

Pathogens Residing Within Similar Intracellular Vacuoles Elicit Discordant Host Responses

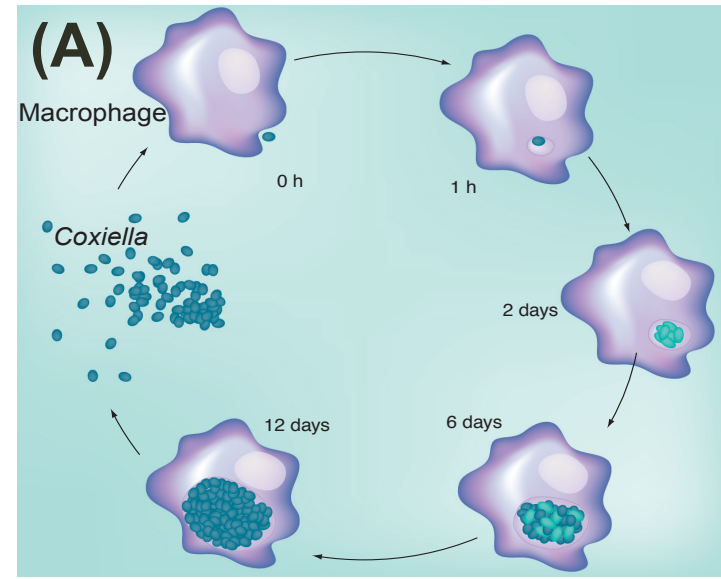
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BACKGROUND

- Coxiella burnetii*, a bacterium and *Leishmania mexicana*, an eukaryote are intracellular pathogens that live within parasitophorous vacuoles (PV) derived from lysosomes¹



- These are the only organisms known to thrive in this acidic environment (~4.5 pH)

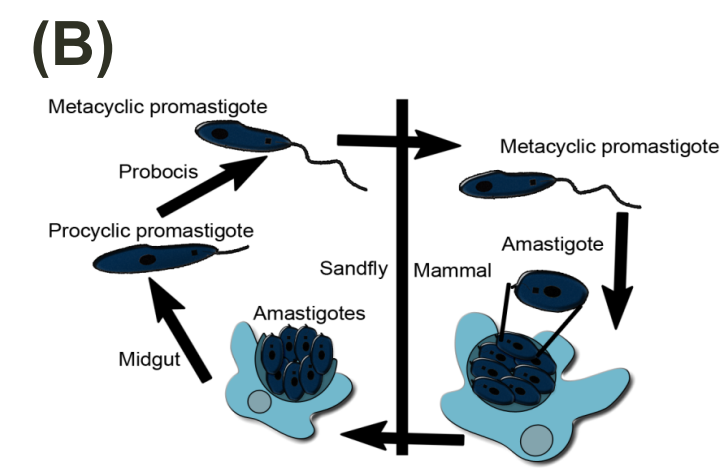


Figure 1: (A) *Coxiella* intracellular growth cycle. (B) *Leishmania* growth cycle³

- Pathogenic strategies that *Coxiella* and *Leishmania* use to inhabit host lysosomes are not well understood

AIMS

- Investigate host response by infecting human macrophage cells with either *C. burnetii* or *L. mexicana* and analyze differences and similarities in host gene expression
- Identify differences in host mRNA isoform expression in response to *C. burnetii* and *L. mexicana* infections
- Identify differences in host miRNA response to *C. burnetii* and *L. mexicana* infections

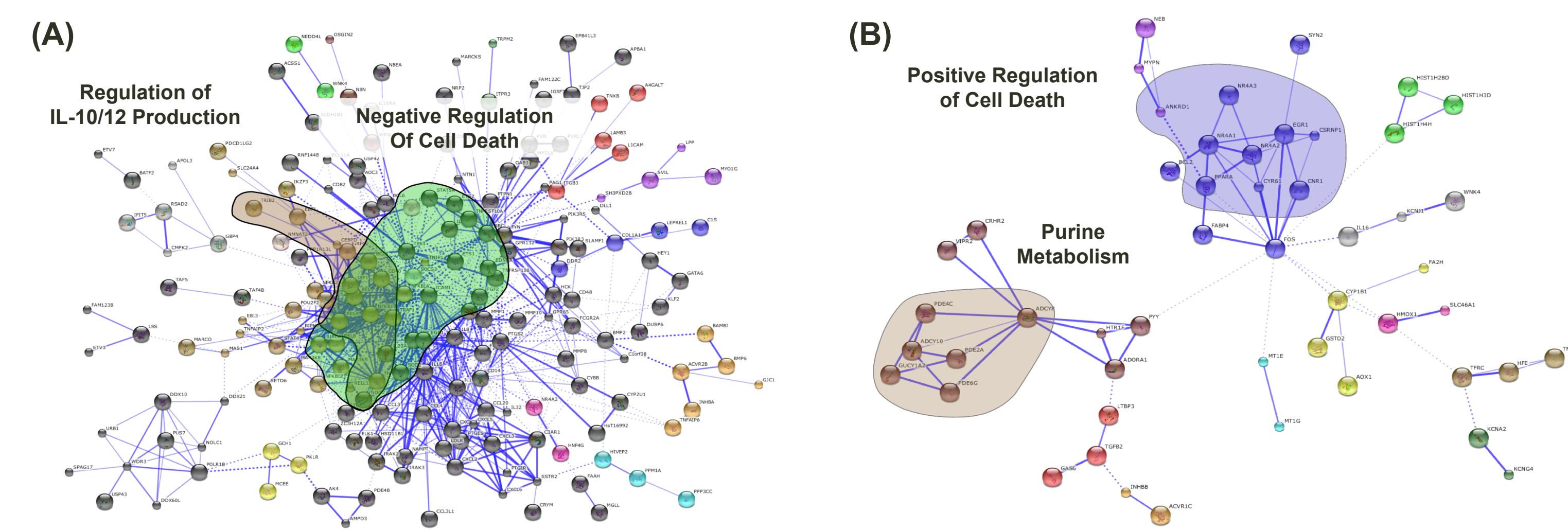
APPROACH

- RNA (5-day post infection) from *C. burnetii* infected THP1 (human macrophage) cells, Vero (monkey kidney) cells, and *C. burnetii* grown in ACCM-2 medium were collected
- RNA from *Leishmania mexicana* infected THP1 cells (5-day post infection) were collected
- RNA-Seq (Illumina), 76 bp reads (~15 million paired end reads) mapped to *Homo sapiens* genome; ~1 million paired end reads mapped to *Leishmania mexicana* genome; ~20 million single end reads mapped to *C. burnetii* genome

RESULTS

1. *C. burnetii* and *L. mexicana* Infections Induce Robust but Non-Overlapping Host Responses

- Only 7% of differentially expressed genes found to overlap
- C. burnetii* infection induces negative regulation of cell death
- L. mexicana* infection induces positive regulation of cell death



	<i>C. burnetii</i> Infected			<i>L. mexicana</i> Infected			
Process	Up	Down	NS ^a	Up	Down	NS ^a	<i>p</i> ^b
Reg. of IL12 Production	6	0	0	1	0	5	0.001
Cell Migration	12	0	0	0	0	12	0.001
Leukocyte Migration	11	0	0	0	0	11	0.001
Neg. Reg. of Cell Death	16	1	3	3	5	12	0.001
Cell Cycle	1	19	4	0	9	15	0.018
Purine Metabolism	0	0	6	6	0	0	0.001
Reg. of Locomotion	0	5	5	8	1	1	0.006
Pos. Reg. of Cell Death	3	10	5	10	0	8	0.004

Figure 2: Protein-protein interaction networks of up-regulated genes in (A) *C. burnetii*-infected and (B) *L. mexicana*-infected THP1 cells visualized in STRING⁴. Colors based on Markov Clustering with an inflation factor of 2. Highlighted clusters are labeled with their GO or KEGG categories.

Table 1: Pathways perturbed by *C. burnetii* and *L. mexicana* infections
^aDifference in expression is not significantly different between samples. ^bFisher's exact test; Bonferroni corrected.

2. Differential Expression of mRNA Isoforms in Infected and Uninfected Cells

- Isoforms are different forms of an mRNA produced from the same gene by alternative splicing
- Only 10% of differentially expressed genes found to overlap
- Cell-wide phenomenon

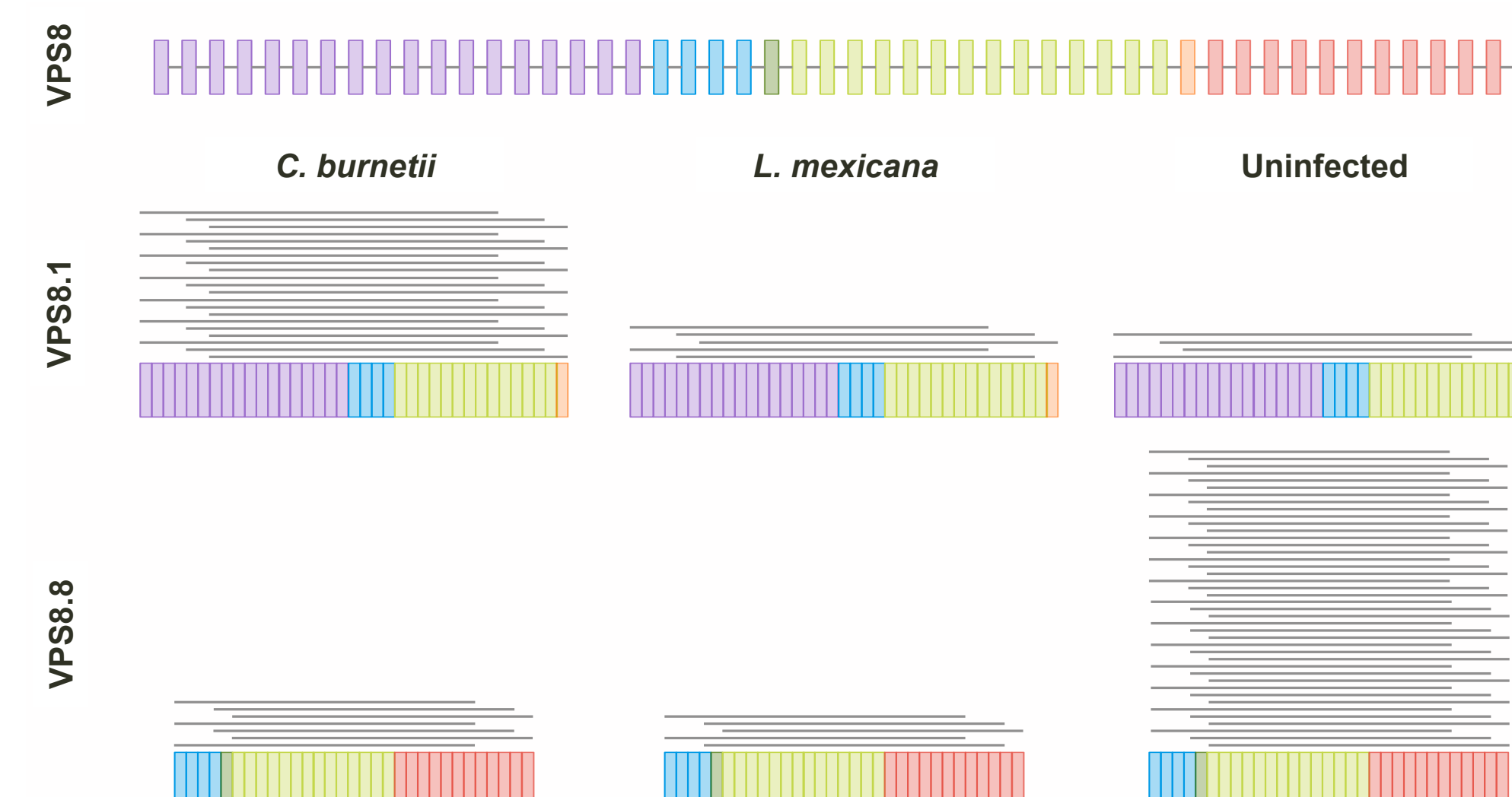


Figure 3: Differential isoform expression of VPS8 in *C. burnetii* infected, *L. mexicana* infected, and uninfected THP1 cells are shown as a representation of isoform analysis. Top panel: The full-length VPS8 gene is depicted with colored bars representing exons. Bottom panels: Isoforms 1 and 8 that have significantly different expression in the three samples are shown. Each grey line above an isoform represents 10 mapped reads.

RESULTS

3. *C. burnetii* and *L. mexicana* Infections Perturb the Expression of Apoptosis-Related miRNAs

- miRNAs regulate expression of genes, play an important role in host cell response to infections
- Some pathogens interfere with the host miRNA to promote growth⁵
- C. burnetii* infection induces more anti-apoptotic miRNAs
- L. mexicana* infection induces more pro-apoptotic miRNAs

Sample	miRNA	FC	<i>p</i>	Regulation	Process
<i>Coxiella</i> -infected	mir-148a-3p	-0.58	0.024	Down	Pro-apoptotic ⁶
	mir-181d-5p	0.78	<0.001	Up	Anti-apoptotic ⁷
	mir-193a-5p	0.81	<0.001	Up	Pro-apoptotic ⁸
	mir-362-5p	0.89	0.015	Up	Anti-apoptotic ⁹
	mir-361-5p	0.95	0.004	Up	Anti-apoptotic ¹⁰
	mir-194-2-5p	1.05	0.024	Up	Anti-apoptotic ¹¹
	mir-28-3p	1.12	0.024	Up	Neither ¹²
<i>Leishmania</i> -infected	mir-28-5p	1.35	<0.001	Up	Pro-apoptotic ¹²
	mir-145-5p	-1.00	0.002	Down	Pro-apoptotic ¹³
	mir-221-5p	-0.62	<0.001	Down	Anti-apoptotic ¹⁴
	mir-15b-5p	0.56	0.035	Up	Pro-apoptotic ¹⁵
	mir-29b-1-3p	1.09	0.002	Up	Pro-apoptotic ¹⁶
	mir-29b-2-3p	1.19	<0.001	Up	Pro-apoptotic ¹⁶

Figure 4: MicroRNAs (miRNAs) perturbed by *Coxiella* and *Leishmania* infections

DISCUSSION

- Host has distinct responses to both types of infection at gene, isoform, and miRNA level
- L. mexicana* apoptosis upregulated, likely aids in cell-to-cell transfer of amastigotes as seen in *L. amazonensis*¹⁷

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ACKNOWLEDGMENTS

This work was supported in part by a grant from American Heart Association to R.R. Travel funds were provided to J.A.M. by the American Society for Microbiology. We thank Drs. Eric Cambronne, Scott Landfear, and Raquel Valdés at OHSU for assistance with *Coxiella* and *Leishmania* infection assays and Christine Sislak, Abraham Moses, and Fenil Kacharia for technical assistance.