Synergistic activation of a NADPH oxidase via Phosphorylation by a CPK and a CIPK

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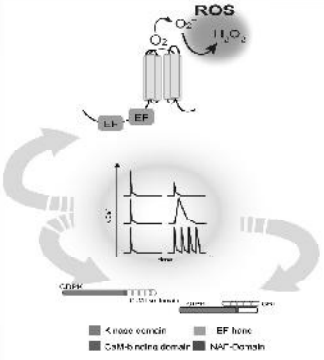
Introduction

Reactive oxygen species (ROS) and Calcium ions (Ca^{2+}) both act as second messengers in plants. Respiratory burst oxidase homologue proteins (RBOHs) are ROS producing NADPH oxidases that are regulated by Ca^{2+} binding to their EF hands and protein phosphorylation. Thereby, they form an interconnection of Ca^{2+} and ROS signaling.
The protein RBOHD is crucial for responses to pathogens, long distance signaling and various other stress responses. RBOHD is known to be phosphorylated by the calcium dependent protein kinase CPK5. Using a ROS production assay in HEK293T cells, we show that RBOHD can be activated by the kinase CIPK26 and the interacting Ca^{2+} sensor CBL1. Furthermore, we show that this activation can be counteracted by a PP2C phosphatase. By manipulating the intracellular Ca^{2+} concentration in theses cells, we found CPK5, CBL1/CIPK26 and both to activate RBOHD at different Ca^{2+} concentrations.
The differential Ca^{2+} dependency of the activation by the two kinases and the synergistic activation of RBOHD by both kinases provides one hint how information encoded in Ca^{2+} signatures can be deciphered, and how the Ca^{2+} signals can be translated into an appropriate, stimulus specific response.



Ca^{2+} signatures

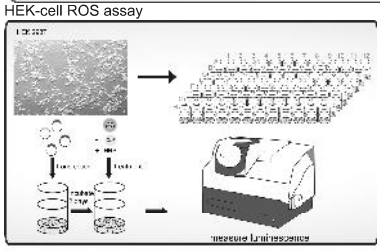
Ca^{2+} ions encode information in their intracellular concentration, their spatial distribution, in the durability of Ca^{2+} peaks and in the frequency of subsequent Ca^{2+} influxes into cells.

Ca^{2+} regulated kinases decode Ca^{2+} signatures

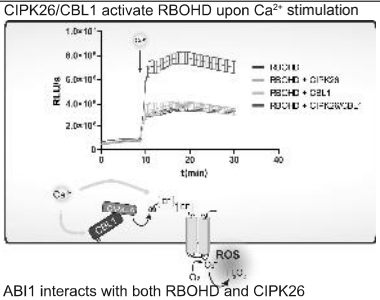
The CIPK/CBL system consists on the one hand of Ca^{2+} sensor proteins that bind Ca^{2+} with their EF hand domains (Calcineurin-B like proteins; CBLs) and on the other hand of interacting kinases. Upon Ca^{2+} binding CBLs change their conformation and bind and activate the interacting kinases (CBL-interacting protein kinases; CIPKs).

Calcium dependent kinases (CDPKs) release an autoinhibitory domain after binding Ca^{2+} ions and are thereby activated.

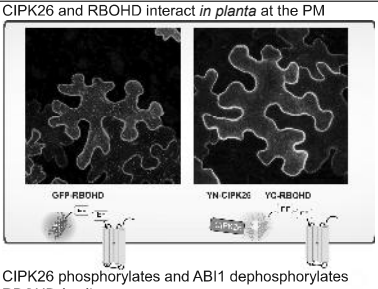
HEK-cell ROS assay



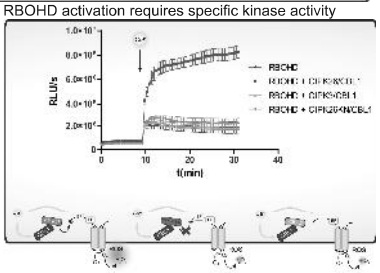
CIPK26/CBL1 activate RBOHD upon Ca^{2+} stimulation



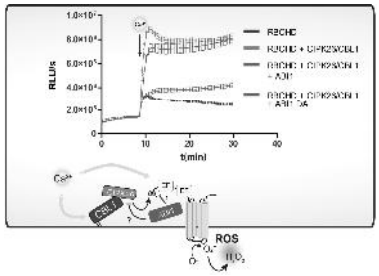
CIPK26 and RBOHD interact *in planta* at the PM



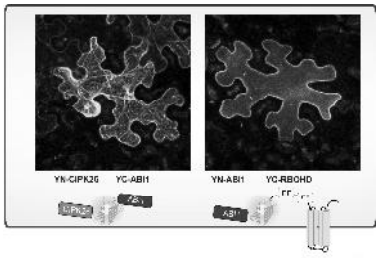
RBOHD activation requires specific kinase activity



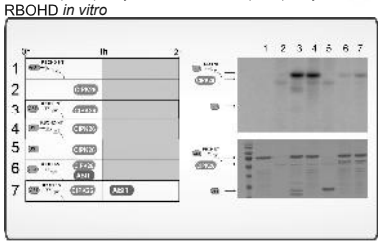
ABI1 counteracts RBOHD activation



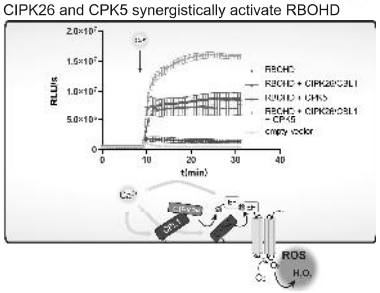
ABI1 interacts with both RBOHD and CIPK26



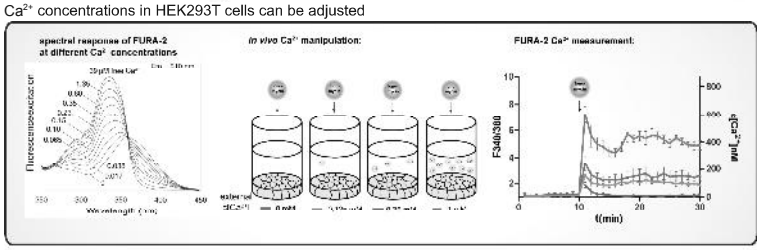
CIPK26 phosphorylates and ABI1 dephosphorylates RBOHD *in vitro*



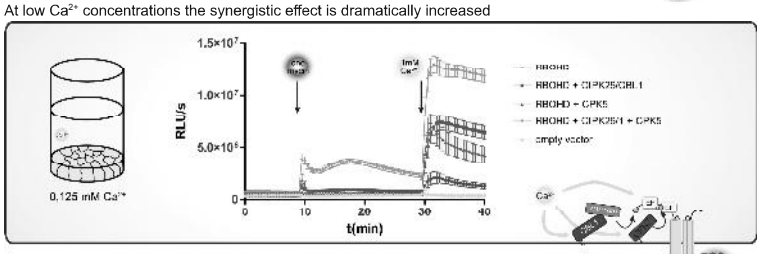
CIPK26 and CPK5 synergistically activate RBOHD



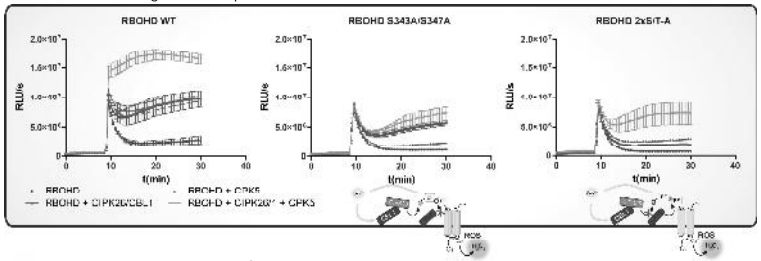
Ca^{2+} concentrations in HEK293T cells can be adjusted



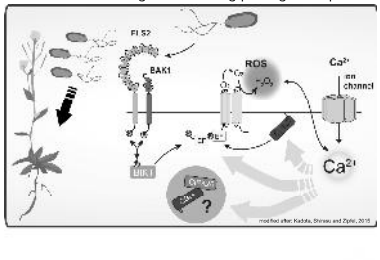
At low Ca^{2+} concentrations the synergistic effect is dramatically increased



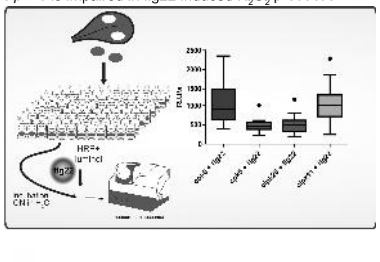
CIPK26 and CPK5 target different p-sites



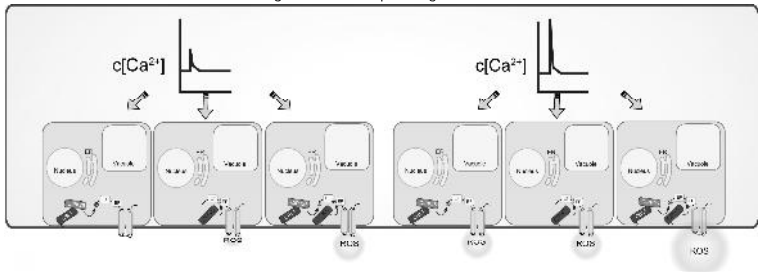
Model of RBOHD regulation during pathogen response



cipk26 is impaired in flg22 induced H_2O_2 production



Model of differential translation of Ca^{2+} signals in cells expressing different kinases



Further reading:

Drerup et al., 2013:
The Calcineurin B-like calcium sensors CBL1 and CBL9 together with their interacting protein kinase CIPK26 regulate the Arabidopsis NADPH oxidase RBOHF. (Mol Plant)

Dubiella et al., 2013:
Calcium-dependent protein kinase NADPH oxidase activation circuit is required for rapid defense signal propagation (PNAS)

Steinhorst and Kudla 2014:
Signaling in cells and organisms - calcium holds the line (Curr Opin Plant Biol.)

Seybold et al., 2014:
Ca^{2+}signalling in plant immune response: from pattern recognitionreceptors to Ca^{2+}decoding mechanisms (New Phyt.)

Kadota, Shirasu and Zipfel, 2015:
Regulation of the NADPH Oxidase RBOHD During Plant Immunity. (Plant Cell Physiol.)

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