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 transcriptomic¹ and genomic² 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 desiccation³-⁶ or cold temperatures⁷, natural factors in soil crust living algae. Ecological influences have been made responsible for fine scaled structuring of genotypes and differentiation of cryptic species⁸,⁹. 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 crenulatum*.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 isopenitenyadenine, 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

Keywords: ABA, abscisic acid, cytokinin signaling, desiccation tolerance, ethylene, green algae

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conservation of the ethylene signaling pathway between conjugating green algae *Spirogyra* (representing the sister group to land plants) and embryophytes. No evidence for gibberelinic acid signaling nor the recently described ABA receptor *S. flaccidum* 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.

**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, Fig. 1). However, no change in cold tolerance by ABA application (1–100 µm) was found experimentally in *K. flaccidum* by. The same discrepancy was earlier also observed in the chlorophyceae *Stigmenocladium cf. tenue*, where ABA had been detected, but exogenously applied ABA caused only a slight growth reduction and promoted senescence in some cases. Experimentally ABA was measured in numerous chlorophytes as well as in *Chlorella foetida*. 

Recently, orthologues of the Arabidopsis 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, while neither algal nor embryophyte 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. crenulatum* (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 isopenetyladenine was identified 2 although at low concentrations. detected cytokinin at very low concentrations (0.29 nmol g DW) in *K. flaccidum*. Isopenetyl adenine was previously chemically identified in the streptophyte green alga *Chara globularis*.

### ABA and Stress Response in *Klebsormidium*

The ABA response is well known in abiotic stress reactions (e.g.) often in interplay with cytokinins. The *Klebsormidium* genome contained a putative ortholog to the GTG protein, 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. The same discrepancy was earlier also observed in the chlorophyceae *Stigmenocladium cf. tenue*, where ABA had been detected, but exogenously applied ABA caused only a slight growth reduction and promoted senescence in some cases. Experimentally ABA was measured in numerous chlorophytes as well as in *Chlorella foetida*. 

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### Table 1

| Pathway/Gene | K. flaccidum gene | Closest A. thaliana homologue | Most similar K. crenulatum Transcript | Differential expressed (control cells vs. desiccation: xfold change/padj) | Additional K. crenulatum transcripts |
|--------------|------------------|-----------------------------|--------------------------------------|--------------------------------------------------|-------------------------------------|
| Cytokinin signaling | | | | | |
| CRE1/AHK | KZ0058_0040 | AT1G27320 | — | — | — |
| AHP | KZ0059_0020 | AT1G03430 | UN031179 | 0.7/0.08 | 0 |
| A-ARR | KZ0060_0050 | AT3G57040 | UN038317 | 2.1/5.0e-06 | 0 |
| B-ARR | KZ0061_0040 | AT4G16110 | UN038143 | 0.5/6.2e-05 | 0 |
| Abscisic acid signaling | | | | | |
| GTG | KZ0032_0060 | AT4G27630 | UN039964 | 5.9/1.7e-26 | 2 |
| PYR | No Blast Hit | — | — | — | — |
| PP2C | KZ0072_0050 | AT1G72770 | UN029018 | 5.9/1.7e-26 | 2 |
| AnRK2 | KZ0097_0360 | AT4G33950 | UN031491 | 2.5/1.5e-09 | 1 |
| AREB | KZ0015_0390 | AT3G56850 | UN032996 | 1.5/0.02 | 0 |
| EREB | KZ0016_0080 | AT3G56850 | UN039695 | 7.8/9.8e-30 | 2 |
| Ethylene signaling | | | | | |
| ETR/ERS/EIN4 | KZ0019_0020 | AT1G6539 | UN031665 | 0.6/0.001 | 1 |
| EIN3 | No definite counterpart | — | — | — | — |
| EIN4 | KZ0021_0020 | AT2G5490 | UN037105 | 0.7/0.18 | 0 |
| EIN5 | KZ0004_0150 | AT3G00770 | UN042380 | 0.1/6.7e-12 | 0 |
by combined GC/MS and appears to be the most common cytokinin in lower green plants including the moss Physcomitrella.\textsuperscript{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\textsuperscript{10} and the evolution of the ethylene receptor family in relation to land plant evolution has recently been summarized.\textsuperscript{17} EST sequences for subfamily I ethylene receptors of \textit{K. flaccidum} are available\textsuperscript{18} and homologues to both plant ethylene receptors subfamilies are encoded in the \textit{Klebsormidium} genome.\textsuperscript{18} However, it is currently not clear how the ethylene signal is transmitted in \textit{Klebsormidium} as the EIN2 protein has not been found in the genome of \textit{Klebsormidium}.\textsuperscript{2} In \textit{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 \textit{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,\textsuperscript{19} suggesting that the observed changes in ethylene receptor transcripts levels serve similar function in \textit{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 acid pathway where only the transcription factor TGA is found upregulated in our transcripts. Taken together, we are convinced that plant phytohormone research in streptophyte 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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