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Underground metabolism: network-level perspective and biotechnological potential

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A key challenge in molecular systems biology is understanding how new pathways arise during evolution and how to exploit them for biotechnological applications. New pathways in metabolic networks often evolve by recruiting weak promiscuous activities of pre-existing enzymes. Here we describe recent systems biology advances to map such 'underground' activities and to predict and analyze their contribution to new metabolic functions. Underground activities are prevalent in cellular metabolism and can form novel pathways that either enable evolutionary adaptation to new environments or provide bypass to genetic lesions. We also illustrate the potential of integrating computational models of underground metabolism and experimental approaches to study the evolution of novel metabolic phenotypes and advance the field of biotechnology.

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Introduction

Studying how new molecular pathways arise during evolution is important to understand why biological networks look the way we find them today and to inform engineering approaches to design new pathways for biotechnological applications. The most well-accepted theory for the emergence of novel metabolic pathways is that pre-existing enzymes are patched together in new combinations [1]. This 'patchwork' model has two main requirements: enzymes should exhibit one or multiple side activities beyond their native metabolite conversions (also termed promiscuous or underground activities [2,3]), and such side activities should be connected to other chemical reactions in the metabolic network (Figure 1). Enzymatic side activities are generally characterized by poor catalytic efficiencies, thus are likely to be coincidental and not the direct result of natural selection. The chemical mechanisms and evolvability of underground activities have received much attention at the level of individual enzymes [3].

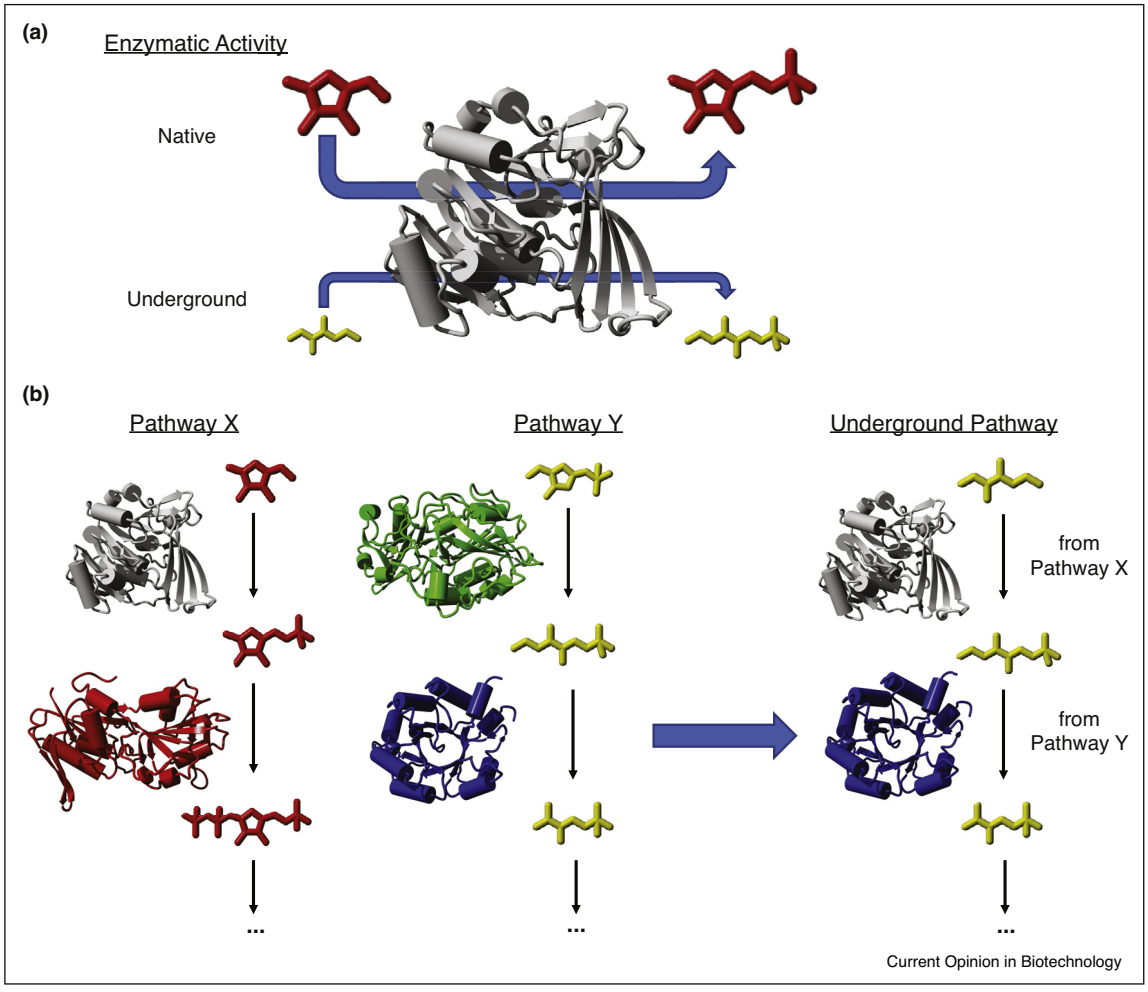
Several lines of empirical evidence indicate that while underground activities are non-evolved, they can have evolutionary and physiological importance. First, enzyme side activities can provide the basis to evolve new nutrient utilization and biosynthetic capabilities both in the laboratory [4,5••,6,7] and in the wild [8–11]. Second, overexpression of enzymes with underground activities can compensate for the deletion of essential enzymes [12]. Third, underground metabolism can produce undesired toxic compounds [13,14], which has led to the evolution of metabolite damage repair systems [15]. For example, it has recently been shown that side activities of two key enzymes of glycolysis produce metabolites that inhibit the pentose phosphate pathway and the production of a regulatory molecule of glycolysis, respectively. A 'repair' enzyme, known as phosphoglycolate phosphatase, dephosphorylates these toxic compounds [14].

Despite various examples illustrating the biological relevance of underground metabolism, it remains largely unknown firstly how many side activities exist, secondly how often they are connected to the existing network to provide new metabolic functions, and finally whether they can be exploited for biotechnological applications. Systems biology provides the necessary framework to explore these open issues via integration of omics techniques and genome-scale metabolic network models (reviewed elsewhere [16,17]). Here we discuss recent advances in mapping and analyzing underground activities on a network-level and survey the biotechnological potential of underground metabolism.

Charting the map of underground metabolism: experimental approaches

Classic empirical discovery of underground activities relies on the quantification of kinetic parameters through

Figure 1



Schematic representation of an underground activity and the 'patchwork' model of pathway evolution. **(a)** An enzyme (grey) with one native (red) and one underground (yellow) metabolite turnover activity. **(b)** The 'patchwork' model of pathway evolution requires at least one underground reaction of a pre-existing enzyme of which the substrates and products can interact with other enzymes in the network. For example, two enzymes (grey and blue) from two different metabolic pathways (X and Y) form a novel pathway, because the native substrate of the enzyme in blue is the product of the underground reaction of the enzyme in grey (see also (a)). Note that new pathways may also be formed by patching together multiple underground activities of existing enzymes.

in vitro enzyme assays. Compared to native activities, underground activities typically have orders of magnitudes lower catalytic efficiency (expressed as k_{cat}/K_m). Hundreds of such enzyme activities have been discovered this way and are now available in public databases such as BRENDA [18]. The large body of biochemical literature has the potential to yield extensive species-specific catalogues of underground activities (Figure 2). For example, in *Escherichia coli*, more than 260 underground reactions have been reported, without known counterparts in the native metabolic network [5••]. This covers approximately 10% of the metabolic reactions [19], which is likely an underestimation of the genuine size of the underground network [3] for several reasons; firstly classic enzyme assays are biased towards particular enzyme families and secondly they typically probe only a small number of substrates. Evidently, there has been an ongoing quest for novel technologies to systematically determine the collection of underground activities (the 'enzyme promiscuome').

So far the most widely applied systematic 'enzyme promiscuome' approach is the scaled-up version of the classic *in vitro* enzyme assays (Figure 2). These medium to large-scale functional profiling efforts typically assay from a handful to hundreds of members from a specific enzyme family for a defined class of chemical transformations [20–22,23•,24]. The most extensive experiment of this kind has carried out substrate profiling with 167 compounds on more than 200 enzymes from the haloalkanoate dehalogenase superfamily [23•]. A universal conclusion from these studies is that enzyme promiscuity in the