Minding the gaps: metabolomics mends functional genomics

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Many have come to regard metabolism as a well-understood house-keeping activity of all cells, functionally compartmentalized away from other biological processes. However, growing reports of unexpected links between a diverse range of disease states and specific metabolic enzymes or pathways have begun to challenge this view. In doing so, such discoveries have exposed more glaring, and neglected, deficiencies in our understanding of cellular metabolism, triggering a broad resurgence of interest in metabolism.

Metabolomics is the newest of the systems-level disciplines and seeks to reveal the physiological state of a given cell or organism through the global and unbiased study of its small-molecule metabolites [1]. Metabolites are the final products of enzymes

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and enzyme networks, the substrates and products of which often cannot be deduced from genetic information and the levels of which reflect the integrated product of the genome, proteome and environment [2]. Metabolomics thus offers a global window into the biochemical state of a cell or organism, made experimentally possible by the unprecedented discriminatory power and sensitivity of modern mass-spectrometry-based technologies (Fig 1). Two recent reports from the Carvalho and Neyrolles groups, published recently in *Proceedings of the National Academy of Science USA* and *Nature Chemical Biology* [3,4], exemplify

the rapidly growing impact of metabolomics-based approaches on tuberculosis research.

Within the field of infectious diseases, the deficiencies in our understanding of microbial metabolism have emerged most prominently in the area of tuberculosis research. Despite the development of the first chemotherapies more than 50 years ago, tuberculosis remains the leading bacterial cause of death worldwide, due in part to a failure to keep pace with the emergence of drug resistance [5]. The causes of this shortfall are multifactorial. However, a key contributing factor is our incomplete understanding of the metabolic properties of *Mycobacterium tuberculosis* (*Mtb*), its aetiological agent. Unlike most bacterial pathogens, *Mtb* infects humans as its only known host and reservoir, within

Figure 1 | Modern mass spectrometry illuminates bacterial metabolism. A comparison of activity-based metabolomic profiling with classic metabolic tracing. See the text for details.

whom it resides largely isolated from other microbes. *Mtb* has thus evolved its metabolism to serve interdependent physiological and pathogenic roles. Yet, more than a century after Koch's initial discovery of *Mtb* and 15 years after the first publication of its genome sequence, knowledge of *Mtb*'s metabolic network remains surprisingly incomplete [6–8].

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As for almost all sequenced microbial genomes, homology-based *in silico* approaches have failed to suggest a function for nearly 40% of *Mtb* genes that, presumably, include a significant number of orphan enzyme activities for which no gene has been ascribed [8]. Such approaches have further neglected the impact of evolutionary selection and its ability to dissociate sequence conservation from biochemical activity and physiological function, in order to help optimize the fitness of a given organism within its specific niche. For *Mtb*, such genes and enzymes represent an especially promising and biologically selective, but untapped, source of potential drug targets.

In the study from the Carvalho group, successful application of a recently developed metabolomics assay—known as activity-based metabolomic profiling (ABMP)—allowed the authors to reassign a putatively annotated nucleotide phosphatase (Rv1692) as a D,L-glycerol 3-phosphate phosphatase [3,9]. ABMP was specifically developed to identify enzymatic activities for genes of unknown function by leveraging the analytical discriminatory power of liquid-chromatography-coupled high-resolution mass spectrometry (LC-MS) to analyse the impact of a recombinant enzyme and potential co-factors on a highly concentrated, small-molecule extract derived from the homologous organism (Fig 1). By monitoring for the matched time and enzyme-dependent depletion and accumulation of putative substrates and products, this assay

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enables the discovery of catalytic activities—rather than simple binding—by using the cellular metabolome as arguably the most physiological chemical library of potential substrates that can be tested, in a label and synthesis-free manner. Moreover, candidate activities assigned by this method can be confirmed by using independent biochemical approaches—such as reconstitution with purified components—and genetic techniques—such as wild-type and genetic knockout, knockdown or overexpression strains. In reassigning Rv1692 as a glycerol phosphate phosphatase, rather than a nucleotide phosphatase, Carvalho and colleagues demonstrate the potential of ABMP to overcome the biochemical challenge of assigning substrate specificity to a member of a large enzyme superfamily—in this case, the haloacid dehydrogenase superfamily. But, perhaps more significantly, they also direct new biological attention to the largely neglected area of *Mtb* membrane homeostasis, in which Rv1692 might play an important role in glycerophospholipid recycling and catabolism.

Neyrolles and colleagues make use of the same metabolomics platform to perform metabolite-tracing studies by using stable-isotope-labelled precursors, which led them to reassign a putatively annotated asparagine transporter (AnsP1) as an aspartate transporter. AnsP1 bears 55% sequence identity and 70% similarity to an orthologue in *Salmonella* that belongs to the amino acid transporter family 2.A.3.1, whereas aspartate transporters are typically members of the dicarboxylate amino acid:cation symporter family 2.A.23 [4]. This study demonstrates the ability of metabolomic platforms to not only characterize the activity of a given protein within its natural physiological milieu, but also revive classical experimental methods by using modern technologies. The availability of stable (non-radioactive) isotopically labelled precursors has now made

it possible to resolve their specific metabolic fates. In this case, such an approach revealed that *Mtb* can use aspartate as both a carbon and nitrogen source, after its uptake through AnsP1. Looking beyond the specific biochemical assignment of AnsP1 as an aspartate—rather than asparagine—transporter, this study illustrates the potential impact of such discoveries on downstream paths of investigation. In this case, the remarkable application of high-resolution dynamic secondary ion mass spectroscopy to provide the first direct biochemical images of the nutritional environment of the *Mtb*-infected phagosome.

New technologies are often developed in the context of specific needs. However, their impact is usually not realized until extended beyond such contexts, sometimes resulting in major paradigm shifts. The above examples highlight just two emerging possibilities of how metabolomics technologies can be extended beyond the context of global comparisons and provide unique biological insights. To the extent that the analytical power of these platforms can be adapted to other functional approaches, metabolomics promises to pay handsome biochemical and physiological dividends.

CONFLICT OF INTEREST

The author declares that he has no conflict of interest.

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