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Evidence for a role of hexokinases as conserved glucose sensors in both monocot and dicot plant species

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The role of the hexokinases (HXKs) as glucose (Glc) sensors has been mainly demonstrated for Arabidopsis (*Arabidopsis thaliana*) HXK1 (*AtHXK1*) but has yet to be shown in other plant species. In our recent publication, we reported that two rice (*Oryza sativa*) HXKs, *OsHXK5* and *OsHXK6*, also function as Glc sensors. These two enzymes harbor both mitochondrial targeting peptides (mTPs) and nuclear localization signals (NLSs), and we confirmed their dual-targeting ability to nuclei and mitochondria using GFP fusion experiments. Consistently, it has been previously known that AtHXK1 is predominantly associated with mitochondria but is also present in nuclei in vivo at appreciable levels. Notably, the expression of *OsHXK5*, *OsHXK6*, or their catalytically inactive mutant alleles complemented the Arabidopsis *glucose insensitive2* (*gin2*) mutant. In addition, transgenic rice plants overexpressing *OsHXK5* or *OsHXK6* exhibited hypersensitive plant growth retardation and enhanced repression of the Rubisco small subunit (*RbcS*) gene in response to glucose treatment. Our results thus provided evidence that *OsHXK5* and *OsHXK6* can function as glucose sensors in rice. Hence, the available current data suggest that the role of the HXKs as Glc sensors may be conserved in both monocot and dicot plant species, and that the nuclear localization of AtHXK1, OsHXK5 and OsHXK6 may be critical for Glc sensing and signaling.

Plants require rigorous sugar sensing and signaling mechanisms to regulate many essential processes, such as photosynthesis, carbon metabolism, growth, senescence and defense responses to biotic and abiotic stresses.[1-6] HXK not only catalyzes the phosphorylation of hexoses but also functions as an evolutionarily conserved Glc sensor in plants, yeast and animals.[2,7,8]

In yeast, the Glc sensor ScHXK2 has an NLS within its N-terminal region, which facilitates its translocation to the nucleus from the cytosol in response to high Glc levels.[9-12] The nuclear sequestration of ScHXK2 is mediated by a direct interaction between the ScHXK2 NLS domain and a transcriptional repressor Mig1 (a zinc-finger DNA-binding transcription factor).[9,13] Nuclear ScHXK2 plays an essential role as a Glc sensor through the formation of regulatory DNA-protein complexes with the cis-acting elements of the Mig1-dependent Glc repressible genes involved in respiration, gluconeogenesis, and the uptake and metabolism of alternative carbon sources.[9,12,13]

The Sucrose non-fermenting1 (Snf1) protein kinase, a homolog of mammalian AMP-activated kinase, is involved in regulating this Glc repression signaling pathway in yeast.[7] Under high Glc conditions, nuclear ScHXK2 strongly interacts with Mig1, thereby inhibiting Mig1 phosphorylation by Snf1 kinase and resulting in the maintenance of repressive conditions for several Glc-regulated genes.[13] In contrast, under low Glc conditions, the ScHXK2 interaction with Mig1 is abolished and the association between Snf1 and Mig1 is subsequently enhanced. This increases the Mig1 phosphorylation state, which causes its translocation from the nucleus to the cytosol. Thus, the absence of the repressor complex in the nucleus activates transcription of several Glc-regulated genes.[13]

Plant HXKs also function as Glc sensors and AtHXK1 is the best-characterized of these.[2,8,14] Whilst the NLS region of ScHKX2 is necessary for its nuclear localization, AtHXK1 is likely to lack an NLS domain. Although an AtHXK1-GFP fusion protein was found to be associated with mitochondria, a small amount of AtHXK1 is also clearly present in nuclei.[14,15] Studies of transgenic plants expressing catalytically inactive *AtHXK1* mutant alleles in the *glucose insensitive2* (*gin2*) mutant background have provided evidence that the catalytic and sensory functions of AtHXK1 are uncoupled in the Arabidopsis plant.[8] Interestingly, nuclear AtHXK1 controls the expression of specific photosynthetic genes as a transcriptional corepressor by creating a nuclear complex core with the vacuolar H^+-ATPase B1 (VHA-B1) and the 19S regulatory particle of proteasome subunit (RPT5B).[14,16]

The rice genome encodes a large HXK family consisting of 10 genes.[17] Recently, we reported that two rice HXKs, *OsHXK5* and *OsHXK6*, function as Glc sensors.[18] Our phylogenetic tree and subcellular localization analyses of rice HXKs further revealed that OsHXK5 and OsHXK6 are closely related to AtHXK1. We found that whereas the OsHXK5-GFP and OsHXK6-GFP fusion proteins are mainly associated with mitochondria, the deletion of their N-terminal mitochondrial targeting peptides (mTPs) resulted in their predominant localization in the nucleus. We also provided evidence that OsHXK5 and OsHXK6 harbor a functional NLS each within their N-terminal regions in a similar manner to ScHXK2. Our Glc repression assay using maize and rice mesophyll protoplasts clearly demonstrated that *OsHXK5*, *OsHXK6*, or indeed their catalytically inactive mutant alleles, possess a conserved Glc sensing and signaling function in monocot plant species. Our analysis of transgenic *gin2-1* plants and rice plants expressing *OsHXK5*, *OsHXK6*, and their mutant alleles further exhibited that *OsHXK5* and *OsHXK6* can replace the role of *AtHXK1* in Arabidopsis and also function as Glc sensors in rice plants.

Our current results will thus further help to understand how Glc signals are sensed and transduced and also elucidate the link between the nuclear localization of these HXKs and sugar sensing mechanisms in plants. It is noteworthy also in this regard that a SCARECROW-like and a MYB-like transcription factor indirectly form a complex with AtHXK1 via VHA-B1 in Arabidopsis.[14] Some other transcription factors such as a zinc-finger protein and a WRKY transcription factor, have also appeared to interact with two members of the SnRK1 (Snf1-related kinse1) family, LOC_Os03g17980 and LOC_Os05g45420 in rice.[19] We speculate that either of these factors might have a similar function to the yeast Mig1 repressor in regulating Glc repression signaling pathways in plants. In addition to these candidates, the identification of transcription factors interacting with OsHXK5 or OsHXK6 using direct screening methods such as a yeast two-hybrid assay and isolation of the in vivo HXK protein complex components, should also help to elucidate HXK-mediated sugar sensing and signaling mechanisms and determine whether a transcriptional repressor similar to yeast Mig1 has evolved in plants.

Chen (2007) raised the possibility in their previous report that *AtHXK2* also has a role in sugar signaling.[16] Previously, we demonstrated in our laboratory that two other rice HXKs, OsHXK4 and OsHXK7, are localized at the chloroplast stroma and cytosol, respectively.[17] It is noteworthy in this regard that the cytosolic HXK isoform in rice, OsHXK7, lacks an N-terminal membrane anchor domain. In dicots, all of the HXKs examined thus far have either a plastidic signal peptide or an N-terminal membrane anchor domain.[20] The *OsHXK7* gene is upregulated under sugar-starvation conditions but downregulated in response to high sugar treatments of the leaf tissues of rice, whilst *OsHXK5* and *OsHXK6* show the opposite regulation.[17] Notably, *OsHXK7* expression is dramatically increased in rice coleoptile tissues under anoxia.[21] Thus, the OsHXK7 protein might be involved in the action of a sugar signaling pathway under stress conditions such as anoxia-driven sugar starvation. In addition, our GFP fusion experiments of the remaining OsHXKs revealed that some are associated with subcellular organelles other than mitochondria (unpublished data), suggesting distinct roles from OsHXK5 and OsHXK6. Ultimately, the characterization of loss-of-function and gain-of-function plants for these rice HXKs will help to determine whether additional HXKs play an important role in sugar signaling pathways.

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