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Tuberculosis: Feeding the Enemy

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The nutrition of intracellular *Mycobacterium tuberculosis* is particularly experimentally intractable. In this issue of *Chemistry and Biology*, using ^{13}C labeling with a new method of data analysis, Beste and colleagues provide direct evidence for the nutrients and pathways used by this ancient enemy of humanity.

Intracellular bacterial pathogens inhabit uniquely challenging environments inside host cells and tissues. They must obtain sufficient nutrients to survive and grow by either adapting their own metabolic pathways or manipulating the metabolism of the host. Understanding the basic biochemistry of these organisms in their intracellular niches is vital to understanding infection and related phenomena, such as bacterial dormancy and phenotypic tolerance to antimicrobials. However, it is particularly difficult to measure the nutrient availability and nutrient utilization within the host.

The genome, enzymatic activities, and growth characteristics in synthetic media each indicate the metabolic potential of bacteria, allowing the construction of a biochemical roadmap. To measure the actual reactions taking place in living cells, stable-isotope (^{13}C) labeled nutrients can be fed to bacteria, where they become incorporated into the metabolome and biomass. Nuclear magnetic resonance or mass spectrometry then allow detection of the pattern of ^{13}C present in the bacteria. A technique called metabolic flux analysis (MFA) combines the ^{13}C information during steady state growth with an in silico model of central carbon metabolism. Iterative fitting of ^{13}C data to the model provides a solution for the precise flux through each pathway (the "traffic" along each mapped road) that accounts for the observed pattern of ^{13}C distribution (Zamboni et al., 2009).

Model organisms such as *Escherichia coli* grown in synthetic medium have been studied extensively by MFA. However, in the case of an intracellular pathogen, the situation is more complex, because the labeled carbon source has to be taken up and potentially transformed by the host before it becomes available to the pathogen. With two interacting organisms, a steady state might not be reached easily, and no attempts at MFA have been made. Highlighting the need to study intracellular pathogens within host cells, transcriptome analysis and gene knockout strategies indicate that there are large differences in gene expression between pathogens grown in broth and cells, reflecting extensive adaptation to intracellular life.

Although system-wide modeling of intracellular growth had not previously been attempted, ^{13}C labeling of intracellular pathogens has led to some important insights into pathogen metabolism within the host. A technique called ^{13}C-isotopologue profiling analysis (IPA) compares ^{13}C incorporation into host and pathogen amino acids. Although flux analysis is not carried out, this method has nevertheless allowed the identification of the nutrients used by three intracellular human enteropathogens (Eisenreich et al., 2010). Usually, a series of mutants of the pathogen is needed to make or validate the predictions.

In this way, glycerol, glucose-6-phosphate, and amino acids were identified as carbon sources for *Listeria monocytogenes* in macrophages, whereas intracellular Salmonella preferentially uses glucose. Among the diversity of intracellular pathogens, *Mycobacterium tuberculosis* (Mtb) stands out as having the largest impact on global health today as well as indications from the genome of a distinctive and extensive long chain fatty acid metabolism.

Mtb is believed to utilize fatty acids derived from host lipids and cholesterol, because genes in the glyoxylate cycle (isocitrate lysase) and gluconeogenesis (phosphoenoylpyruvate carboxykinase) are upregulated inside macrophages and are essential for the infection of mice (Marrero et al., 2010, Muñoz-Elías and McKinney, 2005, Schnappinger et al., 2003). However, despite 130 years of laboratory cultivation, direct evidence was lacking. This is probably due to the slow growth and need for biosafety level 3 containment, which would make the comparison of multiple mutant strains by IPA exceptionally laborious.

Beste and colleagues were the first to tackle the metabolism of Mtb growing on glycerol in vitro by MFA, establishing a full model of central metabolism (Beste et al., 2007, 2011). In this issue of *Chemistry & Biology*, Beste et al. (2013) report ^{13}C glucose labeling of macrophages followed by infection with Mtb. Macrophage and Mtb amino acids were analyzed separately to produce a complex data set. In a novel and innovative approach that they call flux spectral analysis, Beste et al. (2013) first analyzed the ^{13}C profile of macrophage amino acids to deduce the ^{13}C profile of carbon sources within the macrophage. The ^{13}C profile of Mtb amino acids was then analyzed by iterative fitting (similar to MFA) to the in silico metabolic model using combinations of one, two, or three carbon sources from the macrophage. This analysis is considerably more sophisticated than any that has been attempted for intracellular bacteria previously.

Probably the most significant result of the analysis is the direct evidence that intracellular Mtb primarily utilize a C2 carbon source, which could be acetate or acetyl CoA resulting from lipid degradation, supporting the accumulation of indirect evidence for a lipid diet. Unlike other intracellular bacteria, which obtain most amino acids from the host, Mtb incorporates a select few host amino acids into central carbon metabolism but synthesizes the remainder of its amino acids. This observation agrees with years

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of work on auxotrophic strains that are able to grow in vitro but show reduced or completely attenuated virulence in vivo. Besides the likely lipid-derived carbon, amino acids or an unidentified C3 carbon source account for part of the ^{13}C labeling. One of the surprising findings of the MFA of Mtb in vitro was significant incorporation of carbon derived from carbon dioxide. In their latest work, Beste et al. (2013) found that CO_2 incorporation was even more significant inside macrophages.

Their contribution to understanding the biochemistry of intracellular Mtb may help to explain the success of this pathogen and may identify new drug targets or aid the prioritization of existing drug targets, but it also opens up the field for further investigation. Rapid intracellular growth occurs in the acute phase of Mtb infection, but the hallmarks of the disease are chronic infection, periods of clinical latency, and the requirement for long treatment regimens. If the metabolism of intracellularly growing Mtb was mysterious, that of dormant Mtb is even more elusive. Important insights may come from using similar approaches to study Mtb in environments that are nonpermissive for growth, such as activated macrophages or hypoxic foamy macrophages. Beste et al. have also raised the bar for the study of other intracellular pathogens, bringing system-wide models of metabolic flux within reach.

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