ARTICLE ADDENDUM
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Desiccation tolerance in the streptophyte green alga *Klebsormidium*: The role of phytohormones

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The origin of phytohormones has been a puzzling question for decades; however, with the availability of large transcriptomic[1] and genomic[2] datasets of the early branching streptophytic green alga *Klebsormidium* this question can be addressed from a fresh perspective. *Klebsormidium* has recently been examined extensively for physiological and structural reactions to desiccation[3-6] or cold temperatures,[7] natural factors in soil crust living algae. Ecological influences have been made responsible for fine scaled structuring of genotypes and differentiation of cryptic species.[8,9] But how do these organisms sense their changing environments?

In this addendum article, we explore our own data set from a transcriptomic study of severe desiccation stress in *Klebsormidium crenluatum*.[1] The cells were desiccated for 2.5 h under monitored conditions over silicagel at ~10% relative humidity. The relative water content of the desiccated cells was 6.54 ± 1.89%. For the molecular analysis we established a high-coverage reference transcriptome database which contained 24,183 contigs with a mean sequence length of 1,327b (N50 = 1,462). This database was used to evaluate which phytohormone pathways are present in *Klebsormidium* and might be involved for cellular response to desiccation stress. Desiccation is well studied in embryophytes, and the cytokinin, ethylene and abscisic acid (ABA) signaling pathways have been implicated in stress response. Given these facts we wondered whether ABA and/or ethylene and/or cytokinin signaling are involved in desiccation tolerance in *K. crenulatum*, and searched for the most similar transcripts to the *K. flaccidum* proteins reported to be putative orthologues of these 3 (ABA, cytokinin, ethylene) plant phytohormones signaling components. Here, we propose that at least 3 major signaling pathways for land plant hormone response are functional in Klebsormidiophyceae. Based on our transcriptomic data of severe desiccation stress auxin mediated signal transduction seem to be missing and in the case of jasmonic acid (JA) only the receptor JAR1 was found, but the further steps were absent in the analyzed *K. crenulatum* transcriptome.

Phytohormone Signaling in *Klebsormidium* and Other Streptophyte Green Algae

Using the KEGG pathway reconstruction tool we found almost complete pathways for cytokinin signaling, ABA signaling and ethylene response in *K. crenulatum*. Meanwhile the draft sequence of the *K. flaccidum* genome has become available and detected the genes for (nearly) complete signaling pathways for auxin, ABA, cytokinin, salicylic acid and JA.[2] The physical presence of the auxin indole-3-acetic acid, ABA, the cytokinin isopentenyladenine, JA, and salicylic acid in *K. flaccidum* was also confirmed by mass spectrometry. While the ethylene signaling pathway was represented in the genome, no attempt was made to confirm its physical presence in *K. flaccidum*.[2] identified counterparts for the hormone receptors *ABP1, GTG, CRE1* and *ETR* for auxin, ABA, cytokinin and ethylene respectively. Interestingly,[10] reported the

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Table 1. summarizes our results. We detected transcripts for all reported proteins of the 3 signaling pathways. For a few proteins more than one transcript was observed (**Table 1**), the significance of these additional transcripts (that could be explained by alternative splicing or additional isoforms) remains unclear at the moment. Many *Klebsormidium crenulatum* transcripts for components of the plant signaling pathway show significant changes during desiccation with transcripts similar to components of the ABA signaling pathway upregulated up to 9-fold

Pathway/Gene	*K. flaccidum* gene[1]	Closest *A.thaliana* homologue[1]	Most similar *K. crenulatum* Transcript	Differential expressed (control cells vs. desiccation: xfold change/padj)	Additional *K. crenulatum* transcripts
Cytokinin signaling					
CRE1,AHK	Kfl00234_0040	AT1G27320	—	—	
	Kfl00990_0020		—	—	—
	Kfl00564_0090		UN031179	0.7/0.08	0
	Kfl00331_0060		—	—	—
AHP	Kfl00529_0020	AT1G03430	UN038987	2.1/0.0002	0
A-ARR	Kfl00066_0050	AT3G57040	UN038317	2.1/5.0e-06	0
B-ARR	Kfl00133_0040	AT4G16110	UN038143	0.5/6.2e-05	0
Abscisic acid signaling					
GTG	Kfl00326_0060	AT4G27630	UN039964		
PYR	No Blast Hit		—		
PP2C	Kfl00724_0050	AT1G72770	UN029018	5.9/1.7e-26	2
AnRK2	Kfl00097_0360	AT4G33950	UN034191	2.5/1.5e-09	1
AREB	Kfl00015_0390	AT3G56850	UN032996	1.5/0.02	0
	Kfl00100_0080		UN039695	7.8/9.8e-30	2
Ethylene signaling					
ETR/ERS/EIN4	Kfl00196_0020	AT1G66340	UN019655	0.6/0.001	1
	Kfl00385_0110		UN001034	1.9/4.1e-05	1
	Kfl00524_0070		UN023393	2.2/5.9e-06	1
CTR1	Kfl00622_0040	AT5G03730	UN037105	0.6/0.02	0
EIN2	No definite counterpart				
EBF	Kfl00213_0020	AT2G25490	UN037105	0.7/0.18	0
EIN3	Kfl00014_0150	AT3G00770	UN042380	0.1/6.7e-12	0

[1] 5.

conservation of the ethylene signaling pathway between conjugating green algae *Spirogyra* (representing the sister group to land plants) and embryophytes. No evidence for gibbelerinic acid signaling nor the recently described ABA receptor (PYR) and the EIN2 protein essential for the ethylene signaling pathway, were found in the *Klebsormidium* genome.[2]

ABA and Stress Response in *Klebsormidium*

The ABA response is well known in abiotic stress reactions (e.g.[11]) often in interplay with cytokinins.[12] The *Klebsormidium* genome contained a putative ortholog to the GTG protein,[2] which has been proposed as ABA receptor. However recent work has cast doubts, whether this protein really serves as an ABA receptor. Upon desiccation stress the transcript level for the GTG ortholog was decreased by 0.5 times. In contrast the transcripts for the ABA signaling components PP2C as well as SnRK2 were significantly increased, as well as the nuclear AREB protein (**Table 1**, **Fig. 1**). However, no change in cold tolerance by ABA application (1–100 μm) was found experimentally in *K. flaccidum* by.[7] The same discrepancy was earlier also observed in the chlorophyceae *Stigeoclonium* cf. *tenue*, where ABA had been detected, but exogenously applied ABA caused only a slight growth reduction and promoted senescence in some cases.[13] Experimentally ABA was measured in numerous chlorophytes as well as in *Chara foetida*.[13]

Recently, orhologues of the *Arabidopis* protein kinase OST1 (*SnRK2 family protein, regulating stomata closure in guard cells*) and the S-type anion channel gene SLAC1 (required for stomata function) were found in *Klebsormidium nitens*. Interestingly, KnOST1 was able to activate AtSlAC1,[14] while neither algal nor embyrophyte OST1 were able to activate the SLAC1 protein from *K. nitens*. These data suggest that while the intracellular signaling pathway seems to be present early in plant evolution, the target as well as the receptor might have changed.

Cytokinin Signaling

Putative orthologues for cytokinin receptors CRE1/AHK in the plasma membrane, AHP in the cytoplasm as well as the transcription factor A-ARP were all up-regulated upon desiccation stress in *K. cernulatum* (**Fig. 1**, **Table 1**), while B-ARP were down-regulated. Most interesting is the up-regulation of A-ARP, which are under control of the cytokinin signaling pathway in plants (see **Fig. 1**), which provides direct evidence for the involvement of cytokinin signaling in the cellular response to desiccation stress.

In *K. flaccidum* the cytokinin isopentenyladenine was identified[2] although at low concentrations.[15] detected cytokinin at very low concentrations (0.29 nmol g^{1} DW) in *K. flaccidum*. Isopentenyladenine was previously chemically identified in the streptophyte green alga *Chara globularis*

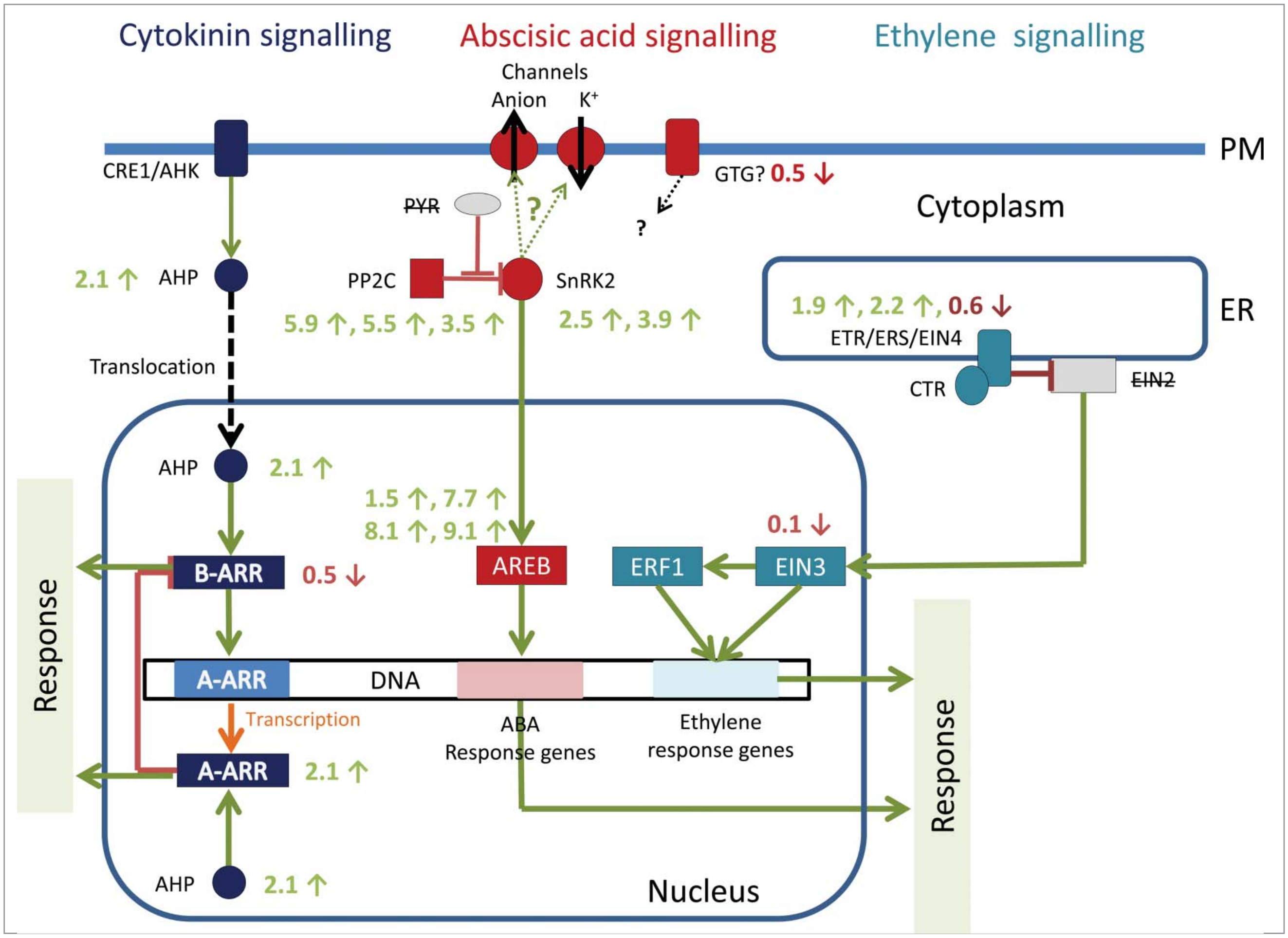

Figure 1. The typical plant signaling pathways for 3 phytohormones with the observed up- and down-regulation upon desiccation stress in *K. crenulatum* mapped onto the pathway. The observed up- and downregulation for the 3 pathways is different (up-or down-regulation, fold change in expression level). While these changes are interesting themselves, they do not represent direct evidence for phytohormone signaling in response to desiccation stress. Most interesting in this respect is the observed up-regulation of the single putative ortholog of type A ARR response regulators, which expression is under direct control of the cytokinin signaling pathway, suggesting that desiccation leads indeed to an activation of cytokinin signaling pathway in *Klebsormidium crenulatum*.

by combined GC/MS and appears to be the most common cytokinin in lower green plants including the moss *Physcomitrella*.[16] No evidence for zeatin, the most common cytokinin of flowering plants has been found in green algae so far.

Highly Conserved Ethylene Response

Ethylene appears to be a highly conserved plant hormone for the last 450 mio years[10] and the evolution of the ethylene receptor family in relation to land plant evolution has recently been summarized.[17] EST sequences for subfamily I ethylene receptors of *K. flaccidum* are available[18] and homologues to both plant ethylene receptors subfamilies are encoded in the *Klebsormidium* genome.[18] However, it is currently not clear how the ethylene signal is transmitted in *Klebsormidium* as the EIN2 protein has not been found in the genome of *Klebsormidium*.[2] In *K. crenulatum* an upregulation of ETR and ERS transcripts (ethylene receptor subfamily 1) were found upon desiccation stress (**Fig. 1**, **Table 1**). Interestingly, the EIN4 homolog (ethylene receptor subfamily 2) was downregulated, suggesting that in *Klebsormidium* the 2 receptor subfamilies serve different functions. All other components of the ethylene signaling machinery showed no or only slight changes in the expression level.

In plants the response to abiotic stress is modulated by changes in ethylene receptor transcript levels,[19] suggesting that the observed changes in ethylene receptor transcripts levels serve similar function in *K. crenulatum*.

Conclusions and Outlook

In terrestrialization events, mechanisms to sense the external environmental situation that might fluctuate and allow cells to react immediately are crucial. Here, we demonstrate that the abiotic stress of severe desiccation regulates the expression of 3 classical phytohormone pathways in the early branching streptophyte algae *K. crenulatum*: Cytokinin, ABA and ethylene signaling. These data further support that Klebsormidiophyceae have the hormonal prerequisites for living on land. The interplay between the different pathways needs further examination, as well as possible roles of e.g., the jasmonic acid receptor JAR1, which is present and upregulated – whereas the further pathway is completely missing. Similar is the case of the salicylic pathway where only the transcription factor TGA is found upregulated in our transcripts. Taken together, we are convinced that plant phytohormone research in streptohyte algae will receive a renewed interest over the next years. Important signaling pathways were already established early in the evolution of plants, which might have been crucial for the colonization of land.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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