Ca^{2+} dependent phosphorylation modulates the activity of the ABA responsive transcription factor ABF2

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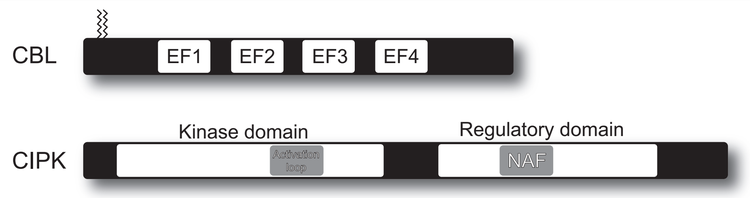
Introduction

Intracellular Ca^{2+} elevation is one of the key events in plant response to various stresses [1]. Besides fast reactions like the regulation of ion homeostasis or ROS, Ca^{2+} dynamics are associated with the control of gene transcription. Several motifs (including ABA responsive elements - ABRE) have been shown to be Ca^{2+} regulated [2,3]. However, the signaling cascades leading to differential gene expression upon Ca^{2+} elevation largely remain unknown.

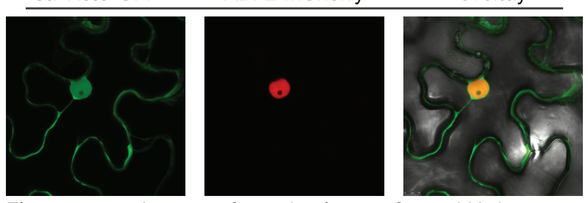
We combined *in vitro* protein biochemical methods and *in vivo* signaling pathway reconstitution methods to investigate the role of CBL/CIPKs in regulating ABRE mediated gene transcription by the transcription factor ABRE binding factor 2 (ABF2). Here we suggest the integration of phosphorylation and dephosphorylation events in the regulation of gene transcription as a convergence point of ABA and Ca^{2+} signaling



Calcineurin B like proteins (CBLs) are a *Bikonta* specific group of Ca^{2+} sensor proteins harboring four potentially Ca^{2+} binding EF-hands. Differential fatty acid modification at the N-terminus is responsible for the subcellular localization of CBLs [4]. They interact and thereby transmit Ca^{2+} signals to their interacting protein kinases (CIPKs). CIPKs consist of a N-terminal kinases domain including the activation loop and a C-terminal regulatory domain. The NAF domain as part of the regulatory domain is a CIPK specific feature that is responsible for CBL interaction. Together CBL/CIPKs form sensor responder modules that regulate diverse Ca^{2+} responses [1].

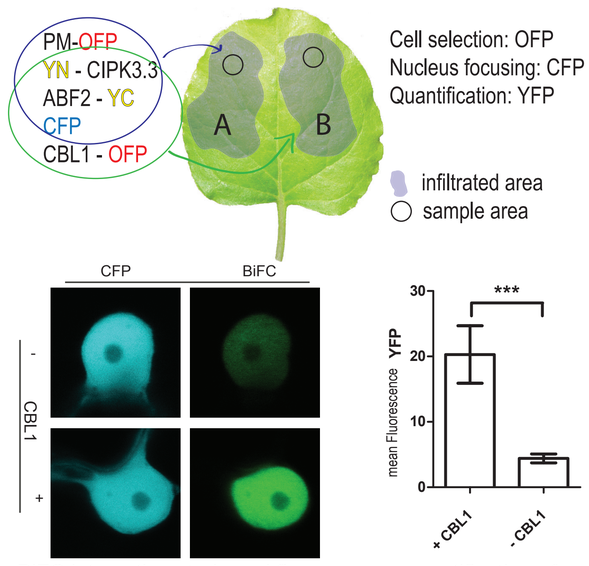
Results

1. CIPK3 and ABF2 localize in the nucleus



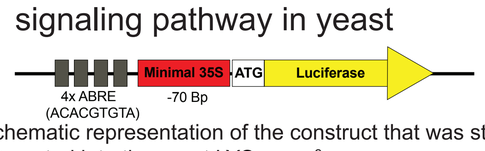
Fluorescence images of transiently transformed N. *benthamiana* epidermal cells.

2. CIPK3 and ABF2 interact CBL1 dependently

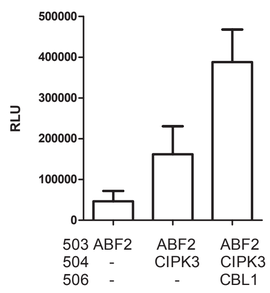


BiFC interaction study and fluorescence quantification of transiently transformed N. *benthamiana* epidermal cells.

3. Reconstitution of a Ca^{2+} dependent signaling pathway in yeast

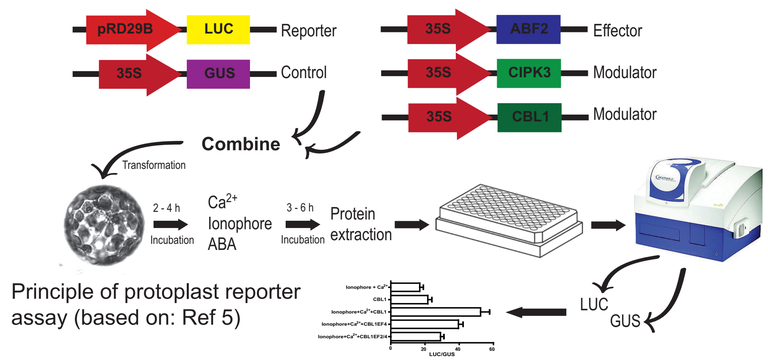


Schematic representation of the construct that was stably integrated into the yeast LYS gene [2].



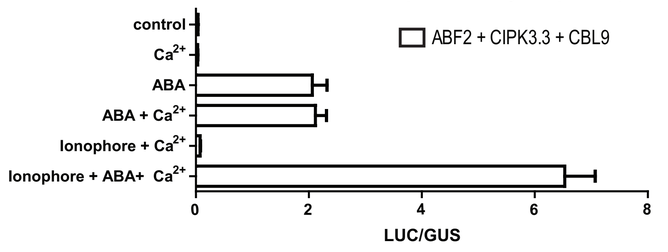
ABF2 is activated by the co expression of CIPK3 and CBL1.

4. Reconstitution of a Ca^{2+} dependent signaling pathway *in vivo*



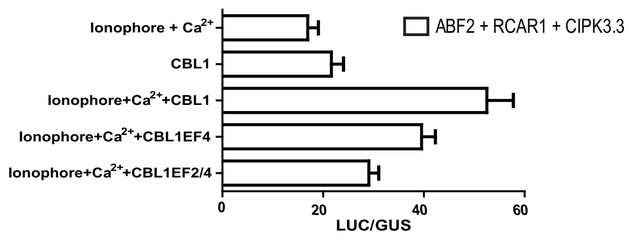
Principle of protoplast reporter assay (based on: Ref 5)

5. CIPK3 activates ABF2 Ca^{2+} dependently in protoplasts

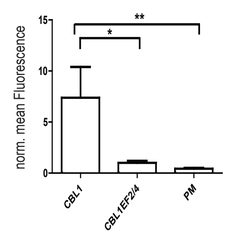


Both, Ca^{2+} elevation and ABA are required for full activation ofABF2 by CIPK3/CBL modules.

6. ABF2 activation by CIPK3/CBL1 requires CBL Ca^{2+} binding

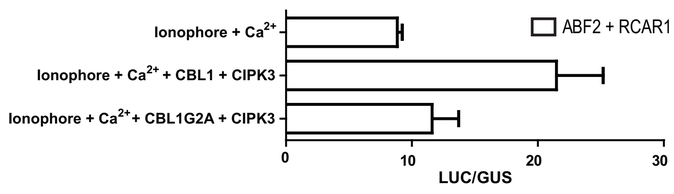


Co-expression of CBL1 variants with mutated EF-hands 2 and 4. Mutations prevent Ca^{2+} binding. Functional EF-hands are required for ABF2 activation.

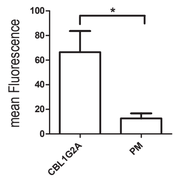


Fluorescence quantification (see 2. for details) of CIPK3 and ABF2 BiFC interaction in dependency of CBL1, CBL1EF2/4 or PM-OFP.

7. CBL1 plasma membrane targeting is required for ABF2 activation by CIPK3 but not for interaction

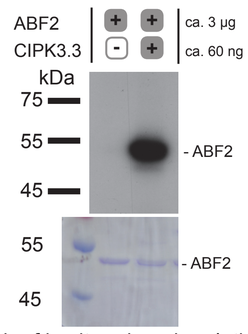


Co-expression of CBL1 variant with mutated lipid modification signal. Mutation prevents myristoylation at the N-terminus and thereby plasma membrane targeting. Lipid modification is required for ABF2 activation.



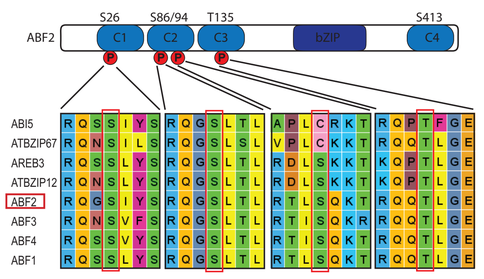
Fluorescence quantification (see 2. for details) of CIPK3 and ABF2 BiFC interaction in dependency of CBL1G2A or PM-OFP. Lipid modification is not required for interaction.

8. CIPK3 phosphorylates ABF2 *in vitro*

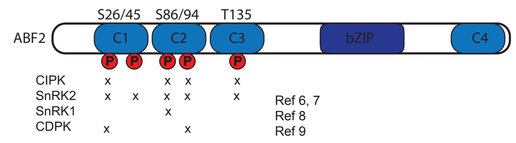


Autoradiograph of in vitro phosphorylation assay and respective CBB staining.

9. CIPK3 phosphorylation sites are conserved in ABF-type transcription factors

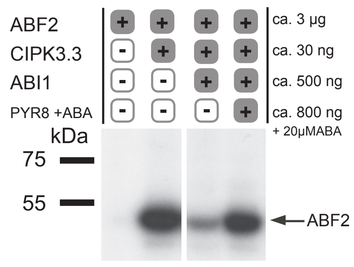


Schematic overview of the four phosphorylation sites found by mass spectrometric analyses and their conservation among related transcription factors. The high conservation suggests further targets for CIPKs.



ABF2 phosphorylation sites are targets for multiple kinase systems.

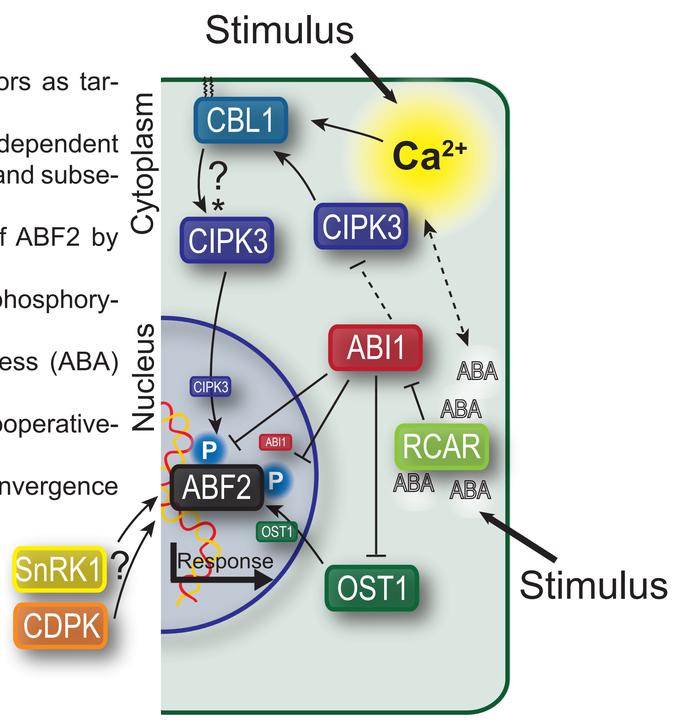
10. ABI1 dephosphorylates ABF2 *in vitro*



Autoradiograph of *in vitro* phosphorylation/dephosphorylation assay. ABI1 is able to dephosphorylate ABF2. Addition of the ABA receptor PYL8 and ABA can inhibit the dephosphorylation.

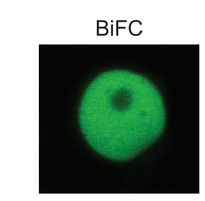
Conclusion and Model

- We identified ABF-type transcription factors as targets of Ca^{2+} dependent phosphorylation.
- We propose a model that involves CBL dependent CIPK activation at the plasma membrane and subsequent translocation to the nucleus.
- Combined, our data support activation of ABF2 by CBL1/CIPK3.
- The ABA regulated phosphatase ABI1 dephosphorylates and inactivates ABF2.
- This suggests that in the absence of stress (ABA) ABI1 keeps ABF2 in an inactive state.
- Upon a stress trigger Ca^{2+} and ABA can cooperatively activate ABF2.
- Together, our data identifies ABF2 as a convergence point of Ca^{2+} and ABA signaling.
- Combined with published data, we propose a dual deactivation mechanism by ABI1 dephosphorylation of the target (ABF2) as well as the kinase (CIPK/SnRK2).

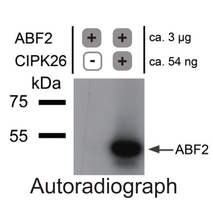


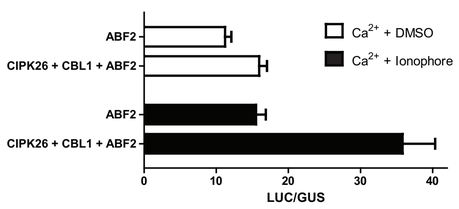
Outlook

- Study the effects of ABF2 p-site modification in phosphorylation and reporter assays
- Establishing and analysing a Ca^{2+} (induction) dependent signaling pathway in yeast
- Which kinase system activates ABF2 under certain conditions?
- How does the signal travel from the plasma membrane localized CBL into the nucleus?
- Analysing *cipk* mutants in terms of ABA and Ca^{2+} related phenotypes → overlapping function (e.g. CIPK26)



cipk3/cipk26 double mutant needed





References

1. Kudla et al., 2010
2. Whalley et al., 2011
3. Whalley, 2013
4. Batistic et al., 2008
5. Yoo et al., 2007
7. Umezawa et al., 2013
8. Zhang et al., 2008
9. Choi et al., 2005
8. Furihata et al., 2006

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