AGC kinases in plant development and defense

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More than 100,000 publications demonstrate that AGC kinases are important regulators of growth, metabolism, proliferation, cell divison, survival and apoptosis in mammalian systems.[1] Mutation and/or dysregulation of these kinases contribute to the pathogenesis of many human diseases, including cancer and diabetes. Although AGC kinases are also present in plants, little is known about their functions. We demonstrated that the AGC kinase OXIDATIVE SIGNAL-INDUCIBLE1 (OXI1/AGC2-1) regulate important developmental processes and defense responses in plants. The summary of recent progress also demonstrates that we are only beginning to understand the role of this kinase pathway in plants.

PDK1 Activates AGC Kinases

In mammalian systems, most of the cellular responses to phosphatidylinositol-3-kinase activation and phosphatidylinositol-3,4,5-trisphosphate production are mediated by the activation of a subgroup of Ser/Thr protein kinases termed AGC (cAMP-dependent protein kinase A/cGMP-dependent protein kinase G/protein kinase C), which play essential roles in cell growth, proliferation, survival, metabolism and apoptosis.[1] Many AGC kinases possess a common upstream activator, namely PDK1 (3-phosphoinositide dependent kinase 1), a master kinase which phosphorylates and thus activates the AGC kinases in response to rises in the levels of the second messenger phosphatidylinositol-3,4,5-trisphosphate.[2,3] Consequently, *pdk1* knock-out mice are embryo-lethal.[4] PDK1 and AGC kinases are also present in plants.[5,6] PDK1 phosphorylates and thus activates AGC kinases such as OXI1 in Arabidopsis[7] and rice,[6] or Adi3 (AvrPto-dependent Pto-interacting protein 3) in tomato.[8] *pdk1* knock-out lines in Arabidopsis and rice are not lethal,[9] and OXI1 can still be activated in Arabidopsis PDK1-RNAi knock-down lines by stimuli such as H_2O_2 and the pathogen-associated molecular pattern (PAMP) flagellin.[10] Therefore, there are additional stimuli that signal to OXI1 via a PDK1-independent pathway.

PDK1 and its Activation by Phosphatidic Acid

An important second messenger in plant signaling is phosphatidic acid (PA) which can be synthesized either by phospholipase D[11] or by a phospholipase C pathway which generates diacylglycerol that is phosphorylated to PA via diacylglycerol kinase.[12] Both lipases are activated in response to many biotic and abiotic stress signals.[11,12] Recently, it was demonstrated that also the beneficial fungus *Piriformospora indica* is able to stimulate PA synthesis in Arabidopsis.[9] Therefore, the second messenger PA may integrate various external signals in plants to activate and coordinate appropriate downstream responses. While mammalian PDK1 integrates signals from receptors that stimulate the production of phosphatidylinositol-3,4,5-trisphosphate, the plant PDK1 binds to different signaling lipids, including the second messenger PA.[13] Thus, although the lipid stimuli are different, animal and plant PDK1 convert phospholipid information into activation of AGC kinases.
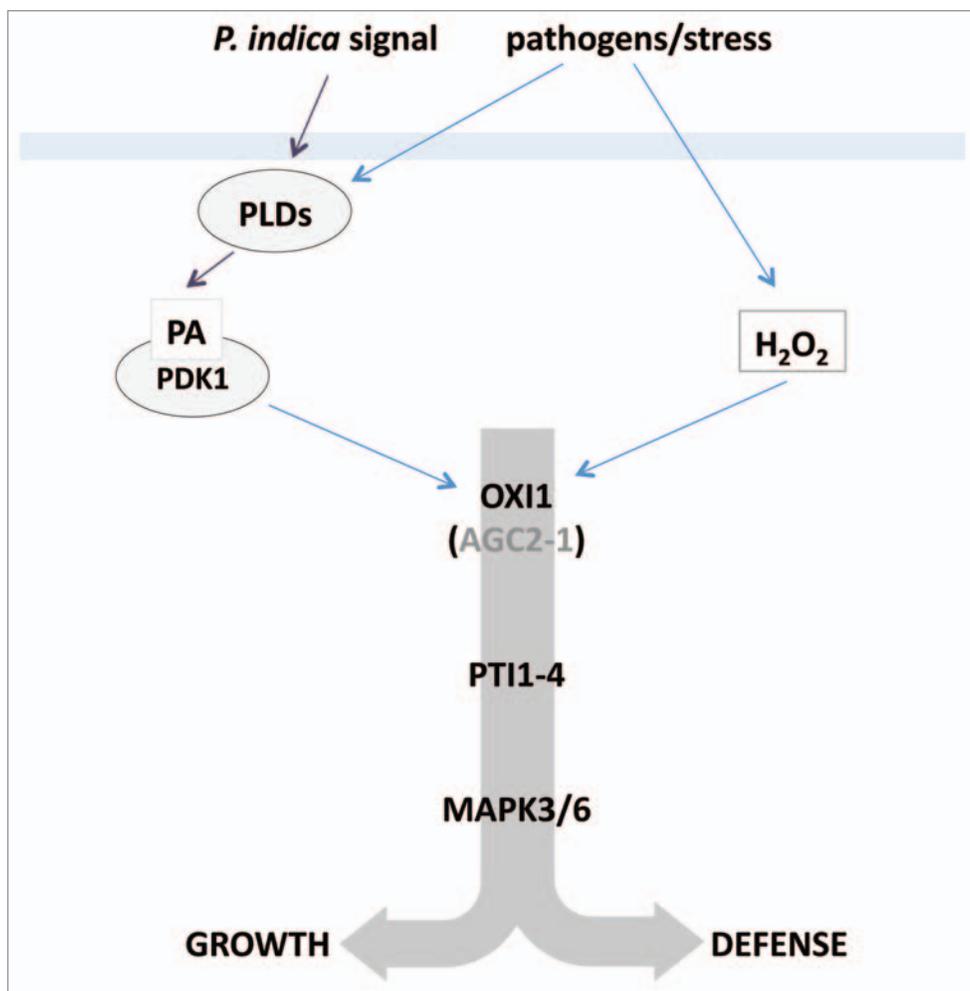
Figure 1. The beneficial fungus *Piriformospora indica* stimulates PA synthesis which activates PDK1 and subsequently OXI1 and AGC2-2. On the other hand, OXI1 can also be activated by environmental challenges, including pathogens in a PDK1-independent manner via H_2O_2. PTI1-4 and the MAPKs MPK3 and 6 are downstream factors of OXI1 and regulate the balance between growth and defense responses.

AGC kinases from Arabidopsis

The Arabidopsis genome encodes 39 members of the AGC protein kinase family[5] and they are involved in various signaling pathways including blue light[14] and auxin signaling.[15-17] Among the AGC kinases, OXI1 was shown to be required for reactive oxygen species (ROS)-mediated responses in Arabidopsis such as root hair elongation and for disease resistance to biotrophic pathogens such as the oomycete *Hyaloperonospora arabidopsidis* and *Pseudomonas syringae* bacteria.[18,19] The kinase activity of OXI1 itself was induced by H_2O_2, wounding, cellulase and various elicitor treatments mimicking pathogen attack.[10,18] Furthermore, as *oxi1* mutant plants are impaired in the activation of mitogen-activated protein kinase (MPK)3 and MPK6 in response to cellular injury and oxidative stress,[18] OXI1 is an upstream regulator of stress-responsive MPKs although its mechanism is still unclear.

In Arabidopsis and rice, kinases of the PTI1 family were identified as interacting partners and kinase targets of OXI1 making them downstream components of OXI1 signaling.[10,20,21] PTI1 proteins are Ser/Thr protein kinases that share strong sequence identity to tomato PTI1 (Pto-interacting 1). In tomato, PTI1 is phosphorylated by the Ser/Thr kinase Pto conferring resistance to *P. syringae* expressing the effector AvrPto and positively regulates the cell death response triggered by Pto.[22,23] On the contrary, rice Pti1a inhibits its disease resistance and cell death and is negatively regulated by OsPDK1-OsOXI1

signaling cascade in response to ROS and PAMP treatments.[6,21,24] Interestingly, Arabidopsis Pti1-4 was recently shown to form protein complexes with MPK3 and MPK6 and could therefore mediate OXI1 regulation of the MPKs[20] and provides a hypothesis how PTI1 proteins could regulate disease resistance.

The endophytic fungus *P. indica*, a cultivable basidiomycete of Sebacinales, colonizes the roots of many plant species including Arabidopsis.[25,26] The fungus stimulates growth, biomass and seed production of the hosts[25-33] and promotes nitrate and phosphate uptake and metabolism.[29,34,35] *P. indica* also confers resistance against abiotic[30,36,37] and biotic stress.[26,38] In a genetic screen for Arabidopsis mutants which do not respond to *P. indica*, we have identified *OXI1* as the responsible

gene for the growth phenotype induced by *P. indica*.[9] Interestingly, whereas OXI1 is required for the long-term interaction between Arabidopsis and *P. indica*, the closest homolog of OXI1, AGC2-2, was found to be important for the early steps in the establishment of the symbiosis. This clearly demonstrates that these two AGC2 kinases participate in *P. indica*-induced growth promotion. Since OXI1 can be activated by H_2O_2 and PDK1, we also tested whether mutants in PDK1.1 or PDK1.2, the two PDK1 genes present in the Arabidopsis genome, are defective in the *P. indica*-induced growth phenotype. We found that single mutants still respond to *P. indica* but not *pdk1.1 pdk1.2* double knock-out mutants.[9] Root colonization by the fungus stimulates PA synthesis in Arabidopsis plants. Phospholipase D (PLD) isoforms synthesize most of the PA in roots in response to stress stimuli. When PA synthesis was reduced by inactivation of PLDα1 or PLDδ, the *P. indica*-induced growth promotion was compromised.[9] These results suggest that *P. indica* stimulates growth by PA-mediated activation of PDK1 which subsequently activates OXI1. ROS production is not stimulated and even inhibited by the beneficial fungus and thus does not play a role in activating OXI1.[9]

Open Questions

Based on these observations, plant AGC kinases seem to regulate the interaction with diverse microbes and to be involved in the control of developmental programs, such as cell death, growth stimulation in response to stimuli from endophytic fungi and auxin functions. The model in **Figure 1** highlights the present knowledge about OXI1 signaling but also points to the open questions: First, what determines the specificity of OXI1 signaling to a given stimulus? Pathogen attack by biotrophic pathogens such as *H. arabidopsidis* or interaction with the friendly *P. indica* requires the activation of different cellular programs in the plant, and a comparative analysis of the two systems might help to understand how OXI1 discriminates and regulates these processes with different outcomes. In this respect, the discrimination may derive from the

activating stimuli/kinase that may lead to the association of OXI1 with different signaling partners. A recent phosphoproteome analysis of Arabidopsis roots identified differentially phosphorylated proteins in *oxi1* seedlings in response to cellulase treatment.[39] Similar approaches could be used to compare potential components of the OXI1 signaling pathway in response to different stimuli. Given that OXI1 shows different expression patterns in roots and aerial tissues,[18] the specificity of OXI1 signaling could also change depending on the plant tissue. Furthermore, in Arabidopsis there are AGC kinases with high sequence homology to OXI1, that also interact with Pti1-4 in yeast[20] and the fact that attempts to obtain double homozygous *oxi1 agc2-2* mutants failed[9] points to certain functional redundancy. Secondly, most biochemical studies on the OXI cascade including phosphorylation and protein/protein interaction assays were performed for defense responses so far but genetic studies and the fact that OXI1 activity is stimulated by growth hormones[7] suggest that the involvement of this pathway in growth regulation deserves further characterization. The functions of AGC2-2 and PTI1-4 have only been analyzed for one model system. Therefore, the relation of OXI1 to other AGC and PTI1 kinases needs to be investigated in the context of different signaling functions. Considering the intensive work on AGC kinases in the mammalian field,[1] which also lead to medicinal applications, the role of this novel kinase pathway is poorly understood in plants. The phenotypes of Arabidopsis mutants in *agc* kinase genes demonstrate the central role of these protein kinases in development and response to environmental signals.

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