Cell, Volume *127*

Supplemental Data

Regulatory Functions of Nuclear Hexokinase1 Complex in Glucose Signaling

Young-Hee Cho, Sang-Dong Yoo, and Jen Sheen

Table S1. Identification of Specific Proteins from the Nuclear HXK1 Complex Using Proteomic Analyses

AGI #	Annotation	Covered protein %	Matched peptide #	Matched peptide sequence
At4g 29130	Hexokinase 1	22% (108/496AA's)	8	KWHGLLPKS RLSAAGIYGILKK KQTSLSSGSLIKW KDILEVPTTSLKM KIISGMYLGEILRR KVVISLCNIIATRG KIKDILEVPTTSLKMRK RQVADAMTVEMHAGLASDGGSK L
At1g 76030	vacuolar H^+-ATPase subunit B1	21% (101/486AA's)	8	KYQEIVNIRL RGQVLEVDGEKA KFVMQGAYDTRN RDFEENGSMERV RTVSGVAGPLVILDKV KRDFEENGSMERV RQIYPPINVLPSLSRL RGYPGYMYTDLATIYERA
At1g 09100	26S proteasome AAA-	23% (99/423AA's)	7	RTNLDLESVKE RADILDPALMRS KGVLLYGPPGTGKT RKIEFPHPTEEARG

	ATPase subunit RPT5B			KQIQELVEAIVLPMTHKE KSPCIIFIDEIDAIGTKRF KDSYLILDTLPSEYDSRV
At1g 50420	scarecrow 3-like protein	16 % (77/482AA's)	6	KALNATQTRT RAILEAMEGEKM RLDCLNVEQLRV RTNNVSEEIHVRR RTDSFLNAIWGLSPKV RKNCALRFQNNPSGVDLQRV
At3g 11280	myb family transcription factor	25% (59/236AA's)	5	KTVFDVMKQ RRFLLGLLKY RNISRNFVVSKT KLEEDVFDIEAGRV RNFVVSKTPTQVASHAQKY

☐ Grey shading marks unique amino acids encoded by each specific gene in the gene family.

L*er* ***gin2***

Figure S1. The Growth Defect in the *gin2* Mutant under High-Light Condition
WT (L*er*) and *gin2* plants were grown in soil under 16/8 h light/dark photoperiod (240 μmol/m^2/s) for 25 days.

Experimental Procedures

Nuclear Fractionation and HXK1-FLAG Complex Isolation

Arabidopsis leaves (100 g) were ground in 1.5 volume of lysis buffer (LB), 20 mM Tris-HCl, pH 7.4, 25% glycerol, 20 mM KCl, 2 mM EDTA, 2.5 mM $MgCl_2$, 250 mM sucrose, at 4 ^oC. The lysates were filtered through four layers of miracloth and centrifuged at 1000 x*g* for 10 min to pellet the nuclei. The pellet was washed three times in nuclei resuspension buffer (NRB), 20 mM Tris-HCl, 25% glycerol, 2.5 mM $MgCl_2$, with 0.5% Triton X-100. The nuclear fraction (500 μg protein in 500 μl) was resuspended in NRB without $MgCl_2$, and then froze and thawed before mixing with 200 μl of the anti-FLAG M2 affinity gel in Tris buffer saline (TBS), 50 mM Tris-HCl, pH 7.4, 150 mM NaCl, and incubated for overnight at 4 ^oC. The gel was washed first with 1 ml of high salt buffer (50 mM Tris-HCl, pH 7.4, 500 mM NaCl, 0.1% Nonidet P40, 0.05% sodium deoxycholate), then 1 ml of low salt buffer (50 mM Tris-HCl, pH 7.4, 0.1% Nonidet P40, 0.05% sodium deoxycholate), and 5 times with 1 ml of TBS containing Complete protein inhibitor cocktail (Roche) before elution with FLAG peptides according the manufacture's instruction (Sigma). The nuclear HXK1-FLAG accounted for about 1% of the total HXK1. The estimation of nuclear HXK1 was based on protein blot analysis with the FLAG antibody (Figure 1A, showing about 7 fold difference in HXK1 abundance between the nuclear and soluble fractions) taking into account the additional 15-fold enrichment during the nuclei isolation process before protein gel loading. Thus, the ratio for HXK1 in the nuclear and soluble fractions is about 1:105.

Proteomic Screens

For MALDI-TOF-MS (Voyger DE-STR/Voyger Biochemistry Version 5.1, Applied Biosystems) analysis according to the manufacture's procedures, we excised around 50 bands (7x1x1 mm^3) after each SDS-PAGE of 0.5 ug of HXK1-FLAG protein complexes. Obtained peptide mass and sequence of each sample was blasted using the publicly available search engines MS-FIT (http://prospector.uscf.edu), Peptident (http://www.expasy.ch/tools/peptident.html), and MOWSE (http://srs.hgmp.mrc.ac.uk/cgi-bin/mowse). Protein/gene identification (48 candidates) was made based on the criterion of at least 5 peptide hits with 10ppm mass tolerance. The proteomic screens were repeated three times and protein/genes candidates were identified from three independent experiments.

Yeast Two-Hybrid Screens

The cDNAs of the identified HXK1 protein complex components were generated by RT-PCR, cloned into both pGDA and pGBK vectors, and transformed into the yeast host strains AH109 and Y187, respectively, for the Y2H screens (Clontech). We first screened 48 candidates for direct interaction with HXK1, and two HXK1 Unconventional Partners were identified. To confirm and validate our clones and their protein interactions, plasmids were extracted and the inserts were sequenced. The inserts were cloned into both vectors again for repeating Y2H screens. All the Y2H procedures were performed according to the manufacture's instruction (Clontech).

Isolation of T-DNA Insertion Lines and Complementation

To identify *vha-B1* and *rpt5b* insertional mutants (SALK_028728 and SALK_069366, respectively), we searched SIGnAL *Arabidopsis* T-DNA collection database (Alonso et al., 2003) and insertion mutant information was obtained from the SIGnAL website at http://signal.salk.edu. The homozygous lines were identified by PCR with primers from the T-DNA vector and *VHA-B1* or *RPT5B* genes (5'caagtgtgttgatggatgtgtgg3' and 5'cagggcctcagcggtagttatt3' for *vha-B1*, 5'cgacacactttcccttcctctga3' and 5'gatggcggaggatacgagctt3' for *rpt5b)*. T-DNA insertional mutants for *vha-H* and *rpt5a* (SALK_139745 and SALK_045623, respectively) were also identified from the same resource. The *vha-C* (*det3*) mutant seeds (CS8554) were obtained from ABRC. The *vha-B1* and *rpt5b* mutants were complemented with WT cDNAs of *VHA-B1* and *RPT5B*, respectively, using *Agrobacterium*-mediated transformation and the minibinary vector (pCB302) as described (Moore et al., 2003).

RT-PCR Primers

The PCR primers are for *CAB* (At1g29920): 5'ATGGCCACTTCAGCAATCCAA3' /5'CACAACTTGACACGCCCATAT3', *CAA* (At5g14740): 5'TGAATACGCTGTCTTGCACC3' /5'TGTGATGGTGGTGGTAGCGA3', and *UBQ*, (ubiquitin10, At4g05320): 5'GATCTTTGCCGGAAAACAATTGGAGGATGGT3' /5'CGACTTGTCATTAGAAAGAAAGAGATAACAGG3'. The *UBQ* primers were included in each PCR as an internal control.