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Discovering radical-dependent enzymes in the human gut microbiota

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Human gut microbes have a tremendous impact on human health, in part due to their unique chemical capabilities. In the anoxic environment of the healthy human gut, many important microbial metabolic transformations are performed by radical-dependent enzymes. Although identifying and characterizing these enzymes has been challenging, recent advances in genome and metagenome sequencing have enabled studies of their chemistry and biology. Focusing on the glycyl radical enzyme family, one of the most enriched protein families in the human gut microbiota, we highlight different approaches for discovering radical-dependent enzymes that influence host health and disease.

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Introduction

The human gut is home to trillions of microorganisms that have a tremendous impact on host health [1]. Members of the human gut microbiota catabolize dietary compounds otherwise inaccessible to the host, metabolize drugs in both beneficial and detrimental ways, influence the host immune system, and even alter nervous system function via the 'gut-brain' axis [2–7]. Despite their importance, we have an extremely limited understanding of the molecular mechanisms underlying these interactions [8]. This knowledge gap limits both our fundamental understanding of the gut microbiota and our ability to intervene therapeutically in the many diseases linked to these organisms.

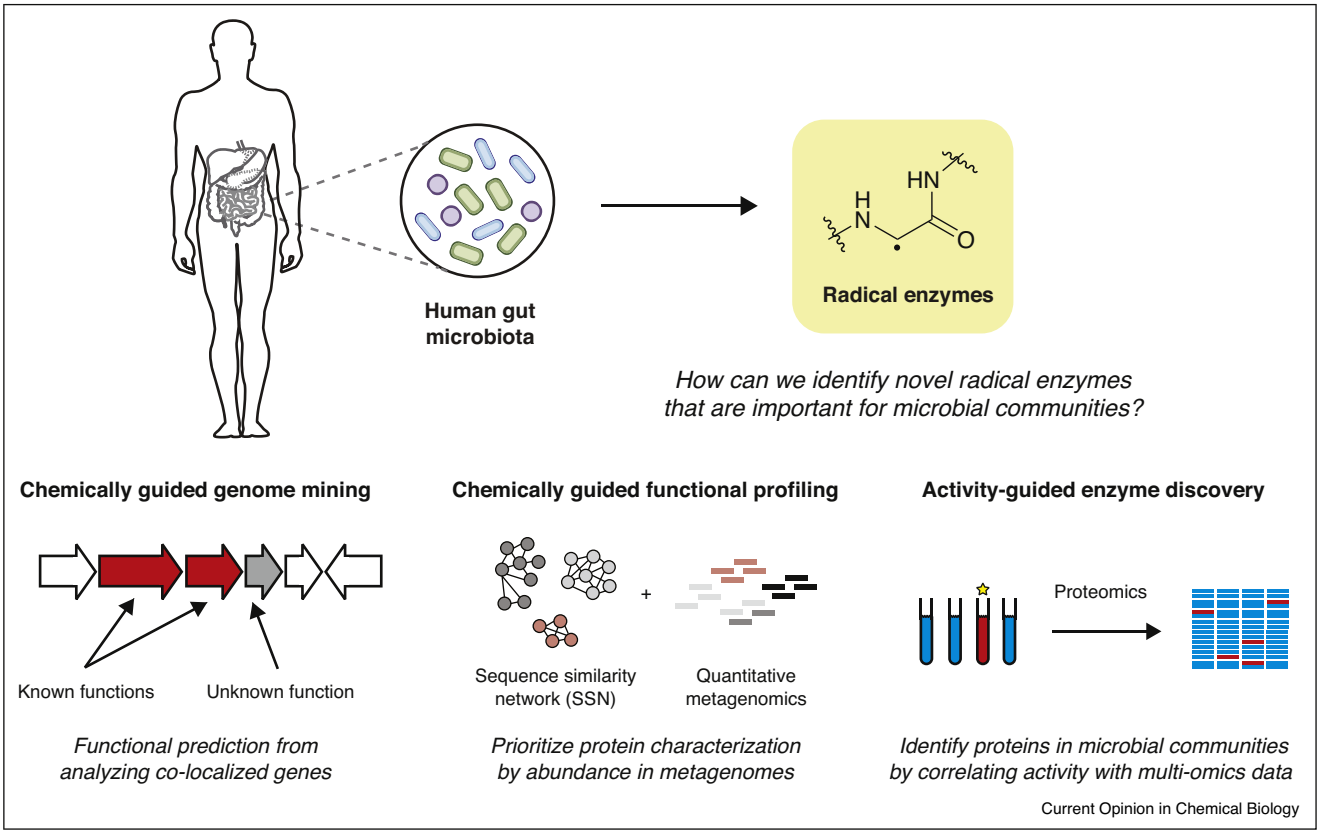
The expanded metabolic capabilities of this microbial community arise from the diverse enzymes collectively encoded by gut microbial genomes. As in other anoxic microbial habitats [9–11], radical-dependent enzymes are particularly abundant in the human gut microbiota as their unique reactivity enables the catalysis of chemically challenging transformations that facilitate anaerobic metabolism [12]. However, the difficulties associated with identifying, expressing, and characterizing these enzymes have limited progress in understanding their specific roles in the human gut microbiota. Recent advances in sequencing technology and bioinformatics now provide an unprecedented opportunity to discover new radical enzymes from these organisms, deepening our understanding of both enzymatic chemistry and the impact of gut microbes on human health [13].

Here, we review recent efforts to discover and characterize new radical-dependent enzymes from the human gut microbiota, with a specific focus on glycyl radical enzymes (GREs) (Figure 1). GREs are encoded by many obligate and facultative anaerobes. Though they represent one of the most abundant and enriched protein families in the human gut microbiota, the full extent of their abundance and diversity in this habitat has been unappreciated. Recent efforts to characterize GREs from the human gut and other microbial habitats illustrate the power of combining modern methods for enzyme discovery with traditional biochemical approaches. We anticipate the lessons drawn from the GREs will enable the discovery of other novel enzymes in the complex environment that is the human gut.

Using chemically guided genome mining to identify choline trimethylamine-lyase and propanediol dehydratase

The GREs form a large protein family that uses conserved chemistry to catalyze a diverse set of reactions [14]. An understanding of GRE catalysis has been used repeatedly to uncover and identify GREs in genomes as well as metagenomes (Figure 2a). In all GREs, an oxygen-sensitive, glycyl radical species is installed in the active site by a cognate activating enzyme (GRE-AE), which is a member of the radical *S*-adenosylmethionine (SAM) enzyme superfamily [15]. This glycyl radical is catalytically essential in all characterized GREs. In addition, all GREs have a conserved cysteine residue positioned between the glycyl radical and the substrate binding site. An initial reaction of the glycyl radical with the cysteine is thought to generate a thiyl radical intermediate. This species can react with substrate to generate a substrate-centered radical, which can react in varying ways to

Figure 1



Overview of different strategies for identifying novel radical-dependent enzymes in the human gut microbiota and other microbial habitats.

generate a product-centered radical. Consecutive hydrogen atom transfers between this species, the conserved cysteine and then the conserved glycine leads to product formation and glycyl radical regeneration. An understanding of the biochemical mechanisms of GREs is crucial for predicting the types of reactions GREs can perform and guiding enzyme discovery.

For example, the identification of choline trimethylamine-lyase (CutC) was enabled by an understanding of GRE chemistry and the chemical logic of microbial metabolism (Figure 2b). Gut microbes have long been known to metabolize choline into trimethylamine (TMA) under anaerobic conditions, and many links between TMA, its oxidized derivative trimethylamine-*N*-oxide (TMAO), and human disease have been reported [16,17]. With this impetus, the Balskus laboratory undertook a search for putative enzymes involved in anaerobic choline metabolism [18]. The first step in choline fermentation is the deamination of choline to TMA and acetaldehyde. The chemical logic of this C–N bond cleavage reaction resembles the first step in ethanolamine metabolism, a C–N bond cleavage carried out by a vitamin B_{12}-dependent enzyme, ethanolamine ammonia-lyase [19]. Recognizing this parallel, the Balskus group searched for homologs of the enzymes from this pathway in the genome of a choline-metabolizing organism. They identified a gene cluster encoding homologs of the acetaldehyde-metabolizing enzymes and microcompartment structural proteins from ethanolamine metabolism, as well as a GRE and a GRE-AE. As GREs were known to catalyze dehydration of 1,2-diols [20], it seemed reasonable that a GRE could catalyze choline deamination. Genetic [21] and *in vitro* biochemical [22,23] experiments verified the activity of CutC, enabling further studies exploring the role of this enzyme in the human gut microbiota [24•].

Another GRE, propanediol dehydratase (PD), was identified in the prominent gut microbe *Roseburia inulinivorans*. Scott *et al.* [25] discovered using shotgun genomic microarrays that when this bacterium is grown on L-fucose, a gene cluster encoding for microcompartment structural proteins, a GRE, and a GRE-AE is upregulated (Figure 2c). As this gene cluster lacked the well-studied B_{12}-dependent propanediol dehydratase (B_{12}-PD) previously found in L-fucose metabolism, the GRE was predicted to perform (*S*)-1,2-propanediol