Article Addendum

G protein α subunit may help zoospore to find the infection site and influence the expression of RGS protein

Chenlei Hua, Xiaobo Zheng and Yuanchao Wang*

Department of Plant Pathology; Nanjing Agricultural University; Nanjing, China

Key words: G protein α subunit, penetrating site, regulator of G protein signaling (RGS), phytophthora

Sensing chemical signal secreted from host root and find the best site for penetration are crucial for initiating infection of Phytophthora zoospore. G protein α subunit of P. sojae participates in not only the chemotaxis to soybean isoflavone, but also finding penetrating site. Furthermore, although calcium signal pathways are influenced by Gα, other signal pathways also influenced by G protein remain to be discovered. In this addendum, we describe an RGS protein, PsRGS6, is expressed downregulated in zoospores of Gα silenced mutant. This result indicates that the expression of Gα and RGS protein may be influenced by each other. Some differences between Gα mutants of P. infestans and P. sojae may be due to the different developmental procedures.

There are many phytopathogenic microbes in Phytophthora genus, which usually cause annual losses worldwide. In the field, fungus-like mycelia of Phytophthora differentiate to form multinucleate asexual sporangia, which could germinate to infect host plant or cleave cytoplasm to form and release uninucleate and nucleate asexual sporangia, which could germinate to infect host fungus-like mycelia of Phytophthora differentiate to form multinucleate asexual sporangia. In this addendum, we described an RGS protein, PsRGS6, is expressed downregulated in zoospores of Gα silenced mutant. This result indicates that the expression of Gα and RGS protein may be influenced by each other. Some differences between Gα mutants of P. infestans and P. sojae may be due to the different developmental procedures.

PsRGS6 silenced mutant encysted randomly to together, while zoospores of Gα silenced mutant encysted randomly on the surface of epidermis without aggregation. However, the same phenomenon was not observed on epidermis of soybean. Zoospores of P. sojae wild-type strain can penetrate soybean cell from either intercellular gap or normal cell wall (Fig. 2), which may be due to the different structure or components of plant cells. This result indicates that Gα has putative function on helping zoospore to find the best site of host for infection.

Gα and RGS Protein

Gα participates the signal transduction pathway, which controls P. sojae zoospore swimming to soybean isoflavone daidzein and some amino acids. In addition, it could help P. sojae zoospore to find best penetration site. We dropped zoospore suspensions of P. sojae Gα silenced mutant and wild-type strain onto the epidermis of onion. After 30 min in 25°C, zoospore encystment on epidermis of onion was observed under microscope. As show in Figure 1, most zoospores of wild-type strain encysted on the gap of two cells, and aggregated together, while zoospores of Gα silenced mutant encysted randomly on the surface of epidermis without aggregation. However, the same phenomenon was not observed on epidermis of soybean. Zoospores of P. sojae wild-type strain can penetrate soybean cell from either intercellular gap or normal cell wall (Fig. 2), which may be due to the different structure or components of plant cells. This result indicates that Gα has putative function on helping zoospore to find the best site of host for infection.

Gα Help P. sojae Zoospore to Find Not Only Host, but also Penetration Site

Gα transmits extracellular signals from G protein coupled receptors, which combined ligands, to downstream targets including adenyl cyclase, phospholipase and ion channels. It has been proven that silencing of Gα can cause upregulation of some calcium binding proteins. In addition, there are also some genes expressed downregulated following silencing of Gα. For example, expression of a gene-coding regulator of G protein signal (RGS), named PsRGS6 was analyzed in Gα silenced mutant and wild-type strain. The result showed that, PsRGS6 was expressed lower in Gα silenced mutant than that in wild-type strain (Fig. 3). RGS proteins have GTPase activity, which could hydrolyze GTP to GDP. That would allow Gα-GTP transforms to Gα-GDP, which reassociates with Gβγ dimer and inhibit Gα mediated signal transduction. Based on our results, PsRGS6 may function as an inhibitor of G protein signal, whose transcription is regulated by Gα.

Different Function of Gα Between P. infestans and P. sojae

P. sojae and P. infestans are two representative Phytophthora species with differences in development and host range. P. infestans is...
a air-borne species, sporangia of which can be separated from sporangiophores, and usually spread by wind or water to new potential sites of infection. Sporangial cleavage of *P. infestans* needs cool and moist conditions. In contrast, *P. sojae* is a soil-borne species, sporangia of which cannot be separated from sporangiophores, and sporangial cleavage does not need cool condition. The most important difference is that *P. sojae* zoospore can be attracted not only by some amino acids, which is the same as that of *P. infestans*, but also by isoﬂavones secreted from soybean roots, which may be partly determined by host range. These differences may be the reason for Gα mutants of *P. infestans* and *P. sojae* have some different phenotypes. There is only one copy of Gα subunit gene in all the sequenced Phytophthora genome. From the results of Northern Blot and RT-PCR, Gα is not expressed in nutrient mycelium, while it is expressed highest in sporangia or sporulating mycelia. However, there are still some different expression patterns of Gα in asexual development between *P. sojae* and *P. infestans*. Zoospore has to encyst to host surface before penetration. Gα are expressed in the same levels between zoospore and cyst in *P. infestans*. However, in *P. sojae*, it is expressed higher in zoospore than that in cyst (Fig. 4). So zoospore of Gα silenced mutant encysts very quickly than that of wild-type strain, while *P. infestans* Gα silenced mutant did not have the same phenotype in zoospore encystment. Gα mutant of *P. sojae* and *P. infestans* reduced their pathogenisity by different mechanisms. *P. sojae* zoospore reduced its ability of cyst germination, while *P. infestans* reduced its ability of appressorium formation. For *P. infestans*, the expression level of Gα in sporangium is much higher than that in zoospore, while for *P. sojae*, the expression levels in those two stages are similar. The reason may be in different functions of Gα in *P. sojae* and *P. infestans*. This indicates that G protein signal pathway in different Phytophthora spp. may participate in different signal transduction pathways.

**Conclusions and Future Directions**

The function of Gα in sporangial cleavage, zoospore motility and pathogenesis of Phytophthora has been found. However, there are no evidence revealed that Gα participates sexual development of Phytophthora. We supposed that, hormone signal could be transmitted independently from Gα mediated signal transduction pathway. There are 24 GPCRs in *P. sojae* genome, 12 of which are fused to a PIPK domain which are similar to Dictyostelium RpkA. These GPCR-PIPKs may transmit extracellular signals to cells which trigger phosphoinositide second messenger synthesis, and activate downstream signaling pathways which control sexual development of *P. sojae*. Although transcription of some putative downstream targets of G protein such as calcium binding proteins and RGS protein were found to be regulated by Gα, more effectors binding with Gα and regulated by Gα need to be found. The G protein involved signal transduction mechanisms also need to be deeply analyzed for disease control.

**References**

1. Erwin DC, Ribeiro OK. Phytophthora Diseases Worldwide: American Phytopathological Society 1996; St. Paul, MN, USA.
2. Tyler BM. Phytophthora sojae: root rot pathogen of soybean and model oomycete. Molec Plant Pathol 2007; 8:1-8.
3. Hua C, Wang Y, Zheng X, Dou D, Zhang Z, Govers F, Wang Y. A Phytophthora sojae G Protein [alpha] Subunit Is Involved In Chemotaxis To Soybean Isoflavones. Eukaryot Cell 2008; In Press.
4. Malbon CC. G proteins in development. Nat Rev Mol Cell Biol 2005; 6:689-701.
5. Wang Y, Ho G, Zhang JJ, Nieuwenhuijsen B, Edris W, Chanda PK, et al. Regulator of G protein signaling Z1 (RGSZ1) interacts with Gαi subunits and regulates Gαi-mediated cell signaling. J Biol Chem 2002; 277:48325-32.

6. Laxalt ML, Latijnhouwers M, van Hulten M, Govers F. Differential expression of G protein alpha and beta subunit genes during development of Phytophthora infestans. Fungal Genet Biol 2002; 36:137-46.

7. Bakthavatsalam D, Meijer HJ, Noegel AA, Govers F. Novel phosphatidylinositol phosphate kinases with a G-protein coupled receptor signature are shared by Dictyostelium and Phytophthora. Trends Microbiol 2006; 14:378-82.