Studying Salmonellae and Yersiniae Host–Pathogen Interactions Using Integrated 'Omics and Modeling

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Abstract *Salmonella* and *Yersinia* are two distantly related genera containing species with wide host-range specificity and pathogenic capacity. The metabolic complexity of these organisms facilitates robust lifestyles both outside of and within animal hosts. Using a pathogen-centric systems biology approach, we are combining a multi-omics (transcriptomics, proteomics, metabolomics) strategy to define properties of these pathogens under a variety of conditions including those that

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mimic the environments encountered during pathogenesis. These high-dimensional omics datasets are being integrated in selected ways to improve genome annotations, discover novel virulence-related factors, and model growth under infectious states. We will review the evolving technological approaches toward understanding complex microbial life through multi-omic measurements and integration, while highlighting some of our most recent successes in this area.

Contents

1 Introduction

The outcome of an intracellular bacterial infection—bacterial replication and host cell death versus host cell containment of the pathogen—is a complex process that involves multiple interactions between the host cell and the attacking bacteria. The mechanisms employed by each participant in these interactions are multifaceted and often difficult to elucidate by traditional methods. This is where a "systems approach" in which detailed data covering the bacterial and/or host transcriptome, proteome, and metabolome are integrated using metabolic and regulatory models is useful.

In this chapter, we describe the systems biology approach we apply to analyze, identify, quantify, model, and predict the overall molecular processes involved in

the pathogenesis by *Salmonella* and *Yersinia* species, two relatively closely related and medically important pathogens within the family Enterobacteria. In humans, the pathogenic *Salmonella* serovars *Salmonella Typhimurium* and *Salmonella Typhi* cause a self-limiting gastroenteritis and frequently fatal typhoid fever, respectively. *Salmonella* infection is a major public health problem, causing up to 3 million cases of infection per year in the US alone (Coburn et al. 2007), and the recent emergence of untreatable, multidrug resistant strains such as phage type DT104 has further increased the threat to public health (Glynn et al. 1998). Also pathogenic to humans, *Y. pseudotuberculosis* and *Y. enterocolitica* induce gastroenteritis in the human host, and *Y. pestis* is the causative agent of plague, an acute and lethal disease responsible for at least three pandemics that killed an estimated 200 million people (Perry and Fetherston 1997).

In addition to each other, *Yersinia* and *Salmonella* are closely related to *E. coli,* one of the best studied model systems for biological research (Brenner et al. 1969; Brenner and Falkow 1971; Brenner 1978; Sharp 1991; Lerat et al. 2003). This phylogenetic relationship is advantageous in that: (1) well-characterized biochemical pathways, (2) protein–protein interaction databases, (3) well-characterized transcriptional regulatory and start sites, and (4) molecular biology tools established for *E. coli* provide baseline information that can be applied to studies of *Salmonella* and *Yersinia*. Our premise is that knowledge gained from coordinated analysis and modeling of these two genera will lead to improved control and therapeutic treatment strategies, not only for these specific pathogens, but also for related gram-negative bacteria in general.

Before delving into the application of our systems-level approach to gain insights into pathogenicity from the perspectives of both these bacteria and the host, as well as into host–pathogen interactions, we introduce the key elements underlying our systems biology approach.

2 Elements of a Systems Approach

Our systems-level approach utilizes iterative and complementary experimental and computational methodologies to obtain sample matched, high-dimensional transcriptome, proteome, and metabolome/lipidome data for developing predictive models of pathogenicity for *Salmonella* and *Yersinia* species.

2.1 Experimental Considerations for a Biological Perspective

Experimental considerations for a systems-based analysis in which the number of components simultaneously quantified is important must balance the desire for in depth measurements and broad analyte coverage with conditions representative of the biological environment. In the majority of scenarios, in vitro culture conditions

that simulate environmental conditions encountered by the pathogen during infection represent a good experimental approach, as they are capable of generating large quantities of samples to simultaneously quantify thousands of components (i.e., transcripts, proteins, and metabolites) for a systems analysis of relevant biological interactions (Coombes et al. 2005; Ansong et al. 2008b; 2009; White et al. 2010; Yoon et al. 2011). For example, when *Salmonella* is grown in an acidic minimal media (low pH, low magnesium, and nutrient-deficient) to partially mimic the host intracellular milieu, expression of many genes required for systemic infection are appropriately regulated (Deiwick et al. 1999; Miao and Miller 2000). Similarly, growth of *Yersinia* at 37 °C in calcium-deficient chemically defined best case scenario (BCS) medium induces the type 3 secretion system (T3SS) required for *Yersinia* virulence (Brubaker 1991; Straley et al. 1993; Perry and Fetherston 1997; Cornelis 1998, 2002). Thus, in vitro growth in media with specific composition can be used as a surrogate of the host environment during infection.

A second experimental approach is based on infection of cultured macrophages (specifically RAW264.7 murine macrophage cell line) in vitro. *Salmonella* remains within professional phagocytic cells during mouse infection, and previous studies have shown that replication in macrophages is directly correlated to the ability to cause systemic infection (Fields et al. 1986). *Yersinia pestis* also displays an intracellular growth phase in macrophages, and the ability of *Yersinia* strains to infect and replicate in macrophages has been correlated with virulence (Cavanaugh and Randall 1959; Straley and Harmon 1984a, b; Fukuto et al. 2010).

The third experimental approach involves whole animal models such as mice. Mouse models of infection for *Salmonella* and *Yersinia* are widely considered to be viable surrogates of pathogenicity in humans. For *Salmonella*, the two most commonly used mouse models are C57BL/6 and Balb/c. Both of these strains are susceptible to *S. Typhimurium* infection and die following either intragastric (i.g.) or intraperitoneal (i.p.) infection with the strain used in our work (14028 s; $LD_{50} \sim 10^5$ i.g., $LD_{50} < 10^1$ i.p.). For *Yersinia,* commonly used susceptible mouse models include Swiss–Webster mice and Balb/c mice, in which intranasal/aerosol challenge and subcutaneous challenges represent pneumonic and bubonic modes of plague infection, respectively. The LD_{50} doses for the subcutaneous (s.c.) and aerosol routes are $<10^1$ and 2×10^4 colony forming units respectively (Welkos et al. 1995, 1997; Worsham et al. 1995). We note that there are many other strains of mice that contain mutations in specific anti-microbiocidal components normally expressed by professional phagocytic cells that represent additional important resources in analyzing host–pathogen interactions (Vidal et al. 2008).

2.2 Foundational Omics Technologies

Omics technologies have transformed molecular biology into a data-rich discipline by enabling scientists to simultaneously measure multiple molecular components (e.g., proteins, metabolites, and nucleic acids) that operate in a network of interactions to

generate cellular functions and phenotypic states (Joyce et al. 2006; Oldiges et al. 2007; Cascante and Marin 2008; Ly et al. 2010; Zhang et al. 2010).

In the context of systems biology, transcriptomics is a critical enabling analytical method due to the high precision and relative ease of data generation. DNA microarray transcriptome analysis platforms are now a common laboratory commodity due to the availability of high quality reagents (e.g., slides, cyanine dyes, etc.), widespread adoption of short oligonucleotide probes (70-mers) and exponential reduction in costs of oligonucleotide synthesis and commercial DNA microarray instrumentation. A limitation of microarrays is that they restrict expression profiling data to specific predicted gene annotations. Overcoming this limitation are the next generation sequencing (NGS) transcriptome analysis platforms that allow biologists to determine the primary sequence and relative abundance of every expressed transcript in a cell (whole transcriptome profiling) at an unprecedented level of sensitivity and accuracy (Wang et al. 2009; Martin and Wang 2011; Ozsolak and Milos 2011). However, even this level of information is insufficient for determining whether the transcript is translated into a protein, the macromolecules that execute biochemical functions in all cellular systems.

Comprehensive knowledge regarding protein abundances in organisms, host cells, and tissues is considered essential to the study of infectious diseases and cellular response to stresses. This information provides a basis for understanding genetic variants, gene functions, and action mechanisms, which are needed to develop the means to diagnose, treat, and protect against infectious disease organisms. While some information about relative protein expression levels may be inferred from high-throughput analysis of the mRNA complement or transcriptome (Adams 1996; Velculescu et al. 1997), measured mRNA levels do not necessarily correlate with either the corresponding activity or abundances of proteins (Anderson and Seilhamer 1997; Haynes et al. 1998; Gygi et al. 2000; Schwanhausser et al. 2011). For example, $\sim$20 % at a minimum and potentially as much as 50 % of the *S. Typhimurium* genome is post-transcriptionally regulated (Sittka et al. 2008; Ansong et al. 2009). Protein functions may also be modulated by post-translational modifications (e.g., phosphorylation, acetylation, etc.) and/or by forming complexes with other biomolecules (e.g., proteins, RNA, lipids, etc.) or small molecules (metabolites, dissolved gases, etc.). This information is not even peripherally available from transcriptome analysis. As such, proteomics—the study of the entire complement of proteins expressed by a cell under a specific set of conditions at a particular time—is another key enabling technology in the emerging science of systems biology.

As proper metabolic function underlies nearly every aspect of pathogenesis, e.g., nutrient acquisition and survival within specialized compartments inside host macrophages, metabolomics plays an important role in developing systems-level understanding. Broadly defined, metabolomics is the quantitative determination of time-related or stimuli-dependent changes in the small molecular weight complement of an integrated biological system, cell, or cell types (Nicholson et al. 1999; Kueger et al. 2012). Metabolomics can be further subdivided based on biochemical class, specifically metabolomic studies selective for lipids is termed

"lipidomics". The metabolome and lipidome are the molecules meant to be directed by the transcriptome and in turn the proteome, with small molecules playing critical roles in energy balance, intercellular communication, membrane dynamics, osmoregulation, and many other life processes.

2.3 Computational Framework for Integrating Biological Information

Extracting 'knowledge' from the volumes of omics data resulting from high-throughput measurements is nontrivial (Palsson and Zengler 2010). Two major network approaches have emerged to extract biological insight from this omics ocean: one is inference based and the other, knowledge based. Both approaches use an interconnected network of biological molecules to interpret omics data; however, there are crucial differences in how the networks are constructed and in the biological questions that can be studied. Inference-based approaches employ statistical methodologies to construct network models from correlation or recurring patterns in omics data (see Refs. (Margolin and Califano 2007; Bonneau 2008; De Smet and Marchal 2010) for reviews). Knowledge-based, which is also referred to as reconstruction based, approaches are essentially two-dimensional genome annotation efforts (Palsson 2004) that construct networks from biochemical and genetic data (reviewed in (Reed et al. 2006; Feist et al. 2009; Hyduke and Palsson 2010; Thiele and Palsson 2010)). Statistical inference methods benefit from incorporation of all data in an omics set to guide hypothesis development related to unknown interactions. However, these methods are complicated by the fact that the component measurements are not independent and that they do not account for biochemical and genetic causality (Margolin and Califano 2007). A major shortcoming of inference-based methods is that they typically solve underdetermined problems, thus they are not guaranteed to provide a unique solution (De Smet and Marchal 2010). Network reconstruction employs established biochemical, genetic, and genomic data (Reed et al. 2006; Feist and Palsson 2008; Oberhardt et al. 2009; Schellenberger et al. 2010) to assemble a knowledge base of an organism's molecular components and interactions (Thiele and Palsson 2010). Because knowledge bases are constructed from biological information, whereas inference methods are based on statistical correlations or information theory, knowledge bases provide a biological context for omics analysis (Lewis et al. 2009). The major shortcoming of the knowledge base approach is that they do not, currently, account for the activities of all genes in a genome, thereby limiting the ability to discover novel relationships important to pathogenesis.

Our systems-level strategy utilizes both inference- and knowledge-based approaches to investigate the molecular mechanisms underlying virulence as both approaches have their own unique strengths that allow us to probe the regulatory influences and biochemical mechanisms associated with virulence.

3 Pathogen Perspective: *Salmonella*

Overview

Our overarching biological approach focuses on elucidating virulence mechanisms necessary for *Salmonella* to cause systemic infection. The approach employs in-silico network reconstructions that integrate omics data into a single coherent, systems-level framework. In this section, we describe key steps in this process to improve annotation of the *Salmonella Typhimurium* genome, develop a *Salmonella Typhimurium* genome-scale metabolic reconstruction, and apply the omics-data constrained *Salmonella* metabolic model for in-silico biology applications.

3.1 Proteogeonomics

Complete and accurate genome annotation is crucial as incorrectly annotated genes and/or unannotated genes confound interpretation of experimental omics analyses and result in non- or dysfunctional computational models. However, determining protein-coding genes for most new genomes is almost completely performed by inference using computational predictions that experience significant error rates (Ansong et al. 2008a; Armengaud 2009; Payne et al. 2010). Compounding this issue is a lack of experimental evidence to support predicted protein-coding regions for the overwhelming majority of annotated genomes. Even when available, experimental evidence is typically based on expressed RNA sequences, such as from microarray or NGS experiments, which do not independently and unequivocally elucidate whether a predicted protein-coding gene is translated into a protein, or provide any reliable information on post-translational processing.

Bottom-up proteomics offers the ability to directly measure peptides arising from expressed proteins representing the current best option for independently and unambiguously identifying at least an important subset of the protein-coding genes in a genome and can be used to experimentally validate and correct in-silico gene annotations (Jaffe et al. 2004; Ansong et al. 2008a; de Groot et al. 2009; Wright et al. 2009). Toward this end, we complemented the current *Salmonella Typhimurium* 14028 in-silico annotation with bottom-up proteomics data (Ansong et al. 2011). The data provide protein-level experimental validation for approximately half of the predicted protein-coding genes in *Salmonella* and suggest revisions to several genes that appear to have incorrectly assigned translational start sites.

The proteomics data also revealed 12 non-annotated genes missed by gene prediction programs and provided evidence that suggested a role for one of these genes in *Salmonella* pathogenesis. Moreover, the data-enabled characterization of post-translational features in the *Salmonella* genome that included chemical modifications and proteolytic cleavages. This information revealed a much larger and more complex repertoire of chemical modifications in bacteria than previously thought and included several novel modifications and more than 130 signal peptide

and N-terminal methionine cleavage events critical for protein function. The refined genome annotation facilitates omics analyses and is useful for developing more complete models of metabolism and regulation.

3.2 Genome-Scale Metabolic Reconstruction

Metabolism arguably has the most complete network in *Salmonella,* relative to for example gene regulatory or protein–protein interaction networks, and its proper function underlies nearly every aspect of pathogenesis, e.g., nutrient acquisition and survival within *Salmonella*-containing vacuoles of macrophages. Thus, understanding metabolism under a variety of growth conditions provides us with key insights and testable hypotheses regarding the molecular mechanisms *Salmonella* employs during host infection. Toward this end, we reconstructed and examined the metabolic network of *S. Typhimurium* (Thiele et al. 2011). A metabolic network reconstruction contains all of the possible metabolic reactions known to occur within an organism, although only a subset of these reactions is likely to be active at any time. The *S. Typhimurium* metabolic reconstruction contains 1270 genes, 1119 biochemically unique intracellular metabolites, and 2201 network reactions. Also considered in this model is the importance of localization and movement of metabolites including distinct compartments for the cytoplasm, periplasm, and inner and outer membranes. The metabolic reconstruction by itself is a useful platform for biological discovery as discussed immediately below; however, integrating global omics measurements relevant to infection constrain the model to growth representative of infection allowing detailed studies on phenotypic behavior and analysis of network properties relevant to pathogenenesis as described in the following sections.

In an initial application, we employed the reconstruction to make a number new of predictions regarding possible therapeutic targets in a synthetic gene deletion analysis (Thiele et al. 2011). A number of 56 synthetic lethal gene pairs were found to disrupt growth of *S. Typhimurium* in silico. Notably, several gene pairs are known to be essential for virulence, but not for growth, and have known inhibitors based on the enzyme database BRENDA, further underscoring the applicability of the network reconstruction for applications such as identification of candidate drug targets.

3.3 Metabolic Model-Guided Analysis of Omics Data

Genome-scale metabolic reconstructions are attractive frameworks for multiomic analysis because they represent metabolism in chemically accurate terms and relate enzyme activities to the genome. To gain insight into the changes in the functional state of *Salmonella*'s metabolic network during infection, we grew *S. Typhimurium*

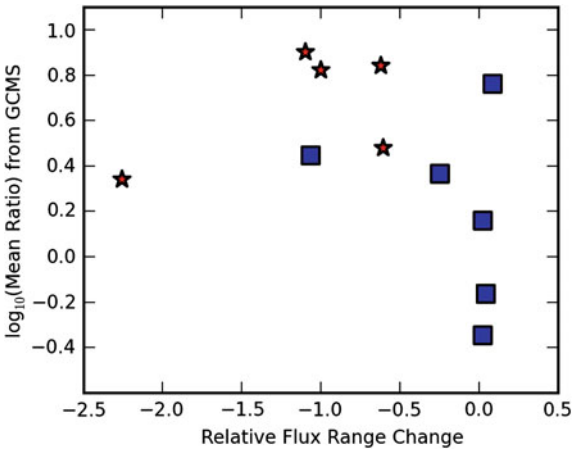


Fig. 1 *Salmonella* preferentially maintains metabolic pathways putatively associated with immunosuppression in minimal media. Omics-data tailored condition-specific models of *Salmonella* metabolism were analyzed with flux variability analysis. The *y*-axis shows the ratio for each metabolite concentration as detected by GC–MS. The *x*-axis depicts the relative change in the allowable ranges of flux through each metabolite as characterized by flux variability analysis. Metabolites capable of suppressing macrophage activation are shown as *blue* boxes. Metabolites capable of supporting macrophage activation are shown as *red* stars

14028 s in rich media and in acidic minimal media defined to mimic the intramacrophage environment after which the *Salmonella* metabolic model was used to analyze sample-matched transcriptomics, proteomics, and metabolomics data generated from these samples. While the transcript and protein data was used to inform reaction flux constraints under the conditions tested, the metabolite data informed on the turnover of intracellular metabolites. This allowed further refinement of the model by requiring that *S. Typhimurium* utilize the detected metabolites in the allowed network states. Small metabolites may play an important role in immunological processes, and we observed a number of metabolites that resulted in modulation of macrophage activation when used as substrates for cellular metabolism. Analysis of sample-matched omics data using the *Salmonella* metabolic model revealed *Salmonella* maintained the metabolic potential for high fluxes of intracellular metabolites postulated to inhibit macrophage activation, presumably allowing for adaptation to the host environment (Fig. 1).

3.4 Inference-Based Analysis of Omics Data

Our reconstruction-based (also known as knowledge-based) network approach is complemented by using an inference-based network approach that employs statistical methodologies to construct network models from correlation or recurring

patterns in omics data. This complementary approach is important as it enables hypothesis development related to unknown interactions.

As a demonstration of the utility of the inference-based modeling approach, the context likelihood of relatedness (CLR) algorithm (Faith et al. 2007), which uses mutual information to infer relationships between genes based on the coordination of their expression profiles across different conditions, was employed to predict proteins important to *Salmonella* pathogenesis (i.e., virulence factors) from sample-matched transcriptomics and proteomics data of *Salmonella* and knockout mutants of 14 regulators required for virulence (Yoon et al. 2011). This approach uncovered many of the known major virulence factors in *Salmonella* recapitulating aspects of known *Salmonella* biology that had taken decades of traditional research to arrive at as well as uncovering several novel network-predicted virulence factors a subset of which importantly were experimentally verified demonstrating the utility of the approach (Yoon et al. 2011).

3.5 Integrated Inference- and Knowledge-Based Analysis of Omics Data

As the metabolic knowledgebase is limited to only those genes associated with metabolism (1271 in the *Salmonella* metabolic reconstruction) it fails to exploit potential clues to virulence programs present in the remaining ~3000 *Salmonella* genes. To overcome this limitation and increase the knowledge extracted from proteome and transcriptome data, we developed an integrated approach that uses the CLR statistical inference method in combination with the *Salmonella* metabolic model (STM_v1.0). In this approach, CLR is utilized to infer a set of candidate 'bottleneck' genes, after which STM_v1.0 is deployed to assess the phenotypic relevance of these genes to growth. A bottleneck gene is frequently (relative to the other genes) found in the shortest path between two genes in the network and they are thought to represent important mediators of system processes (McDermott et al. 2009). The benefit of using CLR inferences with the defined metabolic network is that although CLR does not necessarily infer an actual biological network, it provides information about the influences of all genes measured.

Application of the CLR algorithm to transcriptome data identified potential bottlenecks that were analyzed in the context of the metabolic model to identify the growth conditions in which deletion of a bottleneck would reduce or abrogate growth. We performed in-silico growth simulations using flux balance analysis (FBA) (Feist and Palsson 2010; Orth et al. 2010) or the minimization of metabolic adjustments (MoMA) method (Segre et al. 2002) to assess the impact of gene deletion on growth. Comparison with experimental observations testing the predicted phenotypic effects of the metabolic model and the relevance of the select set of bottleneck genes to virulence showed the FBA method to be less accurate than

Strain	*In Silico* Growth Rate (Relative to WT) FBA	MOMA	*In Vitro* Phenotype
14028s	100%	100%	Growth
ΔatpA	76%	57%	Weak Growth
ΔtpiA	98%	66%	Weak Growth
ΔpurK	100%	100%	Growth
ΔmetN	99%	98%	Growth
ΔmetA	100%	100%	Growth
ΔfrdA	100%	100%	Growth
Δeno	74%	13%	Weak Growth
ΔcyoA	90%	72%	Growth
ΔgpsA	0%	0%	Weak Growth
ΔgapA	66%	0%	No Growth
Δpgk	66%	0%	No Growth
ΔatpA/Δpgk	35%	0%	No Growth
ΔatpA/ΔgapA	35%	0%	No Growth
ΔatpA/ΔtpiA	55%	0%	No Growth

Fig. 2 Experimental phenotypes are consistent with simulated phenotypes for genes identified to be important by coordinated inference and genome-scale metabolic analysis. FBA (quicker to compute) and MoMA (slower to compute) are different approaches for utilizing the metabolic reconstructions to predict phenotypes resulting from knocking out metabolic functions. The growth results for 14 gene deletion mutants relative to "wild type" (WT) parent 14028 s are shown. The *red* boxes indicate poor agreement between growth predictions and the experimentally observed growth result, whereas the *green* boxes indicate good agreement

the MoMA method which showed good agreement with experimental observations (Fig. 2). This finding was not surprising because FBA predicts what the metabolic network could achieve after the organism has evolved to cope with the genetic manipulations while MoMA was developed to identify the growth rate achievable immediately following a perturbation. These results demonstrate the power of leveraging the unique strengths of two different network approaches to increase the amount of knowledge extracted from omics data.

4 Pathogen Perspective: *Yersinia*

Overview

The studies and methodologies focused on Salmonellae provided a foundation from which to study the less understood pathogenic organisms of the *Yersinia* genus. In addition to elucidating virulence mechanisms necessary for *Yersinia* to cause systemic infection, an overarching goal is to understand the differences in disease manifestation among closely related species. In this section, we describe application of the system biology approach described above to gain insight into *Yersinia* biology.

4.1 Proteogeonomics

The concept of annotation refinement introduced above can be extended to include a comparative assessment of genomes across closely related species and the use of multiple omics-data sources further enhancing the value of annotation improvements. Transcriptomic and proteomic data derived from highly similar pathogenic *Yersiniae* (*Y. pestis* CO92, *Y. pestis* Pestoides F, and *Y. pseudotuberculosis* PB1/+) was used to complement the current in-silico annotation for each strain. Peptide and oligo measurements experimentally validated the expression of nearly 40 % of each strain's predicted proteome and revealed 28 novel and 68 previously incorrectly annotated protein-coding sequences (e.g., observed frameshifts, extended start sites, and translated pseudogenes) within the three current *Yersinia* genome annotations (Schrimpe-Rutledge et al. 2012). The refined genome annotations are immediately useful to facilitate omics analyses and develop more complete models of metabolism and regulation.

4.2 Genome-Scale Metabolic Reconstruction

As a framework for integrating and analyzing omics data toward a systems approach to understanding *Yersinia* pathogenesis, we completed a metabolic reconstruction for *Y. pestis* CO92, a strain that is virulent to humans (Charusanti et al. 2011). The metabolic network of *Y. pestis* possesses sufficient flexibility as to endow the organism with the ability to survive and proliferate in its two hosts: (1) the flea insect vector (growth at 26-28 °C) and (2) mammalian vectors such as rodents and humans (growth at 37 °C). The reconstruction contains 815 genes, 678 proteins, 936 unique metabolites, and 1678 reactions, considers localization as for *Salmonella* (see above), and includes two biomass objective functions that account for differences in cellular biomass composition when *Y. pestis* is grown at the two different temperatures.

We employed the reconstruction to analyze gaps in various *Y. pestis* CO92 metabolic pathways. The reconstruction identified two critical gaps in the lysine and fatty acid biosynthesis pathways that needed to be filled in order for model simulations to occur. This necessity prompted a search for alternative genes in *Y. pestis* CO92 that could catalyze the same reactions as those catalyzed by the missing genes. A search for paralogs of YPO0170, the missing gene in lysine biosynthesis, uncovered YPO1962, a potential open reading frame with 59 % nucleotide identity that might have the same catalytic ability; however, there were no apparent paralogs for *fabI*, the missing gene in fatty acid biosynthesis, within the *Y. pestis* CO92 genome.

We searched for alternative enzymes by overlaying global transcript and protein expression data onto the reconstructed metabolic pathways in *Yersinia* as illustrated for the transcript data in Fig. 3. Our reasoning was that any enzyme

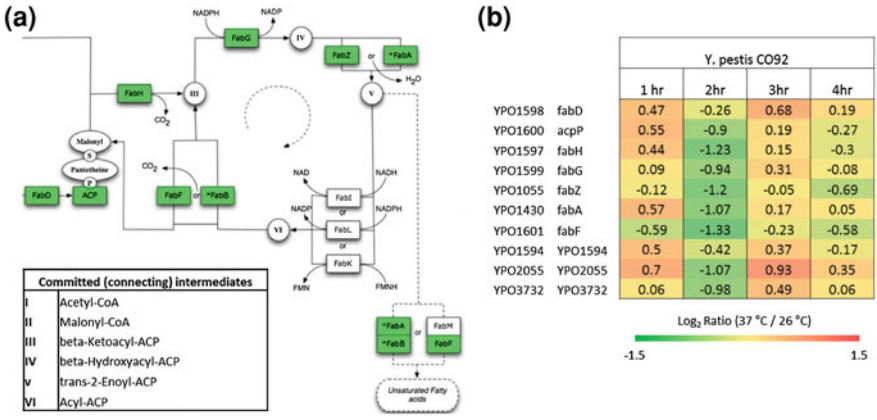


Fig. 3 Visual representation of integrated omics data and reconstruction. Temporal expression pattern of identified fab genes (panel B) in the fab pathway (panel A) are shown including putative fabI candidates YPO1954, YPO3732, and YPO2055. Each column represents ratio of 37 °C/26 °C across time (1 h, 2 h, 4 h, 8 h). The color scale ranges from *green* (total low relative abundance) to *red* (high relative abundance)

having the same catalytic function as FabI should be located near the fatty acid biosynthetic cluster (YPO1595 to YPO1601), exhibit correlated expression with genes in this cluster, and be annotated as hypothetical. The best match based on these criteria turned out to be the hypothetical gene YPO1594. Other genes that showed correlated expression, but were located farther away from the biosynthetic cluster, were YPO3732 and YPO2055.

5 Host Perspective

During infection, pathogens attempt to hijack resources from the host, while host cells attempt to limit the materials available for pathogen reproduction and virulence. The importance of metabolism in host–pathogen interactions is exemplified by the battle over free iron. While the host attempts to limit the iron available to pathogens, pathogens have evolved high-efficiency chelators to scavenge available iron from the active sites of a variety of metabolic enzymes, such as those used in amino acid biosynthesis. However, the extent to which we can accurately measure the complete molecular makeup of a pathogen during infection is limited. Therefore, a systems-level model of host–pathogen interactions possesses the potential to identify a key subset of molecular features that should be measured to unravel the pathogen's molecular decision-making processes during infection.

To better understand the metabolic features of the host during *Salmonella* infection, we completed a genome-scale metabolic reconstruction for the murine RAW 264.7 macrophage cell line (Bordbar et al. Mol Sys Biol 2012—in press). This reconstruction contains 820 genes, 574 unique metabolites, and 1067 reactions. Physiological metabolic rates of the reconciled metabolic network were evaluated for biomass growth, ATP production, and NO synthesis and compared to experimental values. Overall, our results indicate that the reconciled metabolic network is predictive of physiologically relevant experimental rates when in vitro experimental uptake rates are imposed.

In an initial application, the macrophage metabolic model was used to analyze transcriptomics and proteomics data from the time course responses of RAW 264.7 macrophages to lipopolysaccharide (LPS) stimulation. Host cell response(s) to *Salmonella* infection and to LPS treatment are similar in that they both result in expression of multiple antimicrobial factors. This analysis resulted in the identification of metabolites and enzymes associated with immunomodulation. We have also shown this using inference-based modeling of macrophages, which revealed a common response to multiple immune challenges (McDermott et al. 2011a). To determine if nutrient availability could affect macrophage activation, we performed sensitivity analysis for a set of activation phenotypes as a function of in silico medium composition. Our analysis identified a number of nutrients with the potential to modulate macrophage activation such as glutamine, urea, and threonine. This study demonstrates that the role of metabolic processes in regulating host cell activation may be greater than previously anticipated and elucidates underlying metabolic connections between activation and metabolic effectors.

6 Host–Pathogen Interaction

6.1 Integrated Host–Pathogen Model of Metabolism

Computational genome-scale metabolic models of individual pathogens or their respective hosts are undoubtedly useful for integrating omics and physiologic data for systemic, mechanistic analysis of metabolism. However, the next step toward understanding the interactions between a pathogen and its host requires integrated modeling of both host and pathogen metabolic networks. To this end, we pioneered an approach for integrative analysis of host–pathogen interactions that employs in-silico mass-balanced, genome-scale models and tested it using the closely related *Mycobacterium tuberculosis* (M. tb)-human alveolar macrophage interaction as a model system, as resources related to this system were more mature (Bordbar et al. 2010). Briefly, we constructed a cell-specific alveolar macrophage model iAB-AMØ-1410 from the global human metabolic reconstruction, Recon 1 (Duarte et al. 2007). This model was then integrated with an *M. tuberculosis* H37Rv model, iNJ661, to build an integrated host–pathogen genome-scale reconstruction, iAB-AMØ-1410-Mt-661. Importantly,

this integrated host–pathogen network enables simulation of the metabolic changes during infection.

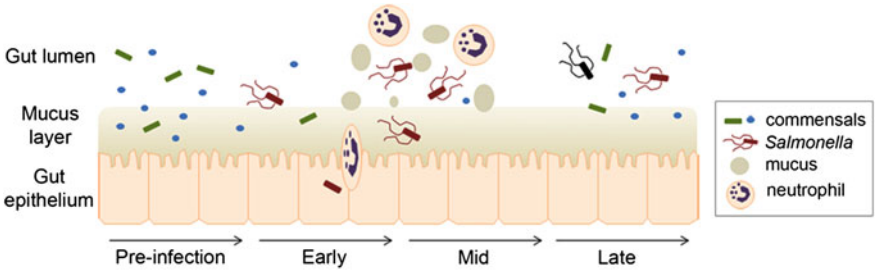
Deployment of the host–pathogen metabolic model to analyze high-throughput data from infected macrophages representing three distinct *M. tuberculosis* infectious states (latent, pulmonary, and meningeal) highlighted differences in metabolism among the three different states (Bordbar et al. 2010). This pioneering effort demonstrates integrated host–pathogen reconstructions can form a foundation upon which understanding the biology and pathophysiology of a variety of infections can be developed. Further, the foundational efforts described above have now been performed to enable this approach with *Salmonella* and *Yersinia* with a mouse macrophage cell line.

6.2 The Host–Pathogen Interface

The interplay between effector proteins secreted by the pathogen and host cells exposed to these effector proteins are relevant to infection in many Enteropathogens and as such can be useful in modeling host–pathogen interactions. An added benefit is that some of these virulence factors may be potential new drug targets.

We applied our systems approach to characterize the *Salmonella* secretome, using omics technologies, inference-based computation and biological experimentation. In this case, we experimentally identified secreted virulence factors by analyzing the extracellular medium from wild type *Salmonella*, a mutant that promotes secretion (ΔSsaL), and a mutant that inhibits secretion (ΔSsaK) (Niemann et al. 2011). Proteomics analysis of the secreted fraction identified the overwhelming majority of known secreted virulence factors and revealed more than 20 new putative secreted virulence factors. In parallel, we utilized SIEVE (SVM-based identification and evaluation of virulence effectors), a machine learning algorithm we developed (Samudrala et al. 2009; McDermott et al. 2011b), to predict novel secreted effectors.

Coupling the SIEVE algorithm with the proteomics data proved to be an efficient way to select novel proteins for characterization. We tested ten proteins based on input from the SIEVE algorithm and proteomics data for secretion into J774 macrophages using CyaA' assays and confirmed that eight of the ten were secreted into the macrophage cytosol. Additional in vivo infection studies demonstrated that deletion mutants of six of the above eight confirmed secreted proteins (Δ*spvD*, Δ*steE*, Δ*gtgE*, Δ*steD*, Δ*sssA* and Δ*ssaB*) were attenuated for virulence. Importantly, these results demonstrate the utility of a systems approach for predicting proteins relevant to understanding host–pathogen interactions.



S. Typhimurium	Absent	Primary proliferation	Secondary proliferation; fucose use	Clearance
Host	Homeostasis	Inflammatory response	Glycan release, inflammation	Clearing pathogen
Commensals	Abundant	Decreasing	Low	Increasing
Metabolites	Homeostasis	Sugar accumulation	Sugar accumulation	Regaining homeostasis

Fig. 4 Model of host–pathogen-commensal interactions during *S. Typhimurium*-induced gastroenteritis. Using a systems biology approach and the available literature, we developed a model of the interplay between the mouse, *S. Typhimurium*, and the commensal population during gastrointestinal infection. Prior to pathogen introduction, the commensal population thrives in the homeostatic gut. Early in infection, *S. Typhimurium* proliferates, stimulates an inflammatory response characterized by neutrophil activation, and disrupts this microbial community. As the commensal population profile changes, so do metabolites in the gut that are normally metabolized by the microbial community such as fucosylated glycans. *S. Typhimurium* senses and responds to fucose availability during gastrointestinal infection, as evidenced by increased expression of fucose utilization proteins. Finally, pathogen clearance from the gut occurs, allowing the gastrointestinal environment to begin to return to pre-infection conditions

6.3 Host–Pathogen Interactions in the Gut Microbiome

The commensal microbiota of the host represents a relatively unexplored contributor to the host–microbe interactions during infection. As a complete understanding of pathogenesis will undoubtedly need to consider the host microbiota, we undertook an exploratory study to investigate the interplay between host, pathogen, and commensal microbes during *S. Typhimurium*-induced gastroenteritis.

For these studies, we chose a mouse model of persistent Salmonellosis, which requires no antibiotic treatment prior to infection and allows *Salmonella* colonization of the gut, allowing us to observe activities of the commensal microbial population. Application of integrated proteomics, metabolomics, metagenomics, and glycomics measurements revealed oral *Salmonella* infection disrupts the commensal population, which allows *S. Typhimurium* to proliferate; concurrently, the host immune system (specifically neutrophil infiltration and release of various inflammatory markers) is activated (Fig. 4). Loss of commensal microbes (likely

due in part to the host inflammatory response) and their associated functions is evident mid-way through infection, when metabolites such as fucose and other sugars normally utilized by commensal bacteria accumulate in the gut. During this time, *Salmonella* thrives, sensing increased host glycan release and utilizing available fucose moieties, among other functions. Resolution of infection by later time points is observed, with a decrease in *S. Typhimurium* abundance, re-establishment of metabolite composition, and outgrowth of indigenous microbiota. Importantly, this model of interactions during *Salmonella*-induced gastroenteritis provides a framework that is both consistent with known factors and provides new insights into infection through integration of omics studies. We anticipate that future endeavors will similarly take advantage of the increased knowledge that can be gained through this systems-level approach.

7 Conclusion and Future Prospects

In this chapter, we have highlighted application of our systems biology approach to investigate interactive host–pathogen mechanisms necessary for two closely related pathogens *Salmonella* and *Yersinia* to cause systemic infection. With the increasing body of knowledge and data arising from high-throughput omics approaches, it is very important that more sophisticated computational approaches be developed to use this information. For example, the integration of inference and knowledge-based modeling approaches discussed above. Comprehensive system models of *Yersinia* and *Salmonella* pathogenesis will have applications for antibiotic development, new strategies for therapeutic treatments, and further understanding of the complex interplay between pathogen and host and the microbiota during infection. In addition to what has been discussed, the reconstruction of other networks including transcriptional regulatory networks and more recently transcription and translation processes (i.e. macromolecular synthesis) are becoming established (Herrgard et al. 2004; Thiele et al. 2009). Methods for their integration with the metabolic models discussed here are in development and should provide a more comprehensive systems-level model enabling systems-level simulations of host–pathogen interactions.

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