The rplU-ysxB-rpmA operon in Bacillus subtilis is auto-regulated by ribosomal protein L21 via an RNA cis-regulatory element.

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Ribosomes perform an essential cellular function and are structurally complex with numerous RNA and protein components. In addition, the rate of ribosome production is growth limiting in many bacterial species. Thus to facilitate proper ribosome assembly and make the best use of cellular resources, ribosome building blocks must be produced in precise stoichiometric ratios. To control the production of ribosome components many bacterial ribosomal protein operons are regulated by cis-regulatory RNAs that occur in 5' untranslated regions. These cis-regulatory RNAs, or leaders, form secondary structures that bind a ribosomal protein from the same operon, leading to repression of translation, or premature transcription termination. This phenomenon is well established in E. coli, a gram-negative bacterium, but little work has been done to assess how stoichiometry is maintained in other bacteria. In the model gram-positive organism B. subtilis several potential mRNA leaders have been identified computationally by comparative genomics, but few have verified regulatory action. One such leader precedes the rplU-yskB-rpmA operon encoding ribosomal proteins L21 and L27 as well as a protease involved in the maturation of L27. The mRNA leader preceding L21 was identified in organisms across the phylum Firmicutes and has a well-defined and conserved structure. Using beta-galactosidase reporter assays and qPCR, over-expression of L21 was found to repress reporter gene expression 2-fold, but have minimal impact on mRNA levels, suggesting translational regulation. In vitro studies confirm binding of the L21 protein to the L21 leader RNA, supporting the in vivo evidence that the rplU-yskB-rpmA operon is cis-regulated by the L21 protein. Together, these studies provide the first experimental evidence for cis-regulation of the L21 operon in Gram-negative species.