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

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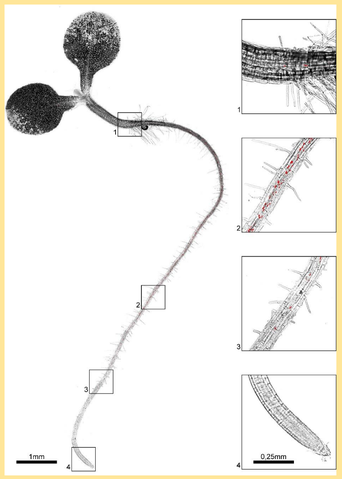
Introduction

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.

I. In seedlings the *AHK1* promoter activates gene expression in the vascular tissue of roots



transgenic *A. thaliana* plant line

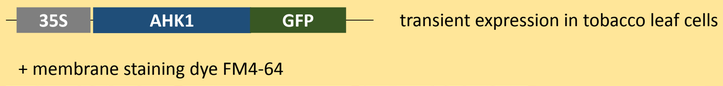
Due to very weak expression of AHK1 the investigation of the tissue specific promoter activity has been difficult.

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.

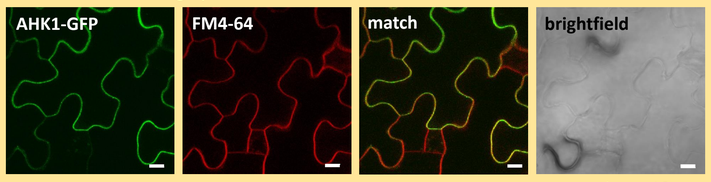
No change in tissue specific expression could be observed after mannitol treatment.

II. AHK1-GFP localizes to the plasma membrane



transient expression in tobacco leaf cells

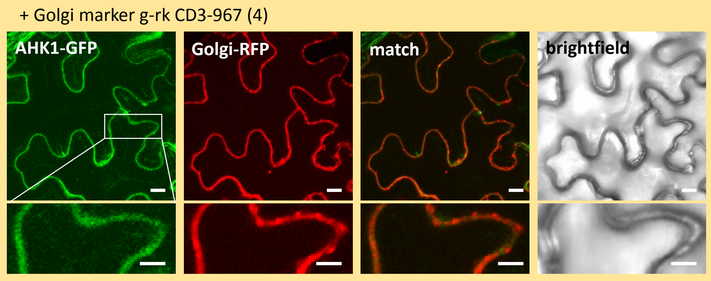
+ membrane staining dye FM4-64



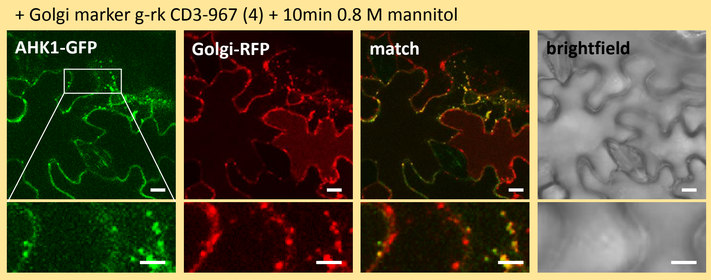
bar = 10µm

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)



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



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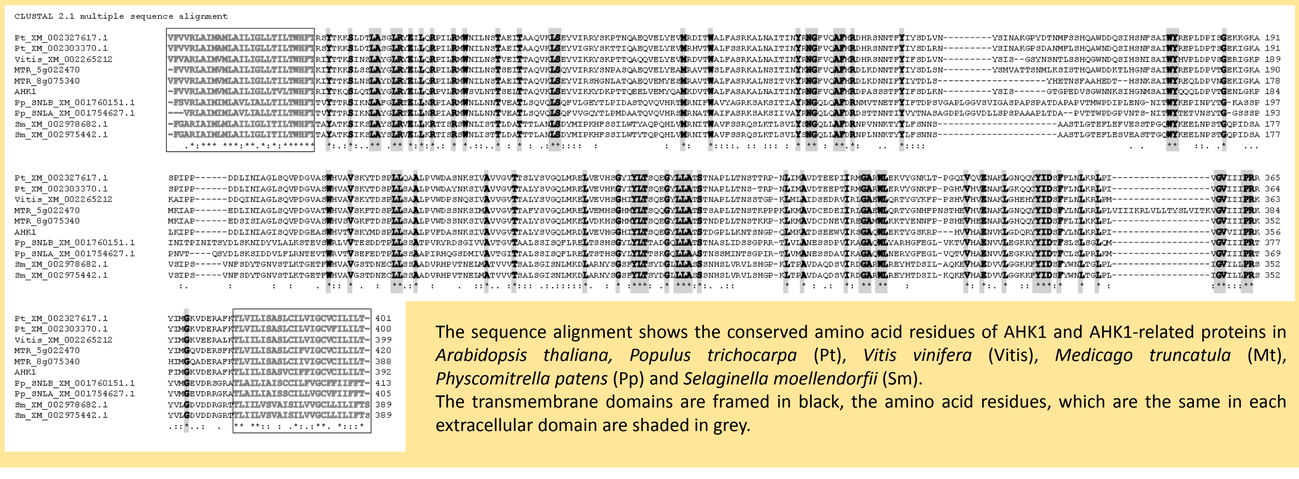
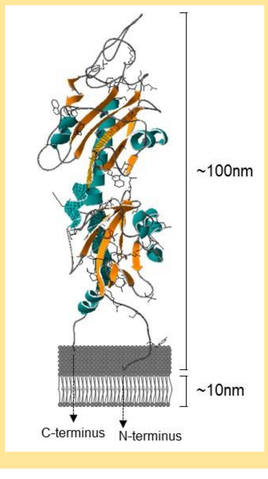
III. The extracellular domain of AHK1 and AHK1-related proteins is highly conserved



The sequence alignment shows the conserved amino acid residues of AHK1 and AHK1-related proteins in *Arabidopsis thaliana, Populus trichocarpa* (Pt), *Vitis vinifera* (Vitis), *Medicago truncatula* (Mt), *Physcomitrella patens* (Pp) and *Selaginella moellendorfii* (Sm).
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



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

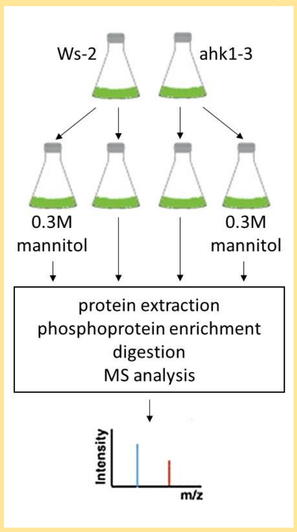


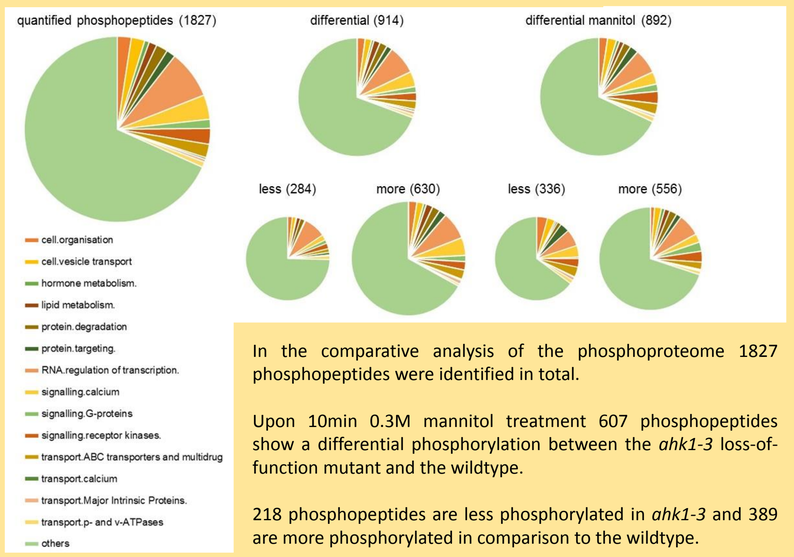
figure corresponding to (6)

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, digested and finally analysed by LC-MS/MS.

VI. The phosphoproteom analysis shows a massive signalling transition from His-to-Asp phosphorelay to Ser/Thr/Tyr-phosphorylation

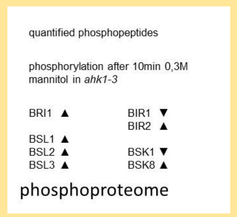


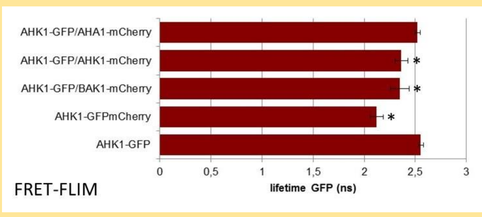
In the comparative analysis of the phosphoproteome 1827 phosphopeptides were identified in total.

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

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

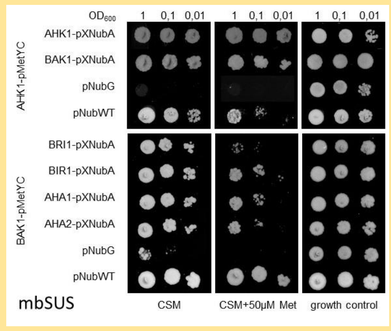
VII. Interaction of AHK1 with BAK1 provides link to some quantified differentially phosphorylated phosphopeptides



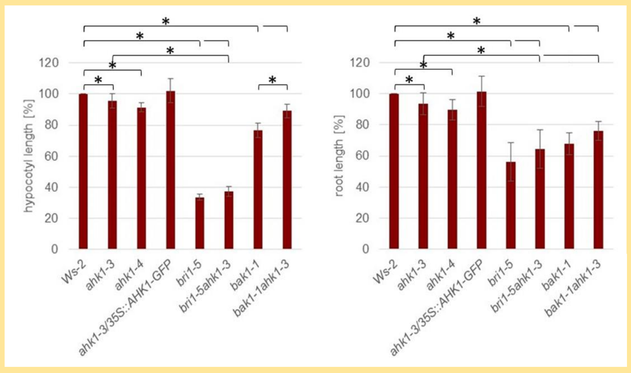


Several peptides of the Brassinosteroid signalling pathway were identified to be differentially phosphorylated in the *ahk1-3* loss-of-function mutant upon 10min treatment with 0.3M mannitol.

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



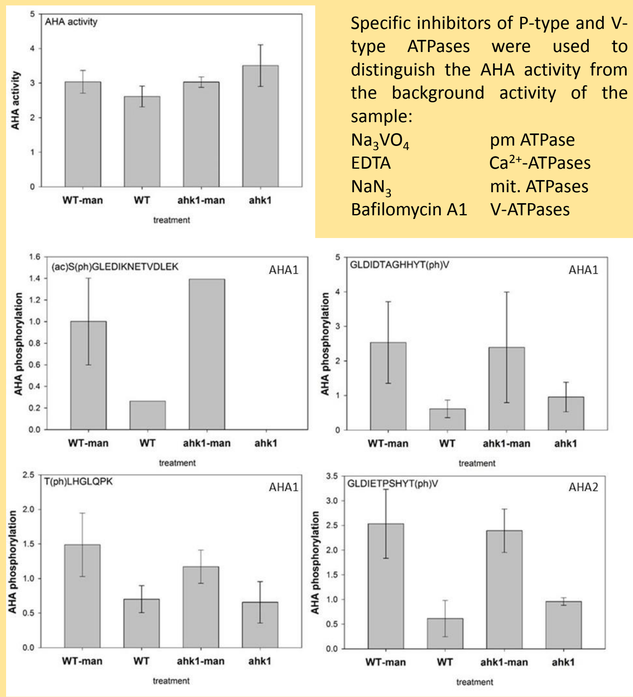
Seeds of the indicated lines were exposed to light for 2 hours and afterwards grown in the dark at 20°C for 3 days on half strength MS salts.

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.

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



Specific inhibitors of P-type and V-type ATPases were used to distinguish the AHA activity from the background activity of the sample:

Na_3VO_4	pm ATPase
EDTA	Ca^{2+}-ATPases
NaN_3	mit. ATPases
Bafilomycin A1	V-ATPases

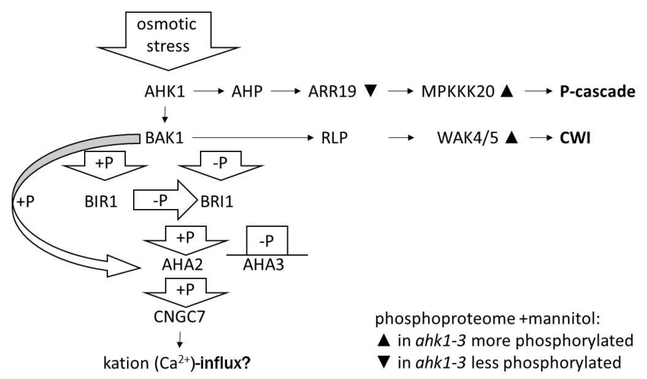
Outlook

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.

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:
(1) Urao *et al.* 1999, Plant Cell 11: 1743-1754; (2) Reiser *et al.* 2003, J. Cell Biol. 161: 1035-1040; (3) Tran *et al.* 2007, Proc. Natl. Acad. Sci. USA 104:20623-20628; (4) Nelson *et al.* 2007, Plant J. 51: 1126-1136;
(5) Wohlbach *et al.* 2008, The Plant Cell 20: 1101–1117; (6) Arsova *et al.* 2012, Front. Plant Sci. 3; (7) Kumar *et al.* 2013, Plant Physiol. 161: 942-953