The AGC Kinase MtIRE
A Link to Phospholipid Signaling During Nodulation?

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Original manuscript submitted: 03/07/07
Manuscript accepted: 03/07/07
Previously published online as a Plant Signaling & Behavior E-publication: http://www.landesbioscience.com/journals/psb/article/4115

KEY WORDS
AGC kinase, nitrogen fixation, nodulation, Medicago truncatula, Sinorhizobium meliloti, infection zone, 3-phosphoinositide-dependent kinase, root hair elongation

ACKNOWLEDGEMENTS
This work was supported by the National Science Foundation (IOB no. 0520728) and University of North Texas Faculty Research Funds to R.D. We thank Janine Sherrier for helpful comments.

Addendum to:
An IRE-Like AGC Kinase Gene, MtIRE, Has Unique Expression in the Invasion Zone of Developing Root Nodules in Medicago truncatula
Pislariu CI, Dickstein R
Plant Physiol 2007; 144:682–94
PMID: 17237187
DOI: 10.1104/pp.106.092494

ABSTRACT

The development of nitrogen fixing root nodules is complex and involves an interplay of signaling processes. During maturation of plant host cells and their endocytosed rhizobia in symbiosomes, host cells and symbiosomes expand. This expansion is accompanied by a large quantity of membrane biogenesis. We recently characterized an AGC kinase gene, MtIRE, that could play a role in this expansion. MtIRE’s expression coincides with host cell and symbiosome expansion in the proximal side of the invasion zone in developing Medicago truncatula nodules. MtIRE’s closest homolog is the Arabidopsis AGC kinase family IRE gene, which regulates root hair elongation. AGC kinases are regulated by phospholipid signaling in animals and fungi as well as in the several instances where they have been studied in plants. Here we suggest that a phospholipid signaling pathway may also activate MtIRE activity and propose possible upstream activators of MtIRE protein’s presumed AGC kinase activity.

ABBREVIATIONS

AGC kinase, cAMP dependent, cGMP dependent, and protein kinase C family; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; CCS52A, plant ortholog of yeast and animal Cdh1/Srw1/Fzr genes; DMI2, Does not Make Infections 2; DMI3, Does not Make Infections 3; dpi, days post inoculation; gusA, β-glucuronidase; IT, infection thread; MtIRE, Medicago truncatula Incomplete Root hair Elongation homolog; PA, phosphatidic acid; PDK1, 3-phosphoinositide-dependent kinase; P3K, Phosphatidylinositol 3-kinase; PLC, phospholipase C; PLD, phospholipase D; PtdIns, phosphatidylinositol; PtdIns3P, phosphatidylinositol 3-phosphate; TC, tentative consensus sequence.

During symbiotic nitrogen-fixing nodule development, both plant cells and rhizobia undergo cell division and expansion.1,3 In legume roots, nodule organogenesis is triggered by rhizobial Nod factor at the emerging root hair zone. In the indeterminate Medicago-Sinorhizobium symbiosis, inner cortical cell divisions form nodule primordia which emerge from the root and differentiate into complex nodule structures. Rhizobia enter the nodules through plant derived conduits, the infection threads (ITs). ITs begin in curled root hairs, grow through several cell layers and end at nodule primordia where rhizobia are deposited into host cell symbiosomes.2 In mature nodules, the meristematic zone I at the nodule apex contains dividing cells. Rhizobia from ITs infect these cells as they exit zone I and enter the infection zone, zone II. The newly released rhizobia, now termed bacteroids, are rod-shaped. In the distal part of zone II, bacteroids divide along with the symbiosome membrane (also called the peribacteroid membrane) that contains them.4 As the plant cells with their internalized bacteroids progress toward the proximal end of zone II, bacteroid division ceases. Bacteroid elongation and expansion of the surrounding symbiosome space and membrane is a feature of the proximal side of zone II.4 Enormous membrane biogenesis accompanies progression through zone II. As the cells exit zone II, both host cells and bacteroids stop expanding. Interzone II-III is characterized by starch accumulation and zone III is where nitrogen fixation takes place.

Members of the protein kinase AGC (for cAMP dependent, cGMP dependent, and protein kinase C) family have been shown to be important in yeast and mammalian signal transduction. The interaction of growth factors with their receptors leads to the activation of phosphatidylinositol (PtdIns) 3-kinase and the phosphorylation of PtdIns species.5
These then activate PDK1 enzymes, 3-phosphoinositide-dependent kinases, also AGC kinases, which then phosphorylate and activate downstream AGC kinases. Several plant AGC kinases have important roles in development and defense, although most plant AGC kinases' functions are still to be discovered. Two Arabidopsis AGC kinases, \textit{IRE} and \textit{AGC2-1} have been shown to have roles regulating root hair elongation.

We recently cloned and characterized a Medicago \textit{IRE}-like AGC kinase gene \textit{MtIRE}, possibly orthologous to the Arabidopsis \textit{IRE} gene, \textit{AtIRE}. Because of \textit{MtIRE}'s homology to \textit{AtIRE} we thought it might function during infection, because infection threads can be viewed as inward root hair growth. However, \textit{MtIRE}'s expression is novel. It is expressed only in nodules and flowers and not in roots or root hairs. During nodule development, its initial expression correlates with the onset of host cell and symbiosome expansion. Expression studies with nodulation mutants demonstrate that \textit{MtIRE} expression correlates with mutant nodules' abilities to support host cell and symbiosome expansion. An \textit{MtIRE} promoter-\textit{gusA} reporter construct (Fig. 1A) shows expression in the proximal part of zone II, the site of continued host cell expansion and bacteroid and symbiosome elongation. RNA interference experiments were unfortunately unsuccessful, probably because of closely related more ubiquitously expressed \textit{IRE} homologs.

We predict that \textit{MtIRE} is part of a signal pathway regulating an aspect of host cell expansion or symbiosome elongation, or both. The \textit{CCS52A} gene has a demonstrated role in host cell expansion, mediating endoreduplication. In contrast to \textit{MtIRE}, its expression is found throughout zone II, as well as zone I, where it acts in cell division. One might expect other genes that regulate host cell expansion to also be expressed throughout zone II, which \textit{MtIRE} is not. A unique feature of the region expressing \textit{MtIRE} is symbiosome elongation. Because of \textit{MtIRE}'s temporal and spatial expression patterns, we favor it having a role in symbiosome expansion, although we cannot rule out a role in the latter stages of host cell expansion.

Figure 1. (A) Localization of \textit{pMtIRE-gusA} expression in wild-type nodulated roots. Composite \textit{M. truncatula} plants with transgenic roots were grown in the presence of \textit{S. meliloti} and stained with X-Gluc (blue) for the localization of \textit{MtIRE} promoter activity. The arrow points to the X-Gluc staining in the proximal side of zone II in a 15 dpi nodule. The arrowhead points to root hairs in which no staining was observed. Bar = 100 \mu M. (B) Phospholipid signaling pathway that may activate \textit{MtIRE} protein's presumed kinase activity.

Signaling pathway for \textit{MtIRE} activation is speculative (Fig. 1B) and based on AGC kinase signaling in other systems. AGC kinases are activated by phosphorylation by phosphoinositide-dependent kinase (PDK1) enzymes, also AGC kinases. We found 4 tentatively conserved sequences (TCs) in the DFCI index (compbio.dfci.harvard.edu) that correspond to PDK1 genes of which 3, TC107335, TC94724 and TC94899, were isolated from expression libraries from roots with developing or mature nodules. PDKs are activated by interaction with lipids. The Arabidopsis PDK1 binds to several signaling lipids, including phosphatidylinositol 3-phosphate (PtdIns3P) and phosphatidic acid (PA). Phosphatidylinositol 3-kinase (PI3K) activity produces PtdIns3P and PI3K genes have been observed to be induced during nodule organogenesis in soybean and in \textit{M. truncatula}. In soybean, two PI3K genes were identified with one specifically expressed during the early stages of nodulation when membrane biogenesis takes place. This gene's predicted protein has potential phosphorylation sites for cAMP dependent kinases and Ca/calmodulin-dependent kinases.

In soybean, PI3K enzymatic activity correlated with membrane proliferation during nodulation. More generally, PI3Ks are implicated in vesicular trafficking and cytoskeletal organization; both are required for host cell and symbiosome elongation. We suggest a model where \textit{MtIRE} kinase activity is activated by PDK1, which is itself regulated by PI3K through the production of PtdIns3P. More speculatively, PI3K could be under the control of the Nod factor signaling pathway Ca/calmodulin-dependent kinase DM13, DM13 is induced during nodulation, with highest expression levels found in the distal side of the infection zone, before expression of \textit{MtIRE}. Expression could persist to the proximal side of this zone, similar to the expression of another Nod factor signaling component, DM2. Alternatively, \textit{MtIRE} could be activated by PA in a PDK1-dependent manner similar to Arabidopsis AGC2-1. PA can be produced by phospholipase C (PLC) or phospholipase D (PLD) pathways, both of which have been implicated in transducing Nod factor signals. Either of these models includes Nod factor signaling in proximal zone II, which has not been well-studied. Expression of rhizobial nod genes has been observed in zone II, making Nod factor signaling in this zone plausible. Further examination of zone II and predicted upstream regulators of \textit{MtIRE} will address this model.

References


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