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Review

News about amino acid metabolism in plant–microbe interactions

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Plants constantly come into contact with a diverse mix of pathogenic and beneficial microbes. The ability to distinguish between them and to respond appropriately is essential for plant health. Here we review recent progress in understanding the role of amino acid sensing, signaling, transport, and metabolism during plant–microbe interactions. Biochemical pathways converting individual amino acids into active compounds have recently been elucidated, and comprehensive large-scale approaches have brought amino acid sensors and transporters into focus. These findings show that plant central amino acid metabolism is closely interwoven with stress signaling and defense responses at various levels. The individual biochemical mechanisms and the interconnections between the different processes are just beginning to emerge and might serve as a foundation for new plant protection strategies.

Highlights

Engaging with beneficial microbes while fending off pathogenic ones is crucial for plant health and crop yield.

Recent findings show that amino acid metabolism is closely linked to plant–microbe interactions, providing signaling molecules, nutrients, and defense compounds.

Great progress has been made in the field of amino-acid-derived secondary metabolites and signaling molecules, leading to the identification of biochemical synthesis pathways, transport, and inactivation mechanisms.

Insight into the mechanisms of amino acid sensing and signaling will be critical to unraveling plant–microbe communication.

Plant amino acid metabolism provides signaling molecules, defense compounds, and nutrients to shape interactions with microbes

In a natural environment outside controlled laboratory conditions, plants interact with complex microbial communities. Microbes usually benefit from the rich supply of organic compounds (including amino acids) in the vicinity of a plant. Some might manipulate plant metabolism to access nutrients, either in return for some kind of service during mutualistic interactions or without benefit for the plant in commensals, whereas pathogens even cause damage to the plant (Figure 1). In any case, microbes need to evade or suppress immune reactions and the plant needs to discriminate between potentially harmful and beneficial interactions to react accordingly. The plant's set of measures may include withdrawing nutrients to starve pathogens (e.g., [1]) or supplying a specific set of compounds to establish beneficial interactions (e.g., [2]). In the case of a pathogen attack, plants also have to activate appropriate defense responses and to alert nonaffected parts of the plant about impending danger to restrict pathogen growth [3].

In this review we discuss how plant amino acid metabolism is involved in shaping these different interactions between plants and microbes. Recent studies have shed light on interspecies communication during first contact, and have demonstrated how plants use amino acids to produce **specialized metabolites** (see Glossary) as a means to selectively promote proliferation of beneficial microbes. Amino acid transport is required for nutrient exchange and, in combination with specific receptors, might be involved in amino acid sensing and signaling mechanisms during interaction with microbes. Amino acid metabolism is also crucial for immune signaling within the plant during the establishment of a systemic immune reaction.

Aromatic amino acids are converted to a set of specialized metabolites involved in shaping the plant microbiome

The aromatic amino acids Tyr, Phe, and Trp are synthesized in plastids and also in the cytosol by the shikimate pathway [4]. In addition to being incorporated into proteins, they serve as

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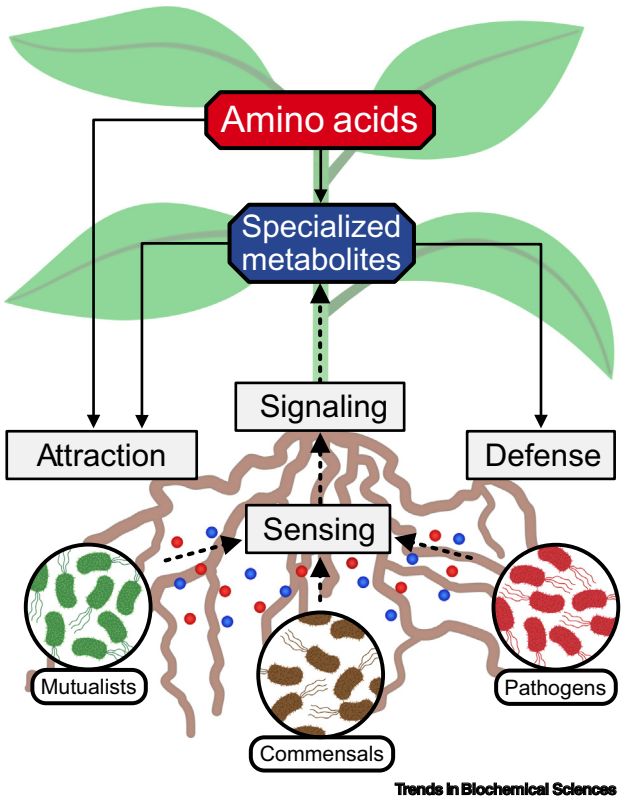


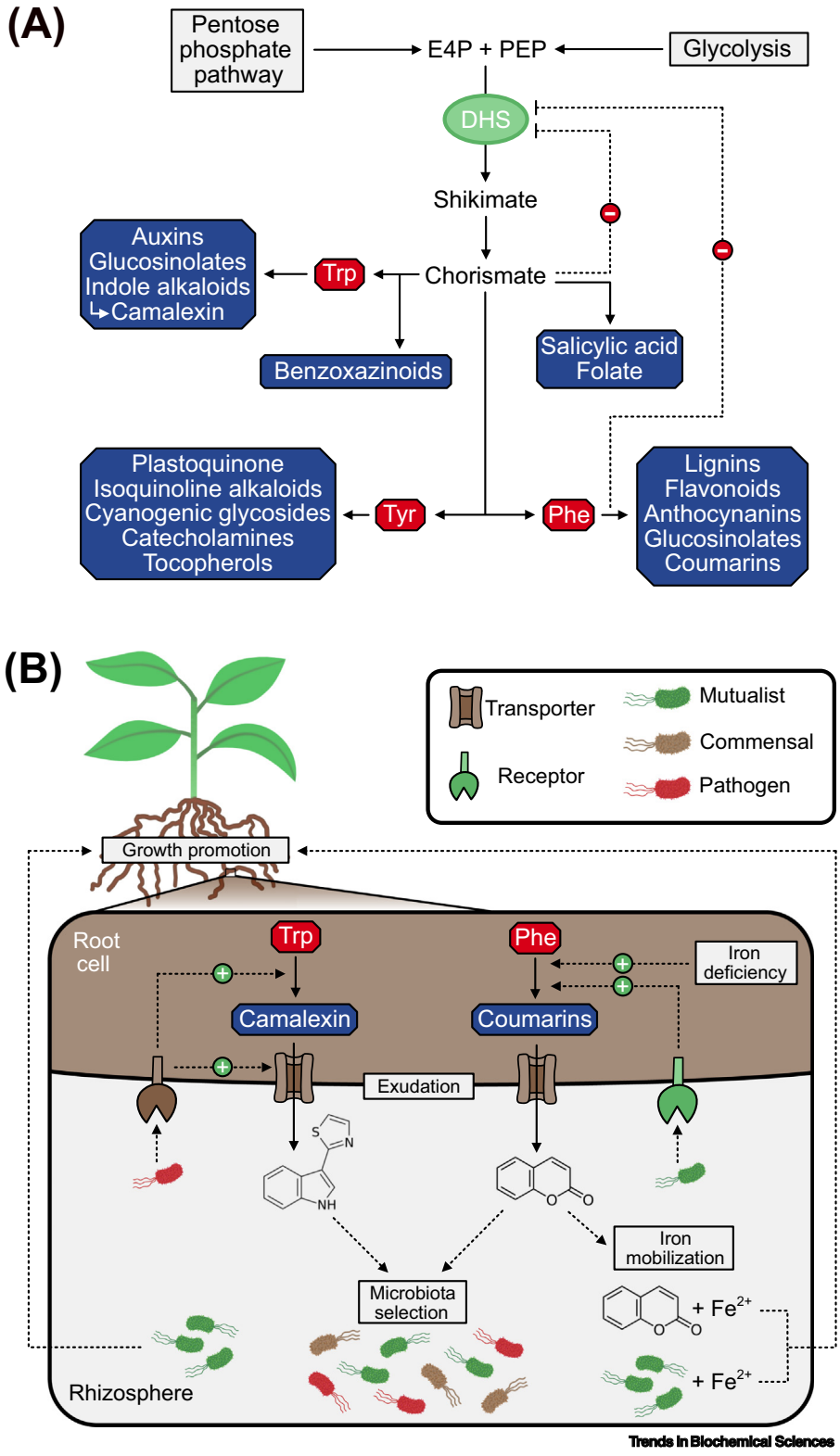
Figure 1. Plant amino acid metabolism provides active compounds for interacting with microbes at different levels. Plants interact with a multitude of microbes, which can be categorized according to their effect on plant health into mutualists (beneficial), commensals (neutral), and pathogens (detrimental). Plants convert amino acids into specialized metabolites that either act as signaling molecules within the plant or are exuded to shape the composition of the microbiome in favor of the plant. The microbes in turn require plant amino acids as a source of nutrients. Experimental results indicate that plants can sense specific patterns in changes in amino acid metabolism and interpret them as a fingerprint of a lurking pathogen. However, the mechanisms of amino acid sensing and signaling are largely unknown.

precursors for a diverse set of specialized metabolites (Figure 2) [5]. A considerable share of carbon flow (≥30%) is directed through the shikimate pathway to produce pigments, defense compounds, and the cell-wall component lignin [6]. Plant specialized metabolites can act as nutrient sources, signaling molecules, or toxins for individual microbial strains, thereby shaping the overall composition of the **microbiome** [7,8]. Recently there has been some significant progress in understanding both the regulation of aromatic amino acid metabolism and the role of individual aromatic phytochemicals in coordinating plant–microbe interactions.

The synthesis rates for the individual aromatic amino acids are regulated by product inhibition of the respective committed step, which is a common scheme in amino acid synthesis in plants [4,6]. However, in addition, the entry reaction of the shikimate pathway – catalyzed by 3-deoxy-d-arabino-heptulosonate 7-phosphate synthase (DHS) – is controlled via a complex pattern of allosteric feedback inhibition in a tissue-specific manner [5]. Notably, all the three DHS isoforms present in arabidopsis (*Arabidopsis thaliana*) are strongly inhibited by caffeate, an intermediate in phenylpropanoid biosynthesis from Phe, indicating that the flux through the shikimate pathway in general is adjusted to meet the demands of specialized metabolite production. A genome-wide **ribosome profiling** approach revealed that during **effector-triggered immunity** (**ETI**) arabidopsis plants specifically induce the biosynthesis pathways for aromatic amino acids and derived specialized metabolites on the level of translation in coordination with increased transcription rates as an additional layer of upregulation [9]. Thus, the metabolism of aromatic amino acids is an important factor in the interaction between plants and microbes and serves as a toolbox for the production of a variety of tailored active compounds.

Glossary

Autoimmune phenotype: natively elevated immunity in plants not exposed to pathogens, commonly accompanied by growth defects.
Auxotrophy: dependence of organisms on taking up certain essential substances from the environment which they are unable to synthesize on their own.
Chemoreceptors: sensory proteins responsive to chemical stimuli.
Damage-associated molecular patterns (DAMPs): endogenous molecules that serve as molecular markers for physical damage on extracellular receptors.
Effector-triggered immunity (ETI): plant immunity elicited by pathogen virulence factors secreted into the plant cell, usually to alter transcription in the host.
Metabolon: a temporary structural–functional complex formed between sequential enzymes of a metabolic pathway.
Microbe-associated molecular patterns (MAMPs): molecular signatures that are highly conserved among microbes but are absent in the host (e.g., flagella).
Microbiome: entirety of microorganisms in a particular environment.
Pattern-triggered immunity (PTI): plant immunity elicited by molecular patterns associated with pathogens or cellular damage.
Promoter profiling: analysis of regulatory genetic elements to predict the expression pattern and function of uncharacterized genes.
Rhizobia: group of symbiotic nitrogen-fixing soil bacteria that infect roots of legumes.
Rhizosphere: the region of soil in the vicinity of plant roots in which the chemistry and microbiology is influenced by the roots' growth, respiration, and nutrient exchange.
Ribosome profiling: technique to determine actively translated mRNA transcripts.
RNAseq: sequencing technique revealing the presence and quantity of RNA in a biological sample at a given moment.
Single-particle cryo-EM: electron microscopy of frozen specimens (e.g., proteins) that allows near-atomic resolutions.
Specialized metabolites: metabolites beyond central (primary) metabolism



with a broad-spectrum chemical diversity serving a multitude of critical functions in plants (e.g., plant defense). Traditionally referred to as secondary metabolites.

Splice variant: alternative recombination of exons allowing one gene to encode multiple proteins.

Systemic acquired resistance (SAR): enhanced immunity of the whole plant following prior exposure to a pathogen.

X-ray crystallography: imaging of crystalline structures facilitated by analysis of diffracting x-rays.

(See figure legend at the bottom of the next page.)

While the protective function of phytoalexins derived from aromatic amino acids during pathogen attack is well established, their role in recruiting beneficial microbes is just beginning to become clear [7,10]. Coumarins are polar phenolic compounds produced from Phe via the general phenylpropanoid pathway, and they are ubiquitous in plants [11]. The synthesis pathway for two coumarins involved in plant–microbe interactions, fratexin and the redox-active sideretin, has been clarified only recently [12,13]. In addition, a suite of new publications has contributed to connecting two of the well established physiological functions of coumarins, namely, improving bioavailability of iron in alkaline soils and defense against pathogens (Figure 2B) [11,14]. Using different coumarin-deficient arabidopsis mutant lines in combination with either selected pathogenic and mutualistic microbes or a synthetic microbial community, they revealed the role of coumarins in shaping the root microbiome to improve plant iron nutrition. Specific coumarins are secreted by the roots of arabidopsis plants, which change the composition of the root microbiome by selectively inhibiting the growth of pathogenic microbes but not beneficial strains [15–17]. This effect is induced in iron-deficient soils and seems to involve redox-mediated toxicity [16].

Camalexin is an antifungal sulfur-containing indolic compound synthesized from Trp. It is specific for Brassicaceae and is the most prominent phytoalexin in arabidopsis [18]. Trp metabolism also produces a series of additional specialized metabolites, including indolic glucosinolates and the auxin indole-3-acetic acid via common intermediates [19]. Efficient camalexin synthesis without release of active intermediates is achieved by the formation of a camalexin-biosynthetic **metabolon**, a cytosolic protein complex attached to the endoplasmic reticulum [20]. The pleiotropic drug-resistance transporters PEN3 and PDR12 function redundantly to mediate camalexin secretion [21]. Camalexin synthesis in the roots is required for recruiting mutualistic microbes to the **rhizosphere** and, interestingly, it is also a prerequisite for actually receiving growth benefits by potentially growth-promoting bacterial strains (Figure 2B) [22]. The mechanism of this interaction has yet to be discovered.

Plants of the Poaceae (such as maize, wheat, and rye) can synthesize large quantities of benzoxazinoids from the Trp precursor indole to regulate belowground as well as aboveground biotic interactions. A number of recent studies highlighted the pivotal role of these heteroaromatic metabolites in shaping the rhizosphere microbiota [23–25]. Benzoxazinoids serve as toxins towards pathogens and, in addition, as chemoattractants for beneficial microbes, affecting both bacterial and fungal root-associated communities. Soil conditioning even persisted into the next growing season and determined biotic interactions and plant performance in the next generation [23].

Plants potentially produce hundreds of thousands of different metabolites, and most of them have not yet been characterized [26]. Even with a focus on compounds derived from amino acids, plant specialized metabolism is highly complex and has the potential to provide a high level of specificity during plant–microbe interactions. Due to the high diversity of metabolites

Figure 2. Specialized metabolites derived from aromatic amino acids shape the root microbiome. (A) The shikimate pathway produces aromatic amino acids and a diverse set of specialized metabolites. The initial step, catalyzed by 3-deoxy-d-arabino-heptulosonate 7-phosphate synthase (DHS), is regulated via feedback inhibition by several intermediates in a complex manner (broken lines) [5]. (B) Camalexin and coumarin shape the root microbiome in *Arabidopsis thaliana* by selectively inhibiting growth of pathogenic microbes. Camalexin synthesis from Trp and its secretion via the pleiotropic drug transporters PEN3 and PDR12 are induced in a coordinated fashion by mitogen-activated protein (MAP) kinases and the downstream WRKY33 transcription factor upon pathogen recognition [21]. During iron starvation, probiotic rhizobacteria induce conversion of Phe to coumarins and their exudation into the rhizosphere via the transcription factor MYB72 [15]. Coumarins as well as coumarin-tolerant microbes increase the bioavailability of iron in iron-limited alkaline soils [15–17]. Abbreviations: E4P, erythrose-4-phosphate; PEP, phosphoenolpyruvate.

across plant species, research on different model and non-model organisms holds the promise of new discoveries.

Amino acid transport controls nutrient exchange between plants and microbes

When a plant pathogen begins proliferating in the apoplast, it is usually nutrient-starved and depends on rapid assimilation of nutrients from the host. The plant in turn may reallocate resources for defense or withdraw nutrients from the site of infection. Thus, adaptations in plant nitrogen metabolism upon pathogen attack represent the combined effects of the plant's defense strategy and manipulation by the pathogen to increase nutrient availability. Plants exude 15% of assimilated nitrogen, and amino acids are a major nitrogen currency [27]. Microbial **chemoreceptors** recognize a broad variety of amino acids and direct the microbes to the nutrient-rich niches surrounding plant roots [28]. The ability to use amino acids supplied by the host plant for nutrition might be crucial for establishing symbiotic interactions. Three independent screening approaches identified **auxotrophy** for specific amino acids as a factor impairing the interaction of growth-promoting Pseudomonas strains with their host arabidopsis [29–31].

Amino acid exudation by the plant requires transport across several membranes: (i) between apoplast and cytoplasm for exudation or uptake, (ii) across membranes of intracellular compartments involved in amino acid synthesis, metabolism, and storage (chloroplasts, mitochondria, vacuole), and (iii) between different cells and plant organs to meet the increased local demand caused by microbial interactions (Figure 3). The arabidopsis genome contains about 100 putative amino acid transporters belonging to three major families [32,33]. Only about 20% of them have been functionally characterized so far [34].

While transporters of the amino acid/auxin permease (AAAP) and amino acid polyamine organocation (APC) families and their role in amino acid uptake and secretion by the roots have been known for some time [35,36], the 'usually multiple acids move in and out transporters' (UMAMIT) family is currently the new center of interest. UMAMITs were originally identified as nodulins required for symbiotic interactions of **rhizobia** with legumes [37,38]. Zhao *et al.* characterized all 47 UMAMIT genes and proteins found in arabidopsis in detail, including tissue and subcellular localization as well as amino acid transport properties [38]. Their results identified a set of particularly stress-responsive UMAMITs as likely candidates for involvement in plant–microbe interactions. UMAMIT14 and UMAMIT18 mediate the radial transport of amino acids in roots and their secretion into the soil [39]. The transcription factor bZIP11 is required for the induction of these two and an additional three UMAMITs [29,31,34] alongside several nitrate and ammonium transporters and might be targeted by pathogens to secure access to nutrients from their hosts [40].

In addition, the stress-responsive W-BOX motif has recently been identified in the promoter regions of 40 amino acid transporter genes, indicating that regulation by WRKY transcription factors could also play a role [33]. Based on the same **promoter profiling** approach, induction by the phytohormone salicylic acid (SA) can be postulated for 34 amino acid transporters. In the systemic (noninfected) leaves of arabidopsis plants locally infected with *Pseudomonas syringae*, the enzymatic pathways catalyzing amino acid synthesis are downregulated on a transcriptional level [41]. The lowered free contents of most amino acids might help to protect the noninfected leaves by reducing their nutritional value and thus making them less attractive for colonization by pathogens. The role of amino acid exudation in shaping the biochemical ecology of the rhizosphere has frequently been discussed in recent reviews [34,36,42,43], but as yet not many mechanistic details are known. Plant amino acid transporters might be targeted by microbes to enhance nutrient availability, and could also be controlled by the plant immune system in order to selectively

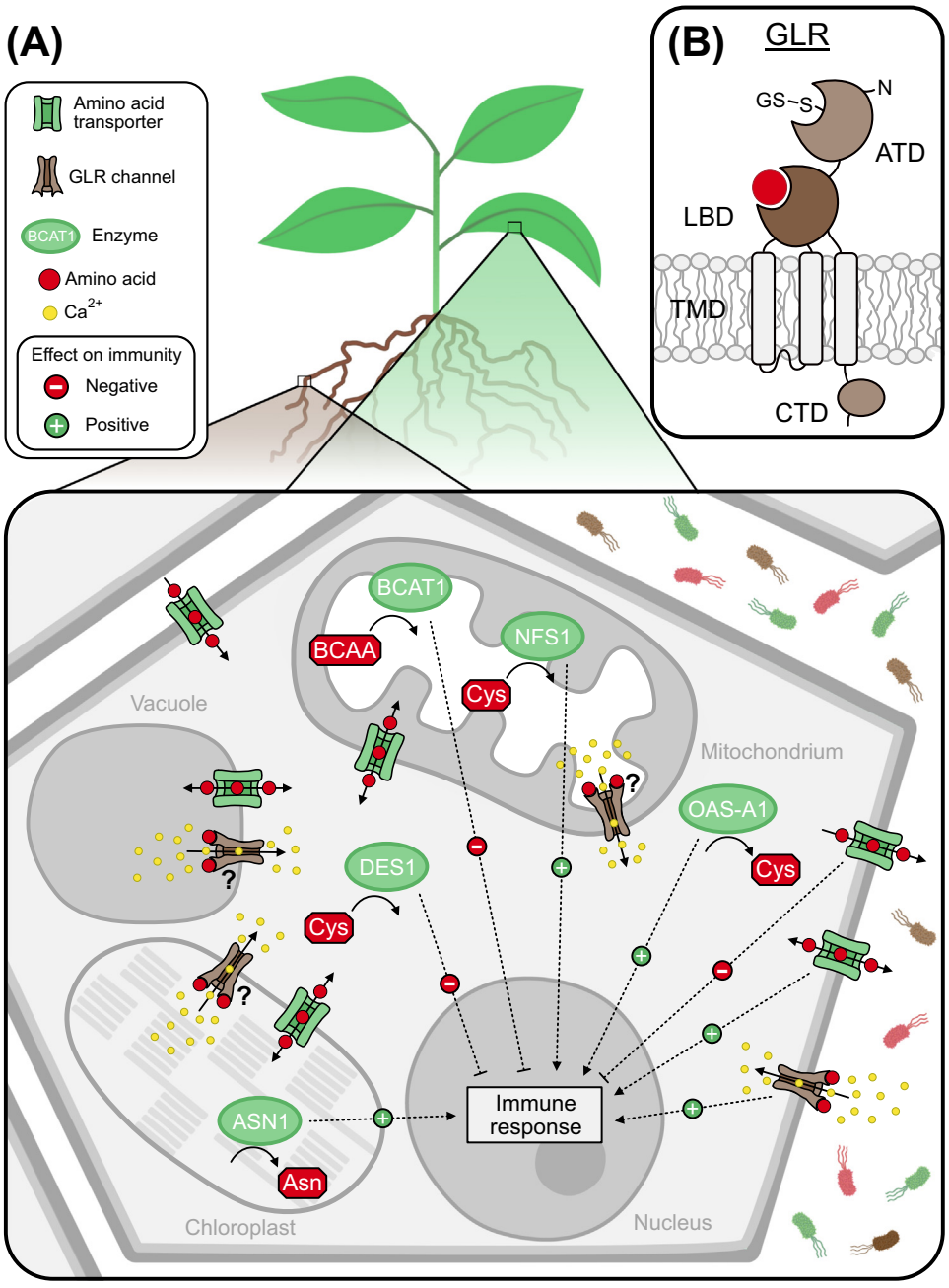


Figure 3. Perturbations in amino acid transport and metabolism can trigger immune signaling. (A) Amino acid metabolism within the plant and exudation into the apoplast requires transport across several membranes. A positive or negative role in plant immunity has been postulated for several amino acid transporters, and also for individual enzymes involved in amino acid metabolism based on the suppression or induction of a pathogen response in mutant plants. Glutamate receptor-like channels (GLRs) might be involved in detecting local fluctuations in specific amino acids and triggering an immune response. The mechanism of amino acid sensing and signaling during plant–microbe interactions is largely unclear. (B) Specific features in the structure of plant glutamate-like receptors provide a basis for a high ligand diversity and additional regulation via glutathionylation of a cysteine residue in the cleft of the amino-terminal domain. Abbreviations: ASN1, asparagine synthetase 1; ATD, amino-terminal domain; BCAA, branched-chain

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feed or starve beneficial or pathogenic microbes, respectively. Thus, an important aspect of future research efforts will be to unravel the connections between plant amino acid transporters and resistance or susceptibility to pathogens and pests.

Specific perturbations in amino acid homeostasis constitutively activate plant defenses

Several lines of evidence indicate that plants monitor their amino acid status and interpret specific alterations in metabolic activity, local amino acid concentrations, or transport activities across membranes as a signature of an attacking pathogen (Figure 3A) [34]. Overexpression of the amino acid exporters UMAMIT14 and glutamine dumper 1 (GDU1), or the importer cationic amino acid transporter 1 (CAT1), leads to constitutive induction of immune signaling in arabidopsis [28,31,44]. By contrast, knockout of lysine histidine transporter 1 (LHT1) or UMAMIT5 (WAT1) increases plant resistance towards a broad spectrum of pathogens [31,45]. However, since both of these amino acid transporters also accept additional substrates – such as auxin or the ethylene precursor aminocyclopropane-1- carboxylic acid (ACC) – a disturbance in hormone signaling might be the primary cause for activating defense responses in the knockout lines [45,46].

An **autoimmune phenotype** has also been reported for mutant lines with different modifications in amino acid metabolic enzymes. An **RNAseq** approach recently identified the mitochondrial branched-chain aminotransferase BCAT1 as a potential regulator of rust infection in wheat [47]. Knockout *bcat1* mutants had moderately increased levels of several amino acids and activated a systemic immune response. Transgenic arabidopsis plants overexpressing pepper asparagine synthetase 1 exhibit enhanced resistance to *P. syringae* pv. tomato DC3000 and *Hyaloperonospora arabidopsidis* [48].

Cysteine can be synthesized and also metabolized in several different compartments of a plant cell, and this compartmentalization seems to be important for signaling functions during abiotic stress [49,50]. Recent results indicate that specific pathways in cysteine metabolism might also be relevant for biotic interactions. Overexpression of the mitochondrial cysteine desulfurase NFS1, which degrades cysteine to provide sulfur during FeS cluster synthesis, results in constitutive upregulation of defense-related genes and increased resistance against *P. syringae* [51]. By contrast, knockout of the cytosolic cysteine desulfhydrase DES1 leads to an autoimmune phenotype whereas knockout lines for OAS1 involved in cysteine synthesis in the cytosol are more susceptible to *P. syringae* [52]. Taken together, these results indicate that increased cysteine degradation in the mitochondria and decreased cysteine degradation in the cytosol both induce a biotic stress response. Homoserine accumulation in the chloroplast due to homoserine kinase deficiency triggers a currently unknown mechanism of downy mildew resistance in arabidopsis [53], whereas threonine accumulation renders the plants unsuitable as an infection substrate for the adapted biotrophic pathogen *H. arabidopsidis* without activating defense responses [54]. Intriguingly, several other defects in amino acid metabolic pathways or transporters also lead to altered amino acid steady state levels without an apparent effect on immune signaling [36]. Even in those lines developing an autoimmune phenotype, there is no clear common trend for any specific amino acids being consistently altered. Thus, local changes in intracellular compartments or specific intermediates of amino acid metabolic pathways might be required to trigger a stress response.

amino acids; BCAT1, branched-chain aminotransferase 1; CTD, carboxyterminal domain; DES1, cysteine desulfhydrase 1; GS-S-, glutathionylated cysteine residue; LBD, ligand-binding domain; NFS1, nitrogen fixation S-like 1; OAS-A1, O-acetylserine(thiol)lyase A1; TMD, transmembrane domain.

A sudden increase in exogenous amino acids could also be interpreted by the plant as a common signature of pathogen-induced cell damage or increased export stimulated by hungry microbes, and thus could be a useful alarm signal. Exogenous treatment with different amino acids has been shown to elicit immune reactions in arabidopsis and rice [55,56]. A diverse set of **damage-associated molecular patterns (DAMPs)** including extracellular ATP has already been identified in arabidopsis [57]. DAMPs are molecules that are normally localized inside the plant cell and elicit an immune response by binding to extracellular receptors. In combination with **microbe-associated molecular patterns (MAMPs)** they can elicit a robust and highly specific local immune response [58]. Some amino acids might be added to the list in the near future (see also next paragraph). Exploring the mechanistic details of amino acid sensing in plants and its role during plant–microbe interactions will be a challenging task, but also offers great potential for identifying central hubs in the integration of plant primary metabolism with biotic stress signaling.

Amino acid activated calcium channels mediate amino acid sensing and signaling in plants

Based on a detailed transcriptome study a set of 39 core immunity response genes was recently defined; these are common to the initial signaling outputs during **pattern-triggered immunity (PTI)** establishment in response to a broad range of patterns in arabidopsis [59]. Strikingly, this set includes two genes for glutamate receptor-like calcium channels (GLRs). GLR proteins, which structurally resemble the neuronal glutamate receptors from metazoans, are involved in long-distance plant defense signaling in response to insect feeding (Figure 3B) [60,61]. In contrast to their animal counterpart, plant GLRs are activated by a broad range of amino acids – including Glu, Gly, Ala, Asn, Ser, Cys, Met, and the tripeptide glutathione [62] – indicating a more general role in the perception of extracellular amino acids. The structural basis for this ligand diversity has been identified using **x-ray crystallography** and **single-particle cryo-EM**, which also revealed some additional plant-specific features in GLR architecture and regulation (Figure 3B) [62,63].

The arabidopsis genome contains 20 GLR homologs belonging to three clades [64]. Most of them are predicted to be localized in the plasma membrane or the vacuole [65], but AtGLR3.4 has also been detected in chloroplasts, and a **splice variant** of AtGLR3.5 was found in the inner mitochondrial membrane [66,67]. Specific isoforms have been implicated in several physiological processes in addition to long-distance wound signaling, such as germination [68], root growth [69], pollen-tube growth [70], and hypocotyl elongation [71]. A general function of GLRs in mediating the calcium influx triggered by selected microbial epitopes has been postulated based on a pharmacological inhibitor study [72]. There is some experimental evidence indicating a role for GLR3.3 and GLR3.6 (the calcium channels required for systemic wound signaling) in plant–microbe interactions [56,73,74]. However, both GLRs in the core set of immunity response genes belong to clade 2, which has not so far been studied in detail [59].The authors demonstrated a functional relevance of clade 2 GLRs in plant immunity by means of a *glr2.7 2.8 2.9* triple mutant. Mutant plants were highly susceptible to the bacterial pathogen *P. syringae* and showed a significantly impaired increase in cytosolic Ca^{2+} concentration after elicitor treatment but not in response to salt or cold.

Since calcium signaling is involved in many different processes in the plant [75,76], identification of the downstream components of the GLR signaling cascade will be crucial for understanding its role in plant–microbe interactions. The 20 GLR isoforms with their potentially broad ligand spectrum may allow the plant to distinguish between different kinds of threat. Amino acid sensing by GLRs might be the mechanism behind observations that specific modifications in amino acid homeostasis induce autoimmune reactions (discussed in the previous paragraph). If localization in membranes of subcellular compartments is confirmed, maybe with splice variants of additional

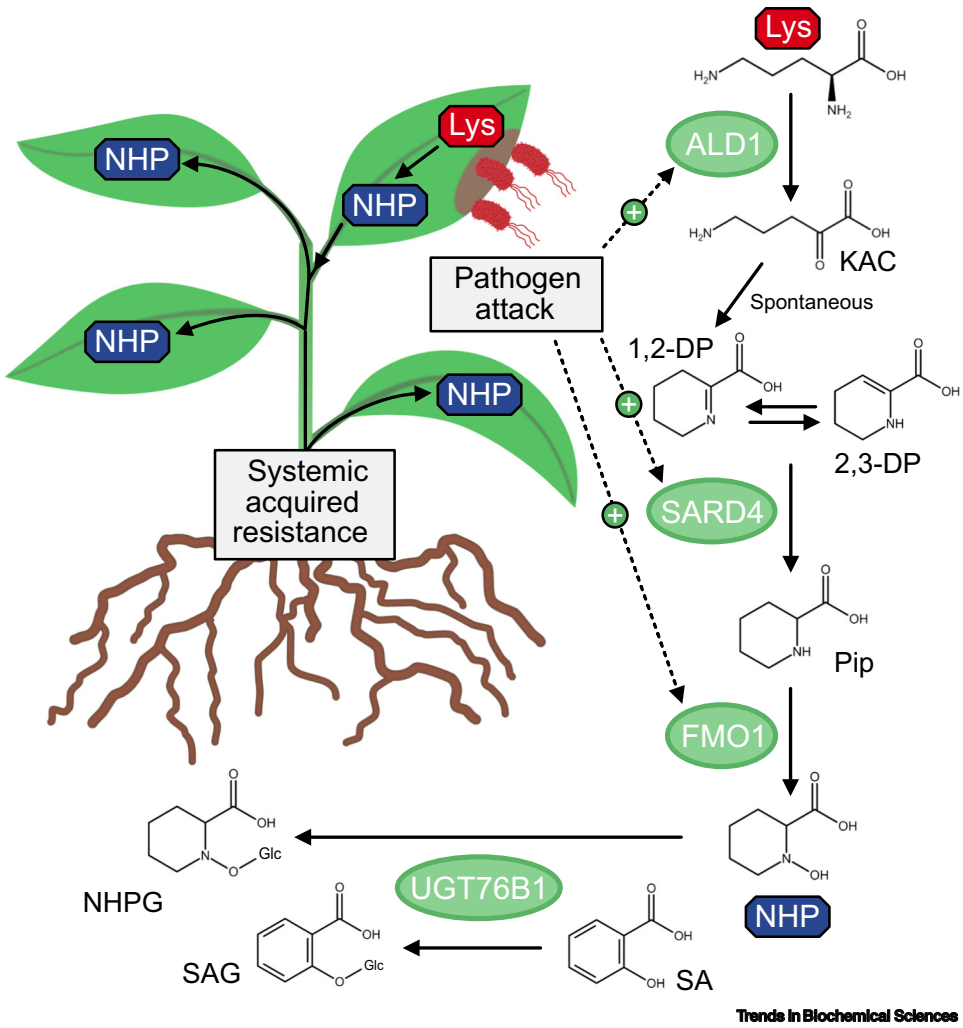

Figure 4. Lysine is converted to the mobile immune signal N-hydroxypipecolic acid to induce systemic acquired resistance. Local pathogen attack induces the synthesis pathway of N-hydroxypipecolic acid (NHP) from Lys catalyzed by three enzymes. NHP is transported to the noninfected systemic parts of the plant and triggers a systemic acquired resistance response that confers long-lasting protection against a broad spectrum of pathogens. The level of NHP is modulated via formation of the inactive β-glucoside catalyzed by the glycosyltransferase UGT76B1, which also accepts salicylic acid (SA) as a substrate. Abbreviations: ALD1, AGD2-like defense response protein 1; DP, dehydropipecolic acid; FMO1, flavin-dependent monooxygenase 1; KAC, α-ketocaproic; NHPG, NHP-N-O-glucoside; Pip, pipecolic acid; SAG, SA-β-glucoside; SARD4, systemic acquired resistance-deficient 4; UGT76B1, UDP-dependent glycosyltransferase 76B1.

GLR isoforms, it is tempting to even speculate about a role in intracellular amino acid sensing and signaling during plant–microbe interactions.

Lysine metabolism is essential for systemic immune signaling

In the past decade, the basic amino acid Lys has attracted research interest as a precursor of a key signaling molecule in plant pathogen response. Pathogen attack elicits local immune responses and also initiates signaling to the noninfected parts of the plant to establish **systemic acquired resistance (SAR)** [77]. Extensive changes in gene transcription triggered during SAR put the plant immune system in a state of alert to prevent the pathogen from spreading over the

entire foliage [78]. The search for the mobile immune signal required for SAR induction recently culminated in the identification of N-hydroxypipecolic acid (NHP) and the final step of its synthesis pathway from Lys [79,80]. All three enzymes required for converting Lys to NHP are strongly induced by biotic stress and have been shown to be essential for SAR induction by means of arabidopsis mutant lines [81]. In a first reaction, the aminotransferase AGD2-like defense response protein 1 (ALD1) deaminates the α-amino group of L-Lys to generate α-ketocaproic acid (KAC), which is unstable and spontaneously reacts to 1,2-dehydropipecolic acid (1,2-DP) and its tautomer 2,3-DP *in planta* (Figure 4) [82–84]. DP is then reduced to pipecolic acid (Pip) by the NAD(P)H-dependent ketimine reductase SAR-deficient 4 (SARD4) [83,84]. Pip accumulates in the leaves of infected plants and external application induces systemic immune reactions [82]. However, N-hydroxylation of Pip to NHP by flavin-dependent monooxygenase 1 (FMO1) is prerequisite for this function, indicating that NHP is the active plant hormone mediating long-distance immune signaling during pathogen defense [79,80].

Defense responses require many resources, so their constitutive induction would impair growth and reproduction [85]. Therefore, the content of active immune signaling molecules must be tightly regulated by fine-tuning their synthesis rates as well as their inactivation [77]. Recently, four independent studies simultaneously showed that UGT76B1 glucosylates NHP to produce the inactive NHP–N–O-glucoside (NHPG) [86–89]. Notably, UGT76B1 also accepts the defense hormone SA as a substrate [86,87]. The metabolic pathways of SA and NHP share multiple common regulatory elements, and both molecules work in concert during SAR induction [77,90].

The SAR signaling pathway was first elucidated in arabidopsis but it is also prevalent in other species such as tobacco, cucumber, tomato and the monocot *Brachypodium dystachyon* [91,92]. Transient expression of UGT76B1 in tomato confirmed glycosylation of NHP and the resulting mitigation of defense signaling [89]. Thus, conversion of the amino acid Lys into the defense hormone NHP seems to be a central component of plant immune response conserved among angiosperms. Changes in Lys metabolism will most likely have immediate consequences for immune signaling, which could be the reason for plants to strictly control the level of free Lys [93,94].

Concluding remarks

Amino acids are central components of protein, energy, and nitrogen metabolism within the plant's primary metabolism. In addition, they are precursors for a variety of active compounds with specific functions in plant–microbe interactions that are far from being fully understood. Most likely, individual amino acids are also involved in signaling events during biotic interactions. In a complex environment plants need to integrate environmental information with intrinsic cues about their nutritional status and trigger appropriate metabolic and cellular responses during initial contact with microbes. A major aim of future research efforts will be to identify the biochemical mechanism of amino acid sensing in plants, including the downstream signaling cascades, but also to unravel the metabolic interactions between plants and microbes (see Outstanding questions). There will most likely be substantial overlap with metabolic adaptations in plants during the interaction with multicellular organisms such as nematodes or mycorrhizal fungi [2,95]. Also, the different organisms interacting with an individual plant compete for the allocation of resources and trigger specific response patterns with reciprocal effects [96]. Thus, integrating advances in these research areas will contribute to a holistic understanding of plant biotic interactions.

Outstanding questions

Due to recent advances in the field, some amino-acid-derived metabolites involved in shaping the root microbiome in arabidopsis and maize have been identified. Are they also the main players in other plant species, and are there additional important compounds?

Which of the 20 GLRs are relevant for plant–microbe interactions? Which amino acids activate them, and what are the components of the downstream signaling cascades?

Are there additional amino acid receptors in plants that are involved in signaling processes during interaction with microbes?

Which amino acid transporters are important for providing nutrients to microbes? How are they regulated, and can they be highjacked by microbes?

Which amino acids are involved in immune signaling? What is the role of intracellular compartments in amino acid signaling?

How do microbes manipulate the amino acid homeostasis of their host plants? Are there effectors that specifically target amino acid metabolism to create an ideal nutrient composition for proliferation?

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Declaration of interests

No interests are declared.

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