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Promiscuity, impersonation and accommodation: evolution of plant specialized metabolism

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Specialized metabolic enzymes and metabolite diversity evolve through a variety of mechanisms including promiscuity, changes in substrate specificity, modifications of gene expression and gene duplication. For example, gene duplication and substrate binding site changes led to the evolution of the glucosinolate biosynthetic enzyme, AtIPMDH1, from a Leu biosynthetic enzyme. BAHD acyltransferases illustrate how enzymatic promiscuity leads to metabolite diversity. The examples 4-coumarate:CoA ligase and aromatic acid methyltransferases illustrate how promiscuity can potentiate the evolution of these specialized metabolic enzymes.

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Introduction

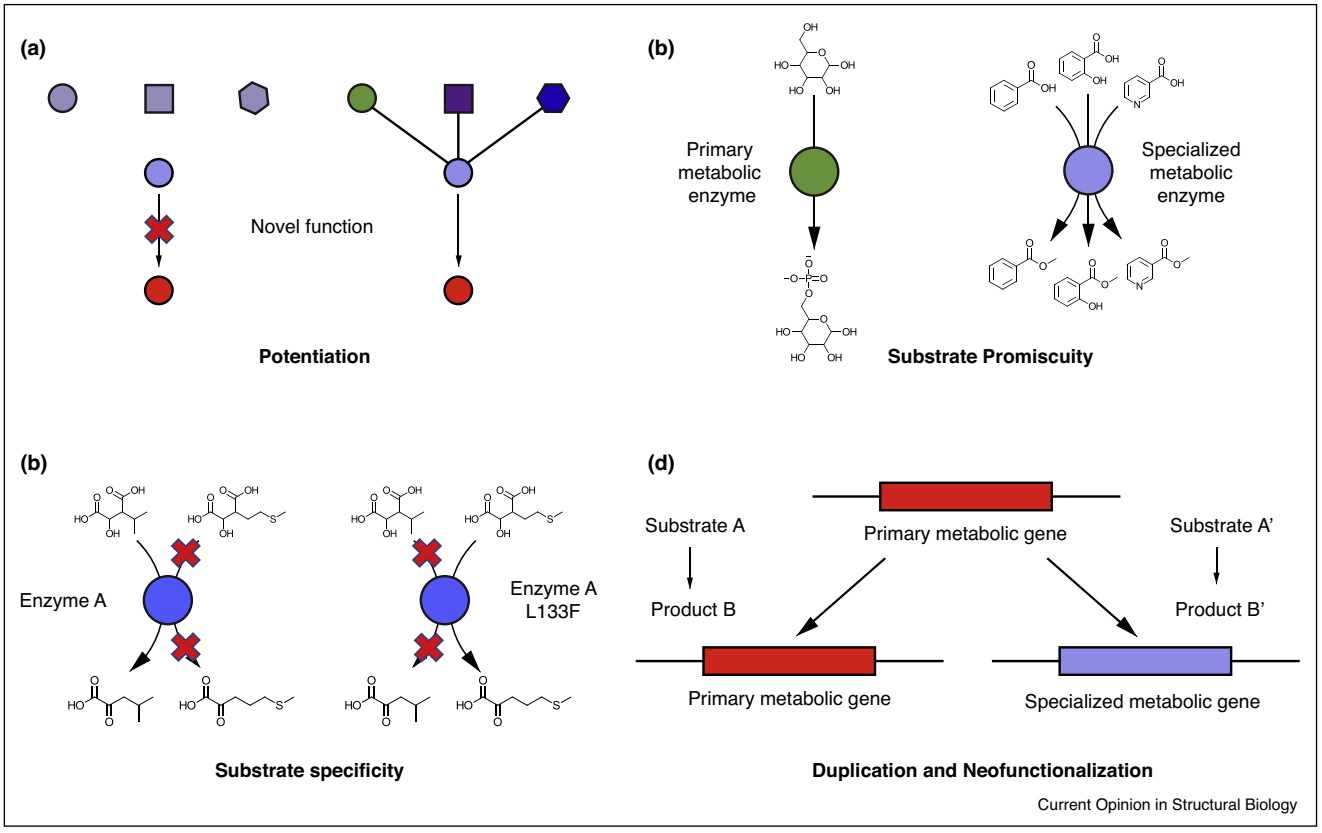
Plant specialized metabolites are lineage-specific compounds, many of which are involved in ecological interactions, such as herbivore defense or pollinator attraction [1,2]. The number of specialized metabolites produced across all plant species is estimated to be in the hundreds of thousands [3]. Specialized metabolic enzymes tend to have lower catalytic efficiency [4] and greater substrate promiscuity [5] than primary metabolic enzymes. This review explores factors involved in enzyme evolution and discusses how these result in metabolite diversity.

We focus on mechanisms that play roles in 'potentiation' (Figure 1a); metabolic examples of what was more generally described by Blount *et al.* [6•] as factors that allow for the realization of a new trait. In recent years, enzymatic promiscuity — the ability of an enzyme to catalyze reaction(s) in addition to its primary reaction — has been documented to 'potentiate' the evolution of new specialized metabolic activities (Figure 1a) [6•]. Substrate promiscuity is documented to play a central role in the evolution of specialized metabolic enzymes (Figure 1b) [5]. Changes in substrate specificity also can result in emergence of novel activities and chemical diversity. Such a shift in substrate specificity can change the primary substrate of an enzyme from an intermediate in an existing biosynthetic pathway to a new substrate, which — in turn — can potentiate novel enzymatic reactions (Figure 1c). Gene duplication and divergence in gene expression patterns or enzyme activities also potentiate the evolution of specialized metabolic enzymes (Figure 1d) [7]. We highlight examples from the past five years in which promiscuity, changes in substrate specificity, gene duplication, and changes in gene expression were shown to play prominent roles in evolution of specialized metabolic enzymes and generation of chemical diversity. These examples illustrate the power of structural analysis — especially in a comparative evolutionary context — to reveal constraints and opportunities to facilitate the modification or engineering of these enzymes.

Gene duplication and changes in substrate specificity in the evolution of a glucosinolate biosynthetic enzyme

Glucosinolates are a group of structurally diverse, amino-acid derived plant specialized metabolites that mediate interactions between crucifers and insects or pathogens [8]. The biosynthesis of methionine-derived glucosinolates involves a repeated three step elongation process similar to Leu biosynthesis: condensation with acetyl-CoA, isomerization, and oxidative decarboxylation to successively add one carbon units to the aliphatic side chain [9]. The glucosinolate oxidative decarboxylation step is catalyzed by the *A. thaliana* isopropylmalate dehydrogenase 1 (AtIPMDH1), while two other *A. thaliana* IPMDH enzymes catalyze the same reaction in Leu biosynthesis; all three enzymes have between 84 and 93% amino acid identity with each other [10,11]. The Leu biosynthetic substrate (3-isopropylmalate) and glucosinolate substrate (3-(2′-methylthio)-ethylmalate), have the same carboxyl and hydroxyl group configuration but differ in side chain length and composition, suggesting similarities in the binding dynamics between the enzymes and substrates in the two pathways (Figure 2a, side chains are in color).

Figure 1



Mechanisms leading to evolution of chemical diversity and enzymatic novelty. **(a)** Potentiation — the different shapes and colors represent factors involved in the evolution of a novel function or enzymatic activity. These factors enable the realization of novel functions or enzymatic activities. In this example, the green circle represents suitable localization of gene expression, the purple square represents substrate availability in the tissue of interest, and the blue hexagon represents the ability of the enzyme to utilize the substrate [6•]. **(b)** Substrate promiscuity — primary metabolic enzymes typically catalyze a specific reaction, while specialized metabolic enzymes tend to be promiscuous and catalyze reactions using multiple substrates. **(c)** Substrate specificity — specific amino acid changes result in alteration of enzyme substrate specificity, resulting in a new enzymatic activity or function. **(d)** Gene duplication and neofunctionalization — a primary metabolic gene is duplicated, facilitating diversification of one isoform into a specialized metabolic function.

The AtIPMDH2 Leu biosynthetic enzyme crystal structure with 3-isopropylmalate (3-IPM) revealed several binding interactions with the polar portion of the substrate [12••]. The structure revealed that residues interacting with the polar groups of 3-IPM are conserved between all IPMDH enzymes (Figure 2b) [13]. This, combined with the similarity of the polar groups of 3-IPM and (3-(2′-methylthio)-ethylmalate), suggested that recognition of the side chain is responsible for substrate discrimination. There are no specific substrate-enzyme interactions between the 3-IPM aliphatic isopropyl side chain and the residues in the largely hydrophobic pocket in the active site (Figure 2b) [12••,13]. Thus, the differences between the glucosinolate biosynthetic enzyme AtIPMDH1 and Leu IPMDH enzymes presumably are responsible for the difference in substrate specificity.

Sequence alignments of Leu IPMDH enzymes with AtIPMDH1 revealed a key feature that affects the ability of the enzyme to discriminate between Leu and glucosinolate substrates. AtIPMDH1 carries a position 137 Leu: Phe change at a site in the hydrophobic pocket that is invariant in the Leu biosynthetic enzymes from bacteria to plants [10,13]. Reciprocal substitutions of residues at this position led to a decrease in the *in vitro* catalytic efficiency with the native substrate — for example, 3-IPM for AtIPMDH2 and AtIPMDH3 and 3-(2′-methylthio)-ethylmalate for AtIPMDH1 — and an increase with the non-native substrate [13]. Because the chemical structures of 3-(2′-methylthio)-ethylmalate and 3-IPM differ only by the length and structure of the side chain, this result demonstrates that the substitution of Leu by Phe is sufficient to facilitate the accommodation of the 3-(2′-methylthio)-ethylmalate side chain in the enzyme [12••].

This example illustrates many themes found throughout the evolution of specialized metabolic enzymes [7]. A