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REVIEW ARTICLE

Location, location! cellular relocalization primes specialized metabolic diversification

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Specialized metabolites are structurally diverse and cell- or tissue-specific molecules produced in restricted plant lineages. In contrast, primary metabolic pathways are highly conserved in plants and produce metabolites essential for all of life, such as amino acids and nucleotides. Substrate promiscuity – the capacity to accept non-native substrates – is a common characteristic of enzymes, and its impact is especially apparent in generating specialized metabolite variation. However, promiscuity only leads to metabolic diversity when alternative substrates are available; thus, enzyme cellular and subcellular localization directly influence chemical phenotypes. We review a variety of mechanisms that modulate substrate availability for promiscuous plant enzymes. We focus on examples where evolution led to modification of the 'cellular context' through changes in cell-type expression, subcellular relocalization, pathway sequestration, and cellular mixing via tissue damage. These varied mechanisms contributed to the emergence of structurally diverse plant specialized metabolites and inform future metabolic engineering approaches.

Introduction

Plants produce hundreds of thousands of structurally diverse specialized metabolites that serve ecological, medicinal, and industrial roles [1]. In contrast to primary – or core – metabolism, specialized metabolites are restricted in their taxonomic distribution, produced in specific cell or tissue types, and often have documented biological activity [2]. Plant primary metabolic pathways provide the building blocks of life including nucleotides, fatty acids, and amino acids, and are more highly conserved than specialized metabolism. As such, primary metabolic pathways are under greater evolutionary constraints, yet can be co-opted into specialized metabolic pathways [3–5]. The expansion and alteration of this core set of ancestral metabolic pathways facilitated the evolution of the vast structural diversity of plant specialized metabolites [6,7].

The classical view of a lock-and-key model for enzyme–substrate interactions first proposed by Emil Fischer and elaborated by Linus Pauling [8] and others – although not inherently wrong – does not allow for the messiness inherent in biology [9]. Enzymes typically can catalyze similar chemical reactions on multiple substrates – a characteristic known as catalytic promiscuity [10–15]. Promiscuity is a common characteristic of enzymes, because selection only requires that a catalyst is good enough to confer a selective advantage to the cell or organism and does not require perfection [16,17].

Abbreviations
ADT, arogenate dehydratase; BIA, benzylisoquinoline alkaloid; CODM, codeinone *O*-demethylase; COR, codeinone reductase; MAM, methylthioalkylmalate synthases; MEP, 2-C-methyl-D-erythritol 4-phosphate pathway; MVA, mevalonate pathway; PvHVS, *Prunella vulgaris* 11-hydroxy vulgarisane synthase; SBS, *Solanum habrochaites* santalene and bergamotene synthase; SpASFF1, *Solanum pennellii* acylsucrose fructofuranosidase 1; T6ODM, thebaine 6-*O*-demethylase; $TyrA_a$, arogenate dehydrogenase; $TyrA_p$, prephenate dehydrogenase.

This provides opportunities for metabolic innovation; however, promiscuous side activities are not physiologically relevant unless alternative (non-native) substrates are available, and promiscuous activities are of high enough catalytic efficiency [10,16,17]. Access to alternative substrates is necessary to unlock the biosynthetic potential of a promiscuous enzyme. While this can occur through various mechanisms, gene duplication plays an especially important role by releasing enzymes from metabolic constraints, priming promiscuous activities for functional innovation.

Paralogous genes emerge through three distinct duplication mechanisms: segmental, tandem, and whole genome [18]. The genetic redundancy created following duplication provides the raw material for functional innovation [19]. Whole-genome duplication is common in plants, with at least five events documented in the Arabidopsis lineage [20,21]. Pseudogenization and gene loss is the most common outcome following duplication [22]. In the rare instances when duplicates are retained, metabolic novelty can occur, either through the alteration of protein function (neofunctionalization) [19,23–25], or the original function can be maintained or split among duplicates (subfunctionalization) [23,26–28]. The recent accumulation of sequence data from divergent organisms enables discovery of the underlying mechanisms involved in gene retention and functional novelty following duplication.

Functional innovation through neofunctionalization occurs via multiple mechanisms, often involving changes in enzymatic activity, which in turn leads to novel products [3]. However, enzymatic activity change is not a prerequisite for metabolic novelty. Another neofunctionalization mechanism involves enzyme relocalization, altering its 'cellular context' while taking advantage of inherently promiscuous enzymes [29–37].

In this review, we describe four different mechanisms by which enzyme relocalization leads to changes in cellular context, in turn potentiating metabolic diversity. First, altered gene regulation modifies cell- or tissue-type enzyme accumulation patterns. Second, alterations in enzyme or pathway subcellular localization occur through loss, gain, or modification of a protein targeting sequence or genes possessing alternative transcription start sites. Third, enzyme relocalization events sequester portions or complete metabolic pathways away from competing reactions and pathways. Fourth, the mixing of cellular compartments through herbivore-induced plant tissue damage generates defensive compound accumulation. These examples highlight the varied evolutionary mechanisms leading to changes in cellular context, which in plants has led to the production of diverse specialized metabolism.

Co-option of an invertase through changes in cell-type expression

Alteration of substrate availability through cell- or tissue-specific gene expression changes in a promiscuous primary metabolic enzyme can prime metabolic innovation. One such example is a trichome-expressed invertase enzyme in the wild tomato *Solanum pennellii*, which produces structurally diverse acylglucoses (Fig. 1).

Acylsugars are defensive compounds produced primarily within the Solanaceae family, consisting of a sugar core decorated with acyl chains [38]. While the sugar core is typically the disaccharide sucrose, some plants accumulate monosaccharide cores including glucose [38]. Cleavage of acylsucroses catalyzed by the cell wall invertase-like enzyme SpASFF1 (*S. pennellii* acylsucrose fructofuranosidase 1) is the final step in *S. pennellii* acylglucose biosynthesis [39]. While homologous invertase enzymes more typically play key roles in plant energy metabolism [40], the larger group of invertases and other glycoside hydrolase enzymes use a broad range of sugar substrates beyond sucrose [41–43].

Relocalization, through trichome gland cell-specific expression, of *SpASFF1* allowed for the emergence of acylglucose biosynthesis in *S. pennellii*. Pyranose ring-acylated acylsucroses accumulate in the trichomes of *S. pennellii,* but not cultivated tomato *Solanum lycopersicum*, which is due to a recent evolutionary alteration in acylsugar biosynthesis [44]. In the trichomes, SpASFF1 can promiscuously cleave pyranose ring-acylated acylsucroses leading to acylglucose production (Fig. 1). Although cultivated tomato possesses an apparently functional ASFF1 ortholog, it does not make acylglucoses; this is partly because tomato ASFF1 does not accumulate in the trichomes [39]. The combination of SpASFF1 relocalization and accumulation of pyranose ring-acylated acylsucroses in the trichomes created a cellular environment that potentiated the evolution of a novel invertase that no longer cleaves sucrose, but instead makes structurally diverse acylglucoses in *S. pennellii*.

Subcellular enzyme relocalization leads to functional innovation in aromatic amino acid biosynthesis

The previous example highlights how sequestration of metabolic activities to specific cell and tissue types can lead to metabolic diversity. Changes in the subcellular localization of metabolic enzymes also can have profound impacts on specialized metabolism, as plant

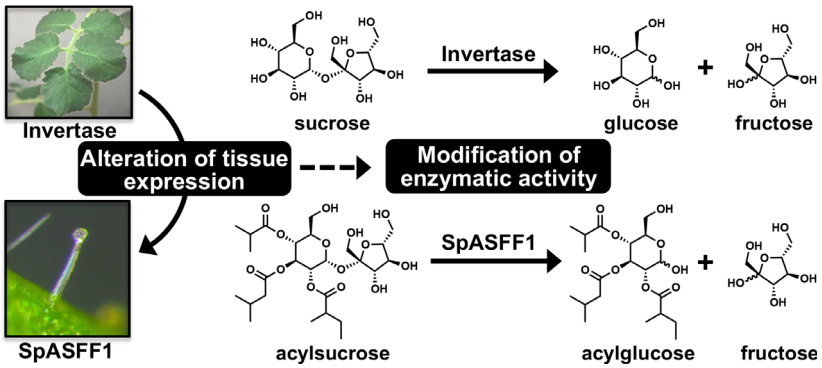

Fig. 1. Alteration of cell-type-specific gene expression of an invertase potentiated acylglucose biosynthesis. Following duplication, gene expression changes and alteration of substrate specificity led to a trichome-specific acylsucrose fructofuranosidase 1 (SpASFF1) that cleaves acylsucroses leading to acylglucose production in *Solanum pennellii*.

aromatic amino acid biosynthesis illustrates. Plant Phe and Tyr biosynthesis and the upstream shikimate pathway occur in the plastids (Fig. 2A), and the branch-point steps are feedback-regulated by the aromatic amino acid end products [45]. The committing steps of Phe and Tyr biosynthesis, arogenate dehydratase (ADT), and arogenate dehydrogenase ($TyrA_a$), respectively, are also plastidic and feedback-regulated by their end products [45,46]. The occurrence of non-feedback-regulated cytosolic isoforms in some plant species is an unusual aspect of aromatic amino acid biosynthesis [47–51]. While the possibility of unique cytosolic Phe and Tyr pathways was raised decades ago [52], these enzymes were only recently confirmed and their *in planta* functions are only now being identified through genetic approaches.

Gene duplication and subsequent subcellular protein relocalization to the cytosol released Phe and Tyr enzymes from plastidic metabolic constraints resulting in functional innovation (Fig. 2). A single petunia *ADT* gene produces both a cytosolic and plastidic ADT, via alternative transcription start sites [53]. Along with the loss of its native substrate, arogenate, cytosolic ADT gained access to the alternative substrate prephenate in the cytosol. Despite ~ 10-fold preference for arogenate over the non-native substrate, prephenate [54], ADT promiscuously converts prephenate into phenylpyruvate; this is further converted into Phe-derived phenylacetaldehyde, a floral scent component involved in pollinator attraction [53,55]. *ADT* is more highly expressed at night than during the day, consistent with diurnally regulated Phe-derived volatile emission in petunia [53]. Following gene family expansion, alteration of substrate accessibility of a promiscuous ADT potentiated the evolution of diverse specialized metabolites in petunia.

Gene duplication and loss of the arogenate dehydrogenase $TyrA_a$ chloroplast transit peptide led to the evolution of cytosolic prephenate dehydrogenase $TyrA_p$ in legumes [56,57]. The relocalization of legume TyrAs altered their cellular environment in two major ways: (a) loss of native substrate availability and (b) diminution of feedback regulation by Tyr, which has lower concentration in the cytosol than plastids [58]. In addition to accepting its native substrate arogenate, cytosolic TyrAs promiscuously catalyze prephenate conversion to 4-hydroxyphenylpyruvate, albeit weakly [56,57]. Following relocalization, legume cytosolic TyrAs diverged in sequence and function: In fact, a single key amino acid mutation results both in a change to prephenate substrate preference and loss of Tyr inhibition (Fig. 2B) [57]. Although the biological role of cytosolic TyrA homologs has yet to be determined, based on analogy to the Phe pathway cytosolic relocalization in petunia, our working hypothesis is that relocalization potentiated Tyr-derived specialized metabolism. These examples suggest that altering promiscuous enzyme–substrate availability through distinct subcellular relocalization mechanisms releases regulatory and substrate availability constraints allowing for the emergence of metabolic novelty.

An example of metabolic innovation without enzyme relocalization but potentiated by alterations in regulatory mechanisms is also found in the Tyr pathway, in the plant order Caryophyllales. The appearance of Tyr-derived betalain pigments evolved multiple times and correlates with the emergence of plastidic $TyrA_a$ enzymes with relaxed Tyr regulation [59] among other metabolic innovations [60]. $TyrA_a$ deregulation allowed for increased Tyr accumulation, potentiating downstream betalain pathway evolution [59]. Taken together, these examples from aromatic amino acid biosynthesis highlight how changes in substrate availability and loss of feedback regulation of promiscuous primary metabolic enzymes – with or without subcellular relocalization – can drive the evolution of specialized metabolic diversity.

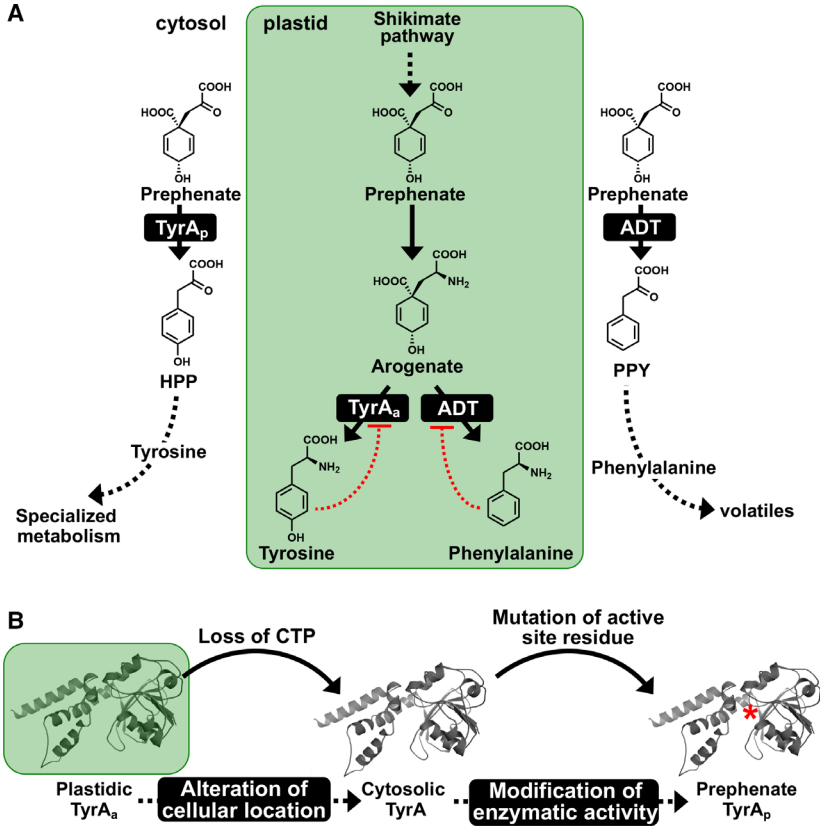

Fig. 2. Subcellular enzyme relocalization leads to diverse specialized metabolism. (A) Tyrosine and phenylalanine biosynthetic pathways and subcellular compartmentalization. Promiscuous, plastidic TyrA$_a$ and ADT enzymes were relocalized to the cytosol where they contribute to diverse metabolic processes. Dotted red lines indicate feedback inhibition. Dotted black lines indicate multiple enzymatic steps. (B) Duplication of plastidic arogenate dehydrogenase (TyrA$_a$) and subsequent loss of the chloroplast transit peptide (CTP) led to cytosolic TyrA. Mutation of a key active site residue (indicated by the red star) of some cytosolic TyrA enzymes led to prephenate-specific TyrA$_p$ (prephenate dehydrogenase) in legumes. Green boxes indicate plastids. ADT, arogenate dehydratase; HPP, 4-hydroxyphenylpyruvate; PPY, phenylpyruvate.

Terpene structural diversity potentiated by enzyme promiscuity and subcellular relocalization

As in Tyr and Phe pathways, there are examples of terpene specialized metabolic diversification being potentiated by subcellular relocalization of promiscuous terpene synthase enzymes providing access to new substrate pools. Terpenes have roles in both primary and specialized metabolism and their structural diversity is derived in part from the catalytic activity of terpene synthases, which create the terpene skeleton from prenyl diphosphate precursors [61,62]. As a group, terpene synthases have broad *in vitro* specificity with some accepting hemi (C5)-, mono (C10)-, sesqui (C15)-, and diterpenoids (C20) [63–66]. Yet, terpene synthases display specificity *in vivo*: Cytosolic terpene synthases generally are involved in sesquiterpene biosynthesis, whereas those in the plastids are usually involved in hemi-, mono-, and diterpenoid biosynthesis [67]. Altering the substrate availability of promiscuous terpene synthases through subcellular relocalization led to the evolution of lineage-specific terpenoids.

Plant terpene synthase prenyl diphosphate substrates are synthesized by distinct organelle-specific pathways: the plastidic 2-C-methyl-D-erythritol 4-phosphate (MEP) and the cytosolic mevalonate (MVA) pathways [67]. This segregates the two potential substrate pools of promiscuous terpene synthases. Extant terpene synthases, including those localized to both the plastids and cytosol, are likely evolutionarily derived from an ancestral plastidic diterpene synthase involved in gibberellin biosynthesis [66,68,69]. Phylogenetic analyses suggest that the relocalization of diterpene synthases from the plastids to the cytosol occurred multiple times [64]. One example of terpene synthase relocalization is the *Prunella vulgaris* diterpene synthase 11-hydroxy vulgarisane synthase (PvHVS), which promiscuously accepts monoterpene, sesquiterpene, and diterpene substrates *in vitro*. However, its plastidic localization prevents access to sesquiterpene precursor pools *in vivo* [64], resulting in specific production of the diterpene vulgarisin in *P. vulgaris* [64]. Another example of a promiscuous terpene synthase increasing metabolite diversity through altered cellular context is the *Solanum habrochaites* santalene and bergamotene synthase (SBS). SBS is localized to the plastids, and phylogenetic analyses show that SBS groups with plastidic diterpene synthases [70]. Like other terpene

synthases, SBS has activity with a range of prenyl diphosphates *in vitro;* however, SBS uniquely produces the sesquiterpenes bergamotene and santalene *in vivo* [70]. Thus, the subcellular relocalization of terpene synthases in plants alters their substrate availability leading to structurally diverse and lineage-specific terpenes.

Pathway sequestration leads to alkaloid diversity in opium poppy

Promiscuity increases the catalytic repertoire of an enzyme and can lead to increased metabolite diversity, but the diversion of pathway intermediates may lead to dead-end products in the presence of multiple substrates [71,72]. Separation of pathway reactions or portions of metabolic pathways within distinct cell types reduces substrate availability, thus decreasing side reactions by promiscuous enzymes. The production of the pharmaceutically important benzylisoquinoline alkaloids (BIAs), such as the analgesic morphine, represents one such example [2]. The BIA intermediate (*S*)-norcoclaurine is modified through tailoring reactions to produce the structural diversity characteristic of BIAs (Fig. 3A). Many of these tailoring enzymes have promiscuous activities (Fig. 3B) [73], and their sequestration in distinct cell types maintains product specificity in the BIA pathway.

Biosynthesis of morphinan-type alkaloids – including morphine and codeine – is separated into two cell types: upstream reactions in the sieve elements and the final three steps in laticifers [74]. The laticifer-localized steps are catalyzed by the promiscuous tailoring enzymes thebaine 6-*O*-demethylase (T6ODM), codeinone reductase (COR), and codeinone *O*-demethylase (CODM) [73,75,76]. A key outcome of pathway separation is that, although CODM and T6ODM have activity with many nonphysiologically relevant BIA intermediates *in vitro*, these substrates are unavailable *in vivo* [75] (Fig. 3A). Pathway sequestration may have provided or potentiated the evolution of novel transport mechanisms, which contribute an additional layer to control cell-type specific substrate availability [77]. Limiting the substrate availability of morphine biosynthetic enzymes in the laticifers allows tailoring enzymes to use their promiscuity to produce structurally diverse BIAs without side reactions. The above examples illustrate how the relocalization of promiscuous enzymes at the organelle, cell, and tissue levels alters their cellular context leading to diverse plant specialized metabolism.

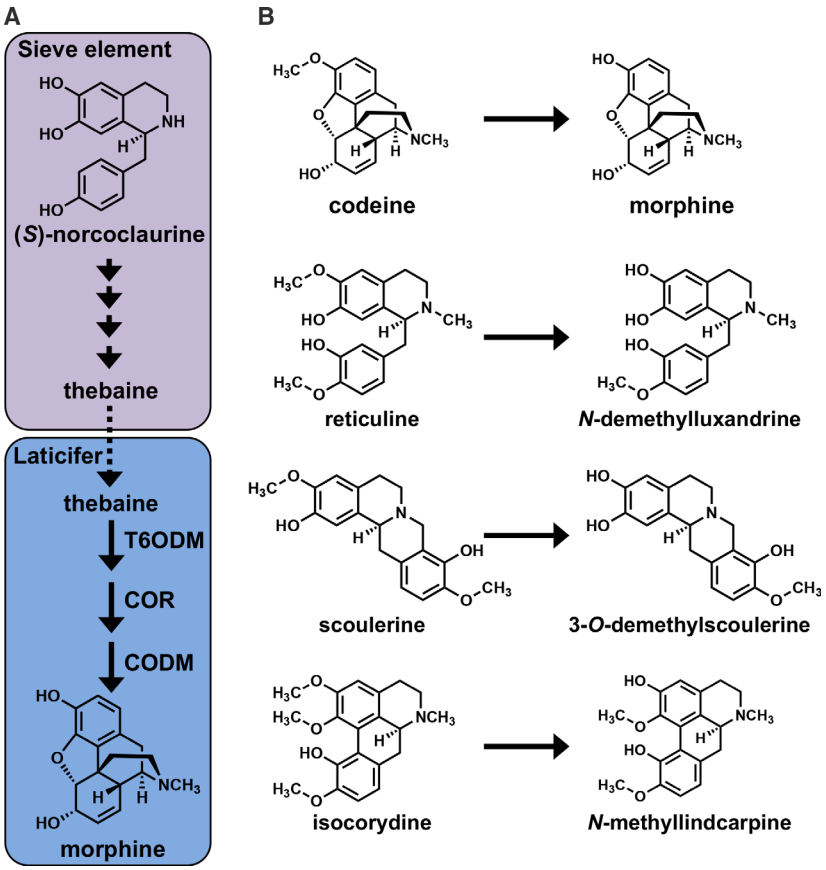

Fig. 3. Sequestration of benzylisoquinoline alkaloid intermediates and enzymatic activities in two cell types. (A) Benzylisoquinoline alkaloids are derived from (*S*)-norcoclaurine. The final three steps of morphine biosynthesis, thebaine 6-*O*-demethylase (T6ODM), codeinone reductase (COR), and codeinone *O*-demethylase (CODM), are localized in laticifer cells (blue), sequestered from the sieve element-localized upstream reactions (purple). A dotted line indicates that the intermediate thebaine is likely transported from sieve elements to laticifers. (B) Some examples of the structurally diverse substrates used - and metabolites produced by —— CODM *in vitro*.

Glucosinolates and release of the 'mustard bomb'

Glucosinolate specialized metabolite synthesis and catabolism provide multiple examples of changes in cellular contexts in modulating structural diversity and biological activity of specialized metabolites. With few exceptions, glucosinolates are produced within plants of the order Capparales, which includes the Brassicaceae family, and upon hydrolysis, glucosinolates produce various defense-related compounds.

Similar to BIA biosynthesis in opium poppy, the stages of glucosinolate synthesis occur in distinct subcellular compartments (Fig. 4). There are three modules: First, amino acids, typically from methionine, are elongated in the plastid; second, the amino acid is converted into a core glucosinolate structure in the cytosol; and third, various modifications can be made to the core structure in the cytosol (Fig. 4B). Plastidic chain elongation sequesters intermediates away from enzymes involved in cytosolic modules (Fig. 4A) [78,79] limiting potential side reactions of promiscuous chain elongation enzymes. Methylthioalkylmalate (MAM) synthases catalyze the first step of chain elongation of methionine-derived glucosinolate precursors in a process analogous to the isopropylmalate synthase committing step in leucine biosynthesis through condensation of acetyl-CoA to the 2-oxoacid of methionine [80–82]. Repeated rounds of elongation are facilitated by the promiscuity of MAM synthases accepting elongated substrates [81]. Pathway sequestration protects against the diversion of nonelongated intermediates entering the core biosynthetic module. This spatial partitioning of metabolic modules facilitates elongated glucosinolate production.

Glucosinolate core biosynthetic enzymes are not only promiscuous and spatially separated from chain elongation steps, but also show tissue-specific expression patterns. For example, CYP83 family enzymes have broad activity with amino acid-derived aldoximes [83], and in *Brassica juncea* CYP83A1, homologs show distinct gene expression patterns in different tissues [84]. *BjuCYP83A1-1* controls the accumulation of aliphatic glucosinolates and is highly expressed in the roots, siliques, and in response to wounding [84]. Tissue-specific expression of the core module further modulates substrate availability and spatial distribution of glucosinolates.

Tissue damage by insect herbivory creates the 'mustard bomb', which is an extreme example of a change

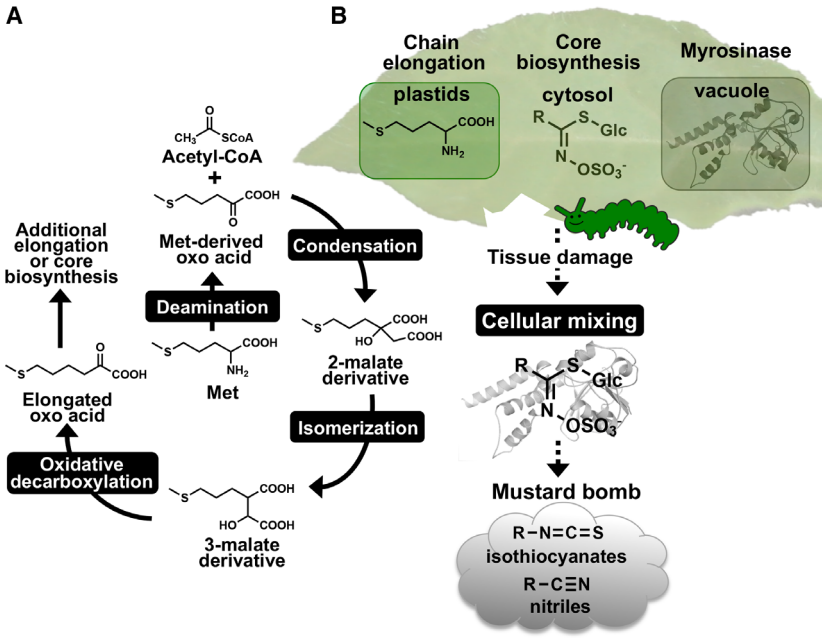

Fig. 4. Enzyme promiscuity in chain elongation coupled with pathway sequestration and cellular mixing lead to glucosinolate diversity and release of the 'mustard bomb'. (A) Plastidic glucosinolate chain elongation pathway. Methylthioalkylmalate synthase catalyzes the initial condensation reaction with an amino acid-derived oxoacid (here methionine (Met)) and acetyl-CoA. Isomerization and oxidative decarboxylation produce an elongated oxoacid that can be further elongated or enter glucosinolate core biosynthesis in the cytosol. (B) Glucosinolate biosynthesis occurs in the plastids (chain elongation) and the cytosol (core biosynthesis) sequestered from vacuolar myrosinase. The mixing of subcellular compartments through tissue damage by an herbivore initiates the mustard bomb as the myrosinase can now interact with its substrate, releasing toxic nitriles and isothiocyanates. Green and gray boxes indicate plastids and vacuole, respectively. R represents the amino acid-derived portion of a glucosinolate.

in cellular context, in this case through mixing of cellular contents. Following tissue damage, vacuolar-confined myrosinase reacts with glucosinolates to release reactive isothiocyanates and nitriles (Fig. 4B) [78,79]. In undamaged tissue, the production of these potentially harmful reaction products is prevented by compartmentalization of the vacuolar myrosinase and glucosinolates in distinct specialized cell types [79]. Maintenance of separate cellular environments through sequestration of glucosinolates away from the catabolic enzyme activity regulates the glucosinolate defense response, mitigating damage to the plant until herbivory causes tissue wounding.

In summary, glucosinolate synthesis and activation show how coordination of varied cellular context-altering mechanisms, including subcellular pathway sequestration, tissue-specific gene expression, and tissue damage, all contribute to the production of specialized defense metabolites.

Future outlook

Not only have enzyme relocalization mechanisms provided opportunities for the evolution of plant specialized metabolism, but engineering of spatial compartmentalization can be used to expand the biosynthetic capacities and reduce metabolic constraints of synthetic biology platforms [85,86]. Recent examples used the high metabolic activity of developing tomato fruit to produce endogenous or novel plant metabolites. Tomato fruit was engineered to accumulate the phenylpropanoid-derived compounds resveratrol and genistein, through tissue-specific co-expression of biosynthetic enzymes and a MYB transcription factor from other plants [87]. Another example produced geraniol and geraniol-derived terpenes in tomato fruit, which enhanced the flavor, through tomato-specific expression of an *Ocimum basilicum* terpene synthase [88]. These examples show that hijacking specific metabolically active tissue types is a successful metabolic engineering approach. The remarkable biosynthetic potential of specialized and core metabolism across the green plant lineage, along with their extraordinary organ-, tissue-, cell-, and subcellular-metabolic capacities, provides unlimited possibilities for future metabolic engineering through hijacking of cellular context.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

CAS and RLL wrote and edited the manuscript.

References

1 Pichersky E & Lewinsohn E (2011) Convergent evolution in plant specialized metabolism. *Annu Rev Plant Biol* **62**, 549–566.
2 Beaudoin GAW & Facchini PJ (2014) Benzylisoquinoline alkaloid biosynthesis in opium poppy. *Planta* **240**, 19–32.
3 Leong BJ & Last RL (2017) Promiscuity, impersonation and accommodation: evolution of plant specialized metabolism. *Curr Opin Struct Biol* **47**, 105–112.
4 Moghe GD & Last RL (2015) Something old, something new: conserved enzymes and the evolution of novelty in plant specialized metabolism. *Plant Physiol* **169**, 1512–1523.
5 Weng J-K (2014) The evolutionary paths towards complexity: a metabolic perspective. *New Phytol* **201**, 1141–1149.
6 Noda-Garcia L, Liebermeister W & Tawfik DS (2018) Metabolite-enzyme coevolution: from single enzymes to metabolic pathways and networks. *Annu Rev Biochem* **87**, 187–216.
7 Weng J-K, Philippe RN & Noel JP (2012) The rise of chemodiversity in plants. *Science* **336**, 1667–1670.
8 Pauling L (1946) Molecular architecture and biological reactions. *Chem Eng News Arch* **24**, 1375–1377.
9 Tawfik DS (2010) Messy biology and the origins of evolutionary innovations. *Nat Chem Biol* **6**, 692–696.
10 Khersonsky O & Tawfik DS (2010) Enzyme promiscuity: a mechanistic and evolutionary perspective. *Annu Rev Biochem* **79**, 471–505.
11 Kuznetsova E, Proudfoot M, Gonzalez CF, Brown G, Omelchenko MV, Borozan I, Carmel L, Wolf YI, Mori H, Savchenko AV *et al.* (2006) Genome-wide analysis of substrate specificities of the *Escherichia coli* haloacid dehalogenase-like phosphatase family. *J Biol Chem* **281**, 36149–36161.
12 Leščić Ašler I, Ivić N, Kovačić F, Schell S, Knorr J, Krauss U, Wilhelm S, Kojić-Prodić B & Jaeger K-E (2010) Probing enzyme promiscuity of SGNH hydrolases. *ChemBioChem* **11**, 2158–2167.

13 McMahon MD & Prather KLJ (2014) Functional screening and *in vitro* analysis reveal thioesterases with enhanced substrate specificity profiles that improve short-chain fatty acid production in *Escherichia coli*. *Appl Environ Microbiol* **80**, 1042–1050.
14 Pandya C, Farelli JD, Dunaway-Mariano D & Allen KN (2014) Enzyme promiscuity: engine of evolutionary innovation. *J Biol Chem* **289**, 30229–30236.
15 Peracchi A (2018) The limits of enzyme specificity and the evolution of metabolism. *Trends Biochem Sci* **43**, 984–996.
16 Copley SD (2015) An evolutionary biochemist's perspective on promiscuity. *Trends Biochem Sci* **40**, 72–78.
17 Bar-Even A, Noor E, Savir Y, Liebermeister W, Davidi D, Tawfik DS & Milo R (2011) The moderately efficient enzyme: evolutionary and physicochemical trends shaping enzyme parameters. *Biochemistry* **50**, 4402–4410.
18 Panchy N, Lehti-Shiu M & Shiu S-H (2016) Evolution of gene duplication in plants. *Plant Physiol* **171**, 2294–2316.
19 Ohno S (1970) Evolution by Gene Duplication. Springer, New York, NY.
20 Bowers JE, Chapman BA, Rong J & Paterson AH (2003) Unravelling angiosperm genome evolution by phylogenetic analysis of chromosomal duplication events. *Nature* **422**, 433–438.
21 Jiao Y, Wickett NJ, Ayyampalayam S, Chanderbali AS, Landherr L, Ralph PE, Tomsho LP, Hu Y, Liang H, Soltis PS *et al*. (2011) Ancestral polyploidy in seed plants and angiosperms. *Nature* **473**, 97–100.
22 Adams KL & Wendel JF (2005) Polyploidy and genome evolution in plants. *Curr Opin Plant Biol* **8**, 135–141.
23 Carretero-Paulet L & Fares MA (2012) Evolutionary dynamics and functional specialization of plant paralogs formed by whole and small-scale genome duplications. *Mol Biol Evol* **29**, 3541–3551.
24 Conant GC & Wolfe KH (2008) Turning a hobby into a job: how duplicated genes find new functions. *Nat Rev Genet* **9**, 938–950.
25 Jensen RA (1976) Enzyme recruitment in evolution of new function. *Annu Rev Microbiol* **30**, 409–425.
26 Dean EJ, Davis JC, Davis RW & Petrov DA (2008) Pervasive and persistent redundancy among duplicated genes in yeast. *PLoS Genet* **4**, e1000113.
27 Force A, Lynch M, Pickett FB, Amores A, Yan YL & Postlethwait J (1999) Preservation of duplicate genes by complementary, degenerative mutations. *Genetics* **151**, 1531–1545.
28 Hanada K, Kuromori T, Myouga F, Toyoda T, Li W-H & Shinozaki K (2009) Evolutionary persistence of functional compensation by duplicate genes in Arabidopsis. *Genome Biol Evol* **1**, 409–414.
29 Blanc G & Wolfe KH (2004) Functional divergence of duplicated genes formed by polyploidy during Arabidopsis evolution. *Plant Cell* **16**, 1679–1691.
30 Byun SA & Singh S (2013) Protein subcellular relocalization increases the retention of eukaryotic duplicate genes. *Genome Biol Evol* **5**, 2402–2409.
31 Hanada K, Kuromori T, Myouga F, Toyoda T & Shinozaki K (2009) Increased expression and protein divergence in duplicate genes is associated with morphological diversification. *PLoS Genet* **5**, e1000781.
32 Kliebenstein DJ (2008) A role for gene duplication and natural variation of gene expression in the evolution of metabolism. *PLoS ONE* **3**, e1838.
33 Liu S-L, Pan AQ & Adams KL (2014) Protein subcellular relocalization of duplicated genes in Arabidopsis. *Genome Biol Evol* **6**, 2501–2515.
34 Roulin A, Auer PL, Libault M, Schlueter J, Farmer A, May G, Stacey G, Doerge RW & Jackson SA (2013) The fate of duplicated genes in a polyploid plant genome. *Plant J* **73**, 143–153.
35 Wang Y, Wang X & Paterson AH (2012) Genome and gene duplications and gene expression divergence: a view from plants. *Ann N Y Acad Sci* **1256**, 1–14.
36 Wapinski I, Pfeffer A, Friedman N & Regev A (2007) Natural history and evolutionary principles of gene duplication in fungi. *Nature* **449**, 54–61.
37 Yim WC, Lee B-M & Jang CS (2009) Expression diversity and evolutionary dynamics of rice duplicate genes. *Mol Genet Genomics* **281**, 483–493.
38 Fan P, Leong BJ & Last RL (2019) Tip of the trichome: evolution of acylsugar metabolic diversity in Solanaceae. *Curr Opin Plant Biol* **49**, 8–16.
39 Leong BJ, Lybrand DB, Lou Y-R, Fan P, Schilmiller AL & Last RL (2019) Evolution of metabolic novelty: a trichome-expressed invertase creates specialized metabolic diversity in wild tomato. *Sci Adv* **5**, eaaw3754.
40 Wan H, Wu L, Yang Y, Zhou G & Ruan Y-L (2018) Evolution of sucrose metabolism: the dichotomy of invertases and beyond. *Trends Plant Sci* **23**, 163–177.
41 Coninck BD, Roy KL, Francis I, Clerens S, Vergauwen R, Halliday AM, Smith SM, Laere AV & Ende WVD (2005) Arabidopsis AtcwINV3 and 6 are not invertases but are fructan exohydrolases (FEHs) with different substrate specificities. *Plant Cell Environ* **28**, 432–443.
42 Ruan Y-L, Jin Y, Yang Y-J, Li G-J & Boyer JS (2010) Sugar input, metabolism, and signaling mediated by invertase: roles in development, yield potential, and response to drought and heat. *Mol Plant* **3**, 942–955.
43 Van den Ende W, Lammens W, Van Laere A, Schroeven L & Le Roy K (2009) Donor and acceptor substrate selectivity among plant glycoside hydrolase family 32 enzymes. *FEBS J* **276**, 5788–5798.
44 Fan P, Miller AM, Liu X, Jones AD & Last RL (2017) Evolution of a flipped pathway creates metabolic

innovation in tomato trichomes through BAHD enzyme promiscuity. *Nat Commun* **8**, 2080.
45 Maeda H & Dudareva N (2012) The shikimate pathway and aromatic amino acid biosynthesis in plants. *Annu Rev Plant Biol* **63**, 73–105.
46 Schenck CA & Maeda HA (2018) Tyrosine biosynthesis, metabolism, and catabolism in plants. *Phytochemistry* **149**, 82–102.
47 Rubin JL & Jensen RA (1985) Differentially regulated isozymes of 3-deoxy-d-arabino-heptulosonate-7-phosphate synthase from seedlings of *Vigna radiata* [L.] Wilczek. *Plant Physiol* **79**, 711–718.
48 Siehl D (1999) The biosynthesis of tryptophan, tyrosine, and phenylalanine from chorismate. In Plant Amino Acids: Biochemistry and Biotechnology (Singh B, ed.), pp. 171–204. CRC Press, New York, NY.
49 Ganson RJ, D'Amato TA & Jensen RA (1986) The two-isozyme system of 3-deoxy-d-arabino-heptulosonate 7-phosphate synthase in *Nicotiana silvestris* and other higher plants. *Plant Physiol* **82**, 203–210.
50 Eberhard J, Ehrler T, Epple P, Felix G, Raesecke H-R, Amrhein N & Schmid J (1996) Cytosolic and plastidic chorismate mutase isozymes from *Arabidopsis thaliana*: molecular characterization and enzymatic properties. *Plant J* **10**, 815–821.
51 Kroll K, Holland CK, Starks CM & Jez JM (2017) Evolution of allosteric regulation in chorismate mutases from early plants. *Biochem J* **474**, 3705–3717.
52 Schmid J & Amrhein N (1995) Molecular organization of the shikimate pathway in higher plants. *Phytochemistry* **39**, 737–749.
53 Qian Y, Lynch JH, Guo L, Rhodes D, Morgan JA & Dudareva N (2019) Completion of the cytosolic post-chorismate phenylalanine biosynthetic pathway in plants. *Nat Commun* **10**, 15.
54 Maeda H, Shasany AK, Schnepp J, Orlova I, Taguchi G, Cooper BR, Rhodes D, Pichersky E & Dudareva N (2010) RNAi suppression of *Arogenate dehydratase1* reveals that phenylalanine is synthesized predominantly via the arogenate pathway in petunia petals. *Plant Cell* **22**, 832–849.
55 Theis N (2006) Fragrance of Canada thistle (*Cirsium arvense*) attracts both floral herbivores and pollinators. *J Chem Ecol* **32**, 917–927.
56 Schenck CA, Chen S, Siehl DL & Maeda HA (2015) Non-plastidic, tyrosine-insensitive prephenate dehydrogenases from legumes. *Nat Chem Biol* **11**, 52–57.
57 Schenck CA, Holland CK, Schneider MR, Men Y, Lee SG, Jez JM & Maeda HA (2017) Molecular basis of the evolution of alternative tyrosine biosynthetic routes in plants. *Nat Chem Biol* **13**, 1029–1035.
58 Szecowka M, Heise R, Tohge T, Nunes-Nesi A, Vosloh D, Huege J, Feil R, Lunn J, Nikoloski Z, Stitt M *et al.* (2013) Metabolic fluxes in an illuminated Arabidopsis rosette. *Plant Cell* **25**, 694–714.
59 Lopez-Nieves S, Yang Y, Timoneda A, Wang M, Feng T, Smith SA, Brockington SF & Maeda HA (2018) Relaxation of tyrosine pathway regulation underlies the evolution of betalain pigmentation in Caryophyllales. *New Phytol.* **217**, 896–908.
60 Timoneda A, Feng T, Sheehan H, Walker-Hale N, Pucker B, Lopez-Nieves S, Guo R & Brockington S (2019) The evolution of betalain biosynthesis in Caryophyllales. *New Phytol* **224**, 71–85.
61 Pichersky E & Raguso RA (2018) Why do plants produce so many terpenoid compounds? *New Phytol* **220**, 692–702.
62 Vranová E, Coman D & Gruissem W (2013) Network analysis of the MVA and MEP pathways for isoprenoid synthesis. *Annu Rev Plant Biol* **64**, 665–700.
63 Crock J, Wildung M & Croteau R (1997) Isolation and bacterial expression of a sesquiterpene synthase cDNA clone from peppermint (*Mentha x piperita*, L.) that produces the aphid alarm pheromone (*E*)-beta-farnesene. *Proc Natl Acad Sci USA* **94**, 12833–12838.
64 Johnson SR, Bhat WW, Sadre R, Miller GP, Garcia AS & Hamberger B (2019) Promiscuous terpene synthases from *Prunella vulgaris* highlight the importance of substrate and compartment switching in terpene synthase evolution. *New Phytol* **223**, 323–335.
65 Pazouki L, Memari HR, Kännaste A, Bichele R & Niinemets Ü (2015) Germacrene A synthase in yarrow (*Achillea millefolium*) is an enzyme with mixed substrate specificity: gene cloning, functional characterization and expression analysis. *Front Plant Sci* **6**, 111.
66 Pazouki L & Niinemets Ü (2016) Multi-substrate terpene synthases: their occurrence and physiological significance. *Front Plant Sci* **7**, 1019.
67 Tholl D (2015) Biosynthesis and biological functions of terpenoids in plants. *Adv Biochem Eng Biotechnol* **148**, 63–106.
68 Hillwig ML, Xu M, Toyomasu T, Tiernan MS, Wei G, Cui G, Huang L & Peters RJ (2011) Domain loss has independently occurred multiple times in plant terpene synthase evolution. *Plant J* **68**, 1051–1060.
69 Chen F, Tholl D, Bohlmann J & Pichersky E (2011) The family of terpene synthases in plants: a mid-size family of genes for specialized metabolism that is highly diversified throughout the kingdom. *Plant J* **66**, 212–229.
70 Sallaud C, Rontein D, Onillon S, Jabès F, Duffé P, Giacalone C, Thoraval S, Escoffier C, Herbette G, Leonhardt N *et al.* (2009) A novel pathway for sesquiterpene biosynthesis from Z, Z-farnesyl pyrophosphate in the wild tomato *Solanum habrochaites*. *Plant Cell* **21**, 301–317.
71 Alcantara J, Bird DA, Franceschi VR & Facchini PJ (2005) Sanguinarine biosynthesis is associated with the

endoplasmic reticulum in cultured opium poppy cells after elicitor treatment. *Plant Physiol* **138**, 173–183.
72 Mackie A, Keseler IM, Nolan L, Karp PD & Paulsen IT (2013) Dead end metabolites – defining the known unknowns of the *E. coli* metabolic network. *PLoS ONE* **8**, e75210.
73 Dastmalchi M, Park MR, Morris JS & Facchini P (2018) Family portraits: the enzymes behind benzylisoquinoline alkaloid diversity. *Phytochem Rev* **17**, 249–277.
74 Onoyovwe A, Hagel JM, Chen X, Khan MF, Schriemer DC & Facchini PJ (2013) Morphine biosynthesis in opium poppy involves two cell types: sieve elements and laticifers. *Plant Cell* **25**, 4110–4122.
75 Farrow SC & Facchini PJ (2013) Dioxygenases catalyze *O*-demethylation and *O,* O-demethylenation with widespread roles in benzylisoquinoline alkaloid metabolism in opium poppy. *J Biol Chem* **288**, 28997–29012.
76 Unterlinner B, Lenz R & Kutchan TM (1999) Molecular cloning and functional expression of codeinone reductase: the penultimate enzyme in morphine biosynthesis in the opium poppy *Papaver somniferum*. *Plant J* **18**, 465–475.
77 Dastmalchi M, Chang L, Chen R, Yu L, Chen X, Hagel J & Facchini PJ (2019) Purine permease-type benzylisoquinoline alkaloid transporters in opium poppy. *Plant Physiol*. https://doi.org/10.1104/pp.19.00565
78 Sønderby IE, Geu-Flores F & Halkier BA (2010) Biosynthesis of glucosinolates–gene discovery and beyond. *Trends Plant Sci* **15**, 283–290.
79 Halkier BA & Gershenzon J (2006) Biology and biochemistry of glucosinolates. *Annu Rev Plant Biol* **57**, 303–333.
80 Benderoth M, Pfalz M & Kroymann J (2009) Methylthioalkylmalate synthases: genetics, ecology and evolution. *Phytochem Rev* **8**, 255.
81 Kumar R, Lee SG, Augustine R, Reichelt M, Vassão DG, Palavalli MH, Allen A, Gershenzon J, Jez JM & Bisht NC (2019) Molecular basis of the evolution of methylthioalkylmalate synthase and the diversity of methionine-derived glucosinolates. *Plant Cell* **31**, 1633–1647.
82 de Kraker J-W & Gershenzon J (2011) From amino acid to glucosinolate biosynthesis: protein sequence changes in the evolution of methylthioalkylmalate synthase in *Arabidopsis*. *Plant Cell* **23**, 38–53.
83 Bak S & Feyereisen R (2001) The involvement of two p450 enzymes, CYP83B1 and CYP83A1, in auxin homeostasis and glucosinolate biosynthesis. *Plant Physiol* **127**, 108–118.
84 Meenu AR, Majee M, Pradhan AK & Bisht NC (2015) Genomic origin, expression differentiation and regulation of multiple genes encoding CYP83A1, a key enzyme for core glucosinolate biosynthesis, from the allotetraploid *Brassica juncea*. *Planta* **241**, 651–665.
85 Alam MT, Olin-Sandoval V, Stincone A, Keller MA, Zelezniak A, Luisi BF & Ralser M (2017) The self-inhibitory nature of metabolic networks and its alleviation through compartmentalization. *Nat Commun* **8**, 16018.
86 Erb TJ, Jones PR & Bar-Even A (2017) Synthetic metabolism: metabolic engineering meets enzyme design. *Curr Opin Chem Biol* **37**, 56–62.
87 Zhang Y, Butelli E, Alseekh S, Tohge T, Rallapalli G, Luo J, Kawar PG, Hill L, Santino A, Fernie AR *et al.* (2015) Multi-level engineering facilitates the production of phenylpropanoid compounds in tomato. *Nat Commun* **6**, 8635.
88 Davidovich-Rikanati R, Sitrit Y, Tadmor Y, Iijima Y, Bilenko N, Bar E, Carmona B, Fallik E, Dudai N, Simon JE *et al.* (2007) Enrichment of tomato flavor by diversion of the early plastidial terpenoid pathway. *Nat Biotechnol* **25**, 899–901.