Front. Biol. China 2008, 3(4): 439–442
DOI 10.1007/s11515-008-0095-x

REVIEW

The language of GABA in pollen tube growth and guidance

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Abstract The achievement of double fertilization in higher plants requires the successful transport of sperm cells to the female gametes, the ovules. Pollen tubes, the tubular structure protruding from pollens, carrying the sperms play an important role in this process. How a pollen tube precisely guides its direction to gain its goal is of mystery. Previous investigation indicated that multiple signal clues from the pistils function as the route signs to regulate the pathway of pollen tube growth. Among the signal clues, γ-aminobutyric acid (GABA) seems to be universal. Its gradient concentration has been found both in tobacco and *Arabidopsis* pollen tube guidance. In the communication of pollens and pistils, what on earth GABA tells pollen tubes is of great interest. The GABA receptors on the pollen membrane are thought to be the hinge in the language conversation. In this review, the mechanism of GABA gradient formation is investigated. The possible GABA receptor on the pollen membrane is examined and its function is discussed. To decipher the possible language of GABA in pollen tube growth and guidance, multiple methods are needed. The combination of transcriptome and proteomics assay is expected to unveil the secret.

Keywords double fertilization, γ-aminobutyric acid (GABA) receptor, pollen tube guidance, signal transduction

1 Introduction

γ-aminobutyric acid (GABA), a four-carbon non-protein amino acid, is ubiquitous in living organisms. In vertebrate, GABA acts as a neurotransmitter to control the excitation and inhibition of the central nervous system (Kaupmann et al., 1997). It exerts its effect on both the ionotropic and metabotropic receptors to regulate the equilibrium of ions (Ganguly et al., 2001; Bettler et al., 2004). In clinical application, its psychotherapeutic effect, such as the treatment of drug addiction and epilepsy, has been the hotspot. In plant kingdom, the chief function is its osmotic adaptation to various stresses, including hypoxia, acidosis, mechanical stress, or cold stress (Shelp et al., 1999). Moreover, increasing evidence demonstrate that GABA may also function as an extracellular and intracellular signaling molecule involved in regulating certain plant physiological processes. For example, GABA may stimulate ethylene biosynthesis in sunflowers as part of the senescence process (Kathiresan et al, 1997). It specifically up-regulates the uptake of nitrate and the expression of the nitrate transporter gene (*BnNrt 2*) in *Brassica napus* and regulates the expression of the 14-3-3s gene family members in *Arabidopsis* seedlings (Beuve et al., 2004; Lancien et al., 2006). More interestingly, the gradient of GABA in the pistils of *Arabidopsis* flowers can regulate pollen growth and orientation (Palanivelu et al., 2003). This finding has broadened our understanding of the role of GABA in plants. On the other hand, how pollen cells sense the GABA gradient to control the direction of growth in fertilization is largely unknown. The present research is aimed to decipher the mechanism underlying the function of GABA in regulating the growth and orientation of pollen tubes. We focused on study of pollen tube growth and guidance in tobacco, another model plant in plant reproduction. Our investigation provides clues for further research in this area.

2 Guidance clues from the female tissues

Pollination and fertilization are the key events in sexual reproduction of higher plants. The alternation of generations between the gametophyte and the sporophyte is accomplished elegantly during this process. Pollen, the male gamete, via the special media such as insects, will be brought onto the stigma of pistil, the female tissue of flowers. On a compatible stigma, the tubular structure, i. e., pollen tubes, will be protruded from the pollen. And then, the pollen tubes, carrying the two gametes, penetrate

Received August 28, 2008; accepted September 17, 2008

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into the stigma and travel the long distance in the transmitting tract within the style and reach the ovules embedded in the ovary (Fig. 1). A mature ovule, the female gamete, contains 8 cells (1 egg cell, 1 central cell, 2 synergid cells and 3 antipodal cells). When the pollen tube is burst up after it penetrates into the micropyle of the ovule, two sperms are delivered and fused with the egg cell and central cell respectively to fulfill double fertilization.

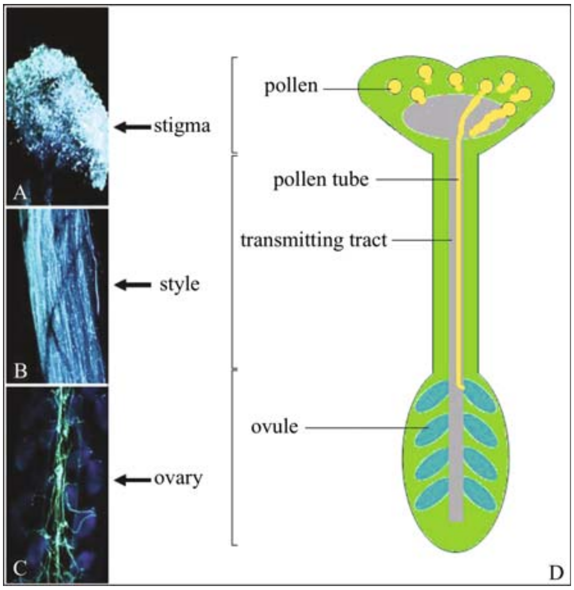

Fig. 1 The pathway for pollen tube growth and guidance. A: Stigma of the pistil. The stigmatic papillae, distributed on the surface of the stigma, are stained with aniline blue. B: Pollen tubes. The bright blue, long filament structures as indicated, travel in the transmitting tract within the style. C: The pollen tubes in the ovary. When pollens reach the ovules, they turn away from the transmitting tract and grow towards the ovules. The bright blue indicates the bundles of pollen tubes, and the light blue indicates the ovules. D: A cartoon simply depicts the long distance of pollen tube growth, corresponding to A, B, and C panels. A, B, and C were obtained from the observation of fluorescence microcopy. D was provided by the courtesy of Dr. Toshiyuki Mori.

The fantastic point in the progamic phase (the phase between the pollination and fertilization) is that a pollen tube must navigate its pathway through the barriers one after another in the pistils, at last exactly through the micropyle to achieve the fertilization. The overwhelming viewpoint is that the guidance signal clues from the female tissues provide the pathfinding for pollen tube pathway (McCormick and Yang, 2005). The lure signal discovered is glycosidoprotein, a signal peptide or the small amino acid (Wu et al., 1995; Kim et al., 2003; Márton et al., 2005; Escobar-Restrepo et al., 2007). Previous investigations indicated that the signal clues for pollen tube guidance secreted from female tissues may be from different sections of the pistil. Arabinogalactan proteins, one kind of glycosidoprotein, for example, existing in the transmitting tract of tobacco style, can induce chemotropic growth of tobacco pollen tube *in vitro* (Wu et al., 1995). In lily hollow style, the secreted chemocyanin fills the style channel, exerting similar effect on lily pollen tubes as arabinogalactan protein (Kim et al., 2003). After the pollen tubes enter the opening of ovules, micropyles, the short distance signal clues, are secreted from the egg apparatus, some from the egg cell, some from the synergid cells, and some from the central cell. Zea mays Egg Apparatus 1 protein, for example, a 94 amino acid peptide, is necessary for the short distance guidance of pollen tube growth of Zea mays (Márton et al., 2005). In *Arabidopsis thaliana*, a kinase from the synergid cells plays a crucial part in the reception of pollen tubes (Escobar-Restrepo et al., 2007). However, there is less evidence to demonstrate how pollen tubes perceive these signal clues. Moreover, most of the signals are discovered from different species. Therefore, the guidance clues are connected with specific uniqueness, while the guidance mechanism is still unclarified.

The discovery of the role of GABA in plant reproduction sheds new light on this topic. Studies have demonstrated that disruption of the gradient of GABA from the sigma to the ovule resulted in mis-guidance of pollen tube growth in *Arabidopsis* (Palanivelu et al., 2003). The target of our study was to decipher the mechanism of GABA in pollen tube guidance as an omnipresent signal. We focused on the fertilization events of another model plant, *Nicotiana tabacum*.

3 Mechanisms controlling the GABA gradient in the flower pistil

In plants, the process of GABA biosynthesis has been elucidated. GABA is synthesized from glutamate through catalysis of glutamate decarboxylase (GAD; EC4.1.1.15), which is the limiting enzyme in GABA production (Fig. 2). The oxidation of GABA is catalyzed by GABA transaminase (GABA-T, EC2.6.1.19) to succinic semialdehyde (SSA), which joins the tricarboxylic acid cycle (TCA). This process is taken place in the mitochondrion.

The gradients of gene expression products usually are important biological factors regulating cell fate. For example, several morphogens form gradients that control early *Drosophila* embryo pattern formation (Lewin, 2004). Generally, gradients can form and develop in one of two basic ways. Firstly, the maintenance of gradients can be achieved by the passive diffusion of gene products in intercellular spaces or by active transmission of morphogen from cell to cell. Secondly, gradients may also be formed by the ordinal degradation of gene products along tissues. A previous investigation showed that the disruption of GABA gradient in pistils resulted from the mutation of the degradation gene, GABA transaminase (or POP2) (Palanivelu et al., 2003).

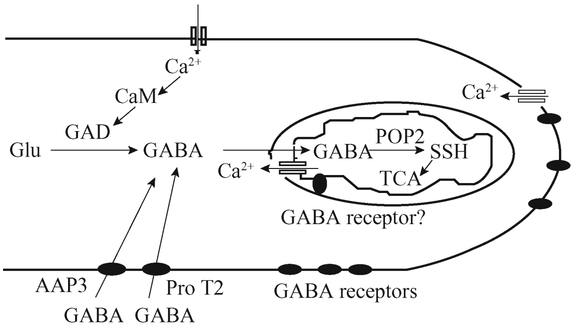

Fig. 2 A hypothetic model of GABA receptor on the plant membrane of tobacco pollen protoplasts and its regulation on the oscillation of Ca^{2+}. Glu: glutamate; AAP3: amino acid permease 3; ProT2: proline transporter; GABA: γ-amino-butyric acid; GAD: glutamate decarboxylase; CaM: calmodulin; POP2: GABA transaminase; SSH: succinic semialdehyde; TCA: tricarboxylic acid cycle.

Consistent with the understanding aforementioned, it is also true that the maintenance of GABA is closely related to its synthesis. The result of high performance liquid chromatography assay shows that the gradient of GABA in the tobacco pistil indeed exists from the top to the bottom, and the concentration of GABA in the bottom is about twice that in the top pistil (Yu, 2006). In line with this, the assay of cryosection and immunofluorescence indicates that GAD is mainly distributed in the parenchyma cells and vascular bundles of the stigma and style, through which pollen tubes may pass, implying the coupling between the synthesis of GABA and its transportation.

4 GABA binding sites detected by quantum dot probes

Chemotropic growth of pollen tubes towards GABA indicates that this event has much more relationship with the GABA receptor on the pollen membrane. However, we know nothing about GABA receptors in plants. The corresponding genes in animal genomes have not been found as the homologs in *Arabidopsis* genome. It is questionable whether GABA receptors are present in plants. Whereas, to understand the mechanism of GABA function in pollen tube guidance, it is of great importance to know whether GABA receptors exist on the membrane surface of pollen.

To detect the possibility of the existence of GABA receptors on pollen membranes, the Quantum Dots technology was employed to synthesize a novel fluorescent probe in our experiments (Yu et al., 2006). For this purpose, water soluble CdSe-ZnS (core-shell) QDs were synthesized. GABA was then bioconjugated to the QDs in the presence of 1-ethyl-3-(3)-dimethylaminopropyl carbodiimide (EDC) and N-hydroxysuccinimide (NHS) to synthesize the fluorescence probe. Using the fluorescence probe, GABA-binding sites were localized on pollen protoplast membrane in tobacco (Fig. 3A, B). Its function of regulation of the level of calcium oscillation was further assayed (Yu et al., 2006). It is notable, however, that the fluorescence signals are not evenly distributed. Perhaps, this asymmetric distribution of GABA binding sites provides the pollen with much more information. It is interesting to test whether this asymmetric distribution is related with the perceiving of the GABA gradient, as the situation of nerve growth cones (Bouzigues et al., 2007).

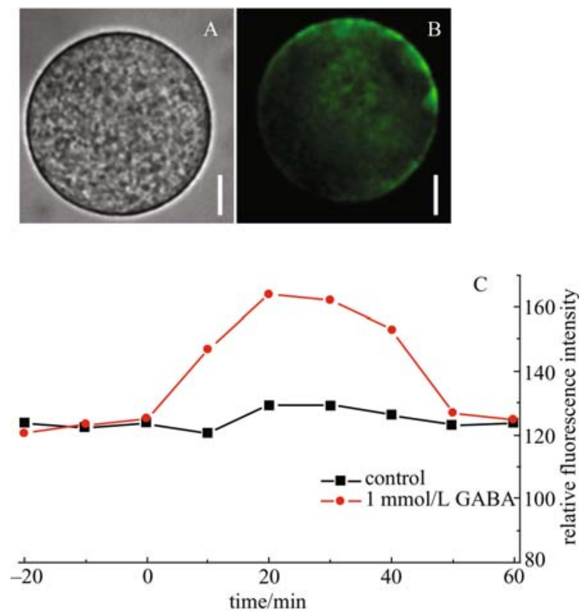

Fig. 3 Detection of GABA binding sites on the membrane of tobacco pollen and the time course of GABA regulation of calcium oscillation. A: Tobacco pollen protoplast observed with bright field microscope. B: The GABA binding sites (green fluorescence as indicated) on the membrane of pollen was detected by quantum dot probes. C: Transient Ca^{2+} oscillation pattern after the addition of GABA. The level of Ca^{2+} was marked by the relative fluorescence intensity of Ca^{2+} probe, Fluo-3/AM. Bars, 2 μm.

Calcium plays a crucial part in the growth of pollen tubes because it acts as a second messenger involved in pollen tube growth regulation. The relationship between the extracellular signal, GABA and the intracellular second messenger, dynamic Ca^{2+} level was assayed via the Ca^{2+} probe Fluo-3/AM. The result showed that GABA could trigger transient enhancement of Ca^{2+} (Fig. 3C). However, it soon returned to a dynamic balance. The possible reason is that higher concentrations of calcium may be harmful to pollen tube growth. The promotion of GABA on pollen tube growth and its regulation of calcium oscillation further confirm the possibility of the existence of GABA receptor on the pollen membrane. The calcium signature response to the GABA signal is probably the relay station to integrate multiple complex signal transduction pathways.

5 Multi-tactics to disrupt the signal transduction of GABA in pollen tube growth and guidance

Although the GABA binding sites have been detected and the extracellular GABA could regulate the calcium oscillation, it is still a myth about the GABA receptor. Much more unexpected, there is no matching sequence in the *Arabidopsis* genomes with animal GABA receptor genes. However, with the development of model molecular biology, it is promising to unveil the mysterious yashmak. In the present situation, multi-tactics should be employed, for example, the combination of the transcriptome and proteomics technology on a large scale, to achieve the goal.

The tip growth of pollen tubes is a complex system involving lots of signal molecules (Feijó et al., 2004; Yu and Sun, 2007). From the viewpoint of transcriptome, emphases should be laid on the genes affected by GABA, no matter whether they are up- or down-regulated. Perhaps the regulation of GABA on pollen tube growth and guidance involves multiple parallel signal transduction pathways. This study at transcriptome level will effectively integrate the related information and provides a comprehensive viewpoint.

Although transcriptome investigation will provide useful information of genes at mRNA level, the product of genes will ultimately be expressed as the function of protein. So proteomics study is a very powerful approach to link the genome and protein functions. The study of protein functions should focus on the dynamics of membrane proteomics because membrane proteins are at the interface between extracellular environment and intracellular compartments and play a significant part in cell signaling, osmoregulation, nutrition, and metabolism (Marmagne et al., 2007). Identification of the genes encoding membrane proteins is a key point for better understanding of the role of GABA in pollen tube growth. The rapid pollen tube growth could be reflected by the dynamic reconstruction of membrane proteins. Therefore, the intrinsic proteins on the pollen membrane greatly affected by GABA should be potential GABA receptors.

Uncovering the secret role of GABA in the process of pollination and fertilization, indeed, needs lots of work. However, it is fascinating to decipher the dialogue between pollen tubes and pistils, which will help understand the mechanisms of GABA involved in the guidance of pollen tubes in the near future.

Acknowledgements This work was supported by the Natural Science Foundation of South-Central University for Nationalities (No. yzz07001) and sponsored by the National Natural Science Foundation of China (Grant No. 30700427) and Key Basic Theoretical Research of Ministry of Science and Technology of the People's Republic of China (No. 2007CB947600).

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