Metabolism and functions of gamma-aminobutyric acid

Barry J. Shelp, Alan W. Bown and Michael D. McLean

Gamma-aminobutyric acid (GABA), a four-carbon non-protein amino acid, is a significant component of the free amino acid pool in most prokaryotic and eukaryotic organisms. In plants, stress initiates a signal-transduction pathway, in which increased cytosolic Ca^{2+} activates Ca^{2+}/calmodulin-dependent glutamate decarboxylase activity and GABA synthesis. Elevated H^+ and substrate levels can also stimulate glutamate decarboxylase activity. GABA accumulation probably is mediated primarily by glutamate decarboxylase. However, more information is needed concerning the control of the catabolic mitochondrial enzymes (GABA transaminase and succinic semialdehyde dehydrogenase) and the intracellular and intercellular transport of GABA. Experimental evidence supports the involvement of GABA synthesis in pH regulation, nitrogen storage, plant development and defence, as well as a compatible osmolyte and an alternative pathway for glutamate utilization. There is a need to identify the genes of enzymes involved in GABA metabolism, and to generate mutants with which to elucidate the physiological function(s) of GABA in plants.

Gamma-aminobutyric acid (GABA), a four-carbon non-protein amino acid, is a significant component of the free amino acid pool. GABA has an amino group on the γ-carbon rather than on the α-carbon, and exists in an unbound form. It is highly soluble in water: structurally it is a flexible molecule that can assume several conformations in solution, including a cyclic structure that is similar to proline[1]. GABA is zwitterionic (carries both a positive and negative charge) at physiological pH values (p*K* values of 4.03 and 10.56).

Typically, GABA levels in plant tissues are low [ranging from 0.03 to 2.00 μmol g^{-1} fresh weight (FW)][2,3], but increase several-fold in response to many diverse stimuli, including heat shock, mechanical stimulation, hypoxia and phytohormones[4–7]. For example, within 5 min of mechanical or cold stimulation, the GABA concentration in soybean leaves rises to 1 to 2 μmol g^{-1} FW, a 20- to 40-fold increase[8]. Anoxia increases the GABA concentrations in rice seedlings by up to 8 μmol g^{-1} FW (Ref. 9.) GABA concentrations of 6 to 39 mM have been documented in suspension cells adapted to water stress[2,10,11], and concentrations of ~0.1 mM occur in root-bleeding sap during drought stress, a 230% increase[12].

Recent studies in the field of GABA metabolism have focused on:

- Enzymes involved in GABA catabolism.
- Regulation of GABA levels by long-term mechanisms of metabolic control (i.e. gene expression, and protein synthesis and turnover).
- Regulation of GABA levels by short-term mechanisms of metabolic control (i.e. pH, Ca^{2+}/calmodulin activation, substrate concentration and feedback inhibition).
- Intracellular and intercellular transport.

In this review, we consider the functions of GABA in pH regulation, nitrogen storage, plant development and defence, as well as a compatible osmolyte and an alternative pathway for glutamate utilization[5–7].

GABA metabolism

GABA shunt

The pathway that converts glutamate to succinate via GABA is called the GABA shunt (Fig. 1). The first step of this shunt is the direct and irreversible α-decarboxylation of glutamate by glutamate decarboxylase (GAD, EC 4.1.1.15). The *in vivo* conversion of [1-^{14}C]glutamate to $^{14}CO_2$ and unlabeled GABA is proof of GAD decarboxylation[13,14]. *In vitro* GAD activity has been characterized in crude extracts from many plant species and tissues[5–7]. GAD is specific for L-glutamate, pyridoxal 5′-phosphate-dependent, inhibited by reagents known to react with sulfhydryl groups, possesses a calmodulin-binding domain, and exhibits a sharp acidic pH optimum of ~5.8. *GAD* genes from *Petunia*[15], tomato[16], tobacco[17] and *Arabidopsis*[18,19] have been identified.

The second enzyme involved in the GABA shunt, GABA transaminase (GABA-T; EC 2.6.1.19), catalyses the reversible conversion of GABA to succinic semialdehyde using either pyruvate or α-ketoglutarate as amino acceptors (Fig. 1). In crude extracts, *in vitro* GABA-T activity appears to prefer pyruvate to α-ketoglutarate[20,21]. However, distinct pyruvate-dependent and α-ketoglutarate-dependent activities are present in crude extracts of tobacco leaf, and these can be separated from each other by ion exchange chromatography[21]. Both activities exhibit a broad pH optimum from 8 to 10 (Refs 20,21). The Michaelis constants (K_m) of a pyruvate-specific mitochondrial GABA-T from tobacco, purified ~1000-fold, are 1.2 mM for GABA and 0.24 mM for pyruvate[21]. The gene(s) encoding plant GABA-Ts has not been identified.

The last step of the GABA shunt is catalysed by succinic semialdehyde dehydrogenase (SSADH; EC 1.2.1.16), irreversibly oxidizing succinic semialdehyde to succinate (Fig. 1). The partially purified plant enzyme has an alkaline pH optimum of ~9; activity is up to 20-times greater with NAD than with NADP (Refs 7,20). The apparent K_m for SSADH, and for NAD and SSA are 166–460 μM and 5–15 μM, respectively[7] (C.S. Walton and B.J. Shelp, unpublished). Plant SSADH has not been purified to homogeneity, and the gene(s) encoding this protein has not been identified.

Regulation via glutamate decarboxylase activity

The *in vitro* activity of purified, recombinant *Petunia* GAD has an acidic pH optimum of ~5.5, with little activity at pH 7.0 in the absence of calmodulin[22]. GAD is a cytosolic enzyme[23], and it has been suggested that stress-induced GABA synthesis is the result of cytosolic acidosis and the consequent stimulation of GAD (Ref. 24). This proposal is supported by *in vivo* experiments

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demonstrating that an increase in cytosolic H^+ levels precedes GABA accumulation[25,26]. These results also support the hypothesis that GABA synthesis in response to H^+ is a pH-regulating mechanism. Thus, both *in vitro* and *in vivo* data indicate that a reduced cytosolic pH stimulates GAD activity and GABA accumulation (Fig. 2).

It is unlikely that the numerous environmental factors that stimulate GABA accumulation are all mediated by a decrease in cytosolic pH. Stress factors such as touch or cold shock, which stimulate GABA levels, are also known to increase cytosolic Ca^{2+} levels[27] (Fig. 2). The first direct evidence for Ca^{2+}-stimulated GAD activity was obtained by screening a *Petunia* cDNA expression library with ^{35}S-labeled calmodulin[15]. Ca^{2+}-dependent binding of calmodulin to a 58 kDa protein from *Petunia*[15] and a 62 kDa protein from fava bean[28] were observed. *In vitro* GAD activity in a variety of species and tissues is stimulated by Ca^{2+}/calmodulin at neutral pH, but not at pH values of <6.5 (Refs 22,28–30). Calmodulin antagonists prevent the stimulation by Ca^{2+}/calmodulin[22,29,31]. In addition, a monoclonal antibody specific for the 26 amino acid, calmodulin-binding, C-terminal region fully activates GAD in the absence of Ca^{2+}/calmodulin[22]. These experiments demonstrate that Ca^{2+}/calmodulin or an antibody binding to an autoinhibitory domain activates GAD.

In vivo evidence for Ca^{2+}/calmodulin activation of GAD was obtained using Ca^{2+} channel-blockers and calmodulin antagonists. Treatment of rice roots for 1 h with these agents blocks the GABA accumulation that is normally observed during the subsequent 3 h of anoxia[32]. A more detailed study investigated the role of Ca^{2+}/calmodulin in cold-shock-stimulated GABA accumulation in isolated mesophyll cells[31]. A fluorescent indicator of cytosolic Ca^{2+} levels shows an increase within 2 s of cold shock. Detectable GABA accumulates within 1 min, but H^+ levels do not increase. In the absence of cold shock, a Ca^{2+} ionophore stimulates cytosolic Ca^{2+} levels and GABA synthesis. Ca^{2+} channel-blockers or calmodulin antagonists inhibit cold-shock-stimulated GABA synthesis, but do not inhibit GABA synthesis in response to cytosolic acidification. Thus, both *in vitro* and *in vivo* data indicate that elevated Ca^{2+} levels stimulate GABA synthesis. Independent increases in either Ca^{2+} or H^+ levels appear sufficient to stimulate GABA synthesis (Fig. 2).

Recently, the regulation of GAD activity by glutamate availability has been investigated *in situ*[33]. The impact of aminoacetonitrile, a glycine decarboxylase inhibitor,

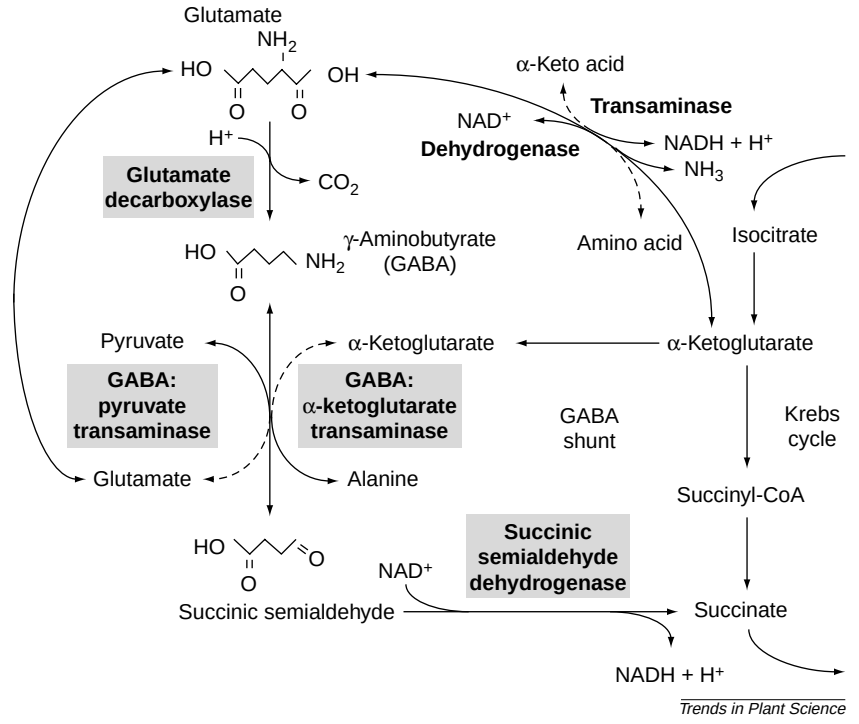

Fig. 1. The gamma-aminobutyric acid (GABA) shunt and its relationship to other metabolic pathways. Enzymes are indicated in bold; those specifically associated with the GABA shunt are in bold and highlighted in grey. Modified from Ref. 53.

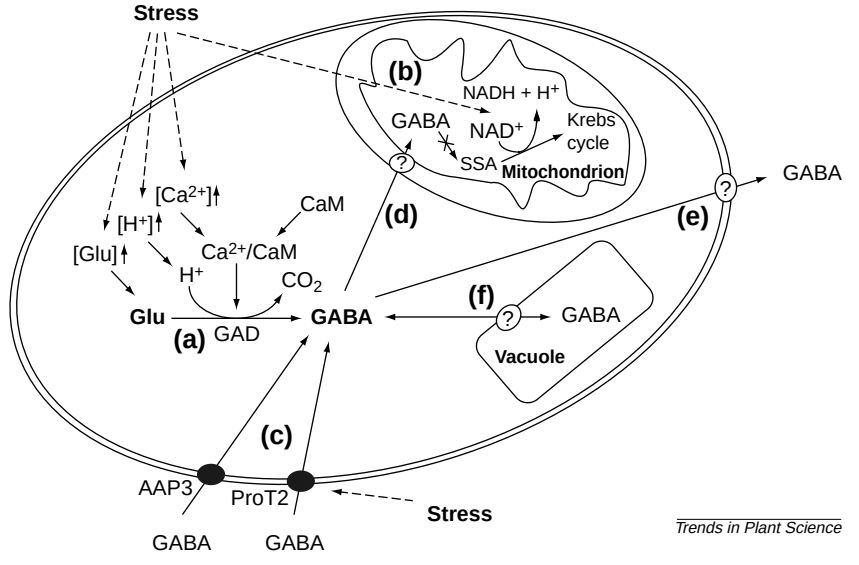

Fig. 2. Regulation of gamma-aminobutyric acid (GABA) levels by biotic and abiotic stresses and intracellular and intercellular transport. Stress might increase (↑) cytosolic Ca^{2+}/calmodulin, H^+ or glutamate levels, which in turn stimulate the production of GABA by glutamate decarboxylase (a). Stress might also decrease the NAD:NADH ratio, thereby limiting or competitively inhibiting succinic semialdehyde dehydrogenase activity and causing the accumulation of succinic semialdehyde, the feedback of which in turn inhibits (×) GABA transaminase (b). In addition stress increases the import of GABA, as well as other compounds (c). GABA accumulation might also result from decreased import into the mitochondrion (d) and export from the cell (e). GABA might also be sequestered in the vacuole (f). Abbreviations: AAP, amino acid permease; CaM, calmodulin; GAD, glutamate decarboxylase; ProT, proline transporter; SSA, succinic semialdehyde; circled question marks indicate that experimental evidence supports the existence of unknown transport steps.

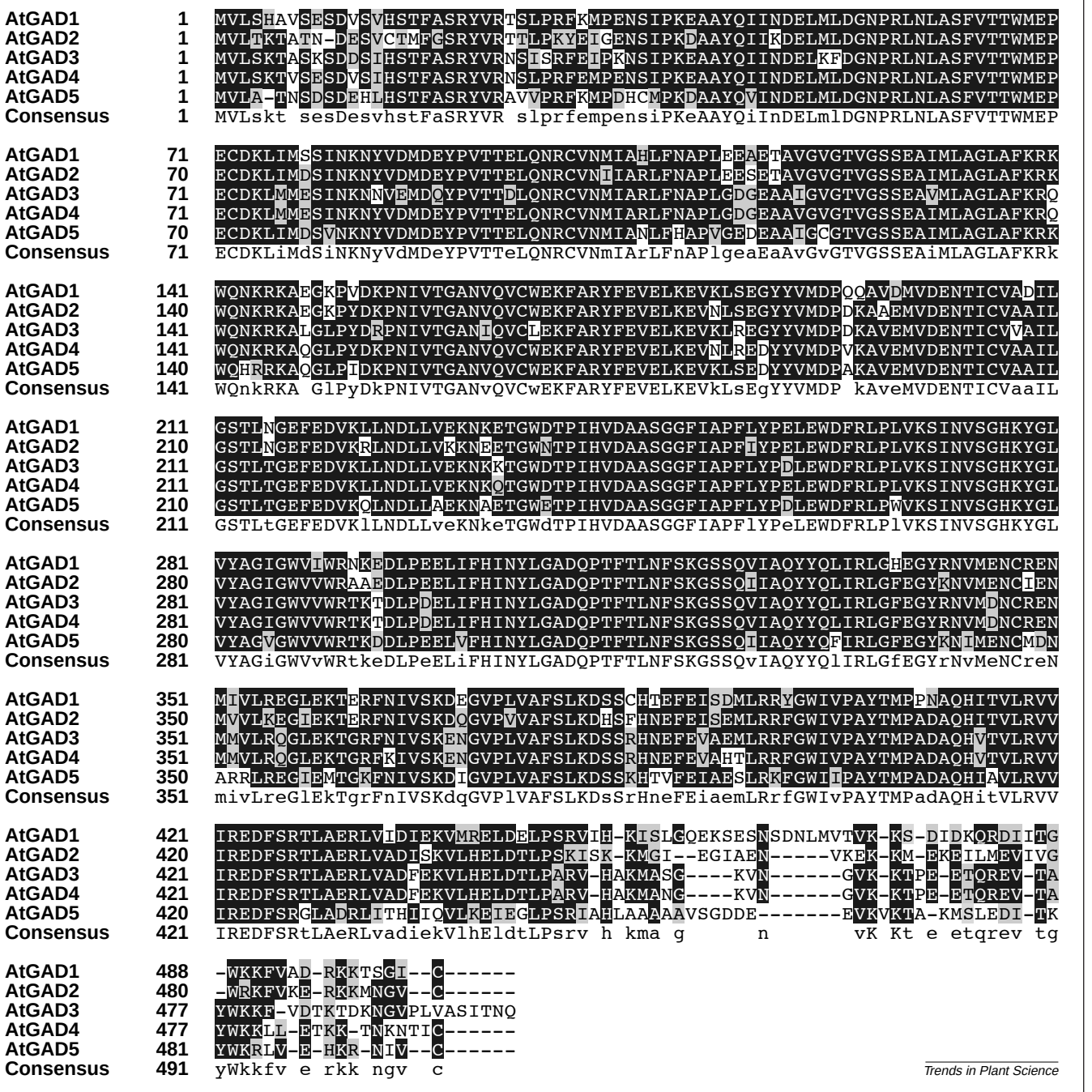

Fig. 3. Comparison of the amino acid sequence of *Arabidopsis* isoforms. Glutamate decarboxylases (GADs) 1–5 correspond to GenBank Accession nos: U10034, U46665 or U49937, AC006532 (gene F14H20.7), AC006532 (gene F14H20.8) and AB026646.1, respectively. The black and grey boxes represent identity and similarity, respectively. The uppercase and lowercase letters in the consensus sequence indicate sites of 100% identity and at least 60% identity or similarity, respectively. The mutiple alignment was performed using the Clustal W program of the A Pack of Molecular Analysis Tools at the website of the University of Adelaide's Dept of Microbiology and Immunology (http://www.microbiology.adelaide.edu.au/learn/index.htm); the figure was prepared using the Boxshade 3.21 program at the Swiss Institute of Bioinformatics website (http://www.isrec.isb-sib.ch:8080/index.html). Pairwise comparisons between GADs were conducted using the ALIGN program at the Institut de Genetique Humaine Genestream website (http://ww2.igh.cnrs.fr/home.html). Pairwise comparisons between calmodulin-binding domains were performed on the terminal 30, 30, 38, 31 and 29 amino acids of GADs 1–5, respectively, after introduction of additional spaces to maximize identity.

on the steady state metabolism of xylem-borne [U-^{14}C]glutamate by young transpiring shoots of tobacco seedlings has been determined. During a 90 min time-course, aminoacetonitrile causes glycine accumulation, decreases the ^{14}C-radioactivity in glutamine, and increases the ^{14}C-radioactivity in glutamate, succinate and other Krebs-cycle organic acids. Furthermore, the early precursor–product relations indicate that succinate is derived primarily via the GABA shunt rather than from α-ketoglutarate. Thus, recyling of photorespiratory NH_3 via glutamine synthetase is restricted by aminoacetonitrile, thereby enhancing the availability of glutamate for use by GAD. This study also provides experimental support for the suggestion that elevated glutamate levels stimulate GABA synthesis in isolated *Asparagus* mesophyll cells[13,31]. Similarly, elevated GABA levels occur under long-term

conditions that limit glutamine synthesis, reduce protein synthesis or enhance protein degradation[7]. These increased GABA levels are probably caused by increases in the substrate levels in the vicinity of the cytosolic GAD. This suggests that *in vivo*, GAD activity is regulated by glutamate concentration as well as by Ca^{2+}/calmodulin and H^+ (Fig. 2).

Although increased GAD activity is probably the major factor stimulating GABA accumulation, decreased catabolism by GABA-T and SSADH cannot be ruled out. *In vitro* activity ratios of GAD:GABA-T are 15–20:1, and GABA-T and SSADH have much higher *in vitro* pH optima than GAD (Ref. 20). This suggests that GABA-T restricts GABA metabolism *in vivo*, contributing to GABA-T accumulation[5]. Under conditions that influence the cells energy status, such as hypoxia, it is possible that a decrease in the NAD:NADH ratio might limit or cause competitive inhibition of SSADH activity[20] (Fig. 2). The resultant succinic semialdehyde accumulation might, in turn, inhibit GABA-T activity[21].

Regulation via GAD levels

The different expression patterns of GAD mRNA and protein in different *Petunia* organs suggest that GAD activity is transcriptionally and translationally regulated[34]. Furthermore, *Arabidopsis* possesses at least two *GAD* isoforms, one that is root-specific (*GAD*1) and another (*GAD*2) that is present in all organs[18,19]. The *GAD*2 transcript level, encoded protein and specific activity are higher in plant leaves supplied with either 10 mM NH_4Cl, 5 mM NH_4NO_3, 5 mM glutamate or 5 mM glutamine as the sole nitrogen source, than in leaves treated with 10 mM KNO_3 (Ref. 18). The impact of other stresses on GAD expression has not been investigated.

A database search has revealed three more putative GAD isoforms in *Arabidopsis* (M.D. McLean and B.J. Shelp, unpublished; Fig. 3). GADs 2–5 possess 75–82% identity with GAD1 over their entire amino acid sequence. The C-terminal residues comprising the putative calmodulin-binding domain are more variable: the C-terminal domain of GADs 2–5 possess 35–43% identity with that of GAD1. Like the *Petunia* GAD (Refs 15,30,35), most of the calmodulin-binding domain of *Arabidopsis* is basic and hydrophobic in nature, and tryptophan is highly conserved. GAD2, unlike the other *Arabidopsis* GADs, does not possess serine, threonine or tyrosine residues, which are potential phosphorylation sites. The single tryptophan residue of the C-terminal region of two separate *Petunia* GAD peptides binds the N- and C-terminal lobes of calmodulin, suggesting that binding Ca^{2+}/calmodulin to GAD dimerizes the protein, which might be necessary for activation[35].

Regulation by intracellular and intercellular transport

Separation of GABA synthesis from GABA catabolism by subcellular compartmentation is another potential mechanism for regulating GABA levels (Fig. 2). A recent study investigated the subcellular localization of GABA shunt enzymes in protoplasts prepared from developing soybean cotyledons[23]. Protoplast lysate was fractionated by differential and continuous percoll-gradient centrifugation to separate the organelle fractions. GAD is located exclusively in the cytosol, whereas GABA-T and SSADH are associated exclusively with the mitochondrial fractions. However, these results might be complicated by the marked instability of α-ketoglutarate-dependent GABA-T activity[21]. Mitochondrial fractions also catabolize [U-^{14}C]GABA to labeled succinate[23]. These results provide convincing evidence for the transport of GABA from the cytosol across the mitochondrial membranes into the matrix. An explanation for the presence of both pyruvate- dependent and α-ketoglutarate-dependent GABA-T activities in mitochondria[21] is not obvious.

Biochemical characterization of GABA transportation into isolated plant mitochondria has been unsuccessful (K.E. Breitkreuz and B.J. Shelp, unpublished), and molecular techniques to isolate transport genes from organelles are not yet available. However, proteins that transport GABA have been cloned. Characterization of known amino acid transporters by heterologous complementation of a plasma membrane GABA-transport-deficient yeast mutant led to the identification of plant H^+-coupled GABA transport proteins[36]. ProT2 and AAP3 from *Arabidopsis* have K_m for GABA of 12.9 and 1.7 mM, respectively. The effect of external pH on the simultaneous transport of [1-^{14}C]GABA and [2,3-^{3}H]proline into yeast expressing *AtProT2* provides evidence that zwitterionic GABA is the preferred form of GABA. ProT2-mediated [1-^{14}C]GABA transport is inhibited by proline, choline and glycine betaine. Direct evidence is provided for the transport of [methyl-^{14}C]choline. In another study, yeast that expresses *LeProT1*, transports GABA and proline with low affinity and transports glycine betaine with high affinity[37]. Thus, the ProTs might represent general transporters for these metabolites in plants.

The cellular location of AAP3 and ProT2 has not been demonstrated *in planta*, but presumably they are part of the plasma membrane. Because *AAP3* and *ProT2* are constitutively expressed in roots and all tissues, respectively, and *ProT2* expression is induced strongly by salt and drought stress[38], it is possible that GABA undergoes intercellular transport during normal and water deficit conditions. Regulation of these transporter proteins, in conjunction with a putative mitochondrial GABA transporter, might affect cytosolic GABA levels by controlling the influx of GABA either into or within the cell. Extracellular GABA presumably arises from its efflux[13], it is a well documented component of the xylem fluid[5,13]. Thus, cellular GABA accumulation might be the result of increased synthesis, decreased catabolism by mitochondrial enzymes and/or intra- or intercellular transport (Fig. 2).

Roles of GABA synthesis

Biochemical pH-stat

Because GAD activity consumes H^+ (Fig. 1), it has been proposed that stress-induced GABA synthesis can contribute to pH regulation[6]. Early *in vivo* NMR spectroscopy data demonstrated that the imposition of anoxia on corn root tips and the corresponding reduction in cytosolic pH involves the transient production of lactate, and a lag in the synthesis of ethanol[39]. However, it was argued that most of the GABA accumulation occurs after the predominant acid-generating reactions have ceased. Two independent investigations have provided direct evidence for GABA accumulation in response to cytosolic acidification. A fluorescent pH probe and an enzymatic assay for GABA were employed to measure cytosolic pH changes and GABA accumulation in photosynthetic asparagus cells exposed to permeant weak acids[26]. Cytosolic pH decreases by 0.6 with a half-time of 2 s, and GABA levels increase by 200–300% within 15 s. It is calculated that after 45 s of weak acid treatment, H^+-consuming GABA production accounts for ~50% of the imposed acid load. *In vivo* ^{31}P and ^{15}N-NMR spectroscopy was employed to monitor cytosolic pH and GABA levels in aerated, cultured carrot cells[25]. The initiation of ammonium assimilation causes a decline in cytosolic pH by 0.2 units, followed by an accumulation of GABA. GAD activity increases with reduced pH, and declines as the pH recovers. Acid-stimulated GABA synthesis does not involve Ca^{2+} flux because of acidification[31]. Thus, GABA accumulation can ameliorate cytosolic acidification.

Krebs cycle bypass

When glutamate C enters the Krebs cycle as α-ketoglutarate, its conversion to succinate requires NAD (Ref. 6; Fig. 1).

Alternatively, glutamate C might enter as succinate via the GABA shunt, bypassing the dehydrogenase or transaminase reaction and the α-ketoglutarate dehydrogenase of the Krebs cycle. Therefore, during certain conditions such as hypoxia, decreases in respiration, and the resultant decrease in the [NAD] to [NADH] ratio, the NAD-dependent SSADH reaction and the entry of carbon into the Krebs cycle is limited, thereby causing GABA to accumulate. This GABA provides an immediate substrate upon recovery from stress[8]. The metabolism of glutamate to succinate via the GABA shunt is energetically less favourable (1 NADH) than via the Krebs cycle (1 NADH + 1 ATP). If glutamate dehydrogenase provides a major entry point into the Krebs cycle, thereby generating an additional NADH, the energetics for the GABA shunt are relatively less favourable.

GABA does not always accumulate under stress. For example, hypoxia rapidly decreases the rate of [U-^{14}C]glutamate catabolism in developing excised soybean cotyledons, and the ^{14}C-content of the GABA pool remains unchanged[20]. Furthermore, under nitrogen limitation, tobacco seedlings rapidly partition more ^{14}C from [U-^{14}C]glutamate into the Krebs-cycle organic acids, but the size and ^{14}C-content of the GABA pool is not enhanced[33]. Glutamate deamination–transamination is apparently not limiting in these organs[20,40]. One interpretation of these results is that metabolism via the GABA shunt is not necessarily associated with stress conditions.

Nitrogen storage

The conversion of glutamate to GABA is increased under conditions that inhibit glutamine synthesis, reduce protein synthesis or enhance protein degradation[7,33]. This prompted the hypothesis that GABA is a temporary nitrogen store. Developing, excised soybean cotyledons rapidly metabolize glutamate to GABA (Ref. 41). The disappearance of nitrogen from glutamate and GABA within 3 h accounts for 49% of that required for protein synthesis during this same period. Furthermore, the glutamate flux through the GABA shunt is comparable to the direct incorporation of glutamate into protein[40]. Evidence also indicates that glutamate and GABA are produced during protein storage and mobilization as a means of recycling arginine-derived nitrogen and carbon[41]. Thus, glutamic acid metabolism via the GABA shunt might be of considerable importance in the nitrogen economy of plants.

Studies using isolated vacuoles show that 50% of glutamate, GABA and alanine is located inside the vacuole[5]. Feeding studies with [U-^{14}C]glutamate indicate that newly synthesized ^{14}C-GABA is not in ready equilibrium with previously synthesized unlabeled GABA (Ref. 13). This suggests that the locations of GABA production and accumulation are not identical, and that accumulated GABA is sequestered within organelles (Fig. 2).

Compatible osmolyte

AtProT2 can be induced by water stress, and AtProT2 and LeProT1 transport GABA as well as other stress-related compounds, such as proline and glycine betaine[36–38]. These findings indicate that GABA might have a role as a compatible osmolyte[42]. All three compounds are zwitterionic at neutral pH, are highly soluble in water, can accumulate to low mM concentrations, and apparently contribute no toxic effects to the cell. At high concentrations (25–200 mM), GABA stabilizes and protects isolated thylakoids against freezing damage in the presence of salt, exceeding the cryoprotective properties of proline[43]. In addition, GABA possesses *in vitro* hydroxyl-radical-scavenging activity, exceeding that of proline and glycine betaine at the same concentrations (16 mM)[44]. GABA might be synthesized from γ-aminobutyraldehyde (a product of the polyamine catabolic pathway[45]) by the chloroplast-localized betaine aldehyde dehydrogenase, which is involved in glycine betaine synthesis[46], but the relative fluxes via polyamines versus glutamate decarboxylation are unknown. Whether GABA has a specific role (i.e. osmolyte or osmoprotectant) under water stress, or is metabolized (e.g. to support the production of known osmolytes, such as proline) is unknown.

Plant development

GAD is one of the most abundant soluble proteins with a Ca^{2+}/calmodulin binding domain[15,47]; it is found in all plant tissues and the level is regulated during development by transcription or post-transcriptional processes[34]. Transgenic tobacco plants expressing a mutant GAD that lacks the auto-inhibitory calmodulin-binding domain, exhibit higher GABA levels, lower glutamic acid levels and less stem elongation[47]. Although growth inhibition might be attributed to lower glutamic acid levels, high GABA concentrations can inhibit stem elongation in *Stellaria*[48]. Separate investigations show that mechanical manipulation transiently increases cytosolic Ca^{2+} (Ref. 27), increases GABA (Ref. 49) and inhibits stem elongation[50]. In the stem-elongation study, growth inhibition in response to manipulation is blocked by Ca^{2+} chelators and calmodulin antagonists. Mechanical stimulation of dark- or light-grown soybean hypocotyl tissue results in >65% growth inhibition within 1 min (A.W. Bown and G. Zhang, unpublished); inhibition is accompanied by rapid fourfold and tenfold increases in GABA levels, respectively.

The hormones α-naphthaleneacetic acid and kinetin induce dedifferentiation of root tissue[51]. ^{15}N-NMR studies demonstrate that these changes are accompanied by enhanced GABA and reduced glutamate levels. GABA accumulation is accompanied by efflux from the cell, suggesting that it might function as an intercellular signalling molecule[13]. Treatment of excised sunflower cotyledons with GABA stimulates ethylene production, mainly by promoting transcript abundance of 1-aminocyclopropane 1-carboxylic acid synthase[48,52]. Thus, the emerging literature suggests that environmental stresses elevate cytosolic Ca^{2+}, which activates GAD and GABA synthesis, which in turn, binds to receptors, thereby regulating growth and development. To date, the existence of GABA receptors remains speculative.

Plant defence

In animals, GABA is an inhibitory neurotransmitter. It hyperpolarizes the neural membrane by stimulating Cl^- influx through $GABA_A$-gated Cl^- channels. Phytophagous activity by insects and other invertebrates destroys vacuolar compartmentation, increases H^+ levels in the cytosol and stimulates GABA synthesis. The hypothesis that the consequent ingestion of GABA inhibits normal growth and development has been investigated[49]. Within ~2 min of simulating the mechanical damage resulting from phytophagous activity, soybean GABA levels increase to ~2 μmol g^{-1} fresh weight, a ten- to 25-fold increase. This level of GABA introduced into the synthetic diet of the phytophagous larvae of the oblique-banded leafroller (*Choristoneura rosaceana*), reduces their rates of growth, development and survival. In addition, the larvae frequent terminal, light-green expanding leaves, which produce lower GABA levels than mature leaves when damaged. Many commercially employed insecticides are antagonists and agonists of the GABA-gated Cl^- current, and are thought to inhibit normal neuromuscular activity; ingested GABA might have a similar effect.

Conclusions and future prospects

Over the past decade, research on GABA has uncovered some of the most exciting findings in the field of plant biology. Evidence indicates that stress initiates a signal-transduction pathway, in

which increased cytosolic Ca^{2+} stimulates Ca^{2+}/calmodulin-dependent GAD activity and GABA synthesis. It is clear that GAD activity is also stimulated by H^+ and substrate levels. Control of GABA accumulation is probably mediated via GAD, although more information is needed on the control of GABA-T and SSADH, and the intracellular and intercellular transport of GABA. Experimental evidence supports the involvement of GABA synthesis and the GABA shunt in various plant processes. There is a need for better characterization of the various metabolic and transport steps involved at both the biochemical and molecular levels, including an investigation of the stress responsiveness of promoter regions and the localization of the GAD isoforms, and the subcellular localization of the GABA shunt enzymes. The potential interactions between the metabolic paths for GABA, proline and betaine synthesis, and the identification and isolation of a GABA receptor should be investigated. More pressing perhaps, is the continued need to generate sense and anti-sense plants and to find knock-out mutants. Only through such efforts can the physiological function(s) of GABA in plants be determined.

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Barry J. Shelp* and Michael D. McLean are at the Dept of Plant Agriculture, Division of Biotechnology, Bovey Bldg, University of Guelph, Guelph, Ontario, Canada N1G 2W1; Alan W. Bown is at the Dept of Biological Sciences, Brock University, St Catharines, Ontario, Canada L2S 3A1.

*Author for correspondence (tel +1 519 824 4120 ext. 3089; fax +1 519 767 0755; e-mail bshelp@evbhort.uoguelph.ca).

Virus resistance and gene silencing: killing the messenger

Peter M. Waterhouse, Neil A. Smith and Ming-Bo Wang

On occassion, virus-derived transgenes in plants can be poorly expressed and yet provide excellent virus resistance, and transgene constructs designed to supplement the expression of endogenous genes can have the effect of co-suppressing themselves and the endogenous genes. These two phenomena appear to result from the same post-transcriptional silencing mechanism, which operates by targeted-RNA degradation. Recent research into RNA-mediated virus resistance and co-suppression has provided insights into the interactions between plant viruses and their hosts, and spawned several models to explain the phenomenon.

The majority of plant-infecting viruses have RNA genomes that contain replication, movement and coat-protein genes. Initially it was thought that over-expressing one or more of these proteins in a normal or a dysfunctional state in transgenic plants would confer protection against the virus from which the transgene was derived. Although there have been some examples where this appears to be true, there are several others in which the transgene appears to have conferred resistance through its mRNA rather than by its encoded protein. This was shown first in 1992 when virus-resistant plants expressing untranslatable coat-protein mRNA were produced[1]. Since then there have been many examples of RNA-mediated resistance (RMVR) and they appear to share several features[2]:

- No transgene protein is required.
- Usually plants contain multiple transgene copies.
- Often associated with a high transcription rate but low steady state levels of transgene mRNA.
- Plants are either resistant to virus infection (no detectable virus replication, spread or symptoms) or initially show virus infection and symptoms, but subsequently produce new growth that is symptomless and resistant to virus infection.
- Usually associated with methylation of transgenes coding regions.
- Plants have resistance only to closely related virus strains.

A few years before RNA-mediated resistance was discovered, co-suppression, a phenomenon that results in the silencing of both a transgene and its homologous endogenous gene, was described. Co-suppression was first uncovered during attempts to over-express *chalcone synthase* (*chs*), a gene encoding a flower intermediate pigment biosynthesis enzyme, in *Petunia*[3,4]. As well as producing plants with purple flowers that are no different from

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