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Amino acid catabolism in plants

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Contents

Zusammenfassung

Aminosäuren erfüllen diverse Funktionen im pflanzlichen Stoffwechsel sowohl als funktionelle Gruppen in Proteinen als auch in ihrer freien Form. Der Fokus dieser kumulativen Habilitationsschrift liegt auf dem Verständnis des katabolen Stoffwechsels von Aminosäuren. Noch immer sind nicht alle biochemischen Stoffwechselwege, die zum Abbau der 20 proteinogenen Aminosäuren in Pflanzen nötig sind, vollständig aufgeklärt. Auch die Annotation des Aminosäurestoffwechsels in Datenbanken und Stoffwechselkarten ist unvollständig und teilweise fehlerhaft, sodass eine umfassende und aussagekräftige Auswertung von Datensätzen aus Proteom- und Transkriptomexperimenten nur schwer möglich ist. Ein grundlegender Beitrag dieser Arbeit liegt daher in der Bereitstellung einer manuell zusammengestellten Übersichtskarte, die das Netzwerk der 136 bisher bekannten Reaktionsschritte aus dem Aminosäurestoffwechsel im Zusammenhang darstellt. Dieser Ansatz zeigt die bestehenden Wissenslücken auf und identifiziert Kandidaten für bisher unbekannte Reaktionen als Ausgangspunkt für weitere Untersuchungen. Darüber hinaus ermöglicht die Auswertung neuer und auch bereits publizierter „Omics“-Datensätze auf der Basis der erweiterten Aminosäurestoffwechselkarte Erkenntnisse zu den unterschiedlichen metabolischen Funktionen des Aminosäureabbaus und ihrer Bedeutung in der pflanzlichen Stressantwort.

Ein weiterer Schwerpunkt dieser Habilitationsschrift ist der Cysteinkatabolismus. Cystein enthält neben der Aminogruppe auch eine Thiolgruppe und verbindet somit Kohlenstoff-, Stickstoff- und Schwefelmetabolismus. Beim Abbau von Cystein wird reduzierter Schwefel in Form von Sulfid oder Persulfiden freigesetzt, die als Signalmoleküle fungieren und durch Oxidation oder Einbau in neue Cysteinmoleküle entfernt werden können. Die vorliegende Arbeit beschreibt einen Cysteinabbauweg, der in der Mitochondrienmatrix lokalisiert ist und die Thiolgruppe durch die Schwefeldioxygenase ETHE1 oxidiert. Dieser Stoffwechselweg ist essentiell für die pflanzliche Embryonalentwicklung und spielt außerdem eine Rolle im Energiestoffwechsel bei Lichtmangelbedingungen. Beim Menschen verursachen Mutationen im ETHE1-Gen die tödlich verlaufende Stoffwechselstörung Ethylmalonsäure Encephalopathie. Sulfid akkumuliert zu toxischen Konzentrationen und führt zu schnell fortschreitenden Nekrosen im Gehirn, chronischem Durchfall und peripheren Durchblutungsstörungen. Diese Symptome können durch eine kombinierte Behandlung mit einem Antibiotikum, das die Sulfidfreisetzung durch Darmbakterien unterdrückt, und einem Vorläufer für die Glutathionsynthese als Puffer für reduzierte Schwefelverbindungen gelindert werden.

Abstract

Amino acids are among the most versatile compounds in plants performing diverse functions within proteins as well as individually. The publications constituting this habilitation thesis focus on different aspects of amino acid catabolism. Not all of the biochemical pathways catalyzing degradation of the twenty proteinogenic amino acids in plants are known, and annotation of amino acid catabolic enzymes in databases and pathway maps is still insufficient for comprehensive evaluation of proteomics or transcriptomics datasets. Thus, a manually assembled pathway map covering 136 reactions involved in amino acid related pathways is presented and used for reevaluation of already published as well as interpretation of new omics datasets. This approach clearly defines gaps in the present knowledge and at the same time postulates candidate proteins for unknown reactions as a starting point for further investigation. In addition, it provides insight into the different metabolic functions of amino acid catabolism and their significance during abiotic and biotic stress response.

A major topic of the work presented here is cysteine catabolism. Cysteine in addition to the amino group also contains a thiol moiety and therefore represents an intersection of carbon, nitrogen and sulfur metabolism. The degradation of cysteine via different enzymatic routes releases reduced sulfur in the form of sulfide or persulfide, which act as signaling molecules and can be further metabolized either by reincorporation into cysteine or by oxidation. This thesis describes a mitochondrial cysteine catabolic pathway that oxidizes the thiol group catalyzed by the sulfur dioxygenase ETHE1. In plants, this pathway is essential during early embryo development and also required for energy metabolism during low light availability. Mutations in the ETHE1 gene in humans cause the metabolic disease ethylmalonic encephalopathy, which is characterized by rapidly progressive encephalopathy, chronic diarrhea, and peripheral microangiopathy caused by accumulation of toxic sulfide levels. These symptoms can be relieved by combined treatment with a bactericide that represses sulfide production by intestinal anaerobes and N-acetylcysteine as a precursor for glutathione, which can act as a buffer for reduced sulfur.

PART A:

Amino acid catabolism in plants – Synopsis

1. Introduction

Amino acids are the building blocks of proteins but in addition they have several physiological functions in plant metabolism and not all of them have even been discovered yet. Amino acid metabolism is relevant for successful plant development and it is closely connected to resistance against biotic as well as abiotic stress. Amino acids are the transport and storage form for organic nitrogen within the plant and they provide precursors for a diverse set of secondary metabolites and metabolic signals. As a consequence, disturbances in amino acid homeostasis can lead to germination defects, premature senescence, and constitutive activation of defense pathways or even be embryo lethal. A detailed knowledge of plant amino acid metabolism is important for fully understanding these physiological processes. It will also contribute to improving different aspects of crop production such as achieving a suitable amino acid composition of food for human nutrition and feedstock, breeding stress and pathogen resistant plants or developing biostimulants to protect crop plants from adverse environmental conditions.

2. Amino acid catabolic pathways and their integration into amino acid metabolism

Despite its central role in plant metabolism, annotation of amino acid related pathways in databases and biochemical pathway maps (such as Mapman, KEGG, BioCyc) is not complete and at present insufficient for comprehensive evaluation of datasets generated by proteomics or transcriptomics approaches. While all enzymes involved in *de novo* synthesis of the 20 proteinogenic amino acids from inorganic carbon, nitrogen, and sulfur compounds are known, there are still many gaps concerning amino acid degradation as well as their conversion to secondary metabolites. In order to provide a sound basis for correct interpretation of existing datasets as well as for identification of interesting future research aims I manually assembled a pathway map covering amino acid catabolism in Arabidopsis on the basis of publications, biochemical databases and sequence homology [8[1]]. An extended version of this map also including amino acid synthesis pathways as well as the initial steps leading to secondary metabolism can be used for analyzing the general direction of the complete system of amino acid related pathways under a particular condition of interest (Fig. 1, Table 1) [3].

[1] Numbers in parentheses refer to publications that are part of this habilitation thesis (Part B).

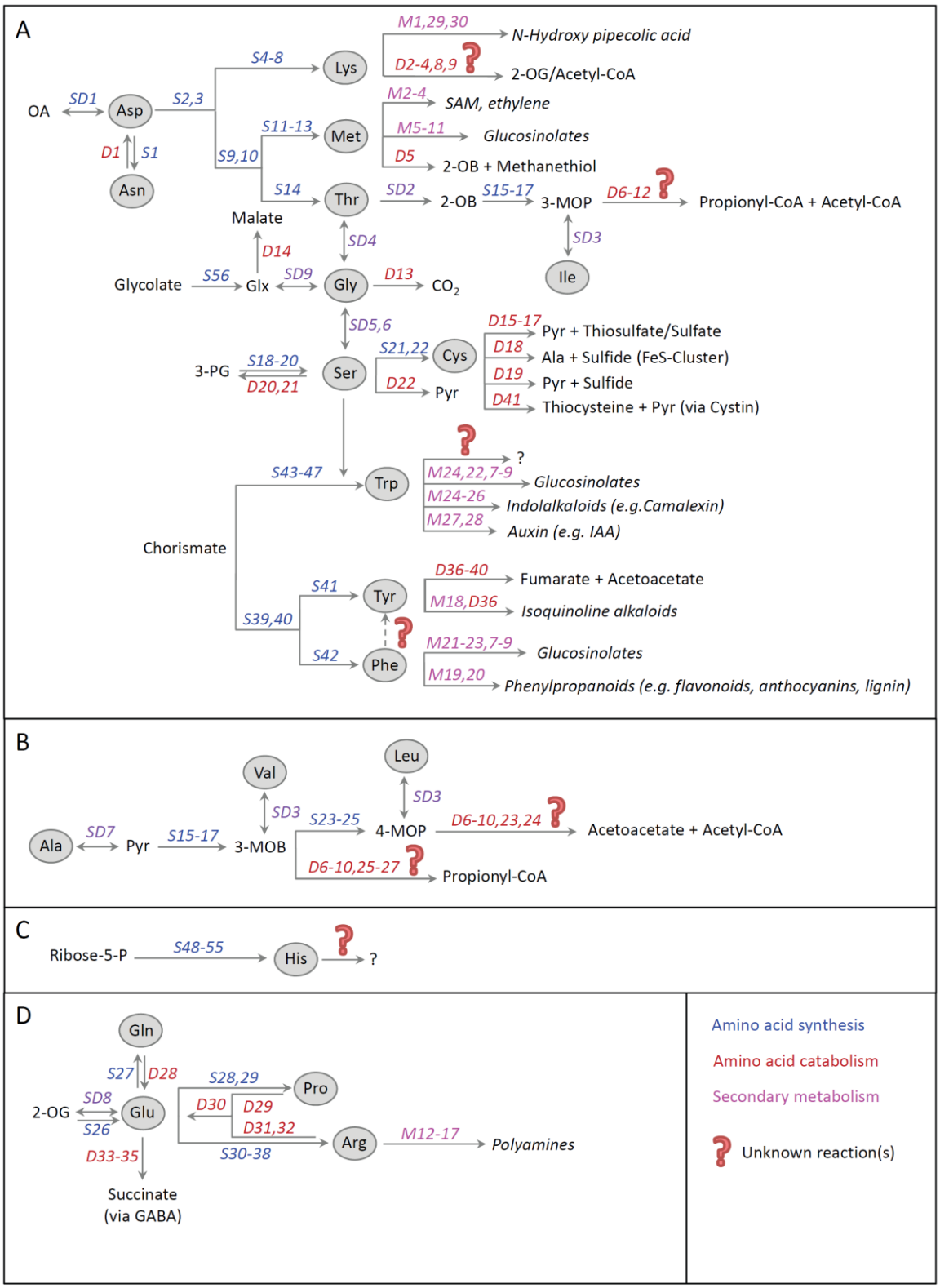

Fig. 1: Amino acid metabolic pathways in plants

Scheme of all currently known reaction steps involved in amino acid synthesis and degradation pathways as well as of the enzymes catalyzing committed steps leading to the synthesis of amino acid derived secondary metabolites (shown in italics). Arrows leading to amino acids represent the synthesis pathways (enzymes S1-S56), arrows pointing away from the amino acid show catabolic reactions (enzymes D1-D41), and metabolites that can be interconverted by a single set of enzymes are connected by arrows pointing in both directions (enzymes SD1-SD9). Initial steps of secondary metabolite synthesis are numbered M1-M30. Pathways and metabolites marked by a question mark are presently unknown. Enzymes are listed in Table 1. 2-OB, 2-oxobutyrate; 2-OG, 2-oxoglutarate; 3-MOB, 3-methyl-2-oxobutanoate; 3-MOP, 3-methyl-2-oxopentanoate; 3-PG, 3-phosphoglycerate; 4-MOP, 4-methyl-2-oxopentanoate; GABA, γ-aminobutyric acid; Glx, glyoxylate; IAA, indole-3-acetic acid; OA, oxaloacetic acid; Pyr, pyruvate; SAM, S-adenosyl methionine. (From [3])

Table 1: **Enzymes involved in amino acid metabolism in Arabidopsis.**
List of all currently known enzymatic steps involved in amino acid synthesis (numbers S1-S56), amino acid degradation (numbers D1-D41), reversible reactions (numbers SD1-SD9), and initial steps of pathways catalyzing the synthesis of secondary metabolites using amino acids as precursors (numbers M1-M30). The corresponding pathway map is shown in Figure 1. BCAA, branched-chain amino acids; DH, dehydrogenase; GLS, glucosinolates; SAM, S-adenosylmethionine. (From [3])

Pathway	Number	Description	Accession
Synthesis			
Asp/Asn	S1	Asparagine synthase	AT3G47340, AT5G65010, AT5G10240
Lys	S2	Monofunctional Asp kinase	AT3G02020, AT5G14060, AT5G13280
	S3	Asp semialdehyde DH	AT1G14810
	S4	Dihydrodipicolinate synthase	AT3G60880, AT2G45440
	S5	Dihydrodipicolinate reductase	AT2G44040, AT3G59890, AT5G52100
	S6	L,L-Diaminopimelate aminotransferase	AT4G33680
	S7	Diaminopimelate epimerase	AT3G53580
	S8	Diaminopimelate decarboxylase	AT3G14390, AT5G11880
Met	S9	Asp kinase/homoserine DH	AT1G31230, AT4G19710
	S10	Homoserine kinase	AT2G17265
	S11	Cystathionine-gamma-synthase	AT3G01120, AT1G33320
	S12	Cystathionine-beta-lyase	AT3G57050
	S13	Met synthase	AT5G17920, AT3G03780, AT5G20980
Thr	S9	Asp kinase/homoserine DH	AT1G31230, AT4G19710
	S3	Asp semialdehyde DH	AT1G14810
	S10	Homoserine kinase	AT2G17265
	S14	Thr synthase	AT1G72810, AT4G29840
BCAA	S15	Acetolactate synthase	AT2G31810, AT5G16290, AT3G48560
	S16	Ketol-acid reductioisomerase	AT3G58610
	S17	Dihydroxyacid dehydratase	AT3G23940
	S23	Isopropylmalate synthase	AT1G18500
	S24	Isopropylmalate isomerase	AT2G43100, AT4G13430, AT2G43090, AT3G58990, AT1G74040
	S25	3-Isopropylmalate DH	AT5G14200, AT1G80560, AT1G31180
Glu	S26	Glutamate synthase	AT5G04140, AT2G41220, AT5G53460
Gln	S27	Glutamine synthase	AT5G37600, AT1G66200, AT3G17820
Pro	S28	D-1-Pyrroline-5-carboxylate synthetase	AT2G39800, AT3G55610
	S29	Pyrroline-5-carboxylate reductase	AT5G14800
Arg	S30	Carbamoylphosphate synthase	AT3G27740, AT1G29900
	S31	Amino acid acetyltransferase	AT2G22910, AT4G37670, AT2G37500
	S32	Acetylglutamate kinase	AT3G57560
	S33	N-acetyl-gamma-glutamyl-phosphate reductase	AT2G19940
	S34	Acetylornithine aminotransferase	AT1G80600
	S35	Acetylornithine deacetylase	AT4G17830, AT2G37500
	S36	Orn carbamoyltransferase	AT1G75330
	S37	Arginosuccinate synthase	AT4G24830
	S38	Argininosuccinate lyase	AT5G10920
Ser	S18	3-Phosphoglycerate DH	AT1G17745, AT3G19480, AT4G34200
	S19	Phosphoserine aminotransferase	AT4G35630, AT2G17630
	S20	3-Phosphoserine phosphatase	AT1G18640
Cys	S21	Serine acetyltransferase	AT1G55920, AT2G17640, AT3G13110, AT4G35640, AT5G56760
	S22	O-acetylserine (thiol) lyase	AT4G14880, AT2G43750, AT3G59760, AT3G04940, AT5G28020
Gly	S56	Glycolate oxidase	AT3G14420, AT3G14415, AT3G14150, AT3G14130, AT4G18360
His	S48	ATP-phosphoribosyl transferase	AT1G58080, AT1G09795
	S49	Histidine biosynthesis bifunctional protein hisIE	AT1G31860
	S50	HisN3	AT2G36230
	S51	Imidazole glycerol phosphate synthase hisHF	AT4G26900
	S52	Imidazoleglycerol-phosphate dehydratase	AT3G22425, AT4G14910

	S53	Histidinol-phosphate aminotransferase	AT5G10330, AT1G71920
	S54	Bifunctional phosphatase IMPL2	AT4G39120
	S55	Histidinol DH	AT5G63890
Tyr	S39	Chorismate mutase	AT3G29200, AT5G10870, AT1G69370
	S40	Prenate aminotransferase	AT2G22250
	S41	Arogenate DH	AT5G34930, AT1G15710
Phe	S39	Chorismate mutase	AT3G29200, AT5G10870, AT1G69370
	S40	Prenate aminotransferase	AT2G22250
	S42	Arogenate dehydratase	AT1G11790, AT3G07630, AT2G27820, AT3G44720, AT5G22630, AT1G08250
Trp	S43	Anthranilate synthase	AT1G25220, AT5G57890, AT5G05730, AT2G29690
	S44	Anthranilate phosphoribosyltransferase	AT5G17990
	S45	N-(5'-phosphoribosyl)anthranilate isomerase	AT1G07780, AT5G05590, AT1G29410
	S46	Indole-3-glycerol phosphate synthase	AT2G04400
	S47	Tryptophan synthase	AT4G02610, AT3G54640, AT5G54810, AT4G27070
Synthesis and catabolism			
Asp	SD1	Aspartate aminotransferase	AT2G30970, AT5G19550, AT5G11520, AT1G62800, AT4G31990, AT2G22250
BCAA	SD2	Threonine dehydratase	AT3G10050
	SD3	Branched-chain amino acid transaminase	AT1G10060, AT1G10070, AT3G49680, AT5G65780, AT1G50110, AT1G50090
Gly	SD4	Threonine aldolase	AT1G08630, AT3G04520
	SD5	Serine hydroxymethyltransferase	AT4G37930, AT5G26780, AT4G32520, AT4G13930, AT4G13890, AT1G22020, AT1G36370
	SD6	Serine-glyoxylate aminotransferase	AT2G13360
Ala	SD7	Alanine aminotransferase	AT1G17290, AT1G72330
Glu	SD8	Glutamate DH	AT5G18170, AT5G07440, AT3G03910
Gly/Ser/Thr	SD9	Alanine-glyoxylate aminotransferase	AT4G39660, AT2G38400, AT3G08860
	SD9	Glutamate-glyoxylate aminotransferase	AT1G23310, AT1G70580
Catabolism			
Arg/Pro	D31	Arginase	AT4G08900, AT4G08870
	D32	Ornithine aminotransferase	AT5G46180
	D29	Proline DH	AT3G30775, AT5G38710
	D30	Delta-1-pyrroline-5-carboxylate DH	AT5G62530
Gln	D28	Glutaminase	AT5G60540
	D33	Glutamate decarboxylase	AT5G17330, AT1G65960, AT2G02010, AT2G02000, AT3G17760
	D34	GABA transaminase	AT3G22200
	D35	Succinate semialdehyde DH	AT1G79440
Asn/Asp	D1	Asparaginase	AT5G08100, AT3G16150
BCAA	D6	Branched-chain alpha-keto acid DH	AT5G09300, AT1G21400, AT3G13450, AT1G55510, AT3G06850, AT1G48030, AT3G17240
	D7	Isovaleryl-CoA DH	AT3G45300
	D8	Electron transfer flavoprotein	AT1G50940, AT5G43430
	D9	Electron-transfer flavoprotein: ubiquinone oxidoreductase	AT2G43400
	D23	Methylcrotonyl-CoA carboxylase	AT1G03090, AT4G34030
	D10	Enoyl-CoA hydratase	AT1G76150, AT4G29010, AT4G16800, AT4G16210
	D24	Hydroxmethylglutaryl-CoA lyase	AT2G26800
	D11	3-hydroxyacyl-CoA DH	AT3G15290, AT4G29010, AT3G06860
	D12	3-ketoacyl-CoA thiolase	AT2G33150, AT1G04710, AT5G48880
	D25	3-hydroxyisobutyryl-CoA hydrolase	AT5G65940, AT2G30650, AT2G30660, AT3G60510, AT4G31810, AT4G13360, AT1G06550
	D26	3-hydroxyisobutyrate DH	AT4G20930
	D27	Methylmalonate-semialdehyde DH	AT2G14170
Lys	D2	Lysine-ketoglutarate reductase/ saccharopine DH	AT4G33150
	D3	Aldehyde DH 7B4	AT1G54100
	D4	D-2-hydroxyglutarate DH	AT4G36400
Met	D5	Methionine gamma-lyase	AT1G64660

Cys	**D15**	Sulfurtransferase	AT1G79230, AT1G16460, AT5G66040, AT5G66170
	D16	Sulfur dioxygenase ETHE1	AT1G53580
	D17	Sulfite oxidase	AT3G01910
	D18	Cysteine desulfurase NifS	AT5G65720, AT1G08490
	D19	Cysteine desulfhydrase	AT3G62130, AT5G28030, AT1G48420, AT3G26115
	D41	Cystine lyase	AT4G23600
Gly/Ser/Thr	**D20**	Glycerate DH	AT1G68010, AT1G79870, AT1G12550
	D21	D-glycerate kinase	AT1G80380
	D22	Serine dehydratase	AT4G11640
	D13	Glycine cleavage system	AT4G33010, AT2G26080, AT2G35370, AT2G35120, AT1G32470, AT1G11860, AT3G17240, AT1G48030
	D14	Malate synthase	AT5G03860
Tyr	**D36**	Tyrosine aminotransferase	AT5G53970, AT5G36160
	D37	4-hydroxyphenylpyruvate dioxygenase	AT1G06570
	D38	Homogentisate 1,2-dioxygenase	AT5G54080
	D39	Maleylacetoacetate isomerase,	AT2G02390
	D40	Fumarylacetoacetase	AT1G12050, AT3G16700, AT4G15940
Secondary metabolism			
Lys to pipecolic acid	**M1**	Lysine aminotransferase	AT2G13810
	M29	Dehydropipecolic acid reductase	AT5G52810
	M30	Flavin monooxigenase	AT1G19250
Met to SAM to ethylene	**M2**	S-adenosylmethionine synthase 1	AT1G02500, AT4G01850, AT2G36880, AT3G17390
	M3	1-aminocyclopropane-1-carboxylate synthase	AT1G01480, AT2G22810, AT5G65800, AT4G11280, AT4G26200, AT4G37770, AT3G49700
	M4	1-aminocyclopropane-1-carboxylate oxidase	AT2G19590, AT1G62380, AT1G12010, AT1G05010, AT1G77330
Met to GLS	**M5**	Monooxygenase	AT1G16410, AT1G16400
	M6	Monooxygenase	AT4G13770
	M7	C-S lyase	AT2G20610
	M8	UDP-glycosyltransferase	AT1G24100
	M9	Sulfotransferase	AT1G74100, AT1G18590, AT1G74090
	M10	Oxidase	AT1G62540, AT1G62560, AT1G62570
	M11	Dioxygenase	AT4G03060, AT4G03050
Arg to polyamines	**M12**	Arginine decarboxylase	AT2G16500, AT4G34710
	M13	Agmatine deiminase	AT5G08170
	M14	N-carbamoylputrescine amidohydrolase	AT2G27450
	M15	Adenosylmethionine decarboxylase	AT3G02470, AT5G15950, AT3G25570, AT5G18930
	M16	Spermidine synthase	AT1G23820, AT1G70310
	M17	Spermine synthase	AT5G53120
Tyr to alkaloids	**M18**	Tyrosine decarboxylase	AT2G20340, AT4G28680
	D32	Tyrosine aminotransferase	AT5G53970, AT5G36160
Phe to propanoids	**M19**	Phenylalanine ammonia lyase	AT2G37040, AT3G53260, AT5G04230, AT3G10340
	M20	Trans-cinnamate 4-monooxygenase	AT2G30490
Phe to GLS	**M21**	Phenylalanine N-monooxygenase	AT5G05260
	M22	Monooxygenase	AT4G13770,
	M23	Gamma-glutamyl-peptidase	AT4G30530, AT4G30540, AT4G30550, AT2G23960, AT2G23970
	M7	S-alkyl-thiohydroximate lyase	AT2G20610
	M8	UDP-glycosyltransferase	AT1G24100, AT2G43840
	M9	Sulfotransferase	AT1G74100, AT1G18590, AT1G74090
Trp to GLS	**M24**	Trp monooxygenase	AT4G39950, AT2G22330
	M22	Monooxygenase	AT4G31500
	M7	S-alkyl-thiohydroximate lyase	AT2G20610
	M8	UDP-glycosyltransferase	AT1G24100, AT2G43840
	M9	Sulfotransferase	AT1G74100, AT1G18590, AT1G74090
Trp to phytoalexin (camalexin)	**M24**	Trp monooxygenase	AT4G39950, AT2G22330
	M25	Indoleacetaldoxime dehydratase	AT2G30770
	M26	Bifunctional dihydrocamalexate synthase/camalexin synthase	AT3G26830
Trp to auxin	**M27**	Tryptophane aminotransferase	AT1G70560, AT1G23320, AT4G24670, AT1G34060, AT1G34040
	M28	Indole 3 pyruvate monooxygenase	AT4G32540, AT4G13260, AT1G04610, AT5G11320, AT5G43890, AT5G25620, AT2G33230, AT4G28720, AT1G04180, AT1G48910

Amino acid and carbohydrate metabolism are tightly linked. Amino acids are synthesized mainly in chloroplasts from intermediates of the Calvin cycle by a set of highly coordinated and tightly regulated pathways (Pratelli and Pilot 2014). The amino group is provided by assimilation of inorganic nitrogen (nitrate or ammonium) into glutamine and asparagine (Masclaux-Daubresse et al. 2010). Sulfur is primarily assimilated into cysteine, which acts as a sulfur donor for methionine and a large variety of additional organic and inorganic sufur compounds (Takahashi et al. 2011). Major groups of amino acids also called families are derived from aspartate and glutamate and additional building blocks are provided by pyruvate and the shikimate pathway (Fig. 1). Amino acid synthesis is mainly regulated on a post-translational level via feedback inhibition of comitted reaction steps (Pratelli and Pilot 2014).

Amino acid degradation results in either precursors or intermediates of the TCA cycle so that the carbon skeleton can be used for mitochondrial ATP production and gluconeogenesis to fulfill the respective needs of the plant. These aspects will be discussed in more detail in section 3. Degradation pathways for all 20 proteinogenic amino acids have been described in animals and prokaryotes (Bender 2012, Nelson & Cox 2013). Many reactions are also present in plants and others can be postulated based on amino acid sequence homology although experimental evidence is still lacking [8]. There are also some plant specific modifications in individual reaction steps.

Amino acid degradation usually starts with the removal of the amino group which can be achieved either by deamination producing 2-oxoacids and ammonium or by transamination. Many aminotransferases use 2-oxoglutarate as an amino group acceptor and produce glutamate, which in turn can be oxidatively deaminated by glutamate dehydrogenase. Glutamate therefore acts as a central branch point between carbon and nitrogen metabolism mediating net nitrogen release from several different amino acids. If required, the ammonium can be reassimilated by glutamine synthase and incorporated into amino acids used for nitrogen storage and transport such as asparagine, glutamine, and arginine.

The degradation pathways of several amino acids are very short. A single transamination reaction for example converts alanine to pyruvate (SD7) or aspartate to oxaloacetate (SD1). Glutamine, arginine, and proline are synthesized from glutamate and can be degraded back to glutamate in three reaction steps at most (Fig. 1D). Threonine, glycine and serine are rapidly interconverted by reactions SD4, 5 and 6 and catabolism is achieved in one step, respectively (SD2, D13, D22). During degradation of methionine and cysteine the fate of the sulfur group also has to be taken into account. Methionine-γ-lyase (D5) produces methanethiol that can be emitted as a gas but is probably also further metabolized in the plant by some presently unknown reaction. Cysteine oxidation is catalyzed by different pathways (D15-19, D41) leading to either sulfide or thiosulfate, and these reactions will be discussed in detail in section 2.1.

The aromatic amino acids, lysine, histidine, and the branched-chain amino acids have a more complex structure and require several degradation steps before they can be integrated into carbohydrate metabolism. Some of these pathways (histidine, tryptophan, phenylalanine) are still completely unknown in plants and for lysine and the branched-chain amino acids only partial reaction sequences have been published so far. Since those of the enzymatic steps that are already known are highly induced during different environmental stress conditions degradation of the complex amino acids might be particularly relevant for stress tolerance (see also section 3.3). We detected many of the established and candidate enzymes in a complexome profile of Arabidopsis mitochondria, which are a major site of amino acid catabolism [5]. Identification of the unknown amino acid catabolic pathways is the topic of ongoing and future research in my group.

2.1 Cysteine catabolism and mitochondrial persulfide metabolism in plants compared to animals

Cysteine has a special position in amino acid metabolism since it connects carbon, nitrogen, and sulfur metabolism. One of my major research topics is to unravel the reactions and regulation of reduced sulfur metabolism in mitochondria. In plants, cysteine can be catabolized by cysteine desulfhydrases (D19) localized in the cytosol and in the mitochondrial matrix as well as by a sulfur dioxygenase dependent mitochondrial pathway (D15-17; Fig. 2A). Two additional processes, namely production of reduced sulfur for iron sulfur clusters by cysteine desulfurases (D18) and cyanide detoxification via β-cyanoalanine synthase, are also associated with the degradation of cysteine. The hydrogen sulfide that is produced during some of these reactions might function as a signaling molecule regulating physiological processes such as stomatal closure and autophagy (Alvarez et al. 2012, Jin et al. 2013). However, sulfide is also a potent inhibitor of mitochondrial cytochrome c oxidase and therefore an efficient detoxification mechanism is required. Incorporation into cysteine is a major means of plant cells to control the free sulfide concentration (Fig. 2A) [9]. In contrast to the synthesis pathways of other amino acids that are mainly restricted to the chloroplasts, isoforms of serine acetyltransferase and O-acetlyserine-(thiol)-lyase required for sulfide fixation into cysteine are present in chloroplasts, mitochondria, as well as in the cytosol and regulated in a complex manner [14]. However, this sulfide cycle is based on a balance between cysteine catabolic processes producing sulfide and its removal by cysteine synthesis. Net degradation of cysteine requires oxidation of the thiol group, which can be achieved by the sulfur dioxygenase ETHE1 (Fig. 2A) [7][9][10]. ETHE1 is part of a cysteine catabolic pathway localized in the mitochondrial matrix [7]. After transamination to 3-mercaptopyruvate by a presently unknown aminotransferase, the sulfhydryl group from L-cysteine is transferred to glutathione by sulfurtransferase 1 (D15) and oxidized to sulfite by the sulfur dioxygenase ETHE1 (D16).

Sulfite is then converted to thiosulfate by addition of a second persulfide group by sulfurtransferase 1 (D15).

Persulfide homeostasis is essential during early embryo development in Arabidopsis since knockout of the sulfur dioxygenase is embryo lethal and the knockdown line *ethe1-1* with less than 1 % of residual sulfur dioxygenase activity has a severe delay in embryo development (Holdorf et al. 2012) [2][10]. Seeds of sulfurtransferase deficient plants (*str1-1*) and the double mutant line *ethe1-1* x *str1-1* even develop highly irregular shapes (Mao et al. 2011) [7]. These developmental defects might be caused by toxic effects of accumulating reactive intermediates such as persulfides or cyanide [7]. Sulfur dioxygenase deficiency also leads to a general disturbance in seed amino acid homeostasis during early development with a strong increase in alanine and glycine content [2][10]. The defects in endosperm cellularization observed in the mutant might be connected to a potential role of ETHE1 in abscisic acid (ABA) signaling since ABA sensitivity is decreased in germinating *ethe1-1* seeds [2].

In leaves, ETHE1 expression is induced during carbohydrate starvation for example when photosynthesis rates are low due to drought stress or shading [3][10]. *Ethe1-1* plants show early senescence under short-day growth conditions indicating a potential role in the use of amino acids as alternative substrates for ATP production when carbohydrate supply is insufficient. Co-expression analysis indicates a close connection between sulfur and branched-chain amino acid catabolism, which is well in line with metabolomics datasets. We detected an accumulation of cysteine, glutathione and serine, which are directly involved in cysteine metabolism, but also strongly increased amounts of valine, leucine, and isoleucine in *ethe1-1* plants grown under short-day conditions or subjected to extended darkness [7][10].

Since glutathione persulfide is the substrate of the sulfur dioxygenase ETHE1, the phenotypic and metabolic effects observed in *ethe1-1* mutant plants could be mediated by a disturbance in persulfide homeostasis. Formation of cysteine persulfides in proteins, a process also referred to as persulfidation, has recently been recognized as a posttranslational modification similar to nitrosylation (Sen 2017). Persulfidation occurs at specific reactive cysteine residues with a low pKa and has been shown to influence localization, stability and activity of the target protein. In humans and mouse models aberrant persulfidation patterns have been observed under several pathological conditions such as heart disease and neurodegenerative diseases. However, the physiological relevance of this post-translational modification has not been fully established yet and the biochemical mechanisms of protein persulfidation and de-persulfidation are largely unknown. In Arabidopsis, 2015 persulfidated proteins involved in diverse physiological processes were identified in rosette leaves of plants grown under long-day control conditions (Aroca et al. 2017).

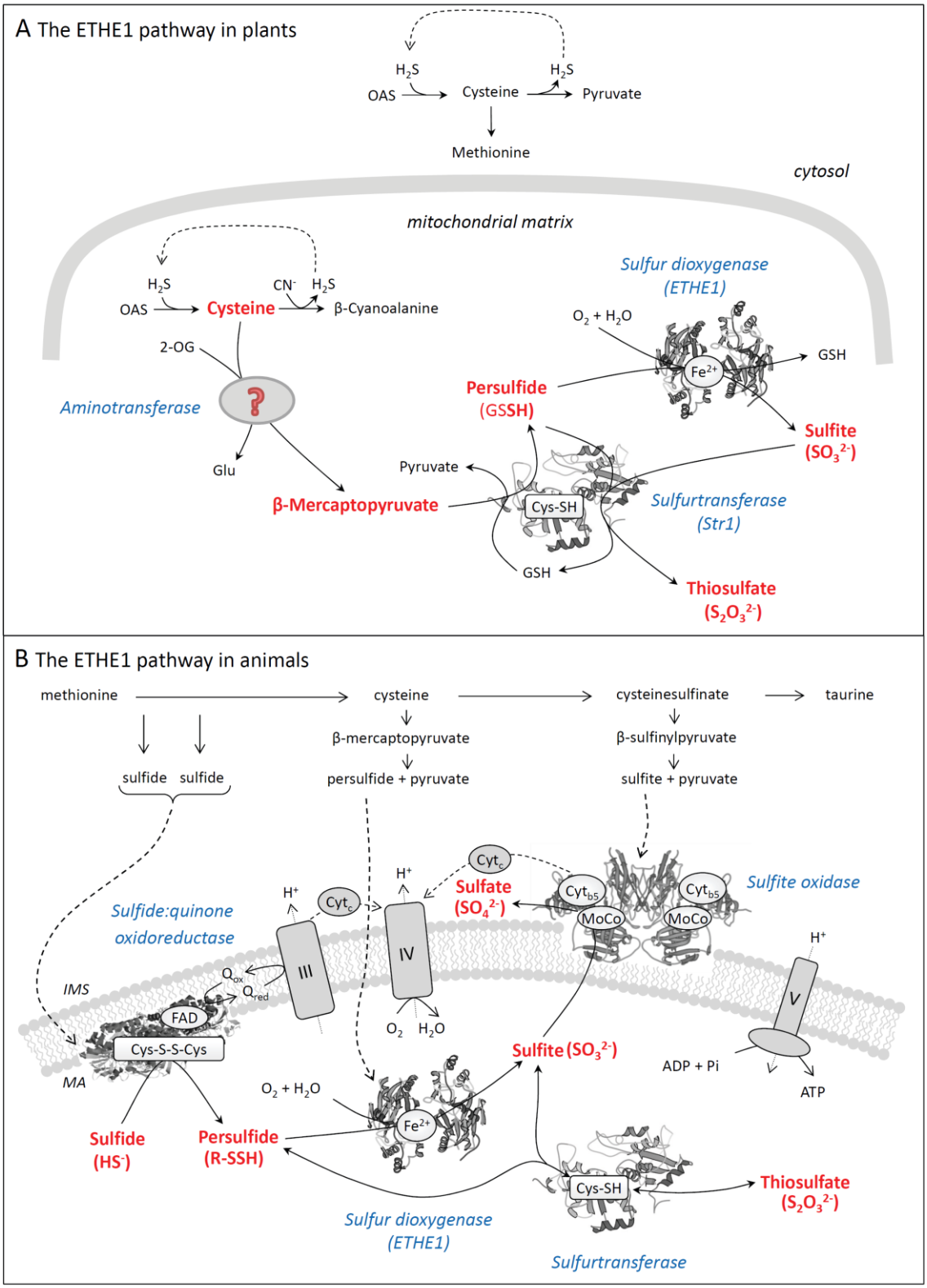

Fig.2: Mitochondrial persulfide metabolism in plants and animals

(A) Mitochondrial cysteine degradation in plants is initiated by a transamination step to 3-mercaptopyruvate catalyzed by a presently unknown aminotransferase. The sulfhydryl group is then transferred to glutathione by sulfurtransferase 1 (Str1) and oxidized to sulfite by the sulfur dioxygenase ETHE1. Sulfite is converted to thiosulfate by addition of a second persulfide group by sulfurtransferase 1. Additional cytosolic and reactions that release and reassimilate hydrogen sulfide are indicated. (B) In animals, the ETHE1 pathway catalyzes the oxidation of hydrogen sulfide. Sulfide:quinone oxidoreductase (SQR) oxidizes sulfide to persulfide and transfers the electrons to ubiquinone. The sulfur dioxygenase ETHE1 oxidizes the persulfides to sulfite, which is either oxidized to sulfate by sulfite oxidase or metabolized to thiosulfate by addition of a second persulfide catalyzed by the mitochondrial sulfurtransferase rhodanese.

Activity tests showed that persulfidation reversibly regulates the functions of individual plant enzymes in a manner similar to that described in animals (Aroca et al. 2015). Unraveling the potential role of the different cysteine catabolic pathways in persulfide signaling will be a topic of my future research.

The mitochondrial sulfur dioxygenase ETHE1 is also present in animals and catalyzes the same reaction as in plants, the oxidation of glutathione persulfide to sulfite [15][16]. However, integration into sulfur metabolism is quite different due to specific requirements of the animal compared to the plant system. In contrast to plants, animals are not able to synthesize the sulfur amino acids but they have to be provided in sufficient amounts via food. Methionine can be converted to cysteine in a reversion of the transsulfuration pathway used for methionine synthesis in plants. Cysteine catabolism mainly proceeds via cysteine sulfinate catalyzed by a cysteine dioxygenase that is not present in plants (Fig. 2B). Degradation via 3-mercaptopyruvate is also possible but this reaction is localized in the cytosol and most likely does not produce the main substrate for the mitochondrial sulfur dioxygenase (Yin et al. 2016). Hydrogen sulfide signaling is involved in the regulation of several physiological processes in animals such as vasorelaxation and insulin secretion (Li et al. 2011). Since there is no sulfate assimilation pathway sulfur amino acids are the only substrate for the production of hydrogen sulfide and in contrast to plants it cannot be detoxified via reincorporation into cysteine. Thus, a major function of the ETHE1 dependent pathway in animals is the oxidation and detoxification of hydrogen sulfide, which requires two additional enzymes (Fig. 2B) [16]. The first reaction step is catalyzed by sulfide:quinone oxidoreductase (SQR), a flavoenzyme bound to the inner mitochondrial membrane. SQR oxidizes sulfide to persulfide and transfers the electrons to ubiquinone, so that sulfide can be used as an inorganic substrate for mitochondrial ATP production. The sulfur dioxygenase ETHE1 oxidizes the persulfides to sulfite, which is either oxidized to sulfate by sulfite oxidase or metabolized to thiosulfate by addition of a second persulfide catalyzed by the mitochondrial sulfurtransferase rhodanese. This pathway is redox regulated and highly active in animals adapted to sulfide containing habitats such as the lugworm *Arenicola marina* living in the intertidal flats of the wadden sea [12][16][17]. However, ETHE1 dependent sulfide detoxification is also essential for human health as demonstrated by the severe symptoms of ethylmalonic encephalopathy, a metabolic disease caused by mutations in the ETHE1 gene [15]. Patients suffer from peripheral microangiopathy, chronic diarrhea and rapidly progressive encephalopathy, and they excrete unusually high amounts of ethylmalonic acid and thiosulfate. These symptoms are caused by accumulation of toxic sulfide levels and can be relieved by combined treatment with a bactericide that reduces sulfide production by intestinal anaerobes and N-acetylcysteine as a precursor of sulfide-buffering glutathione [13]. Increased levels of reduced sulfur compounds might interfere with persulfidation and thus lead to defects in post-translational protein regulation [11]. Sulfide toxicity also contributes to the pathophysiology of coenzyme Q deficiency

syndrome, since low concentrations of ubiquinone in the respiratory chain cause a reduction in SQR level and activity [6].

3. The physiological role of amino acid catabolism in plants

3.1. General metabolic functions of amino acid catabolism in plants

3.1.1 Producing hormones and secondary metabolites

Some amino acids are required as precursors for secondary metabolites and non-protein amino acids (Fig. 1). The aromatic amino acids can be converted to a highly diverse set of metabolites including isoquinoline and indole alkaloids, phenylpropanoids, and glucosinolates (Tzin and Galili 2010). Polyamines such as spermidine and putrescine are derived from arginine, and N-hydroxy-pipecolic acid required for long distance signaling during the establishment of systemic acquired resistance is produced from lysine (Alcázar et al. 2006, Chen et al. 2018, Hartmann et al. 2018). Thus, the demand for a specific subset of amino acids can be strongly increased during abiotic or biotic stress conditions. A continous flux of methionine through the S-adenosylmethionine cycle provides C1-units for methylation reactions and also the precursor for synthesizing the phytohormone ethylene (Amir 2010). Ethylene regulates seed germination, plant growth and senescence as well as abscission of flowers and leaves and induces fruit ripening in climacteric fruits (Buchanan et al. 2015). The auxin indol-3-acetic acid (IAA) is synthesized from tryptophan via several different routes. Auxins are involved in the regulation of a number of physiological processes including cell division, initiation of root growth, shoot elongation, flower production, tropism, and apical dominance. The amount of active auxin can be controled by conjugation to various amino acids such as alanine, aspartate, glutamate, methionine and tyrosine (Buchanan et al. 2015).

3.1.2 Adjusting amino acid pool sizes

Proteins represent the largest pool of amino acids in a plant cell. An average leaf with a protein content of about 20 mg/g fresh weight contains 200 nmol/mg fresh weight amino acids in proteins but only about 10 nmol/mg fresh weight in the free amino acid pool (Fig. 3) [8]. The average content of the 20 amino acids within proteins varies by less than a factor of 10 with leucine (9.2 %) being the most abundant and tryptophan (1.2 %) the least abundant amino acid (Fig. 3 right). In contrast, steady state levels of free amino acids are highly diverse. Some amino acids that serve as nitrogen stores such as glutamine, glutamate, asparagine, and aspartate or as compatible osmolytes such as proline are present in millimolar concentrations. Others have 1000fold lower levels, for example lysine, the branched-chain, the aromatic, and the sulfur containing amino acids. These low abundant amino acids are characterized by either a complex structure requiring multiple reaction steps during synthesis as

well as degradation (Fig. 1), or a high reactivity which makes them potentially toxic (Jacob et al. 2003, Park and Imlay 2003).

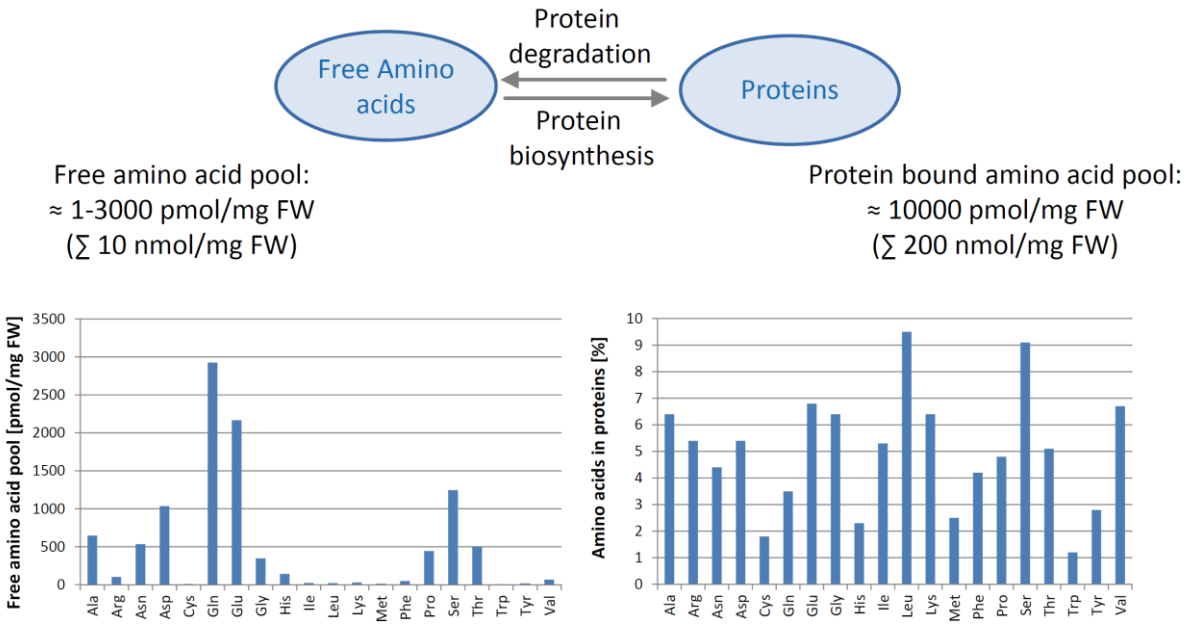

Fig. 3: Pool sizes of free and protein bound amino acids in Arabidopsis
Left: Leaf amino acid content of Arabidopsis plants grown under short-day conditions in the middle of the light period. Right: Frequencies of amino acids in the theoretical Arabidopsis proteome calculated from all protein sequences listed in TAIR10. FW, fresh weight. (adapted from [8])

The pools of free and protein bound amino acids are obviously closely connected. Efficient protein synthesis requires an adequate supply with all 20 amino acids, so that the low abundant ones will be rate limiting. On the other hand, the effect of protein breakdown is much stronger regarding low abundant than high abundant amino acids. For example, degradation of the average Arabidopsis proteome (Fig. 3) leading to a moderate 10 % increase in glutamine would increase the free leucine concentration by 3500 % [3]. Thus, an important physiological function of amino acid catabolism is to adjust the levels of the individual amino acids under conditions of increased protein turnover in order to maintain homeostasis.

3.1.3 Producing ATP

Amino acids can serve as alternative substrates for mitochondrial ATP production when the supply of carbohydrates is insufficient. After removal of the amino group the carbon skeletons of the amino acids are converted to pyruvate, acetyl-CoA, or TCA cycle intermediates. Specific dehydrogenases in the individual catabolic pathways as well as the oxidation steps of the TCA cycle provide NADH and $FADH_2$ for mitochondrial ATP production via oxidative phosphorylation (Fig. 4) [8]. In addition, some of the enzymes involved in the oxidation of branched-chain amino acids, lysine, and proline directly feed

electrons into the mitochondrial respiratory chain (Araújo et al. 2010, Engqvist et al. 2009, Schertl et al. 2014).

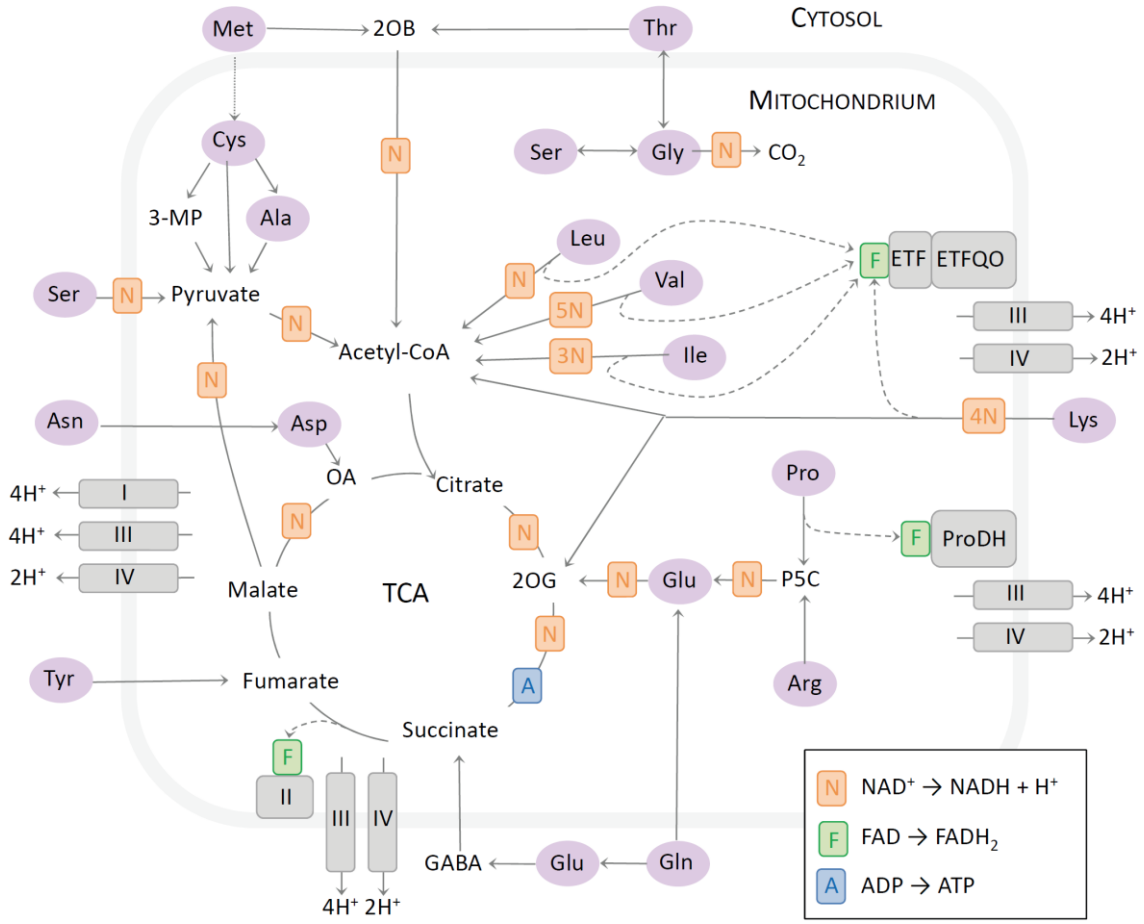

Fig. 4: Subcellular localization and energy yield of amino acid catabolic pathways
Amino acids (highlighted in purple) are degraded to precursors or intermediates of the TCA cycle. Most reaction steps of the catabolic pathways occur either in the mitochondria or in the cytosol. Additional isoforms with different localizations have been omitted from this scheme for clarity. 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. Abbreviations: 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. (From [8])

The ATP yield of amino acid oxidation can be estimated by counting NAD- and FAD dependent reaction steps, which lead to translocation of 10 and 6 protons across the inner mitochondrial membrane and thus provide energy for the synthesis of 2.5 and 1.5 molecules of ATP, respectively (Fig. 4, Tab. 2). In addition, the number of ATPs produced by substrate-level phosphorylation in the TCA cycle has to be taken into account. Complete oxidation of glycine results in the synthesis of only 2.5 ATP molecules in

contrast to tyrosine, leucine, lysine, and isoleucine catabolism, which leads to the production of about 30 ATPs per amino acid degraded and is therefore comparable to the oxidation of glucose as a substrate. The physiological relevance of amino acids as alternative respiratory substrates has been repeatedly demonstrated under extended darkness, drought, and short light periods (Araújo et al. 2010, Däschner et al. 2001, Ishizaki et al. 2005, Engqvist et al. 2011) [10]. This aspect will be discussed in detail in section 3.3.

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 (From [8])

Amino acid	NADH	$FADH_2$	Direct ATP	∑ ATP*
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
Aspartat, 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

* assuming 4 protons required per ATP

3.1.4 Producing carbohydrates (gluconeogenesis)

During germination storage proteins have to be converted to glucose for the synthesis of cell walls in the rapidly growing seedling. This process is presently not completely understood. In order to serve as a substrate for gluconeogenesis amino acids have to be metabolized to phosphoenolpyruvate in a reaction bypassing the irreversible final reaction step of glycolysis. Phosphoenolpyruvate carboxykinase (PEPCK) fulfills this pivotal function during gluconeogenesis from lipids (Theodoulou & Eastmond 2012). Amino acids that are degraded to acetyl-CoA or intermediates of the TCA cycle can most likely be metabolized via the same route (Fig. 5). An additional gluconeogenetic gateway has recently been identified for conversion of amino acids degraded to pyruvate into carbohydrates (Eastmond et al. 2015). Pyruvate orthophosphate dikinase (PPDK) catalyzes phosphorylation of pyruvate via a diphosphorylated enzyme intermediate and is required for efficient recovery of carbon from storage proteins during Arabidopsis seedling establishment. However, there are still many open questions regarding the intermediate steps of the respective pathways. The degradation products of

several amino acids can directly be converted to malate via the TCA cycle whereas valine, leucine, isoleucine, and lysine produce acetyl-CoA and therefore require reactions of the glyoxylate cycle for combining the C2-units (Fig. 5). Since amino acid catabolism is localized in the mitochondria and the glyoxylate cycle in the peroxisomes there has to be some interaction between the two compartments. There are several possible options for combining branched-chain amino acid degradation with the critical reaction steps of the glyoxylate cycle and this topic is currently being investigated in my group.

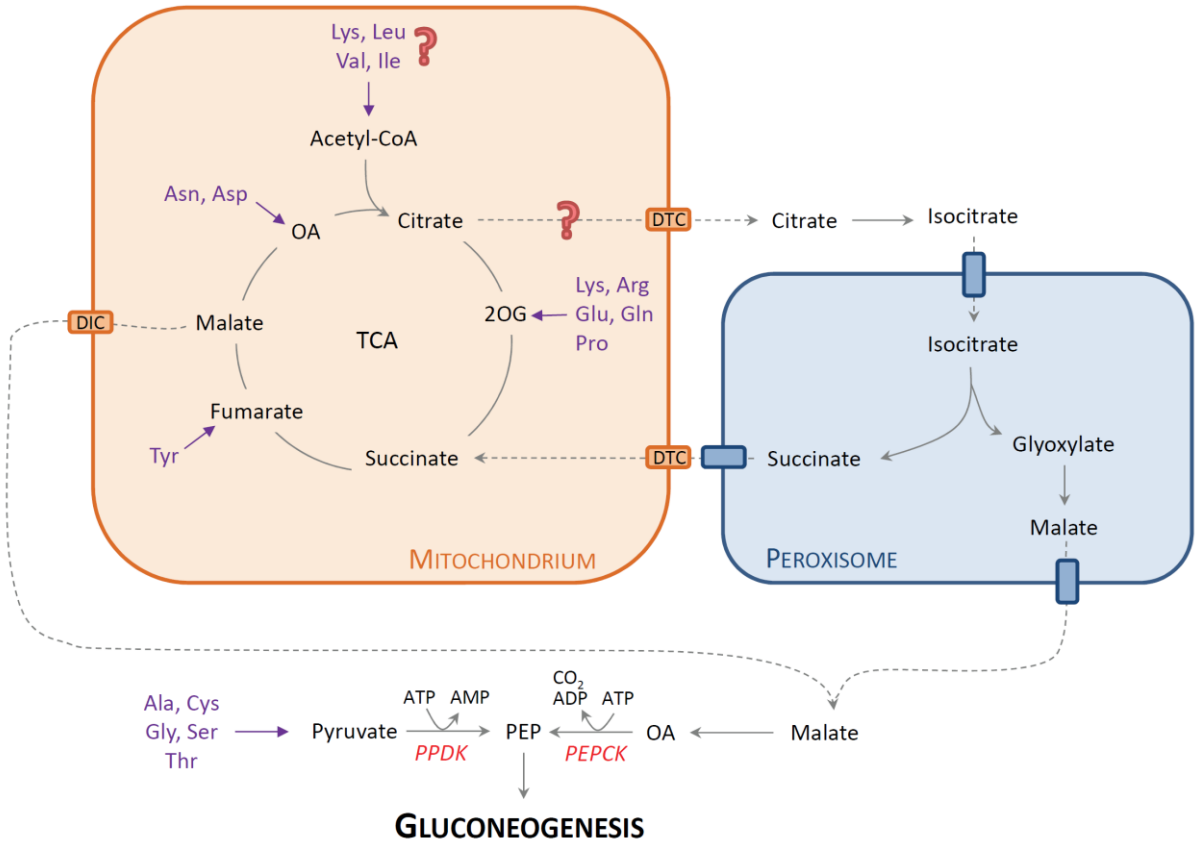

Fig. 5: Current model of pathways involved in gluconeogenesis from amino acids during germination
Amino acids that are degraded to pyruvate (Ala, Cys, Gly, Ser, Thr) require pyruvate orthophosphate dikinase (PPDK) activity for production of phosphoenolpyruvate (PEP). Gluconeogenesis from lipids as well as from amino acids that are degraded to acetyl-CoA (Lys, Leu, Val, Ile) or TCA cycle intermediates (Lys, Arg, Glu, Gln, Pro, Tyr, Asn, Asp) is mediated by phosphoenolpyruvate carboxykinase (PEPCK). A critical step for integrating the C2 units derived from acetyl-CoA into carbohydrates is conversion to malate via the glyoxylate cycle localized in peroxisomes. The exact route of this pathway is not known yet. 2-OG, 2 oxoglutarate; DIC, dicarboxylate carrier; DTC, dicarboxylate/tricarboxylate carrier; OA, oxaloacetate; PEP, phosphoenolpyruvate; TCA, tricarboxylic acid cycle. (modified after Eastmond et al. 2014)

3.2 Amino acid catabolism during development

Large scale nutrient recycling is required during several stages in a plant's life cycle. Germination initially occurs in the absence of light, so that ATP, reducing power, and also carbohydrates for the synthesis of cell walls have to be provided by degradation of storage compounds. Catabolism of seed storage proteins contributes amino acids that can be used for the synthesis of new proteins required for seedling establishment and also be the substrates for ATP production as well as gluconeogenesis

(see above). Thus, amino acid metabolism and the associated pathways are induced and highly active in the germinating seed (Galili et al. 2014). Mutant lines with defects in branched-chain amino acid catabolism such as *etfqo*, *ivdh* and *mmsd* require an external supply of sucrose for successful germination, which nicely illustrates the physiological function of amino acid degradation for early seedling establishment (Gipson et al. 2017).

During vegetative growth under optimal conditions, photosynthesis is the main source of energy and carbohydrates. However, bulk nutrient recycling in a process called autophagy has been shown to be relevant for supporting plant growth during the night (Izumi et al. 2013). Autophagosomes enclose cytosolic constituents and even complete organelles and deliver them to the vacuoles for degradation (Avila-Ospina et al. 2014). A diverse set of proteases finally catalyzes complete hydrolysis of the proteins into amino acids (Roberts et al. 2012).

During senescence, nutrients have to be reallocated from the leaves to the developing seeds (Watanabe et al. 2013). Chloroplasts contain the most abundant proteins in plant cells such as RuBisCO and the photosynthetic apparatus and therefore constitute important reservoirs of the plant's resources. They are degraded relatively early during the senescence process leading to reduced photosynthetic capacity. Since mitochondria stay intact until very late stages of senescence amino acid catabolism contributes considerably to the energy status of an aging leaf (Chrobok et al. 2016). In addition to serving as an alternative substrate for mitochondrial respiration amino acids are also transported to the seeds as precursors for the synthesis of proteins required during seed development as well as for storage proteins. Transport in the phloem is not equally efficient for all 20 amino acids but there seems to be a preference for glutamine, glutamate, and asparagine (Masclaux-Daubresse et al. 2010). These amino acids are non-toxic, have a high N/C ratio, and they are suitable substrates for efficient synthesis of the other amino acids in the seed tissues.

Amino acid metabolism in the endosperm and embryo is not known in detail yet. A potential function of amino acids as alternative substrates for ATP production has been postulated (Galili et al. 2014). However, since the mother plant supplies sucrose to the developing seeds the physiological context is clearly different from leaves. We addressed this question by analyzing amino acid profiles and protein abundances during Arabidopsis seed development in shaded siliques compared to control conditions and did not find any evidence for a specific role of amino acids as alternative respiratory substrates [2]. Seeds developed normally in siliques covered with aluminum foil and accumulated amino acids that are imported from the mother plant (asparagine and glutamine) rather than those typically increasing in leaves in the dark as a consequence of protein degradation (lysine, branched-chain and aromatic amino acids). Also, amino acid catabolic enzymes were not induced in the dark grown seeds.

Several studies indicate a complex regulation of seed amino acid homeostasis. We found changes in the contents of most amino acids in developing seeds of the *ethe1-1* mutant with a defect in mitochondrial cysteine catabolism (see above) [2, 10]. Similar results have been reported from several knockout lines for different enzymes involved in the degradation of branched-chain amino acids (Gu et al. 2010, Peng et al. 2015). Attempts to increase the seed content of the essential amino acids lysine and methionine by either inducing their synthesis of inhibiting their degradation were only moderately successful since plants were able to adapt by induced catabolism or inhibition of the synthesis pathway, respectively. If an increase in free lysine content was forced by a combination of both approaches the energy status of the seeds was severely affected leading to germination defects (Galili & Amir 2013). The abnormal development of seeds with defects in 3-hydroxyisobutyryl-CoA hydrolase involved in valine degradation or in ETHE1 provides additional evidence for a pivotal role of amino acid metabolism in embryogenesis (Gipson et al. 2017, Holdorf et al. 2012) [2][7][10]. My group continues studying the role of amino acid metabolism during seed development using mutants with defects in different catabolic pathways. An additional topic of interest is the contribution of the different pathways to the nutrient remobilization efficiency during senescence and seed production, which can be analyzed using different Arabidopsis ecotypes with contrasting nitrogen remobilization efficiencies.

3.3 Amino acid catabolism in abiotic stress response

Environmental conditions such as aridity, extreme temperatures (heat, cold, freezing), and high salinity strongly affect plant growth and agricultural yield. Metabolic adaptations to these adverse growth conditions are often associated with massive rearrangements in the free amino acid pool (Fig. 6). In order to understand the potential function of specific metabolic changes the physiological effects of the different abiotic stress conditions have to be considered. For example, high salt concentrations lead to ionic stress and secondary deficiencies in K^+ and NO_3^-, during cold stress plants increase the proportion of unsaturated fatty acids in the membranes to lower the transition temperature and stabilize the membranes against freezing injuries, and heat affects the stability of different cellular components such as proteins, membranes and structural elements (Mahajan & Tuteja 2005, Hasanuzzaman et al. 2013). However, some effects are more general and can result from several different abiotic stresses. For example, dehydration, high salinity, and freezing ultimately lead to desiccation of the cell and osmotic imbalance. Plants accumulate compatible osmolytes, which can be sugars but also amino acids or proteins, to prevent a loss of turgor and to protect proteins from denaturation (Slama et al. 2015). In addition, cell walls are adapted to the low osmotic potential by increasing their elasticity and strength (Le Gall et al. 2015). In an attempt to minimize water loss plants close their stomata in response to low water availability in the soil. The resulting decline in intracellular CO_2 leads to reduced photosynthesis rates and an increase in oxidative stress (Mahajan & Tuteja 2005).

As a consequence, plant growth is restricted primarily in the aerial parts and the available resources are redirected to expanding the root system to improve the water supply (Wu & Cosgrove 2000).

In order to gain a comprehensive overview about amino acid pools and the direction of their metabolism during stress response I systematically analyzed published transcriptomics and metabolomics datasets from different abiotic stress conditions on the basis of the comprehensive pathway map shown in Figure 1 [3]. This metastudy revealed that stress conditions leading to energy deficiency and/or an osmotic imbalance such as drought, high salinity, and extended darkness had the strongest effect on amino acid metabolism in Arabidopsis (Fig. 6).

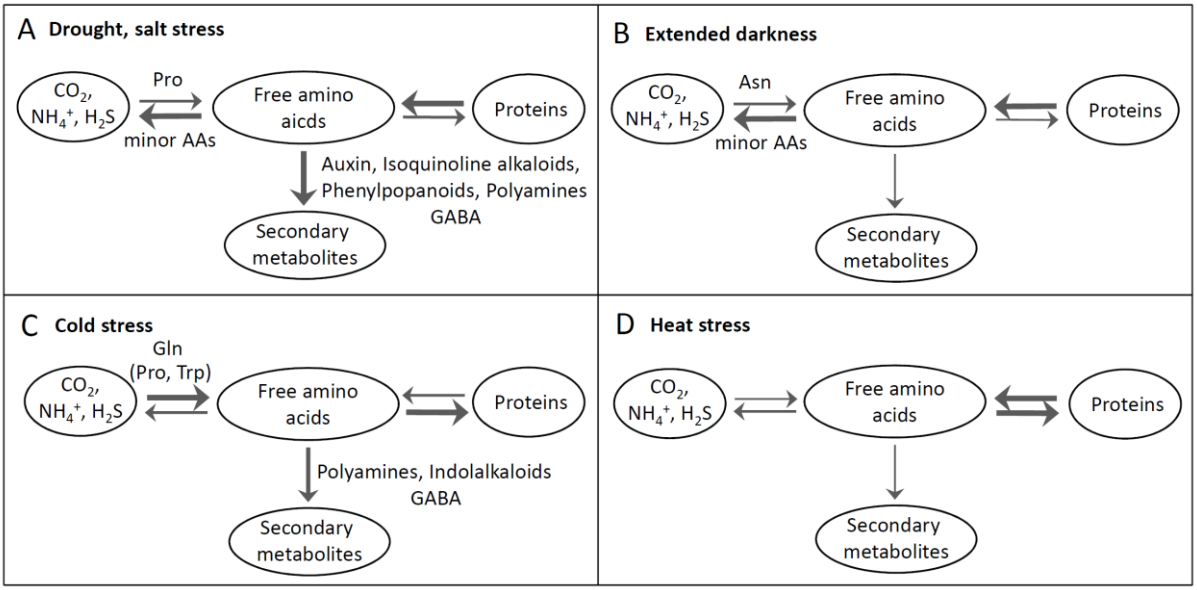

Fig. 6: Summary of changes in the free amino acid pool during abiotic stress conditions
The intensity of the arrows indicates the flux through the respective pathway. (A) During drought and salt stress free amino acids are mainly produced by protein degradation. Low abundant amino acids are degraded and proline is synthesized as compatible osmolyte. Also, amino acids are used as precursors for the synthesis of secondary metabolites. (B) Extended darkness leads to protein degradation for the production of amino acids as an alternative substrate for mitochondrial respiration. Asparagine is used for N-storage. (C) Cold stress leads to net protein synthesis, N-fixation into glutamine, and the synthesis of GABA, polyamines and indolalkaloids from amino acids. (D) During heat stress protein degradation and synthesis are approximately balanced so that only minor changes in the free amino acid pool occur. (From [3])

In a recent study we also experimentally addressed the role of amino acid metabolism in Arabidopsis tolerance to salt stress and low water potential using an *in vitro* approach [1]. Both studies indicate a strong induction of protein degradation to provide amino acids as alternative respiratory substrates for ATP production (Fig. 6A, Fig. 7) [1][3]. Proline synthesis is strongly induced during salt and drought stress and proline accumulates to high millimolar concentrations. It is non-toxic and acts as a compatible osmolyte and as a buffer of the cellular redox status (Bhaskara et al. 2015, Shinde et al. 2016). Proline synthesis requires glutamate as a substrate (Fig. 1, reactions S28, S29), which can be produced by transamination of many other amino acids. During severe dehydration the amino acids resulting from protein degradation are massively channeled into proline synthesis so that proline

becomes the most abundant free amino acid and constitutes up to 80 % of the total pool (Fig. 6A, Verslues & Sharma 2010) [1]. The non-protein amino acid γ-aminobutyric acid (GABA) and polyamines are additional metabolites produced from glutamate during osmotic stress (Krasensky & Jonak 2012). Aromatic amino acids are required as precursors for the synthesis of a diverse set of secondary metabolites from the groups of isoquinoline alkaloids and phenylpropanoids so that their synthesis pathways are induced during severe stress [1]. In contrast, the strong stress-induced increase in the free contents of other low abundant amino acids such as branched-chain amino acids and lysine was clearly a consequence of massive protein degradation. Our datasets show a strong induction of their degradation pathways while the synthesis was down-regulated [1][3]. These findings are in line with experimental results reported by Huang and Jander (2017). Inhibition of the synthesis pathways had no effect on the accumulation of branched-chain amino acids during drought stress whereas protease inhibitors led to a significant decrease.

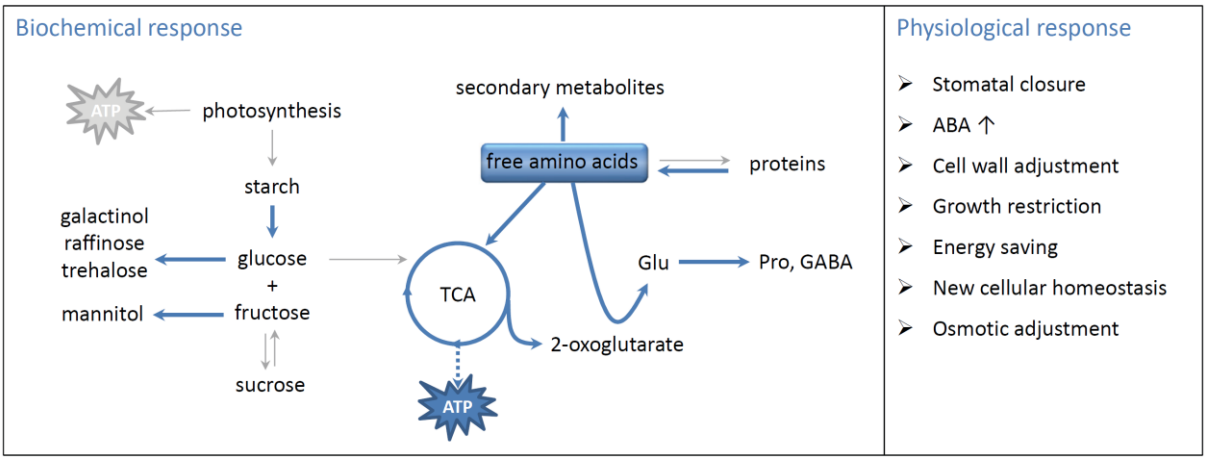

Fig. 7: Biochemical and physiological aspects of plant response to drought stress
Water deficiency leads to carbohydrate starvation due to decreased photosynthesis rates and increased production of sugars as osmolytes. Free amino acids resulting from protein degradation are used as alternative substrates for mitochondrial ATP production and they are precursors for the synthesis of proline and secondary metabolites. Blue arrows indicate pathways that are induced by drought stress.

Stress tolerance requires efficient metabolic adaptation to the stressful condition but also a fast recovery process allowing the plant to resume active growth as soon as environmental conditions have improved. Therefore, we particularly addressed the role of amino acid metabolism in the recovery period from low water potential or high salinity [1]. Protein abundance of the photosynthetic apparatus was severely decreased after the stress and slowly regenerated in the following recovery phase of 18 hours. As a consequence, amino acids were still required as alternative mitochondrial substrates for ATP production. Enzymes involved in proline metabolism were strongly induced during stress and recovery, indicating post-translational regulation of metabolite fluxes. Synthesis of secondary metabolites and their amino acid precursors was up-regulated specifically after stress release. In general, we observed a gradual return of amino acid levels to the non-stressed steady state.

However, some amino acids that might have toxic effects such as lysine, leucine, and methionine were degraded rapidly.

The effect of decreased photosynthesis rates can be studied without the additional stress factors associated with low water potential by keeping plants in the dark for several days. The main effect of this treatment on amino acid metabolism is a massive induction of protein degradation to provide amino acids as alternative respiratory substrates (Fig. 6B) [3]. My group frequently uses extended darkness as an experimental tool to study amino acid catabolism in the context of ATP production. We have identified a potential function of mitochondrial cysteine degradation in the carbohydrate starvation response of Arabidopsis leaves [7][10] (section 2.1) and detected a completely different pattern of amino acid metabolism in developing seeds [2] (section 3.2). In addition, our results indicate that not only amino acids but also glucosinolates, which are secondary metabolites derived from amino acid metabolism, are degraded during carbohydrate starvation induced by extended darkness [4].

In contrast to osmotic stress or darkness, which lead to increased proteolysis, extreme temperatures induce protein synthesis (Fig. 6 C,D) [3]. When exposed to low temperatures plants synthesize large amounts of proteins without enzymatic functions to decrease their freezing point and prevent ice formation (Guy 1990). Heat-shock proteins are required for preserving or reestablishing the native structure of proteins and in addition antioxidative enzymes are highly induced during heat stress (Mahajan & Tuteja 2005, Hasanuzzaman et al. 2013). Amino acids with a high nitrogen content (asparagine and glutamine) are used for storage and transport of reduced nitrogen under stress conditions that do not require massive synthesis of proline as a compatible osmolyte.

3.4 Amino acid catabolism in plant pathogen response

Plants have developed complex constitutive and inducible defense mechanisms to counteract infection by pathogenic microbes (Fig. 8). Conserved microbial structures can be detected by plant pattern recognition receptors triggering a general transcriptional activation of defense-related gene expression (PAMP-triggered immunity, PTI, Hacquard et al. 2017). Resistance proteins mediate a pathogen-specific immune reaction (effector-triggered immunity, ETI, Cui et al. 2015), which is usually associated with rapid localized cell death and a massive burst of reactive oxygen species at the pathogen inoculation site (hypersensitive response, HR).

A local microbial infection can also increase resistance of the entire plant to a broad spectrum of phytopathogens via a process called systemic acquired resistance (SAR). SAR leads to increased systemic expression of antimicrobial proteins as well as specific changes in the metabolite profile and also primes the plant to react more quickly and intensely to subsequent pathogen attacks (Navarova et al. 2012). The primed stage, which confers increased defensive capacities without a concomitant

induction of specific defense genes, usually persists longer than SAR and can via epigenetic changes even be transmitted to the next generation (Mauch-Mani et al. 2017). Since constitutive production of defense proteins and signaling molecules is energetically costly, a tight control of the different stages of defense is essential in order to avoid a decrease in overall plant fitness.

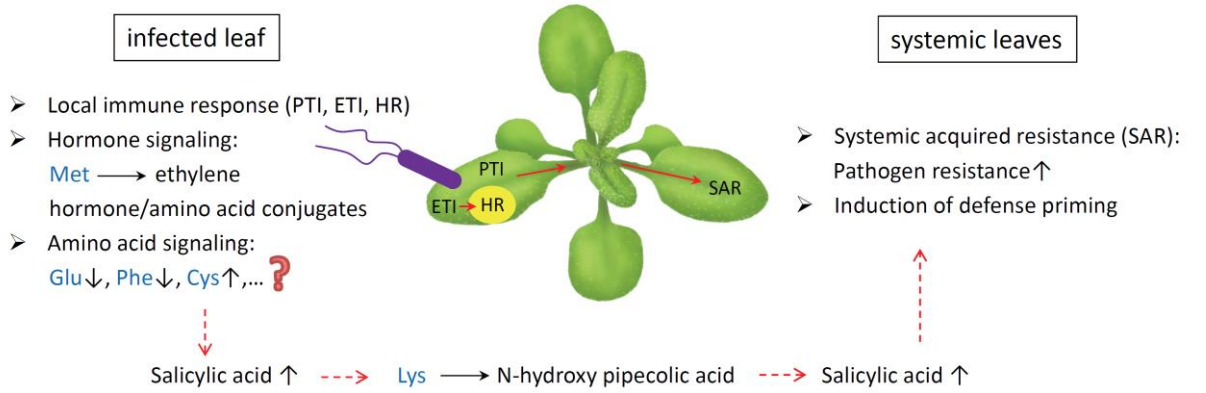

Fig. 8: Amino acids in local and systemic reactions of plant pathogen response.
A primary local infection initiates PAMP-triggered immunity (PTI) and effector triggered immunity (ETI), which is often associated with a local hypersensitive response (HR) leading to programmed cell death in the infected area. Amino acids are precursors for the synthesis of phytohormones as well as for additional secondary metabolites with signaling functions during the immune response. Amino acid homeostasis is also relevant for the induction of systemic defense reactions. Increased concentrations of salicylic acid induce the conversion of lysine to the phloem mobile immune signal N-hydroxyl pipecolic acid, which in turn leads to a signal amplification cascade in the non-infected, systemic leaves to establish systemic acquired resistance (SAR) and defense priming.

Amino acids are precursors for a diverse set of secondary plant metabolites with antimicrobial or toxic activities such as cyanogenic glucosides, glucosinolates, alkaloids, and phenolics (Bennett & Wallsgrove 1994). Not surprisingly, defense signaling also is closely associated with plant amino acid metabolism (reviewed by Zeier 2013). The phytohormone salicylic acid (SA), a critical regulator of both local and systemic resistance responses, originates from the shikimate pathway catalyzing aromatic amino acid biosynthesis (Wildermuth et al. 2001). Methionine is the precursor for the gaseous hormone ethylene, which together with jasmonic acid (JA) activates defense pathways against necrotrophic pathogens and is mostly antagonistic to SA signaling (Robert-Seilaniantz et al. 2011). The crosstalk between SA and JA signaling seems to be connected to branched-chain amino acid catabolism via isoleucic acid, a postulated side product of isoleucine degradation (von Saint-Paul et al. 2011). To modify their stability, activity, and transport efficiency some phytohormones such as JA, indole-3-acetic acid (IAA), and probably also SA can be conjugated to amino acids via acylation reactions. Isoleucine, leucine, valine, methionine, and alanine conjugates have been identified as the endogenous signaling active derivatives of JA (Yan et al. 2016).

Conversion of lysine into the cyclic, non-protein amino acid pipecolic acid (Pip) by the aminotransferase ALD1 is an essential step during SAR establishment. Pip acts as a phloem-mobile long-distance signal,

is hydroxylated to N-hydroxy pipecolic acid (NHP) by the flavin monooxygenase FMO1, and initiates a feedback amplification cycle in the systemic leaves leading to increased expression of a set of defense genes and specific changes in the metabolite profile that increase pathogen resistance (Chen et al. 2018, Hartmann et al. 2018). In addition, Pip induces defense priming enabling the plant to react more quickly and vigorously to subsequent pathogen attacks (Bernsdorff et al. 2016, Navarova et al. 2012). Reactive oxygen species (ROS) mediate the local hypersensitive pathogen response and also act as signaling molecules during the establishment of SAR as well as defense priming (Alvarez et al. 1998). Polyamines, which are synthesized from arginine and ornithine, can contribute to extracellular hydrogen peroxide production by the action of polyamine oxidases and thus participate in immune signaling (Hussain et al. 2011).

Several studies indicate that amino acid homeostasis is also relevant for the regulation of SAR, and specific changes in the amino acid profile either induce or inhibit immune reactions. Disruption of the amino acid transporter LHT1 as well as increased excretion of glutamine from leaf hydathodes leads to constitutive activation of defense pathways. Analysis of the respective Arabidopsis mutant lines revealed that glutamine deficiency activates the SA dependent pathogen response by moderation of the cellular redox status (Liu et al. 2010, Pilot et al. 2004). A similar effect has been described for the depletion of phenylalanine due to massive synthesis of phenolic defense compounds during pathogen infection. Uncharged $tRNA_{Phe}$ serves as a sensor for phenylalanine deficiency and triggers translation of TBF1, a key transcription factor during growth-to-defense transition (Pajerowska-Mukhtar et al. 2012).

A metabolome study detected a general decrease in free amino acids after treatment of Arabidopsis plants with the chemical priming compound β-aminobutyric acid (BABA) as well as after inoculation with avirulent *Pseudomonas syringae* bacteria. As a clear exception the sulfur containing amino acids cysteine and methionine were consistently and sustainedly increased during defense priming (Pastor et al. 2014). Analysis of mutants with defects in cytosolic cysteine synthesis or degradation also indicated a function of cysteine homeostasis during initiation of the hypersensitive response as well as in immune signaling (Alvarez et al. 2012). Our data revealed an interaction of the ETHE1 dependent cysteine degradation pathway with hormone signaling [2] (section 2.1). However, specific targets, the signaling molecules involved, as well as the underlying biochemical mechanism remain completely unknown and are subject of ongoing research projects in my group.

4. Conclusions

Amino acid homeostasis is crucial to a plant for completing its normal life cycle and also for stress tolerance. Previous findings have greatly improved our understanding of various aspects such as stress signaling, nitrogen and energy metabolism. However, there are still many open questions in plant amino acid metabolism such as identifying the basic reaction sequences of the more complex degradation pathways, defining specific functions of individual standard and non-protein amino acids, and unraveling amino acid dependent signaling pathways. In addition, metabolic re-adaptation after stress release and the role of amino acids in stress priming will be interesting aspects that need to be studied in order to fully understand plant amino acid metabolism in a physiological context.

5. References

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PART B:

Amino acid catabolism in plants – Publications

1. Batista-Silva W, Heinemann B, Rugen N, Nunes-Nesi A, Araújo WL, Braun HP & **Hildebrandt TM*** (2019) The role of amino acid metabolism during abiotic stress release. Plant Cell Environ 42, 1630-1644. doi: 10.1111/pce.13518

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