Assigning Enzyme Function from the Metabolic Milieu

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DOI 10.1016/j.chembiol.2010.04.001

In this issue of *Chemistry & Biology*, Rhee and co-workers develop a metabolic profiling technique that probes a mycobacterial small molecule extract and use it to correctly assign enzyme function to a previously mis-annotated gene product of *Mycobacterium tuberculosis* (de Carvalho et al., 2010).

With the advent of whole-genome sequencing comes the daunting task of assigning cellular and molecular function to the thousands of proteins encoded in a single genome. This challenge has inspired the creation of a new field termed 'functional genomics', in which researchers set out to assign function to a gene whose physiological role is not known. Historically, the function of an unknown gene was putatively assigned based on the similarity of the gene product's amino acid sequence with sequences of proteins whose functions are known (Lee et al., 2007). Although this method has proven useful in successfully annotating the function of a large number of genes, several shortcomings still persist. For example, protein sequence similarity does not necessarily equate to cellular or molecular functional similarity (Saghatelian and Cravatt, 2005). Furthermore, it is estimated that an error rate of 5%–40% exists in the functional annotation of enzymes, where the large range in reported error arises from varying definitions of the term "function" (Furnham et al., 2009; Valencia, 2005).

In order to unambiguously assign protein function to a particular gene found within the genome of *Mycobacterium tuberculosis* (*Mtb*), Rhee and co-workers (de Carvalho et al., 2010) devised an ingenious technique to analyze and assign protein function in the presence of *Mtb*'s metabolic milieu. By incubating the recombinant protein of interest with a mycobacterial cellular extract, the protein is presented with the most physiologically germane and unbiased collection of substrates. Successful assignment of protein function in this work, achieved by detection of both consumption and production of two metabolites in the presence of the *Mtb* metabolome, was largely attributed to the advances in liquid chromatography (LC) separation techniques as well as the increased sensitivity and accuracy of mass spectrometry (MS) instruments (Buchholz et al., 2001).

The protein of interest in this study was the gene product of *Rv1248c* from *Mtb*, which was originally assigned, based on sequence homology, as the α-ketoglutarate (α-KG) decarboxylase (E1) component of an α-KG dehydrogenase complex (Cole et al., 1998). Although recombinant Rv1248c was shown in vitro to produce succinic semialdehyde (SSA) via decarboxylation of α-KG (Tian et al., 2005), Rhee and coworkers show that when Rv1248c is presented with its physiological substrate pool, SSA is in fact a product of a slower side reaction and not the physiologic product of Rv1248c.

To unveil the true, physiological function of Rv1248c, the authors incubate recombinant protein and potential cofactors with a small molecule extract of *Mycobacterium bovis* and analyze the time-dependant consumption and production of small molecules using LC-MS. They refer to this technique as Activity Based Metabolomic Profiling (ABMP) and argue its merit for use in identifying the functions of proteins encoded by essential genes in microorganisms. Analysis of the LC-MS experiments showed only two small molecules whose abundance changed over time. These changes, which required the presence of the Rv1248c enzyme, Mg^{2+}, and thiamine diphosphate, included the decrease in abundance of α-KG and concomitant increase in abundance of 5-hydroxylevulinic acid (HLA).

The authors propose the catalytic mechanism of HLA formation as shown in Figure 1A. Once bound to the protein-sequestered thiamine diphosphate (TDP) cofactor, the activated aldehyde α-KG intermediate undergoes condensation with a second substrate, glyoxylate (GLX), to form the unstable β-keto acid, 2-hydroxy-3-oxoadipate (HOA), which then nonenzymatically decarboxylates to form HLA. Confirmation of this mechanism was achieved via several rigorous experiments. First, recombinant Rv1248c was incubated with pure substrates (α-KG and GLX) and cofactors (TDP and Mg^{2+}), and the reaction progress was monitored by LC-MS; both consumption of α-KG and production of HLA occurred only when all reaction components were present. Next, the authors further confirmed the proposed mechanism by trapping the HOA intermediate via methylation (using trifluoromethanesulfonate) to provide dimethyl HOA (Figure 1B), which, unlike HOA, was detectable by LC-MS. Finally, the time-dependant disappearance of α-KG and production of HLA were monitored by ^{1}H-NMR and found to be conditional on the inclusion of protein, substrates and cofactors. Collectively, these biochemical studies suggest that the Rv1248c gene product be referred to as an HOA synthase.

The novel work presented by Rhee and coworkers in this issue of *Chemistry & Biology* is sure to have an impact on the field of functional genomics and its imperative quest to unambiguously assign cellular function to gene products. Not only did the authors apply their developed ABMP method to assign the correct function to a previously mis-annotated gene of *Mtb*, but they also demonstrated that ABMP could be implemented in assigning function to essential genes in

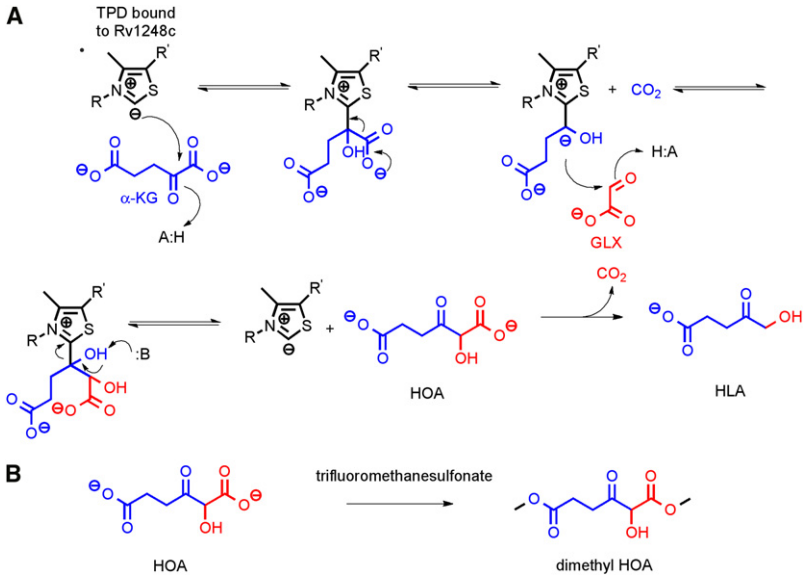

Figure 1. Proposed Catalytic Mechanism of Rv1248c
(A) Following decarboxylation, the activated aldehyde derivative of α-ketoglutarate (α-KG) undergoes condensation with glyoxylate (GLX) to form 2-hydroxy-3-oxoadipate (HOA). HOA spontaneously decarboxylates to form 5-hydroxylevulinate (HLA).
(B) Trapping method used to observe the HOA intermediate.

which knockout mutants can not be created. Since essential microbial gene products have often been targeted for therapeutic development, this profiling method will ultimately aid in both the assignment of promising, novel antimicrobial protein targets and deciphering the vital biochemical function of those targets.

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How to Manipulate Cellular O_2 Sensing

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DOI 10.1016/j.chembiol.2010.04.004

Insufficiency of oxygen supply to mammalian cells activates the transcription factor complex "hypoxia inducible factor" (HIF). In this issue of *Chemistry and Biology*, Smirnova et al. (2010) report on the results of a high throughput screen, which they have used to identify low molecular weight compounds that activate HIF even in the presence of oxygen.

A number of proteins are subjected to a variety of posttranslational modifications, such as phosphorylation, ubiquitination, sumoylation, acetylation, and others. Thus, in many cases, the requirements of cell metabolism can be met by subtle changes of the protein structure. Hydroxylation, a posttranslational modification that introduces hydroxyl groups (-OH) into an amino acid side chain, is now recognized to be of major importance. The first protein shown to undergo hydroxylation was collagen, where hydroxyproline and hydroxylysine residues add to the mechanical stability of collagen fibrils. A list of proteins documented to be hydroxylated has grown significantly over the last decade and includes, most importantly, a master transcriptional regulator termed hypoxia-inducible factor (HIF). The α-subunit of the transcription factor complex HIF is modified in an oxygen-dependent manner by three prolyl hydroxylase domain proteins, PHD1–3 (Epstein et al., 2001) and the asparaginyl hydroxylase factor inhibiting HIF-1, FIH-1, (Lando et al., 2002). While modification of either of two distinct proline residues leads to binding of the von-Hippel-Lindau protein and rapid proteasomal destruction, asparaginyl hydroxylation abrogates binding of the transcriptional coactivator p300/CBP and thus inhibits function of