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Amino Acid Catabolism in Plants

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ABSTRACT

Amino acids have various prominent functions in plants. Besides their usage during protein biosynthesis, they also represent building blocks for several other biosynthesis pathways and play pivotal roles during signaling processes as well as in plant stress response. In general, pool sizes of the 20 amino acids differ strongly and change dynamically depending on the developmental and physiological state of the plant cell. Besides amino acid biosynthesis, which has already been investigated in great detail, the catabolism of amino acids is of central importance for adjusting their pool sizes but so far has drawn much less attention. The degradation of amino acids can also contribute substantially to the energy state of plant cells under certain physiological conditions, e.g. carbon starvation. In this review, we discuss the biological role of amino acid catabolism and summarize current knowledge on amino acid degradation pathways and their regulation in the context of plant cell physiology.

Keywords: adenosine triphosphate, amino acid catabolism, carbon starvation, energy metabolism, enzyme regulation

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INTRODUCTION

Plant cells contain low levels of protein in comparison with animal cells mainly because of the high amount of carbohydrate (cellulose and others) that compose most of a plant's structure. However, the importance of proteins and amino acids, the building blocks for proteins, cannot be overlooked. Besides their role as protein constituents, amino acids are also involved in a plethora of cellular reactions and therefore they influence a number of physiological processes such as plant growth and development, intracellular pH control, generation of metabolic energy or redox power, and resistance to both abiotic and biotic stress (Moe, 2013; Watanabe et al., 2013; Zeier, 2013; Fagard et al., 2014; Galili et al., 2014; Häusler et al., 2014; Pratelli and Pilot, 2014). Furthermore, a role for amino acids during signaling in plants has recently been discussed (Szabados and Savoure, 2010; Häusler et al., 2014). As a consequence, one can expect that the regulation of amino acid catabolism involves a wide set of both general and specific regulators and shows significant differences among plant species, tissues, and developmental stages (Okumoto and Pilot, 2011; Tegeder, 2012; Galili et al., 2014; Häusler et al., 2014). It should be noted that the importance of protein metabolism has been revisited in recent years (Araújo et al., 2011; Nelson et al., 2014). The function of specific amino acids and their degradation has been extensively investigated in different plant organs (Glawischnig et al., 2001; Götz et al., 2007; Fait et al., 2008; Gu et al., 2010; Riebeseel et al., 2010; Kochevenko et al., 2011; Krüßel et al., 2014). However, our current knowledge concerning amino acid catabolism in general remains rather fragmented. In contrast, the regulation of amino acid biosynthesis has received considerable attention in the past, mainly in the context of biotechnological approaches aiming at increasing the concentration of essential amino acids in crop plants (Binder et al., 2007; Binder, 2010; Kirma et al., 2012; Miret and Munné-Bosch, 2014).

Here, we discuss the currently available information on amino acid catabolism and its biological multifaceted regulation, an overlooked aspect in plants in general. Several amino acids serve as precursors for the synthesis of secondary metabolites, e.g. glucosinolates can be produced from methionine, alanine, and branched-chain and aromatic amino acids (Halkier and Gershenzon, 2006). However, since we consider these reactions to be relevant for the synthesis processes rather than for the removal of the substrate amino acids, they are clearly beyond the scope of this review and are mentioned without further details. Particular emphasis is placed on recent studies involved in several aspects of plant metabolism, and how amino acid catabolism might be important not only during normal senescence but also in stress tolerance in land plants. In this context, we also discuss how the degradation of amino acids, which represents an association of carbon and nitrogen

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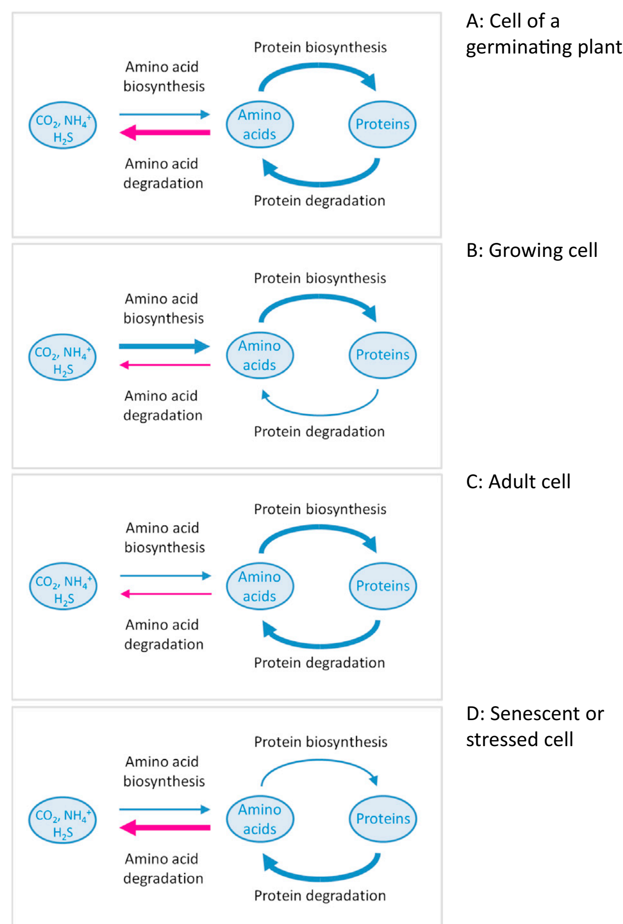

Figure 1. Amino Acid and Protein Biosynthesis and Degradation Are Differentially Regulated During Plant Cell Development.
Germination, which initially takes place in the absence of light, simultaneously requires protein and amino acid degradation as well as protein biosynthesis for the early plant (bold arrows). In growing and differentiating cells, amino acid and protein biosynthesis is especially high. In contrast, amino acids for protein biosynthesis largely can be provided by protein degradation in adult cells, at least in the absence of stress. During senescence, protein degradation and amino acid degradation is especially high. Similarly, this situation can also occur during carbon starvation or stress.

metabolism, may provide an energetic connection allowing plants to cope with suboptimal conditions. We anticipate that additional efforts using genetic engineering approaches may be able to improve the balance of synthesis and catabolism of amino acids, allowing the survival of plants under prolonged stress conditions as well as the biofortification of crop plants particularly with essential amino acids.

THE BIOLOGICAL ROLE OF PROTEIN CATABOLISM

The flux through amino acid catabolic pathways can be expected to change massively throughout the life cycle of a plant (Figure 1). During germination, which initially occurs in the absence of light, seed storage proteins are degraded to provide amino acids for the biosynthesis of the proteins required by the growing plant (Figure 1A). In addition, the energy demand of the young seedling has to be covered by amino acid oxidation and degradation of other storage compounds, such as fatty acids and starch, until the photosynthetic apparatus is fully functional (Galili et al., 2014).

In growing photosynthetically active cells, amino acid biosynthesis is up-regulated to provide substrates for the highly active protein synthesis (Figure 1B). Protein turnover and amino acid degradation are less important during this stage. In non-meristematic tissue, amino acids for protein biosynthesis can mainly be provided by protein turnover (Figure 1C), at least in the absence of stress. However, it also has to be considered that amino acids have several functions in addition to their role as a protein constituent, which require tightly controlled steady state levels. In fact, some amino acids (e.g., serine, proline, and leucine) have been shown to act as signaling molecules themselves and others are precursors for the synthesis of phytohormones or other secondary metabolites with signaling function (Hannah et al., 2010; Szabados and Savoure, 2010; Timm et al., 2012; Häusler et al., 2014; Ros et al., 2014). In addition, cysteine is very reactive and therefore toxic if it is allowed to accumulate above a certain level.

During senescence, nutrients are reallocated from the source leaves to sink tissues such as developing seeds, and therefore protein and amino acid degradation is especially high (Figure 1D; Watanabe et al., 2013). Similarly, in conditions leading to scarcity of amino acids in plants, proteins constitute reservoirs of amino acids that catabolic programs, such as proteasome-mediated degradation and autophagy, mobilize (Araújo et al., 2010). Amino acids are subsequently recycled and allocated for the synthesis of specific proteins required under nutrient limitation. Furthermore, during carbon starvation or the normal life cycle, proteins are degraded, and the complete oxidation of their amino acids produces the energy required to fuel the particular needs of certain organs (e.g. stressed leaves or roots). Hence, the accurate sensing of amino acid levels seems to be a key point for the efficient regulation of protein and amino acid synthesis and catabolism, as well as for the control of energy production. In this context, regulation of amino acid content, fluxes, and transport through the plant are critical for plant adaptation to carbon and nitrogen status, development, and defense (Zeier, 2013; Pratelli and Pilot, 2014). The molecular mechanisms underlying the regulation of plant amino acid catabolism are largely unknown but can be expected to be very complex.

AUTOPHAGY AND PROTEIN DEGRADATION

Large-scale nutrient recycling observed during several stages of a plant's life cycle (e.g. seed production, developmental and stress-induced senescence) is achieved by a process called autophagy (Lv et al., 2014; Avin-Wittenberg et al., 2015). Interestingly, autophagy has also been shown to be relevant for the vegetative phase of plant development, as it provides energy for growth during the night (Izumi et al., 2013). Cytosolic constituents and whole organelles are enclosed by autophagosomes and subsequently delivered to the vacuoles

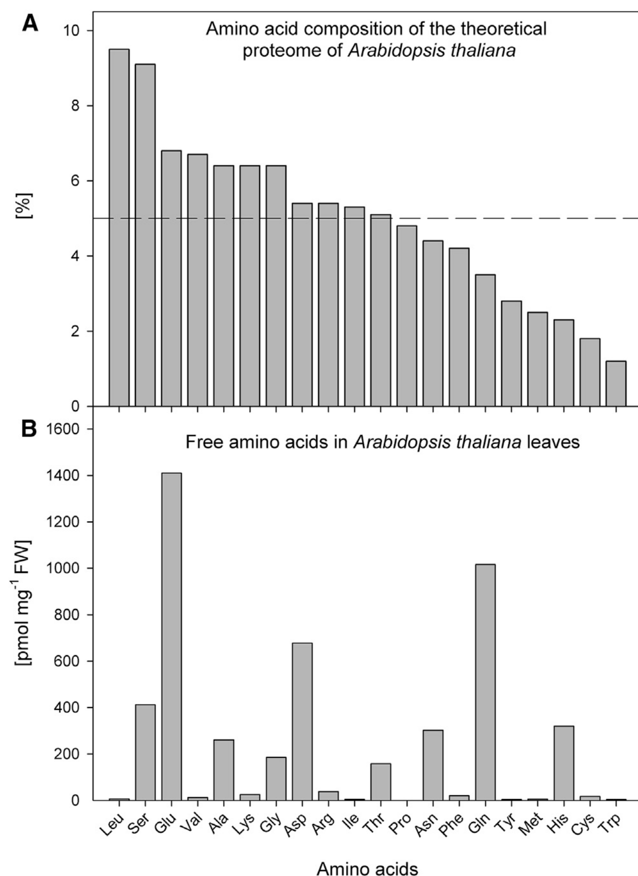

Figure 2. Protein-Bound and Free Amino Acids in *Arabidopsis thaliana*.

(A) Frequencies of amino acids in the theoretical proteome were calculated based on all protein sequences defined by the Arabidopsis TAIR10 protein database (ftp://ftp.arabidopsis.org/home/tair/proteins/).

(B) Concentrations of free amino acids are from Watanabe et al. (2013), Supplemental Table 4 (*Arabidopsis* Col0 cultivated on soil in a growth chamber under short-day light condition [8-h day, 140–160 mmol m^{-2} s^{-1}, 20°C; 16-h night, 16°C] for 14 days, transferred to a greenhouse for an additional growth period under short-day conditions [8-h day, 20°C; 16-h night, 16°C]. Material: base of the leaf; leaf harvested 4 h after onset of light).

for degradation (Avila-Ospina et al., 2014; Michaeli and Galili, 2014). The breakdown of proteins into amino acids or peptides is performed by different classes of proteases. Studies on *Arabidopsis thaliana* reported the predominant involvement of cysteine and serine proteases (Roberts et al., 2012) but also a role for the proteasome in the degradation of carbonylated proteins, which are accumulated during senescence (Jain et al., 2008). Notably, the genes coding for the major proteases involved in senescence-associated protein degradation are largely unknown (Distelfeld et al., 2014).

POOL SIZES OF PROTEIN-BOUND AND FREE AMINO ACIDS IN PLANTS

Considering the manifold functions of the individual amino acids, their pool sizes are of critical importance. Pool sizes of free amino acids not only depend on the ratio of amino acid biosynthesis and degradation but also on the biosynthesis and the breakdown of proteins (Figure 1). To understand more how this actually happens, we first asked: what is the interdependence of the pools of free amino acids on one hand and the pools of the protein-bound amino acids on the other? To answer this question, we need to consider that, if the total protein content of the leaf accounts for about 2% of the fresh weight, each individual protein-bound amino acid accounts, on average, for 0.1% of the leaf fresh weight. Considering a molecular mass of ~100 Da for an average amino acid, this corresponds to pool sizes of the protein-bound amino acids in the range of 10 nmol/mg fresh weight. In fact, pool sizes of the 20 amino acids only vary by less than a factor of 10. Within the theoretical proteome of *Arabidopsis thaliana*, leucine represents the most abundant amino acid (9.2%), while tryptophan is least abundant (1.2%; Figure 2A). Interestingly, the proportions of protein-bound amino acids of the theoretical proteome nicely correlate with the average proportions determined for plant-based foodstuff (Supplemental Figure 1).

By contrast, pools sizes of the free amino acids are much smaller and highly diverse. In expanding *Arabidopsis* leaves, concentrations of free amino acids vary between <5 pmol/mg fresh weight (tryptophan, tyrosine, and isoleucine) and >1000 pmol/mg fresh weight (glutamate) (Figure 2B; Watanabe et al., 2013). These large differences reflect the various functional roles of the individual amino acids. In leaves, glutamate, glutamine, aspartate, and asparagine are the primary products of nitrogen assimilation and therefore pools are large, especially in the light (Lam et al., 1995; Coruzzi, 2003). Furthermore, many amino acids represent precursors for the biosynthesis of other nitrogenous compounds such as nucleotides, phytohormones, or secondary metabolites. In addition, the serine content can be high under conditions of increased photorespiration. Finally, pools of all amino acids are much induced during stress. Proline is known to significantly increase during the stress response in several plants and considered to represent a compatible osmolyte (Verbruggen and Hermans, 2008; Szabados and Savoure, 2010; Jacoby et al., 2011). Moreover, branched-chain amino acids are also much induced during various stresses (Zhao et al., 1998; Joshi et al., 2010). A meta-study on the metabolic response of *Arabidopsis* to abiotic stresses revealed that lysine and threonine are much induced under several stress situations (Obata and Fernie, 2012). Furthermore, virtually all pools of free amino acids increase substantially during leaf senescence (Watanabe et al., 2013).

In summary, pools of free amino acids are on average 100- to 1000-fold smaller than the corresponding pools of protein-bound amino acids. At the same time, they may change dynamically and substantially in response to either environmental factors or developmental stages. Pool sizes of free amino acids are thus of great importance, not only because they are required for protein biosynthesis but also due to the numerous additional functions of amino acids for other metabolic pathways and in the frame of signal transduction processes. Precisely adjusted pool sizes of free amino acids are reached by the regulated interplay of amino acid and protein biosynthesis on one side and protein and amino acid degradation on the other (Figure 1).

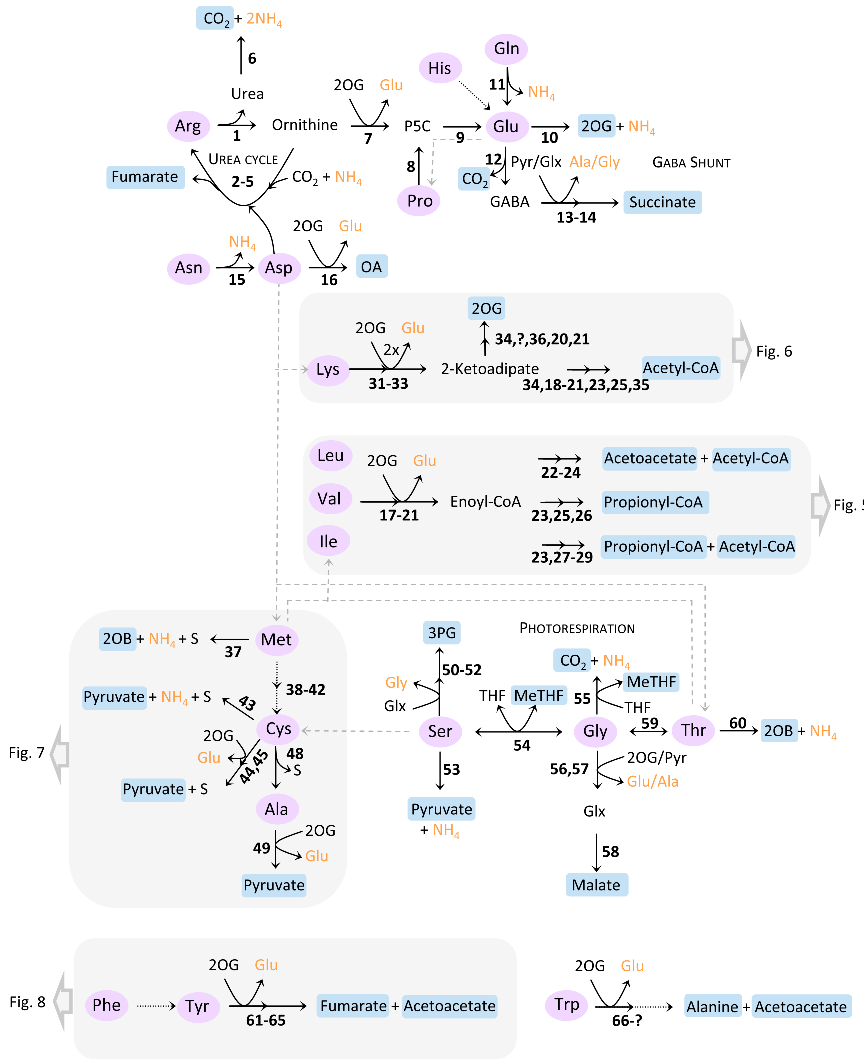

Figure 3. Amino Acid Catabolic Pathways in Plants.
Amino acids (highlighted in purple) are degraded to precursors or intermediates of the TCA cycle (highlighted in blue). The nitrogen-containing products are given in orange letters. Enzymatic steps are numbered from 1 to 66, and enzyme names can be found in Table 1 together with some further information. Dashed gray arrows indicate synthesis pathways of amino acids that require other amino acids as a precursor. Complex pathways involving several reaction steps are shown in detail in Figures 6–8 (indicated by the gray boxes). 2OB, 2-oxobutyrate; 2OG, 2-oxoglutarate; 3PG, 3-phosphoglycerate; GABA, γ-aminobutyric acid; Glx, glyoxylate; MeTHF, methylene tetrahydrofolate; OA, oxaloacetate; P5C, 1-pyrroline-5-carboxylate; Pyr, pyruvate; S, sulfur-containing product; THF, tetrahydrofolate.

PATHWAYS OF AMINO ACID CATABOLISM IN PLANTS

Complete degradation pathways for all 20 proteinogenic amino acids have been described in animals, and there are several alternative pathways present in prokaryotes (Bender, 2012; Nelson and Cox, 2013). In plants, corresponding knowledge so far is limited. Notwithstanding, several reaction steps involved in plant amino acid degradation have been described. Others can be postulated based on predicted enzymes identified by sequence homology in enzymes of other groups of organisms. Interestingly, some plant-specific variations are also apparent and are discussed below. Figure 3 shows an overview of our current knowledge of amino acid catabolic pathways in land plants. Enzymes are numbered from 1 to 66, and information such as *Arabidopsis* accession numbers, subcellular localization, experimental evidence, and expression characteristics is provided (Table 1 and Supplemental Table 1).

Step 1: Nitrogen Is Removed as Ammonium and Transferred to Storage Compounds

Nitrogen can be removed from amino acids by deamination reactions producing 2-oxoacids and ammonium. The mitochondrial matrix enzyme glutamate dehydrogenase (reaction 10) catalyzes the oxidative deamination of glutamate to 2-oxoglutarate and free ammonium transferring electrons to NAD^+ or $NADP^+$. Serine and threonine can also be directly deaminated by dehydratases (reactions 53 and 60), and the amino groups of methionine and cysteine are released as ammonium during the reactions performed by methionine-γ-lyase (reaction 37) and cysteine desulfhydrase (reaction 43; Alvarez et al., 2010; Fujitani et al., 2006; Goyer et al., 2007; Joshi et al., 2006; Rébeillé et al., 2006). In contrast to the reaction performed by glutamate dehydrogenase, these reactions are not linked to NADH production. Free ammonium is also produced during oxidative decarboxylation of glycine (reaction 55; Bauwe et al., 2010). The amino groups of the remaining amino acids, which cannot be directly deaminated, are transferred to 2-oxoglutarate producing glutamate (Liepman and Olsen, 2004). Several aminotransferases with different substrate specificities have been described (reactions 7, 13, 16, 17, 49, 50, 56, 57, 61, 66). However, the enzymes catalyzing transamination of cysteine (reaction 44) and α-aminoadipate (reaction 33) remain to be identified. Since the glutamate dehydrogenase shunt enables the netto release of carbon skeletons from several amino acids, it acts as a branch point between carbon and nitrogen metabolism. As such, it is particularly relevant for the maintenance of respiration during carbohydrate limitation (Fontaine et al., 2012).

If required for further metabolism, ammonium can be re-assimilated by glutamine synthase and incorporated into amino acids with high nitrogen content. In particular, asparagine, glutamine, and arginine are used as nitrogen storage and transport compounds (Miflin and Habash, 2002). In sink tissues, the amide groups of asparagine and glutamine and the guanidine group of arginine are hydrolyzed by asparaginase (reaction 15), glutaminase (reaction 11), and arginase (reaction 1),

respectively, to remobilize the nitrogen (Tambasco-Studart et al., 2007; Ivanov et al., 2012; Shi and Chan, 2013).

Step 2: Carbohydrates Are Remobilized to Sink Tissues or Oxidized during Respiration

The carbon skeletons of amino acids are generally converted to precursors or intermediates of the tricarboxylic acid (TCA) cycle. Thus, they contribute to mitochondrial metabolism and ATP production. In addition, the oxidation of some amino acids (leucine, isoleucine, valine, lysine, and proline) directly feeds electrons into the mitochondrial electron transport chain (Engqvist et al., 2009; Araújo et al., 2010; Schertl et al., 2014). In order to estimate the maximal energy yield that can be achieved from the oxidation of the individual amino acids, we counted all oxidation steps (Figure 4 and Table 2). NAD-dependent reactions can lead to the translocation of 10 protons from the mitochondrial matrix into the intermembrane space if electrons enter the respiratory chain at complex I (four protons) and are transferred to oxygen via complex III (four protons) and complex IV (two protons). Accordingly, when electrons are fed into the respiratory chain at the level of ubiquinone, the maximal number of translocated protons is six. This is the case for the FAD-dependent enzymes succinate dehydrogenase and proline dehydrogenase (reaction 8), as well as for isovaleryl-CoA dehydrogenase (reaction 19) and D-2-hydroxyglutarate dehydrogenase (reaction 36), which transfer electrons to ubiquinone via the electron-transfer flavoprotein/electron-transfer flavoprotein oxidoreductase system (reactions 20 and 21). ATP production was then estimated assuming that three protons are required for the synthesis of each ATP molecule plus one additional proton for the ADP/ATP antiport across the inner mitochondrial membrane (Table 2). The number of ATPs resulting directly from substrate-level phosphorylation in the TCA cycle was added. Energy yield ranges from 2.5 ATP for glycine up to 34 ATP for tyrosine, which is comparable to the oxidation of glucose as a substrate. Our results show that ATP production from branched-chain amino acids (BCAA) and lysine catabolism is particularly high (Table 2). Remarkably, carbohydrate starvation induces these pathways exactly, and mutants of enzymes associated show an accelerated senescence phenotype under extended darkness (Supplemental Table 1; Däschner et al., 2001; Ishizaki et al., 2005; Araújo et al., 2010). The physiological relevance of amino acids as alternative respiratory substrates has also been demonstrated during less severe stress conditions experienced by most plants at some stage during their life cycle, such as drought or short light periods (Araújo et al., 2010; Engqvist et al., 2011; Krüßel et al., 2014).

Aspartate, Alanine, Asparagine, and Glutamine

Catabolic pathways for some amino acids can be very short. For instance, transamination of aspartate produces oxaloacetate (reaction 16), and alanine is directly converted to pyruvate by alanine aminotransferases (reaction 49). Also, asparagine and glutamine are metabolized to aspartate and glutamate, respectively (reactions 15 and 11). Others are rather more complicated and are discussed in the following sections.

Arginine

Arginine is used for nitrogen storage and transport in many plants. Thus, mobilization of nitrogen from source tissues requires arginine degradation, and the enzymes involved are induced during senescence and germination (Witte, 2011). Arginine is hydrolyzed in the mitochondria by arginase (reaction 1) producing urea and ornithine. Further hydrolysis of urea catalyzed by cytosolic urease (reaction 6) yields ammonia and carbamate, which rapidly decays non-enzymatically forming a second molecule of ammonia and carbon dioxide. Urease is the only nickel-containing enzyme known in plants and requires three accessory proteins for activation (Witte et al., 2005). Complete degradation of arginine proceeds by transamination of ornithine to 1-pyrroline-5-carboxylate (P5C; reaction 7) and subsequent oxidation to glutamate via glutamic semialdehyde by $NAD(P)^+$dependent 1-pyrroline-5-carboxylate dehydrogenase (reaction 9) in the mitochondrial matrix (Funck et al., 2008). Alternatively, for nitrogen recycling, ornithine can be transferred to the plastids and re-enter arginine synthesis (reactions 2–5; Slocum, 2005). These reactions are equivalent to the urea cycle, which eliminates excess nitrogen in animals. Indeed, urea strongly accumulates during leaf senescence and is used for long-distance nitrogen transport in the phloem, indicating a similar function in plants (Bohner et al., 2015). After decarboxylation, arginine and ornithine are also metabolized to polyamines such as putrescine, spermidine, and spermine (Alcazar et al., 2006).

Proline

Proline acts as an osmolyte and a chemical chaperone and is therefore accumulated by plants under various stress conditions (Szabados and Savoure, 2010). Its catabolism takes place in the mitochondria and proceeds via two oxidation steps to glutamate. Proline dehydrogenase (reaction 8) converts proline to P5C, which is also produced by transamination of ornithine (see above), and transfers electrons to ubiquinone (Schertl et al., 2014). Oxidation of P5C to glutamate is then catalyzed by 1-pyrroline-5-carboxylate dehydrogenase (reaction 9).

Glutamate

As described above, the breakdown of glutamate can be achieved by oxidative deamination to 2-oxoglutarate (reaction 10). In addition, decarboxylation of glutamate by cytosolic glutamate decarboxylases (reaction 12) produces γ-aminobutyrate (GABA), which is transaminated to succinic semialdehyde (reaction 13) and oxidized to succinate by succinate semialdehyde dehydrogenase (reaction 14) in the mitochondria (reviewed by Shelp et al., 1999; Bouche and Fromm, 2004; Fait et al., 2008; Michaeli and Fromm, 2015). The so-called GABA shunt bypasses two steps of the TCA cycle and carries a high metabolic flux in illuminated leaves, whereas the classical TCA cycle is prevalent during heterotrophic plant metabolism at night (Michaeli and Fromm, 2015). The physiological significance of GABA, which accumulates up to millimolar concentrations in certain tissues and under stress conditions, remains largely unclear. Several functions have been suggested, such as the regulation of cytosolic pH and osmolarity, defense against insects, and signaling (Shelp et al., 1999; Bouche and Fromm, 2004). Recent evidence from metabolome and transcriptome studies also revealed a close association of GABA with central metabolism under carbon limitation, indicating that it might play a role in coordinating carbon–nitrogen balance and even mediate a starvation response in plant cells (Batushansky et al., 2014; Michaeli and Fromm, 2015).

Pathway	No.	Description	Accession numbers	Localization
Arg/Pro	1	Arginase	AT4G08870, AT4G08900	mi
	2	Carbamoyl-phosphate synthase	AT1G29900, AT3G27740	pl
	3	Ornithine carbamoyltransferase	AT1G75330	pl
	4	Argininosuccinate synthase	AT4G24830	pl
	5	Argininosuccinate lyase	AT5G10920	pl
	6	Urease + accessory proteins	AT1G67550, AT2G35035, AT1G21840, AT2G34470	cy
	7	Ornithine aminotransferase	AT5G46180	mi
	8	Proline dehydrogenase	AT3G30775, AT5G38710	mi
	9	δ-1-Pyrroline-5-carboxylate dehydrogenase	AT5G62530	mi
Glu	10	Glutamate dehydrogenase	AT5G18170, AT5G07440, AT3G03910	mi
Gln	11	Glutaminase	AT5G60540	cy
	12	Glutamate decarboxylase	AT5G17330, AT1G65960, AT2G02000, AT2G02010, AT3G17760	cy
	13	GABA transaminase	AT3G22200	mi
	14	Succinate semialdehyde dehydrogenase	AT1G79440	mi
Asn/Asp	15	Asparaginase	AT5G08100, AT3G16150	cy, pe
	16	Aspartate aminotransferase	AT2G30970, AT4G31990, AT2G30970, AT2G22250, AT1G62960, AT4G31990, AT5G11520, AT5G19550, AT2G22250, AT1G62800, AT1G62800	pl, mi, pe, cy
BCAA	17	Branched-chain amino acid transaminase	AT1G10060, AT1G10070, AT3G49680, AT3G19710, AT5G65780, AT1G50110, AT3G05190, AT5G27410, AT1G50090	mi, pl, cy
	18	Branched-chain α-keto acid dehydrogenase complex	AT5G09300, AT3G13450, AT3G06850, AT3G17240, AT1G48030, AT1G21400, AT1G55510	mi
	19	Isovaleryl-CoA-dehydrogenase	AT3G45300	mi
	20	Electron transfer flavoprotein (ETF)	AT1G50940, AT5G43430	mi
	21	ETF:ubiquinoneoxidoreductase	AT2G43400	mi
	22	Methylcrotonyl-CoA carboxylase	AT1G03090, AT4G34030	mi
	23	Enoyl-CoA hydratase	AT1G76150, AT4G29010, AT4G16800, AT4G16210	pe, mi
	24	Hydroxmethylglutaryl-CoA lyase	AT2G26800	mi
	25	3-Hydroxyacyl-CoA dehydrogenase	AT3G15290, AT4G29010, AT3G06860	pe
	26	3-Ketoacyl-CoA thiolase	AT2G33150, AT5G48880, AT1G04710	pe, mi
	27	Hydroxyacyl-CoA hydrolase	AT5G65940, AT1G06550, AT2G30650, AT2G30660, AT3G60510, AT4G31810, AT4G13360	pe, mi
	28	3-Hydroxyisobutyrate dehydrogenase	AT4G20930, AT4G29120	mi
	29	Methylmalonate-semialdehyde dehydrogenase	AT2G14170	mi
	30	Acyl-CoA oxidase	AT4G16760, AT5G65110, AT1G06290, AT3G51840, AT2G35690, AT1G06310	pe
Lys	31	Lysine-ketoglutarate reductase/saccharopine dehydrogenase	AT4G33150	cy
	32	Aldehyde dehydrogenase 7B4	AT1G54100	cy
	34	2-Oxoglutarate dehydrogenase complex	AT5G65750, AT3G55410, AT3G17240, AT4G26910, AT5G55070, AT1G48030	mi
	35	Acetyl-CoA acetyltransferase	AT5G48230, AT5G47720, AT5G48230	cy
	36	D-2-Hydroxyglutarate dehydrogenase	AT4G36400	mi

Table 1. List of Enzymes Involved in Amino Acid Catabolism.

(Continued on next page)

Pathway	No.	Description	Accession numbers	Localization
Met	37	Methionine γ-lyase	AT1G64660	cy
	38	*S*-Adenosylmethionine synthase	AT1G02500, AT4G01850, AT2G36880, AT3G17390	cy, nu
	39	*S*-Adenosylmethionine-dependent methyltransferase	AT5G38780, AT5G38100, AT5G37970, AT5G37990	cy
	40	Adenosylhomocysteinase	AT4G13940, AT3G23810	cy
	41	Cystathionine β-synthase homolog	AT4G14880, AT3G22460	cy
	42	Cystathionine γ-lyase homolog	AT3G57050, AT3G01120	pl
	43	Cysteine desulfhydrase	AT5G28030, AT1G48420, AT3G26115	cy, mi
	45	Sulfurtransferase	AT1G79230, AT1G16460, AT4G01050, AT5G66040, AT5G66170, AT3G25480, AT1G09280, AT2G40760, AT1G17850, AT2G42220, AT3G08920, AT5G19370, AT4G24750, AT5G55130, AT4G27700, AT4G35770, AT2G17850, AT2G21045	cy, mi, pl, nu, er
Cys	46	Sulfur dioxygenase	AT1G53580	mi
	47	Sulfite oxidase	AT3G01910	pe
	48	Cysteine desulfurase	AT5G65720, AT1G08490, AT3G62130	mi, pl, cy
Ala	49	Alanine aminotransferase	AT1G17290, AT1G72330	mi
Gly/Ser/ Thr	50	Serine-glyoxylate aminotransferase	AT2G13360	pe
	51	Glycerate dehydrogenase	AT1G79870, AT1G68010, AT1G12550	cy, mi, pe
	52	D-Glycerate kinase	AT1G80380	cy
	53	Serine dehydratase	AT4G11640	cy
	54	Serine hydroxymethyltransferase	AT4G37930, AT4G32520, AT4G13930, AT5G26780, AT1G22020, AT1G36370, AT4G13890	mi, pl, cy
	55	Glycine cleavage system	AT4G33010, AT1G32470, AT2G35370, AT2G35120, AT2G26080, AT1G11860, AT3G17240, AT1G48030	mi
	56	Alanine-glyoxylate aminotransferase	AT2G38400, AT4G39660, AT3G08860	mi
	57	Glutamate-glyoxylate aminotransferase	AT1G23310, AT1G70580	pe
	58	Malate synthase	AT5G03860	pe
	59	Threonine aldolase	AT1G08630, AT3G04520	cy
	60	Threonine dehydratase	AT3G10050	pl
Aromatic	61	Tyrosine aminotransferase	AT4G23600, AT5G53970, AT5G36160	cy
	62	4-Hydroxyphenylpyruvate dioxygenase	AT1G06570	cy
	63	Homogentisate 1,2-dioxygenase	AT5G54080	cy
	64	Maleylacetoacetate isomerase	AT2G02390	cy
	65	Fumarylacetoacetase	AT1G12050, AT3G16700, AT4G15940	cy, mi
	66	Tryptophan aminotransferase	AT1G70560, AT1G23320, AT4G24670, AT1G34060, AT1G34040	cy, va

The enzymes are numbered according to Figures 3–8. Subcellular localizations were taken from SUBA3 or, if available, from experimental evidence in the literature. cy, cytosol; er, endoplasmic reticulum; mi, mitochondrion; nu, nucleus; pe, peroxisome; pl, plastid; va, vacuole. Additional information is available in Supplemental Table 1.

Table 1. *Continued*

Histidine

Histidine is metabolized to glutamate by four enzymatic steps in animals (histidine ammonia-lyase, urocanatehydratase, imidazole propionase, and formimidoylglutamase). However, to our knowledge, this pathway has not yet been investigated at all in plants.

Leucine, Isoleucine, and Valine

BCAA catabolism is rather complex and has not been completely unraveled in plants (Binder, 2010; Araújo et al., 2011). The initial steps in the degradation pathways of leucine, isoleucine, and valine are identical (Figure 5). First, branched-chain 2-oxoacids are produced by transamination (reaction 17). Seven different isoforms of BCAA transaminase have been identified in *Arabidopsis* so far, localized in different compartments. They also catalyze the final step in BCAA synthesis, and the mitochondrial isoform BCAT2 has been shown to be especially relevant for degradation (Angelovici et al., 2013). The oxidative decarboxylation of α-ketoacids producing acyl-CoAs is catalyzed by the branched-chain α-ketoacid dehydrogenase

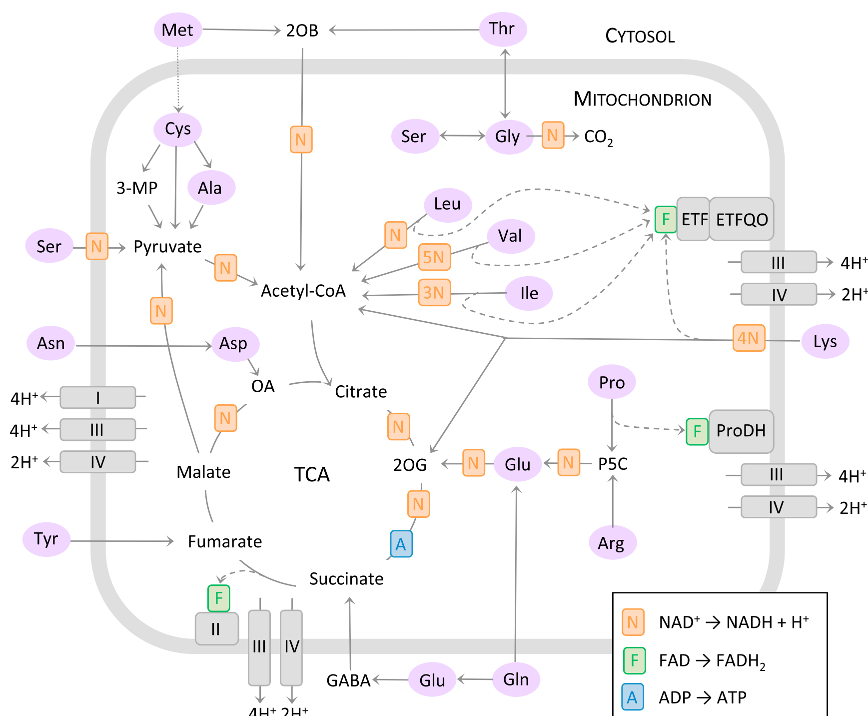

Figure 4. Subcellular Localization and Energy Yield of Amino Acid Catabolic Pathways.

Amino acids (highlighted in purple) are degraded to precursors or intermediates of the TCA cycle. Most reaction steps of the catabolic pathways occur either in the mitochondria or cytosol. Additional isoforms with different localizations have been omitted from this scheme for the sake of clarity but can be found in Table 1. Note that for threonine dehydratase, which converts Thr to 2OB, only a plastid localized isoform has been described so far (plastid not shown in the figure). In order to estimate the amount of ATP that can be produced from the degradation of the individual amino acids, oxidation steps are marked with an orange "N" for NAD-dependent dehydrogenases, and with a green "F" for FAD-dependent dehydrogenases. The blue "A" indicates ATP production via substrate-level phosphorylation. The number of protons translocated across the inner mitochondrial membrane by respiratory chain complexes I, III, and IV is also shown. A calculation of the possible energy yield from complete oxidation of the individual amino acids can be found in Table 2. 2OB, 2-oxobutyrate; 2OG, 2-oxoglutarate; 3MP, 3-mercaptopyruvate; ETF, electron-transfer flavoprotein; ETFQO, electron-transfer flavoprotein:ubiquinone oxidoreductase; GABA, γ-aminobutyric acid; OA, oxaloacetate; P5C, 1-pyrroline-5-carboxylate; ProDH, proline dehydrogenase; TCA, tricarboxylic acid cycle.

complex (reaction 18), which is functionally and structurally similar to pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase and shares the same E3 subunit (Mooney et al., 2002). Subsequently, isovaleryl-CoA dehydrogenase (reaction 19) oxidizes isovaleryl-CoA derived from leucine catabolism, but probably also 2-methylbutanoyl-CoA and 2-methylpropanoly-CoA from isoleucine and valine degradation, transferring the electrons into the mitochondrial electron transport chain at the level of ubiquinone via electron transfer flavoprotein (ETF, reaction 20) and ETF:ubiquinone oxidoreductase (ETFQO, reaction 21) (Däschner et al., 2001; Araújo et al., 2010). Leucine catabolism proceeds with a well-characterized carboxylation step catalyzed by biotin-containing methylcrotonyl-CoA carboxylase (reaction 22) to produce 3-methyl-glutaconyl-CoA (Alban et al., 1993; Anderson et al., 1998).

The subsequent steps can be inferred from homology to the animal system, but the respective enzymes and their physiological functions have not been analyzed in plants to date. 3-Methyl-glutaconyl-CoA as well as the enoyl-CoAs derived from isoleucine and valine catabolism are probably converted to hydroxyacyl-CoAs by enoyl-CoA hydratase (reaction 23). Afterward, the three pathways split. 3-Hydroxymethylglutaryl-CoA lyase (reaction 24) produces acetoacetate and acetyl-CoA in the final reaction of leucine degradation. The conversion of acetoacetate to acetyl-CoA is catalyzed by acetoacetyl-CoA synthase and acetyl-CoA acetyltransferase (reaction 35) in animals; however, for the first reaction, no plant homolog has been identified so far. Breakdown of 2-methyl-3-hydroxybutyryl-CoA derived from isoleucine catabolism to acetyl-CoA and propionyl-CoA requires two enzymatic steps: oxidation by 3-hydroxyacyl-CoA dehydrogenase (reaction 25) and thiolytic cleavage by 3-ketoacyl-CoA thiolase (reaction 26). 3-Hydroxyisobutyryl-CoA from valine oxidation is probably hydrolyzed to 3-hydroxyisobutyrate (reaction 27) followed by an oxidation step (reaction 28). Finally, methylmalonate-semialdehyde dehydrogenase (reaction 29) catalyzes the oxidative decarboxylation of methylmalonate semialdehyde yielding propionyl-CoA.

Breakdown of propionyl-CoA leads to acetyl-CoA in plants and thus requires a different set of enzymes than the propionyl-CoA carboxylase pathway, which converts propionyl-CoA to succinyl-CoA in animals. The enzymes involved in this pathway have not been identified so far. Interestingly, by using radioactive labeling, a sequence of reactions completely analogous to the conversion of isobutyryl-CoA to propionyl-CoA has been demonstrated for propionyl catabolism in peanut (*Arachis hypogaea*) mitochondria (Giovanelli and Mudd, 1971). Thus, it seems plausible that this final part of the pathway could be catalyzed by the same set of enzymes (reactions 23 and 27–29; see Figure 5), except isovaleryl-CoA dehydrogenase, which does not use propionyl-CoA as a substrate and therefore has to be replaced by a different acyl-CoA dehydrogenase (reaction 30) (Lucas et al., 2007). It is still a matter of debate whether the entire BCAA degradation pathway is localized in the mitochondria (Binder, 2010; Kochevenko et al., 2012). Some reaction steps are also required for peroxisomal fatty acid β oxidation, and localization of the involved enzyme isoforms is unclear.

Amino acid	NADH	$FADH_2$	Direct ATP	∑ATP[a]
Tyrosine	11	3	2	34
Leucine	10	4	1	32
Lysine	10	3	2	31.5
Isoleucine	9	3	2	29
Proline	8	3	1	25.5
Arginine	8	2	1	24
Valine	8	2	1	24
Glutamate, glutamine	7	2	2	22.5
Methionine, threonine	6	1	1	17.5
Aspartate, asparagine	4	1	1	12.5
Alanine, serine, cysteine	4	1	1	12.5
Glycine	1	0	0	2.5
Glucose	10	2	4	32

Table 2. Possible Energy Yield from Complete Oxidation of Amino Acids.

NADH/$FADH_2$, number of NAD/FAD molecules reduced during the pathway; direct ATP, ATP produced by substrate-level phosphorylation in the TCA cycle.

[a]Assuming four protons required per ATP.

Lysine

A complete degradation pathway for lysine has not yet been identified in plants (Figure 6). However, the initial steps are already well characterized (Azevedo and Arruda, 2010; Medici et al., 2015). Conversion of lysine to saccharopine and subsequently to 2-aminoadipic-6-semialdehyde is catalyzed by two functionally independent domains in the bifunctional cytosolic enzyme lysine-ketoglutarate reductase (LKR)/saccharopine dehydrogenase (SDH) (reaction 31). This enzyme is regulated at the post-translational level via calcium and phosphorylation and, together with the committed step in the synthesis pathway, controls lysine homeostasis (Arruda et al., 2000). Lysine can also be converted to L-pipecolate, a regulator of inducible plant immunity (Figure 6, dark gray box; Navarova et al., 2012). Since L-pipecolate is catabolized by oxidation to L-Δ^1-pipeidine-6-carboxylate, which spontaneously isomerizes to 2-aminoadipic-6-semialdehyde, this route has been suggested as a possible bypass of the LKR/SDH reaction during lysine degradation (Araújo et al., 2010; Engqvist et al., 2014).

The next steps in the catabolic pathway are still highly speculative (Figure 6, light gray box). Labeling studies in different crop species identified glutamate as a product of lysine degradation (Nigam and McConnell, 1963; Sodek and Wilson, 1970; Moller, 1976). This finding led to the conclusion that lysine is probably converted to acetyl-CoA as in animals, then further metabolized to 2-oxoglutarate in the TCA cycle and transaminated to glutamate (Moller, 1976). Interestingly, an alternative route, which directly produces 2-oxoglutarate via D-2-hydroxyglutarate dehydrogenase (reaction 36), has recently been demonstrated in plants (Engqvist et al., 2009; Araújo et al., 2010; Engqvist et al., 2011).

The conversion of 2-aminoadipic-6-semialdehyde to acetyl-CoA probably requires eight reaction steps. First, cytosolic aldehyde dehydrogenase 7B4 (reaction 32) catalyzes the oxidation to 2-aminoadipate, which is transaminated to 2-ketoadipate (reaction 33) and then oxidatively decarboxylated to glutaryl-CoA (reaction 34). Since this reaction is equivalent to the oxidative decarboxylation of 2-oxoglutarate in the TCA cycle, it might be catalyzed by the 2-oxoglutarate dehydrogenase complex. A specific glutaryl-CoA dehydrogenase has not been identified in plants. However, isovaleryl-CoA dehydrogenase has been shown to be involved in lysine catabolism (Araújo et al., 2010) and could catalyze the oxidation of glutaryl-CoA to glutaconyl-CoA (reaction 19). In animals, glutaconyl-CoA metabolism then proceeds via crotonyl-CoA, 3-hydroxybutyryl-CoA, and acetoacetyl-CoA (reactions 23, 25, and 35; Moller, 1976). Interestingly, feeding experiments with isovaleryl-CoA dehydrogenase-deficient *Arabidopsis* mutants recently provided evidence for a slight modification of this reaction sequence in plants (Araújo et al., 2010). 2-Oxopentanoate (= α-ketovalerate) accumulated strongly indicating that the terminal carboxyl group of 2-ketoadipate is probably removed prior to the oxidation step catalyzed by isovaleryl-CoA dehydrogenase (Figure 6, left branch). However, enzymes catalyzing the decarboxylation of either glutaconyl-CoA or 2-ketoadipate have not yet been identified, and experimental evidence proving the functional significance for this part of the pathway in plants is still missing.

D-2-Hydroxyglutarate has been identified as an intermediate of lysine catabolism in plants, but the route of its production remains elusive (Engqvist et al., 2009; Araújo et al., 2010; Engqvist et al., 2011). One possibility to generate D-2-hydroxyglutarate from glutaryl-CoA would be a reaction sequence analogous to the conversion of succinyl-CoA to malate in the TCA cycle. Alternatively, D-2-hydroxyglutarate could be produced from glutaconyl-CoA by an unknown enzyme activity. Only the final reaction step in this alternative lysine catabolic pathway, oxidation of D-2-hydroxyglutarate to 2-oxoglutarate by D-2-hydroxyglutarate dehydrogenase (reaction 36), is well characterized (Engqvist et al., 2009, 2011). Electrons are transferred to the mitochondrial respiratory chain via the ETF/ETFQO complex (reactions 20 and 21).

Glycine, Serine, and Threonine

The aspartate family pathway synthesizes, through several different metabolic branches, lysine, threonine, methionine, and isoleucine (Azevedo et al., 2006). Furthermore, methionine and threonine are precursors of isoleucine synthesis, and cysteine is produced from serine (Figure 3, dashed lines). In plants, it is clear that this pathway is of high importance from a nutritional perspective given that it culminates with the synthesis of the four essential amino acids described above. However, these reactions are metabolically expensive and are not likely to play a role during amino acid catabolism. In contrast, threonine, glycine, and serine can be interconverted easily, so that the reactions shown (reactions 54 and 59) can be relevant for synthesis of the product amino acid as well as degradation of the substrate amino acid.

The complete degradation of glycine to carbon dioxide and ammonium is catalyzed by the mitochondrial multi-enzyme system glycine decarboxylase (reaction 55). A methyl group is transferred

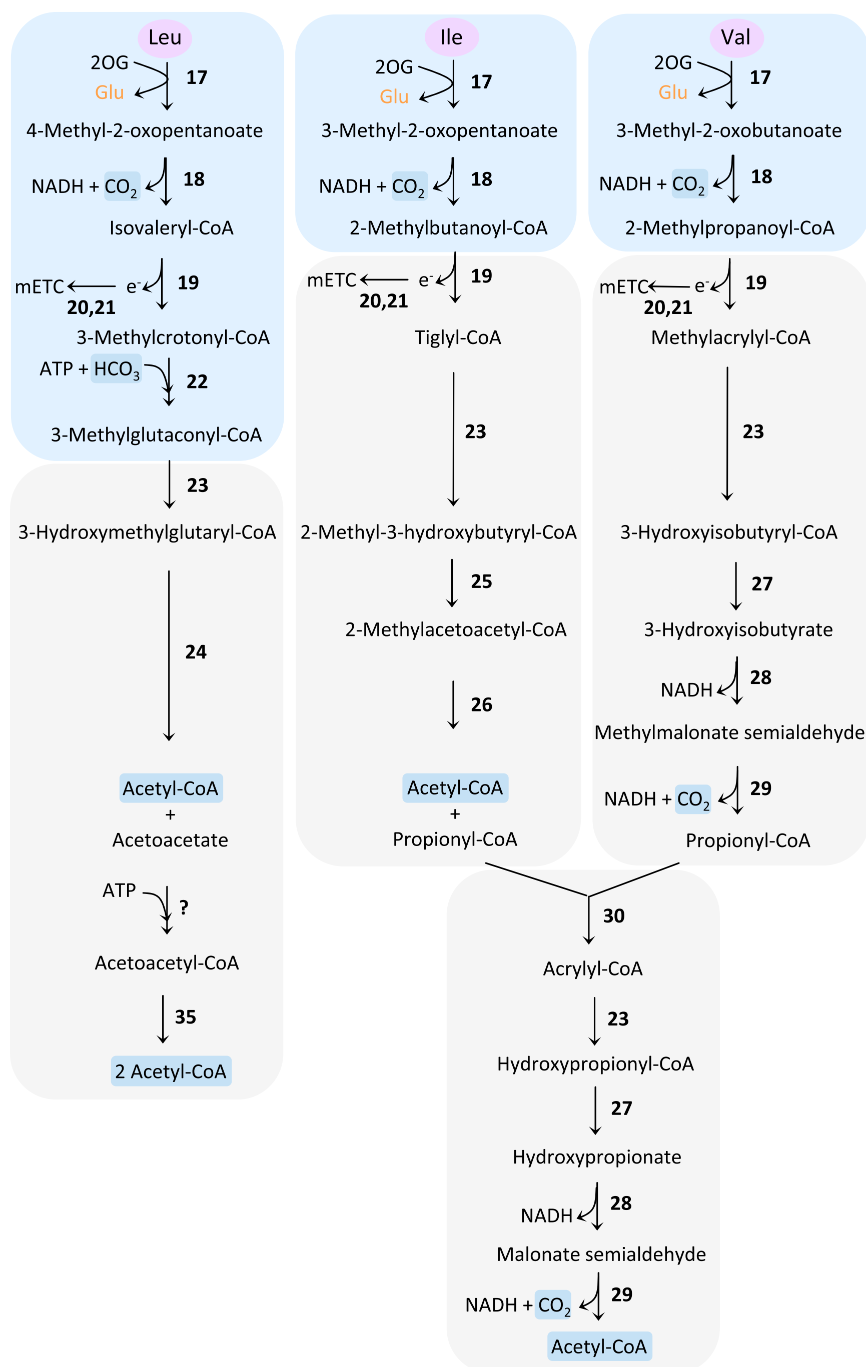

Figure 5. Branched-Chain Amino Acid Catabolism.
The degradation of leucine, isoleucine, and valine finally leads to acetyl-CoA and CO_2 (marked in blue). The amino groups are transferred to glutamate (orange letters). Enzymes catalyzing the initial steps of the three pathways have already been characterized in plants (indicated by the light blue boxes). Subsequent reactions (shown in gray boxes) have been deduced from homology and indirect experimental evidence, and further confirmation is still required. For enzyme names and further information please see Table 1 and Supplemental Table 1. 2OG, 2-oxoglutarate; e^-, electrons; mETC, mitochondrial electron transport chain.

to tetrahydrofolate and thus contributes to C1 metabolism. It is still a matter of debate whether the four enzymes involved in the reaction sequence known as P, H, L, and T protein form a stable complex (Oliver et al., 1990; Douce et al., 2001). The glycine cleavage system is required for photorespiration in C_3 plants and closely interacts with serine hydroxymethyltransferase (reaction 54), which methylates glycine to serine using methylene tetrahydrofolate as a cofactor (for further details, see Bauwe et al., 2010). Due to their role in photorespiration, these enzymes are strongly induced by light and can become the most abundant mitochondrial matrix proteins during the day (Douce et al., 2001). The photorespiratory cycle proceeds via transamination of serine to hydroxypyruvate catalyzed by serine-glyoxylate aminotransferase (reaction 50) and NADH-dependent reduction to glycerate (reaction 51) in the peroxisomes. Phosphorylation by glycerate kinase (reaction 52) in the plastids then generates 3-phosphoglycerate, which can re-enter the Calvin–Benson cycle or be reduced to glyceraldehyde 3-phosphate and used for glycolytic degradation or carbohydrate synthesis (Douce et al., 2001). The alternative route of serine degradation via pyruvate requires serine dehydratase activity, shown to be present in the cytosolic enzyme serine racemase (reaction 53; Fujitani et al., 2006). Glycine can also be transaminated by alanine-glyoxylate aminotransferase (reaction 56) or glutamate-glyoxylate aminotransferase (reaction 57), and the resulting glyoxylate can be acetylated in the peroxisomes to produce malate (reaction 58; Mazelis, 1980). However, whether this route is relevant for glycine catabolism has not been tested.

Threonine aldolase (reaction 59) catalyzes the reversible conversion of threonine to glycine plus acetaldehyde (Joshi et al., 2006). The strong (50-fold) accumulation of threonine in threonine aldolase 1 knockout mutants demonstrates its physiological role in threonine catabolism. The alternative reaction, deamination of threonine to 2-oxobutyrate (reaction 60), is part of the isoleucine synthesis pathway and therefore regulated by feedback inhibition. A threonine dehydrogenase, which is involved in threonine catabolism in animals and prokaryotes, has not yet been identified in plants.

Cysteine

The carbon skeleton of cysteine is converted to pyruvate via removal of the amino and sulfhydryl groups. So far, three different routes have been described for the catalysis of these reactions (Figure 7). Cytosolic cysteine desulfhydrase

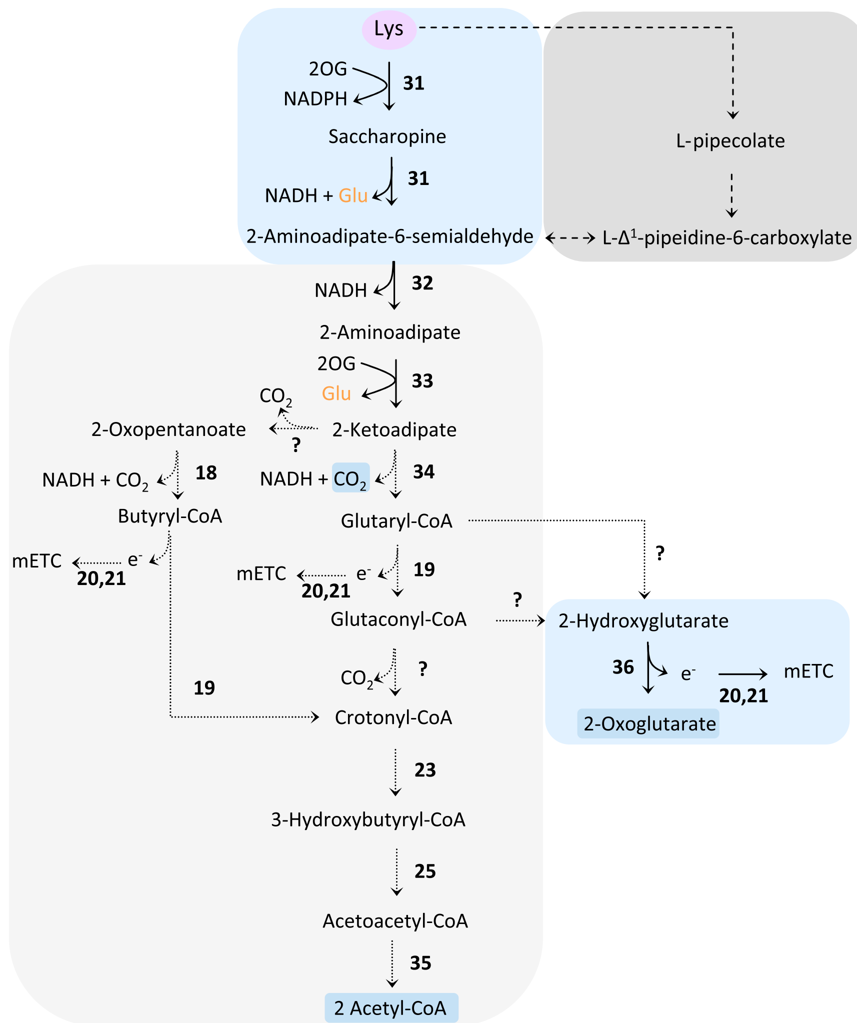

Figure 6. Lysine Catabolism.

Lysine can be catabolized to either acetyl-CoA or 2-oxoglutarate (marked in blue), and both amino groups are transferred to glutamate (orange letters). Individual reaction steps have already been extensively characterized in plants (indicated by the light blue boxes). However, the major part of the pathway (shown in the light gray box) still requires experimental confirmation, and some steps are highly speculative (dotted arrows). A potential bypass of the reaction catalyzed by the bifunctional enzyme lysine-ketoglutarate reductase/saccharopine dehydrogenase (reaction 31) via L-pipecolate is also indicated (dark gray box). For enzyme names and further information, please see Table 1 and Supplemental Table 1. 2OG, 2-oxoglutarate; e⁻, electrons; mETC, mitochondrial electron transport chain.

(reaction 43) deaminates cysteine producing ammonia, pyruvate, and hydrogen sulfide. Knockout of this enzyme leads to an accumulation of cysteine indicating a function in cysteine homeostasis (Alvarez et al., 2010). In addition, alterations in autophagosome formation and stomatal opening were detected, which could be reversed by exogenous supplementation with hydrogen sulfide (H_2S). Thus, cysteine desulfhydrase also regulates production of the signaling molecule H_2S (Alvarez et al., 2012; Jin et al., 2013). A D-cysteine-specific desulfhydrase is localized in the mitochondria and has also been implicated in cysteine catabolism (Riemenschneider et al., 2005). All isoforms of this enzyme are induced during senescence, suggesting a role during nutrient mobilization. Reduced sulfur for the synthesis of iron–sulfur clusters, biotin, and thiamin is further produced by cysteine desulfurases, also known as NifS-like proteins (reaction 48). Different isoforms are present in the mitochondria, cytosol, and plastids. However, since cysteine desulfurases exclusively transfer the sulfur to iron–sulfur cluster scaffold proteins, they are most likely not relevant for cysteine catabolism (Couturier et al., 2013). An additional mitochondrial pathway catalyzing complete degradation of cysteine begins with the transamination to 3-mercaptopyruvate by a presently unknown aminotransferase (reaction 44; Krüßel et al., 2014). A sulfurtransferase (reaction 45) then transfers the sulfhydryl group to glutathione, and the resulting glutathione persulfide is oxidized to sulfite by the sulfurdioxygenase ETHE1 (reaction 46). Sulfite is either converted to thiosulfate by addition of a persulfide group (reaction 45) or transferred to the peroxisomes and oxidized to sulfate by sulfite oxidase (reaction 47). ETHE1 is highly induced by carbohydrate starvation, and mutants show premature senescence under light-limiting growth conditions indicating that this pathway is functionally significant for the use of amino acids as an energy source.

Methionine

A significant amount of methionine is converted to *S*-adenosylmethionine (SAM) by *S*-adenosylmethionine synthase (reaction 38; Amir, 2010). SAM serves as a methyl donor in transmethylation reactions and is a precursor for the synthesis of ethylene, biotin, and polyamines. Methylation of methionine results in *S*-methylmethionine (SMM), a possible storage and transport form of methionine. Both metabolites can be recycled back to methionine. Methionine feeding leads to an increase in SAM and SMM levels, and a reduction in the expression level of *S*-adenosylmethionine synthase significantly increases methionine levels, showing that this pathway is also relevant for methionine homeostasis (Rébeillé et al., 2006; Amir, 2010). However, it is doubtful whether the reverse transsulfuration pathway (reactions 39–42), which converts SAM to cysteine during methionine catabolism in animals, also takes place in plants (Figure 7). The first two steps, transfer of the methyl group to an acceptor (reaction 39) and cleavage of the adenosyl group (reaction 40), are required for the recycling of SAM to methionine in the methyl cycle and clearly occur in plants. Cystathionine-β-synthase (reaction 41) and cystathionine-γ-lyase (reaction 42) subsequently convert homocysteine to cysteine in animals, and homologs of both genes are present in the *Arabidopsis* genome (Supplemental Table 1). However, earlier studies found only limited capacity to metabolize methionine to

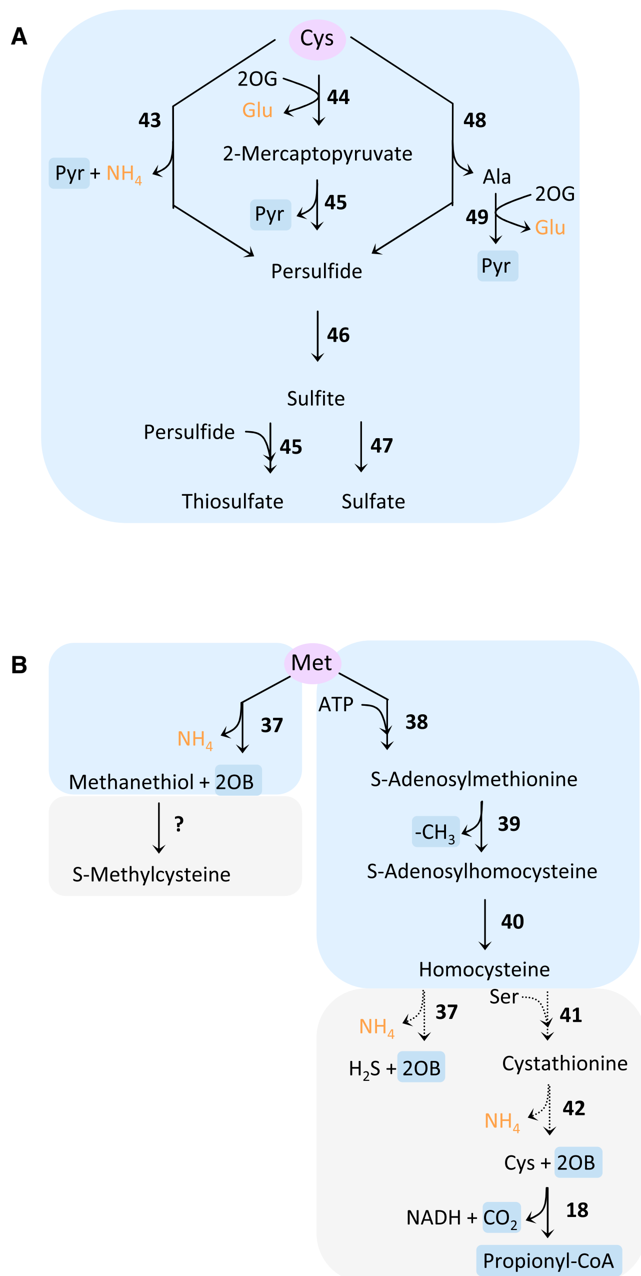

Figure 7. Sulfur Amino Acid Catabolism.
(A) Cysteine catabolism. Three different routes have been described that convert cysteine to pyruvate (marked in blue). The amino group is either released as ammonium or transferred to glutamate (orange letters). The thiol group is oxidized to thiosulfate or sulfate.
(B) Methionine catabolism. Methionine can be converted to methanethiol and 2-oxobutyrate or to homocysteine via *S*-adenosylmethionine, and the respective enzymes are known in plants (indicated by the light blue boxes). The subsequent steps shown in gray boxes are still rather speculative. Products of the carbon skeleton are highlighted in blue and the nitrogen-containing products are given in orange letters. For enzyme names and further information, please see Table 1 and Supplemental Table 1. 2OB, 2-oxobutyrate; 2OG, 2-oxoglutarate; pyr, pyruvate.

cysteine in *Spinacia oleracea* (spinach) and *Lemna paucicostata* (duckweed) (Giovanelli and Mudd, 1971; Datko and Mudd, 1984).

An alternative pathway for methionine degradation that is not present in animals has been identified in plants, (Rébeillé et al., 2006; Goyer et al., 2007). Methionine-γ-lyase (reaction 37) converts methionine to 2-oxobutyrate, ammonium, and methanethiol in the cytosol. 2-Oxobutyrate can be used as a precursor for isoleucine synthesis or degraded via oxidation to acetyl-CoA. The further fate of methanethiol is not yet entirely clear. Part of it is probably emitted as a volatile compound, and the remaining is converted to *S*-methylcysteine, which could play a role as a storage compound (Rébeillé et al., 2006). However, given that these results have been obtained by feeding an *Arabidopsis* cell suspension culture with 2 mM methionine in the presence of sucrose, they might rather be a detoxification reaction of excess methionine. Recombinant methionine-γ-lyase also has high activity with homocysteine as a substrate (Goyer et al., 2007), indicating that complete degradation of methionine could proceed alternatively via this route (Figure 7). A physiological role of methionine-γ-lyase for the remobilization of sulfur under sulfate limitation and in seed production has been shown in *Arabidopsis* (Goyer et al., 2007; Joshi and Jander, 2009).

Tyrosine

Dixon and Edwards (2006) demonstrated that the complete degradation pathway of tyrosine, known from the mammalian system (Figure 8), is also functionally present in *Arabidopsis*. Transamination of tyrosine (reaction 61) produces 4-hydroxyphenylpyruvate, which is converted to homogentisate by 4-hydroxyphenylpyruvate dioxygenase (reaction 62). In plants, homogentisate is the precursor of tocopherols such as vitamin E and plastoquinones. Catabolism proceeds via oxidative ring cleavage catalyzed by homogentisate-1,2-dioxygenase (reaction 63). The product maleylacetoacetate is isomerized to fumarylacetoacetate by glutathione-*S*-transferase zeta 1 (reaction 64), which has maleylacetoacetate isomerase activity. The final step in tyrosine degradation is the hydrolysis of fumarylacetoacetate to fumarate and acetoacetate (reaction 65). Thus, an association of tyrosine and fumarate metabolism inside and outside the mitochondria should be considered.

Phenylalanine

Phenylalanine is hydroxylated to tyrosine prior to degradation in animals. However, the catabolic pathway for phenylalanine in plants remains largely elusive since no phenylalanine hydroxylase homolog has been found in the *Arabidopsis* genome. Furthermore, evidence from feeding experiments with different species of higher plants indicates that phenylalanine is not degraded to any great extent (Mazelis, 1980). However, it is important to mention that a unique folate-dependent phenylalanine hydroxylase localized in chloroplasts has been identified in non-flowering plants (Pribat et al., 2010).

Tryptophan

Studies on tryptophan metabolism in plants have focused on its role as a precursor for the synthesis of diverse secondary metabolites such as auxin, phytoalexins, glucosinolates, and alkaloids (Radwanski and Last, 1995). Although research efforts on herbicides have allowed significant advances in the understanding of the synthesis of aromatic amino acids (Tzin and Galili, 2010; Maeda and Dudareva, 2012), to our

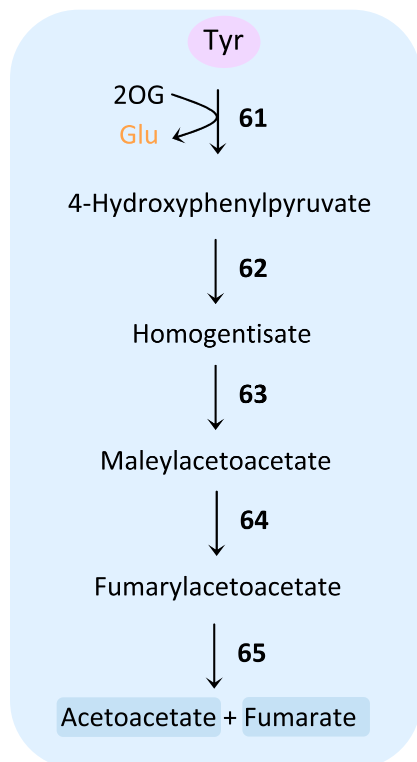

Figure 8. Tyrosine Catabolism.
The complete pathway oxidizing tyrosine to acetoacetate and fumarate (marked in blue) has been fully characterized in plants. The amino group is transferred to glutamate (orange letters). For enzyme names and further information, please see Table 1 and Supplemental Table 1. 2OG, 2-oxoglutarate.

knowledge, the degradation pathway of tryptophan has not been studied in plants so far.

SUBCELLULAR COMPARTMENTALIZATION OF AMINO ACID CATABOLIC PATHWAYS

Amino acid synthesis takes place mainly in the chloroplasts, where reducing power as well as ATP can be provided directly. In contrast, most reactions of the catabolic pathways are localized in the mitochondria or the cytosol (Table 1). From a metabolic perspective, this localization makes sense, since on the one hand the carbohydrate skeletons are converted to substrates of the TCA cycle. On the other hand, amino acid catabolism is highly relevant for the senescence process, and chloroplasts are degraded very early in this process, whereas mitochondria remain fully functional until the end (Avila-Ospina et al., 2014). For some steps (e.g. reactions 25 and 30 from BCAA oxidation or reactions 57 and 58 from glycine metabolism), transfer of the intermediates into the peroxisomes might be necessary, since the enzymes involved are also required for peroxisomal pathways such as fatty acid β-oxidation and the glyoxylate cycle. However, dual localization of individual enzymes or isoforms is also likely and has to be tested experimentally.

REGULATION OF AMINO ACID CATABOLISM

Local amino acid concentrations depend on the synthesis and degradation rates of proteins as well as amino acids (Figure 1) and on transport processes. Protein synthesis and degradation are obviously not suitable to control the level of individual amino acids. These processes rather modify the total pool size, which is especially relevant when bulk degradation takes place (e.g. during germination and senescence) leading to a massive increase in free amino acid concentrations (see the section on Pool Sizes of Protein-Bound and Free Amino Acids in Plants). However, protein synthesis is energetically expensive and complex. Accordingly, cells must sense intracellular amino acid pools to couple their abundance to their usage. Amino acid transport is relevant for the distribution of nitrogen within the plant and is regulated mainly at the transcriptional level. However, a potential regulatory protein, GDU1, has also been described recently (Okumoto and Pilot, 2011; Tegeder, 2012). To maintain normal metabolic function, homeostasis of individual amino acids has to be tightly regulated due to their specific functions in signaling, stress response, and the production of secondary metabolites. Also, during senescence or carbohydrate starvation, steady state levels of amino acids change dramatically with a relative increase in branched-chain and aromatic amino acids, which can be used most efficiently as alternative respiratory substrates (Table 2). In this regard, synthesis and degradation rates of individual amino acids are probably the most relevant parameters for adjusting their concentrations.

Less and Galili (2008) concluded from bioinformatics analysis of a large microarray dataset that free amino acid concentrations are regulated by a combination of transcriptional and post-translational control. Amino acid biosynthesis is mainly modulated via allosteric feedback inhibition of individual enzymes by the end product, and therefore transcript levels are relatively stable. In contrast, transcription of catabolic enzymes changes considerably in response to different stress conditions. Interestingly, genes of the BCAA degradation pathway also show an oscillating diurnal transcription pattern with induction during the night, when they are required for energy metabolism (Peng et al., 2015). Some details are already available on transcription factors involved in the induction of proline catabolic enzymes during darkness and stress relief (Szabados and Savoure, 2010). As already described, the first enzyme of lysine catabolism (LKR/SDH, reaction 31) is a clear exception to this regulation pattern, since it is extensively regulated via post-translational modifications (Arruda et al., 2000).

Labeling experiments using $^{13}CO_2$ revealed that *de novo* biosynthesis rates of amino acids largely differ (Szecowka et al., 2013; Ishihara et al., 2015). While alanine was labeled rapidly (>70% after 1 h labeling pulse), phenylalanine, glycine, and serine were only moderately and several others (e.g., glutamate, threonine, lysine, leucine, isoleucine, proline, and asparagine) were very slowly (<10% after 1 h) labeled. It can be anticipated that labeling rates for individual amino acids in addition to their biosynthesis rates depend on their pool size, their frequencies within the proteome, and their turnover rates, as well as cellular protein biosynthesis and degradation rates.

It is also important to highlight that a growing body of evidence suggests that there is not always a strict correlation between transcript levels and changes in the abundance of the encoded proteins (Bourgis et al., 2011; Baerenfaller et al., 2012; Fernie and Stitt, 2012). This apparent discordance is not only due to limitations in the analysis procedures for each component but can also be explained by dynamic rates of protein turnover (Fernie and Stitt, 2012), which makes understanding the regulation of protein and amino acid catabolism a rather exciting research field. Recent research into plant metabolism has often included the usage of modeling and correlation-based approaches (Fukushima et al., 2011; Arnold et al., 2015; Toubiana et al., 2015). Since protein and amino acid degradation pathways are involved in a complex metabolic network, integration of the results obtained using different post-genomic techniques will be important for this research field in the future. Notably, when revisiting amino acid catabolism, we realized that a large-scale comparison of relevant transcript abundance (encoding specific enzymes and metabolite transporters) with metabolic fluxes has yet to be made. Given the recognized importance of amino acids, this is rather surprising and highlights that further examination of the catabolic enzymes (described above) should provide significant insights into an integrated overview of the metabolic connections of the catabolic pathways of amino acids in higher plants.

PERSPECTIVES

The last decade has seen a significant increase in our knowledge of the role that amino acids and their breakdown products play in virtually every aspect of plant metabolism. These novel insights into the biochemical pathways underpinning metabolic regulation raise the exciting prospect that these mechanisms can be manipulated for biotechnological benefit. Amino acid biosynthesis is often of pivotal importance in several ways. The current challenge is to elucidate the diverse mechanisms by which amino acid catabolizing pathways are regulated, interacting with other pathways and influencing plant physiology at the whole-plant level and affecting stress responses. This is no small task, for as highlighted earlier, amino acid catabolism influences plant cell behavior in a myriad of ways. However, the identification of major response elements involved in the system (i.e. mTOR, autophagy, senescence-related genes, amino acid catabolites) represents a promising target for future studies on the signaling function of several amino acids. This approach would not only shed light on the question whether several amino acids are signaling molecule in plants but it would also help to understand how growth and development is regulated by metabolite signals.

As discussed earlier, a combination of post-genomic approaches (transcriptome, proteome, and metabolome) for analyses of mutant, transgenic, and wild-type plants will help to unravel detailed mechanisms of amino acid signaling. These technologies have already aided our understanding of amino acid catabolism at a systems level through additions to the parts list of the metabolic network. However, they are poised to become more useful still through the discovery of new interactions and the provision of the quantitative data required for the construction of predictive models of whole metabolism in land plants (Fukushima et al., 2011; Arnold et al., 2015). So far, neither interaction partners nor *cis* or *trans* elements of amino acid-triggered gene regulation have been identified. Deciphering these signatures and understanding the downstream signaling cascades governing amino acid catabolism remains a great challenge for future research.

SUPPLEMENTAL INFORMATION

Supplemental Information is available at *Molecular Plant Online*.

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