

## How plants sense the continuing CO<sub>2</sub> increase to narrow stomatal pores and regulate water loss

Julian I. Schroeder<sup>1</sup>, Jingbo Zhang<sup>1</sup>, Po-Kai Hsu<sup>1</sup>, Yohei Takahashi<sup>1</sup>, Shintaro Munemasa<sup>2</sup>, Rainer Waadt<sup>3</sup>, Nuo Wang<sup>1</sup>, Yinglong Miao<sup>1</sup>, J. Andrew McCammon<sup>1</sup>, Felix Hauser<sup>1</sup>, Wouter-Jan Rappel<sup>1</sup>

<sup>1</sup>University of California, San Diego, La Jolla CA, USA

<sup>2</sup>Okayama University, Okayama, Japan

<sup>3</sup>University of Heidelberg, Heidelberg, Germany

Stomatal pores in plants control CO<sub>2</sub> influx and water loss. Respiration in the dark and atmospheric [CO<sub>2</sub>] elevation cause an increase in leaf CO<sub>2</sub> (C<sub>i</sub>), causing reduction in stomatal pore apertures. CO<sub>2</sub>-induced reduction in stomatal conductance could enhance water use efficiency of plants. However, the underlying CO<sub>2</sub> sensing mechanisms remain unknown.

Research has suggested that bicarbonate (HCO<sub>3</sub><sup>-</sup>) may directly up-regulate SLAC1 anion-channels, which is required for stomatal closing. However, whether this HCO<sub>3</sub><sup>-</sup> regulation is relevant remained unknown. We computationally predicted candidate bicarbonate-binding sites in SLAC1 through molecular dynamics simulations. Gas exchange and patch-clamp experiments with complemented *slac1* mutant plants expressing SLAC1 proteins mutated at a critical site for HCO<sub>3</sub><sup>-</sup> regulation suggest that SLAC1 can function as a direct “secondary” bicarbonate/CO<sub>2</sub> sensor. An additional primary CO<sub>2</sub> sensor is needed for protein kinase activation.

[CO<sub>2</sub>] elevation and the plant hormone abscisic acid (ABA) trigger stomatal closure. However, it remains unclear whether [CO<sub>2</sub>]-triggered stomatal closure is mediated via activation of ABA signaling. Data using multiple approaches including newly designed real-time FRET protein kinase-nanosensors and ABA-nanosensors provide evidence for a new model of how CO<sub>2</sub> signaling and ABA signaling merge in stomatal control.

Advances at dissecting CO<sub>2</sub>-specific signaling in the grass species *Brachypodium distachyon* will be presented. A new critical component that functions in transmitting osmotic/drought stress and abscisic acid signal transduction will be presented, leading to a new model for early ABA signal transduction mechanisms that control stomatal movements.