

# Evolutionary origin and functional diversification of aminotransferases

Received for publication, January 12, 2022, and in revised form, June 6, 2022. Published, Papers in Press, June 11, 2022.  
<https://doi.org/10.1016/j.jbc.2022.102122>

Kaan Koper<sup>1</sup>, Sang-Woo Han<sup>2</sup>, Delia Casas Pastor<sup>4</sup>, Yasuo Yoshikuni<sup>2,3,5</sup>, and Hiroshi A. Maeda<sup>1,\*</sup>

From the <sup>1</sup>Department of Botany, University of Wisconsin–Madison, Madison, Wisconsin, USA; <sup>2</sup>The US Department of Energy Joint Genome Institute, and <sup>3</sup>Environmental Genomics and Systems Biology Division, Lawrence Berkeley National Laboratory, Berkeley, California, USA; <sup>4</sup>Unaffiliated, Barcelona, Spain; <sup>5</sup>Global Center for Food, Land, and Water Resources, Research Faculty of Agriculture, Hokkaido University, Hokkaido, Japan

Edited by Joseph Jez

**Aminotransferases (ATs) are pyridoxal 5'-phosphate-dependent enzymes that catalyze the transamination reactions between amino acid donor and keto acid acceptor substrates. Modern AT enzymes constitute ~2% of all classified enzymatic activities, play central roles in nitrogen metabolism, and generate multitude of primary and secondary metabolites. ATs likely diverged into four distinct AT classes before the appearance of the last universal common ancestor and further expanded to a large and diverse enzyme family. Although the AT family underwent an extensive functional specialization, many AT enzymes retained considerable substrate promiscuity and multifunctionality because of their inherent mechanistic, structural, and functional constraints. This review summarizes the evolutionary history, diverse metabolic roles, reaction mechanisms, and structure–function relationships of the AT family enzymes, with a special emphasis on their substrate promiscuity and multifunctionality. Comprehensive characterization of AT substrate specificity is still needed to reveal their true metabolic functions in interconnecting various branches of the nitrogen metabolic network in different organisms.**

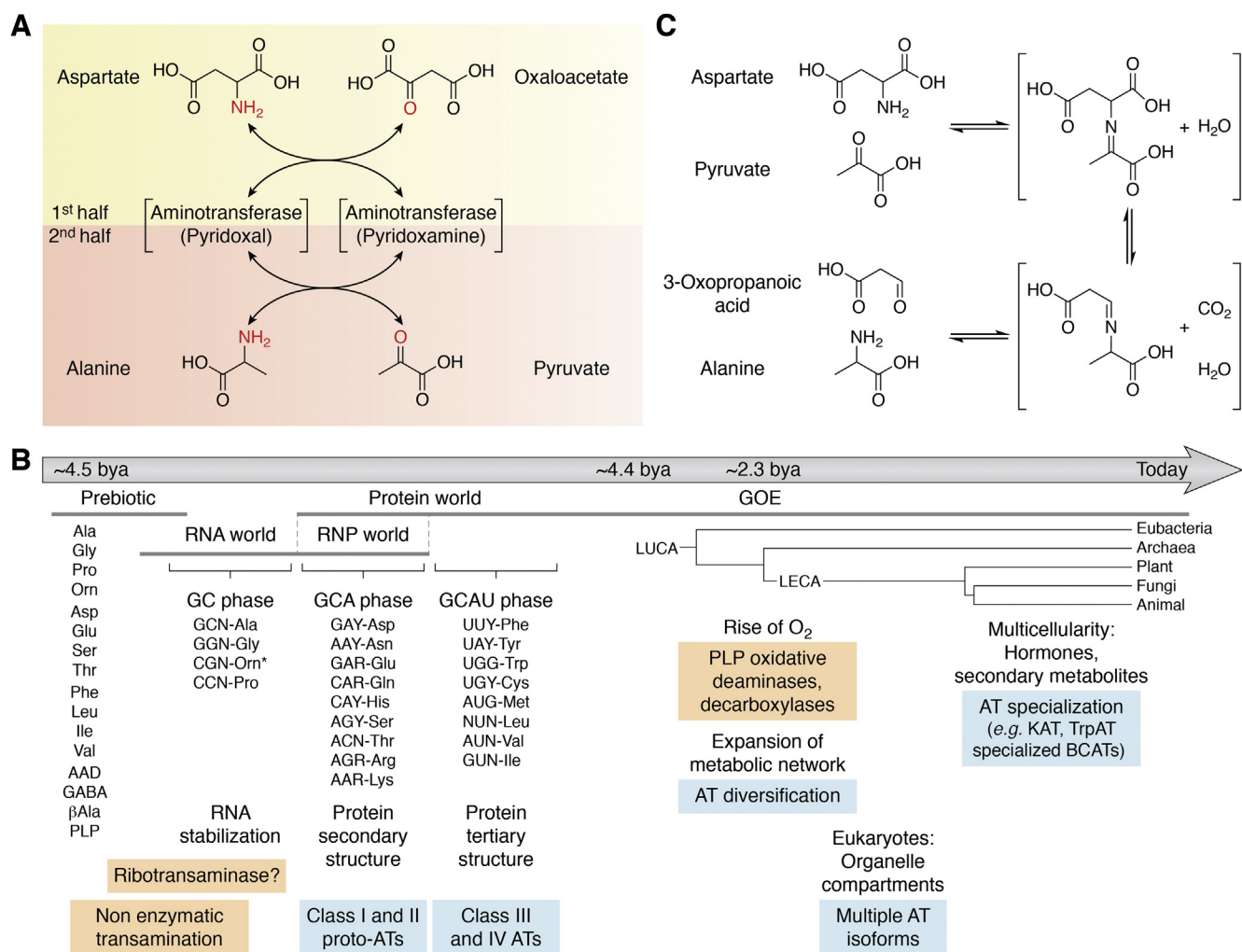
Aminotransferases (ATs), also known as transaminases (Enzyme Commission [EC] 2.6.1.-), are a large family of pyridoxal 5'-phosphate (PLP)-dependent enzymes that catalyze the transamination reactions between amino acid donor and keto acid acceptor substrates (Fig. 1A) (1–4). AT reactions make up ~50% of all PLP-dependent reactions, which equate to ~2% of all classified enzymatic activities (5). ATs are functionally diverse and ubiquitous to all kingdoms of life (6, 7). Since free and protein-bound amino acids constitute the predominant form of organic nitrogen, the amino group represents a major nitrogen source for cellular metabolism (8). Consequently, ATs play crucial roles in distributing reduced nitrogen to different branches of both primary and secondary metabolism. For example, plants, fungi, and bacteria assimilate nitrogen in the form of glutamate by glutamine synthetase

(GS)/glutamate synthase (also known as glutamine oxoglutarate amidotransferase [GOGAT]) (9–11) and further distribute the reduced nitrogen to other keto acid acceptors by a variety of ATs, such as aspartate ATs (1, 8). In mammals, alanine AT is a critical enzyme of the alanine–glucose cycle that transports nitrogen and carbon between muscle and liver (12–14). ATs can also participate in secondary metabolism, such as some isoforms of plant branched-chain amino acid ATs (BCATs) that deaminate methionine to synthesize 4-methylthio-2-oxobutanoate (4MTOB), a precursor of defense compounds, aliphatic glucosinolates (15–17). In halophilic bacteria, 2,4-diaminobutanoate AT catalyzes the intermediary step of biosynthesis of osmoregulant ectoine from aspartate (18). ATs can also act as conduits between different metabolic branches, such as *Arabidopsis* aromatic AT that transfers amino group from methionine to tryptophan, the precursors of phytohormones, ethylene and auxin, respectively (19). Therefore, AT enzymes participate in various metabolic pathways of amino acids, secondary metabolites, vitamins and cofactors, as well as gluconeogenesis, detoxification, assimilation, and transport of carbon and nitrogen (6, 20–22).

Origins of ATs can be traced back to at least to the last universal common ancestor (LUCA), which already had all four distinct AT classes (Fig. 1B) (7, 23–26). Notably, unlike other primary metabolic enzymes, most AT enzymes show substrate ambiguity, which might have originated as an evolutionary constraint because of the mechanistic and structural properties of AT enzymes. Today, the unique versatility of ATs having broad substrate specificity potentially plays important roles in metabolic plasticity and environmental adaption (27). However, the substrate ambiguity can complicate our full understanding of AT enzyme functionality, and there are still many uncharacterized ATs even in model organisms. In addition, multiple, yet unrealized, physiological substrates may still be present for previously characterized enzymes. Despite the critical roles ATs play in the metabolism of all organisms, the most recent reviews on AT enzymes date back to 1990s (2, 3). This review article provides critical updates on this essential enzyme family and highlights new perspectives on AT enzyme evolution and promiscuity and their potential roles in the metabolic network properties.

\* For correspondence: Hiroshi A. Maeda, [maeda2@wisc.edu](mailto:maeda2@wisc.edu).

Present address for Delia Casas Pastor: Stat-Dx Life S.L, Barcelona, Spain.  
Stat-Dx Life S.L. does not support any content or opinion stated in this research article.



**Figure 1. Enzymatic and nonenzymatic transamination reactions, and the evolutionary history of the amino acid metabolism, proteinogenesis, and transamination.** A, two half reactions of PLP-dependent transamination. In the first half reaction, an amino group from aspartate is transferred onto PLP, which generates oxaloacetate and PMP. In the second half, the amino group on PMP is transferred to pyruvate, forming alanine and regenerating PLP. B, evolution of transamination reactions since the origin of life. Transamination reactions were likely nonenzymatic initially and later catalyzed by hypothetical ribotransaminases during the RNA world, where the two-letter GC coded for a few amino acids. Additional amino acids were recruited after the genetic code expanded to three letters in the GCA phase during the RNA protein (RNP) world, when class I and II proto-ATs might have appeared and streamlined the amino acid metabolism. The subsequent GCAU phase and expansion of proteinogenic amino acids recruited class IV and class III ATs. About 4 billion years ago, LUCA inherited a diverse set of ATs and passed them down to its descendants. ATs underwent additional diversification during and after the Great Oxidation Event (GOE) and the appearance of eukaryotes and multicellularity. C, a nonenzymatic transamination reaction between aspartate and pyruvate, where aspartate is converted into an aldehydic acid (3-oxopropanoic acid), rather than keto acid (oxaloacetic acid). AT, aminotransferase; PLP, pyridoxal 5'-phosphate; PMP, pyridoxamine 5'-phosphate; RNP, ribonucleoprotein.

### Rise of PLP-dependent transamination reaction

All living organisms degrade, interconvert, and synthesize compounds through elaborate chains of chemical reactions to fulfill biological needs, which all together constitutes metabolism (28, 29). The earliest forms of life likely had simpler metabolism consisting of much fewer reactions than extant organisms. The early metabolism expanded over time because of the selective pressure to better utilize new or existing external nutrients, biosynthesize depleted compounds, and generate energy efficiently (28–36). Biocatalysts were most likely recruited to these metabolic pathways later and accelerated and provided specificity to the chemical reactions (30, 33).

### Nonproteinaceous transamination

Amino acids were likely abundant in the primordial soup and could be abiotically replenished by the conditions present on the early earth (29, 30, 37–41). Therefore, the earliest forms of life likely used nonenzymatic reactions to metabolize amino acids (Fig. 1B) (30, 34, 42–51). Several nonenzymatic transamination reactions have also been described (43, 52, 53). For instance, Bishop *et al.* (43) showed nonenzymatic decarboxylative transamination between amino and keto acids that generates analogous amino and aldehydic acids (Fig. 1C). In addition, PLP is capable of catalyzing nonenzymatic transamination reactions though 10<sup>7</sup> to 10<sup>9</sup> times slower than those carried out by PLP-dependent enzymes, where PLP is

covalently linked to the active-site lysine *via* a Schiff base (1–3, 7, 53, 54). Spontaneous formation of pyridoxine-like compounds from components present in the primordial soup is thermodynamically feasible and has been experimentally demonstrated (35, 36). Therefore, it is possible that transamination reactions were initially catalyzed by free PLP, and more complex PLP-dependent apoenzymes might have evolved later, increasing the specificity and catalytic rate (7). As RNA is thought to be an earlier form of biocatalysts (32, 55, 56), the presence of a since-lost ribozyme-mediated PLP-dependent transaminase can be speculated in the RNA world (Fig. 1B) (57–59). However, no natural or artificial “ribo-transaminase” is known to date.

### Transition to proteinaceous transamination

The transition from an RNA to protein world likely occurred in a stepwise manner through an intermediary ribonucleoprotein (RNP) world (Fig. 1B), which is supported by the properties of the genetic code, ribosomes, and some basal proteins (58, 60–63). The appearance of a protein-encoding system—the genetic code—was likely accompanied by the expansion of the amino acid biosynthesis, in which ATs likely played key roles (58). Hartman and Smith (60, 61) proposed the elegant three-step evolutionary scheme (GC → GCA → GCA/U) for the expansion of genetic code and proteinogenesis (Fig. 1B); the very first ribosome-translated proteins were coded by permutations of two nucleotides, guanine (G) and cytosine (C): GG, GC, CC, and CG encoding glycine, alanine, proline, and likely ornithine with a positively charged side chain, respectively. These earliest amino acids could be easily produced from intermediates of central carbon metabolism (*i.e.*, pyruvate and glyoxylate, Fig. 2) (58, 60) and most likely formed random cationic peptides (58, 60) that could interact with and stabilize RNAs. The earlier two-letter coding system is still embedded inside the three-letter codons of glycine (GGN), alanine (GCN), and proline (CCN), where N can be any nucleobase (Fig. 1B). Notably, however, CGN today encodes for arginine whose biosynthesis is too complicated to be an early proteinogenic amino acid (Fig. 2). Instead, CGN might have originally coded for an easier-to-produce positively charged amino acid, such as ornithine, a precursor to arginine (58, 60). Ornithine having a shorter side chain than arginine (or lysine) facilitates RNA–peptide interactions and discourages the formation of  $\alpha$ -helices (60, 64). In later evolutionary stages, however, arginine became advantageous as a proteinogenic amino acid over ornithine for the formation of  $\alpha$ -helices in protein-based functional folds (60).

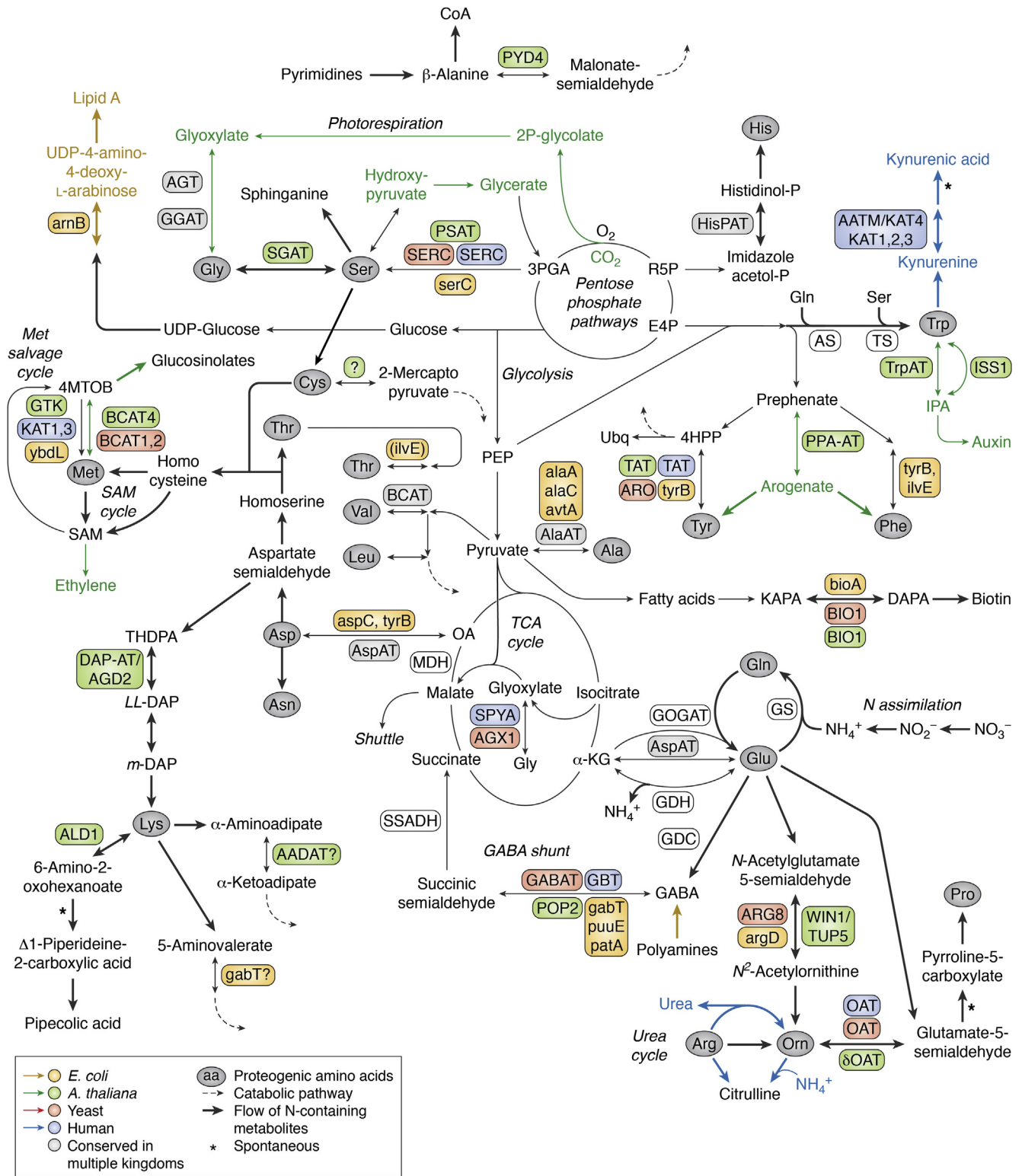
Next step in RNP evolution was the recruitment of polar amino acids—aspartate, asparagine, glutamate, glutamine, threonine, serine, histidine, arginine, and lysine—that allowed the peptide portion of the RNP to attain structure (*i.e.*,  $\alpha$ -helices) and participate in catalysis (58). The addition of these new amino acids became possible with the incorporation of adenosine (A), expanding to three-nucleotide codons (*i.e.*, GCA) (58, 63) (Fig. 1B). All GCA phase amino acids could be

produced within a few steps of central metabolism (Fig. 2), except for histidine derived from nucleotides—the precursors for RNA (58)—and lysine, which most likely appeared in late GCA phase (58). Notably, the highly conserved residues of ATs (see “Conserved residues and structural features of AT classes” section) are composed almost entirely of GC and GCA phase amino acids, suggesting that proto-ATs might have appeared during the GCA phase. The early proto-ATs lacking lysine might have employed a PLP–ornithine Schiff base. Alternatively, free vitamin B<sub>6</sub> might have acted as a cosubstrate, like seen in the extant PLP-independent pyridoxamine (PM) ATs (see “Nitrogen transfer catalyzed by PLP-independent enzymes” section) (65–67); then PLP became a prosthetic group only after the addition of lysine to the genetic code. Interestingly, promiscuous class I and II ATs could hypothetically catalyze all transamination reactions for AT-dependent GCA phase amino acids (see “Evolution of four classes of AT enzymes” section) and could be the first ATs to emerge (Fig. 2).

Hydrophobic amino acids—leucine, isoleucine, valine, cysteine, methionine, phenylalanine, tyrosine, and tryptophan—were perhaps not proteinogenic in the early GCA phase, since their large nonpolar side chains on a polar amide backbone create intramolecular repulsion that distorts overall structure. However, the establishment of the secondary structure (*i.e.*,  $\alpha$ -helices) by the end of the GCA phase stabilized backbone conformation (58), which set the stage for the introduction of hydrophobic amino acids encoded by GCAU that includes uracil (U) (Fig. 1B). Since these GCAU phase amino acids are synthesized through complex biosynthetic pathways (Fig. 2) (62, 68), the emergence of additional class III and IV proto-ATs during the GCAU phase likely played critical roles in their biosynthesis and recycling pathways, together with the further divergence of the existing class I and II proto-ATs (Fig. 1B). Thus, the late emergence of hydrophobic amino acid ATs, such as aromatic ATs and BCATs, may reflect their wide distributions across different AT classes (see “Functional diversification of AT enzymes” section). Hydrophobic motifs were a structural breakthrough allowing proteins to form tertiary folds and enter into the membrane that is inaccessible to RNA (58, 60, 69). Thus, the rise of the GCAU amino acids likely marked the end of the RNA’s dominance and allowed the cellular metabolism to predominantly utilize proteins, except for few indispensable relics of the RNA and RNP worlds, such as the ribosome (70) and spliceosome (71). Around this time, ornithine was likely removed from proteins, as ornithine’s benefits over arginine and lysine for interacting with RNA (64) did not justify an inapt fit for complex protein structures in a “protein world.”

ATs most likely emerged and underwent initial diversification between the GCA phase of the RNP world (Fig. 1B) and the time of the LUCA, from which all extant organisms have evolved (25). Recent genome reconstruction studies predicted that all AT classes and the majority of PLP-dependent enzyme types were already found among 355 protein families (57) and 3018 protein-coding genes (25) that were predicted to be





**Figure 2. Metabolic roles of AT reactions in human, yeast, *Arabidopsis*, and *E. coli*.** A metabolic map depicting AT enzyme functions in representative species. Proteogenic amino acids are shown in gray ellipses. Thick solid and dashed arrows dictate the flow of nitrogen containing metabolites and catabolic pathways, respectively. ATs and pathways from *E. coli*, *Arabidopsis*, yeast, and human are shown with yellow, green, red, and blue, respectively. The AT enzyme abbreviations are listed in Table 1. AS, anthranilate synthase; AT, aminotransferase; DAPA, 7,8-diaminopelargonic acid; E4P, erythrose 4-phosphate; GDC, glutamate decarboxylase; GDH, glutamate dehydrogenase; GOGAT, glutamine oxoglutarate amidotransferase; GS, glutamine synthase; 4HPP, 4-hydroxyphenylpyruvate; KAPA, 7-keto-8-aminopelargonic acid;  $\alpha$ -KG, alpha-ketoglutarate; L,L-DAP, L,L-2,6-diaminopimelic acid; *m*-DAP, meso-2,6-diaminopimelic acid; MDH, malate dehydrogenase; 4MTOB, 4-methylthio-2-oxobutanoic acid; OA, oxaloacetate; PEP, phosphoenolpyruvate; 3PGA, 3-phosphoglyceric acid; R5P, ribose 5-phosphate; SSADH, succinic semialdehyde dehydrogenase; THDPA, tetrahydrodipicolinate; TS, tryptophan synthase; Ubq, ubiquinone.

present in LUCA. Since the inference of the gene sequences that predate LUCA is inherently imprecise, it is difficult to phylogenetically reconstruct and analyze the initial stages of PLP-dependent enzyme evolution. Nevertheless, a deep analysis of codon structure of ancient PLP-dependent enzymes, similar to studies conducted on aminoacyl-tRNA synthetases and ribosomes (60, 72, 73), could link proteinogenesis and the appearance of proto-ATs.

### Diversification of PLP-dependent enzymes with the rise of oxygen

Although LUCA already had diverse PLP-dependent enzymes and ATs, these and other enzymes further expanded during and after the Great Oxidation Event (GOE), when atmospheric O<sub>2</sub> levels rapidly increased at ~2.3 billion years ago (Fig. 1B) (74–77). As the carbanionic intermediate of the PLP-dependent reactions is reactive with oxygen, GOE likely led to the appearance of novel PLP-dependent enzymes that catalyze oxygenic reactions, such as oxidative deamination or decarboxylation (78) (Fig. 1B). In bacteria (*i.e.*, *Streptomyces*), PLP-dependent arginine oxidases utilize oxygen in the biosynthesis of heterocycles (78). PLP-dependent amino acid decarboxylases are also reactive with oxygen (78–81); for example, amino acid aldehyde synthases catalyze oxidative deamination followed by decarboxylation (78), converting phenylalanine to volatile phenylacetaldehyde in petunia flowers (82) and 3,4-dihydroxyphenylalanine to 3,4-dihydroxyphenylacetaldehyde in insects for flexible cuticle formation (83). The increased availability of ATPs through aerobic respiration also made the synthesis of ATP-demanding amino acids (84)—tryptophan, phenylalanine, tyrosine, and arginine—more affordable. Notably, most of the oxygen utilizing PLP-dependent enzymes (*e.g.*, amino acid aldehyde synthase) act on many of those same amino acids (78, 85). Thus, GOE contributed to a tremendous expansion of many metabolic pathways and networks (85), which include biosynthesis of certain amino acids and alkaloids, as well as nitrogen metabolism that incorporated novel PLP-dependent enzymes.

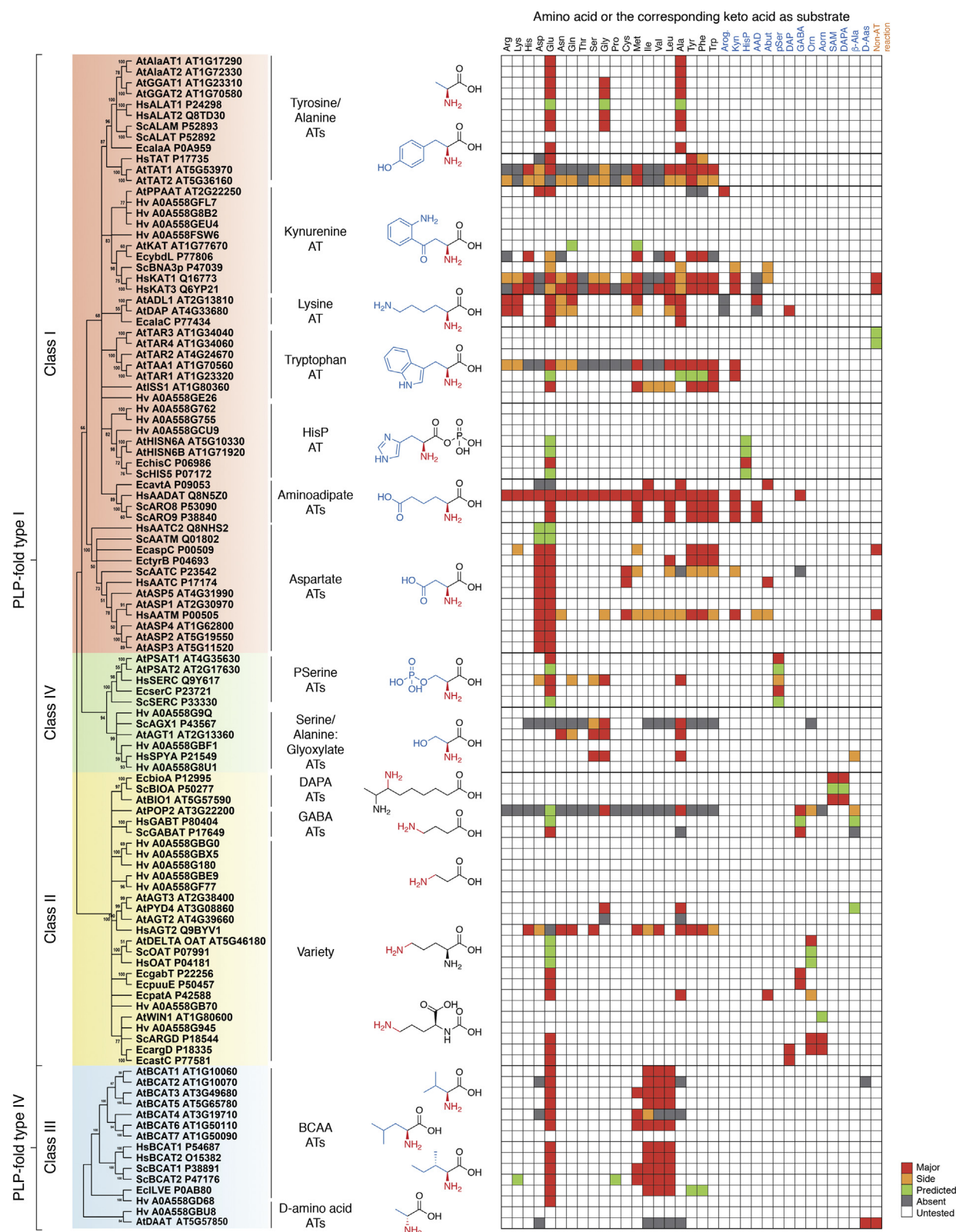
The expansion of PLP-dependent enzymes after the GOE could also be an indirect consequence of the appearance of complex eukaryotic life derived from the last eukaryotic common ancestor (Fig. 1B). For instance, a general expansion of ATs occurred for all eukaryotes, where the appearance of organelles required multiple isoforms in different subcellular compartments (Fig. 1B). Later expansion of multicellularity, such as in animals and the land plants, also required ATs with tissue or developmental stage-specific functions (Fig. 1B). For instance, in the human brain, kynurenine is converted into kynurenic acid for tryptophan degradation, primarily by kynurenine aminotransferase 2 (KAT2) and to a lesser by KAT1, KAT3, and KAT4 (Fig. 2) (86–90). While KAT1 and KAT3 are phylogenetically related with each other, KAT2 is closely related to aminoadipate AT and KAT4 to aspartate AT, which gained KAT activity (Fig. 3). *Arabidopsis* plants also have at least three different tryptophan AT isoforms having

specific roles: TAA1, TAR1, and TAR2 generate indole-3-pyruvate, the precursor of auxin, in certain developmental stages or tissue types (Fig. 2) (91–94).

### Nitrogen transfer catalyzed by PLP-independent enzymes

It is important to note that nitrogen transfer reactions can be also catalyzed by PLP-independent enzymes. Pyridoxamine:pyruvate aminotransferase (PM-AT; EC 2.6.1.30) and pyridoxamine 5'-phosphate (PMP): $\alpha$ -ketoglutarate aminotransferase (PMP-AT; EC 2.6.1.54), so far found in bacteria (*i.e.*, *Mesorhizobium loti* (65) and *Clostridium kainantoi* (95)) and plant (*i.e.*, *Nicotiana tabacum* (66)), catalyze reversible transamination reactions between vitamin B<sub>6</sub> and keto acids in the absence of PLP. PM and PMP, two natural forms of vitamin B<sub>6</sub>, act as amino donors, and their deaminated products are released from the active site, which is contrary to PLP-dependent transamination where PLP functions as a prosthetic group (65–67, 96). PM-ATs are, however, related to PLP-dependent enzymes based on their similar structural and mechanistic features. For example, the crystal structure of PM-AT from *M. loti* shows typical domain characteristic of fold type I PLP-dependent enzyme and class IV ATs (97), with the presence of a Schiff base linkage of K197-pyridoxal as well as a hydrogen bond network between the enzyme D171 residue and N1 of the pyridine ring as observed in the internal aldimine intermediate of PLP-dependent enzymes. Although the biological roles of PM-AT and PMP-AT are currently unclear, both enzymes are considered to participate in the metabolism of vitamin B<sub>6</sub> (65, 66).

Glutamine amidotransferases are PLP-independent enzymes that are known to be involved in AT-like nitrogen transfer. However, unlike ATs that have a single active site with the PLP cofactor that directly transfers an amino group from amino and keto substrates, glutamine amidotransferases first hydrolyze the amido group of glutamine at the glutaminase domain and then channel the released ammonia to a separate synthase or transferase domain (98, 99). Glutamate synthase, also known as GOGAT, is an NAD(P)H or ferredoxin-dependent oxidoreductase (EC 1.4.1.14, 1.4.1.13, and 1.4.7.1, respectively) that converts  $\alpha$ -ketoglutarate to glutamate using glutamine as amido donor (100, 101). Together with ATP-dependent GS, the GS–GOGAT cycle is responsible for nitrogen assimilation in plants, bacteria, and fungi (Fig. 2) (9, 11, 100). Other glutamine amidotransferases include ATP-dependent asparagine synthetases (EC 6.3.5.4) (102, 103), anthranilate synthase (EC 4.1.3.27) (104), PLP synthase (EC 4.3.3.6) (105), and imidazole glycerol phosphate synthase (EC 4.3.2.10) (106). Interestingly, glutamine-fructose-6-phosphate amidotransferases (EC 2.6.1.16), which are involved with amino-sugar metabolism (107), are competitively inhibited by PLP through its reversible binding to an active-site lysine (pyridoxylation) (108). Amidotransferases are particularly abundant in nucleotide metabolism, such as the purine metabolic enzyme glutamine phosphoribosylpyrophosphate amidotransferase (also known as



**Figure 3. Phylogeny and reported activities of ATs from representative organisms.** Phylogenetic relationship of ATs from human (Hs), yeast (Sc), *Arabidopsis* (At), *Escherichia coli* (Ec), and *Halobacterium volcanii* (Hv). Multiple sequence alignment was performed using MAFFT-DASH (138), and the analysis was performed under default setting in MEGA 11 (343) neighbor-joining method (344, 345) with partial deletion site coverage set to 50% and 1000 bootstraps (346). Analysis was done separately for class III ATs from class I, II, and IV ATs because of their distinct evolutionary origins. ATs formed at least 12 distinct clades whose major substrates are shown. Activities detected in the literature for each enzyme are shown on the right. Red, orange, green, gray, and white denote major, side, predicted, absent, and untested activities, respectively. AT, aminotransferase.



amidophosphoribosyltransferases or ATase; EC 2.4.2.14) that catalyze the conversion of 5-phosphoribosyl-1-pyrophosphate into 5-phosphoribosyl-1-amine (109).

Certain amino acid dehydrogenases release or incorporate free ammonium ion from or to the C $\alpha$  of amino acids through NAD(P)<sup>+</sup>/NAD(P)H-dependent oxidative deamination or reductive amination, respectively (110, 111). For instance, glutamate dehydrogenases (EC 1.4.1.2) play critical roles at the interface of carbon and nitrogen metabolism, such as by releasing ammonium ion from glutamate at the entry of the urea cycle (112) or by assimilating ammonium ion to generate glutamate from  $\alpha$ -ketoglutarate (111) (Fig. 2). These certain PLP-independent dehydrogenases can transfer free nitrogen between metabolites but cannot catalyze transamination of the  $\alpha$ -amino group of amino acids. The apparent indispensable role of PLP in transamination of  $\alpha$ -amino group, at least in extant AT enzymes, could come from the hypothesis of “principle of many users” (113–115), where it becomes very difficult to lose or replace a particular component (*i.e.*, a cofactor) that participates in multiple critical metabolic processes. The replacement of such a component would nullify various critical metabolic pathways, which would significantly decrease fitness (115). Hypothetically, alternative routes could evolve synchronously, but multiple examples suggest that the complete replacement is rare even if the alternative route is more efficient or better suited (*i.e.*, RuBisCO *versus* phosphoenolpyruvate carboxylase) (116). Thus, the PLP cofactor used by modern ATs today likely represents a molecular relic of an ancient metabolic state (59, 113).

## Evolution of four classes of AT enzymes

Modern ATs can be phylogenetically classified into four distinct AT *classes*, three of which (class I, II, and IV) were diversified from an ancestral fold type I PLP-dependent enzyme (Box 1 describing different fold types of PLP-

dependent enzymes), whereas AT class III evolved independently from a fold type IV PLP enzyme (Fig. 3) (2, 7, 117).

## Phylogeny of ATs

Structure-guided phylogenetic analysis of 109 AT sequences from representative organisms from different kingdoms—*Homo sapiens* (animal), *Saccharomyces cerevisiae* (fungi), *Arabidopsis thaliana* (plant), *Escherichia coli* (bacterium), and *Halobacterium volcanii* (archaeon)—further supports the evolutionary relationship of four AT classes (Fig. 3) (2, 3, 6, 7, 21). The presence of all four class AT members in all domains of life—Eubacteria, Archaeobacteria, and Eukarya—is a testament to the heritage of ATs that can be traced back to LUCA (25, 26) (Fig. 1B). Within each class, certain ATs form distinct clades and utilize similar substrates, though there are many exceptions as discussed later (Fig. 3).

Class I is the largest and the most functionally diverse class of ATs and utilizes substrates, such as aspartate, aromatic amino acids, histidinol phosphate, kynurenine, and diaminopimelate (Fig. 3). Class I ATs form a robust monophyletic clade, which also includes certain non-AT enzymes, such as 1-amino-cyclopropane-1 carboxylate synthases (ACC) and some carbon–sulfur lyases (not shown in Fig. 3), involved in the synthesis of ethylene precursor ACC (118) and cysteine metabolism (119, 120), respectively. Class II is a small class of ATs that utilize  $\gamma$ -aminobutyric acid (GABA), ornithine, acetylornithine, 7,8-diaminopelargonic acid (DAPA), and  $\omega$ -amino acid (Fig. 3). Unlike other classes of ATs that transaminate  $\alpha$ -amino/keto groups, class II ATs can act on non- $\alpha$  amino acids (Fig. 3). These ATs are in the same phylogenetic clade with non-AT enzyme, glutamate-1-semialdehyde 2,1-aminomutase, that is involved in the biosynthesis of tetrapyrroles (121). Class IV is another relatively small AT class that utilizes serine and phosphoserine. Non-ATs, cysteine desulfurases, and molybdenum cofactor sulfurase are also found in class IV clade. Class III has D-amino acid and BCATs and is the

### Box 1. Seven distinct fold types of PLP-dependent enzymes

PLP-dependent enzymes were initially classified into five distinct types based on their overall structure folds that independently adopted PLP through the course of evolution (23). However, as some could not be placed in any of these five types (23), PLP-dependent enzymes are now categorized to seven fold types I to VII (24). AT enzymes are found in the fold type I and IV of PLP-dependent enzymes, which are briefly summarized here.

Fold type I (23) (or  $\alpha$  family (7)) is the largest family of PLP-dependent enzymes, mostly ATs (except D-amino acid and BCATs) and some non-AT enzymes involved in amino acid metabolism: amino acid decarboxylases (*i.e.*, prokaryotic ornithinedecarboxylase), aminomutases (*i.e.*, glutamate-1-semialdehyde aminomutase), lyases (*i.e.*, cystathionine- $\beta$ -lyase), synthases (*i.e.*, DAPA synthase), and hydroxymethyltransferases (*i.e.*, serine hydroxymethyltransferase) (130, 341, 342). Aspartate AT is one of the well-characterized enzymes of this family (Fig. 5) (7, 23). Type I enzymes commonly have their Schiff base lysine closer to the C terminus compared with the glycine-rich region, and a hydrophobic  $\beta$ -strand is present downstream of the lysine (23). An invariant aspartate residue is present 20 to 50 amino acids downstream of the lysine and interacts with the nitrogen of pyridoxal ring (23). In addition, two other residues that interact with PLP are also conserved; an aromatic residue (corresponding to W130 in aspartate AT) and another residue (corresponding to A213 in aspartate AT) that is one of alanine, serine, threonine, valine, isoleucine, proline, or methionine (23). Overall, the structure of type I enzymes consists of a large N-terminal domain of seven-stranded  $\beta$ -sheet and a small C-terminal domain of three or four stranded  $\beta$ -sheet with helices on one side (133, 134). Fold type I enzymes typically form homodimers or homotetramers, which is required for activity as their active site is located at the subunit–subunit interface (133–136).

Fold type IV (23) (or D-alanine AT family (7)) is another small family containing D-alanine ATs, BCAT, and 4-amino-4-deoxychorismate lyase. D-alanine AT is the prototype enzyme of this family (7). Type IV enzymes consist of two domains of dissimilar sizes. The smaller N-terminal domain contains a six-stranded antiparallel  $\beta$ -sheet that is flanked by two  $\alpha$  helices on one side. The larger C-terminal domain is made of four-stranded pseudo- $\beta$ -barrel with a few surrounding helices and contains the active site lysine (133). Importantly, PLP binds to the active site in opposite direction compared with type I, explaining the unique substrate stereospecificity of type IV enzymes. A glutamic acid molecule interacts with the ring nitrogen of PLP (133). These enzymes are usually active as homodimers, but BCAT further oligomerizes into a hexamer (130, 133).

most structurally distinct AT class that belongs to the independently evolved fold type IV PLP-dependent enzymes (Box 1). This class also contains the non-AT enzyme 4-amino-4-deoxychorismate lyase that is involved with folate biosynthesis. Interestingly, *Arabidopsis* D-amino acid AT also exhibits 4-amino-4-deoxychorismate lyase activity (122, 123).

### Mechanisms of AT-catalyzed reactions

Transamination reactions catalyzed by ATs proceed through two half-reactions employing “ping-pong bi-bi” kinetic mechanism (4, 124, 125). In the first half of the reaction, the PLP at the active site of the enzyme reacts with the amino acid substrate to form PMP, and the corresponding keto acid product is released. The second half of the reaction is essentially the reverse of the first half, where a keto acid substrate reacts with PMP to regenerate PLP and forms the corresponding amino acid (Fig. 1A) (4). During the transamination reaction, the cofactor PLP acts as an electron sink, storing electrons from cleaved bonds of the substrate and later dispersing them for the formation of new bonds (4, 6), whereas the protein portion of the ATs (apoenzyme) limits the unintended side reactions and facilitates the main reaction (4, 6, 7, 53).

Most AT-catalyzed reactions are reversible, but sometimes, there is a preferred reaction direction determined by the kinetic properties of the AT enzyme, or through the rapid consumption of one of the end products by a downstream enzyme or process (1, 6, 21, 126, 127). For example, amination of glyoxylate to glycine by glutamate:glyoxylate ATs is physiologically irreversible because of the exceedingly low affinity of glutamate:glyoxylate ATs toward glycine for the reverse reaction (126). Also, deamination of kynurenine by KATs is irreversible since the keto acid product, 4-(2-aminophenyl)-2,4-dioxobutanoate, is unstable and rapidly cyclized to kynurenic acid (Fig. 2).

#### Reaction mechanisms of class I, II, and IV ATs

Reaction mechanism of class I, II, and IV ATs is well known; thanks to the detailed characterization of aspartate ATs (4) (Fig. 4). Like other PLP-dependent enzymes, the formation of an internal aldimine in which PLP forms a Schiff-base linkage with an active-site lysine residue is prerequisite for AT activity (128). In the first half-reaction, an unprotonated amino acid substrate binds to a protonated internal aldimine, or a protonated amino acid substrate binds to an unprotonated internal aldimine (4, 124), where the extra proton is mutually transferable. The reactive Michaelis complex requires a protonated internal aldimine and an unprotonated amino group (4, 129), leading to the nucleophilic addition of the substrate amino group to the C4' of PLP and the formation of the first geminal diamine intermediate. The proton transfer between the two geminal nitrogen further results in the second geminal diamine, which subsequently collapses to form the external aldimine and displaces the active-site lysine as a free base (Fig. 4). Until the external aldimine formation, the reactions are rapid and are often represented as a single step (4).

Next, the external aldimine is deprotonated at the C $\alpha$ -H bond by the free base lysine and forms the carbanionic intermediate that has three major resonance contributors. The most catalytically potent resonance form is known as the quinonoid intermediate, which has the electron pair from the C $\alpha$ -H bond delocalized onto the pyridine ring nitrogen. As the reaction can either revert to the original external aldimine or form the ketimine intermediate (blue arrows in Fig. 4), a highly conserved aspartic or glutamic acid residue interacts with the ring nitrogen of PLP to keep it protonated and encourages forward progress. After the formation of the ketimine intermediate, a water molecule is added to the C $\alpha$  by the active-site lysine, which generates the first carbinolamine intermediate. The proton transfer from the lysine to the C4' nitrogen forms the second carbinolamine intermediate. Finally, the hydroxyl group of the second carbinolamine intermediate is deprotonated by the active-site lysine, and the intermediate collapses into PMP and a free keto acid product (Fig. 4). The second half reaction progresses through the reversal of the first half reaction, where a keto acid substrate reacts with the PMP to form the corresponding amino acid product (4).

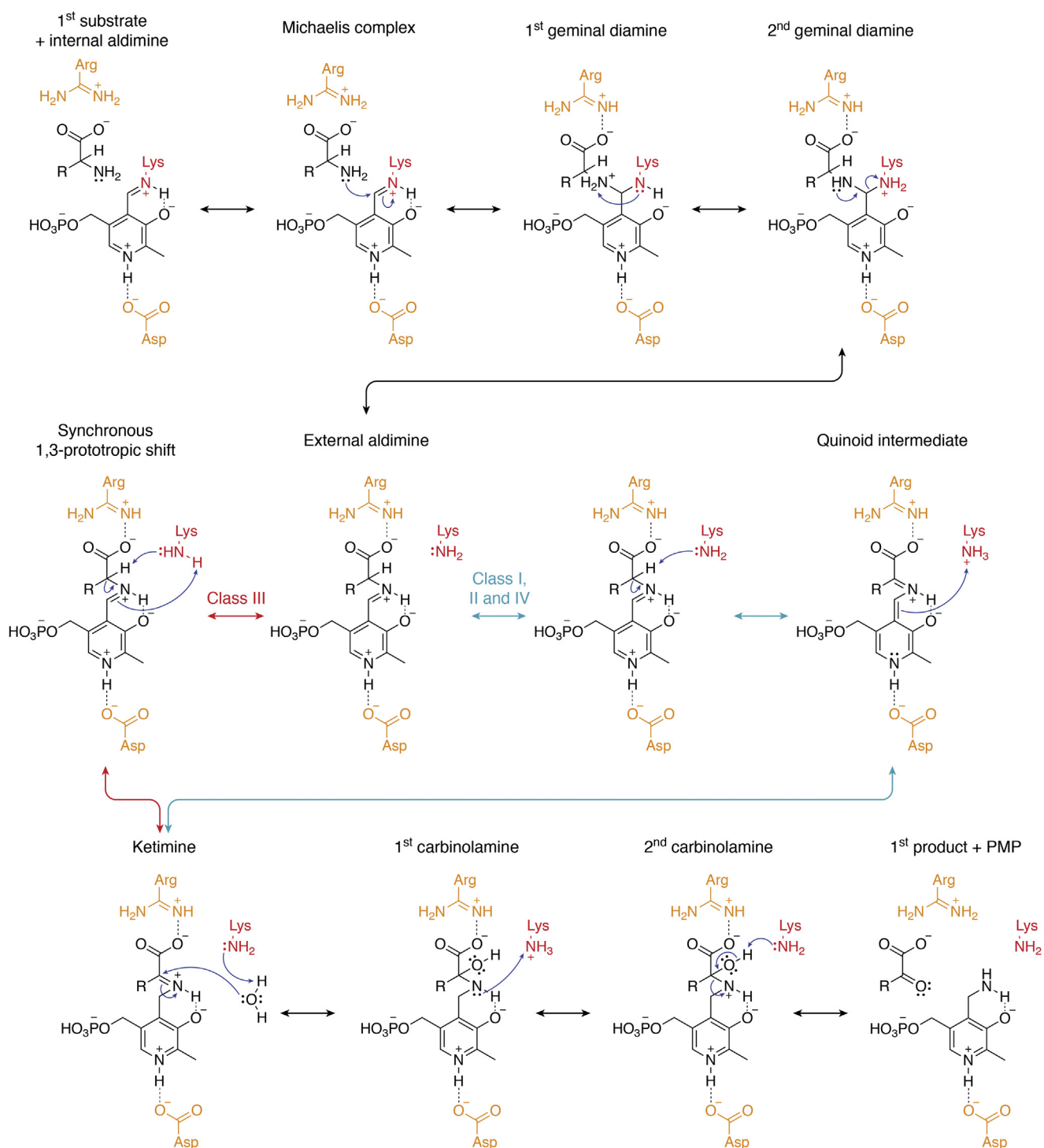
#### Reaction mechanisms of class III ATs

Although class III ATs evolved independently from classes I, II, and IV, their reaction mechanisms are remarkably similar because of convergent evolution. This suggests that PLP-dependent transamination likely has certain evolutionary constraints, such as the formation of external aldimine (Fig. 4) (7, 130). Class III transamination follows the same enzymatic steps as class I, II, and IV ATs until the formation of the external aldimine, but how the reaction proceeds forward differs (red arrows, Fig. 4). Unlike in classes I, II, and IV ATs, analysis of the *Mycobacterium tuberculosis* IlvE (structural gene E of isoleucine, valine-leucine operon, also known as transaminase B) reaction did not detect the formation of the reactive quinoid intermediate even under short reaction times (~3 ms) (131, 132). Instead, the reactions go through a synchronous cleavage of the substrate C $\alpha$ -H bond and the protonation of PLP C4' via 1,3-prototropic shift mechanism to form ketimine intermediate (131). The steps after the ketimine intermediate are identical between classes I, II, IV, and class III ATs (131).

### Conserved residues and structural features of AT classes

Class I, II, and IV ATs, which belong to PLP fold type I, share similar topology and three-dimensional structures, whereas class III ATs belong to PLP fold type IV and have distinct structures (Fig. 5). Class I, II, and IV ATs have a large N-terminal domain of seven-stranded  $\beta$ -sheet (green region in Fig. 5, A and B) and a small C-terminal domain of three or four stranded  $\beta$ -sheet with  $\alpha$ -helices on one side (blue region in Fig. 5, A and B) (133, 134). The active site is located at the subunit-subunit interface, whereas the active-site lysine is located at the N-terminal domain (Fig. 5A). Furthermore, the active enzymes form dimers or tetramers (133–136). On the

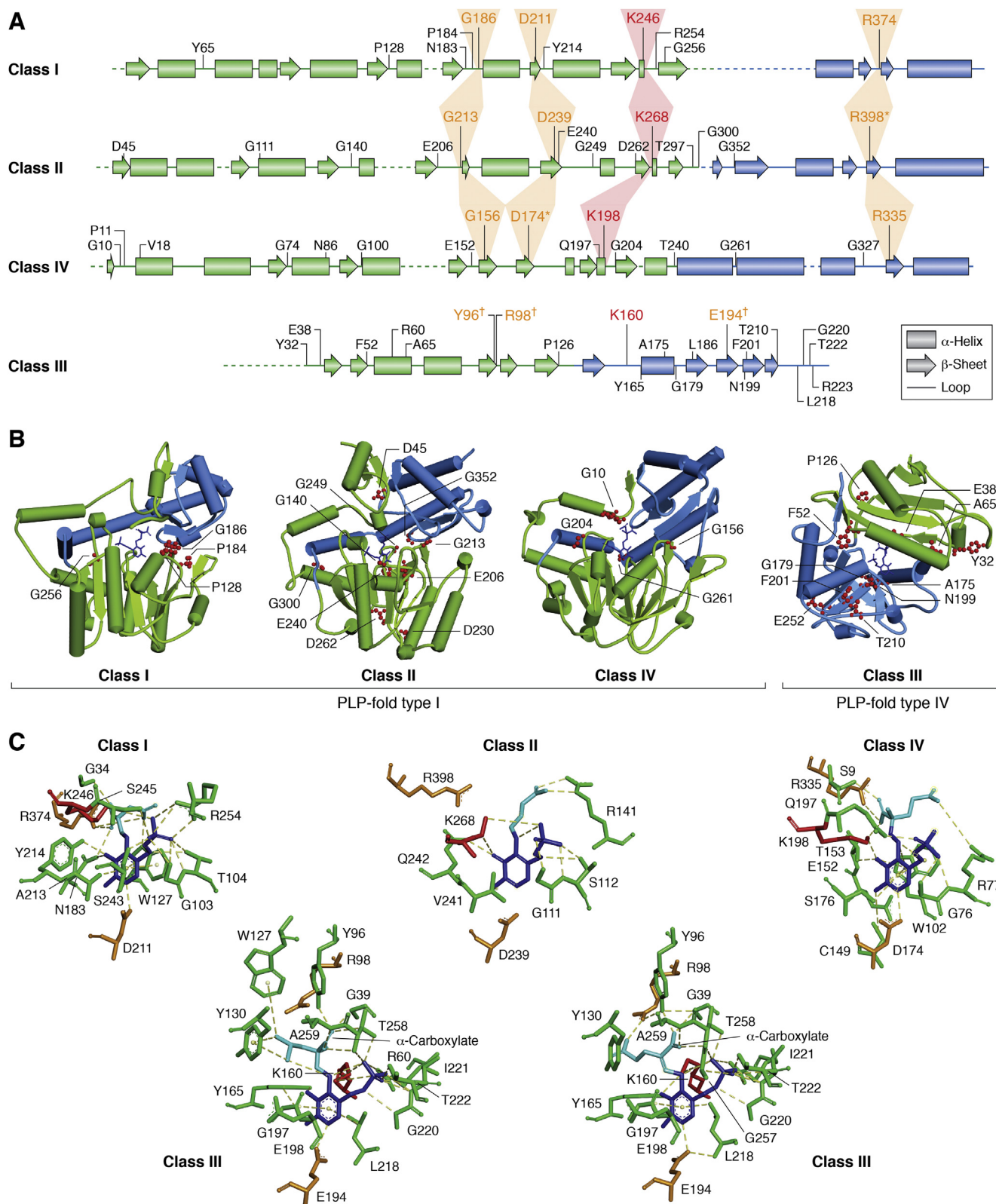




**Figure 4. Reaction mechanisms of AT-catalyzed transamination reaction.** The first half-reaction mechanisms for class I, II, IV, and class III ATs are shown starting from the first amino acid substrate and PLP–enzyme complex (internal aldimine). After the formation of the Michaelis complex, the reaction proceeds through the formation of first and second geminal diamines and external aldimine. In class I, II, and IV ATs, the external aldimine first forms quinoid intermediate, which is subsequently converted to ketamine (blue arrows). In contrast, in class III ATs, external aldimine is directly converted to the ketimine through 1,3-prototropic shift mechanism (red arrows). Ketimine is subsequently converted to first and second carbinolamine intermediates and collapses to form PMP plus the first keto acid product. In the second half-reaction, PMP reacts with second keto acid substrate in the reverse direction to form second amino acid product and PLP. AT, aminotransferase; PLP, pyridoxal 5'-phosphate; PMP, pyridoxamine 5'-phosphate.

other hand, class III ATs have a smaller N-terminal domain of six-stranded antiparallel  $\beta$ -sheet that is flanked by two  $\alpha$ -helices on one side and a larger C-terminal domain of four-stranded pseudo- $\beta$ -barrel with few surrounding helices (Fig. 5, A and B). The active-site lysine is located on the

C-terminal domain of class III ATs (133) and binds to PLP in the opposite direction compared with class I, II, and IV ATs (Fig. 5C), explaining their unique substrate stereospecificity. Class III ATs are usually active as homodimers, but BCATs further oligomerize into hexamer (130, 133) (Box 1). Although



**Figure 5. Structure and conserved residues of four classes of ATs.** A, simplified secondary structures of AT polypeptides showing the overall topology of each class AT, which include  $\alpha$ -helices (boxes),  $\beta$ -sheets (arrows), and loops (straight lines). Black residues are conserved for >90% of aligned sequences within each class, except for the ones marked with a star, which are conserved for ~70%. The Schiff-base lysine is shown in red and traced by red traces. Functionally conserved glycine, glutamate/aspartate, and arginine are shown in orange and traced by orange traces. Functional conservation of residues marked with † is inferred from the crystal structure. N- and C-terminal domains are shown in green and blue, respectively. B, overall structures of *Escherichia coli* class I aspartate AT (AAT; Protein Data Bank [PDB] ID: 1ARG (139)), class II GABA AT (GABT; PDB ID: 1SFF (347)), class IV phosphoserine AT (PSAT, PDB ID: 1BJO (141)), and class III BCAT (IlvE; 1i1I (142)). Non-active-site residues that are conserved within each AT class are labeled in red. C, conserved active-site residues (green) of the substrate (cyan)-PLP (dark blue) complex of four AT classes. The Schiff-base lysine is shown in red. IlvE is shown in complexes with

the quaternary structure of ATs can affect enzyme-cofactor stability (137), little is known about the dynamics of the quaternary AT structures and if subunits function synergistically or monomers of different ATs can form heteromultimers.

A structure-guided sequence alignment with MAFFT-DASH (138) shows the active-site lysine, which forms a Schiff-base linkage with cofactor PLP (red in Fig. 5A) is absolutely conserved for all ATs with catalytic activity. Two more active-site residues, aspartate and arginine (orange in Fig. 5A) are also conserved among all ATs, except for class III that convergently evolved glutamate and arginine (or tyrosine), correspondingly (Fig. 5A). The aspartic (or glutamic) acid (orange in Fig. 5C) interacts with and maintains the protonation of the pyridine ring nitrogen of PLP, which is essential for the forward progress of AT reaction from the external aldimine to ketimine intermediates (see “Mechanisms of AT-catalyzed reactions” section, Fig. 4) (4). The arginine (or tyrosine) residue of class III ATs (orange in Fig. 5C) coordinates the  $\alpha$ -carboxylate of their substrates. Class I, II, and IV ATs, but not class III ATs, also have a structurally conserved glycine (orange in Fig. 5A), which is found outside the active site and at the interface of N- and C-terminal domains (Fig. 5B).

Among class I ATs, seven more residues are highly conserved ( $\sim 90\%$ , Fig. 5). N183 and Y214 interact with the pyridine ring, and R254 interacts with the phosphate group of PLP and stabilizes the cofactor (Fig. 5C), whereas the other conserved active-site residue, P184, is likely structural (Fig. 5B). Y65 at the subunit interface likely also contributes to the stability of PLP, but of the other subunit, through interaction with phosphate groups, whereas P128 and G256 are a part of the solvation shell and core of the enzyme, respectively (Fig. 5B). Based on the crystal structure of *E. coli* AAT (or aspC; P00509) (139), additional residues interact with the phosphate group (G103, T104, S243, and S245) and the pyridine ring (W127 and A214) of PLP, though these residues are not highly conserved ( $\sim 50\text{--}85\%$ ) among class I ATs.

Class II ATs have 10 other highly conserved ( $\sim 90\%$ ) residues (Fig. 5). The active-site residue G111 interacts with the phosphate of PLP (Fig. 5C), whereas the two other conserved residues, G140 and E206, are likely structural (Fig. 5B). T297 is found at the subunit interface and interacts with the phosphate group from the PLP of the other subunit, whereas G249, D262, and G352 are parts of the solvation shell and D45, E240, and G300 are found at the core of the enzyme (Fig. 5B). Based on the crystal structure of *E. coli* GABA AT (gabT, P22256) (140) (Fig. 5), an additional S112 residue interacts with the phosphate group, and V241 and Q242 interact with the pyridine ring of PLP (Fig. 5C). These three residues, however, are not highly conserved ( $\sim 50\text{--}70\%$ ) among class I ATs. Interestingly, R398 that is highly conserved among all fold type I ATs has relatively low conservation ( $\sim 70\%$ ) among class II ATs. For

example, DAPA ATs from yeast, *A. thaliana*, and *E. coli* lack R398 that is replaced by a tyrosine residue (140). According to the crystal structure of *E. coli* gabT, the role of R398 in interacting with the substrate could be less strict compared with other fold type I ATs, as the  $\alpha$ -carboxylate of a GABA substrate analog, aminooxyacetate, faces away from R398 and, instead, interacts with R141 that is at the opposite end of the active site (Fig. 5C). Considering most class II ATs mainly utilize non  $\alpha$ -amino/keto acid substrates, such as  $\gamma$ -amino acid (*i.e.*, GABA),  $\beta$ -amino acid (*i.e.*,  $\beta$ -alanine), and diamino acids (*i.e.*, ornithine and DAPA), the substrate  $\alpha$ -carboxylate is distantly located from their amino group that attaches to PLP and interacts with a different arginine residue (R141 instead of R398, Fig. 5C). The presence of both arginine residues in most class II ATs may allow the use of both  $\alpha$ - and non  $\alpha$ -amino/keto acid substrates. Mutagenesis and structural studies of different class II AT with both substrate types can further test this hypothesis.

Class IV ATs have 12 additional highly conserved ( $\sim 90\%$ ) residues (Fig. 5). At the active site, Q197 interacts with the phosphate of PLP (Fig. 5C), whereas G10 and P11 are likely structural (Fig. 5B). G204 and T240 are at the subunit interface and likely assist dimerization and interact with the phosphate group of the PLP of the other subunit, respectively. G100 and G261 are parts of the solvation shell of the enzyme (Fig. 5B). Based on the structure of *E. coli* phosphoserine AT 1 (SerC or Psat; P23721) (141) (Fig. 5), additional active-site residues, G76 and R77, interact with the phosphate, and W102, C149, T153, and S176 interact with the pyridine ring of PLP (Fig. 5C), though they are not highly conserved ( $\sim 50\text{--}80\%$ ) among class IV ATs.

Class III ATs have 18 additional conserved residues, which are more than other AT classes. This is likely because of more recent evolution (see “Transition to proteinaceous transamination” section) and less diversification of class III having only two subtypes (*i.e.*, BCATs and  $\text{D}$ -amino acid ATs). Based on *E. coli* BCAT (IlvE; P0AB80) (142), the E/D194, R98/Y96, and Schiff-base K160 residues, highly conserved among all ATs (Fig. 5), do not align at primary sequences but have identical roles as in class I, II, and IV ATs based on structural analyses (142, 143) (Fig. 5C). Notably, however, R98 of class III ATs interacts with the side-chain carboxylate of acidic substrates (144) (*i.e.*, glutamate, Fig. 5C) and Y96 instead interacts with the substrate  $\alpha$ -carboxylate (142), allowing the use of both acidic and hydrophobic substrates by BCATs (Fig. 5C). Y96 is highly conserved among BCATs but not in  $\text{D}$ -amino acid ATs from *A. thaliana* and *Bacillus* sp. potentially because of the difference in the stereochemistry of  $\text{D}$ -amino acid substrates. Active-site residues R60, L218, G220, and T222 function for interacting with the phosphate of PLP, Y165 interacts with the ring of PLP, and E38 and N199 are likely structural. L186 is found at the subunit interface, whereas Y32, P126, G179, R223, and E252 are parts of the solvation shell and F52, A65, A175, F201, and T210

both glutamate (right) and leucine (left). Note that in class I and IV, conserved arginine (R374 in AAT and R335 in PSAT) interacts with  $\alpha$ -carboxylate of the substrate. The weakly conserved R in class II (R398 in GABT) does not interact with  $\alpha$ -carboxylate of the substrate; instead another conserved arginine (R141 in GABT) fulfills this duty. In class III, a conserved tyrosine (Y96 in IlvE) interacts with  $\alpha$ -carboxylate group of both glutamate and leucine, whereas the conserved arginine (R98 in IlvE) only interacts with the acidic side-chain carboxylate of glutamate. AT, aminotransferase; GABA,  $\gamma$ -aminobutyric acid; GABT, GABA AT; PLP, pyridoxal 5'-phosphate.



are found at the core of the enzyme, respectively. According to the crystal structure of *E. coli* IlvE (P0AB80) (142), additional active-site residues G39, I221, and T258 interact with PLP phosphate group, G197 and E198 interact with PLP ring, whereas Y130, W127, and A259 stabilize the bound substrate. However, these residues are not highly conserved (~40–80%) among class III ATs.

Among PLP fold type I ATs, analogous roles for several active-site residues could be defined based on spatial configuration. G103 and T104 of class I ATs, G111 and S112 of class II ATs, and G76 of class IV ATs interact with phosphate of PLP (Fig. 5C). N183 and N242 of class I and class II ATs, respectively, and A213 and S126 of class I and class IV ATs, respectively, similarly interact with the pyridine ring of PLP (Fig. 5C). The aromatic ring W127 and W102 of class I and IV ATs, respectively, facilitate pi stacking with the pyridine ring of PLP (Fig. 5C). Overall, conserved residues are concentrated around the AT active site, likely because of the strong selective pressure to maintain the active-site conformation and catalytic activity. However, the conserved residues found inside the core, on the surface, or between monomers point to the importance of maintaining the tertiary and quaternary structures of ATs.

### Functional diversification of AT enzymes

This section will describe various functions of AT enzymes from human (animal), yeast (fungi), *Arabidopsis* (plant), and *E. coli* (bacterium), which represent the majority of published research (145). Although functions of archaeal ATs remain poorly described, we in addition included *H. volcanii* (archaeon) that is a moderate halophile and can grow under conditions similar to *E. coli* and *S. cerevisiae* (146), unlike many other archaea that adapted to extreme environments. We will discuss each AT group, which forms a well-supported phylogenetic clade within each AT class, highlighting their substrate specificity and promiscuity, though their full substrate specificity is still largely uncharacterized (*open boxes* in Fig. 3). Representative AT functions and reactions were also mapped onto the metabolic networks (Fig. 2). Different gene/enzyme nomenclatures have been introduced in the literature even for functional orthologs from different organisms, and we kept these original gene/enzyme names here (e.g., Biosynthesis of Nicotinic Acid protein 3 [BNA3p] from yeast and KAT from plants and animals, and ydbL from *E. coli*; all of them belong to the KAT clade, Table 1). A comprehensive dataset on the nomenclature and properties of ATs from human, yeast, *Arabidopsis*, and *E. coli* that are listed in Table 1 is provided at <https://nfluxmap.github.io/resources/>.

#### Class I ATs

Class I ATs can be categorized into several phylogenetic clades (Fig. 3) and participate in a wide range of pathways including metabolism of many proteinogenic amino acids (Fig. 2).

##### Alanine/tyrosine AT clade

Alanine/tyrosine ATs make up a functionally diverse clade that mainly contains ATs acting on alanine/glutamate and/or

aromatic amino acids, which are generally hydrophobic except for glutamate, a major AT amino donor. Within this clade, the ATs that primarily act on alanine/glycine/glutamate, such as alanine AT and glutamate:glyoxylate AT, are more closely related to each other than to the tyrosine ATs that mainly act on aromatic amino acids (Fig. 3). Interestingly, *H. volcanii* has no ATs in this clade, and these essential activities must be provided by other uncharacterized ATs in this archaeon.

The alanine/glutamate branch of this clade contains human ALAT1 and ALAT2 (also known as SGPT1 and SGPT2; serum glutamate:pyruvate transaminase), yeast ALAT and ALAM (alanine transaminase), *Arabidopsis* AlaAT1, AlaAT2, GGAT1, and GGAT2, and *E. coli* AlaA. All these enzymes have glutamate:pyruvate or alanine:α-ketoglutarate AT activity and are mostly involved in alanine metabolism to pyruvate, the product of glycolysis, or vice versa (12, 147–154) (Fig. 2). In animals, alanine ATs are involved in the alanine–glucose cycle, in which alanine produced in the muscle tissue by glutamate:pyruvate AT activity is transported to the liver, where alanine is converted back to pyruvate and glutamate by alanine:α-ketoglutarate AT activity of hepatic ALATs (12–14). Then, the liver deaminates glutamate for the urea cycle and uses gluconeogenesis to convert pyruvate to glucose, which can be shuttled back to the muscle (14). *Arabidopsis* enzymes also show alanine:glyoxylate (155) and glutamate:glyoxylate activities (156), which are involved in photorespiration and overlap with the activity of class II and class IV alanine:glyoxylate ATs (see later sections). In some C4 plants (i.e., NAD-malic enzyme type carrying out C4 photosynthesis), alanine generated by alanine ATs are transferred from bundle sheath to mesophyll cells as a part of the C4 carbon fixation cycle (157). Notably, extreme redundancy exists in alanine AT activity of *E. coli* as the sextuple mutant of *alaA avtA alaC ilvE tyrB aspC* was still not auxotrophic to alanine (158).

The tyrosine AT branch of this clade contains human TAT and *Arabidopsis* TAT1 and TAT2, which primarily deaminate tyrosine in the initial step of tyrosine degradation (21, 159). Human TAT has narrow substrate specificity and is the only known AT that can discriminate between tyrosine and phenylalanine (160). Although human and other mammalian TATs can also use 3,4-dihydroxyphenylalanine or 3-O-methyl-dopa, their physiological significance is unclear (161–163). *Arabidopsis* TATs, on the other hand, are highly promiscuous and can utilize other aromatic amino acids (164), which may be involved in rebalancing of aromatic amino acids (21, 165). They can also use methionine efficiently (164), though its metabolic and physiological roles remain to be examined. Notably, non-AT enzymes, such as carbon–sulfur lyases (SUR1 (119) and COR13 (120)) involved in glucosinolate-specialized metabolism, evolved through recent divergence from TAT enzymes and within the Brassicales (mustard) order (21). To note, *E. coli* and yeast ATs having tyrosine AT activities are found in a different clade of class I ATs (see later).

##### Kynurenine AT (KAT) clade

KAT orthologs are found in all kingdoms (Fig. 3) and characterized by their highly promiscuous nature. Human

**Table 1**The list of previously reported AT enzymes from *Arabidopsis*, human, yeast, and *E. coli*

Class	Genes	Associated activity	References	
<i>Arabidopsis thaliana</i>				
I	ASP1-5	Aspartate AT	(221–224)	
	PPAAT	Prephenate AT	(189, 190)	
	TAA1, TAR1-4	Tryptophan AT	(91–94)	
	ISS1	Aromatic amino acid AT	(19, 231)	
	TAT1, 2	Tyrosine AT	(21, 164)	
	HisN6A, B	Histidinol phosphate (HisP) AT	(195, 196)	
	KAT	Methionine AT	(184)	
	?	Amino adipate AT		
	AlaAT1, 2	Alanine AT	(148)	
	GGAT1, 2	Glutamate:glyoxylate AT	(155, 156)	
	ALD1	Lysine AT	(191)	
	AGD2	L,L-Diaminopimelate AT	(192)	
	II	POP2	$\gamma$ -Aminobutyric acid (GABA) AT	(260)
		WIN1	Acetylornithine AT	(285)
$\delta$ OAT		Ornithine AT	(271)	
BIO1		7,8-Diaminopelargonic acid AT	(251)	
PYD4		$\beta$ -Alanine AT	(294)	
AGT2, 3		Alanine:glyoxylate AT	(156)	
III	BCAT1–7	Branched-chain amino acid/methionine AT	(15–17, 20, 307)	
	PSAT1, 2	Phosphoserine AT	(232, 240)	
IV	AGT1/SGAT	Serine:glyoxylate AT	(246, 247)	
<i>Homo sapiens</i> (human)				
I	AATC, AATM	Aspartate AT	(90, 217)	
	ALAT1, 2	Alanine AT	(12–14)	
	TAT	Tyrosine AT	(160–163)	
	KAT1, 3, AATM/KAT4	Kynurenine AT	(88, 167–169)	
	AADAT/KAT2	Amino adipate AT	(86, 208)	
	II	GABT	$\gamma$ -Aminobutyric acid (GABA) AT	(255, 257)
OAT		Ornithine AT	(274, 275)	
AGT1, 2		Alanine:glyoxylate AT	(287–293)	
III	BCAT1, 2	Branched-chain amino acid AT	(301)	
	IV	SERC	Phosphoserine AT	(236, 237)
		SPYA	Serine:pyruvate AT	(243)
<i>Saccharomyces cerevisiae</i> (yeast)				
I	AATC, AATM	Aspartate AT	(218–220)	
	ALAT, ALAM	Alanine AT	(154)	
	HIS5	Histidinol phosphate (HisP) AT	(199)	
	ARO8, 9	Aromatic amino acid AT	(182, 203–205)	
	II	Uga1	$\gamma$ -Aminobutyric acid (GABA) AT	(258)
OAT		Ornithine AT	(274)	
ARG8		Acetylornithine AT	(278, 279)	
BIOA		7,8-Diaminopelargonic acid AT	(252)	
III		BCAT1, 2	Branched-chain amino acid/methionine AT	(206)
	IV	SERC	Phosphoserine AT	(234)
		AGX	Alanine:glyoxylate AT	(244, 245)
<i>Escherichia coli</i>				
I	aspC, tyrB	Aspartate AT	(226, 227)	
	tyrB, aspC, ilvE	Tyrosine/phenylalanine AT	(226, 227)	
	hisC	Histidinol phosphate (HisP) AT	(200)	
	ybdL	Methionine AT	(185, 186)	
	alaA, alaC, avtA, ?	Alanine AT	(158)	
	II	gabT, puuE, patA	$\gamma$ -Aminobutyric acid (GABA) AT	(264–269)
bioA		7,8-Diaminopelargonic acid AT	(253)	
astC, argD		Acetylornithine AT	(281, 282)	
III		ilvE	Branched-chain amino acid AT	(308–310)
	IV	serC	Phosphoserine AT	(241, 242)
Sugar AT		arnB	UDP-4-amino-4-deoxyarabinose-ketoglutarate AT	(297)

Since many AT enzymes have multiple EC numbers, the complete list of their activities is provided in Figure 3 and the additional table at <https://nfluxmap.github.io/resources/>.

KAT1 and KAT3 have activity toward glutamine—hence also named as glutamine transaminase of kidney or liver (GTK and GTL), for KAT1 and KAT3, respectively (88, 166)—as well as a wide range of other amino acid substrates, particularly aromatic amino acids and kynurenine (88, 167–169). In mammals, KATs are involved in the tryptophan catabolic pathway by catalyzing the irreversible transamination of kynurenine to a highly unstable keto acid product, which is spontaneously converted to kynurenic acid (170–173). Although KAT activity is present in many tissues and cell types (*i.e.*, liver, heart, lungs, leucocytes, astrocytes, and microglia) (166, 170), its role in the brain is particularly important since kynurenic acid is a

neuroactive compound that is antagonistic to many excitatory amino acid receptors, such as *N*-methyl-D-aspartate subtype glutamate receptor (174) and  $\alpha$ 7-nicotinic acetylcholine receptor (170–173, 175). In the brain, KAT1/GTK acts as methionine AT of the SAM cycle to regenerate methionine from 4MTOB using glutamine as the amino donor (Fig. 2). KAT1/GTK can also prevent buildup of neurotoxic phenyl pyruvate by converting it into phenylalanine, which can be ring hydroxylated to tyrosine for degradation (176, 177). Besides their AT activity, KAT1 and KAT3 also has secondary  $\beta$ -lyase activity toward drugs and natural products that contain *S*-conjugates of cysteine and *Se*-conjugates of

L-selenocysteine, which may be important for detoxification of halogenated xenobiotics (178–180).

In yeast, BNA3p shows structural and sequence homology to KATs from other organisms and is demonstrated to have KAT activity *in vitro* (181). However, BNA3p unlikely contributes to KAT activity *in vivo*, as the double knockout of ARO8 and ARO9, amino adipate AT clade enzymes with KAT activity in yeast (182) (see *later section*), resulted in the complete loss of the apparent KAT activity *in vivo* (181). Further characterization could reveal the function and the main substrate, other than kynurenine, of BNA3p in yeast.

*Arabidopsis* KAT, despite its annotation based on phylogeny (183), has not been demonstrated to have KAT activity. Gene coexpression data indicate that *Arabidopsis* KAT is expressed with the gene encoding 5-methylthioribose kinase of the methionine salvage cycle (184) (Fig. 2). Biochemical characterization of tomato and maize KAT orthologs (initially named after mammalian GTKs) identified 4MTOB and glutamine as the preferred amino acceptor and donor, respectively (184). Therefore, plant KAT orthologs mediate the cryptic methionine AT within the methionine salvage cycle, which is also linked to biosynthesis of a plant hormone, ethylene (Fig. 2) (184). Notably, KATs work together with  $\omega$ -amidase that converts  $\alpha$ -ketoglutarate, the keto acid of glutamine, into  $\alpha$ -ketoglutarate (184), thus directing the reaction toward methionine formation, unlike typical reversible AT reactions. ybdL is KAT homolog in *E. coli*, but KAT activity has not been demonstrated. ybdL mainly functions not only as a methionine AT *in vitro* but also has side activities with histidine, aromatic amino acids, leucine, and glutamine (185, 186). Although its *in vivo* function remains to be examined, ybdL may be also involved in the SAM cycle. Thus, plant KATs and *E. coli* ybdL may be best to be referred to as methionine ATs.

In this clade, *Arabidopsis* in addition has prephenate AT (PPAAT), which converts prephenate into aroenate (187, 188). Aroenate is the immediate precursor for phenylalanine and tyrosine biosynthesis in plants (189, 190), unlike in many microbes, where prephenate is the last common precursor, and hence PPAAT is not required for phenylalanine and tyrosine biosynthesis. Thus, the evolution of PPAAT enzymes rerouted these aromatic amino acid pathways *via* the aroenate intermediate in the plant kingdom (187, 188). Interestingly, *H. volcanii* has four enzymes, three of which are closely related to plant PPAATs, with unknown function (Fig. 3). Some of them may have prephenate AT activity, if *H. volcanii* uses the aroenate pathway for phenylalanine and tyrosine biosynthesis, like in some microbes (190).

#### Lysine AT clade

Lysine AT clade contains *Arabidopsis* ADL1 (AGD2-Like defense response protein 1) and DAP-AT/AGD2 (L,L-2,6-diaminopimelate AT also known as Aberrant Growth and Death 2). Genetic and biochemical evidence support that ADL1 converts lysine to 6-amino-2-oxohexanoate (the keto acid analog of lysine), which spontaneously cyclize into  $\Delta^1$ -

piperidine-2-carboxylic acid, an intermediate of the plant defense signal pipercolic acid (191) (Fig. 2). Heterozygous *agd2* mutants show mild dwarfism but have elevated levels of defensive compound salicylic acid, whereas homozygous *ald1* mutants showed increased susceptibility to *Pseudomonas syringae* infection (192). Interestingly, homozygous DAP-AT/AGD2 mutation causes embryonic lethality, suggesting an essential role in plant development and likely in lysine biosynthesis (192). ALD1 and DAP-AT could be operating in lysine catabolism and anabolism, respectively (Fig. 2) (192). While *E. coli* alaC phylogenetically associates with the lysine AT clade (Fig. 3), it is involved in alanine biosynthesis (152).

#### Tryptophan AT clade

ATs in the tryptophan AT clade are found almost exclusively in plants and function in deaminating tryptophan into indole-3-pyruvate, which is further converted to a plant hormone auxin, indole acetic acid (91, 193) (Fig. 2). In *Arabidopsis*, tryptophan AT activity has been demonstrated for TAA1 (Tryptophan Aminotransferase of Arabidopsis 1) and TAR1 (Tryptophan Aminotransferase Related 1) and implied for TAR2 based on genetic evidence (92), whereas TAR3 and TAR4, which form a distinct subclade (Fig. 3), remain to be characterized. TAA1 and TAR1 are promiscuous, especially with other aromatic amino acids (91–94), and are the only known plant ATs having KAT activity. TAR3 and TAR4 have a putative domain of alliinase, the  $\beta$ -lyase involved in the formation of volatile compounds uniquely produced in the genus *Allium*, such as garlic and onions (194). *In vivo* roles of the enzymes in the TAR3 and TAR4 subclade, other than alliinases, are currently unknown.

#### Histidinol-phosphate (HisP) AT clade

Histidinol-phosphate (HisP) ATs act on HisP, a major intermediate of the *de novo* histidine biosynthesis (Fig. 2). ATs in the HisP AT clade are found in all kingdoms except animals, which depend on dietary intake of histidine. Yeast HIS5, *Arabidopsis* HISN6A and HISN6B (195, 196), and *E. coli* hisC are found within this clade. Of the three *H. volcanii* enzymes within the HisP AT clade, the loss of A0A558GCU9 results in histidine auxotrophy (197), like of *Corynebacterium glutamicum* HisC (198) and yeast HIS5 (199). While the activity of yeast, *Arabidopsis*, and *E. coli* HisP ATs with substrates other than HisP is not known (200), HisC of the hyperthermophile *Thermotoga maritima* and *Bacillus subtilis* works as a bifunctional HisP and aromatic AT (201, 202). Therefore, promiscuity might have emerged in some lineages within the HisP AT clade.

#### Amino adipate AT clade

The amino adipate AT clade contains promiscuous ATs from yeast, human, and *E. coli*, but not from *Arabidopsis*. Yeast ARO8 and ARO9 show AT activity toward aromatic amino acids, methionine, leucine, kynurenine, and  $\alpha$ -amino adipate (182, 203). ARO8 is involved in the biosynthesis of tyrosine, phenylalanine (182, 203, 204), and lysine (205), and salvage of methionine (206), whereas ARO9 is mainly



involved in tryptophan catabolism (203, 204). However, the *aro8* deficiency can be compensated by the presence of ARO9, and only the *aro8/aro9* double mutant show phenylalanine and tyrosine auxotrophy (182, 203). For tyrosine metabolism to 4-hydroxyphenylpyruvate and then eventually to ubiquinone or coenzyme Q biosynthesis (Fig. 2), ARO8 and ARO9, as well as BNA3p, BCATc (or Bat2), and AATC (or Aat2), are redundantly involved based on their quintuple yeast mutant (207). Human AADAT (or KAT2) shows activity toward a wide range of amino and keto acids but uses  $\alpha$ -aminoadipate and kynurenine as preferred amino acid substrates (86). In kidney and liver, AADAT/KAT2 deaminate  $\alpha$ -aminoadipate into  $\alpha$ -ketoadipate as a part of lysine catabolism (86, 208) (Fig. 2). The analogous lysine catabolic pathway exists in plants (209), but the aminoadipate AT activity is likely mediated by an unknown AT(s) from another clade or class. In the brain, AADAT/KAT2 is also important in deaminating  $\alpha$ -aminoadipate that is toxic to glial cells and also converts kynurenine to kynurenic acid, together with KAT1 and KAT3 from the KAT clade (see aforementioned section) (86, 210–213). *E. coli* *avtA* in this clade is involved in alanine biosynthesis and transaminates pyruvate into alanine using either valine or homoalanine as amino donors (152, 214, 215).

#### Aspartate AT clade

The aspartate AT clade is sister to all other class I AT, and their members are involved in transamination between aspartate and oxaloacetate, which is often coupled to glutamate for further assimilation of nitrogen into aspartate and their derived amino acids (Fig. 2). Aspartate ATs also function as a part of the malate–aspartate shuttle, where oxaloacetate is reduced by NADH and malate dehydrogenase to malate, which can then be transported across organelle compartments as a reducing equivalent (Fig. 2) (216), as seen for human cytosolic AATC and mitochondrial AATM (217). In addition, AATM is able to irreversibly catalyze the synthesis of kynurenic acid from kynurenine in the brain and hence has the alternative name of KAT4 (90). Humans also have one additional aspartate AT homolog, AATC2, that however lacks the conserved active-site arginine and remains to be functionally characterized. Yeast has a similar pair of aspartate ATs, AATC and AATM, which localize to peroxisome and mitochondria, respectively, and are also involved in the malate–aspartate shuttle based on biochemical and genetic evidence (218–220).

*Arabidopsis* contains five aspartate AT homologs (ASP1–ASP5) with different subcellular localizations: mitochondrial ASP1, cytosolic ASP2 and ASP4, and plastidial ASP3 and ASP5 (221–223). ASP2 plays the major role in nitrogen assimilation and transport, as the *asp2* loss-of-function mutant shows 80% reduced aspartate transported in the phloem with compromised growth (222, 224). Other *asp* mutants of *Arabidopsis* show little change in amino acid profile or growth suggesting their functional redundancy (222).

*E. coli* has two enzymes in this clade *aspC* (also known as AAT) and *tyrB*, which are likely derived from a shared

ancestral enzyme through a recent duplication (225). In fact, *aspC/AAT* has high  $K_M$  and low activity toward aromatic amino acids tyrosine and phenylalanine (226, 227), and substantial tyrosine AT activity can be obtained by DNA shuffling and *in vivo* selection of *AspC/AAT* (225, 228). *aspC/AAT* oversees aspartate synthesis and coordinate cell cycle in *E. coli* (229). *TyrB* exhibits AT activity toward tyrosine as well as other aromatic amino acids and (3*S*)-2-oxo-3-phenylbutanoate (230). The triple mutants of *tyrB*, *aspC*, and *ilvE* (a class III enzyme) are auxotrophic to tyrosine and phenylalanine, suggesting that these enzymes from three distinct clades are together responsible for tyrosine AT activity in *E. coli* (226), similar to the situation in yeast (207). Interestingly, *H. volcanii* has no aspartate AT homolog and may have a novel aspartate AT(s) in other AT clades or classes.

#### Other class I ATs

Class I contains several ATs that form single enzyme clades, which appear to have distal evolutionary relationship to other class I ATs. *Arabidopsis* aromatic amino acid AT, ISS1, clearly belongs to class I but does not associate with any specific sister clade and shows activity with a variety of aromatic amino acids (19, 231). The phenotype of the *iss1* mutant supports that, together with TAA1 and TAR enzymes from the tryptophan AT clade, ISS1 plays a role in homeostasis of tryptophan and its derived hormone auxin *in planta* (19, 231). A *H. volcanii* enzyme A0A558GE26 in class I does not associate with any sister clade either (Fig. 3), and its function is currently unknown.

#### Class IV ATs

Class IV contains alanine/serine AT and serine/phosphoserine AT clades (Fig. 3) and are mainly involved in the metabolism of serine, glycine, and alanine.

#### Phosphoserine AT clade

The phosphoserine AT clade contains enzymes from human (SERC), yeast (SERC), *Arabidopsis* (PSAT1 and PSAT2), and *E. coli* (*serC*), but none from *H. volcanii*. These enzymes catalyze the reversible glutamate:3-phosphohydroxy-pyruvate transamination that forms *O*-phospho-L-serine in the phosphorylated pathway of serine biosynthesis (Fig. 2) (232–235). Human SERC (also known as PSAT) plays a critical role in the brain, since serine cannot be transported efficiently across the blood–brain barrier (236, 237). Indeed, mutations in *SERC* lead to phosphoserine AT deficiency (PSATD) and Neu-Laxova syndrome 2 (237, 238). *Arabidopsis* has two isoforms, PSAT1 and PSAT2, both of which localize to the plastids and are involved in serine biosynthesis *via* the phosphoserine pathway (Fig. 2) (232). Although photorespiration is the major source of serine in photosynthetic tissues (239), silencing of PSAT1, but not PSAT2, resulted in the strong growth retardation, which can be rescued by serine supplementation, highlighting the importance of nonphotorespiratory synthesis of serine *via* PSAT1 (232). The PSAT1-silenced plants also showed disturbed ammonia assimilation in roots likely because of

reduced recycling of  $\alpha$ -ketoglutarate to be used in the GS–GOGAT cycle (232, 240). In addition to serine biosynthesis, *E. coli* serC can also catalyze glutamate:3-hydroxy-4-phospho-hydroxy- $\alpha$ -ketobutrate transamination that forms O-phospho-4-hydroxy-L-threonine for the synthesis of its own cofactor PLP (241, 242).

#### Alanine/serine AT clade

The alanine/serine:glyoxylate AT clade contains human SPYA (serine:pyruvate AT), yeast AGX1 (alanine:glyoxylate aminotransferase), and *Arabidopsis* AGT1 (alanine:glyoxylate aminotransferase), which are not closely related to functionally similar class I alanine AT and glutamate:glyoxylate ATs or class II  $\beta$ -alanine/L-alanine ATs from *Arabidopsis* and human. Human SPYA is present in peroxisomes and involved in detoxification of glyoxylate to glycine. The loss-of-function mutations of SPYA lead to the hereditary kidney stone disease, primary hyperoxaluria type 1, because of increased excretion and accumulation of oxalate from glyoxylate oxidation (243). Yeast AGX1 converts glyoxylate to glycine using alanine as an amino donor and participates in glycine biosynthesis, as a side branch of the glyoxylate shunt (Fig. 2) (244, 245). *Arabidopsis* AGT1 (or serine-glyoxylate AT [SGAT]) can catalyze transamination reactions between various substrates, such as serine:glyoxylate, alanine:glyoxylate, serine:pyruvate, and asparagine:glyoxylate AT activities (246, 247). AGT1/SGAT is localized in the peroxisome and is essential for photorespiration in photosynthetic tissues (246). Although serine is its preferred amino donor, AGT1 can also use asparagine (248) and is involved in metabolism of serine, glycine, and asparagine in roots (247, 249). This clade contains no enzymes of *E. coli*, as most bacteria primarily convert glyoxylate to malate but has three uncharacterized *H. volcanii* enzymes, two of which are closely related to human SPYA (Fig. 3).

#### Class II ATs

Human, yeast, *Arabidopsis*, and *E. coli* ATs within class II can be categorized into several clades based on their phylogenetic relationship (Fig. 3). AT reactions catalyzed by class II ATs are mainly involved with the metabolism of non-proteinogenic amino acids (Fig. 2).

#### 7,8-Diaminopelargonic acid (DAPA) AT (BIO1) clade

7,8-Diaminopelargonic acid (DAPA) ATs are involved in biosynthesis of biotin, an essential enzyme cofactor known as vitamin B<sub>7</sub>, and are found in yeast (BIOA), *Arabidopsis* (BIO1), and *E. coli* (bioA) but not in animals that lack biotin biosynthesis. In plants and fungi, BIO1 is a part of a bifunctional enzyme and fused with a dethiobiotin synthetase (also known as BIO3) (250–252), whereas in bacteria, BIO1 and BIO3 are separate enzymes (253). Yeast, *Arabidopsis*, and *E. coli* BIO1 catalyzes the conversion of 7-keto-8-aminopelargonic acid to DAPA (Fig. 2), using specifically SAM as the amino donor. DAPA AT is the only AT known to use SAM as an amino donor to date (250–253), but some bacterial DAPA ATs can

utilize other unusual amino donors such as lysine, instead of SAM (*i.e.*, *B. subtilis*) (254).

#### $\gamma$ -Aminobutyric acid (GABA) AT clade

GABA ATs form a small clade that contains yeast GABAT and human GABT and transaminates GABA to succinic semialdehyde (255, 256). However, *Arabidopsis* and *E. coli* GABTs (*i.e.*, AtPOP, EcgabT, EcpuuE, and EcpatA, Fig. 3) are not part of the GABA AT clade (see *later section*). Human GABT, also known as ABAT, is an enzyme of the GABA shunt that deaminates and controls the level of the neurotransmitter GABA (255) (Fig. 2). Human GABT is also able to use  $\beta$ -alanine as a substrate (257), a reaction that does not occur in yeast GABAT (256). Yeast GABAT, also known as Uga1 (Utilization of GABA 1), deaminates GABA as a source of nitrogen (258), which can also enhance oxidative stress tolerance by increasing the NADPH pool through succinic semialdehyde dehydrogenase (GABAT in Fig. 2) (259).

#### Other class II ATs

In addition, class II contains a variety of ATs with different metabolic roles that do not confidently associate with a certain clade. Interestingly, though, they all have activities with non-proteinogenic and nonalpha amino acids (Fig. 3).

*Arabidopsis* and *E. coli* also have GABA ATs but distantly related to yeast GABAT and human GABT (Fig. 3). *Arabidopsis* POP2 is localized in mitochondria (260, 261) and recycles amino acids and nitrogen *via* the GABA shunt pathway together with glutamate decarboxylase and succinic semialdehyde dehydrogenase (succinic semialdehyde dehydrogenase, Fig. 2) (262, 263). POP2 specifically uses GABA as the only amino donor among 21 other amino acids tested, whereas both pyruvate and glyoxylate, but not  $\alpha$ -ketoglutarate, can act as amino acceptors (260). In contrast, *E. coli* gabT is required for GABA utilization as its sole nitrogen source (264) and also act as the 5-aminovalerate AT for degradation of lysine (265). puuE also deaminates GABA but is induced in the presence of putrescine, which can be converted to GABA and used as the sole nitrogen source (266). Putrescine can be also degraded by patA through an alternative pathway *via*  $\gamma$ -aminobutyraldehyde, which can be spontaneously cyclized to  $\Delta^1$ -pyrroline or enzymatically oxidized to GABA (267, 268). patA also transaminates other alkane- $\alpha$ - $\omega$ -diamines, including the cadaverine and spermidine for polyamine degradation (268, 269) (Fig. 2). *E. coli* puuE, patA, and gabT are closely related phylogenetically and likely evolved from a promiscuous ancestral enzyme but have been co-opted to function in different steps of polyamine and GABA metabolic pathways (Fig. 3).

Ornithine AT enzymes catalyze the conversion of ornithine to glutamate-5-semialdehyde (270, 271), which spontaneously cyclizes into pyrroline-5-carboxylate (271), an intermediate of arginine and proline metabolism (272, 273) (Fig. 2). While human and yeast OATs function in the synthesis of pyrroline-5-carboxylate (274, 275), *Arabidopsis*  $\delta$ OAT is involved in the recycling of nitrogen during

arginine catabolism *via* ornithine (271). Although the diamino acid ornithine has an  $\alpha$ -amino group, ornithine ATs transaminate the side-chain  $\delta$ -amino group but not the  $\alpha$ -amino group (Fig. 3).

Acetylornithine ATs convert *N*-acetylglutamate-5-semialdehyde into *N*<sup>2</sup>-acetylornithine (276), as a part of *de novo* arginine biosynthesis (277–279) (Fig. 2). Yeast ARG8 is essential for arginine biosynthesis (278, 279), and *E. coli*, argD is a bifunctional enzyme having both acetylornithine AT and *N*-succinyl-L,L-diaminopimelate: $\alpha$ -ketoglutarate AT activities involved in arginine and lysine biosynthesis, respectively (280). In contrast, *E. coli* astC, paralogous to ArgD (Fig. 3), utilizes succinylornithine and ornithine as substrates (281, 282) and functions in arginine catabolism *via* the arginine succinyltransferase pathway (283, 284). Phylogenetically related *Arabidopsis* WIN1 (HopW1 Interacting Protein 1 also known as Tumor Prone 5, TUP5, Fig. 3) can complement the arginine auxotrophy of yeast ARG8 mutant (285), and its *Arabidopsis* mutant exhibits reduced arginine accumulation. Therefore, WIN1/TUP5 is likely responsible for the acetylornithine AT activity in plants, which has been detected in soybean plastid fraction (286), though its biochemical characterization remains to be conducted.

$\beta$ -Alanine/L-alanine AT subclade is represented by human AGT2 and *Arabidopsis* AGT2, AGT3, and PYD4 (pyrimidine 4). Human AGT2 is a promiscuous mitochondrial enzyme that was first discovered to be an AGT (287) but can use a number of other substrates including *N*<sup>G</sup>,*N*<sup>G</sup>-asymmetric dimethylarginine (ADMA), a potent inhibitor of renal nitric-oxide synthase essential for the regulation of blood pressure by kidneys (288–291). Human AGT2 also acts as  $\text{D}$ - $\beta$ -aminoisobutyrate:pyruvate AT for thymine catabolism (292, 293). The function of *Arabidopsis* AGT2 and AGT3 is poorly understood, and recombinantly expressed AGT2 lacks alanine:glyoxylate AT activity (156). PYD4 coexpresses strongly with PYD1, PYD2, and PYD3 of the reductive pyrimidine nucleotide degradation pathway and is predicted to catalyze the  $\beta$ -alanine AT step (thus also known as BAT) (294–296). PYD4 showed alanine:glyoxylate AT activity *in vitro*, although its physiological significance is unclear (294), and can also functionally complement alanine and  $\beta$ -alanine deficient *E. coli* mutants (294). Importantly, both human and *Arabidopsis* harbor another alanine:glyoxylate AT from class IV—human SPYA and *Arabidopsis* AGT1 (Fig. 3, see “Class IV ATs” section)—, which is more active in glyoxylate metabolism than class II alanine:glyoxylate ATs.

*H. volcanii* has seven enzymes in class II but, except for one (A0A558G945) related to acetylornithine ATs, none are closely related to enzymes from other organisms (Fig. 3). Thus, ATs in this family might have diverged relatively recently, perhaps, at least after the divergence of archaea and eubacteria.

#### Other PLP fold type I ATs

Sugar ATs, like *E. coli* UDP-4-amino-4-deoxy-L-arabinose:oxoglutarate AT (arnB), form a functionally and structurally distinct AT group within PLP fold type I enzymes (Box 1).

These sugar ATs are typified by nucleotide interacting loops that are not found in other types of ATs (297–299). arnB homologs from other bacteria have been characterized as sugar ATs that produce amino-sugars, such as UDP-4-amino-4-deoxy-L-arabinose (297), which is incorporated into lipid A from the outer membrane lipopolysaccharide (300) (Fig. 2). *H. volcanii* also contains a sugar AT, annotated as A0A558GCH4, with unknown function. Because of the distal evolutionary relationship of sugar ATs to other PLP fold type I ATs, they are not included in the phylogeny in Figure 3.

#### Class III ATs

Class III ATs mainly contain  $\text{D}$ -amino acid AT and BCAT enzymes (Fig. 3) and are mainly involved with the metabolism of amino acids with unusual stereochemistry, such as branched-chain and  $\text{D}$ -amino acids (Fig. 2).

##### *Branched-chain amino acid AT (BCAT) clade*

BCATs found in this clade are present in all five kingdoms and transaminate valine, leucine, and isoleucine. However, some yeast and *Arabidopsis* BCAT isoforms can also utilize methionine (15), whereas *E. coli* IlvE can utilize phenylalanine (226).

In human, BCATs are catabolic enzymes that degrade the essential branched-chain amino acids and synthesize glutamate, although the reaction is generally reversible. Human BCAT2 is mitochondrial and thus is also known as BCATm (301). However, one splice variant of BCAT2 lacks the signal peptide and is cytoplasmic (302), whereas another variant has a 12-amino acid deletion and is localized in nuclei and mitochondria (303). Human BCAT1 is cytoplasmic (hence BCATc) and is mostly present in the peripheral nervous system of brain, ovaries, and testes (301). In the rodent brain, BCATc and BCATm are both involved in nitrogen transfer between astrocytes and neurons where branched-chain amino acids are used as amino donors for neurotransmitter biosynthesis (304–306).

Yeast BCAT1 (mitochondrial, also known as Bat1p) is involved in the synthesis of branched-chain amino acids and also of methionine from 4MTOB in the methionine salvage pathway using branched-chain amino acids as amino donors (206) (Fig. 2). Yeast BCAT2 (cytoplasm, also known as Bat2p) mainly functions in the degradation of branched-chain amino acids and methionine salvage pathway. BCAT2 is more promiscuous than BCAT1 and uses branched-chain amino acids as well as lysine and proline as amino donors (206).

*Arabidopsis* has seven BCAT paralogs, though BCAT7 has no detectable transcript and is likely a pseudogene (20, 307). The remaining six BCATs include mitochondrial BCAT1, plastidial BCAT2, BCAT3, BCAT5, and cytosolic BCAT4, BCAT6 (20, 307). All of them, except BCAT4, show strong BCAT activity *in vitro* and are able to rescue the yeast auxotrophic mutant of branched-chain amino acids (307). The mitochondrial and plastidial BCATs are typically involved in the catabolism and biosynthesis of BCAAs, respectively (307). Notably, BCAT4 shows the highest activity toward 4MTOB



and 4MTOB, intermediates of the methionine-derived glucosinolate biosynthesis, and only has residual activity with leucine and its keto acid, 4-methyl-2-oxopentanoic acid (16). BCAT3 and BCAT6 also have substantial activity toward 4MTOB, the keto acid of methionine (15, 17). Thus, BCAT3, BCAT4, and BCAT6 are also involved in methionine metabolism (Fig. 2 pathway) as their knockout mutants of *Arabidopsis* have altered levels of methionine and methionine-derived specialized metabolites, such as glucosinolates (15–17).

*E. coli* ilvE reversibly catalyzes the final steps of isoleucine, valine, and leucine biosynthesis and can also catalyze transamination of tyrosine and phenylalanine though inefficiently (308–310). *H. volcanii* BC61-TAm is most closely related to *E. coli* ilvE, although the biological function is unclear. Whereas BCAT activity has not been tested, this archaea enzyme can utilize (*R*)-methylbenzylamine as an amino donor with  $\alpha$ -ketoglutarate being the acceptor (311). Consistent with the lifestyle of halophilic mesophile *H. volcanii*, BC61-TAm is most active at 50 °C with 1 M NaCl (146, 311).

#### *D*-Amino acid AT clade

*D*-Amino acids are the *D*-enantiomers of proteinogenic amino acids that are found on microbial cell walls and certain bioactive peptides like bacterial antibiotics and the venoms and toxins of various animals (312). *D*-Amino acids are typically produced from their *L*-enantiomers by racemases (313). *Arabidopsis* and *H. volcanii*, but not yeast, human, or *E. coli*, have ATs in this *D*-amino acid AT clade that are the only representative of this clade. However, *D*-amino acid AT orthologs are present in certain bacteria (*i.e.*, *Bacillus* species (314)) and fungi (*i.e.*, *Aspergillus* species (315)). *Arabidopsis* DAAT is the major AT involved in metabolism of *D*-amino acids, which stimulate ethylene production through unknown mechanisms (123). *Arabidopsis* DAAT prefers *D*-methionine among many other *D*-amino acid substrates that can be used and utilizes both pyruvate and, to a lesser extent,  $\alpha$ -ketoglutarate as amino acceptors (forming *D*-alanine and *D*-glutamate, respectively) (123, 316). *Arabidopsis* DAAT also functions as 4-amino-4-deoxychorismate lyase for the synthesis of a folate precursor, aminobenzoate (122). *E. coli* facilitates *D*-amino acid metabolism by racemases that can interconvert *L*- and *D*-amino acids (317), whereas yeast and humans use *D*-amino acid oxidases to deaminate *D*-amino acids (318).

#### Potential modes and mechanisms of AT diversification

An interesting property of many ATs is their apparent substrate promiscuity even from the incomplete biochemical data (Fig. 3). For instance, *Arabidopsis* TAT1 catalyzes transamination between glutamate and tyrosine but can also use phenylalanine, tryptophan, histidine, methionine, and leucine (Fig. 3) (164). Similarly, human KAT2 prefers kynurenine and amino adipate as substrates but can also use 16 other amino acids (86). Substrate specificity can differ considerably, even among closely related ATs, and often accompanies only subtle differences in active-site conformation that affect a substrate-

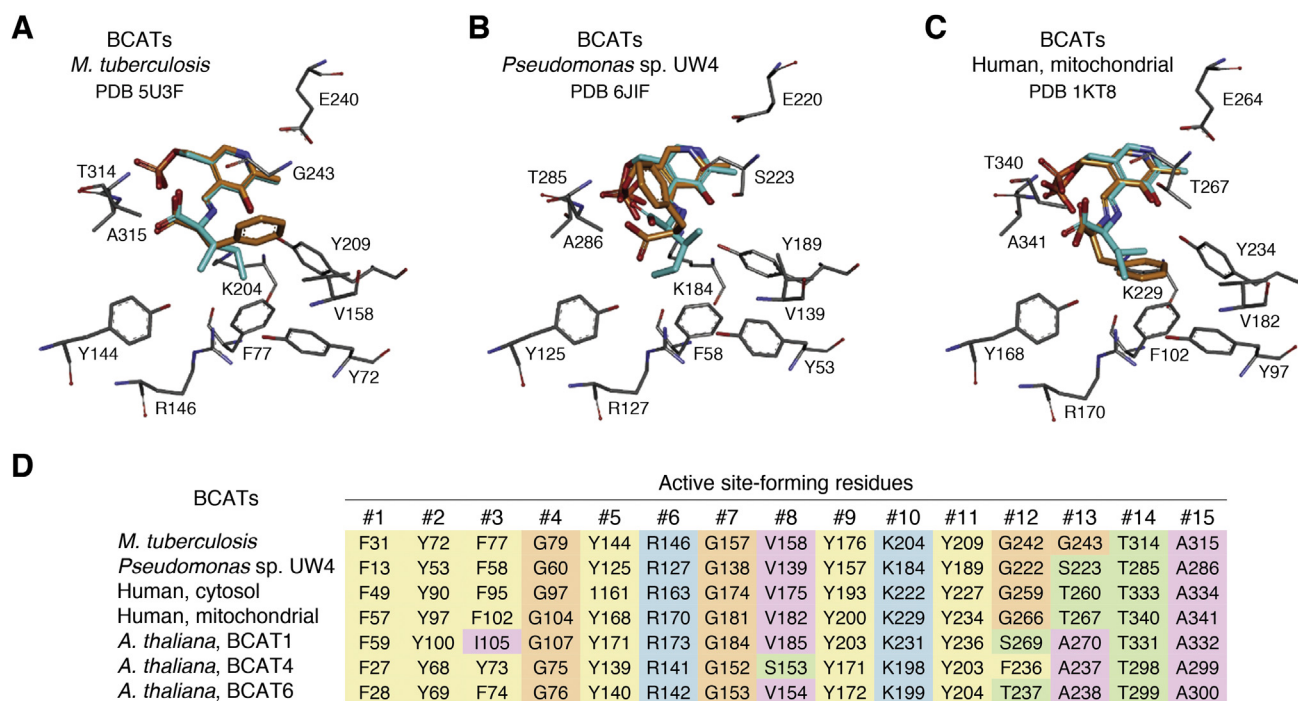
binding and a catalytic process (319). Consequently, mutations of active-site residues can significantly influence catalytic properties of ATs and drive AT evolution (320–323).

#### Mechanical and structural basis of AT promiscuity

During AT-catalyzed reactions, two substrates, often with different chemical properties or structures, need to bind to the same location on the active site sequentially (as discussed in “Mechanisms of AT-catalyzed reactions” section). In most organisms, nitrogen shuttles are constructed around glutamate (30, 37), aspartate (324), and alanine (325), since their corresponding keto acids— $\alpha$ -ketoglutarate, oxaloacetate, and pyruvate, respectively—are gluconeogenic and abundant intermediates of glycolysis and tricarboxylic acid cycles, which allow efficient coregulation of carbon and nitrogen metabolism. Therefore, many ATs evolved to utilize at least one of these three amino/keto acids as substrates. For example, in plants, assimilated ammonia is transferred mainly as glutamate or aspartate, and the amino group is transferred to other keto acid substrates by ATs (1, 8, 10). Similarly, in animals, excess amino acids are first converted to glutamate by different ATs and subsequently deaminated oxidatively by glutamate dehydrogenase to yield ammonia (326). Consequently, many ATs that act on uncharged hydrophobic (*e.g.*, leucine) and aromatic (*e.g.*, tyrosine) substrates must also be able to bind to a negatively charged hydrophilic substrate (glutamate).

AT active sites utilize two mechanisms to accommodate two or more substrates having side chains of different sizes and chemical properties (327). In one example, aromatic amino acid ATs induce a rearrangement of the hydrogen bond network through conformational changes, allowing the formation of charged pockets for acidic side chains and neutral pockets for aromatic side chains at the same location (327). In the case of BCATs, however, a hydrophobic pocket that is implanted with hydrophilic islands behaves much like a “lock-key” model and is capable of housing both hydrophobic and acidic side chains without undergoing conformational change (142, 319, 327). It is important to note that these two mechanisms are not mutually exclusive, and many other ATs, such as *E. coli* HisP AT and *Thermus thermophilus* glutamine AT and acetylornithine AT, can achieve multisubstrate specificity by a mixture of the two solutions (142, 327–329).

Computational approaches such as molecular modeling have been employed to comprehend the molecular basis of substrate promiscuity (130). While the structural basis of multisubstrate specificity achieved by conformational rearrangement of the active site remains difficult to simulate without crystallization of individual enzymes at different reaction stages, enzymes that use the “lock-key” mechanism, such as BCATs, can be computationally simulated with reasonable accuracy. In our protein–ligand docking analyses of external aldimine intermediates with isoleucine and phenylalanine, *M. tuberculosis* BCAT (Protein Data Bank [PDB] ID: 5U3F, Fig. 6A) showed nearly the same binding orientation regardless of substrates, whereas *Pseudomonas* BCAT (PDB ID: 6JIF, Fig. 6B) and human mitochondrial BCAT (PDB ID:



**Figure 6. Sequence and structure features determining the substrate specificity of BCATs.** Molecular modeling to predict binding modes of external aldimine intermediates of isoleucine (cyan) and phenylalanine (orange) with three homologous BCATs: *A*, *Mycobacterium tuberculosis* (PDB ID: 5U3F). *B*, *Pseudomonas* sp. UW4 (PDB ID: 6JIF). *C*, human mitochondria (PDB ID: 1KT8). *D*, putative amino acid residues of several BCATs consisting of the active sites deduced from sequence alignment. Color shading indicates physicochemical properties of amino acids: aliphatic/hydrophobic (pink), aromatic (yellow), glycine (orange), hydrophilic (green), and positive (blue). BCAT, branched-chain amino acid AT; PDB, Protein Data Bank.

1KT8, Fig. 6C) with phenylalanine, but not isoleucine, triggered a tilt on the entire intermediate structure presumably induced by S223 and T267, respectively (#13 position in Fig. 6D). This result suggests that the tilt can deteriorate the proton abstraction by 1,3-prototropic shift, an important mechanism to proceed transamination reactions in BCATs (131), by weakening the interaction of pyridine ring N-E264 and the H-bond networks between C $\alpha$ -H, pyridine ring O, catalytic Lys, and Y234 of the human mitochondrial BCAT (Fig. 6C). The sequence analysis of active-site residues in BCATs supports the modeling result and shows the highest divergence at the residue involved (*i.e.*, position #13 that corresponds to T267 of human mitochondrial BCAT, Fig. 6D).

Structural and/or mechanistic changes could easily eliminate promiscuity of one half-reaction, such as in the case of aminoacyl-tRNA amidotransferase GatCAB that has separate binding sites for the amido donor glutamine and amido acceptor Glu-tRNA<sup>Gln</sup>, and uses an ammonia channel to transfer and form Gln-tRNA<sup>Gln</sup> (330). While glutamine and asparagine are structurally and chemically very similar,  $k_{cat}/K_M$  of GatCAB for glutamine is ~140-fold higher than asparagine (331). However, if an AT gained a similarly high preference for a substrate of one-half reaction, the active site could inadvertently not be able to effectively recognize the substrate of the other half-reaction, which would reduce fitness. Therefore, the evolution of AT substrate specificity is shaped by a tight balance to bind the substrates of both half-reactions. The tradeoff could be the inability to prevent side reactions of substrates that are chemically or structurally similar to, or in-between of,

the main substrates. Indeed, multisubstrate specificity is observed for many ATs, especially with related substrates, for example, tyrosine, phenylalanine, and tryptophan used by many aromatic amino acid ATs, and alanine, serine, and glycine utilized by alanine:glyoxylate ATs (15, 21, 246, 247). Thus, both mechanistic and functional constraints likely contributed to the apparent substrate promiscuity of ATs, which in turn provided a unique opportunity for ATs to mediate multiple reactions *in vivo*, unlike often highly specific primary metabolic enzymes.

#### At multisubstrate specificity for metabolic plasticity and robustness

The substrate promiscuity of ATs is not necessarily a drawback and can provide advantages in new or changing environments (321, 322). Many AT reactions with secondary cosubstrates could provide metabolic plasticity, when the availability of the main cosubstrate is low, preventing a certain AT-catalyzed reaction to seize up. For instance, TAA1 can continue to deaminate tryptophan into indole-3-pyruvate for auxin production even in the limited availability of its main amino acceptor pyruvate (91), such as under hypoxia with active glycolysis, as TAA1 can also use phenylpyruvate or 4-hydroxyphenylpyruvate (94). Collectively, the substrate promiscuity or side activities of many AT enzymes could form an “underground” (320, 322) amino acid metabolic network, which may have negligible influence on overall metabolism under optimal conditions but can rebalance amino acid levels, such as

under various stresses. Therefore, the multisubstrate specificity of AT enzymes may play key roles in metabolic plasticity and robustness in response to environmental changes.

AT substrate promiscuity could lead to multifunctionality if a certain side activity confers enhanced fitness and is positively selected for while also maintaining primary activity. For instance, human KAT2, or AADAT, mainly functions as detoxifying amino acid in the liver and kidney (86, 208) while degrading both amino acid and kynurenine in the brain (see “Class I ATs” section) (86, 210–213). Since yeast amino acid ATs (ARO8 and ARO9) are also promiscuous with aromatic amino acids including kynurenine (182), the promiscuous kynurenine AT activity was likely present in an ancestral eukaryotic amino acid AT and positively selected for to have a role in the brain, while maintaining the primary amino acid AT activity. Ultimately, the ability to act on many substrates is advantageous for detoxification enzymes like KAT2 (332, 333)

It is important to note, however, that AT substrate promiscuity can be selected against in some cases. Certain substrates should not be consumed by ATs in other pathways, even when the alternative substrate is structurally or chemically similar. For example, human TAT, unlike other tyrosine ATs (see “Class I ATs” section), is highly specific toward tyrosine but not phenylalanine (160), which only differ by a 4-hydroxy group on the aromatic ring. While humans cannot synthesize any of the aromatic amino acids, tyrosine is deemed conditionally essential because excess phenylalanine is converted to tyrosine by phenylalanine 4-hydroxylase (334, 335). Thus, human TAT, which mainly functions to breakdown excess tyrosine (160), needs to reject phenylalanine not to inadvertently deplete the phenylalanine pool and interfere with phenylalanine 4-hydroxylase to produce tyrosine.

## Conclusion and future perspectives

Transamination reactions have always been an essential part of the cellular metabolic network. Pre-LUCA life may have initially achieved transamination nonenzymatically, or through free PLP, followed by nonproteinogenic biocatalysts (*i.e.*, ribotransaminase), which were then gradually replaced by proteinogenic ATs. ATs evolved independently from two distinct PLP-dependent enzyme fold type I (for class I, II, and IV ATs) and fold type IV (for class III ATs). AT family enzymes were already diversified before LUCA and further expanded during and after the GOE with the appearance of complex eukaryotic life. Reconstructing and characterizing ATs at ancestral nodes, including those of LUCA and last eukaryotic common ancestor, can potentially recapitulate evolutionary pathways of AT enzymes and the nitrogen metabolic networks throughout life history.

Class I, II, IV ATs and class III ATs convergently evolved active sites that can catalyze AT reactions using nearly identical mechanisms, likely because of the universal utilization of PLP in biological transamination reactions. The ping-pong bi-bi mechanisms of AT reactions between two distinct substrates structurally predisposed ATs to maintain substrate promiscuity, which provided a starting point for the evolution

of new enzymes and pathways (*i.e.*, biosynthesis of new amino acids and specialized metabolites). Although AT reaction mechanisms have been previously characterized in detail (4), the structural basis of the promiscuity is not yet fully understood. AT enzymes crystallized with a diverse set of substrates could reveal structural features that influence substrate promiscuity, which in turn can facilitate computational prediction and redesign of the function of ATs. In addition, detailed characterization of the subunit-subunit dynamics of AT multimers could further reveal some of the poorly understood properties of ATs, such as subunit synergy and potential heteromultimer formations between different ATs (137, 336, 337).

Many AT enzymes have been characterized, but the functions of some AT enzymes are still unknown even in model organisms (*i.e.*, human, yeast, *Arabidopsis*, *E. coli*). Given that a certain AT reaction can be mediated by ATs from different clades or even different classes depending on organisms, predicted functions of AT enzymes from nonmodel organisms based solely upon sequence alignments should be considered tentative and confirmed experimentally. Considering the rapid evolvability of ATs because of their inherent substrate promiscuity, unlike other primary metabolic enzymes, it would be interesting to examine potential differences in AT substrate specificity between different species even within the same kingdom (*e.g.*, C3 versus C4 plants having different nitrogen use efficiency, carnivorous versus herbivorous animals having different dietary nitrogen) and assess their impacts on overall nitrogen metabolic network.

Since most studies “looked for” AT enzymes that can catalyze a certain reaction of one’s interest, the potential side activities of AT enzymes have not been tested in many cases. These promiscuous AT reactions may be physiologically insignificant under optimal growth conditions but can provide metabolic plasticity and robustness such as under changing environmental conditions. Testing all substrate combinations—that is, 20 amino acid and 19 keto acid substrates give 380 combinations—is challenging with traditional methods; therefore, the development of high-throughput methods capable of characterizing the full spectrum of AT reactivity is needed. In the meantime, computational approaches, such as molecular modeling (338, 339) and deep/machine learning (340), can accelerate the functional mapping and sequence-structure-function analyses of ATs. Together, comprehensive characterization of AT substrate specificity will reveal the true functionality of AT enzymes and their roles in interconnecting different branches of nitrogen metabolic networks, which are surely much more complex than we currently understand.

---

*Acknowledgments*—This work was supported by the US Department of Energy (DOE), Office of Science, Office of Biological and Environmental Research, Genomic Science Program (grant no. DE-SC0020390), and the Joint Genome Institute (award no.: CSP-503757). The work conducted by the US DOE Joint Genome Institute, a DOE Office of Science User Facility, is supported under contract no. DE-AC02-05CH11231.

*Author contributions*—K. K., Y. Y., and H. A. M. conceptualization; K. K., S.-W. H., and H. A. M. methodology; K. K., D. C. P., S. W. H.,



Y. Y., and H. A. M. writing—original draft; K. K., S.-W. H., D. C. P., Y. Y., and H. A. M. writing—review & editing.

**Conflict of interest**—The authors declare that they have no conflicts of interest with the contents of this article.

**Abbreviations**—The abbreviations used are: AADAT, amino adipate AT; AGT, alanine:glyoxylate AT; AT, aminotransferase; BCAT, branched-chain amino acid AT; BNA3p, Biosynthesis of Nicotinic Acid protein 3; DAPA, 7,8-diaminopelargonic acid; GABA,  $\gamma$ -aminobutyric acid; GABT, GABA AT; GGAT, glutamate:glyoxylate aminotransferase; GOE, Great Oxidation Event; GOGAT, glutamine oxoglutarate amidotransferase; GS, glutamine synthetase; GTK, glutamine transaminase of kidney or liver; HisP, histidinol phosphate; KAT, kynurenine aminotransferase; LUCA, last universal common ancestor; PDB, Protein Data Bank; PLP, pyridoxal 5'-phosphate; PM, pyridoxamine; PM-AT, PM:pyruvate aminotransferase; PMP, pyridoxamine 5'-phosphate; PPAAT, prephenate AT; PSAT, phosphoserine AT; PYD4, pyrimidine 4; RNP, ribonucleoprotein.

## References

- Givan, C. V. (1980) 8 - aminotransferases in higher plants. In Mifflin, B. J., ed., *Amino Acids and Derivatives* 329–357. Academic Press, Cambridge, MA
- Mehta, P. K., Hale, T. I., and Christen, P. (1993) Aminotransferases: demonstration of homology and division into evolutionary subgroups. *Eur. J. Biochem.* **214**, 549–561
- Jensen, R. A., and Gu, W. (1996) Evolutionary recruitment of biochemically specialized subdivisions of Family I within the protein superfamily of aminotransferases. *J. Bacteriol.* **178**, 2161–2171
- Toney, M. D. (2014) Aspartate aminotransferase: an old dog teaches new tricks. *Arch. Biochem. Biophys.* **544**, 119–127
- Percudani, R., and Peracchi, A. (2003) A genomic overview of pyridoxal-phosphate-dependent enzymes. *EMBO Rep.* **4**, 850–854
- Liepmann, A. H., and Olsen, L. J. (2004) Genomic analysis of aminotransferases in *Arabidopsis thaliana*. *Crit. Rev. Plant Sci.* **23**, 73–89
- Christen, P., and Mehta, P. K. (2001) From cofactor to enzymes. The molecular evolution of pyridoxal-5'-phosphate-dependent enzymes. *Chem. Rec.* **1**, 436–447
- Mifflin, B. J., and Lea, P. J. (1980) 4 - ammonia assimilation. In Mifflin, B. J., ed., *Amino Acids and Derivatives* 169–202. Academic Press, Cambridge, MA
- Guillamón, J. M., van Riel, N. A. W., Giuseppin, M. L. F., and Verrips, C. T. (2001) The glutamate synthase (GOGAT) of *Saccharomyces cerevisiae* plays an important role in central nitrogen metabolism. *FEMS Yeast Res.* **1**, 169–175
- Stewart, G. R., Mann, A. F., and Fentem, P. A. (1980) 7 - enzymes of glutamate formation: Glutamate dehydrogenase, glutamine synthetase, and glutamate synthase. In Mifflin, B. J., ed., *Amino Acids and Derivatives* 271–327. Academic Press, Cambridge, MA
- van Heeswijk, W. C., Westerhoff, H. V., and Booger, F. C. (2013) Nitrogen assimilation in *Escherichia coli*: putting molecular data into a systems perspective. *Microbiol. Mol. Biol. Rev.* **77**, 628–695
- Karmen, A., Wróblewski, F., and LaDue, J. S. (1955) Transaminase activity in human blood. *J. Clin. Invest.* **34**, 126–133
- Felig, P. (1973) The glucose-alanine cycle. *Metabolism* **22**, 179–207
- Dashty, M. (2013) A quick look at biochemistry: Carbohydrate metabolism. *Clin. Biochem.* **46**, 1339–1352
- Lächler, K., Imhof, J., Reichelt, M., Gershenzon, J., and Binder, S. (2015) The cytosolic branched-chain aminotransferases of *Arabidopsis thaliana* influence methionine supply, salvage and glucosinolate metabolism. *Plant Mol. Biol.* **88**, 119–131
- Schuster, J., Knill, T., Reichelt, M., Gershenzon, J., and Binder, S. (2006) Branched-chain AMINOTRANSFERASE4 is part of the chain elongation pathway in the biosynthesis of methionine-derived glucosinolates in *Arabidopsis*. *Plant Cell* **18**, 2664–2679
- Knill, T., Schuster, J., Reichelt, M., Gershenzon, J., and Binder, S. (2008) *Arabidopsis* branched-chain aminotransferase 3 functions in both amino acid and glucosinolate biosynthesis. *Plant Physiol.* **146**, 1028–1039
- Ono, H., Sawada, K., Khunajakr, N., Tao, T., Yamamoto, M., Hiramoto, M., et al. (1999) Characterization of biosynthetic enzymes for ectoine as a compatible solute in a moderately halophilic eubacterium, *Halomonas elongata*. *J. Bacteriol.* **181**, 91–99
- Zheng, Z., Guo, Y., Novák, O., Dai, X., Zhao, Y., Ljung, K., et al. (2013) Coordination of auxin and ethylene biosynthesis by the aminotransferase VAS1. *Nat. Chem. Biol.* **9**, 244–246
- Binder, S. (2010) Branched-chain amino acid metabolism in *Arabidopsis thaliana*. *Arabidopsis Book* **8**
- Wang, M., and Maeda, H. A. (2018) Aromatic amino acid aminotransferases in plants. *Phytochem. Rev.* **17**, 131–159
- Nakayasu, M., Umemoto, N., Akiyama, R., Ohya, K., Lee, H. J., Miyachi, H., et al. (2021) Characterization of C-26 aminotransferase, indispensable for steroidal glycoalkaloid biosynthesis. *Plant J.* **108**, 81–92
- Grishin, N. V., Phillips, M. A., and Goldsmith, E. J. (1995) Modeling of the spatial structure of eukaryotic ornithine decarboxylases. *Protein Sci.* **4**, 1291–1304
- Percudani, R., and Peracchi, A. (2009) The B6 database: a tool for the description and classification of vitamin B6-dependent enzymatic activities and of the corresponding protein families. *BMC Bioinformatics* **10**, 273
- Huang, X., Albou, L. P., Mushayahama, T., Muruganujan, A., Tang, H., Thomas, P. D., et al. (2019) Ancestral genomes: a resource for reconstructed ancestral genes and genomes across the tree of life. *Nucl. Acids Res.* **47**, D271–D279
- Weiss, M. C., Sousa, F. L., Mrnjavac, N., Neukirchen, S., Roettger, M., Nelson-Sathi, S., et al. (2016) The physiology and habitat of the last universal common ancestor. *Nat. Microbiol.* **1**, 1–8
- Aharoni, A., Gaidukov, L., Khersonsky, O., McQ Gould, S., Roodveldt, C., et al. (2005) The 'evolvability' of promiscuous protein functions. *Nat. Genet.* **37**, 73–76
- Scossa, F., and Fernie, A. R. (2020) The evolution of metabolism: how to test evolutionary hypotheses at the genomic level. *Comput. Struct. Biotechnol. J.* **18**, 482–500
- Maeda, H. A., and Fernie, A. R. (2021) Evolutionary history of plant metabolism. *Annu. Rev. Plant Biol.* **72**, 185–216
- Cunchillos, C., and Lecointre, G. (2003) Evolution of amino acid metabolism inferred through cladistic analysis. *J. Biol. Chem.* **278**, 47960–47970
- Oparin, A. I. (1924) The origin of life, trad. A. Synge, republished. In: Bernal, J. D., ed. (1967) *The Origin of Life*, Weidenfeld and Nicolson, London: 199–234
- Lazcano, A., and Miller, S. L. (1996) The origin and early evolution of life: prebiotic chemistry, the pre-RNA world, and time. *Cell* **85**, 793–798
- Fani, R. (2012) The origin and evolution of metabolic pathways: why and how did primordial cells construct metabolic routes? *Evo. Edu. Outreach.* **5**, 367–381
- Orgel, L. E. (1998) The origin of life—a review of facts and speculations. *Trends Biochem. Sci.* **23**, 491–495
- Austin, S. M., and Waddell, T. G. (1999) Prebiotic synthesis of vitamin B6-type compounds. *Orig. Life Evol. Biosph.* **29**, 287–296
- Aylward, N., and Bofinger, N. (2006) A plausible prebiotic synthesis of pyridoxal phosphate: vitamin B6 – a computational study. *Biophys. Chem.* **123**, 113–121
- Cleaves, H. J., II (2010) The origin of the biologically coded amino acids. *J. Theor. Biol.* **263**, 490–498
- Kitadai, N., and Maruyama, S. (2018) Origins of building blocks of life: a review. *Geosci. Front.* **9**, 1117–1153
- Johnson, A. P., Cleaves, H. J., Dworkin, J. P., Glavin, D. P., Lazcano, A., Bada, J. L., et al. (2008) The miller volcanic spark discharge experiment. *Science* **322**, 404

40. Miller, S. L. A. (1953) Production of amino acids under possible primitive earth conditions. *Science* **117**, 528–529
41. Parker, E. T., Cleaves, H. J., Dworkin, J. P., Glavin, D. P., Callahan, M., Aubrey, A., *et al.* (2011) Primordial synthesis of amines and amino acids in a 1958 Miller H<sub>2</sub>S-rich spark discharge experiment. *Proc. Natl. Acad. Sci. U. S. A.* **108**, 5526–5531
42. Degani, C., and Halmann, M. (1967) Chemical evolution of carbohydrate metabolism. *Nature* **216**, 1207
43. Bishop, J. C., Cross, S. D., Waddell, and prebiotic, T. G. (1997) Transamination. *Orig. Life Evol. Biosph.* **27**, 319–324
44. Keller, M. A., Kampjut, D., Harrison, S. A., and Ralser, M. (2017) Sulfate radicals enable a non-enzymatic Krebs cycle precursor. *Nat. Ecol. Evol.* **1**, 1–9
45. Wächtershäuser, G. (2014) The place of RNA in the origin and early evolution of the genetic machinery. *Life (Basel)* **4**, 1050–1091
46. Orgel, L. E. (2008) The implausibility of metabolic cycles on the prebiotic earth. *PLoS Biol.* **6**, e18
47. Zubarev, D. Y., Rappoport, D., and Aspuru-Guzik, A. (2015) Uncertainty of prebiotic scenarios: the case of the non-enzymatic reverse tricarboxylic acid cycle. *Sci. Rep.* **5**, 1–7
48. Kauffman, S. A. (1986) Autocatalytic sets of proteins. *J. Theor. Biol.* **119**, 1–24
49. Hordijk, W. (2013) Autocatalytic sets: from the origin of life to the economy. *BioScience* **63**, 877–881
50. Arsène, S., Ameta, S., Lehman, N., Griffiths, A. D., and Nghe, P. (2018) Coupled catabolism and anabolism in autocatalytic RNA sets. *Nucl. Acids Res.* **46**, 9660–9666
51. Alberts, B., *et al.* (2002) The RNA world and the origins of life. In *Molecular Biology of the Cell*, 4th edition, Garland Science, New York City, NY
52. Nakada, H. I., and Weinhouse, S. (1953) Non-enzymatic transamination with glyoxylic acid and various amino acids. *J. Biol. Chem.* **204**, 831–836
53. Zabinski, R. F., and Toney, M. D. (2001) Metal ion inhibition of nonenzymatic pyridoxal phosphate catalyzed decarboxylation and transamination. *J. Am. Chem. Soc.* **123**, 193–198
54. Johnson, L. N., Hu, S.-H., and Barford, D. (1992) Catalytic mechanism of glycogen phosphorylase. *Faraday Discuss.* **93**, 131–142
55. Gilbert, W. (1986) Origin of life: the RNA world. *Nature* **319**, 618
56. Ralser, M. (2014) The RNA world and the origin of metabolic enzymes. *Biochem. Soc. Trans.* **42**, 985–988
57. Weiss, M. C., Preiner, M., Xavier, J. C., Zimorski, V., and Martin, W. F. (2018) The last universal common ancestor between ancient earth chemistry and the onset of genetics. *PLoS Genet.* **14**, e1007518
58. Kubyskin, V., and Budisa, N. (2019) The alanine world model for the development of the amino acid repertoire in protein biosynthesis. *Int. J. Mol. Sci.* **20**, 5507
59. Chen, X., Li, N., and Ellington, A. D. (2007) Ribozyme catalysis of metabolism in the RNA world. *Chem. Biodivers.* **4**, 633–655
60. Hartman, H., and Smith, T. F. (2014) The evolution of the ribosome and the genetic code. *Life (Basel)* **4**, 227–249
61. Smith, T. F., and Hartman, H. (2015) The evolution of Class II Aminoacyl-tRNA synthetases and the first code. *FEBS Lett.* **589**, 3499–3507
62. Klipcan, L., and Safro, M. (2004) Amino acid biogenesis, evolution of the genetic code and aminoacyl-tRNA synthetases. *J. Theor. Biol.* **228**, 389–396
63. Hartman, H., and Smith, T. F. (2010) GTPases and the origin of the ribosome. *Biol. Direct* **5**, 36
64. Padmanabhan, S., York, E. J., Stewart, J. M., and Baldwin, R. L. (1996) Helix propensities of basic amino acids increase with the length of the side-chain. *J. Mol. Biol.* **257**, 726–734
65. Yoshikane, Y., Yokochi, N., Ohnishi, K., Hayashi, H., and Yagi, T. (2006) Molecular cloning, expression and characterization of pyridoxamine-pyruvate aminotransferase. *Biochem. J.* **396**, 499–507
66. Huang, S., Zhang, J., Wu, M., Wu, Q., and Huang, L. (2013) Enzymatic transamination of pyridoxamine in tobacco plants. *Plant Sci.* **212**, 55–59
67. Tani, Y., Ogata, K., Ukita, M., Nakamatsu, T., and Izumi, Y. (1972) Studies on vitamin B6 metabolism in microorganisms: Part IX. Microbial phosphorylation of vitamin B6 through a new phosphotransferring reaction (5) phosphorylation of pyridoxine by several phosphatases Part X. Further purification and characterization of pyridoxamine 5'-Phosphate- $\alpha$ -ketoglutarate transaminase from *Clostridium kainantoi* Part XI. Extracellular formation of vitamin B6 by marine and terrestrial microorganisms and its control. *Agric. Biol. Chem.* **36**, 173–197
68. Fournier, G. P., and Alm, E. J. (2015) Ancestral reconstruction of a pre-LUCA aminoacyl-tRNA synthetase ancestor supports the late addition of trp to the genetic code. *J. Mol. Evol.* **80**, 171–185
69. Vlassov, A. (2005) How was membrane permeability produced in an RNA world? *Orig. Life Evol. Biosph.* **35**, 135–149
70. Cech, T. R. (2000) The ribosome is a ribozyme. *Science* **289**, 878–879
71. Fica, S. M., Tuttle, N., Novak, T., Li, N. S., Lu, J., Koodathingal, P., *et al.* (2013) RNA catalyses nuclear pre-mRNA splicing. *Nature* **503**, 229–234
72. Johnson, D. B. F., and Wang, L. (2010) Imprints of the genetic code in the ribosome. *PNAS* **107**, 8298–8303
73. Hartman, H., and Smith, T. F. (2019) Origin of the genetic code is found at the transition between a thioester world of peptides and the phosphoester world of polynucleotides. *Life (Basel)* **9**, 69
74. Margulis, L., and Sagan, D. (1997) *Microcosmos: Four Billion Years of Microbial Evolution*, University of California Press, Oakland, CA
75. Dismukes, G. C., Klimov, V. V., Baranov, S. V., Kozlov, Y. N., DasGupta, J., Tyryshkin, A., *et al.* (2001) The origin of atmospheric oxygen on Earth: The innovation of oxygenic photosynthesis. *Proc. Natl. Acad. Sci. U. S. A.* **98**, 2170–2175
76. Marais, D. J. D. (2000) When did photosynthesis emerge on earth? *Science* **289**, 1703–1705
77. Schirrmester, B. E., Vos, J. M. de, Antonelli, A., and Bagheri, H. C. (2013) Evolution of multicellularity coincided with increased diversification of cyanobacteria and the great oxidation event. *Proc. Natl. Acad. Sci. U. S. A.* **110**, 1791–1796
78. Hoffarth, E. R., Rothchild, K. W., and Ryan, K. S. (2020) Emergence of oxygen- and pyridoxal phosphate-dependent reactions. *FEBS J.* **287**, 1403–1428
79. Bertoldi, M., and Borri Voltattorni, C. (2000) Reaction of dopa decarboxylase with L-aromatic amino acids under aerobic and anaerobic conditions. *Biochem. J.* **352**, 533–538
80. Sakai, K., Miyasako, Y., Nagatomo, H., Watanabe, H., Wakayama, M., Moriguchi, M., *et al.* (1997) L-ornithine decarboxylase from *Hafnia alvei* has a novel L-ornithine oxidase activity. *J. Biochem.* **122**, 961–968
81. Bertoldi, M., Carbone, V., and Borri Voltattorni, C. (1999) Ornithine and glutamate decarboxylases catalyse an oxidative deamination of their alpha-methyl substrates. *Biochem. J.* **342**, 509–512
82. Kaminaga, Y., Schnepf, J., Peel, G., Kish, C. M., Ben-Nissan, G., Weiss, D., *et al.* (2006) Plant phenylacetaldehyde synthase is a bifunctional homotetrameric enzyme that catalyzes phenylalanine decarboxylation and oxidation. *J. Biol. Chem.* **281**, 23357–23366
83. Vavricka, C., Han, Q., Huang, Y., Erickson, S. M., Harich, K., Christensen, B. M., *et al.* (2011) From L-dopa to dihydroxyphenylacetaldehyde: a toxic biochemical pathway plays a vital physiological function in insects. *PLoS One* **6**, e16124
84. Arnold, A., and Nikoloski, Z. (2014) Bottom-up metabolic reconstruction of Arabidopsis and its application to determining the metabolic costs of enzyme production. *Plant Physiol.* **165**, 1380–1391
85. Raymond, J., and Segrè, D. (2006) The effect of oxygen on biochemical networks and the evolution of complex life. *Science* **311**, 1764–1767
86. Han, Q., Cai, T., Tagle, D. A., Robinson, H., and Li, J. (2008) Substrate specificity and structure of human amino acid aminotransferase/kynurenine aminotransferase II. *Biosci. Rep.* **28**, 205–215
87. Okuno, E., Nakamura, M., and Schwarcz, R. (1991) Two kynurenine aminotransferases in human brain. *Brain Res.* **542**, 307–312
88. Han, Q., Li, J., and Li, J. (2004) pH dependence, substrate specificity and inhibition of human kynurenine aminotransferase I. *Eur. J. Biochem.* **271**, 4804–4814

89. Guidetti, P., Amori, L., Sapko, M. T., Okuno, E., and Schwarcz, R. (2007) Mitochondrial aspartate aminotransferase: A third kynurenate-producing enzyme in the mammalian brain. *J. Neurochem.* **102**, 103–111
90. Han, Q., Robinson, H., Cai, T., Tagle, D. A., and Li, J. (2011) Biochemical and structural characterization of mouse mitochondrial aspartate aminotransferase, a newly identified kynurenine aminotransferase-IV. *Biosci. Rep.* **31**, 323–332
91. Tao, Y., Ferrer, J. L., Ljung, K., Pojer, F., Hong, F., Long, J. A., et al. (2008) Rapid synthesis of auxin *via* a new tryptophan-dependent pathway is required for shade avoidance in plants. *Cell* **133**, 164–176
92. Stepanova, A. N., Robertson-Hoyt, J., Yun, J., Benavente, L. M., Xie, D. Y., Dolezal, K., et al. (2008) TAA1-Mediated auxin biosynthesis is essential for hormone crosstalk and plant development. *Cell* **133**, 177–191
93. He, W., Brumos, J., Li, H., Ji, Y., Ke, M., Gong, X., et al. (2011) A small-molecule screen identifies 1-kynurenine as a competitive inhibitor of TAA1/TAR activity in ethylene-directed auxin biosynthesis and root growth in Arabidopsis[C][W]. *Plant Cell* **23**, 3944–3960
94. Lynch, J. H., Qian, Y., Guo, L., Maoz, I., Huang, X. Q., Garcia, A. S., et al. (2020) Modulation of auxin formation by the cytosolic phenylalanine biosynthetic pathway. *Nat. Chem. Biol.* **16**, 850–856
95. Tani, Y., Ukita, M., and Ogata, K. (1972) Studies on vitamin B<sub>6</sub> metabolism in microorganisms Part X. *Agric. Biol. Chem.* **36**, 181–188
96. Wada, H., and Snell, E. E. (1962) Enzymatic transamination of pyridoxamine: II. Crystalline pyridoxamine-pyruvate transaminase. *J. Biol. Chem.* **237**, 133–137
97. Yoshikane, Y., Yokochi, N., Yamasaki, M., Mizutani, K., Ohnishi, K., Mikami, B., et al. (2008) Crystal structure of pyridoxamine-pyruvate aminotransferase from *Mesorhizobium loti* MAFF303099. *J. Biol. Chem.* **283**, 1120–1127
98. Mouilleron, S., and Golinelli-Pimpaneau, B. (2007) Conformational changes in ammonia-channeling glutamine amidotransferases. *Curr. Opin. Struct. Biol.* **17**, 653–664
99. Korolev, S., Skarina, T., Evdokimova, E., Beasley, S., Edwards, A., Joachimiak, A., et al. (2002) Crystal structure of glutamine amidotransferase from *Thermotoga maritima*. *Proteins* **49**, 420–422
100. Temple, S. J., Vance, C. P., and Gantt, J. S. (1998) Glutamate synthase and nitrogen assimilation. *Trends Plant Sci.* **3**, 51–56
101. Nigro, D., Blanco, A., Anderson, O. D., and Gadaleta, A. (2014) Characterization of ferredoxin-dependent glutamine-oxoglutarate amidotransferase (Fd-GOGAT) genes and their relationship with grain protein content QTL in wheat. *PLoS One* **9**, e103869
102. Patterson, M. K., and Orr, G. R. (1968) Asparagine biosynthesis by the Novikoff Hepatoma isolation, purification, property, and mechanism studies of the enzyme system. *J. Biol. Chem.* **243**, 376–380
103. Larsen, T. M., Boehlein, S. K., Schuster, S. M., Richards, N. G., Thoden, J. B., Holden, H. M., et al. (1999) Three-dimensional structure of *Escherichia coli* asparagine synthetase B: a short journey from substrate to product. *Biochemistry* **38**, 16146–16157
104. Baker, T. I., and Crawford, I. P. (1966) Anthranilate synthetase: partial purification and some kinetic studies ON the enzyme from *Escherichia coli*. *J. Biol. Chem.* **241**, 5577–5584
105. Zein, F., Zhang, Y., Kang, Y. N., Burns, K., Begley, T. P., Ealick, S. E., et al. (2006) Structural insights into the mechanism of the PLP synthase holoenzyme from *Thermotoga maritima*. *Biochemistry* **45**, 14609–14620
106. Klem, T. J., and Davison, V. J. (1993) Imidazole glycerol phosphate synthase: the glutamine amidotransferase in histidine biosynthesis. *Biochemistry* **32**, 5177–5186
107. Badet, B., Vermoote, P., Haumont, P. Y., Lederer, F., and LeGoffic, F. (1987) Glucosamine synthetase from *Escherichia coli*: purification, properties, and glutamine-utilizing site location. *Biochemistry* **26**, 1940–1948
108. Golinelli-Pimpaneau, B., and Badet, B. (1991) Possible involvement of Lys603 from *Escherichia coli* glucosamine-6-phosphate synthase in the binding of its substrate fructose 6-phosphate. *Eur. J. Biochem.* **201**, 175–182
109. Messenger, L. J., and Zalkin, H. (1979) Glutamine phosphoribosylpyrophosphate amidotransferase from *Escherichia coli*. Purification and properties. *J. Biol. Chem.* **254**, 3382–3392
110. Smith, H. Q., Li, C., Stanley, C. A., and Smith, T. (2019) J. Glutamate dehydrogenase, a complex enzyme at a crucial metabolic branch point. *Neurochem. Res.* **44**, 117–132
111. Voss, C. M., Arildsen, L., Nissen, J. D., Waagepetersen, H. S., Schousboe, A., Maechler, P., et al. (2021) Glutamate dehydrogenase is important for ammonia fixation and amino acid homeostasis in brain during hyperammonemia. *Front. Neurosci.* **15**, 646291
112. Karaca, M., Martin-Levilain, J., Grimaldi, M., Li, L., Dizin, E., Emre, Y., et al. (2018) Liver glutamate dehydrogenase controls whole-body energy partitioning through amino acid-derived gluconeogenesis and ammonia homeostasis. *Diabetes* **67**, 1949–1961
113. White, H. B. (1976) Coenzymes as fossils of an earlier metabolic state. *J. Mol. Evol.* **7**, 101–104
114. Ellington, A. D. (2012) Origins for everyone. *Evo. Edu. Outreach.* **5**, 361–366
115. Wong, J. T.-F. (2009) *Prebiotic Evolution and Astrobiology*, CRC Press, Boca Raton, FL
116. Kubis, A., and Bar-Even, A. (2019) Synthetic biology approaches for improving photosynthesis. *J. Exp. Bot.* **70**, 1425–1433
117. Mehta, P. K., and Christen, P. (2000) The molecular evolution of pyridoxal-5'-phosphate-dependent enzymes. *Adv. Enzymol. Relat. Areas Mol. Biol.* **74**, 129–184
118. Yamagami, T., et al. (2003) Biochemical diversity among the 1-aminocyclopropane-1-carboxylate synthase isozymes encoded by the Arabidopsis gene family. *J. Biol. Chem.* **278**, 49102–49112
119. Mikkelsen, M. D., Naur, P., and Halkier, B. A. (2004) Arabidopsis mutants in the C-S lyase of glucosinolate biosynthesis establish a critical role for indole-3-acetaldoxime in auxin homeostasis. *Plant J.* **37**, 770–777
120. Jones, P. R., Manabe, T., Awazuhara, M., and Saito, K. (2003) A new member of plant CS-lyases. A cystine lyase from *Arabidopsis thaliana*. *J. Biol. Chem.* **278**, 10291–10296
121. Ilag, L. L., Kumar, A. M., and Söll, D. (1994) Light regulation of chlorophyll biosynthesis at the level of 5-aminolevulinate formation in *Arabidopsis*. *Plant Cell* **6**, 265–275
122. Basset, G. J. C., Ravanel, S., Quinlivan, E. P., White, R., Giovannoni, J. J., Rébeillé, F., et al. (2004) Folate synthesis in plants: the last step of the p-aminobenzoate branch is catalyzed by a plastidial aminodeoxychorismate lyase. *Plant J.* **40**, 453–461
123. Suarez, J., Hener, C., Lehnhardt, V. A., Hummel, S., Stahl, M., and Kolkusaoglu, Ü. (2019) AtDAT1 is a key enzyme of D-amino acid stimulated ethylene production in *Arabidopsis thaliana*. *Front. Plant Sci.* **10**, 1609
124. Cleland, W. W. (1963) The kinetics of enzyme-catalyzed reactions with two or more substrates or products: I. Nomenclature and rate equations. *Biochim. Biophys. Acta* **67**, 104–137
125. John, R. A. (1995) Pyridoxal phosphate-dependent enzymes. *Biochim. Biophys. Acta* **1248**, 81–96
126. Nakamura, Y., and Tolbert, N. E. (1983) Serine: glyoxylate, alanine: glyoxylate, and glutamate:glyoxylate aminotransferase reactions in peroxisomes from spinach leaves. *J. Biol. Chem.* **258**, 7631–7638
127. Leegood, R. C., Lea, P. J., Adcock, M. D., and Häusler, R. E. (1995) The regulation and control of photorespiration. *J. Exp. Bot.* **46**, 1397–1414
128. Oliveira, E. F., Cerqueira, N. M. F. S. A., Fernandes, P. A., and Ramos, M. J. (2011) Mechanism of formation of the internal aldimine in pyridoxal 5'-phosphate-dependent enzymes. *J. Am. Chem. Soc.* **133**, 15496–15505
129. Hayashi, H., and Kagamiyama, H. (1997) Transient-state kinetics of the reaction of aspartate aminotransferase with aspartate at low pH reveals dual routes in the enzyme-substrate association process. *Biochemistry* **36**, 13558–13569
130. Liang, J., Han, Q., Tan, Y., Ding, H., and Li, J. (2019) Current advances on structure-function relationships of pyridoxal 5'-phosphate-dependent enzymes. *Front. Mol. Biosci.* **6**, 4



131. Amorim Franco, T. M., Hegde, S., and Blanchard, J. S. (2016) Chemical mechanism of the branched-chain aminotransferase IlvE from *Mycobacterium tuberculosis*. *Biochemistry* **55**, 6295–6303
132. Amorim Franco, T. M., and Blanchard, J. S. (2017) Bacterial branched-chain amino acid biosynthesis: structures, mechanisms, and drugability. *Biochemistry* **56**, 5849–5865
133. Schneider, G., Käck, H., and Lindqvist, Y. (2000) The manifold of vitamin B6 dependent enzymes. *Structure* **8**, R1–R6
134. Han, Q., Ding, H., Robinson, H., Christensen, B. M., and Li, J. (2010) Crystal structure and substrate specificity of *Drosophila* 3,4-dihydroxyphenylalanine decarboxylase. *PLoS One* **5**, e8826
135. Milano, T., Paiardini, A., Grgurina, I., and Pascarella, S. (2013) Type I pyridoxal 5'-phosphate dependent enzymatic domains embedded within multimodular nonribosomal peptide synthetase and polyketide synthase assembly lines. *BMC Struct. Biol.* **13**, 26
136. Salvo, M. L. di, Remesh, S. G., Vivoli, M., Ghatge, M. S., Paiardini, A., D'Aguzzo, S., et al. (2014) On the catalytic mechanism and stereospecificity of *Escherichia coli* l-threonine aldolase. *FEBS J.* **281**, 129–145
137. Börner, T., Remesh, S. G., Vivoli, M., Ghatge, M. S., Paiardini, A., D'Aguzzo, S., et al. (2017) Explaining operational instability of amine transaminases: substrate-induced inactivation mechanism and influence of quaternary structure on enzyme–cofactor intermediate stability. *ACS Catal.* **7**, 1259–1269
138. Rozewicki, J., Li, S., Amada, K. M., Standley, D. M., and Katoh, K. (2019) MAFFT-DASH: integrated protein sequence and structural alignment. *Nucl. Acids Res.* **47**, W5–W10
139. Graber, R., Kasper, P., Malashkevich, V. N., Sandmeier, E., Berger, P., Gehring, H., et al. (1995) Changing the reaction specificity of a pyridoxal-5'-phosphate-dependent enzyme. *Eur. J. Biochem.* **232**, 686–690
140. Sandmark, J., Eliot, A. C., Famm, K., Schneider, G., and Kirsch, J. F. (2004) Conserved and nonconserved residues in the substrate binding site of 7,8-diaminopelargonic acid synthase from *Escherichia coli* are essential for catalysis. *Biochemistry* **43**, 1213–1222
141. Hester, G., Stark, W., Moser, M., Kallen, J., Marković-Housley, Z., and Jansonius, J. N. (1999) Crystal structure of phosphoserine aminotransferase from *Escherichia coli* at 2.3 Å resolution: comparison of the unligated enzyme and a complex with  $\alpha$ -methyl-l-glutamate11 Edited by R. Huber. *J. Mol. Biol.* **286**, 829–850
142. Okada, K., Hirotsu, K., Hayashi, H., and Kagamiyama, H. (2001) Structures of *Escherichia coli* branched-chain amino acid aminotransferase and its complexes with 4-methylvalerate and 2-methylleucine: induced fit and substrate recognition of the enzyme. *Biochemistry* **40**, 7453–7463
143. Tremblay, L. W., and Blanchard, J. S. (2009) The 1.9 Å structure of the branched-chain amino-acid transaminase (IlvE) from *Mycobacterium tuberculosis*. *Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun.* **65**, 1071–1077
144. Goto, M., Miyahara, I., Hayashi, H., Kagamiyama, H., and Hirotsu, K. (2003) Crystal structures of branched-chain amino acid aminotransferase complexed with glutamate and glutarate: true reaction intermediate and double substrate recognition of the enzyme. *Biochemistry* **42**, 3725–3733
145. Dietrich, M. R., Ankeny, R. A., and Chen, P. M. (2014) Publication trends in model organism research. *Genetics* **198**, 787–794
146. Hartman, A. L., Norais, C., Badger, J. H., Delmas, S., Haldenby, S., Madupu, R., et al. (2010) The complete genome sequence of *haloferax volcanii* DS2, a model archaeon. *PLoS One* **5**, e9605
147. Heazlewood, J. L., Tonti-Filippini, J. S., Gout, A. M., Day, D. A., Whelan, J., and Millar, A. H. (2004) Experimental analysis of the Arabidopsis mitochondrial proteome highlights signaling and regulatory components, provides assessment of targeting prediction programs, and indicates plant-specific mitochondrial proteins. *Plant Cell* **16**, 241–256
148. Miyashita, Y., Dolferus, R., Ismond, K. P., and Good, A. G. (2007) Alanine aminotransferase catalyses the breakdown of alanine after hypoxia in *Arabidopsis thaliana*. *Plant J.* **49**, 1108–1121
149. Carrie, C., Venne, A. S., Zahedi, R. P., and Soll, J. (2015) Identification of cleavage sites and substrate proteins for two mitochondrial intermediate peptidases in *Arabidopsis thaliana*. *J. Exp. Bot.* **66**, 2691–2708
150. Anemaet, I. G., Metón, I., Salgado, M. C., Fernández, F., and Baanante, I. V. (2008) A novel alternatively spliced transcript of cytosolic alanine aminotransferase gene associated with enhanced gluconeogenesis in liver of *Sparus aurata*. *Int. J. Biochem. Cell Biol.* **40**, 2833–2844
151. Glinghammar, B., Rafter, I., Lindström, A. K., Hedberg, J. J., Andersson, H. B., Lindblom, P., et al. (2009) Detection of the mitochondrial and catalytically active alanine aminotransferase in human tissues and plasma. *Int. J. Mol. Med.* **23**, 621–631
152. Kim, S. H., Schneider, B. L., and Reitzer, L. (2010) Genetics and regulation of the major enzymes of alanine synthesis in *Escherichia coli*. *J. Bacteriol.* **192**, 5304–5311
153. Peña-Soler, E., Fernandez, F. J., López-Esteva, M., Garcés, F., Richardson, A. J., Quintana, J. F., et al. (2014) Structural analysis and mutant growth properties reveal distinctive enzymatic and cellular roles for the three major L-alanine transaminases of *Escherichia coli*. *PLoS One* **9**, e102139
154. Peñalosa-Ruiz, G., Aranda, C., Ongay-Larios, L., Colon, M., Quezada, H., and Gonzalez, A. (2012) Paralogous ALT1 and ALT2 retention and diversification have generated catalytically active and inactive aminotransferases in *Saccharomyces cerevisiae*. *PLoS One* **7**, e45702
155. Niessen, M., Krause, K., Horst, I., Staebler, N., Klaus, S., and Gaertner, S. (2012) Two alanine aminotransferases link mitochondrial glycolate oxidation to the major photorespiratory pathway in Arabidopsis and rice. *J. Exp. Bot.* **63**, 2705–2716
156. Liepman, A. H., and Olsen, L. J. (2003) Alanine aminotransferase homologs catalyze the glutamate:glyoxylate aminotransferase reaction in peroxisomes of Arabidopsis. *Plant Physiol.* **131**, 215–227
157. von Caemmerer, S., and Furbank, R. T. (2003) The C4 pathway: an efficient CO2 pump. *Photosynthesis Res.* **77**, 191
158. Iwasaki, T., Miyajima-Nakano, Y., Fukazawa, R., Lin, M. T., Matsushita, S. I., Hagiuda, E., et al. (2021) *Escherichia coli* amino acid auxotrophic expression host strains for investigating protein structure–function relationships. *J. Biochem.* **169**, 387–394
159. Hunziker, N. (1980) Richner-hanhart syndrome and tyrosinemia type II. *DRM* **160**, 180–189
160. Sivaraman, S., and Kirsch, J. F. (2006) The narrow substrate specificity of human tyrosine aminotransferase—the enzyme deficient in tyrosinemia type II. *FEBS J.* **273**, 1920–1929
161. Waterhouse, M. J., Chia, Y. C., and Lees, G. J. (1979) Inhibition of human and rat hepatic aminotransferase activity with L-3,4-dihydroxyphenylalanine by inhibitors of peripheral aromatic amino acid decarboxylase. *Mol. Pharmacol.* **15**, 108–114
162. Maeda, T., and Shindo, H. (1976) Metabolic pathway of L-3-methoxy-4-hydroxyphenylalanine (3-O-methylDOPA)-participation of tyrosine aminotransferase and lactate dehydrogenase. *Chem. Pharm. Bull. (Tokyo)* **24**, 1104–1106
163. Maeda, T., and Shindo, H. (1978) Metabolic difference between 3,4-dihydroxyphenylpyruvic acid (DHPP) and 3-methoxy-4-hydroxyphenylpyruvic acid (MHPP). *Chem. Pharm. Bull. (Tokyo)* **26**, 2054–2057
164. Wang, M., Toda, K., and Maeda, H. A. (2016) Biochemical properties and subcellular localization of tyrosine aminotransferases in Arabidopsis thaliana. *Phytochemistry* **132**, 16–25
165. Yoo, H., Widhalm, J. R., Qian, Y., Maeda, H., Cooper, B. R., Jannasch, A. S., et al. (2013) An alternative pathway contributes to phenylalanine biosynthesis in plants via a cytosolic tyrosine:phenylpyruvate aminotransferase. *Nat. Commun.* **4**, 2833
166. Cooper, A. J. L., and Meister, A. (1974) Isolation and properties of a new glutamine transaminase from rat kidney. *J. Biol. Chem.* **249**, 2554–2561
167. Yang, C., Zhang, L., Han, Q., Liao, C., Lan, J., Ding, H., et al. (2016) Kynurenine aminotransferase 3/glutamine transaminase L/cysteine conjugate beta-lyase 2 is a major glutamine transaminase in the mouse kidney. *Biochem. Biophys. Res. Commun.* **478**, 234–241
168. Rossi, F., Garavaglia, S., Montalbano, V., Walsh, M. A., and Rizzi, M. (2008) Crystal structure of human kynurenine aminotransferase II, a drug target for the treatment of schizophrenia\*. *J. Biol. Chem.* **283**, 3559–3566

169. Han, Q., Robinson, H., Cai, T., Tagle, D. A., and Li, J. (2009) Biochemical and structural properties of mouse kynurenine aminotransferase III. *Mol. Cell Biol.* **29**, 784–793
170. Nadvi, N. A., Salam, N. K., Park, J., Akladios, F. N., Kapoor, V., Collyer, C. A., *et al.* (2017) High resolution crystal structures of human kynurenine aminotransferase-I bound to PLP cofactor, and in complex with aminoxyacetate. *Protein Sci.* **26**, 727–736
171. Han, Q., Robinson, H., Cai, T., Tagle, D. A., and Li, J. (2009) Structural insight into the inhibition of human kynurenine aminotransferase I/ Glutamine transaminase K. *J. Med. Chem.* **52**, 2786–2793
172. Han, Q., Cai, T., Tagle, D. A., and Li, J. (2010) Structure, expression, and function of kynurenine aminotransferases in human and rodent brains. *Cell Mol Life Sci* **67**, 353–368
173. Yu, P., Li, Z., Zhang, L., Tagle, D. A., and Cai, T. (2006) Characterization of kynurenine aminotransferase III, a novel member of a phylogenetically conserved KAT family. *Gene* **365**, 111–118
174. Ganong, A. H., Lanthorn, T. H., and Cotman, C. W. (1983) Kynurenic acid inhibits synaptic and acidic amino acid-induced responses in the rat hippocampus and spinal cord. *Brain Res.* **273**, 170–174
175. Schwieler, L., and Erhardt, S. (2003) Inhibitory action of clozapine on rat ventral tegmental area dopamine neurons following increased levels of endogenous kynurenic acid. *Neuropsychopharmacology* **28**, 1770–1777
176. Cooper, A. J. L. (1978) Purification of soluble and mitochondrial glutamine transaminase K from rat kidney: use of a sensitive assay involving transamination between l-phenylalanine and  $\alpha$ -keto- $\gamma$ -methylolbutyrate. *Anal. Biochem.* **89**, 451–460
177. Cooper, A. J. L. (2004) The role of glutamine transaminase K (GTK) in sulfur and  $\alpha$ -keto acid metabolism in the brain, and in the possible bioactivation of neurotoxicants. *Neurochem. Int.* **44**, 557–577
178. Commandeur, J. N. M., Andreadou, I., Rooseboom, M., Out, M., de Leur, L. J., and Groot, E. (2000) Bioactivation of selenocysteine Se-conjugates by a highly purified rat renal cysteine conjugate  $\beta$ -lyase/glutamine transaminase K. *J. Pharmacol. Exp. Ther.* **294**, 753–761
179. Cooper, A. J. L., Krasnikov, B. F., Niatsetskaya, Z. V., Pinto, J. T., Callery, P. S., Villar, M. T., *et al.* (2011) Cysteine S-conjugate  $\beta$ -lyases: important roles in the metabolism of naturally occurring sulfur and selenium-containing compounds, xenobiotics and anticancer agents. *Amino Acids* **41**, 7–27
180. Pinto, J. T., Krasnikov, B. F., Alcutt, S., Jones, M. E., Dorai, T., Villar, M. T., *et al.* (2014) Kynurenine aminotransferase III and glutamine transaminase L are identical enzymes that have cysteine S-conjugate  $\beta$ -lyase activity and can transaminate l-selenomethionine\*. *J. Biol. Chem.* **289**, 30950–30961
181. Wogulis, M., Chew, E. R., Donohoue, P. D., and Wilson, D. K. (2008) Identification of formyl kynurenine formamidase and kynurenine aminotransferase from *Saccharomyces cerevisiae* using crystallographic, bioinformatic and biochemical evidence. *Biochemistry* **47**, 1608–1621
182. Iraqui, I., Vissers, S., Cartiaux, M., and Urrestarazu, A. (1998) Characterisation of *Saccharomyces cerevisiae* ARO8 and ARO9 genes encoding aromatic aminotransferases I and II reveals a new aminotransferase subfamily. *Mol. Gen. Genet.* **257**, 238–248
183. Gaudet, P., Livstone, M. S., Lewis, S. E., and Thomas, P. D. (2011) Phylogenetic-based propagation of functional annotations within the Gene Ontology consortium. *Brief. Bioinformatics* **12**, 449–462
184. Ellens, K. W., Richardson, L. G., Frelin, O., Collins, J., Ribeiro, C. L., Hsieh, Y. F., *et al.* (2015) Evidence that glutamine transaminase and omega-amidase potentially act in tandem to close the methionine salvage cycle in bacteria and plants. *Phytochemistry* **113**, 160–169
185. Dolzan, M., Johansson, K., Roig-Zamboni, V., Campanacci, V., Tegoni, M., Schneider, G., *et al.* (2004) Crystal structure and reactivity of YbdL from *Escherichia coli* identify a methionine aminotransferase function. *FEBS Lett.* **571**, 141–146
186. Martin, J., Eisoldt, L., and Skerra, A. (2018) Fixation of gaseous CO<sub>2</sub> by reversing a decarboxylase for the biocatalytic synthesis of the essential amino acid l-methionine. *Nat. Catal.* **1**, 555–561
187. Graindorge, M., Giustini, C., Jacomin, A. C., Kraut, A., Curien, G., and Matringe, M. (2010) Identification of a plant gene encoding glutamate/aspartate-prephenate aminotransferase: The last homeless enzyme of aromatic amino acids biosynthesis. *FEBS Lett.* **584**, 4357–4360
188. Maeda, H., Yoo, H., and Dudareva, N. (2011) Prephenate aminotransferase directs plant phenylalanine biosynthesis via arogenate. *Nat. Chem. Biol.* **7**, 19–21
189. Dornfeld, C., Weisberg, A. J., K C, R., Dudareva, N., Jelesko, J. G., and Maeda, H. A. (2014) Phylobiochemical characterization of class-ib aspartate/prephenate aminotransferases reveals evolution of the plant arogenate phenylalanine pathway. *Plant Cell* **26**, 3101–3114
190. Schenck, C. A., Men, Y., and Maeda, H. A. (2017) Conserved molecular mechanism of TyrA dehydrogenase substrate specificity underlying alternative tyrosine biosynthetic pathways in plants and microbes. *Front. Mol. Biosci.* **4**, 73
191. Ding, P., Rekhter, D., Ding, Y., Feussner, K., Busta, L., Haroth, S., *et al.* (2016) Characterization of a pipercolic acid biosynthesis pathway required for systemic acquired resistance. *Plant Cell* **28**, 2603–2615
192. Song, J. T., Lu, H., and Greenberg, J. T. (2004) Divergent roles in *Arabidopsis thaliana* development and defense of two homologous genes, aberrant growth and DEATH2 and AGD2-LIKE defense response PROTEIN1, encoding novel aminotransferases. *Plant Cell* **16**, 353–366
193. Mano, Y., and Nemoto, K. (2012) The pathway of auxin biosynthesis in plants. *J. Exp. Bot.* **63**, 2853–2872
194. Turnaev, I. I., Gunbin, K. V., and Afonnikov, D. A. (2015) Plant auxin biosynthesis did not originate in charophytes. *Trends Plant Sci.* **20**, 463–465
195. Mo, X., Zhu, Q., Li, X., Li, J., Zeng, Q., Rong, H., *et al.* (2006) The hpa1 mutant of *Arabidopsis* reveals a crucial role of histidine homeostasis in root meristem maintenance. *Plant Physiol.* **141**, 1425–1435
196. Muralla, R., Sweeney, C., Stepansky, A., Leustek, T., and Meinke, D. (2007) Genetic dissection of histidine biosynthesis in *Arabidopsis*. *Plant Physiol.* **144**, 890–903
197. Conover, R. K., and Doolittle, W. F. (1990) Characterization of a gene involved in histidine biosynthesis in *Halobacterium (haloferax) volcanii*: isolation and rapid mapping by transformation of an auxotroph with cosmid DNA. *J. Bacteriol.* **172**, 3244–3249
198. Kulis-Horn, R. K., Persicke, M., and Kalinowski. (2014) J. Histidine biosynthesis, its regulation and biotechnological application in *Corynebacterium glutamicum*. *Microb. Biotechnol.* **7**, 5–25
199. Satoshi, H., Sidhu, R. S., Akio, T., and Yasuji, O. (1981) Cloning of the HIS5 gene of *Saccharomyces cerevisiae* by yeast transformation. *Gene* **16**, 335–341
200. Grisolia, V., Carlomagno, M. S., Nappo, A. G., and Bruni, C. B. (1985) Cloning, structure, and expression of the *Escherichia coli* K-12 hisC gene. *J. Bacteriol.* **164**, 1317–1323
201. Fernandez, F. J., Vega, M. C., Lehmann, F., Sandmeier, E., Gehring, H., Christen, P., *et al.* (2004) Structural studies of the catalytic reaction pathway of a hyperthermophilic histidinol-phosphate aminotransferase\*. *J. Biol. Chem.* **279**, 21478–21488
202. Nester, E. W., and Montoya, A. L. (1976) An enzyme common to histidine and aromatic amino acid biosynthesis in *Bacillus subtilis*. *J. Bacteriol.* **126**, 699–705
203. Urrestarazu, A., Vissers, S., Iraqui, I., and Grenson, M. (1998) Phenylalanine- and tyrosine-auxotrophic mutants of *Saccharomyces cerevisiae* impaired in transamination. *Mol. Gen. Genet.* **257**, 230–237
204. Kradolfer, P., Niederberger, P., and Hütter, R. (1982) Tryptophan degradation in *Saccharomyces cerevisiae*: Characterization of two aromatic aminotransferases. *Arch. Microbiol.* **133**, 242–248
205. Karsten, W. E., Reyes, Z. L., Bobyk, K. D., Cook, P. F., and Chooback, L. (2011) Mechanism of the aromatic aminotransferase encoded by the Aro8 gene from *Saccharomyces cerevisiae*. *Arch. Biochem. Biophys.* **516**, 67–74
206. Pirkov, I., Norbeck, J., Gustafsson, L., and Albers, E. (2008) A complete inventory of all enzymes in the eukaryotic methionine salvage pathway. *FEBS J.* **275**, 4111–4120
207. Robinson, K. P., Jochem, A., Johnson, S. E., Reddy, T. R., Russell, J. D., Coon, J. J., *et al.* (2021) Defining intermediates and redundancies in coenzyme Q precursor biosynthesis. *J. Biol. Chem.* **296**, 100643

208. Okuno, E., Tsujimoto, M., Nakamura, M., and Kido, R. (1993) 2-Amino adipate-2-oxoglutarate aminotransferase isoenzymes in human liver: a plausible physiological role in lysine and tryptophan metabolism. *Enzyme Protein* **47**, 136–148
209. Hildebrandt, T. M., Nunes Nesi, A., Araújo, W. L., and Braun, H.-P. (2015) Amino acid catabolism in plants. *Mol. Plant* **8**, 1563–1579
210. Alkondon, M., Pereira, E. F., Yu, P., Arruda, E. Z., Almeida, L. E., Guidetti, P., et al. (2004) Targeted deletion of the kynurenine aminotransferase ii gene reveals a critical role of endogenous kynurenic acid in the regulation of synaptic transmission via alpha7 nicotinic receptors in the hippocampus. *J. Neurosci.* **24**, 4635–4648
211. Yu, P., Di Prospero, N. A., Sapko, M. T., Cai, T., Chen, A., Melendez-Ferro, M., et al. (2004) Biochemical and phenotypic abnormalities in kynurenine aminotransferase II-deficient mice. *Mol. Cell Biol.* **24**, 6919–6930
212. Sapko, M. T., Guidetti, P., Yu, P., Tagle, D. A., Pellicciari, R., and Schwarcz, R. (2006) Endogenous kynurenate controls the vulnerability of striatal neurons to quinolinate: Implications for Huntington's disease. *Exp. Neurol.* **197**, 31–40
213. Olney, J. W., de Gubareff, T., and Collins, J. F. (1980) Stereospecificity of the gliotoxic and anti-neurotoxic actions of alpha-amino adipate. *Neurosci. Lett.* **19**, 277–282
214. Whalen, W. A., and Berg, C. M. (1982) Analysis of an avtA::mu d1(ap lac) mutant: metabolic role of transaminase C. *J. Bacteriol.* **150**, 739–746
215. Yoneyama, H., Hori, H., Lim, S. J., Murata, T., Ando, T., Isogai, E., et al. (2011) Isolation of a mutant auxotrophic for L-alanine and identification of three major aminotransferases that synthesize L-alanine in Escherichia coli. *Biosci. Biotechnol. Biochem.* **75**, 930–938
216. Borst, P. (2020) The malate-aspartate shuttle (Borst cycle): How it started and developed into a major metabolic pathway. *IUBMB Life* **72**, 2241–2259
217. Lu, M., Zhou, L., Stanley, W. C., Cabrera, M. E., Saidel, G. M., Yu, X., et al. (2008) Role of the malate-aspartate shuttle on the metabolic response to myocardial ischemia. *J. Theor. Biol.* **254**, 466–475
218. Verleur, N., Elgersma, Y., Van Roermund, C. W., Tabak, H. F., and Wanders, R. J. (1997) Cytosolic aspartate aminotransferase encoded by the AAT2 gene is targeted to the peroxisomes in oleate-grown Saccharomyces cerevisiae. *Eur. J. Biochem.* **247**, 972–980
219. Morin, P. J., Subramanian, G. S., and Gilmore, T. D. (1992) AAT1, a gene encoding a mitochondrial aspartate aminotransferase in Saccharomyces cerevisiae. *Biochim. Biophys. Acta* **1171**, 211–214
220. Easlon, E., Tsang, F., Skinner, C., Wang, C., and Lin, S.-J. (2008) The malate-aspartate NADH shuttle components are novel metabolic longevity regulators required for calorie restriction-mediated life span extension in yeast. *Genes Dev.* **22**, 931–944
221. Schultz, C. J., and Coruzzi, G. M. (1995) The aspartate aminotransferase gene family of Arabidopsis encodes isoenzymes localized to three distinct subcellular compartments. *Plant J.* **7**, 61–75
222. Schultz, C. J., Hsu, M., Miesak, B., and Coruzzi, G. M. (1998) Arabidopsis mutants define an *in vivo* role for isoenzymes of aspartate aminotransferase in plant nitrogen assimilation. *Genetics* **149**, 491–499
223. Wilkie, S. E., and Warren, M. J. (1998) Recombinant expression, purification, and characterization of three isoenzymes of aspartate aminotransferase from Arabidopsis thaliana. *Protein Expr. Purif.* **12**, 381–389
224. Miesak, B. H., and Coruzzi, G. M. (2002) Molecular and physiological analysis of Arabidopsis mutants defective in cytosolic or chloroplastic aspartate aminotransferase. *Plant Physiol.* **129**, 650–660
225. Rothman, S. C., and Kirsch, J. F. (2003) How does an enzyme evolved *in vitro* compare to naturally occurring homologs possessing the targeted function? Tyrosine aminotransferase from aspartate aminotransferase. *J. Mol. Biol.* **327**, 593–608
226. Gelfand, D. H., and Steinberg, R. A. (1977) Escherichia coli mutants deficient in the aspartate and aromatic amino acid aminotransferases. *J. Bacteriol.* **130**, 429–440
227. Onuffer, J. J., Ton, B. T., Klement, I., and Kirsch, J. F. (1995) The use of natural and unnatural amino acid substrates to define the substrate specificity differences of Escherichia coli aspartate and tyrosine aminotransferases. *Protein Sci.* **4**, 1743–1749
228. Hayashi, H., Kuramitsu, S., and Kagamiyama, H. (1991) Replacement of an interdomain residue Val39 of Escherichia coli aspartate aminotransferase affects the catalytic competence without altering the substrate specificity of the Enzyme1. *J. Biochem.* **109**, 699–704
229. Liu, F., Qimuge, Hao, J., Yan, H., Bach, T., Fan, L., et al. (2014) AspC-mediated aspartate metabolism coordinates the Escherichia coli cell cycle. *PLoS One* **9**, e92229
230. Huang, Y.-T., Lyu, S. Y., Chuang, P. H., Hsu, N. S., Li, Y. S., Chan, H. C., et al. (2009) *In vitro* characterization of enzymes involved in the synthesis of nonproteinogenic residue (2S,3S)-beta-methylphenylalanine in glycopeptide antibiotic mannopeptimycin. *Chembiochem* **10**, 2480–2487
231. Pieck, M., Yuan, Y., Godfrey, J., Fisher, C., Zolj, S., Vaughan, D., et al. (2015) Auxin and tryptophan homeostasis are facilitated by the ISS1/VAS1 aromatic aminotransferase in Arabidopsis. *Genetics* **201**, 185–199
232. Wulfert, S., and Krueger, S. (2018) Phosphoserine Aminotransferase1 is part of the phosphorylated pathways for serine biosynthesis and essential for light and sugar-dependent growth promotion. *Front. Plant Sci.* **9**, 1712
233. Baek, J. Y., Jun, D. Y., Taub, D., and Kim, Y. H. (2003) Characterization of human phosphoserine aminotransferase involved in the phosphorylated pathway of l-serine biosynthesis. *Biochem. J.* **373**, 191–200
234. Melcher, K., Rose, M., Künzler, M., Braus, G. H., and Entian, K.-D. (1995) Molecular analysis of the yeast SER1 gene encoding 3-phosphoserine aminotransferase: Regulation by general control and serine repression. *Curr. Genet.* **27**, 501–508
235. Belhumeur, P., Fortin, N., and Clark, M. W. X. V. (1994) Yeast sequencing reports. A gene from Saccharomyces cerevisiae which codes for a protein with significant homology to the bacterial 3-phosphoserine aminotransferase. *Yeast* **10**, 385–389
236. Smith, Q. R., Momma, S., Aoyagi, M., and Rapoport, S. I. (1987) Kinetics of neutral amino acid transport across the blood-brain barrier. *J. Neurochem.* **49**, 1651–1658
237. Hart, C. E., Race, V., Achouri, Y., Wiame, E., Sharrard, M., Olpin, S. E., et al. (2007) Phosphoserine aminotransferase deficiency: a novel disorder of the serine biosynthesis pathway. *Am. J. Hum. Genet.* **80**, 931–937
238. Acuna-Hidalgo, R., Schanze, D., Kariminejad, A., Nordgren, A., Kariminejad, M. H., Conner, P., et al. (2014) Neu-laxova syndrome is a heterogeneous metabolic disorder caused by defects in enzymes of the L-serine biosynthesis pathway. *Am. J. Hum. Genet.* **95**, 285–293
239. Ros, R., Muñoz-Bertomeu, J., and Krueger, S. (2014) Serine in plants: biosynthesis, metabolism, and functions. *Trends Plant Sci.* **19**, 564–569
240. Benstein, R. M., Ludewig, K., Wulfert, S., Wittek, S., Gigolashvili, T., Frerigmann, H., et al. (2013) Arabidopsis phosphoglycerate Dehydrogenase1 of the phosphoserine pathway is essential for development and required for ammonium assimilation and tryptophan biosynthesis[C][W][OPEN]. *Plant Cell* **25**, 5011–5029
241. Lam, H. M., and Winkler, M. E. (1990) Metabolic relationships between pyridoxine (vitamin B6) and serine biosynthesis in Escherichia coli K-12. *J. Bacteriol.* **172**, 6518–6528
242. Drewke, C., Klein, M., Clade, D., Arenz, A., Müller, R., and Leistner, E. (1996) 4-O-Phosphoryl-l-threonine, a substrate of the pdxC(serC) gene product involved in vitamin B 6 biosynthesis. *FEBS Lett.* **390**, 179–182
243. Fargue, S., Lewin, J., Rumsby, G., and Danpure, C. J. (2013) Four of the most common mutations in primary hyperoxaluria type 1 unmask the cryptic mitochondrial targeting sequence of alanine:glyoxylate aminotransferase encoded by the polymorphic minor allele. *J. Biol. Chem.* **288**, 2475–2484
244. Takada, Y., and Noguchi, T. (1985) Characteristics of alanine: glyoxylate aminotransferase from Saccharomyces cerevisiae, a regulatory enzyme in the glyoxylate pathway of glycine and serine biosynthesis from tricarboxylic acid-cycle intermediates. *Biochem. J.* **231**, 157–163
245. Schlösser, T., Gätgens, C., Weber, U., and Stahmann, K.-P. (2004) Alanine: glyoxylate aminotransferase of Saccharomyces cerevisiae-encoding gene AGX1 and metabolic significance. *Yeast* **21**, 63–73
246. Liepman, A. H., and Olsen, L. J. (2001) Peroxisomal alanine: glyoxylate aminotransferase (AGT1) is a photorespiratory enzyme with multiple substrates in Arabidopsis thaliana. *Plant J.* **25**, 487–498



247. Zhang, Q., Lee, J., Pandurangan, S., Clarke, M., Pajak, A., and Marsolais, F. (2013) Characterization of Arabidopsis serine:glyoxylate aminotransferase, AGT1, as an asparagine aminotransferase. *Phytochemistry* **85**, 30–35
248. Modde, K., Timm, S., Florian, A., Michl, K., Fernie, A. R., and Bauwe, H. (2017) High serine:glyoxylate aminotransferase activity lowers leaf daytime serine levels, inducing the phosphoserine pathway in Arabidopsis. *J. Exp. Bot.* **68**, 643–656
249. Wang, R., Yang, L., Han, X., Zhao, Y., Zhao, L., Xiang, B., *et al.* (2019) Overexpression of AtAGT1 promoted root growth and development during seedling establishment. *Plant Cell Rep.* **38**, 1165–1180
250. Cobessi, D., Dumas, R., Pautre, V., Meinguet, C., Ferrer, J. L., and Alban, C. (2012) Biochemical and structural characterization of the Arabidopsis bifunctional enzyme dethiobiotin synthetase–diaminopelargonic acid aminotransferase: evidence for substrate channeling in biotin synthesis [C][W]. *Plant Cell* **24**, 1608–1625
251. Muralla, R., Chen, E., Sweeney, C., Gray, J. A., Dickerman, A., Nikolau, B. J., *et al.* (2008) A bifunctional locus (BIO3-BIO1) required for biotin biosynthesis in Arabidopsis. *Plant Physiol.* **146**, 60–73
252. Phalip, V., Kuhn, L., Lemoine, Y., and Jeltsch, J. M. (1999) Characterization of the biotin biosynthesis pathway in *Saccharomyces cerevisiae* and evidence for a cluster containing BIO5, a novel gene involved in vitamer uptake. *Gene* **232**, 43–51
253. Stoner, G. L., and Eisenberg, M. A. (1975) Purification and properties of 7, 8-diaminopelargonic acid aminotransferase. *J. Biol. Chem.* **250**, 4029–4036
254. Van Arsdell, S. W., Perkins, J. B., Yocum, R. R., Luan, L., Howitt, C. L., Chatterjee, N. P., *et al.* (2005) Removing a bottleneck in the *Bacillus subtilis* biotin pathway: bioA utilizes lysine rather than S-adenosylmethionine as the amino donor in the KAPA-to-DAPA reaction. *Bio-technol. Bioeng.* **91**, 75–83
255. Yoon, C. S., Kim, D. W., Jang, S. H., Lee, B. R., Choi, H. S., Choi, S. H., *et al.* (2004) Cysteine-321 of human brain GABA transaminase is involved in intersubunit cross-linking. *Mol. Cells* **18**, 214–219
256. Andersen, G., Andersen, B., Dobritzsch, D., Schnackerz, K. D., and Piskur, J. (2007) A gene duplication led to specialized gamma-aminobutyrate and beta-alanine aminotransferase in yeast. *FEBS J.* **274**, 1804–1817
257. Blancquaert, L., Baba, S. P., Kwiatkowski, S., Stautemas, J., Stegen, S., Barbaresi, S., *et al.* (2016) Carnosine and anserine homeostasis in skeletal muscle and heart is controlled by  $\beta$ -alanine transamination. *J. Physiol.* **594**, 4849–4863
258. Ramos, F., El Guezzer, M., Grenson, M., and Wiame, J.-M. (1985) Mutations affecting the enzymes involved in the utilization of 4-aminobutyric acid as nitrogen source by the yeast *Saccharomyces cerevisiae*. *Eur. J. Biochem.* **149**, 401–404
259. Coleman, S. T., Fang, T. K., Rovinsky, S. A., Turano, F. J., and Moye-Rowley, W. S. (2001) Expression of a glutamate decarboxylase homologue is required for normal oxidative stress tolerance in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **276**, 244–250
260. Clark, S. M., Di Leo, R., Dhanoa, P. K., Van Cauwenberghe, O. R., Mullen, R. T., and Shelp, B. J. (2009) Biochemical characterization, mitochondrial localization, expression, and potential functions for an Arabidopsis  $\gamma$ -aminobutyrate transaminase that utilizes both pyruvate and glyoxylate. *J. Exp. Bot.* **60**, 1743–1757
261. Van Cauwenberghe, O. R., Makhmoudova, A., McLean, M. D., Clark, S. M., and Shelp, B. J. (2002) Plant pyruvate-dependent gamma-aminobutyrate transaminase: identification of an Arabidopsis cDNA and its expression in *Escherichia coli*. *Can. J. Bot.* **80**, 933–941
262. Uzma Jalil, S., Khan, M. I. R., and Ansari, M. I. (2019) Role of GABA transaminase in the regulation of development and senescence in Arabidopsis thaliana. *Curr. Plant Biol.* **19**, 100119
263. Ansari, M. I., Hasan, S., and Jalil, S. U. (2014) Leaf senescence and GABA shunt. *Bioinformation* **10**, 734–736
264. Schneider, B. L., Ruback, S., Kiupakis, A. K., Kasbarian, H., Pybus, C., and Reitzer, L. (2002) The *Escherichia coli* gabDTPC operon: specific  $\gamma$ -aminobutyrate catabolism and nonspecific induction. *J. Bacteriol.* **184**, 6976–6986
265. Knorr, S., Sinn, M., Galetskiy, D., Williams, R. M., Wang, C., Müller, N., *et al.* (2018) Widespread bacterial lysine degradation proceeding via glutarate and L-2-hydroxyglutarate. *Nat. Commun.* **9**, 5071
266. Kurihara, S., Kato, K., Asada, K., Kumagai, H., and Suzuki, H. (2010) A putrescine-inducible pathway comprising PuuE-YneI in which  $\gamma$ -aminobutyrate is degraded into succinate in *Escherichia coli* K-12. *J. Bacteriol.* **192**, 4582–4591
267. Schneider, B. L., and Reitzer, L. (2012) Pathway and enzyme redundancy in putrescine catabolism in *Escherichia coli*. *J. Bacteriol.* **194**, 4080–4088
268. Samsonova, N. N., Smirnov, S. V., Altman, I. B., and Ptitsyn, L. R. (2003) Molecular cloning and characterization of *Escherichia coli* K12 yjgG gene. *BMC Microbiol.* **3**, 2
269. Jorge, J. M. P., Pérez-García, F., and Wendisch, V. F. (2017) A new metabolic route for the fermentative production of 5-aminovaleate from glucose and alternative carbon sources. *Bioresour. Technol.* **245**, 1701–1709
270. Roosens, N. H. C. J., Thu, T. T., Iskandar, H. M., and Jacobs, M. (1998) Isolation of the ornithine- $\delta$ -aminotransferase cDNA and effect of salt stress on its expression in Arabidopsis thaliana. *Plant Physiol.* **117**, 263–271
271. Funck, D., Stadelhofer, B., and Koch, W. (2008) Ornithine- $\delta$ -aminotransferase is essential for arginine catabolism but not for proline biosynthesis. *BMC Plant Biol.* **8**, 40
272. Verbruggen, N., Villarroel, R., and Van Montagu, M. (1993) Osmoregulation of a pyrroline-5-carboxylate reductase gene in Arabidopsis thaliana. *Plant Physiol.* **103**, 771–781
273. Krumpelman, P. M., Freyermuth, S. K., Cannon, J. F., Fink, G. R., and Polacco, J. C. (1995) Nucleotide sequence of Arabidopsis thaliana arginase expressed in yeast. *Plant Physiol.* **107**, 1479–1480
274. Degols, G., Jauniaux, J.-C., and Wiame, J.-M. (1987) Molecular characterization of transposable-element-associated mutations that lead to constitutive l-ornithine aminotransferase expression in *Saccharomyces cerevisiae*. *Eur. J. Biochem.* **165**, 289–296
275. Shen, B. W., Hennig, M., Hohenester, E., Jansonius, J. N., and Schirmer, T. (1998) Crystal structure of human recombinant ornithine aminotransferase11Edited by R. Huber. *J. Mol. Biol.* **277**, 81–102
276. Albrecht, A. M., and Vogel, H. (1964) J. Acetylornithine  $\delta$ -transaminase partial purification and repression behavior. *J. Biol. Chem.* **239**, 1872–1876
277. Slocum, R. D. (2005) Genes, enzymes and regulation of arginine biosynthesis in plants. *Plant Physiol. Biochem.* **43**, 729–745
278. Jauniaux, J. C., Urrestarazu, L. A., and Wiame, J. M. (1978) Arginine metabolism in *Saccharomyces cerevisiae*: subcellular localization of the enzymes. *J. Bacteriol.* **133**, 1096–1107
279. Heimberg, H., Boyen, A., Crabeel, M., and Glansdorff, N. (1990) *Escherichia coli* and *Saccharomyces cerevisiae* acetylornithine aminotransferases: Evolutionary relationship with ornithine aminotransferases. *Gene* **90**, 69–78
280. Ledwidge, R., and Blanchard, J. S. (1999) The dual biosynthetic capability of N-acetylornithine aminotransferase in arginine and lysine biosynthesis. *Biochemistry* **38**, 3019–3024
281. Billheimer, J. T., Carnevale, H. N., Leisinger, T., Eckhardt, T., and Jones, E. E. (1976) Ornithine delta-transaminase activity in *Escherichia coli*: Its identity with acetylornithine delta-transaminase. *J. Bacteriol.* **127**, 1315–1323
282. Newman, J., Seabrook, S., Surjadi, R., Williams, C. C., Lucent, D., Wilding, M., *et al.* (2013) Determination of the structure of the catabolic N-succinylornithine transaminase (AstC) from *Escherichia coli*. *PLoS One* **8**, e58298
283. Schneider, B. L., Kiupakis, A. K., and Reitzer, L. J. (1998) Arginine catabolism and the arginine succinyltransferase pathway in *Escherichia coli*. *J. Bacteriol.* **180**, 4278–4286
284. Fraley, C. D., Kim, J. H., McCann, M. P., and Matin, A. (1998) The *Escherichia coli* starvation gene cstC is involved in amino acid catabolism. *J. Bacteriol.* **180**, 4287–4290
285. Frémont, N., Riefler, M., Stolz, A., and Schmölling, T. (2013) The Arabidopsis tumor prone5 gene encodes an acetylornithine

- aminotransferase required for arginine biosynthesis and root meristem maintenance in blue light. *Plant Physiol.* **161**, 1127–1140
286. Jain, J. C., Shargool, P. D., and Chung, S. (1987) Compartmentation studies on enzymes of ornithine biosynthesis in plant cells. *Plant Sci.* **51**, 17–20
  287. Noguchi, T., Okuno, E., Takada, Y., Minatogawa, Y., Okai, K., and Kido, R. (1978) Characteristics of hepatic alanine-glyoxylate aminotransferase in different mammalian species. *Biochem. J.* **169**, 113–122
  288. Rodionov, R. N., Murry, D. J., Vaulman, S. F., Stevens, J. W., and Lentz, S. R. (2010) Human alanine-glyoxylate aminotransferase 2 lowers asymmetric dimethylarginine and protects from inhibition of nitric oxide production. *J. Biol. Chem.* **285**, 5385–5391
  289. Caplin, B., Wang, Z., Slaviero, A., Tomlinson, J., Dowsett, L., Delahaye, M., et al. (2012) Alanine-glyoxylate aminotransferase-2 metabolizes endogenous methylarginines, regulates no, and controls blood pressure. *Arterioscler. Thromb. Vasc. Biol.* **32**, 2892–2900
  290. Vallance, P., and Leiper, J. (2004) Cardiovascular biology of the asymmetric dimethylarginine:dimethylarginine dimethylaminohydrolase pathway. *Arterioscler. Thromb. Vasc. Biol.* **24**, 1023–1030
  291. Ogawa, T., Kimoto, M., and Sasaoka, K. (1990) Dimethylarginine:pyruvate aminotransferase in rats. Purification, properties, and identity with alanine-glyoxylate aminotransferase 2. *J. Biol. Chem.* **265**, 20938–20945
  292. Kontani, Y., Kaneko, M., Kikugawa, M., Fujimoto, S., and Tamaki, N. (1993) Identity of D-3-aminoisobutyrate-pyruvate aminotransferase with alanine-glyoxylate aminotransferase 2. *Biochim. Biophys. Acta* **1156**, 161–166
  293. Rodionov, R. N., Jarzebska, N., Weiss, N., and Lentz, S. R. (2014) AGXT2: a promiscuous aminotransferase. *Trends Pharmacol. Sci.* **35**, 575–582
  294. Parthasarathy, A., Adams, L. E., Savka, F. C., and Hudson, A. O. (2019) The Arabidopsis thaliana gene annotated by the locus tag At3g08860 encodes alanine aminotransferase. *Plant Direct.* **3**, e00171
  295. Zrenner, R., Riegler, H., Marquard, C. R., Lange, P. R., Geserick, C., Bartosz, C. E., et al. (2009) A functional analysis of the pyrimidine catabolic pathway in Arabidopsis. *New Phytol.* **183**, 117–132
  296. Wu, S., Alseekh, S., Cuadros-Inostroza, Á., Fusari, C. M., Mutwil, M., Kooke, R., et al. (2016) Combined use of genome-wide association data and correlation networks unravels key regulators of primary metabolism in Arabidopsis thaliana. *PLoS Genet.* **12**, e1006363
  297. Hwang, B.-Y., Lee, H.-J., Yang, Y.-H., Joo, H.-S., and Kim, B.-G. (2004) Characterization and investigation of substrate specificity of the sugar aminotransferase WecE from E. coli K12. *Chem. Biol.* **11**, 915–925
  298. Sonnhammer, E. L. L., Eddy, S. R., and Durbin, R. (1997) Pfam: a comprehensive database of protein domain families based on seed alignments. *Proteins* **28**, 405–420
  299. Wang, F., Singh, S., Xu, W., Helmich, K. E., Miller, M. D., Cao, H., et al. (2015) Structural basis for the stereochemical control of amine installation in nucleotide sugar aminotransferases. *ACS Chem. Biol.* **10**, 2048–2056
  300. Breazeale, S. D., Ribeiro, A. A., and Raetz, C. R. H. (2003) Origin of lipid A species modified with 4-Amino-4-deoxy-l-arabinose in polymyxin-resistant mutants of Escherichia coli: an aminotransferase (ArnB) that generates UDP-4-AMINO-4-DEOXY-l-ARABINOSE\*. *J. Biol. Chem.* **278**, 24731–24739
  301. Conway, M. E., and Hutson, S. M. (2016) BCAA metabolism and NH3 homeostasis. In Schousboe, A., Sonnewald, U., eds., *The Glutamate/GABA-Glutamine Cycle: Amino Acid Neurotransmitter Homeostasis* 99–132. Springer International Publishing, New York City, NY
  302. Than, N. G., Sümegi, B., Than, G. N., Bellyei, S., and Molecular, Bohn, H. (2001) Cloning and characterization of placental tissue protein 18 (PP18a)/human mitochondrial branched-chain aminotransferase (BCATm) and its novel alternatively spliced PP18b variant. *Placenta* **22**, 235–243
  303. Lin, H. M., Kaneshige, M., Zhao, L., Zhang, X., Hanover, J. A., and Cheng, S. Y. (2001) An isoform of branched-chain aminotransferase is a novel co-repressor for thyroid hormone nuclear receptors. *J. Biol. Chem.* **276**, 48196–48205
  304. Yudkoff, M., Daikhin, Y., Grunstein, L., Nissim, I., Stern, J., Pleasure, D., et al. (1996) Astrocyte leucine metabolism: significance of branched-chain amino acid transamination. *J. Neurochem.* **66**, 378–385
  305. Rothman, D. L., De Feyter, H. M., Maciejewski, P. K., and Behar, K. L. (2012) Is there *in vivo* evidence for amino acid shuttles carrying ammonia from neurons to astrocytes? *Neurochem. Res.* **37**, 2597–2612
  306. Yudkoff, M. (2017) Interactions in the metabolism of glutamate and the branched-chain amino acids and ketoacids in the CNS. *Neurochem. Res.* **42**, 10–18
  307. Diebold, R., Schuster, J., Däschner, K., and Binder, S. (2002) The branched-chain amino acid transaminase gene family in Arabidopsis encodes plastid and mitochondrial proteins. *Plant Physiol.* **129**, 540–550
  308. Adelberg, E. A., and Umbarger, H. E. (1953) Isoleucine and valine metabolism in Escherichia coli. V. alpha-Ketoisovaleric acid accumulation. *J. Biol. Chem.* **205**, 475–482
  309. Kline, E. L., Manross, D. N., and Warwick, M. L. (1977) Multivalent regulation of isoleucine-valine transaminase in an Escherichia coli K-12 *ilvA* deletion strain. *J. Bacteriol.* **130**, 951–953
  310. Lee-Peng, F.-C., Hermodson, M. A., and Kohlhaw, G. B. (1979) Transaminase B from Escherichia coli: quaternary structure, amino-terminal sequence, substrate specificity, and absence of a separate valine-alpha-ketoglutarate activity. *J. Bacteriol.* **139**, 339–345
  311. Kelly, S. A., Magill, D. J., Megaw, J., Skvortsov, T., Allers, T., McGrath, J. W., et al. (2019) Characterisation of a solvent-tolerant haloarchaeal (R)-selective transaminase isolated from a Triassic period salt mine. *Appl. Microbiol. Biotechnol.* **103**, 5727–5737
  312. Ollivaux, C., Soyec, D., and Toullec, J.-Y. (2014) Biogenesis of d-amino acid containing peptides/proteins: where, when and how? *J. Pept. Sci.* **20**, 595–612
  313. Martínez-Rodríguez, S., Martínez-Gómez, A. I., Rodríguez-Vico, F., Clemente-Jiménez, J. M., and Las Heras-Vázquez, F. J. (2010) Natural occurrence and industrial applications of d-amino acids: An overview. *Chem. Biodiversity* **7**, 1531–1548
  314. Fotheringham, I. G., Bledig, S. A., and Taylor, P. P. (1998) Characterization of the genes encoding d-amino acid transaminase and glutamate racemase, two d-glutamate biosynthetic enzymes of Bacillus sphaericus ATCC 10208. *J. Bacteriol.* **180**, 4319–4323
  315. Schätzle, S., Mahesh, D. P., Sharad, S., Hyunwoo, J., Geon-Hee, K., Taresh, P., et al. (2011) Enzymatic asymmetric synthesis of enantiomerically pure aliphatic, aromatic and arylaliphatic amines with (R)-selective amine transaminases. *Adv. Synth. Catal.* **353**, 2439–2445
  316. Funakoshi, M., Sekine, M., Katane, M., Furuchi, T., Yohda, M., Yoshikawa, T., et al. (2008) Cloning and functional characterization of Arabidopsis thaliana d-amino acid aminotransferase – d-aspartate behavior during germination. *FEBS J.* **275**, 1188–1200
  317. Doublet, P., van Heijenoort, J., Bohin, J. P., and Mengin-Lecreux, D. (1993) The murI gene of Escherichia coli is an essential gene that encodes a glutamate racemase activity. *J. Bacteriol.* **175**, 2970–2979
  318. Pollegioni, L., Piubelli, L., Sacchi, S., Pilone, M. S., and Molla, G. (2007) Physiological functions of D-amino acid oxidases: from yeast to humans. *Cell Mol. Life Sci.* **64**, 1373–1394
  319. Bezsudnova, E. Yu., Boyko, K. M., and Popov, V. O. (2017) Properties of bacterial and archaeal branched-chain amino acid aminotransferases. *Biochem. Mosc.* **82**, 1572–1591
  320. Notebaart, R. A., Szappanos, B., Kintsjes, B., Pál, F., Györkei, Á., Bogos, B., et al. (2014) Network-level architecture and the evolutionary potential of underground metabolism. *Proc. Natl. Acad. Sci. U. S. A.* **111**, 11762–11767
  321. Khersonsky, O., and Tawfik, D. S. (2010) Enzyme promiscuity: a mechanistic and evolutionary perspective. *Annu. Rev. Biochem.* **79**, 471–505
  322. Notebaart, R. A., Kintsjes, B., Feist, A. M., and Papp, B. (2018) Underground metabolism: network-level perspective and biotechnological potential. *Curr. Opin. Biotechnol.* **49**, 108–114
  323. Jensen, R. A. (1976) Enzyme recruitment in evolution of new function. *Annu. Rev. Microbiol.* **30**, 409–425
  324. Li, Y., Ogola, H. J. O., and Sawa, Y. (2012) L-Aspartate dehydrogenase: features and applications. *Appl. Microbiol. Biotechnol.* **93**, 503–516

325. Dave, U. C., and Kadeppagari, R.-K. (2019) Alanine dehydrogenase and its applications – a review. *Crit. Rev. Biotechnol.* **39**, 648–664
326. Berg, J. M., Tymoczko, J. L., and Stryer, L. (2002) The first step in amino acid degradation is the removal of nitrogen. In *Biochemistry*, 5th edition, W H Freeman, New York City, NY
327. Hirotsu, K., Goto, M., Okamoto, A., and Miyahara, I. (2005) Dual substrate recognition of aminotransferases. *Chem. Rec.* **5**, 160–172
328. Haruyama, K., Nakai, T., Miyahara, I., Hirotsu, K., Mizuguchi, H., Hayashi, H., *et al.* (2001) Structures of *Escherichia coli* histidinol-phosphate aminotransferase and its complexes with histidinol-phosphate and N-(5'-phosphopyridoxyl)-L-glutamate: double substrate recognition of the enzyme. *Biochemistry* **40**, 4633–4644
329. Goto, M., Omi, R., Miyahara, I., Hosono, A., Mizuguchi, H., Hayashi, H., *et al.* (2004) Crystal structures of glutamine:phenylpyruvate aminotransferase from *thermus thermophilus* HB8 induced fit and substrate recognition. *J. Biol. Chem.* **279**, 16518–16525
330. Nakamura, A., Yao, M., Chimnarong, S., Sakai, N., and Tanaka, I. (2006) ammonia channel couples glutaminase with transamidase reactions in GatCAB. *Science* **312**, 1954–1958
331. Sheppard, K., Akochy, P.-M., Salazar, J. C., and Söll, D. (2007) The *Helicobacter pylori* amidotransferase GatCAB is equally efficient in glutamine-dependent transamidation of asp-tRNA<sup>Asn</sup> and glu-tRNA<sup>Gln</sup>. *J. Biol. Chem.* **282**, 11866–11873
332. Copley, S. D. (2015) An evolutionary biochemist's perspective on promiscuity. *Trends Biochem. Sci.* **40**, 72–78
333. Atkins, W. M. (2020) Mechanisms of promiscuity among drug metabolizing enzymes and drug transporters. *FEBS J.* **287**, 1306–1322
334. Scriver, C. R., Hurtubise, M., Konecki, D., Phommarinh, M., Prevost, L., Erlandsen, H., *et al.* (2003) PAHdb 2003: what a locus-specific knowledgebase can do. *Hum. Mutat.* **21**, 333–344
335. MacDonald, A., Singh, R. H., Rocha, J. C., and van Spronsen, F. J. (2019) Optimising amino acid absorption: essential to improve nitrogen balance and metabolic control in phenylketonuria. *Nutr. Res. Rev.* **32**, 70–78
336. Heckmann, C. M., Gourlay, L. J., Dominguez, B., and Paradisi, F. (2020) An (R)-Selective transaminase from *thermomycetes stellatus*: stabilizing the tetrameric form. *Front. Bioeng. Biotechnol.* **8**, 707
337. Huang, X.-Q., Li, R., Fu, J., and Dudareva, N. (2022) A peroxisomal heterodimeric enzyme is involved in benzaldehyde synthesis in plants. *Nat. Commun.* **13**, 1352
338. Waterhouse, A., Bertoni, M., Bienert, S., Studer, G., Tauriello, G., Gumienny, R., *et al.* (2018) SWISS-MODEL: homology modelling of protein structures and complexes. *Nucl. Acids Res.* **46**, W296–W303
339. Jumper, J., Evans, R., Pritzel, A., Green, T., Figurnov, M., Ronneberger, O., *et al.* (2021) Highly accurate protein structure prediction with AlphaFold. *Nature* **596**, 583–589
340. Rives, A., Meier, J., Sercu, T., Goyal, S., Lin, Z., Liu, J., *et al.* (2021) Biological structure and function emerge from scaling unsupervised learning to 250 million protein sequences. *Proc. Natl. Acad. Sci. U. S. A.* **118**, e2016239118
341. Steffen-Munsberg, F., Vickers, C., Kohls, H., Land, H., Mallin, H., Nobili, A., *et al.* (2015) Bioinformatic analysis of a PLP-dependent enzyme superfamily suitable for biocatalytic applications. *Biotechnol. Adv.* **33**, 566–604
342. Kappes, B., Tews, I., Binter, A., and Macheroux, P. (2011) PLP-dependent enzymes as potential drug targets for protozoan diseases. *Biochim. Biophys. Acta* **1814**, 1567–1576
343. Tamura, K., Stecher, G., and Kumar, S. (2021) MEGA11: molecular evolutionary genetics analysis version 11. *Mol. Biol. Evol.* **38**, 3022–3027
344. Saitou, N., and Nei, M. (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**, 406–425
345. Zuckerkandl, E., and Pauling, L. (1965) Evolutionary divergence and convergence in proteins. In Bryson, V., Vogel, H. J., eds., *Evolving Genes and Proteins* **97–166**. Academic Press, Cambridge, MA
346. Felsenstein, J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791
347. Liu, W., Peterson, P. E., Carter, R. J., Zhou, X., Langston, J. A., Fisher, A. J., *et al.* (2004) Crystal structures of unbound and aminooxyacetate-bound *Escherichia coli*  $\gamma$ -aminobutyrate aminotransferase. *Biochemistry* **43**, 10896–10905