

Fireworks of salt stress: The role of H⁺/OH⁻ channels in saline pathology of *Chara australis*

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The motif of spatially separated proton pumps and H⁺/OH⁻ channels generating acid and alkaline zones can be found in many branches of plant kingdom. Characeae (Spear, Barr & Barr, 1969; Lucas & Smith, 1973) employ such pH banding to increase carbon assimilation and photosynthetic efficiency. In salt sensitive *Chara australis* the transporters participating in the pH banding pattern are strongly affected by salinity stress. Comparatively mild salinity of 50 - 100 mM NaCl inhibits the proton pump and opens putative H⁺/OH⁻ channels all over the cell surface (Shepherd *et al.*, 2008). These channels were thought to open transiently at first, causing a characteristic noise in membrane potential difference (PD), and after longer exposure to salinity to remain open with a typical current-voltage (I/V) profile (Al Khazaaly *et al.*, 2009; Beilby & Al Khazaaly, 2009). The addition of 1 mM ZnCl₂, the main known blocker of animal H⁺ channels (Decoursey, 2003), abolished both the PD noise and the latter H⁺/OH⁻ I/V characteristics in salt stressed cells (Al Khazaaly & Beilby, 2012). The final proof needed was to observe changes in pH around the cell at the time of salt stress.

Chara cells were imaged with confocal microscopy, using fluorescein isothiocyanate (FITC) coupled to dextran to illuminate the pH changes around cells in artificial pond water (APW) and in 50 mM NaCl/0.1 mM Ca²⁺ Saline medium. FITC fluorescence becomes brighter with pH increase. In the early saline exposure we observed bright spots appearing and disappearing. After longer exposure the spots became more numerous and fixed in space.

The pH imaging not only confirmed the electrophysiological data at the time of saline stress, but also revealed self-organisation aspect of H⁺/OH⁻ channels at the time of pH band formation.

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