Wholly *Rickettsia*! Metabolic Profile of the Quintessential Bacterial Parasite of Eukaryotic Cells

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Background

Bacteria in the genus *Rickettsia* (Rickettsiaceae; *Alphaproteobacteria*) are obligate intracellular microbes with fragmented genomes and diminished metabolic capabilities. They are found widely distributed among vertebrate hosts and include several serious human pathogens. Although the metabolic intractability of the rickettsiae has been studied for decades, *few details are known about how rickettsiae acquire the metabolites required for their survival*. In this study, we integrate recent genomic data with historical analyses to construct a **comprehensive, genus-level metabolic and transport network for the rickettsiae**.

Conclusions

Collectively, our work provides a detailed metabolic profile of rickettsial parasites and highlights aspects of reductive genome evolution and addiction to host cell metabolites. We show that **the core biosynthesis hubs in *Rickettsia* have been supplanted by elaborate thievery of host metabolites** to complement a patchwork metabolic network. We also reveal a **startling degree of functional conservation** across all 84 sequenced *Rickettsia* genomes regardless of host or pathogenicity. Finally, we identify areas of future research aimed at understanding the host/parasite interface and elucidating the chemical dependency that defines the *Rickettsia* obligate intracellular lifestyle.

Results

Our metabolic reconstruction (**Fig. 1**) identified **51 host metabolites (including 21 previously characterized) required to compensate for the patchwork *Rickettsia* metabolic network** (**Fig. 2**). Fatty acid and glycerophospholipid synthesis initiate from host precursors (**Fig. 3**), and the import of both isoprenes and terpenoids is required for the synthesis of ubiquinone and the lipid carrier of Lipid I and O-antigen (data not shown). Unlike bacterial symbionts of arthropods, rickettsiae lack the capability for *de novo* synthesis of B vitamins or most other cofactors (**Fig. 4**); consequently, we stress the use of "**endoparasite**" over "endosymbiont" for non-pathogenic rickettsiae. Cell envelope glycoconjugates are synthesized from three imported host sugars (**Fig. 5A**), with a range of additional host-acquired metabolites fueling the TCA cycle (**Fig. 5B**). **Six biosynthetic pathways contain pathway holes** (**Fig. 6A**; see **Methods**), and similar patterns in taxonomically diverse bacteria (**Fig. 6B**) suggest atypical chemistry or utilization of novel metabolites. A paucity of characterized and predicted transporters (see **Fig. 2**) emphasizes the **gap in our knowledge concerning how rickettsiae import host metabolites**, many of which are large and not known to be transported by any described bacteria.

Acknowledgements

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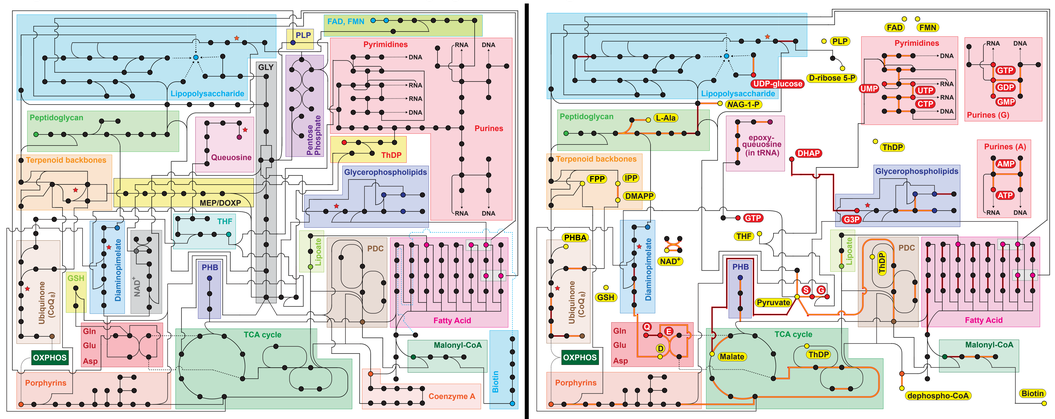
Figure 1. *Rickettsia* metabolic network reconstruction highlights **reductive genome evolution** and an **addiction to host cell metabolites**.



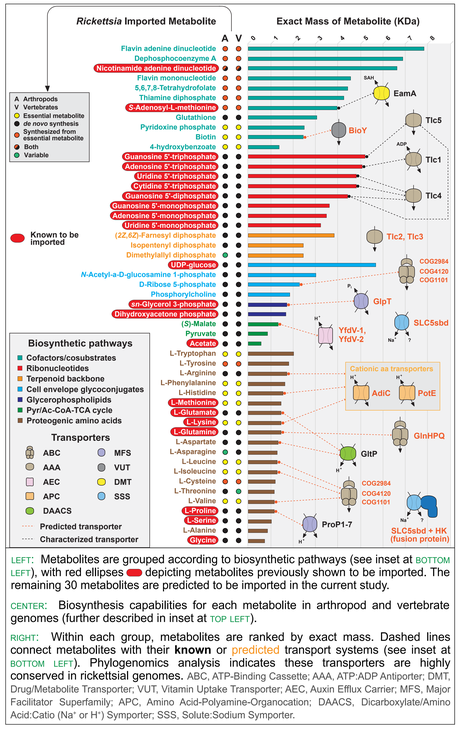
LEFT: **Theoretical *Rickettsia* metabolic network** in the absence of imported host metabolites. *Rickettsia* metabolic pathways have been "supplemented" with typical Gram-negative biosynthetic pathways to create a complete metabolic network.

RIGHT: **Reconstructed *Rickettsia* metabolic network** including imported metabolites. Pathways removed from LEFT have been purged from *Rickettsia* genomes throughout evolution, a consequence of metabolite pilfering from the eukaryotic host.

The network focuses on the biosynthetic pathways discussed in the text. For brevity, the pathways for most amino acids have been omitted. Red stars ★ indicate six pathway holes found in this study (see **Fig. 6**). Red ellipses ⬬ indicate metabolites known to be imported by *Rickettsia* species. Yellow ellipses ⬬ indicate metabolites predicted to be imported based on this study. Import of *S*-Adenosyl-L-methionine, phosphorylcholine, and the majority of amino acids are not included in the reconstruction. Pathway lines are highlighted red to indicate cofactors that are synthesize directly from imported metabolites. Yellow pathway lines with red highlighting indicate cofactors that are directly imported from the host.

Abbreviations: FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide; PLP, pyridoxal phosphate; GLY, glycolysis; ThDP, thiamine diphosphate; MEP/DOXP, non-mevalonate terpenoid biosynthesis; THF, 5,6,7,8-tetrahydrofolate; GSH, glutathione; NAD, nicotinamide adenine dinucleotide; PHB, polyhydroxybutyrate; PDC, pyruvate dehydrogenase complex.

Figure 2. Synopsis of known and predicted metabolites imported from the eukaryotic cytoplasm by rickettsiae.



LEFT: Metabolites are grouped according to biosynthetic pathways (see inset at BOTTOM LEFT), with red ellipses ⬬ depicting metabolites previously shown to be imported. The remaining 30 metabolites are predicted to be imported in the current study.

CENTER: Biosynthesis capabilities for each metabolite in arthropod and vertebrate genomes (further described in inset at TOP LEFT).

RIGHT: Within each group, metabolites are ranked by exact mass. Dashed lines connect metabolites with their **known** or predicted transport systems (see inset at BOTTOM LEFT). Phylogenomics analysis indicates these transporters are highly conserved in rickettsial genomes. ABC, ATP-Binding Cassette; AAA, ATP:ADP Antiporter; DMT, Drug/Metabolite Transporter; VUT, Vitamin Uptake Transporter; AEC, Auxin Efflux Carrier; MFS, Major Facilitator Superfamily; APC, Amino Acid-Polyamine-Organocation; DAACS, Dicarboxylate/Amino Acid:Catio (Na$^+$ or H$^+$) Symporter; SSS, Solute:Sodium Symporter.

Methods

Orthologous gene families (n=3,576) were constructed from 84 sequenced rickettsial genomes using *fastortho*, a modified version of OrthoMCL, and a subset of 149 completely conserved single-copy families was used to estimate a genus-level phylogeny. All protein sequences (n=109,191) were submitted to BlastKOALA and KEGG classifications were subsequently extracted (*kcompile*), transferred across orthologous families (*ktransfer*), and used for metabolic network reconstruction (*kreconstruct*). Rickettsial **pathway holes** (missing gene in an otherwise complete metabolic pathway) were confirmed by *blastp,* and their taxonomic distribution assessed by converting into feature vectors and pattern matching against all complete bacterial genomes in KEGG (*khole*). Transporter systems were assembled in an iterative process involving bioinformatic predictions (TransportDB and TCDB), manual evaluation, and comparative genomics analyses. Custom software packages developed for this study are freely available from the *keggerator* repository on github.

Figure 3. Fatty acids and glycerophospholipids are synthesized from host precursors dephospho-CoA and biotin, DHAP and G3P.

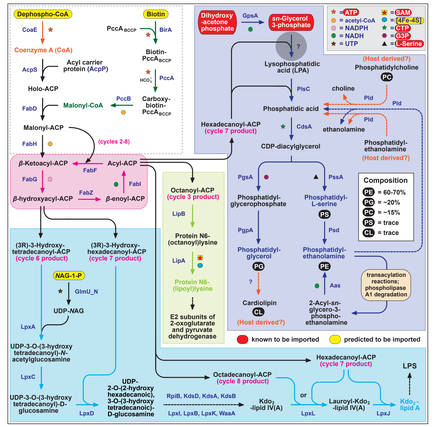


Figure 4. Rickettsiae lack the capacity for *de novo* folate synthesis, but may use FolE to initiate queuosine synthesis from imported GTP.

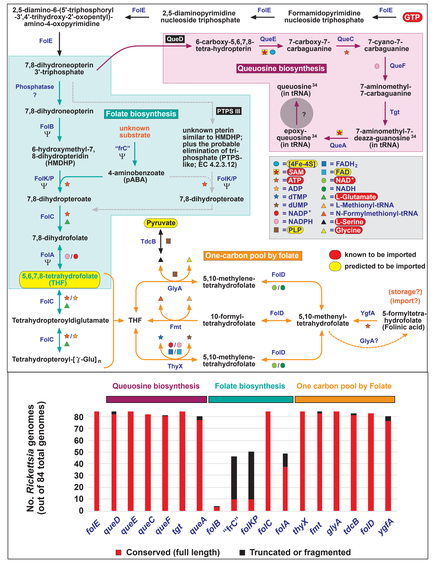


Figure 5A. Cell envelope glycoconjugates are synthesized from imported host sugars D-ribose 5-P, UDP-glucose, and *NAG*-1-P.

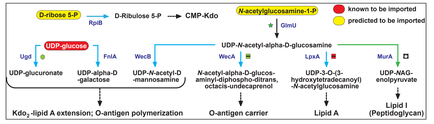


Figure 5B. Rickettsiae possess a complete TCA cycle, but require a range of host-acquired cofactors to fuel it (ATP, CoA, Glx, *etc*).

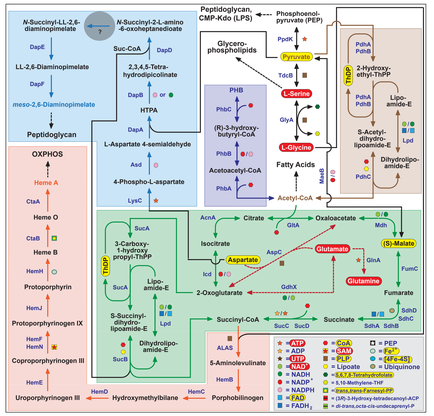


Figure 6A. Rickettsiae contain six **pathway holes** in otherwise conserved metabolic pathways, suggesting alternative chemistries.

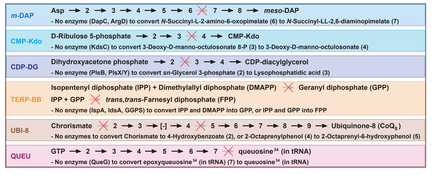
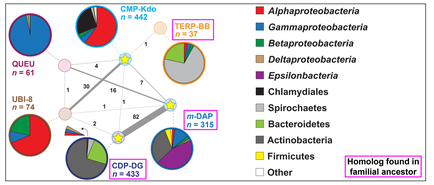


Figure 6B. Several rickettsial **pathway holes** (★) are also common across a taxonomically diverse set of bacteria.



This work is dedicated to the late Dr. Herbert H. Winkler (1939-2016, former Professor of Microbiology and Immunology at the University of South Alabama College of Medicine), whose pioneering work on *Rickettsia* metabolism paved the way forward, and in memory of Chris Cornell (1964-2017), author of "Black Hole Sun".