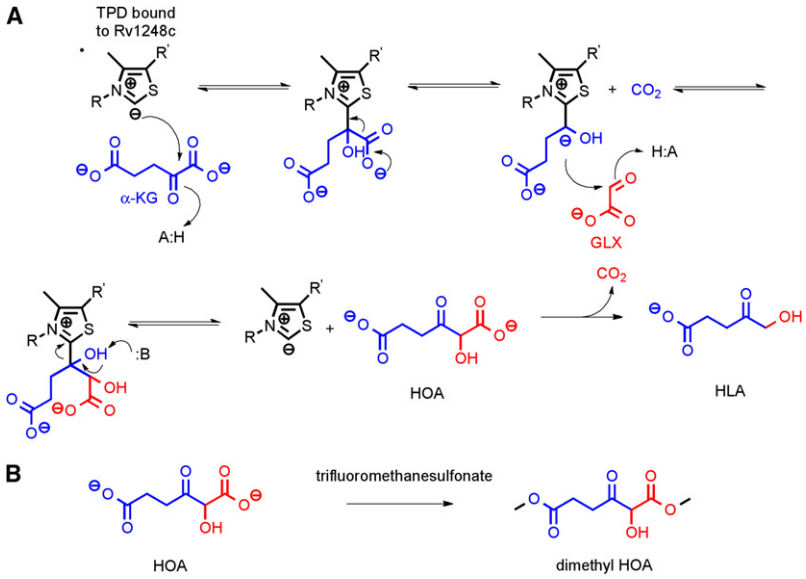

Figure 1. Proposed Catalytic Mechanism of Rv1248c
(A) Following decarboxylation, the activated aldehyde derivative of α-ketoglutarate (α-KG) undergoes condensation with glyoxylate (GLX) to form 2-hydroxy-3-oxoadipate (HOA). HOA spontaneously decarboxylates to form 5-hydroxylevulinate (HLA).
(B) Trapping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

the C-terminal transactivation domain. This means that two distinct modes of HIF activity regulation act in parallel, both of which are oxygen-dependent (Webb et al., 2009).

The PHDs and FIH-1 belong to a superfamily of 2-oxoglutarate-dependent dioxygenases. These enzymes differ with respect to their peptide recognition sequence and thus their primary substrate. However, all of them adopt the conformation of non-heme Fe^{2+} binding dioxygenases and use molecular oxygen and ascorbate as cosubstrates. Another important distinction between the enzymes of this superfamily is their affinity to molecular oxygen (Hirsila et al., 2003). For example, the collagen hydroxylases have a relatively high O_2 affinity, making them rather insensitive to changes in tissue oxygenation. They appear to be more sensitive to a lack of ascorbate, which causes scurvy. In contrast, HIF-α prolyl hydroxylases have a lower affinity to oxygen, so that a reduction of tissue oxygen supply reduces their activity. This leads to an increased stability of HIF-α. Actually, low oxygen affinity makes these enzymes apt to function as cellular oxygen sensors.

Hypoxia, i.e., a situation when cells are not supplied with sufficient amounts of oxygen, can occur in physiology. For example, hypoxia is a significant stimulus for blood vessel growth in the embryo (Dunwoodie, 2009). In clinical medicine, hypoxia is very relevant in many conditions. For example, in anemia, the oxygen transport capacity of the blood is reduced. Tissue hypoxia is also caused when blood flow is impaired, as in atherosclerosis, or when rapidly proliferating cells outgrow the oxygen supply provided, e.g., in a malignant tumor. Strikingly, normal as well as malignant cells respond to a lack of oxygen with the activation of HIF. This transcription factor complex is composed of two subunits: the β-subunit is constitutively expressed, while the α-subunit is constantly produced but has a very short half-life in the presence of oxygen. When oxygen becomes limiting, the PHDs are inactivated, which allows translocation of HIF-α to the nucleus and transcriptional activation of a celltype-dependent set of target genes. The effects of HIF activation include the release of erythropoietin that stimulates the production of red blood cells. HIF also promotes angiogenesis, and a shift of cellular energy metabolism away from mitochondrial respiration and in favor of an increase in glycolysis and anaerobic ATP generation. With respect to pharmacological intervention, the multitude of HIF effects hold promise and threat at the same time. On the one hand, it would be desirable to have a HIF-activating compound to stimulate erythropoiesis and angiogenesis in anemia and ischemic disease, respectively. On the other hand, however, it would be attractive to limit angiogenesis and thus blood supply to tumors via HIF inhibition (Semenza, 2009).

In this issue, Smirnova et al. (2010) report their results on the screen for HIF activators that included 85,000 low molecular weight compounds. For the screen, the authors made use of the molecular switch, which causes degradation of HIF-α in the presence of oxygen. The DNA sequence of the oxygen dependent degradation domain (ODD) of HIF-α was fused to the firefly *luciferase* gene, i.e., the feature of oxygen-dependent instability was transferred to the luciferase protein. When they transfected this artificial DNA into human neuroblastoma cells, they were able to use ODD-luciferase expression as an indicator of PHD activity. Any inhibitor of PHD-dependent HIF degradation could now be identified by an increased luciferase activity in the presence of oxygen. With this approach, Smirnova et al. (2010) identified novel PHD inhibitors that work efficiently at low micromolar concentration. Using in silico modeling, the authors provide compelling evidence that their best compounds act preferentially on PHDs as compared to FIH-1 or other enzymes of the same class. Clearly, these results are encouraging.

One note of caution is, however, that substrates of the PHDs other than HIF-α have been identified, such as IκB kinase-β (Cummins et al., 2006), and the large subunit of RNA polymerase II (Mikhaylova et al., 2008). Because the cell physiological relevance of hydroxylation of alternative PHD substrates is somewhat unclear so far, careful experimentation is warranted to define whether PHD inhibition is indeed a viable approach in vivo. Definitely, the results reported by Smirnova et al. (2010) represent an important step in this direction.

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