Extracellular γ-Aminobutyrate Mediates Communication between Plants and Other Organisms[1]

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Plants exhibit mutually beneficial and antagonistic interactions with a variety of prokaryotic and eukaryotic organisms. We argue that these interactions are mediated in part by extracellular plant-derived γ-aminobutyrate (GABA).

GABA is a ubiquitous four-carbon, nonprotein amino acid that is synthesized from Glu in a reaction catalyzed by the cytosolic enzyme Glu decarboxylase (GAD; Bown and Shelp, 1997; Shelp et al., 1999; Bouché and Fromm, 2004). It is noteworthy that plant GAD possesses a calmodulin (CaM)-binding domain that enables in vitro activity at pH 7.0 to 7.5 to be stimulated by the Ca^{2+}/CaM complex. In contrast, activity at acidic pH values is unaffected by Ca^{2+}/CaM and has a sharp pH optimum at 5.8. Subsequently, the GABA carbon is converted into succinic semialdehyde, and then to succinate, in reactions catalyzed by two mitochondrial enzymes, GABA transaminase and succinic semialdehyde dehydrogenase, respectively.

In invertebrates (Sattelle, 1990) and vertebrates (Bormann, 2000), GABA is a potent inhibitory neurotransmitter. In plants, rapid GABA accumulation occurs in response to a variety of stresses (Bown and Shelp, 1997; Shelp et al., 1999; Kinnersley and Turano, 2000); however, the function(s) of that accumulation remains contentious. In many studies, accumulation results in the appearance of extracellular GABA, either in the apoplast or external medium (Secor and Schrader, 1985; Chung et al., 1992; Crawford et al., 1994; Solomon and Oliver, 2001). Extracellular GABA could result from exocytosis across the plasma membrane using a mechanism similar to that found in animal neurons (McIntire et al., 1997). In addition, wounding and associated disruption of cellular compartmentation release the acidic vacuolar contents to the cytosol, thereby activating GAD and stimulating GABA release from the wound to the external medium (Bown et al., 2006).

Several papers demonstrate that plant-derived extracellular GABA mediates communications between plants and animals, fungi, bacteria, and other plants. To our knowledge, the earliest known example involves the crustose red algae (e.g. *Lithothamnium* spp.) and the planktonic larvae of the gastropod mollusk *Haliotis rufescens* Swainson, the large red abalone of the eastern Pacific. Applied GABA triggers transitional developmental processes bridging the motile larval and benthic juvenile stages (Morse et al., 1979). The larvae are induced to settle, attach to substrata, and metamorphose by chemosensory recognition of GABA-mimetic molecules uniquely associated with the algal surface (Morse and Morse, 1984). Subsequently, GABA receptors were characterized in *H. rufescens* and identified as those receptors controlling metamorphosis (Trapido-Rosenthal and Morse, 1986). Juveniles gain nutrients through nondestructive grazing of the algal surface and algae are kept relatively free of overgrowth by epibionts (Morse and Morse, 1984).

A more recent example of GABA-mediated interactions involves plants and invertebrate pests. Insect larvae raised on synthetic diets containing elevated GABA concentrations exhibit reduced growth and survival rates, and increased time to pupation (Ramputh and Bown, 1996). Crawling of tobacco budworm (*Heliothis virescens* Fabricus) and oblique-banded leafroller (*Choristoneura rosaceana* Harris) larvae on plant-attached tobacco (*Nicotiana tabacum*) and soybean (*Glycine max* L. Merr.) leaves, respectively, increases GABA concentrations by 4- to 12-fold within 5 to 10 min (Bown et al., 2002). This result is attributed to wounding of the leaf surface by a perimeter row of hook-like, gripping crotchets attached to larval feet (Hall et al., 2002). The ingestion of elevated GABA concentrations in transgenic tobacco plants overexpressing a full-length GAD or a truncated GAD lacking the autoinhibitory CaM-binding domain reduces feeding by tobacco budworm larvae (MacGregor et al., 2003) and impairs the normal development of the northern root-knot nematode (McLean et al., 2003). Since GABA is an inhibitory neurotransmitter in animals and there is no barrier between the nervous system and neurotransmitters present in the hemolymph of insect larvae, it has been proposed that excess GABA

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activates chloride channels at neuromuscular junctions, thereby disrupting normal physiological and developmental processes of invertebrate pests (Bown et al., 2006).

GABA functions in communication between tomato (*Lycopersicon esculentum* var *commune* Bailey) plants and the fungus *Cladosporium fulvum* (Oliver and Solomon, 2004). Hyphae of *C. fulvum* do not enter the tomato cell and are restricted to the apoplast; therefore, the fungus is dependent on the contents of the apoplast for its nutrients. During infection, the GABA concentration in the apoplast increases from about 0.8 mM to 2 to 3 mM, a result that can be attributed to the stimulation of GAD activity by decreased pH and increased cytosolic calcium, which are associated with pathogen attack (Solomon and Oliver, 2001). Furthermore, GABA transaminase and succinic semialdehyde dehydrogenase are induced in the fungus, indicating that GABA is being utilized as a nutrient source (Solomon and Oliver, 2002). Thus, the fungus somehow alters the physiology of the plant, resulting in the enhanced production of GABA, which in turn plays a signaling role in the induction of fungal enzymes responsible for its degradation.

GABA also mediates communication between plants and bacteria. Chevrot et al. (2006) investigated the role of GABA during the infection of plants by *Agrobacterium tumefaciens*. The addition of GABA or wounded tomato stems to an Agrobacterium culture induces the expression of the *attKLM* operon and stimulates the production of lactonase AttM, which in turn inactivates *N*-(3-oxooctanoyl) homoserine lactone. The lactone is implicated in the control of conjugation of the Ti plasmid and severity of tumoral symptoms. The researchers also demonstrated that extracellular GABA enters the pathogenic bacterium via the GABA transporter Bra; wounding rapidly decreases the concentration of Glu in tomato stems with a corresponding increase in GABA; and transgenic tobacco plants with elevated GABA levels are less susceptible to *A. tumefaciens* infection than wild-type plants. These findings indicate that wound-induced GABA accumulation reduces Agrobacterium virulence.

A gradient of GABA is required for the guidance of the pollen tube through the apoplastic spaces within the *Arabidopsis thaliana* L. Heynh pistil to the female gametophyte (Palanivelu et al., 2003). While this observation has been interpreted as evidence for the role of GABA in cell-to-cell communication within plants (Bouché et al., 2003; Palanivelu et al., 2003), it is also an example of GABA-mediated communication between cells from separate plants. In wild-type plants, GABA concentrations increase along the pollen tube path from 20 μM in the stigma, 60 μM in the style, 110 μM in ovary walls, and 160 μM in the septum to 500 μM in integument cells. GABA transaminase mutants lack the ability to degrade GABA and have similar concentrations of GABA in the pistil and ovule (approximately 1,500 μM), where integuments accumulate even more GABA (24 mM). In these mutants, pollen tube growth is arrested or misdirected. These findings are supported by pollen tube elongation in vitro at low GABA concentrations (1–10 mM) and inhibition of elongation at higher concentrations. It is unknown whether extracellular GABA influences pollen tube growth via a plasma membrane-located uptake system (Meyer et al., 2006) or receptor (Bouché et al., 2003). Yu et al. (2006) recently provided evidence for $GABA_B$ receptors on the plant cell membrane. They used a fluorescence probe of quantum dots to detect the presence of GABA-binding sites on the protoplast membrane of both pollen and somatic cells of tobacco, and demonstrated that these sites function in the regulation of endogenous Ca^{2+} level.

With the exception of GABA-mediated metamorphosis of abalone, the examples discussed above were uncovered within the last 5 years. Collectively, they suggest a recurring motif in which extracellular GABA is employed to mediate plant communication with other organisms. Further examples can be expected. Other research revealed that extracellular GABA modulates plant growth (Kathiresan et al., 1998) and mineral acquisition (Kinnersley and Lin, 2000). Recently, Beuvé et al. (2004) confirmed that GABA is translocated in phloem, and demonstrated that changes in phloem GABA are positively correlated with nitrate influx during nitrogen deprivation and over the growth cycle of rape (*Brassica napus*). Furthermore, extracellular GABA induces expression of a plasma membrane-located nitrate transporter and stimulates $^{15}NO_3$ influx by the root system. While these observations are consistent with the involvement of GABA in signaling, it is not clear whether they reflect a mechanism involving plant-derived extracellular GABA.

The mechanisms by which GABA functions in communication appear to be diverse. In abalone and insect larvae, GABA activates GABA neuronal receptors, whereas in C. *fulvum* GABA induces the synthesis of enzymes involved in its utilization. In Agrobacterium, GABA induces the synthesis of enzymes that modulate the infection process. Thus, it appears that plant-derived extracellular GABA mediates communication with other organisms via multiple mechanisms, in much the same fashion as GABA influences various processes within the plant (Bown and Shelp, 1997; Shelp et al., 1999; Kinnersley and Turano, 2000; Bouché et al., 2003).

Note Added in Proof

In a recent article, Lancien and Roberts (**Lancien M, Roberts MR** [2006] Regulation of *Arabidopsis thaliana* 14-3-3 gene expression by γ-aminobutyric acid. Plant Cell Environ **29**: 1430–1436) reported that GABA influences the expression of 14-3-3 genes, which are important players in the regulation of carbon and nitrogen metabolism in plants. The results indicate that GABA specifically down-regulates the expression of a large subset of 14-3-3 gene family members in Arabidopsis seedlings grown in the presence of high

external concentrations of calcium. This repression is dependent on functional ethylene and abscisic acid signaling pathways.

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