes require both B12 and ethanolamine (Fig. 1B). These data indicated B12 is required to activate eut expression and suggested that the B12 riboswitch does not prevent transcription of the eut locus in the presence of B12, as might be expected for a classical riboswitch (11).

To clarify whether the riboswitch has a role in the B12-dependent regulation of eut expression, we examined transcription of the riboswitch locus (Fig. 1C) in response to B12 and ethanolamine. We were unable to detect any long transcript, which might extend into the downstream eut locus. However, we did detect a ~450-nucleotide (nt) transcript, Rli55 (10, 12), in the absence of B12 (Fig. 1D), and a smaller (~200-nt) transcript that accumulated in the presence of B12 (Fig. 1D). This result suggested that the riboswitch mediates transcription termination of the ~450-nt Rli55 transcript in response to B12, and any remaining long transcript is rapidly degraded (fig. S2A). A strain with a deletion in the B12 riboswitch (Aribo) constitutively expressed the long Rli55 transcript under all conditions (Fig. 1E and fig. S2B), which confirmed B12-dependent regulation by the riboswitch. Conversely, neither the long or short Rli55 transcripts were detected in a strain (∆rli55) in which the entire rli55 locus was deleted (Fig. 1E and fig. S2B). Thus, the B12 riboswitch determines whether Rli55 is expressed as a long or short transcript in response to B12.

We reasoned that Rli55 might act as a regulatory RNA controlling expression of the eut locus. To test this hypothesis, we examined expression of eut genes by qRT-PCR in the wild-type, Aribo, and ∆rli55 strains. In the wild-type strain, eut expression was elevated only when both ethanolamine and B12 were present in the media (Fig. 1F and fig. S3, A and B), whereas in the Aribo strain, which constitutively expresses the long Rli55, eut genes were never expressed under any conditions. In contrast, in the ∆rli55 strain, expression of the eut genes was high in the presence of ethanolamine alone in addition to B12.

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Fig. 1. Control of ethanolamine utilization (eut) genes by a B12 riboswitch–regulated ncRNA. (A) The eut locus. Green arrow denotes B12 riboswitch. Vertical arrows denote positions of ANTAR elements (13). (B) RNA-seq coverage of eut operon. (C) Regions deleted in the Δrli55 and Δripo strains are indicated by solid black lines. Northern blot of the rli55 transcript in the (D) wild-type strain with probe indicated by thick dashed line in schematic or (E) wild-type (wt), Δripo, or Δrli55 strains with probe indicated by thin dashed line in schematic. Ethidium bromide staining of ribosomal RNA is shown as a loading control. Quantification of bands from three experiments is shown below each blot. Expression of the (F) eutV, eutC, and eutT genes was evaluated by qRT-PCR in the indicated strains. LB, Luria broth. Values represent means ± SEM, n = 3; *P < 0.05; ns, not significant.

Fig. 2. Ethanolamine utilization and pathogenesis. BALB/c mice were injected intravenously with ~4500 colony-forming units (CFU) of the indicated strain. Mice were killed at (A) and (D) 24 hours, (B) and (E) 48 hours, or (C) and (F) 72 hours, and spleens and livers were removed to assess bacterial load per organ. Results represent two independent experiments with three or four mice per group in each experiment. *P < 0.05, ns, not significant.
to ethanolamine and B₁₂ together. Together, these data support a model in which Rli55 prevents the expression of the eut locus in the absence of B₁₂, ensuring that the eut genes are expressed only in the presence of both ethanolamine (substrate) and B₁₂ (cofactor) (fig. S3C).

We tested whether defects in ethanolamine utilization, or its regulation, impacted L. monocytogenes virulence by examining the wild-type, Δribo, and Δrli55 strains in a mouse intravenous infection model. We also tested a mutant lacking the eutB gene encoding an ethanolamine lyase subunit, which is unable to catabolize ethanolamine. The Δribo and ΔeutB strains both had significantly reduced bacterial loads at 24 hours after infection compared with the wild-type strain in the spleen and liver. Thus, these differences increased at 48 hours and 72 hours postinfection (~10- to 50-fold) (Fig. 2, A to F). In contrast, the Δrli55 strain, in which eut expression is not inhibited, was present in amounts comparable to those in the wild-type strain in the spleen and liver. Thus, defects in ethanolamine utilization or activation of eut expression significantly attenuate L. monocytogenes virulence.

In E. faecalis, ethanolamine is sensed by the sensor-kinase EutW, which subsequently phosphorylates the response regulator EutV (7). Phosphorylated EutV in turn binds ANTAR elements (AmiR and NasR transcriptional antiterminator regulator) in the 5′ untranslated regions of actively transcribed eut mRNAs, which prevents the formation of a transcription terminator and consequently activates eut expression (13). In L. monocytogenes, ANTAR sites were identified upstream of the eutA and eutV genes, and a third site was identified in the rli55 locus upstream of the eutG gene and downstream of the B₁₂ riboswitch (13). We also identified a second ANTAR site in the rli55 locus (Fig. 3A), which suggested that one or both of these ANTAR elements could be transcribed as part of the 3′ end of Rli55 RNA. Indeed, our RNA-seq data showed that, in the absence of B₁₂, high levels of Rli55 are transcribed as a long transcript encompassing the first ANTAR element (Fig. 3A). In contrast, rli55 transcription terminates abruptly after the riboswitch in the presence of B₁₂, which indicates that the riboswitch determines whether Rli55 is transcribed with or without an ANTAR element. This notion was supported by the detection of putative Rli55 orthologs in E. faecalis and Streptococcus sanguinis (fig. S4). In the latter, the riboswitch, in conjunction with a single ANTAR element, has undergone an inversion relative to the adjacent eutV gene, which suggests that the riboswitch and the first ANTAR element are functionally linked (fig. S4B) and that this is also the case in L. monocytogenes.

To test if the ANTAR elements are involved in Rli55-mediated regulation, we complemented the Δrli55 strain with chromosomally integrated rli55 alleles carrying mutations in the ANTAR elements and examined which ones restored Rli55-mediated regulation of eut expression (Fig. 3, B and C, and fig. S5). A strain with an empty
construct (\(\Delta rli55:\)Empty) could not prevent expression of the \(eut\) genes in ethanolamine alone (as in the parental \(Arli55\) strain), whereas a strain with a wild-type copy of \(rli55\) (\(\Delta rli55:\)\(rli55\)) fully restored \(Rli55\)-mediated inhibition. However, a strain with a deletion in the riboswitch (\(\Delta rli55:\)\(rib\)) inhibited \(eut\) expression in all conditions, as the riboswitch can no longer terminate \(rli55\) transcription in response to \(B_{12}\). In strain \(Arli55::rli55::M1\), wherein four uridine residues in the first \(ANTAR\) site were mutated (Fig. 3D, AM1), inhibition of \(eut\) expression by \(B_{12}\) was abolished in the presence of ethanolamine alone. In contrast, in strain \(\Delta rli55::rli55::M1/M2\), where compensatory mutations were made to the opposite side of the \(ANTAR\) stem-loop (Fig. 3D, AM2), wild-type regulation of \(eut\) expression was restored. Mutations of the six nucleotides in the stem-loop of the second \(ANTAR\) element (Fig. 3D, AM3, \(\Delta rli55::rli55::M3\)) had no significant effect on \(Rli55\)-mediated regulation. Thus, the first \(ANTAR\) element is necessary and sufficient for \(Rli55\)-mediated regulation.

The long form of \(Rli55\) containing an \(ANTAR\) element might bind and sequester \(EutV\) and be sufficient for \(Rli55\)-mediated regulation. We first showed that expression of \(EutV\)FLAG bacteria, grown in the presence of ethanolamine alone, although we saw no enrichment of cell lysates from these two strains (Fig. S6, B and C), followed by RNA-seq analysis (Fig. 3, E and F), showed that \(Rli55\) is enriched by co-immunoprecipitation with \(EutV\)FLAG primarily when bacteria are grown in the presence of ethanolamine alone, although we saw no enrichment in a parallel immunoprecipitation with the \(EutV\)\(NODFLAG\) strain (Fig. 3E). In contrast, the \(ANTAR\) element upstream of the \(eutV\) gene (Fig. 3F) is enriched by coimmunoprecipitation of lysates from \(EutV\)\(NODFLAG\) bacteria, but not \(EutV\)\(NODFLAG\) bacteria, grown in the presence of ethanolamine and \(B_{12}\) together but not from lysates of bacteria grown in ethanolamine alone. To a lesser extent, the \(ANTAR\)-containing region upstream of \(eutA\) and the entire \(eutA\)-\(Q\) locus are enriched under the latter condition (fig. S7). These data support a model in which the majority of \(EutV\) is bound and sequestered by \(Rli55\) in the presence of ethanolamine alone. Conversely, in the presence of ethanolamine and \(B_{12}\), the riboswitch produces short truncated \(Rli55\) transcripts, which cannot bind \(EutV\), and so allows \(EutV\) to bind \(eut\) mRNAs and to activate \(eut\) expression (Fig. 3G).

This riboregulatory mechanism coordinates expression of the ethanolamine utilization (\(eut\)) locus with the availability of \(B_{12}\), the essential cofactor for ethanolamine catabolism. Previously, ethanolamine utilization has been shown to be important after oral infection by \(Salmonella enterica\) serovar Typhimurium and enterohemorrhagic \(Escherichia\) coli (3, 4, 16); however, the contribution of ethanolamine utilization to \(L.\) monoxygenes pathogenesis in an intravenous mouse infection model suggests that ethanolamine utilization is important outside of the intestine and possibly in the intracellular environment. This study also extends the role of riboswitches in the regulation of noncoding RNAs (15, 16). Finally, our data show that \(Rli55\) represents a new member of the small family of regulatory RNAs that function by sequestering a protein, which also includes the 6S and CsrB/C RNAs (17), and highlights a distinctive means of signal integration in bacterial gene regulation.

REFERENCES AND NOTES


IMMUNOGENETICS

Chromatin state dynamics during blood formation

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Chromatin modifications are crucial for development, yet little is known about their dynamics during differentiation. Hematopoiesis provides a well-defined model to study chromatin state dynamics; however, technical limitations impede profiling of homogeneous differentiation intermediates. We developed a high-sensitivity indexing-first chromatin immunoprecipitation approach to profile the dynamics of four chromatin modifications across 16 stages of hematopoietic differentiation. We identify 48,415 enhancer regions and characterize their dynamics. We find that lineage commitment involves de novo establishment of 17,035 lineage-specific enhancers. These enhancer repertoire expansions foreshadow transcriptional programs in differentiated cells. Combining our enhancer catalog with gene expression profiles, we elucidate the transcription factor network controlling chromatin dynamics and lineage specification in hematopoiesis. Together, our results provide a comprehensive model of chromatin dynamics during development.

Chromatin plays a major regulatory role in cell-type-specific functions and response (1, 2). The current dogma of cellular differentiation suggests that there is a progressive closing of the regulatory potential of the genome. According to this model, differentiation is a gradual transition from an open chromatin state in multipotent stem cells to a compacted chromatin state in differentiated cells. However, genome-wide histone modification profiling of embryonic stem cells and terminally differentiated cells is not fully compatible with this model.
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A dual-action RNA switch for expression

Riboswitches are short segments of RNA that bind small molecules and switch between two different conformations, thereby regulating gene expression (see the Perspective by Chen and Gottesman). DebRoy et al. and Mellin et al. find a new class of riboswitches—in two different species of bacteria—that are both part of and regulate the production of a noncoding RNA. Each riboswitch ensures that a particular metabolic pathway is only activated in the presence of an essential small-molecule cofactor. In the absence of the cofactor, the full-length non-coding RNA is made and binds a regulator protein, preventing the regulator protein from inappropriately activating the metabolic pathway. Science, this issue p. 937 and p. 940; see also p. 876