



Localization, intracellular dynamics and cellular function of the Arabidopsis thaliana histidine kinase AHK1.

<u>Rebecca Dautel¹</u>, Katharina Caesar, Waltraud X. Schulze², Michael Hothorn³, Klaus Harter¹

¹Center for Plant Molecular Biology, Department of Plant Physiology, University of Tübingen, Germany ²Institute of Plant Physiology and Biotechnology, University of Hohenheim, Germany ³Department for Botany and Plant Biology, University of Geneva, Switzerland

Introduction

The Arabidopsis thaliana histidine kinase 1 (AHK1) is part of the multistep phosphorelay network in plants in which signals are transduced by a His-to-Asp phosphorelay.

AHK1 is 135 kDa hybrid histidine kinase with two transmembrane domains of 23 amino acids each, an extracellular domain of 347 amino acids and an intracellular part of 738 amino acids comprising the histidine kinase and receiver domain.

Arabidopsis ahk1 loss-of-function mutants show a better recovery after drought stress and a higher tolerance to osmotic stress (5, 7). In yeast, AHK1 complements the osmotic stress sensitive double mutant lacking the histidine kinases SLN1 and SHO1 which induce the HOG pathway upon osmotic stress. In this pathway a switch from the His-to-Asp phosphorelay to classical Ser/Thr/Tyr phosphorylation takes place (1, 2, 3). Accordingly, AHK1 is suggested as putative osmosensor.





To overcome this, the promoter of AHK1 was fused to mCherry with a nuclear localization signal (NLS) to enhance the signal output.

In 5 day old seedlings the AHK1 promoter shows activity in the vascular tissue of roots except for the root meristem, the elongation and differentiation zone.

No change in tissue specific expression could be observed after mannitol treatment.



 $bar = 10 \mu m$

AHK1-GFP fusions were used to investigate the subcellular localization of AHK1. AHK1 with an N-terminal GFP fusion remains in the ER and is not able to complement ahk1 loss-of-function mutants (data not shown) whereas AHK1 with a C-terminal GFP localizes to the plasma membrane and complements (see fig. VIII.). Treatment of transiently transfected tobacco leafs with 0,8M mannitol leads to AHK1-

GFP localizing to the golgi and other compartments which have to be further analysed.

+ Golgi marker g-rk CD3-967 (4) + 10min 0.8 M mannitol



 $bar = 10 \mu m$

III. The extracellular domain of AHK1 and AHK1-related proteins is highly conserved

CLUSTAL	2.1	multiple	sequence	alignment

Pp_SNLB_XM_001760151.1

Pp_SNLA_XM_001754627.1

Sm_XM_002978682.1

Sm_XM_002975442.1

Pt_XM_002327617.1 Pt_XM_002303370.1 Vitis_XM_002265212 MTR_5g022470	VFVVRLAIMMIAILIGLITILWHFT RSYTKKSLDTLASGLRYSILORPILRMWNILNSTAEITAAQVKLSEYVIRRYSKPTNQAEQVELYEVMRDITWALFASRKALNAITINYRNGFVQAFHRDHRSNNTFYIYSDLVNYSINAKGPYDTNMFSSHQAWDDQSIHSNFSAIWYREPLDPISGEKKGKA 191 VFVVRLAIMMIAILIGLITILWHFT RSYTKKSLDTLASGLRYSLLORPILRMWNILNSTAEITAAQVKLSEYVIGRYSKTTIQAEQVELYEVMRDTWALFSSRKALNAITINYRNGFVQAFHRDHRSNNTFYIYSDLVNYSINAKGPYDTNMFSSHQAWDDQSIHSNFSAIWYREPLDPISGEKKGKA 191 VFVRLAIMMIAILIGLATILWHFT RIYTKSINSLAYGLRYSLLORPILRMWNILNSTVEITTAQVKLSEYVIGRYSKTTIQAEQVELYEVMRDVTWALFASRKALNAITINYRNGFVQAFHRDHRSNNTFYIFSDLVNYSINAKGPYDANMFLSHQAWDDQSIHSNFSAIWYREPLDPISGEKKGKA 193 -FVVRLAIMMIAILIGLATILWHFT RIYTTKSINSLAYGLRYSLLORPILRMWNILNSTVEITTAQVKLSEYVIRRYSKPTOAQVELYEVMRDVTWALFASRKALNAITINYRNGFVQAFHRDHRSNNTFYIFSDLVNYSISGSYNSNTLSSHQGWNDQSIHSNISAIWYRPLDPVSGERIGKP 189 -FVVRLAIMMIAILIGLATILWHFT KIYTKKSLSSLAYGLRYSLLORPILRMWNILNSTSEITTAQVKLSQVVIRRYSNPASQAEQVELYEAMRAVTWSLFASRKALNSITINYRNGFVQAFHRDHRDNTFYIYSDLSNYSMVATTSNMLKSISTHQAWDDKTLHGNFSAIWYREPLDPVTGEKIGKA 190
MTR_8g075340 AHK1 Pp_SNLB_XM_001760151.1 Pp_SNLA_XM_001754627.1 Sm XM 002978682.1	VFVREALWIATING ALTER KIYTTKSLNSLAYDLKYSLORPILRMWNILNSTAEITTAQVKLSEYVIRSHGNLATQAEQVEMYESNRAVTWALFASRKALNSITVKYRGFVQAFHRDLKDNNIFYIYTDLSYHETNSFAAHEDTHSNKSAIWYREQLDPVNGEKIGKA 178 -FVREALWIATING ALTER VIEWET RIYTKOLQUL YGLRYSLLORPULRWWSVLNT SELTAQVKLSEYVIKKYDKPTTQEELVEMYQAMKDVTWALFASRKALNAITINYRGFVQAFHRDPASSSTFYIFSDLKNYSISGTGPEDVSGWNNKSIHGNMSAIWYQQLDPVTGENLGKP 184 -FSVRLAININAAVILLUHHFT TVYTTRSIKNLAFGLRTELLNRPIARMWNLLNNTVEATLSQVOLSQFVLGEYTLPIDASTQVQVHRTMRNIFWAVYAGRKSAKSITIAYRGQLQAFDRNMVTNETFYIFTDPSVGAPLGGVSVIGASPAPSPATDAPAPVTMWPDIPLENG-NITWYKEPINPYTG-KASSP 197
sm_XM_002975442.1	-FGARIAIMIMAILIGLITILIMHFTTAYATKSIKSLAYSLRVELLKRTISRSWNLISTTLDATTLANLSDYMIPKHFSSILWTYTQPQHLVMRNITWAVFSSRQSLKTLSVLYSNGQLLAFDRNPLNNKTYYLFSNNSAASTLGTEFLESVEASTTGQWYKEELNPSTGQPIDSA 177 .*:*** ***:****************************
Pt_XM_002327617.1 Pt_XM_002303370.1 Vitis_XM_002265212 MTR 5g022470	SPIPPDLINIAGLSQVPDGVASWHVAVSKYTDSPLLQAALPVWDASNKSIVAVVGVTTSLYSVGQLMRELVEVHSGYIYLTSQEGYLLATSTNAPLLTNSTTRP-NLIMAVDTEEPIIRMGARWLEKVYGNKLT-PGQIVQVENAKLGNQQYYIDSFFLNLKRLPIVGVIIIPR 365 SPIPPDDLINIAGLSQVPDGVASWHVAVSKYTDSPLLSAALPVWDAYNKSIVAVVGVTTALYSVGQLMRELVEVHKGYIYLTSQEGYLLATSTNAPLLTNS-TRP-NLIMAVDTEEPIIRMGARWLERVYGNKFP-PGHVVHVENAKLGKQQCYIDSFFLNLKRLPIVGVIIIPR 364 KAIPPDDQINIAGLSQVPDGVASWHVAVSKYTDSPLLSAALPVWDPSNQSIVAVVGVTTALYSVGQLMKELVEVHSGHIYLTSQEGYLLATSTNAPLLTNSSTGP-KLMLAIDSEDRVIRLGAEWLQRTYGYKFP-PSHVVHVENAKLGHEHYYIDSFFLNLKRLPMVGVIIIPR 363 MKIAPEDLINIAGLSQVPDGVATWHVAVSKFTDSPLLSAALPVWDSSNKSIMAVVGVTTAFYSVGQLMRELVEMHSGHMYLTSQCGYLLATSTSAPLLTNSTKPPFKLKMAVDCEDEIIRLGAEWLQRTYGNHFPNSTHEVHVENAKLGNQQYYIDSFYLNLKRLPLVIIIKRLVLLTYSLVITKVGVIIIPR 364
MTR_8g075340 AHK1 Pp_SNLB_XM_001760151.1 Pp_SNLA_XM_001754627.1	MKIAPEDSISIAGLSQVPDGVASWHVSVGKFTDSPLLSAALEVWDSSNKSIVAVVGVTTALYSVGQLMKELVDKHSGHMYLTSQEGYLLATSTNDPLLTNSTKKP-KLKMAVDCDNEVIREGAMWLKKTYENNFP-PSHEVHEENARLGHQQYYIDSFFLILKKLPLVGVIIIPRK 352 LKIPPDDLINIAGISQVPDGEASWHVTVSKYMDSPLLSAALEVFDASNKSIVAVVGVTTALYSVGQLMRDLVEVHGGHIYLTSQEGYLLATSTDGPLLKNTSNGP-OLMKATDSEEWVIKSGAQWLEKTYGSKRPHVVHAENVKLGDQRYYIDSFYLNLKRLPIVGVVIIPRK 356 INITPINITSYDLSKNIDYVLALKSTEVSWRLVVTESDDTPLLSSATEVRYRDSGIVVAVTGVTAALSSISQFLRELTSSHSGYIYLTTADGOLLATSTNASLIDSSGPR-TLVIANESSDPVIKAGAQWLYARHGFEGL-VKTVVHAENVVLEGKRYYIDTFSLSLSGLQM
sm_XM_002978682.1 sm_XM_002975442.1	VSIPSVNFSDYTGNVSTLKTGETFWHVAVGSTDNECLLSSAADVRHPVTNELMATVVVTSALSGISNLMKDLARNYSGSFYLTSYDGLLLASSSNHSLVRVLSHGP-KLTPAVDAQDSVIRDGARWLREYHTDSIL-AQKEVHABDVVLGGKKFYIDSFYWNLTGLPL
Pt_XM_002327617.1 Pt_XM_002303370.1 Vitis_XM_002265212 MTR_5g022470 MTR_8g075340 AHK1	YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGQUDERSFRTIVILISASLCILVIGCVCILIT- HIMGQADERAFRTIVILISASLCILVIGCVCILIT- FIMGRVDERAFRTIVILISASLCILVIGCVCILIT- HIMGQADERAFRTIVILISASLCILVIGCVCILIT- S199 VIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YIMGRVDERAFRTIVILISASLCILVIGCVCILIT- YYMGRVDERAFRTIVILISASLCILVIGCVCILIT- YYMGRVDERAFRTIVILISASLCILVIGCVCILIT- YYMGRVDERAFRTIVILISASLCILVIGCVCILIT- YYMGRVDERAFRTIVILISASLCILVIGCVCILIT- YYMGRVDERAFRTIVILISASLCILVIGCVCILIT- YYMGRVDERAFRTIVILISASLCILVIGCVCILIT- YYMGRVDERAFRTIVILISASLCILVIGCVCILIT- YYMGRVDERAFRTIVILY YYMGRVDERAFRTIVILY YYMGRVDERAFRTIVILY YYMGRVDERAFRTIVILY YYMG

Physcomitrella patens (Pp) and *Selaginella moellendorfii* (Sm).

ell.organisation

lipid metabolism.

signalling.receptor kinases.

transport.calcium

- others

transport.ABC transporters and multidrug

transport.Major Intrinsic Proteins.

transport.p- and v-ATPases

The transmembrane domains are framed in black, the amino acid residues, which are the same in each extracellular domain are shaded in grey.

IV. Homology model of the extracellular domain of AHK1

~100nm

~10nm

C-terminus N-terminus

The extracellular domain of AHK1 comprises 347 amino acids (aa 100-447).

Despite of just 13% similarity to AHK4, a homology–based model of the AHK1 extracellular domain could be derived.

 α -helices are shown in tourquoise, β -sheets are shown in orange. The conserved amino acids from the sequence alignment are shown at their distinct position in black.

It remains to be elucidated, how the structure od AHK1 really looks like and which signal is sensed.

Therefore the extracellular domain has been codon-optimized for bacterial expression. The expressed and purified protein can now be used for cristallization approaches and for a mass spectrometric ligand identification.

V. Comparative analysis of the phosphoproteome in wildtype and the *ahk1-3* loss-of-function mutant in the absence and presence of osmotic stress

YVMGEVDRSGRATIAILIAISCCILFVGCFFIIFFT- 413

VMGEVDRRGKATLAILIAISSCILLVGCVFIIFFT- 405

VULGDVDDRGRTTLIILVSVAISILVVGCLLILIFTS 389

** **:: : .*:.:**. *:::*

YVLGDVDDRGRTTLIILVSVAISILVVGCLLILIFTS 389



Seedlings of wildtype (Ws) and the *ahk1-3* loss-of-function mutant were cultivated in liquid culture under constant light conditions for 14 days.

After 10min treatment with 0.3M mannitol or mock, the seedlings were harvested and instantly frozen in liquid nitrogen.

The proteins were extracted, the phosphoproteins enriched, digesprotein.targeting. ted and finally analysed by LC-



VI. The phosphoproteom analysis shows a massive signalling transition

Upon 10min 0.3M mannitol treatment 607 phosphopeptides show a differential phosphorylation between the ahk1-3 loss-offunction mutant and the wildtype.

218 phosphopeptides are less phosphorylated in ahk1-3 and 389 are more phosphorylated in comparison to the wildtype.

VII. Interaction of AHK1 with BAK1 provides link to some quantified differentially phosphorylated phosphopeptides



phosphorylated in the ahk1-3 lossof-function mutant upon 10min treatment with 0.3M mannitol.

The interaction of AHK1 with BAK1 provides the molecular link to Ser/Thr phophorylation.



VIII.Interaction of AHK1 and BAK1 shows physiologic relevance during skotomorphogenesis



MS/MS.

IX. Differential phosphorylation of AHAs does not result in a general change in AHA activity



Specific inhibitors of P-type and Vtype ATPases were used to distinguish the AHA activity from

Outlook

The huge phosphoproteome dataset gives new insights into the tremendous change in the Ser/Thr/Tyr phosphorylation pattern at normal conditions and upon mannitol stress as well as differences between wildtype and the *ahk1-3* loss-of-function mutant.



Seeds of the indicated lines were exposed to light for 2 hours and afterwards grown in the dark at 20°C for 3 days on half strength MS salts.

AHK1-GFP complements *ahk1-3* in the hypocotyl and the root.

The *bri1-5ahk1-3* double mutant looks like *bri1-5*.

The loss of AHK1 in the *bak1-1* background partially rescues the phenotype in the hypocotyl.



In regard to the number of known interaction partners of BAK1 it can be assumed that BAK1 might be a part of a protein complex which comprises AHAs and ion channels as well as different receptors.

As AHK1 interacts with BAK1 and as there are several components of described complexes of BAK1 found, the following model suggests how the signalling pathway of AHK1 might work.

Unfortunately AHA3 could so far not be cloned, which still has to be done to complete the model. It might also be of interest to check if the AHA activity is differently regulated in hypocotyl and root tissue. Additionally the tissue specific composition of a putative BAK1 super complex should be investigated.





References:

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