PNAS
www.pnas.org

Supplementary Information for

RibU is an essential determinant of *Listeria* pathogenesis that mediates acquisition of FMN and FAD during intracellular growth

Rafael Rivera-Lugo[a], Samuel H. Light[a,1], Nicholas E. Garelis[a], and Daniel A. Portnoy[a, b, *]

[a]Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA 94720.

[b]Department of Plant and Microbial Biology, University of California, Berkeley, Berkeley, CA 94720.

[1]Present address: Department of Microbiology, University of Chicago, Chicago, IL 60607.

*Corresponding author: Daniel A. Portnoy

Email: portnoy@berkeley.edu

This PDF file includes:

Figures S1 to S3
Tables S1
SI References

Supplementary Information Figures

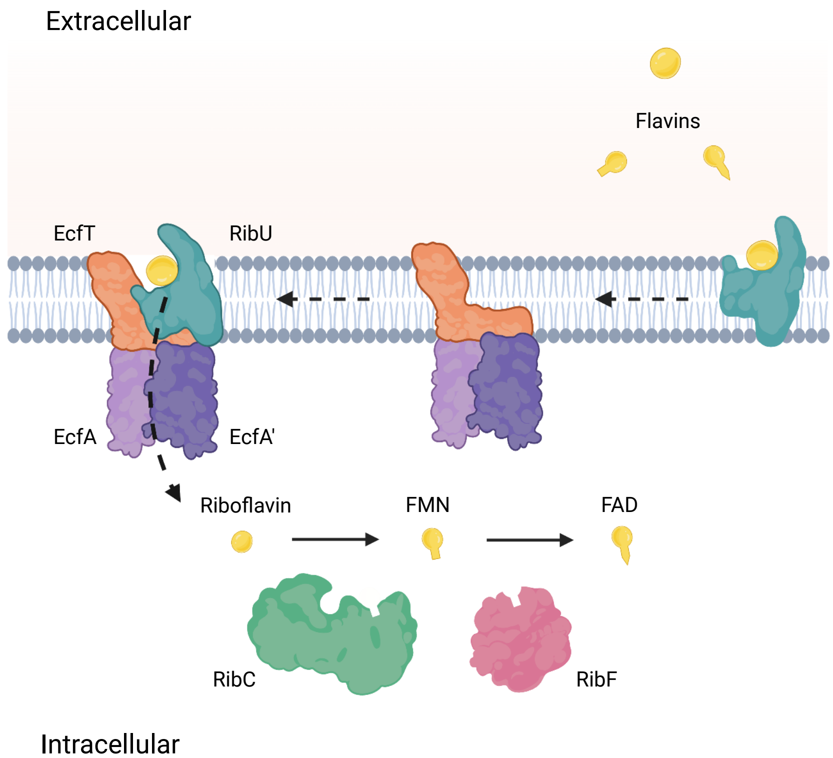


Fig. S1. Flavin metabolism in *L. monocytogenes*. *L. monocytogenes* encodes the energy-coupling factor (ECF) transporter RibU which is composed of four subunits: RibU, the substrate binding subunit; EcfT, the transmembrane coupling subunit; and EcfA and EcfA', two ATPase subunits. RibU binds and transports riboflavin, as well as FMN and FAD (this study). The RibU subunit is dissociated in the membrane in its flavin unbound form and upon capturing a flavin molecule it interacts with the transport module (EcfT:EcfA:EcfA') to import the flavin across the membrane using energy from ATP hydrolysis (1). Upon riboflavin import, the bifunctional enzyme RibC catalyzes the phosphorylation of riboflavin to generate FMN and the conversion of FMN to FAD by attaching an adenosine monophosphate molecule (adenylylation) to FMN. The enzyme RibF can also adenylylate FMN to synthesize FAD (2, 3).

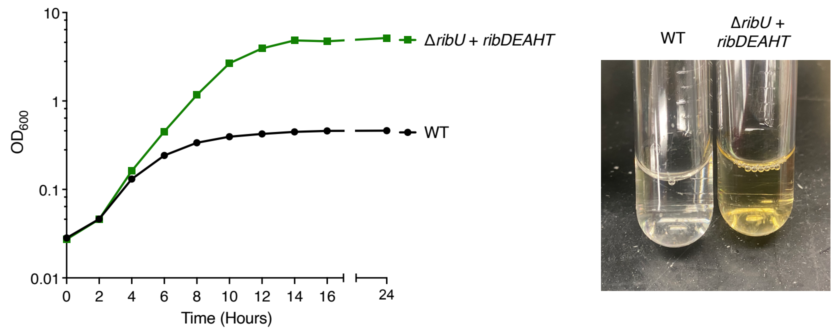
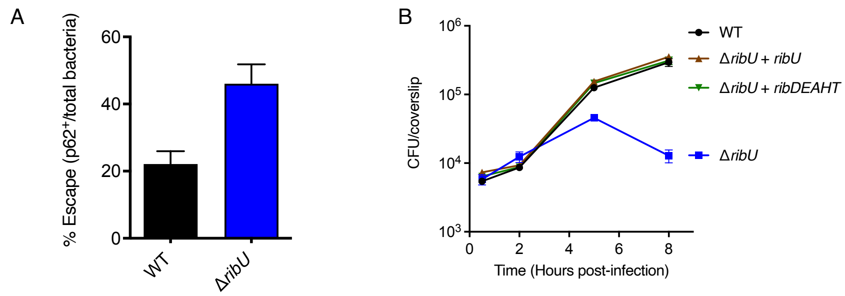
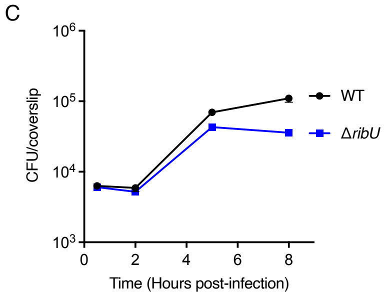


Fig. S2. The Δ*ribU* strain producing riboflavin grows in chemically defined media lacking flavins. Broth growth curve of *L. monocytogenes* strains grown in chemically defined media lacking flavins. OD_{600} was used to determine cell density. The data show the means and standard deviations of three independent experiments. In chemically defined media lacking flavins, wild-type *L. monocytogenes* grows until it depletes its flavin pool. In contrast, the Δ*ribU* + *ribDEAHT* strain grows to higher densities. The picture shows the media supernatant of wild-type (left) and the Δ*ribU* + *ribDEAHT* strain (right) after 24 h of growth at 37 °C shaking. The change in color from colorless to bright yellow, the natural color of flavins, suggests the Δ*ribU* + *ribDEAHT* strain is producing riboflavin and allowing it to grow in media lacking flavins.





D

	Generation time (minutes) of *L. monocytogenes* strains in BMMs	
	+ riboflavin	- riboflavin
WT	51.5	48.6
Δ*ribU*	95.6	-724.2

Fig. S3. Growth dynamics of the Δ*ribU* mutant in BMMs. (A) Percentage of bacteria that colocalized with the autophagy receptor p62 in infected BMMs. In BMMs treated with cytochalasin D, bacteria that escape phagosomes are tagged with p62. Percent phagosomal escape is calculated by counting the number of $p62^{+}$ bacteria of total bacteria. The data show the means and standard errors of the mean of two independent experiments. (B-C) Intracellular growth curves of *L. monocytogenes* strains in BMMs. BMMs were infected at an MOI of 0.1 and CFUs were enumerated at the indicated times. (B) Intracellular growth curve of indicated *L. monocytogenes* strains in wild-type BMMs. The data show the means and standard errors of the mean of two independent experiments. (C) Intracellular growth curve of indicated flavin-starved *L. monocytogenes* strains in riboflavin-deprived wild-type BMMs (for 3 h) supplemented with 1 µM riboflavin just prior to infection. The data represent the means and standard errors of the mean of three independent experiments. (D) Generation time of intracellularly growing bacteria in riboflavin-sufficient and -deprived BMMs between 2 to 5 h. Negative values indicate that the number of recoverable bacteria was decreasing over time.

Table S1. Bacterial strains used in this study

Strains	*Strain number*	*Reference*
Listeria monocytogenes		
Wild type	10403S	4
Δ*ribU*	DP-L7376	This study
Δ*ribU* + pPL2-pNative *ribU*	DP-L7377	This study
Δ*ribU* + pPL2-pNative *ribDEAHT*	DP-L7378	This study
Δ*hly*	DP-L2161	5
Wild type + pPL2-Holin/Lysin	DP-L5961	6
Wild type + pPL2-*L.p. flaA*	DP-L5964	7
Δ*ribC*	DP-L7379	This study
Δ*ribF*	DP-L7380	This study
Δ*ribC/* Δ*ribF*	DP-L7381	This study
Δ*ribC/* Δ*ribF* + pPL2-pNative *ribC*	DP-L7382	This study
Escherichia coli		
SM10 pPL2-pNative *ribU*	DP-E7383	This study
SM10 pPL2-pNative *ribDEAHT*	DP-E7384	This study
SM10 pPL2-pNative *ribC*	DP-E7385	This study
SM10 pKSV7 *ribU*	DP-E7204	This study
SM10 pKSV7 *ribC*	DP-E7386	This study
SM10 pKSV7 *ribF*	DP-E7387	This study

SI References

1. N. K. Karpowich, J. M. Song, N. Cocco, D.-N. Wang, ATP binding drives substrate capture in an ECF transporter by a release-and-catch mechanism. *Nat. Struct. Mol. Biol.* **22**, 565–571 (2015).
2. A. Matern, D. Pedrolli, S. Großhennig, J. Johansson, M. Mack, Uptake and metabolism of antibiotics roseoflavin and 8-demethyl-8-aminoriboflavin in riboflavin-auxotrophic Listeria monocytogenes. *J. Bacteriol.* **198**, 3233–3243 (2016).
3. M. Sebastián, S. Arilla-Luna, J. Bellalou, I. Yruela, M. Medina, The Biosynthesis of Flavin Cofactors in Listeria monocytogenes. *J. Mol. Biol.* **431**, 2762–2776 (2019).
4. C. Bécavin, *et al.*, Comparison of Widely Used Listeria monocytogenes Strains EGD, 10403S, and EGD-e Highlights Genomic Differences Underlying Variations in Pathogenicity. *MBio* **5** (2014).
5. S. Jones, D. A. Portnoy, Characterization of Listeria monocytogenes Pathogenesis in a Strain Expressing Perfringolysin O in Place of Listeriolysin O. *Infect. Immun.*, 5608–5613 (1994).
6. J. D. Sauer, *et al.*, Listeria monocytogenes Triggers AIM2-Mediated Pyroptosis upon Infrequent Bacteriolysis in the Macrophage Cytosol. *Cell Host Microbe* **7**, 412–419 (2010).
7. J.-D. Sauer, *et al.*, Listeria monocytogenes engineered to activate the Nlrc4 inflammasome are severely attenuated and are poor inducers of protective immunity. *Proc. Natl. Acad. Sci.* **108**, 12419–12424 (2011).