The genetic proteome: Using genetics to inform the proteome of mycobacterial pathogens

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Abstract

Mycobacterial pathogens pose a sustained threat to human health. There is a critical need for new diagnostics, therapeutics, and vaccines targeting both tuberculous and nontuberculous mycobacterial species. Understanding the basic mechanisms used by diverse mycobacterial species to cause disease will facilitate efforts to design new approaches toward detection, treatment, and prevention of mycobacterial disease. Molecular, genetic, and biochemical approaches have been widely employed to define fundamental aspects of mycobacterial physiology and virulence. The recent expansion of genetic tools in mycobacteria has further increased the accessibility of forward genetic approaches. Proteomics has also emerged as a powerful approach to further our understanding of diverse mycobacterial species. Detection of large numbers of proteins and their modifications from complex mixtures of mycobacterial proteins is now routine, with efforts of quantification of these datasets becoming more robust. In this review, we discuss the “genetic proteome,” how the power of genetics, molecular biology, and biochemistry informs and amplifies the quality of subsequent analytical approaches and maximizes the potential of hypothesis-driven mycobacterial research. Published proteomics datasets can be used for hypothesis generation and effective post hoc supplementation to experimental data. Overall, we highlight how the integration of proteomics, genetic, molecular, and biochemical approaches can be employed successfully to define fundamental aspects of mycobacterial pathobiology.

Introduction

Mycobacterial species have coevolved with humans over thousands of years [1]. Of the 188 distinct mycobacterial species, many are clinically relevant or emerging pathogens [2]. *Mycobacterium tuberculosis* is an obligate human pathogen that causes tuberculosis [3]. Several environmental mycobacterial species cause disease in humans and animals. While *M. tuberculosis* alone caused 1.5 million deaths in 2018 [4], infections involving other nontuberculous environmental species of pathogenic mycobacteria (nontuberculous mycobacteria (NTM)) are
increasing, and in some countries including the United States, NTM infections outnumber those by *M. tuberculosis* [5,6]. These largely opportunistic pathogens, including *M. abscessus*, *M. kansasii*, and the *M. avium* complex (MAC), cause lung disease and other systemic infections sometimes associated with medical implants [6,7].

The bacillus Calmette–Guérin (BCG) vaccine is the only vaccine against *M. tuberculosis* infection, and its effectiveness in adults is poor [8]. There are no vaccines against NTMs, and diagnostic and treatment options are limited. *M. tuberculosis* has become increasingly resistant to the antibiotic cocktails used to treat tuberculosis [4]. NTM pathogens harbor natural antibiotic resistance to many of the drugs used to treat tuberculosis [9,10]. *M. abscessus* isolates, for example, are intrinsically resistant to tetracycline at concentrations 500-fold higher than observed for *M. tuberculosis* [11]. There remains a critical need for new diagnostic, therapeutic, and vaccine targets, which benefits from understanding the basic physiology and virulence mechanisms in mycobacteria.

The proteome is the collection of proteins associated with a cell, tissue, or organism under a set of conditions. Recent advances in mass spectrometry (MS) and data-processing have enabled an expansion from static snapshots of the proteome to one that is more temporal, quantitative, spatial, and targeted. Technical and methodological advancements in mass spectrometers, protein fractionation/separations, preparation/enrichment and informatics have made proteomics a fast and reliable discipline for whole proteome studies. The strength of these approaches lies in the ability to generate large datasets biased predominately by protein abundance and targeted enrichment. Hypothesis-driven biological research provides the context to these deep and global approaches.

Proteomic datasets can be powerful tools for generating new hypotheses or testing existing ones. Quantitative proteomics is an encompassing term for several isotopic and non-isotopolog–based strategies to quantify liquid chromatography–mass spectrometry (LC-MS) data [12–15], which are routinely used to address changes in protein levels in pathogenic mycobacterial studies. Quantifying LC-MS data is typically performed by comparing the intensity or peak area for proteotypic peptides across conditions and replicates. These approaches have been widely applied to microbial studies and mycobacteria [16–18]. The proteomic analysis of hypo- and hypervirulent strains of *M. tuberculosis* identified differential protein expression after mouse infection providing new insight into mycobacterial virulence factors [19]. Numerous other analyses of the secreted proteins of *M. tuberculosis* identified protein virulence factors present at higher levels in hypervirulent strains compared with *M. tuberculosis* laboratory strains [20–22]. These “census-style” experiments report the presence and/or abundance of proteins for comparative and declarative values. Although there are several ways to develop context for proteomic experiments, we think using comparative proteomics in infection models and combining the strength of genetics and genetic content to augment the proteome are the most productive approaches.

Physiological context can be obtained through the use of classical bacteriological approaches, including molecular genetics and biochemistry, and more recently “-omics” approaches. Our concept of the “genetic proteome” is underscored by the deliberate use of genetics, or other classical approaches, to alter the measurable proteome (Fig 1). The genetic proteome differs from multi-omics and proteo-genetics approaches. Multi-omics combines multiple “omes” to provide depth, while proteo-genetics typically relies on existing natural genetic diversity and brute-force computation to define the search space for MS-based proteomics [23–25]. We are suggesting that incorporating classical and proteomic approaches improves our understanding of organismal physiology, more so than either approach independently [26]. By leveraging the awesome power of genetics, mycobacterial researchers using proteomics, as other microbial proteomics fields, enjoy near-perfect controls for their analyses.
Exploiting both mycobacterial genetics and proteomics has allowed researchers to link changes in single or global protein levels or modifications to specific mycobacterial genes and pathways. In this review, we consider several types of proteomic analyses in mycobacteria, as well as the contributions these approaches have made.

**Exploiting the awesome power of mycobacterial genetics**

Researchers using *M. tuberculosis* and some NTMs benefit from robust genetic approaches for dissecting the role of specific genes and pathways in disease. Despite differences in environmental niche, host range, and disease etiology, fundamental mechanisms of virulence are well conserved between *M. tuberculosis* and NTMs [27–30]. Both *M. tuberculosis* and NTMs also share physiological pathways with the nonpathogenic mycobacterial species *M. smegmatis* which is similarly genetically tractable, and also exhibit conjugation [31,32]. Therefore, studies in nonpathogenic and nontubercular pathogenic mycobacterial species as well as those in *M. tuberculosis* contribute to defining fundamental aspects of mycobacterial biology [9]. Many mycobacterial species are robustly and efficiently transformed with DNA, with several available markers for antibiotic resistance. Genetic knockouts and knockdowns, as well as gene overexpression, are now routine manipulations in several (but not all) mycobacterial species. Numerous knockout approaches include suicide plasmid [33–37] and phage-mediated allelic exchange [38], CRISPR interference (CRISPRi) [39–41] oligonucleotide-mediated recombineering followed by Bxb1 integrase targeting (ORBIT) [42], and recombineering approaches [43]. There are also several robust transposon platforms available that have been widely exploited to understand mycobacteriology both in vitro and in host models for infection [44–46]. Foundational examples of using this approach include the transposon site hybridization
(TraSH) and signature tagged mutagenesis (STM) transposon platforms. An example of TraSH in *M. tuberculosis* identified essential and conditionally essential genes, and those required during host infection [44,47,48]. An example of STM in *M. tuberculosis* revealed roles for phthiocerol dimycocerosate (PDIM), a cell wall–associated lipid [46], and the ESAT-6 system -1 (ESX-1) secretion system in mycobacterial virulence [49]. The suicide plasmid or phage-based allelic exchange approaches and ORBIT ideally result in unmarked deletions that impact the expression of a single mycobacterial gene [42]. Numerous studies reporting targeted unmarked deletions of genes in pathogenic and nonpathogenic mycobacterial species have identified roles of individual genes in various physiological and virulence processes (some exemplar publications include [50–55]). CRISPRi approaches rely on targeting the dead Cas protein (dCas) complex to prevent or reduce gene expression and can be polar on downstream genes [39]. CRISPRi silencing can be applied to conditionally silence individual or multiple genes or be used for generating high-density silenced libraries [39,56]. Importantly, the dCas system is inducible and can be used to study essential mycobacterial genes, for example, to define their role in physiology or as potential therapeutic targets [57–59].

To demonstrate that the genetic knockout caused the observed phenotypes, there are several options for genetic complementation by restoration of gene expression, including plasmids that integrate at 2 different phage attachment sites and episomal plasmids with both low-level and high-level constitutive promoters [60–62]. There are also several inducible promoter systems and riboswitch platforms that can be used for genetic complementation or to perform depletion studies to study essential mycobacterial genes [63–71]. Essential mycobacterial genes are often studied as potential drug targets. For example, the genetic depletion of the *M. tuberculosis* Rho transcription termination factor using anhydrotetracycline inducible repression led to widespread transcriptional dysregulation and bacterial growth defects, demonstrating that Rho is essential and a potential target of novel antitubercular drugs [72]. Therefore, there are several forward genetic approaches that are accessible to the mycobacterial community and facilitate understanding how specific genes contribute to basic mycobacterial physiology and virulence. These genetic approaches have been applied to alter the measured proteome (Fig 1).

**Targeted and comprehensive proteomic approaches define the mycobacterial proteome and its modifications**

MS-based proteomics is a diverse set of approaches applied to study individual proteins, groups of proteins, or the entire proteome of the bacterial cell (Fig 2). So-called “bottom-up” proteomics, the most applied method for proteomics studies, creates peptides from proteins typically using a protease like trypsin [73]. The mass-to-charge ratio of the peptides are measured (MS1) before further fragmentation in a tandem manner to generate MS-MS daughter ions. Peptides fragment by predictable mechanisms, and the masses of the fragment ions are used to infer the protein from which it derived. This process is performed most commonly by spectral mass-matching, and occasionally by de novo sequencing [73]. The bottom-up approach has been used as a building block for both discovery and targeted proteomics and widely applied to basic mycobacterial research [52,74,75]. These discovery-based methods have aided in profiling clinical isolates of *M. tuberculosis* and distinguishing the relationship between protein synthesis and active disease [19,76–78].

Targeted proteomics is an approach where identification/detection of specific proteins/peptides is known prior to LC-MS/MS acquisition. It provides substantial gains in sensitivity and specificity (signal-to-noise ratio) at the expense of target density [79–82]. Because quantitative data obtained from targeted proteomics is more accurate and precise, it is the preferred
### Approach

| Purpose                                                                                                                                                                                                 | Examples                                                                                                                                 |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|
| **Riboseq**                                                                                                                                                                                             | • Generate ‘multi-omics maps’ from mycobacteria to reveal novel open reading frames (ORFs), internal transcription start sites, and noncoding RNA  
  • ORFs used to reannotate genomes for protein reading frames                                                                                                                                 | (77), (143), (144), (145), (146), (147), (148)                                                                                               |
| **MALDI-MS**                                                                                                                                                                                            | • Surface and secreted proteomics  
  • Imaging MS  
  • Phenotypic identification of mycobacteria  
  • Rapid assessment of novel therapeutics  
  • Peptide mass-fingerprinting (PMF)                                                                                                                                                        | (17), (127)                                                                                                                                 |
| **Genetic Proteomics**                                                                                                                                                                                  | • Define secreted and cell-wall associated virulence factors  
  • Enrich proteins to detect post-translational modifications (PTMs)  
  • Identify mycobacterial envelope proteins and their molecular interactions  
  • Investigate gene contributions to mycobacterial physiology and virulence                                                                                                                                 | (33), (34), (35), (36), (37), (38), (39), (40), (41), (42), (43), (44), (45), (63), (64)                                                 |
| **Discovery-based Proteomics**                                                                                                                                                                          | • Compare protein levels across mycobacterial strains  
  • Define relationships between protein synthesis and disease  
  • Identify and study the relationship between PTMs and virulence  
  • Understand mechanisms of resistance and identify potential therapeutic targets                                                                                                                                 | (19), (20), (21), (22), (76), (77), (78), (89), (90), (91)                                                                                       |
| **Targeted Proteomics (SRM/PRM/MRM)**                                                                                                                                                                  | • Library generation for selective reaction monitoring assays that span the majority of the proteome  
  • Use libraries to track and quantify PTMs of virulence factors  
  • Identify PTMs responsible for protein turnover in virulent mycobacteria  
  • Characterise the mechanism of virulence associated proteasomal degradation  
  • Targeted quantitation of mycobacterial proteotypic peptides                                                                                                                                 | (79), (80), (82), (95), (98), (99), (100), (101), (102), (103), (104), (105), (106), (107)                                                  |

**Fig 2. Proteome-centric approaches used in the study of mycobacteria described in this review.** Listed are 5 major approaches, their associated measurements, and representative references. Riboseq methods measure the “translatome” and have been applied to annotate and define the mycobacterial genome landscape. MALDI-MS–based approaches are more rapid and are used in screening approaches. Genetic proteomics uses alterations in the genome to manipulate the measured proteome. Discovery and targeted proteomics approaches measure and quantify proteins and their modification from complex and enriched cellular material. Examples list numbers of relevant references. MALDI-MS, matrix-assisted laser desorption/ionization mass spectrometry; MRM, multiple reaction monitoring; ORFs, open reading frames; PMF, peptide mass-fingerprinting; PRM, parallel reaction monitoring; PTMs, posttranslational modifications; Riboseq, ribosome sequencing; SRM, selected reaction monitoring.

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approach when determining absolute quantitative abundance using stable heavy-isotope dilution Absolute Quantification (AQUA) style approaches [83,84]. Stable heavy isotope dilution relies on MS acquisition referred to as selected/multiple reaction monitoring (SRM/MRM), or parallel reaction monitoring (PRM). Although numerous MS instruments are capable of
performing targeted proteomics, triple quadrupoles are the most common, sensitive, and precise platform for performing these experiments, and they are extensively applied to study mycobacterial pathogenesis [18,79,80,82]. Targeted proteomics is the dominant form of LC-MS/MS in clinical proteomics used for diagnostic, drug, and detection [85–87].

Both targeted and non-targeted proteomics methods have identified protein posttranslational modifications (PTM) in mycobacteria. Mycobacteria use PTMs to regulate protein function and activity. Glycosylation, phosphorylation, lipidation, formylation, pupylation, acetylation, and methylation have been identified in *M. tuberculosis* [88,89]. Several studies have elucidated extensive networks of reversible (S/T/Y) protein phosphorylation in mycobacteria [90–93], during mycobacterial infection [91,94], and the identification of irreversible protein N-terminal acetylation as an abundant PTM in mycobacteria associated with virulence and survival [17,95].

Targeted approaches have made it possible to create a global *M. tuberculosis* library which contains SRM assays covering 97% of all annotated proteins for *M. tuberculosis* and 72% of the entire proteome [82,96]. Libraries such as these allow other researchers access to deeper mechanistic insight into *M. tuberculosis* treatments that directly benefit tuberculosis patients. In previous work, libraries of MRM/SRM transitions to quantify mycobacterial protein levels have allowed targeted approaches to identify and measure posttranslational modifications of virulence factors that could be clinically relevant signatures of infection [79,80].

In addition to modification, protein turnover by proteasomal degradation is essential for *M. tuberculosis* virulence in mice [97] and during stress under nitrogen starvation and DNA damage in *M. smegmatis* [98–100]. Protein turnover is regulated by both protein structure and protein modifications [101–103]. Affinity purification, 2D gel electrophoresis (2DGE), and targeted proteomics in *M. tuberculosis* led to the identification of pupylation, which is the addition of the prokaryotic ubiquitin-like protein (Pup) to protein lysine residues which targets proteins for proteasomal degradation [104–107]. Emerging proteomics techniques continue to increase throughput in the detection of a variety of PTM events.

The genetic proteome defines secreted and cell wall–associated virulence factors

Proteomes can be fractionated to study proteins localized to specific compartments within the cell or those secreted by the bacteria to the cell surface or into the extracellular environment. Fundamentally, secreted proteomes or "secretomes" are a "natural fraction" of the proteome (Fig 2). Secretomes are inherently less complex than the whole cell proteome and often enriched for proteins important for physiology and virulence. As such, secretomes have been widely exploited to generate dense biological context in particular for mycobacteria. One of the largest challenges in the area of secreted proteomics in mycobacteria is the tendency for a subset of the bacteria to lyse during routine culturing. Lysis contaminates the secreted proteome with cytosolic contents. This has been addressed by the careful control of growth when proteomics is intended and subtractive controls for cytosolic contaminants [16,108–110].

Proteomic studies on culture filtrates (proteins secreted into the culture media during mycobacterial growth in vitro), when combined with powerful and precise genetic approaches, were primarily responsible for the elucidation of the several of known ESX (type VII)-secreted proteins, which are essential for mycobacterial pathogenesis [79,80,111–114]. A powerful example of the applied genetic–proteome approach has been altering the secreted proteome with genetics to identify the secreted substrates associated with specific secretory systems. For example, defining the secretome in the presence and absence of the *esx-1* genes encoding the ESX-1 secretory system, numerous conserved ESX-1-dependent substrates were identified
including EspA, EspF, EspJ, EspK, and PPE68 [79,80,111], as well as other substrates unique to the nontubercular species [112,114,115]. Similar strategies have been widely applied to identify and study the substrates of other cell-associated and alternative secretion systems in mycobacteria, including but not limited to the SecA2 system [116–118]. It is important to note that many bacterial proteins are not amenable to detection using antibodies. Moreover, epitope tagging and fusion to ESX-1 substrates prevents secretion through the ESX-1 system or diminishes function to an unacceptable degree. Therefore, proteomics approaches are in some cases the only way to identify these important virulence factors.

Another key finding regarding mycobacterial protein secretion was that protein secretion is not “on or off.” The advent of label-free quantitative (LFQ) proteomics approaches allowed for the increased detection of low abundance peptides in secreted fractions, revealing the potential for intermediate secretion phenotypes in transposon mutant strains [80,111].

One limitation to using proteomics approaches when studying the secreted proteins of mycobacterial pathogens is the difficulty in detecting mycobacterial proteins in the host. Instead, there have been several clever approaches to contextualize existing proteomics data and verify that proteins secreted by mycobacteria in vitro are also secreted in the host [119–121]. These approaches could also reveal mycobacterial proteins only secreted within the host and not when mycobacteria are grown in vitro.

In addition to the secretome, proteins associated with the cell envelope and the cell surface, as well as those in secreted microvesicles, are essential for mycobacterial physiology and pathogenesis [122]. Genetic–proteome approaches have yielded important information into the protein content of mycobacterial membranes and how these proteins interact with each other [123–126]. Whole colony proteomics approaches contributed to measuring virulence factors associated with the mycobacterial cell surface, in the presence and absence of mutations or deletions in genes encoding secretory systems [127]. Defining the cell wall proteome has involved biochemical approaches including cell surface enzymatic [117,128,129] or hydrolysis shaving [130] followed by MS to identify surface-exposed mycobacterial proteins. However, cell surface techniques can be ineffective. Biochemical cell wall fractionation is not only often more robust, but can also be more challenging than modifying the cell surface. Coupling cellular fractionation of both wild-type and SecA2-deficient M. tuberculosis strains with LFQ MS revealed that the mycobacterial cell wall proteins and solute transporters require the SecA2 system for localization [118]. One promising emerging approach is ascorbate peroxidase (APEX) proximity-based biotinylation to selectively label and enrich proteins within the cell wall. This approach has been coupled with proteomics approaches to identify protein–protein interactions and inform molecular pathways to promote cell wall biogenesis [131–133]. Likewise, chemical probes for detecting direct interactions between proteins in the mycobacterial cell envelope have been coupled to quantitative proteomics. These approaches have revealed more than 100 envelope proteins and their binding partners in M. smegmatis [134]. Another interesting example of the genetic proteome is a recent study in which genetics were used to alter the proteome of microvesicles produced by M. tuberculosis. By using genetic deletions that alter M. tuberculosis signal transduction followed by microvesicle isolation and proteomics, the authors defined specific proteins regulated by the Pst1/SenX/RegX signal transduction system that are targeted to microvesicles [135].

### The genetic proteome and antibiotic susceptibility and resistance

In addition to defining virulence mechanisms, using genetics to alter the proteome can also provide insight into antibiotic sensitivity and resistance. For example, protein overexpression can alter the proteome and impact mycobacterial drug sensitivity and resistance. A recent...
example of this type of study was performed in *M. bovis*. A latency-related universal stress protein (USP, BCG_2013) was overexpressed in *M. bovis*, which increased the efficacy of isoniazid (INH) [136]. Subsequent quantitative proteomics analysis revealed that USP overexpression resulted in 50 up-regulated proteins, including catalase peroxidase KatG, which is required for INH activation in the mycobacterial cell [136]. Similar overexpression coupled with proteomics approaches have been used to study lipid metabolism and vancomycin resistance in *M. smegmatis* [137].

Interestingly, clinical mycobacterial strains carry frameshift and nonsense mutations in the essential *rpoB* gene (for example, [137–139]). Strains with mutations in essential genes should be nonviable. However, frameshift and nonsense mutations in the *rpoB* gene, which encodes the β-subunit of RNA polymerase, cause resistance to rifampicin, because rifampicin kills bacteria by interacting with RpoB and blocking transcription [140,141].

By coupling genetic and proteomic approaches, it was recently shown that frameshifting of the *rpoB* gene is a mechanism used by pathogenic mycobacteria to generate resistance to antibiotics [142]. Importantly, LC/MS/MS was applied to demonstrate low levels of frameshift suppression of *rpoB* mutations, indicating that sometimes, what is encoded in the genome does not necessarily predict the resulting proteome [142]. The decoupling of the proteome from the genome underscores the necessity of combining approaches that alter the genome and measure the proteome.

**The genetic proteome and multi-omics reveal new insight into transcription and translation in mycobacteria**

Combining classical and -omics approaches have shed new light on the genetic structure and the fundamental mechanisms of transcription and translation in mycobacteria. There are also several recent examples of integrative multi-omics methods combining transcriptomic, proteomic, and metabolomic approaches with genetics to interrogate mycobacterial physiology.

A recent study using differential RNA-seq, ribosome profiling, and proteomics of *M. abscessus* under different clinically relevant conditions generated “multi-omics maps,” revealing regulation by novel short open reading frames (ORFs), internal transcription start sites, and noncoding RNAs [143]. These short ORFs have been identified in *M. tuberculosis* and NTMs for individual genes and used to reannotate existing genomes for protein reading frames [77,144–148]. The majority of translation events occur on mRNA transcripts with a 5′ untranslated region (UTR) which includes a Shine–Delgarno ribosome binding site. mRNA transcripts lacking a 5′UTR and a ribosome binding site are referred to as “leaderless.” However, recent studies in *M. smegmatis* have investigated protein translation initiation through transcription start site mapping and selective detection of protein N-termini by MS, ultimately illustrating widespread leaderless translation in mycobacteria [144,145,147].

Transcription factors and mechanisms of RNA degradation also remain topics of intense investigation by mycobacterial researchers. Commonly used approaches include the genetic perturbation of a specific transcription factor gene followed by studying the resulting changes in gene expression (exemplar publications include [52,149,150]). Alternatively, transcriptional regulons have been defined using whole genome approaches including transcription factor overexpression followed by chromatin immunoprecipitation (ChIP) (exemplar publications include [151,152]). However, advances in biochemical, molecular, and proteomic approaches has improved the inverse of this process—the selective identification of proteins responsible for modulating transcriptional regulation through the direct binding to DNA or RNA. In this approach, rather than genetics, biochemistry is coupled to proteomics. Recent work in *M. smegmatis* used an affinity purification–mass spectrometry (AP-MS) approach to globally...
identify transcription-associated proteins. Here, chemical crosslinking and affinity purification of the RNA polymerase β-subunit led to the global identification of 275 transcription-associated proteins [153]. Of these proteins, 20 were not previously associated with nucleic acids [153]. The function of these transcription associated proteins could then be confirmed by coupling genetics and transcriptomics approaches as indicated above. This work exemplifies the utility of proteomics and molecular approaches in providing foundational regulatory classifications for mycobacterial proteins globally. In a similar approach, DNA affinity chromatography and proteomics approaches were used to enrich and identify proteins specifically bound to a promoter, resulting in the identification of a novel transcription factor required for the regulation of genes by the ESX-1 secretion system [150]. In this study, multi-omics techniques with traditional reverse genetics methodologies uncovered multitiered regulatory phenomena controlling a key protein transport system in mycobacteria.

Protein–protein interactions are commonly critical for the regulation of physiological processes and pathogenesis within the host. Targeted immunoprecipitation of single proteins has been used to assemble regulatory pathways across mycobacterial physiology and aid in protein characterization; these studies span many aspects of mycobacterial physiology, including virulence, metabolism, and persistence [154,155]. Immunoprecipitation and MS have long been used to identify novel binding partners of essential ATPases, illustrating the utility of affinity enrichment to discover protein–protein interactions necessary for cell survival and pathogenesis [79,156].

Finally, as part of a multi-omics approach, proteomics has been used to complement transcriptomics to define the molecular composition of the machinery that degrades RNA in *M. tuberculosis*, known as the “degradosome.” In this study, RNA–protein complexes were enriched, and components of the degradosome were identified by proteomics. Targeted genetic approaches were applied together with transcriptomics to confirm the role of putative degradosome complex components in global RNA turnover [157].

**What’s next in applying the genetic proteome to mycobacterial research?**

As discussed above, harnessing the power of proteomics to its full extent requires effective integration with other approaches, database curation, and network correlation. Proteomic datasets generated from published studies are useful data mining resources that can be directly leveraged to inform future research. These datasets provide a wealth of information that can be used to generate new hypotheses that can be tested using classical approaches or supplement existing experimental data. Examples in recent literature illustrated the utility of tandem MS dataset mining in validating genomic mutations leading to chimeric protein production in *M. tuberculosis* clinical isolates [56,158]. However, variation in proteomics methodology, sample preparation, and physiological context can lead to ineffective data utilization. Database cura
tion is 1 way to circumvent these issues. Data repositories and libraries, like the MRMaid database and SRMAtlas, have been constructed to store experimental data from SRM and MRM assays [159,160]. An *M. tuberculosis* proteome library has been stored on SRMAtlas encompassing SRM assays for 97% of all annotated *M. tuberculosis* proteins [82]. Table 1 describes representative datasets and repositories of SRM/MRM (targeted), bottom-up (non-targeted), and mRNA/ribosome sequencing (Ribo-seq (profiling))–based approaches from pathogenic mycobacteria utilizing proteo-genetic methodologies.

**Conclusions**

Proteomics has considerably advanced the field of mycobacterial research in recent years. Concomitant advancements in genetics approaches for generating mutant strains and
biochemical approaches in targeted enrichment have broadened proteomics applications. This suite of tools currently at our disposal has the capacity to answer questions at every step of information transfer in mycobacteria from genes to protein localization and behavior. Leveraged properly, classical approaches with proteomics gain critical insight into mycobacterial physiology and pathogenesis.

References

1. Chisholm RH, Trauer JM, Curnoe D, Tanaka MM. Controlled fire use in early humans might have triggered the evolutionary emergence of tuberculosis. Proc Natl Acad Sci U S A. 2016; 113(32):9051–6. Epub 2016/07/28. https://doi.org/10.1073/pnas.1603224113 PMID: 27457933; PubMed Central PMCID: PMC4987778.

2. Gupta RS, Lo B, Son J. Phylogenomics and Comparative Genomic Studies Robustly Support Division of the Genus Mycobacterium into an Emended Genus Mycobacterium and Four Novel Genera. Front Microbiol. 2018; 9:67. Epub 2018/03/03. https://doi.org/10.3389/fmicb.2018.00067 PMID: 29497402; PubMed Central PMCID: PMC5819568.

3. Koch R. [Tuberculosis etiology]. Dtsch Gesundheitsw. 1884; 7(15):457–65. PMID: 12988652.

4. World Health Organization. Global tuberculosis report 2019. 2019.

5. Donohue MJ. Increasing nontuberculous mycobacteria reporting rates and species diversity identified in clinical laboratory reports. BMC Infect Dis. 2018; 18(1):163. Epub 2018/04/11. https://doi.org/10.1186/s12879-018-3043-7 PMID: 29631541; PubMed Central PMCID: PMC5891905.

6. Johansen MD, Herrmann JL, Kremer L. Non-tuberculous mycobacteria and the rise of Mycobacterium abscessus. Nat Rev Microbiol. 2020; 18(7):392–407. Epub 2020/02/23. https://doi.org/10.1038/s41579-020-0331-1 PMID: 32086501.

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**Table 1. Mycobacterial genetic proteome databases and datasets.**

| Name/Type | Location | Reference |
|-----------|----------|-----------|
| **Targeted Databases** | | |
| Peptide Atlas (M.tb) | SRM Atlas http://www.srmatlas.org/ | Schubert et al., 2013 (82) |
| SRM Atlas (M. tb) | Peptide Atlas http://www.peptideatlas.org/ | Schubert et al., 2015 (18) |
| MRM/SRM profiling of ESX-1 mutants in culture filtrate and cytosol | https://pubs.acs.org/doi/10.1021/pr500484w | Champion et al., 2014 (80) |
| **Non-targeted Datasets** | | |
| High-resolution proteogenomic analysis of M. tb H37Rv | Peptide Atlas PAe001767 | Kelkar et al., 2011 (23) |
| Lineage-specific proteomes: virulence etc. large comprehensive MS/MS dataset | Proteome Xchange PXD020383 | Yimer et al., 2020 (56) |
| **mRNA / Ribosomal Profiling** | | |
| Ribo-seq of M. smeg and M. Tb | EMBL-EBI E-MTAB-2929 | Shell et al., 2015 (144) |
| RNA-seq, RNA-seq of 5’PPP, proteomics | EMBL-EBI E-MTAB-1616 | Cortes et al., 2013 (147) |
| | Proteome Xchange PXD000483 | |

*These are comprehensive MRM/SWATH datasets for targeted detection of M. tb in cells, host and filtrates*

*These are representative datasets from large-scale MS/MS bottom-up profiling*

*These studies identified the extensive leaderless and small ORF’s population within Mycobacteria*

Representative datasets and databases from *M. tuberculosis* (*M. tb*) studies, organized by proteome approach. A brief description, the type, reference, and current raw file location is included.

MRM, multiple reaction monitoring; MS, mass spectrometry; ORFs, open reading frames; Ribo-seq, ribosome sequencing; RNA-seq, RNA sequencing; SRM, selected reaction monitoring.

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biochemical approaches in targeted enrichment have broadened proteomics applications. This suite of tools currently at our disposal has the capacity to answer questions at every step of information transfer in mycobacteria from genes to protein localization and behavior. Leveraged properly, classical approaches with proteomics gain critical insight into mycobacterial physiology and pathogenesis.
22. Cornejo-Granados F, Zatarain-Barron ZL, Cantu-Robles VA, Mendoza-Vargas A, Molina-Romero C, Sanchez F, et al. Secretome Prediction of Two M. tuberculosis Clinical Isolates Reveals Their High Antigenic Density and Potential Drug Targets. Front Microbiol. 2017; 8:128. Epub 2017/02/23. https://doi.org/10.3389/fmicb.2017.00128 PMID: 28223967; PubMed Central PMCID: PMC5293778.

23. Kelkar DS, Kumar D, Kumar P, Balakrishnan L, Muthusamy B, Yadav AK, et al. Proteogenomic analysis of Mycobacterium tuberculosis by high resolution mass spectrometry. Mol Cell Proteomics. 2011;
26. Zhang B, Kuster B. Proteomics Is Not an Island: Multi-omics Integration Is the Key to Understanding

27. Ansong C, Purvine SO, Adkins JN, Lipton MS, Smith RD. Proteogenomics: needs and roles to be filled by proteomics in genome annotation. Brief Funct Genomic Proteomic. 2008; 7(1):50–62. Epub 2008/03/13. https://doi.org/10.1093/bfgp/ein010 PMID: 18334489.

28. Cook GM, Berney M, Gebhard S, Heinemann M, Cox RA, Danilchanka O, et al. Physiology of mycobacteria and Mycoplasma. Mol Cell Proteomics. 2019; 18(8 suppl 1):S1–S4. Epub 2019/08/11. https://doi.org/10.1074/mcp.E119.001693 PMID: 31399542; PubMed Central PMCID: PMC6692779.

29. Melly G, Purdy GE. MmpL Proteins in Physiology and Pathogenesis of Mycobacterium tuberculosis. Microorganisms. 2019; 7(3). Epub 2019/03/08. https://doi.org/10.3390/microorganisms7030070 PMID: 30841535; PubMed Central PMCID: PMC6463170.

30. Parsons LM, Jankowski CS, Derbyshire KM. Conjugal transfer of chromosomal DNA in Mycobacterium smegmatis. Mol Microbiol. 1995; 16(4):755–60. Epub 1995/05/01. https://doi.org/10.1111/j.1365-2958.1995.tb02345.x PMID: 7476169.

31. Aldovini A, Husson RN, Young RA. The uraA locus and homologous recombination in Mycobacterium bovis BCG. Mol Microbiol. 1995; 16(4):755–60. Epub 1995/05/01. https://doi.org/10.1111/j.1365-2958.1995.tb02345.x PMID: 7476169.

32. Pelvicic V, Reyrat JM, Gicquel B. Comparative pathogenesis of Mycobacterium marinum and Mycobacterium tuberculosis. Cell Microbiol. 2000; 10(5):1027–39. https://doi.org/10.1111/j.1462-5822.2000.01133.x PMID: 12896367.

33. Parson T, Stoker NG. Use of a flexible cassette method to generate a double unmarked targeted gene disruption in Mycobacterium tuberculosis. J Bacteriol. 1993; 175(22):7282–9. Epub 1993/11/01. https://doi.org/10.1128/jb.175.22.7282-7289.1993 PMID: 8226675. PubMed Central PMCID: PMC206871.

34. Pelvicic V, Reyram JM, Gicquel B. Generation of unmarked directed mutations in mycobacteria, using sucrose counter-selectable suicide vectors. Mol Microbiol. 1996; 20(5):919–25. Epub 1996/06/01. https://doi.org/10.1111/j.1365-2958.1996.tb02533.x PMID: 8809745.

35. Pelvicic V, Reyram JM, Gicquel B. Positive selection of allelic exchange mutants in Mycobacterium bovis BCG. FEMS Microbiol Lett. 1996; 144(2–3):161–6. Epub 1996/11/01. https://doi.org/10.1111/j.1574-6968.1996.tb08524.x PMID: 8900059.

36. Bardarov S, Bardarov S, Pavelka MS, Sambandamurthy V, Larsen M, Tufariello J, et al. Specialized transduction: an efficient method for generating marked and unmarked targeted gene disruptions in Mycobacterium tuberculosis, M. bovis BCG and M. smegmatis. Microbiology. 2002; 148(Pt 10):3007–17. Epub 2002/10/09. https://doi.org/10.1099/00221287-148-10-3007 PMID: 12368434.

37. Rock JM, Hopkins FF, Chavez A, Diallo M, Chase MR, Gerrick ER, et al. Programmable transcriptional repression in mycobacteria using an orthogonal CRISPR interference platform. Nat Microbiol. 2017; 2:16274. https://doi.org/10.1038/nmicrobiol.2016.274 PMID: 28165460; PubMed Central PMCID: PMC5302332.

38. Sun B, Yang J, Yang S, Ye RD, Chen D, Jiang Y. A CRISPR-Cpf1-Assisted Non-Homologous End Joining Genome Editing System of Mycobacterium smegmatis. Biotechnol J. 2018; 13(9):e1700588. Epub 2018/07/25. https://doi.org/10.1002/biot.201700588 PMID: 30039229.

39. Yan MY, Yan H, Ren G, Zhao JP, Guo XP, Sun YC. CRISPR-Cas12a-Assisted Recombining in Bacteria. Appl Environ Microbiol. 2017; 83(17). Epub 2017/06/25. https://doi.org/10.1128/AEM.00947-17 PMID: 28646112; PubMed Central PMCID: PMC5561284.
42. Murphy KC, Nelson SJ, Nambi S, Papavinassundaram K, Baer CE, Sassetti CM. ORBIT: a New Paradigm for Genetic Engineering of Mycobacterial Chromosomes. MBio. 2018; 9(6). Epub 2018/12/13. https://doi.org/10.1128/mBio.01467-18 PMID: 30538179; PubMed Central PMCID: PMC6299477.

43. van Kessel JC, Haftull GF. Recombining in Mycobacterium tuberculosis. Nat Methods. 2007; 4 (2):147–52. Epub 2006/12/21. https://doi.org/10.1038/nmeth996 PMID: 17179933.

44. Sassetti CM, Boyd DH, Rubin EJ. Comprehensive identification of conditionally essential genes in mycobacteria. Proc Natl Acad Sci U S A. 2001; 98(22):12712–7. https://doi.org/10.1073/pnas.231275498 PMID: 11606763.

45. Gao LY, Groger R, Cox JS, Beverley SM, Lawson EH, Brown EJ. Transposon mutagenesis of Mycobacterium marinum identifies a locus linking pigmentogenesis and intracellular survival. Infect Immun. 2003; 71(2):922–9. https://doi.org/10.1128/aii.71.2.922-929.2003 PMID: 12540574.

46. Cox JS, Chen B, McNeil M, Jacobs WR Jr.. Complex lipid determines tissue-specific replication of Mycobacterium tuberculosis in mice. Nature. 1999; 402(6757):79–83. https://doi.org/10.1038/47042 PMID: 10573420.

47. Sassetti CM, Rubin EJ. Genetic requirements for mycobacterial survival during infection. Proc Natl Acad Sci U S A. 2003; 100(22):12989–94. https://doi.org/10.1073/pnas.2134250100 PMID: 14569030.

48. Sassetti CM, Boyd DH, Rubin EJ. Genes required for mycobacterial growth defined by high density mutagenesis. Mol Microbiol. 2003; 48(1):77–84. https://doi.org/10.1046/j.1365-2958.2003.03425.x PMID: 12657046.

49. Stanley SA, Raghavan S, Hwang WW, Cox JS. Acute infection and macrophage subversion by Mycobacterium tuberculosis require a specialized secretion system. Proc Natl Acad Sci U S A. 2003; 100 (22):13001–6. https://doi.org/10.1073/pnas.2235593100 PMID: 14557536.

50. Bottai D, Majlessi L, Simeone R, Frigui W, Laurent C, Lenormand P, et al. ESAT-6 secretion-independent impact of ESX-1 genes espF and espG1 on virulence of Mycobacterium tuberculosis. J Infect Dis. 2011; 203(8):1155–64. https://doi.org/10.1093/infdis/ijq889 PMID: 21196469.

51. Lewis KN, Liao R, Guinn KM, Hickey MJ, Smith S, Behr MA, et al. Deletion of RD1 from Mycobacterium tuberculosis mimics bacille Calmette-Guerin attenuation. J Infect Dis. 2003; 187(1):117–23. https://doi.org/10.1086/345862 PMID: 12508154.

52. Bosserman RE, Nguyen TT, Sanchez KG, Chirakos AE, Ferrell MJ, Thompson CR, et al. WhiB6 regulation of ESX-1 gene expression is controlled by a negative feedback loop in Mycobacterium marinum. Proc Natl Acad Sci U S A. 2017. https://doi.org/10.1073/pnas.1710167114 PMID: 29180415.

53. Bottai D, Frigui W, Sayes F, Di Luca M, Spadoni D, Pawlik A, et al. TbD1 deletion as a driver of the evolutionary success of modern epidemic Mycobacterium tuberculosis lineages. Nat Commun. 2020; 11 (1):684. Epub 2020/02/06. https://doi.org/10.1038/s41467-020-14508-5 PMID: 32019932; PubMed Central PMCID: PMC7000671.

54. Papavinassundaram KG, Chan B, Chung JH, Colston MJ, Davis EO, Av-Gay Y. Deletion of the Mycobacterium tuberculosis pknH gene confers a higher bacillary load during the chronic phase of infection in BALB/c mice. J Bacteriol. 2005; 187(16):5751–60. Epub 2005/08/04. https://doi.org/10.1128/JB.187.16.5751-5760.2005 PMID: 16077122; PubMed Central PMCID: PMC196067.

55. Beites T, O’Brien K, Tiwari D, Engelhart CA, Walters S, Andrews J, et al. Plasticity of the Mycobacterium tuberculosis respiratory chain and its impact on tuberculosis drug development. Nat Commun. 2019; 10(1):4970. Epub 2019/11/02. https://doi.org/10.1038/s41467-019-12956-2 PMID: 31672993; PubMed Central PMCID: PMC6823465.

56. Yimer SA, Kalayou S, Homberset H, Birhanu AG, Riaz T, Zageye ED, et al. Lineage-Specific Prototypic Signatures in the Mycobacterium tuberculosis Complex Reveal Differential Abundance of Proteins Involved in Virulence, DNA Repair, CRISPR-Cas, Bioenergetics and Lipid Metabolism. Front Microbiol. 2020; 11:550760. Epub 2020/10/20. https://doi.org/10.3389/fmicb.2020.550760 PMID: 33072011; PubMed Central PMCID: PMC7536270.

57. Choudhary E, Sharma R, Kumar Y, Agarwal N. Conditional Silencing by CRISPR/Cas Reveals the Role of DNA Gyrase in Formation of Drug-Tolerant Persister Population in Mycobacterium tuberculosis. Front Cell Infect Microbiol. 2019; 9:70. Epub 2019/04/19. https://doi.org/10.3389/fcimb.2019.00070 PMID: 30972304; PubMed Central PMCID: PMC6443821.

58. McNeil MB, Cook GM. Utilization of CRISPR Interference To Validate MmpL3 as a Drug Target in Mycobacterium tuberculosis. Antimicrob Agents Chemother. 2019; 63(8). Epub 2019/06/05. https://doi.org/10.1128/AAC.00629-19 PMID: 31160289; PubMed Central PMCID: PMC6658742.

59. McNeil MB, Ryburn HK, Harold LR, Tirados JF, Cook GM. Transcriptional Inhibition of the F1Fo-Type ATP Synthase Has Bactericidal Consequences on the Viability of Mycobacteria. Antimicrob Agents Chemother. 2020; 64(8). Epub 2020/05/20. https://doi.org/10.1128/AAC.00492-20 PMID: 32423951; PubMed Central PMCID: PMC7526845.
60. Jacobs WR Jr., Tuckman M, Bloom BR. Introduction of foreign DNA into mycobacteria using a shuttle phasmid. Nature. 1987; 327(6122):532–5. Epub 1987/06/11. https://doi.org/10.1038/327532a0 PMID: 3473289.

61. Cosma CL, Humbert O, Ramakrishnan L. Superinfecting mycobacteria home to established tuberculous granulomas. Nat Immunol. 2004; 5(8):828–35. Epub 2004/06/29. https://doi.org/10.1038/ni1091 PMID: 15220915.

62. George KM, Yuan Y, Sherman DR, Barry CE, 3rd. The biosynthesis of cyclopropated mycolic acids in Mycobacterium tuberculosis. Identification and functional analysis of CMAS-2. J Biol Chem. 1995; 270(45):27292–8. https://doi.org/10.1074/jbc.270.45.27292 PMID: 7592990.

63. Ehrt S, Guo XV, Hickey CM, Ryou M, Monteleone M, Riley LW, et al. Controlling gene expression in mycobacteria with anhydrotetracycline and Tet repressor. Nucleic Acids Res. 2005; 33(2):e21. Epub 2005/02/03. https://doi.org/10.1093/nar/gni013 PMID: 15687379; PubMed Central PMCID: PMC548372.

64. Klotzsche M, Ehrt S, Schnappinger D. Improved tetracycline repressors for gene silencing in mycobacteria. Nucleic Acids Res. 2009; 37(6):1778–88. https://doi.org/10.1093/nar/gkp015 PMID: 19174563.

65. Mahenthiralingam E, Draper P, Davis EO, Colston MJ. Cloning and sequencing of the gene which encodes the highly inducible acetamidase of Mycobacterium smegmatis. J Gen Microbiol. 1993; 139(3):575–83. Epub 1993/03/01. https://doi.org/10.1099/00221287-139-3-575 PMID: 8473863.

66. Forti F, Crosta A, Ghisotti D. Pristinamycin-inducible gene regulation in mycobacteria. J Biotechnol. 2009; 140(3–4):270–7. Epub 2009/05/12. https://doi.org/10.1016/j.jbiotec.2009.02.001 PMID: 19428723.

67. Blokpoel MC, Murphy HN, O'Toole R, Wiles S, Runn ES, Stewart GR, et al. Tetracycline-inducible gene regulation in mycobacteria. Nucleic Acids Res. 2005; 33(2):e22. Epub 2005/02/03. https://doi.org/10.1093/nar/gni023 PMID: 15687380; PubMed Central PMCID: PMC548381.

68. Pandey AK, Raman S, Proff R, Joshi S, Kang CM, Rubin EJ, et al. Nitrile-inducible gene expression in mycobacteria. Tuberculosis (Edinb). 2009; 89(1):12–6. Epub 2008/09/20. https://doi.org/10.1016/j.tube.2008.07.007 PMID: 18801704; PubMed Central PMCID: PMC2845969.

69. Van Vlack ER, Topp S, Seeliger JC. Characterization of Engineered PreQ1 Riboswitches for Inducible Gene Regulation in Mycobacteria. J Bacteriol. 2017; 199(6). Epub 2017/01/11. https://doi.org/10.1128/JB.00656-16 PMID: 28069821; PubMed Central PMCID: PMC5331669.

70. Van Vlack ER, Seeliger JC. Using riboswitches to regulate gene expression and define gene function in mycobacteria. Methods Enzymol. 2015; 550:251–65. Epub 2015/01/22. https://doi.org/10.1016/bse.mie.2014.10.034 PMID: 25605389; PubMed Central PMCID: PMC5154175.

71. Seeliger JC, Topp S, Sogi KM, Previti ML, Gallivan JP, Bertozzi CR. A riboswitch-based inducible gene expression system for mycobacteria. PLoS ONE. 2012; 7(1):e29266. Epub 2012/01/27. https://doi.org/10.1371/journal.pone.0029266 PMID: 22279533; PubMed Central PMCID: PMC3261144.

72. Botella L, Vaubourgeois J, Livny J, Schnappinger D. Depleting Mycobacterium tuberculosis of the transcription termination factor Rho causes pervasive transcription and rapid death. Nat Commun. 2017; 8:14731. Epub 2017/03/30. https://doi.org/10.1038/ncomms14731 PMID: 28348398; PubMed Central PMCID: PMC5379054.

73. Washburn MP, Wolters D, Yates JR, III. Large-scale analysis of the yeast proteome by multidimensional protein identification technology. Nat Biotechnol. 2001; 19(3):242–7. Epub 2001/03/07. https://doi.org/10.1038/85686 PMID: 11231557.

74. Jungblut PR, Schaible UE, Molkenkopf HJ, Zinny-Arndt U, Raupach B, Mattow J, et al. Comparative proteome analysis of Mycobacterium tuberculosis and Mycobacterium bovis BCG strains: towards functional genomics of microbial pathogens. Mol Microbiol. 1999; 33(6):1103–17. Epub 1999/10/06. https://doi.org/10.1046/j.1365-2958.1999.01549.x PMID: 10510226.

75. Gengenbacher M, Mourtisan J, Schubert OT, Aebersold R, Kaufmann SHE. Mycobacterium tuberculosis in the Proteomics Era. Microbiol Spectr. 2014; 2(2). Epub 2014/04/01. https://doi.org/10.1128/microbiolspec.MGM2-0020-2013 PMID: 26105825.

76. Gopinath V, Raghunanjan S, Gomez RL, Jose L, Surendran A, Ramachandran R, et al. Profiling the Proteome of Mycobacterium tuberculosis during Dormancy and Reactivation. Mol Cell Proteomics. 2015; 14(8):2160–76. https://doi.org/10.1074/mcp.M115.051151 PMID: 26025969; PubMed Central PMCID: PMC4528245.

77. Esterhuyse MM, Weiner J 3rd, Caron E, Loxton AG, Iannaccone M, Wagman C, et al. Epigenetics and Proteomics Join Transcriptomics in the Quest for Tuberculosis Biomarkers. MBio. 2015; 6(5):e01187–15. Epub 2015/09/17. https://doi.org/10.1128/mBio.01187-15 PMID: 26374119; PubMed Central PMCID: PMC4600108.
78. Mateos J, Estevez O, Gonzalez-Fernandez A, Anibarro L, Pallares A, Relic R, et al. High-resolution quantitative proteomics applied to the study of the specific protein signature in the sputum and saliva of active tuberculosis patients and their infected and uninfected contacts. J Proteome. 2019; 195:41–52. Epub 2019/01/21. https://doi.org/10.1016/j.jprot.2019.01.010 PMID: 30660789.

79. Champion PA, Champion MM, Manzanillo P, Cox JS. ESX-1 secreted virulence factors are recognized by multiple cytosolic AAA ATPases in pathogenic mycobacteria. Mol Microbiol. 2009; 73(5):950–62. https://doi.org/10.1111/j.1365-2958.2009.06821.x PMID: 19682254.

80. Champion MM, Williams EA, Pinapati RS, Champion PA. Correlation of Phenotypic Profiles Using Targeted Proteomics Identifies Mycobacterial Esx-1 Substrates. J Proteome Res. 2014; 3(11):5151–64. https://doi.org/10.1021/pr500484w PMID: 25106450.

81. Aebersold R, Burlingame AL, Bradshaw RA. Western Blots vs. SRM Assays: Time to turn the tables? Mol Cell Proteomics. 2013; 12(9):2381–2. https://doi.org/10.1074/mcp.E113.031658 PMID: 23756428.

82. Schubert OT, Moursifis J, Ludwig C, Rost HL, Rosenberger G, Arthur PK, et al. The Mtb proteome library: a resource of assays to quantify the complete proteome of 

83. Mycobacterium tuberculosis. Cell Host Microbe. 2013; 13(5):602–12. https://doi.org/10.1016/j.chom.2013.04.008 PMID: 23684311.

84. Gerber SA, Rush J, Stemman O, Kirschner MW, Gygi SP. Absolute quantification of proteins and phosphoproteins from cell lysates by tandem MS. Proc Natl Acad Sci U S A. 2020; 117(25):15475–80. Epub 2020/05/27. https://doi.org/10.1073/pnas.2001371117 PMID: 32470866.

85. Kennedy GM, Hooley GC, Champion MM, Medie FM, Champion PA. A novel ESX-1 locus reveals that surface associated ESX-1 substrates mediate virulence in 

86. Mycobacterium marinum. J Bacteriol. 2014; 196(10):1877–88. https://doi.org/10.1128/JB.01902-14 PMID: 24610712.

87. Mehaffy C, Kruh-Garcia NA, Graham B, Jarlsberg LG, Willyerd CE, Borisov A, et al. Identification of 

88. Mycobacterium tuberculosis Peptides in Serum Extracellular Vesicles from Persons with Latent Tuberculosis Infection. J Clin Microbiol. 2020; 58(6). Epub 2020/04/05. https://doi.org/10.1128/JCM.00393-20 PMID: 32245831; PubMed Central PMCID: PMC7269374.

89. Kruh-Garcia NA, Wolfe LM, Chaisson LH, Worodria WO, Nahid P, Schorey JS, et al. Detection of 

90. Mycobacterium tuberculosis peptides in the exosomes of patients with active and latent M. tuberculosis infection using MRM-MS. PLoS ONE. 2014; 9(7):e103811. Epub 2014/08/01. https://doi.org/10.1371/journal.pone.0103811 PMID: 25080537; PubMed Central PMCID: PMC4078798.

91. Kim SH, Lee NE, Lee JS, Shin JH, Lee JY, Ko JH, et al. Identification of Mycobacterial Antigens in Human Urine by Use of Immunoglobulin G Isolated from Sera of Patients with Active Pulmonary Tuberculosis. J Clin Microbiol. 2016; 54(6):1631–7. Epub 2016/03/18. https://doi.org/10.1128/JCM.00236-16 PMID: 26984972; PubMed Central PMCID: PMC4879297.

92. van Els CA, Corbiere V, Smits K, van Gaans-van den Brink JA, Poelen MC, Mascart F, et al. Toward Understanding the Essence of Post-Translational Modifications for the Mycobacterium tuberculosis Immunoproteome. Front Immunol. 2014; 5:361. Epub 2014/06/27. https://doi.org/10.3389/fimmu.2014.00361 PMID: 25157294; PubMed Central PMCID: PMC4127798.

93. Xie L, Wang X, Zeng J, Zhou M, Duan X, Li Q, et al. Proteome-wide lysine acetylation profiling of the human pathogen Mycobacterium tuberculosis. Int J Biochem Cell Biol. 2015; 59:193–202. Epub 2014/12/03. https://doi.org/10.1016/j.biocel.2014.11.010 PMID: 25456444.

94. Prsic S, Dankova S, Schwartz D, Chou MF, Locasale JW, Kang CM, et al. Extensive phosphorylation with overlapping specificity by Mycobacterium tuberculosis serine/threonine protein kinases. Proc Natl Acad Sci U S A. 2010; 107(16):7521–6. Epub 2010/05/11. https://doi.org/10.1073/pnas.0913482107 PMID: 20368441; PubMed Central PMCID: PMC2867705.

95. Budzick JM, Swaney DL, Jimenez-Morales D, Johnson JR, Garelis NE, Repasy T, et al. Dynamic post-translational modification profiling of Mycobacterium tuberculosis-infected primary macrophages. eLife. 2020; 9. Epub 2020/01/18. https://doi.org/10.7554/eLife.51461 PMID: 31951200; PubMed Central PMCID: PMC7030789.

96. Ganguly N, Giang PH, Basu SK, Mir FA, Siddiqui I, Sharma P. Mycobacterium tuberculosis 6-kDa early secreted antigenic target (ESAT-6) protein downregulates lipopolysaccharide induced c-myc expression by modulating the extracellular signal regulated kinases 1/2. BMC Immunol. 2007; 8:24. Epub 2007/10/05. https://doi.org/10.1186/1471-2172-8-24 PMID: 17915024; PubMed Central PMCID: PMC2082026.

97. Kusebauch U, Ortega C, Ollodart A, Rogers RS, Sherman DR, Moritz RL, et al. Mycobacterium tuberculosis supports protein tyrosine phosphorylation. Proc Natl Acad Sci U S A. 2014; 111(25):9265–70. Epub 2014/06/14. https://doi.org/10.1073/pnas.1323894111 PMID: 24927537; PubMed Central PMCID: PMC4078798.

98. Xu JY, Zhao L, Xu Y, Li B, Zhai L, Tan M, et al. Dynamic Characterization of Protein and Posttranslational Modification Levels in Mycobacterial Cholesterol Catabolism. mSystems. 2020; 5(1). Epub
95. Thompson CR, Champion MM, Champion PA. Quantitative N-Terminal Footprinting of Pathogenic Mycobacteria Reveals Differential Protein Acetylation. J Proteome Res. 2018; 17(9):3246–58. Epub 2018/08/07. https://doi.org/10.1021/acs.jproteome.8b00373 PMID: 30080413.

96. Lange V, Picotti P, Domon B, Aebersold R. Selected reaction monitoring for quantitative proteomics: a tutorial. Mol Syst Biol. 2008; 4:222. Epub 2008/10/16. https://doi.org/10.1038/msb.2008.61 PMID: 18854821; PubMed Central PMCID: PMC2583086.

97. Gandotra S, Schnappinger D, Monteleone M, Hillen W, Ehrl S. In vivo gene silencing identifies the Mycobacterium tuberculosis proteasome as essential for the bacteria to persist in mice. Nat Med. 2007; 13(12):1515–20. https://doi.org/10.1038/nm1683 PMID: 18059281.

98. Elharar Y, Roth Z, Hermelin I, Moon A, Peretz G, Shenkerman Y, et al. Survival of mycobacteria depends on proteasome-mediated amino acid recycling under nutrient limitation. EMBO J. 2014; 33(16):1802–14. Epub 2014/07/06. https://doi.org/10.1002/embj.201387076 PMID: 24986881; PubMed Central PMCID: PMC4195762.

99. Fascellaro G, Petrera A, Lai ZW, Nanni P, Grossmann J, Burger S, et al. Comprehensive Proteomic Analysis of Nitrogen-Starved Mycobacterium smegmatis Deltapup Reveals the Impact of Pupylation on Nitrogen Stress Response. J Proteome Res. 2016; 15(8):2812–25. Epub 2016/07/06. https://doi.org/10.1021/acs.jproteome.6b00378 PMID: 27378031.

100. Muller AU, Imkamp F, Weber-Ban E. The Mycobacterial LexA/RecA-Independent DNA Damage Response Is Controlled by PafBC and the Pup-Proteasome System. Cell Rep. 2018; 23(12):3551–64. Epub 2018/06/21. https://doi.org/10.1016/j.celrep.2018.05.073 PMID: 29924998.

101. Raju RM, Jedrzychowski MP, Wei JR, Pinkham JT, Park AS, O’Brien K, et al. Post-translational regulation via Clp protease is critical for survival of Mycobacterium tuberculosis. PLoS Pathog. 2014; 10(3):e1003994. Epub 2014/03/08. https://doi.org/10.1371/journal.ppat.1003994 PMID: 24603869; PubMed Central PMCID: PMC3946367.

102. Barandun J, Delley CL, Weber-Ban E. The pupylation pathway and its role in mycobacteria. BMC Biol. 2012; 10:95. Epub 2012/12/04. https://doi.org/10.1186/1741-7007-10-95 PMID: 23198822; PubMed Central PMCID: PMC3511204.

103. Pearce MJ, Mintseris J, Ferreyra J, Gygi SP, Darwin KH. Ubiquitin-like protein involved in the proteasome pathway of Mycobacterium tuberculosis. Science. 2008; 322(5904):1104–7. Epub 2008/10/04. https://doi.org/10.1126/science.1163885 PMID: 18932610.

104. Festa RA, McAllister F, Pearce MJ, Mintseris J, Burns KE, Gygi SP, et al. Prokaryotic ubiquitin-like protein (Pup) proteome of Mycobacterium tuberculosis [corrected]. PLoS ONE. 2010; 5(1):e5859. Epub 2010/01/13. https://doi.org/10.1371/journal.pone.0005859 PMID: 20066936; PubMed Central PMCID: PMC2797603.

105. Mba Medie F, Champion MM, Williams EA, Champion PAD. Homeostasis of N-alpha terminal acetylation of EsxA correlates with virulence in Mycobacterium marinum. Infect Immun. 2014; 82(11):4572–86. Epub 2014/07/22. https://doi.org/10.1128/IAI.02153-14 PMID: 25135684.

106. Burns KE, Liu WT, Boshoff HI, Dorrestein PC, Barry CE, 3rd. Proteasomal protein degradation in Mycobacteria is dependent upon a prokaryotic ubiquitin-like protein. J Biol Chem. 2009; 284(5):3069–75. Epub 2008/11/26. https://doi.org/10.1074/jbc.M908032200 PMID: 19028697; PubMed Central PMCID: PMC2631945.

107. Salgame P. PUPylation provides the punch as Mycobacterium tuberculosis battles the host macrophage. Cell Host Microbe. 2008; 4(5):415–6. Epub 2008/11/11. https://doi.org/10.1016/j.chom.2008.10.009 PMID: 18996341; PubMed Central PMCID: PMC3202434.

108. Mba Medie F, Champion MM, Williams EA, Champion PAD. Homeostasis of N-alpha terminal acetylation of EsxA correlates with virulence in Mycobacterium marinum. Infect Immun. 2014; 82(11):4572–86. Epub 2014/07/22. https://doi.org/10.1128/IAI.02153-14 PMID: 25135684.

109. Chen J, Zhang M, Rybniker J, Basterra L, Dhar N, Tischler AD, et al. Phenotypic Profiling of Mycobacterium tuberculosis EsxA Point-mutants Reveals Blockage of ESAT-6 and CFP-10 secretion in vitro Does Not Always Correlate with Attenuation of Virulence. J Bacteriol. 2013; 195(24):5421–30. https://doi.org/10.1128/JB.00967-13 PMID: 24078612.

110. Champion PA. Disconnecting in vitro ESX-1 secretion from mycobacterial virulence. J Bacteriol. 2013; 195(24):5418–20. https://doi.org/10.1128/JB.01145-13 PMID: 24123823.

111. Fortune SM, Jaeger A, Sarracino DA, Chase MR, Sassetti CM, Sherman DR, et al. Mutually dependent secretion of proteins required for mycobacterial virulence. Proc Natl Acad Sci U S A. 2005; 102(30):10676–81. https://doi.org/10.1073/pnas.0504922102 PMID: 16030141.
112. Bosserman RE, Nicholson KR, Champion MM, Champion PA. A New ESX-1 Substrate in Mycobacterium marinum That Is Required for Hemolysis but Not Host Cell Lysis. J Bacteriol. 2019; 201(14). Epub 2019/03/06. https://doi.org/10.1128/JB.00760-18 PMID: 30833360.

113. Phan TH, van Leeuwen LM, Kuijl C, Ummels R, van Stempvoort G, Rubio-Cañalejas A, et al. EspH is a hypervirulence factor for Mycobacterium marinum and essential for the secretion of the ESX-1 substrates EspE and EspF. PLoS Pathog. 2018; 14(8):e1007247. Epub 2018/08/14. https://doi.org/10.1371/journal.ppat.1007247 PMID: 30102741; PubMed Central PMCID: PMC6107294.

114. Abdallah AM, Weerdenburg EM, Guan Q, Ummels R, Borggreve S, Adroub SA, et al. Integrated transcriptomic and proteomic analysis of pathogenic mycobacteria and their esx-1 mutants reveal secreted-dependent regulation of ESX-1 substrates and WhiB6 as a transcriptional regulator. PLoS ONE. 2019; 14(1):e0211003. Epub 2019/01/24. https://doi.org/10.1371/journal.pone.0211003 PMID: 30673778.

115. Bosserman RE, Thompson CR, Nicholson KR, Champion PA. Esx Paralogs Are Functionally Equivalent to ESX-1 Proteins but Are Dispensable for Virulence in Mycobacterium marinum. J Bacteriol. 2018; 200(11):e00726–17. https://doi.org/10.1128/JB.00726-17 PMID: 29555701.

116. Diaz G, Wolfe LM, Kruh-Garcia NA, Dobos KM. Changes in the Membrane-Associated Proteins of Exosomes Released from Human Macrophages after Mycobacterium tuberculosis Infection. Sci Rep. 2016; 6:37975. Epub 2016/11/30. https://doi.org/10.1038/srep37975 PMID: 27897253; PubMed Central PMCID: PMC5126699.

117. Sayes F, Blanc C, Ates LS, Deboosere N, Orgeur M, Le Chevalier F, et al. Multiplexed Quantitation of Intraphagocyte Mycobacterium tuberculosis Secreted Protein Effectors. Cell Rep. 2018; 23(4):1072–16. Epub 2018/04/03. https://doi.org/10.1016/j.celrep.2018.03.125 PMID: 29513378; PubMed Central PMCID: PMC4458716.

118. Feltcher ME, Gunawardena HP, Zulauf KE, Malik S, Griffin JE, Sassetti CM, et al. Label-free Quantitative Proteomics Reveals a Role for the Mycobacterium tuberculosis SecA2 Pathway in Exporting Solute Binding Proteins and Mce Transporters to the Cell Wall. Mol Cell Proteomics. 2015; 14(6):1501–16. Epub 2015/03/31. https://doi.org/10.1074/mcp.M114.044685 PMID: 25813378; PubMed Central PMCID: PMC4458716.

119. Malone KM, Rue-Albrecht K, Magee DA, Conlon K, Schubert OT, Nalpas NC, et al. Comparative ‘omics analyses differentiate Mycobacterium tuberculosis and Mycobacterium bovis and reveal distinct macrophage responses to infection with the human and bovine tubercle bacilli. Microb Genom. 2018; 4(3). Epub 2018/03/21. https://doi.org/10.1099/mgen.0.000163 PMID: 29557774; PubMed Central PMCID: PMC5885015.

120. Bosserman RE, Champion PA. ESX systems and the Mycobacterial Cell Envelope: What’s the connection? J Bacteriol. 2017; 199 (17):e00131–17. https://doi.org/10.1128/JB.00131-17 PMID: 28461452.

121. Jain M, Cox JS. Interaction between polyketide synthase and transporter suggests coupled synthesis and export of virulence lipid in M. tuberculosis. PLoS Pathog. 2005; 1(1):e2. https://doi.org/10.1371/journal.ppat.0010002 PMID: 16201014; PubMed Central PMCID: PMC1238737.

122. Pandey AK, Sassetti CM. Mycobacterial persistence requires the utilization of host cholesterol. Proc Natl Acad Sci U S A. 2008; 105(11):4376–80. https://doi.org/10.1073/pnas.0711159105 PMID: 18334639; PubMed Central PMCID: PMC2393810.

123. Minier MD, Chang JC, Pandey AK, Sassetti CM, Sherman DR. Role of cholesterol in Mycobacterium tuberculosis infection. Indian J Exp Biol. 2009; 47(6):407–11. Epub 2009/07/29. PMID: 19634704.

124. Williams EA, Mba Medie F, Bosserman RE, Johnson BK, Reyna C, Ferrell MJ, et al. A Nonsense Mutation in Mycobacterium marinum That Is Suppressible by a Novel Mechanism. Infect Immun. 2017; 85(2). https://doi.org/10.1128/IAI.00653-16 PMID: 27789543; PubMed Central PMCID: PMC5278160.

125. Champion MM, Williams EA, Kennedy GM, Champion PA. Direct detection of bacterial protein secretion using whole colony proteomics. Mol Cell Proteomics. 2012; 11:596–604. https://doi.org/10.1074/mcp.M112.017533 PMID: 22580590.
128. He Z, De Buck J. Localization of proteins in the cell wall of Mycobacterium avium subsp. paratuberculosis K10 by proteomic analysis. Proteome Sci. 2010; 8:21. Epub 2010/04/10. https://doi.org/10.1186/1477-5965-8-21 PMID: 20377898; PubMed Central PMCID: PMC2859856.

129. He Z, De Buck J. Cell wall proteome analysis of Mycobacterium smegmatis strain MC2 155. BMC Microbiol. 2010; 10:121. Epub 2010/04/24. https://doi.org/10.1186/1471-2180-10-121 PMID: 20412585; PubMed Central PMCID: PMC2867950.

130. van der Woude AD, Mahendran KR, Ummels R, Piersma SR, Pham TV, Jimenez CR, et al. Differential detergent extraction of mycobacterium marinum cell membrane proteins identifies an extensively modified threonine-rich outer membrane protein with channel activity. J Bacteriol. 2013; 195(9):2050–9. https://doi.org/10.1128/JB.02236-12 PMID: 23457249.

131. Ganapathy US, Bai L, Wei L, Eckartt KA, Previti ML, et al. Compartment-Specific Labeling of Bacterial Periplasmic Proteins by Peroxidase-Mediated Biotinylation. ACS Infect Dis. 2018; 4(6):918–25. Epub 2018/05/01. https://doi.org/10.1021/acsinfecdis.8b00044; PMID: 29708735; PubMed Central PMCID: PMC6767932.

132. Olson MG, Widner RE, Jorgenson LM, Lawrence A, Lagundzin D, Woods NT, et al. Proximity Labeling To Map Host-Pathogen Interactions at the Membrane of a Bacteria-Containing Vacuole in Chlamydia trachomatis-Infected Human Cells. Infect Immun. 2019; 87(1). Epub 2019/08/14. https://doi.org/10.1128/IAI.00537-19 PMID: 31405957; PubMed Central PMCID: PMC6803327.

133. Santin YG, Doan T, Lebrun R, Espinosa L, Journet L, Cascales E. In vivo TssA proximity labelling during type VI secretion biogenesis reveals TagA as a protein that stops and holds the sheath. Nat Microbiol. 2018; 3(11):1304–13. Epub 2018/10/03. https://doi.org/10.1038/s41564-018-0234-3 PMID: 30275513.

134. Kavunja HW, Biegas KJ, Banahene N, Stewart JA, Piligian BF, Groenevelt JM, et al. Photocrosslinkable Glycolipid Probes for Identifying Mycolate-Protein Interactions in Live Mycobacteria. J Am Chem Soc. 2020; 142(17):7725–31. Epub 2020/04/16. https://doi.org/10.1021/jacs.0c01065 PMID: 32293873.

135. White DW, Elliott SR, Odean E, Bemis LT, Tischler AD. Mycobacterium tuberculosis Pst/SenX3-RegX3 Regulates Membrane Vesicle Production Independently of ESX-5 Activity. MBio. 2018; 9(3). Epub 2018/06/14. https://doi.org/10.1128/mBio.00778-18 PMID: 29895636; PubMed Central PMCID: PMC6016242.

136. Hu X, Li X, Huang L, Chan J, Chen Y, Deng H, et al. Quantitative proteomics reveals novel insights into isoniazid susceptibility in mycobacteria mediated by a universal stress protein. J Proteome Res. 2015; 14(3):1445–54. Epub 2015/02/11. https://doi.org/10.1021/pr5011058 PMID: 25664397.

137. Wu Z, Wei W, Zhou Y, Guo H, Zhao J, Liao Q, et al. Integrated Quantitative Proteomics and Metabolome Profiling Reveal MSMEG_6171 Overexpression Perturbing Lipid Metabolism of Mycobacterium smegmatis Leading to Increased Vancomycin Resistance. Front Microbiol. 2020; 11:1572. Epub 2020/08/15. https://doi.org/10.3389/fmicb.2020.01572 PMID: 32793136; PubMed Central PMCID: PMC7393984.

138. Jnawali HN, Hwang SC, Park YK, Kim H, Lee YS, Chung GT, et al. Characterization of mutations in multi- and extensive drug resistance among strains of Mycobacterium tuberculosis clinical isolates in Republic of Korea. Diagn Microbiol Infect Dis. 2013; 76(2):187–96. Epub 2013/04/09. https://doi.org/10.1016/j.diagmicrobio.2013.02.035 PMID: 23561273.

139. Wang Q, Yue J, Zhang L, Xu Y, Chen J, Zhang M, et al. A newly identified 191A/C mutation in the Rv2629 gene that was significantly associated with rifampin resistance in Mycobacterium tuberculosis. J Proteome Res. 2007; 6(12):4565–71. Epub 2007/11/01. https://doi.org/10.1021/pr070242z PMID: 17970586.

140. McClure WR, Cech CL. On the mechanism of rifampcin inhibition of RNA synthesis. J Biol Chem. 1978; 253(24):8949–56. Epub 1978/12/25. PMID: 363713.

141. Honore N, Cole ST. Molecular basis of rifampin resistance in Mycobacterium leprae. Antimicrob Agents Chemother. 1993; 37(3):414–8. Epub 1993/03/01. https://doi.org/10.1128/aac.37.3.414 PMID: 8460911; PubMed Central PMCID: PMC187686.

142. Huseby DL, Brandis G, Praski Alzrigat L, Hughes D. Antibiotic resistance by high-level intrinsic suppression of a frameshift mutation in an essential gene. Proc Natl Acad Sci U S A. 2020; 117(6):3185–91. Epub 2020/01/30. https://doi.org/10.1073/pnas.1919390117 PMID: 31992637; PubMed Central PMCID: PMC7022156.

143. Miranda-CasoLuengo AA, Staunton PM, Dinan AM, Lohan AJ, Loftus BJ. Functional characterization of the Mycobacterium abscessus genome coupled with condition specific transcriptomics reveals conserved molecular strategies for host adaptation and persistence. BMC Genomics. 2016; 17:553. Epub 2016/08/09. https://doi.org/10.1186/s12866-016-2668-y PMID: 27495169; PubMed Central PMCID: PMC4974804.
144. Shell SS, Wang J, Lapiere P, Mir M, Chase MR, Pyle MM, et al. Leaderless Transcripts and Small Proteins Are Common Features of the Mycobacterial Translational Landscape. PLoS Genet. 2015; 11 (11):e1005641. Epub 2015/11/05. https://doi.org/10.1371/journal.pgen.1005641 PMID: 26536359; PubMed Central PMCID: PMC4633059.

145. Canestrari JG, Lasek-Nesselquist E, Upadhyay A, Rofaeil M, Champion MM, Wade JT, et al. Polycysteine-encoding leaderless short ORFs function as cysteine-responsive attenuators of operonic gene expression in mycobacteria. Mol Microbiol. 2020; 114(1):93–108. Epub 2020/03/18. https://doi.org/10.1111/mmi.14498 PMID: 32181921.

146. Jungblut PR, Muller EC, Mattow J, Kaufmann SH. Proteomics reveals open reading frames in Mycobacterium tuberculosis H37Rv not predicted by genomics. Infect Immun. 2001; 69(9):5905–7. Epub 2001/08/14. https://doi.org/10.1128/iai.69.9.5905-5907.2001 PMID: 11500470; PubMed Central PMCID: PMC387110.

147. Cortes T, Schubert OT, Rose G, Amvig KB, Comas I, Aebersold R, et al. Genome-wide mapping of transcriptional start sites defines an extensive leaderless transcriptome in Mycobacterium tuberculosis. Cell Rep. 2013; 5(4):1121–31. Epub 2013/11/26. https://doi.org/10.1016/j.celrep.2013.10.031 PMID: 24268774; PubMed Central PMCID: PMC3898074.

148. Ignatov D, Malakhov S, Majorov K, Skvortsov T, Apt A, Azhikina T. RNA-Seq analysis of Mycobacterium avium non-coding transcriptome. PLoS ONE. 2013; 8(9):e74209. Epub 2013/09/26. https://doi.org/10.1371/journal.pone.0074209 PMID: 24066122; PubMed Central PMCID: PMC3774663.

149. Chen Z, Hu Y, Cumming BM, Lu P, Feng L, Deng J, et al. Mycobacterial WhiB6 Differentially Regulates ESX-1 and the Dos Regulon to Modulate Granuloma Formation and Virulence in Zebrafish. Cell Rep. 2016; 16(9):2512–24. https://doi.org/10.1016/j.celrep.2016.07.080 PMID: 27545883.

150. Sanchez KG, Ferrell MJ, Chirakos AE, Nicholson KR, Abramovitch RB, Champion MM, et al. EspM Is a Conserved Transcription Factor That Regulates Gene Expression in Response to the ESX-1 System. MBio. 2020; 11(1). Epub 2020/02/02. https://doi.org/10.1128/mBio.02807-19 PMID: 32019792.

151. Rustad TR, Minch KJ, Ma S, Winkler JK, Hobbs S, Hickey M, et al. Mapping and manipulating the Mycobacterium tuberculosis transcriptome using a transcription factor overexpression-derived regulatory network. Genome Biol. 2014; 15(11):502. https://doi.org/10.1186/PREACCEPT-170163804134699 PMID: 25380655; PubMed Central PMCID: PMC4249609.

152. Minch KJ, Rustad TR, Peterson EJ, Winkler J, Reiss DJ, Ma S, et al. The DNA-binding network of Mycobacterium tuberculosis. Nat Commun. 2015; 6:5829. https://doi.org/10.1038/ncomms6829 PMID: 25581030; PubMed Central PMCID: PMC4301838.

153. Kriel NL, Heunis T, Sampson SL, Gey van Pittius NC, Williams MJ, Warren RM. Identifying nucleic acid-associated proteins in Mycobacterium smegmatis by mass spectrometry-based proteomics. BMC Mol Cell Biol. 2020; 21(1):19. Epub 2020/04/16. https://doi.org/10.1186/s12860-020-00261-6 PMID: 32293251; PubMed Central PMCID: PMC7092591.

154. Guo J, Wang C, Han Y, Liu Z, Wu T, Liu Y, et al. Identification of Lysine Acetylation in Mycobacterium abscessus Using LC-MS/MS after Immunoprecipitation. J Proteome Res. 2016; 15(8):2567–78. Epub 2016/08/22. https://doi.org/10.1021/acs.jproteome.6b00116 PMID: 27323652.

155. Wang L, Wu J, Li J, Yang H, Tang T, Liang H, et al. Host-mediated ubiquitination of a mycobacterial protein suppresses immunity. Nature. 2020; 577(7792):682–8. Epub 2020/01/17. https://doi.org/10.1038/s41586-019-1915-7 PMID: 31942069.

156. Whitaker M, Ruecker N, Hartman T, Klever T, Andrews J, Kim J, et al. Two interacting ATPases protect Mycobacterium tuberculosis from glycerol and nitric oxide toxicity. J Bacteriol. 2020. Epub 2020/06/03. https://doi.org/10.1128/JB.00202-20 PMID: 32482725.

157. Plocinski P, Macios M, Houghton J, Niemiec E, Plocinska R, Brzostek A, et al. Transcriptomic experiments reveal an essential role of RNA degradosome complexes in shaping the transcriptome of Mycobacterium tuberculosis. Nucleic Acids Res. 2019; 47(11):5892–905. Epub 2019/04/09. https://doi.org/10.1093/nar/gkz251 PMID: 30957850; PubMed Central PMCID: PMC6582357.

158. Sampson BA, Misra R, Benson SA. Identification and characterization of a new gene of Escherichia coli K-12 involved in outer membrane permeability. Genetics. 1989; 122(3):491–501. PMID: 2547691.

159. Mead JA, Bianco L, Ottone V, Barton C, Kay RG, Lilley KS, et al. MRMap, the web-based tool for designing multiple reaction monitoring (MRM) transitions. Mol Cell Proteomics. 2009; 8(4):696–705. Epub 2008/11/18. https://doi.org/10.1074/mcp.M800192-MCP200 PMID: 19011259; PubMed Central PMCID: PMC2667351.

160. Kusebauch U, Campbell DS, Deutsch EW, Chu CS, Spicer DA, Brusniak MY, et al. Human SRMAtlas: A Resource of Targeted Assays to Quantify the Complete Human Proteome. Cell. 2016; 166(3):766–78. Epub 2016/07/28. https://doi.org/10.1016/j.cell.2016.06.041 PMID: 27453469; PubMed Central PMCID: PMC5245710.