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Short communication

F-actin reorganization upon de- and rehydration in the aeroterrestrial green alga *Klebsormidium crenulatum*

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ABSTRACT

Filamentous actin (F-actin) is a dynamic network involved in many cellular processes like cell division and cytoplasmic streaming. While many studies have addressed the involvement of F-actin in different cellular processes in cultured cells, little is known on the reactions to environmental stress scenarios, where this system might have essential regulatory functions. We investigated here the de- and rehydration kinetics of breakdown and reassembly of F-actin in the streptophyte green alga *Klebsormidium crenulatum*. Measurements of the chlorophyll fluorescence (effective quantum yield of photosystem II [ΔF/Fm']) via pulse amplitude modulation were performed as a measure for dehydration induced shut down of physiological activity, which ceased after 141 ± 15 min at ~84% RH. We hypothesized that there is a link between this physiological parameter and the status of the F-actin system. Indeed, 20 min of dehydration (ΔF/Fm' = 0) leads to a breakdown of the fine cortical F-actin network as visualized by Atto 488 phalloidin staining, and dot-like structures remained. Already 10 min after rehydration a beginning reassembly of F-actin is observed, after 25 min the F-actin network appeared similar to untreated controls, indicating a full recovery. These results demonstrate the fast kinetics of F-actin dis- and reassembly likely contributing to cellular reorganization upon rehydration.

1. Introduction

The filamentous actin (F-actin) cytoskeleton is a dynamic network of polymeric proteins, motors and associated proteins. Among the first plant cells, where F-actin was visualized is the streptophyte green alga *Nitella flexilis* (Palevitz et al., 1974). F-actin contributes to the determination of cell shape in green algae (Meindl et al., 1994; Holzinger and Lütz-Meindl, 2001), serves for myosin-based cytoplasmatic streaming and was pharmacologically perturbed to study its function (eg. Foissner and Wasteneys, 2007; Wasteneys et al., 1996). However, in algae little is known on the role of F-actin in environmentally relevant stress scenarios like dehydration. This is particularly interesting, as poikilohydric ancestors of land plants had to cope with fluctuating water conditions, which has been addressed in several ecophysiological studies (for summary see Holzinger and Karsten, 2013). While the development of land plants from a charophyte green algal ancestor is a major event in earthís history (e.g. Becker and Marin, 2009; Leliaert et al., 2012), little is known on the cellular adaptations that allowed conquering land, and adaptation to desiccation.

We selected the basal streptophyte green alga *Klebsormidium crenulatum* for the present investigation, as this alga is growing on alpine soil crusts where it can be potentially exposed to desiccation in its natural habitat. Numerous data are already available on its ecophysiological performance (Karsten et al., 2010), cellular adaptations to desiccation (Holzinger et al., 2011), and osmotic stress (Kaplan et al., 2012). Moreover, investigations on transcriptomic alterations upon severe desiccation have been performed (Holzinger et al., 2014). Relative volume changes as a consequence of exposure to different relative air humidities (RHs) have been reported for this alga (Lajos et al., 2016). Analysis of the composition of the cell wall showed a remarkable proportion of callose, which increased upon desiccation stress and allowed a flexible shrinkage process (Herburger and Holzinger, 2015). With the knowledge of a dynamic reaction to water loss in *Klebsormdium* (Karsten and Holzinger, 2012, 2014,; Karsten et al., 2016), it was particularly interesting, how cytoskeletal elements would react to this environmental stress. A breakdown of F-actin was observed in *K. crenulatum* upon severe desiccation at 5% RH, and only dot-like

Abbreviations: 3N MBBM, Bold's Basal Medium with triple nitrate concentration; ΔF/Fm', effective quantum yield of photosystem II (=YII); EGTA, ethylene glycol tetraacetic acid; F-actin, filamentous actin; MBS, *m*-maleimidobenzoyl *n*-hydroysuccinimide ester; PAM, pulse amplitude modulation; PIPES, piperazine-N,N'-bis 2-ethane sulfonic acid; RH, relative air humidity; YII, effective quantum yield of photosystem II (=ΔF/Fm').

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batches remained (Holzinger et al., 2011). However, this treatment was not sufficient for an immediate recovery (Holzinger et al., 2014), calling for studies with a milder dehydration.

In the present study, we investigated the effects of physiologically monitored de- and rehydration on the F-actin cytosekelton, visualized by fluorescent phalloidin staining (Pflügl-Haill et al., 2000; Holzinger and Blaas, 2016). We hypothesized that the breakdown of F-actin during desiccation is correlated to the reduction of the effective quantum yield (YII), which is used as physiological measure. Moreover, we wanted to investigate the kinetics of F-actin reorganization after rehydration, which was not studied before in *K. crenulatum*.

2. Material and methods

1.1 Algal cultures and desiccation experiments

Klebsormidium crenulatum (SAG 2415) was isolated previously from an alpine soil crust at mount Schönwieskopf (Tyrol, Austria; Karsten et al., 2010), purified and established as unialgal culture. Stock cultures were grown on 1.5% agar plates containing modified Bold's Basal Medium at low light conditions (30–35 μmol photons m^{-2} s^{-1}, light dark regime 16:8 h, at 20 °C, Karsten et al., 2010).

Desiccation and rehydration experiments were performed according to Karsten et al. (2014) in a transparent 735 mL polystyrol box filled at its bottom with 150 mL saturated KCl solution (Merck, Darmstadt, Germany) or *A. bidest*., respectively. The temperature inside the chamber during the desiccation experiment was between 22 °C and 23.6 °C. Small amounts of algal biomass (20–28 d old) were placed by tweezers in 10 μL drops of *A. bidest.* (n = 8) on Parafilm® stripes. After checking their uniform appearance, quarters of Whatman GF/C glass microfiber filters (Whatman, Dassel, Germany; ø 47 mm) were placed onto the algal samples which were soaked up immediately. Filter pieces were positioned on coverslips (Menzel, Braunschweig, Germany; 24 × 24 mm) in the measuring box around the PCEMSR145S-TH mini data logger (PCE Instruments, Meschede, Germany). The distance between the samples and the inner side of the chamber lid was 3 mm. RH, temperature and ΔF/Fm' (PAM 2500, Heinz Walz GmbH, Effeltrich, Germany) were determined in intervals of 5 min during desiccation. When the signal remained for 20 min at YII = 0, samples were rehydrated by adding 180 μL of *A. bidest*. to each sample, the KCl solution was replaced by *A. bidest* and measurements were continued. Three different conditions for the subsequent F-actin staining were chosen: untreated cells (n = 4), desiccated cells with YII = 0 for 20 min (n = 4) and cells rehydrated for different periods: 3 s (n = 3), 10 min (n = 3) and 25 min (n = 4).

2.1. F-actin visualization

Atto-phalloidin staining of F-actin followed the modified protocol after Pflügl-Haill et al. (2000). Briefly, cells were incubated for 15 min at room temperature in fixative comprising 0.60 mM *M*-maleimidobenzoyl *N*-hydroysuccinimide ester (MBS; Pierce; 300 mM stock solution in DMSO) for cross-linking F-actin, 3.70% formaldehyde and 0.07% glutaraldehyde in PIPES buffer (12.50 mM piperazine-N,N'-bis 2-ethane sulfonic acid [PIPES], 2.50 mM EGTA, 1.25 mM $MgCl_2 \cdot 6H_2O$; pH 7). After a washing step in PIPES buffer the cells were labeled for 75 min in darkness, at room temperature with 2.44% Atto 488 Phalloidin (Sigma; stock solution: 10 nM in 500 μL methanol) and 0.50% Triton X-100 (15% stock solution) in PIPES buffer. After a second washing step, cells were mounted in PIPES buffer. Samples were analyzed microscopically immediately after the staining experiment.

Stained F-actin was visualized by confocal laser scanning microscopy (CLSM) on an inverted Zeiss Axiovert 200 M microscope (Carl Zeiss AG, Oberkochen, Germany) equipped with a Zeiss Pascal software. Samples were excited with an argon laser beam (488 nm) and emission was collected in two separate channels at 505–550 nm and long pass (LP) 560 nm. Using a Zeiss 63x immersion oil objective (NA = 1.4), z-stacks of confocal images (512 × 512 pixel, mean of two images) with slide-height of 1.0 μm were generated. Raw images, covering the whole width of samples, were further processed with the visualization software package ImageJ (Rasband 1997–2016). Raw images were imported as 2-channel z-stacks, z-projections for each channel were created (projection type: standard deviation) and then split in order to have one z-projection per channel. Further, z-projections were thresholded against the backgrounds (method: RenyiEntrop) and brightness and contrast in z-projection from 560 nm LP channel (chlorophyll autofluorescence) were adjusted manually. In z-projections from the 505–550 nm channel (Atto-phalloidin stained F-actin) the minimal gray values – as they were responsible for the noisy background below the thin filamentous structures – were measured and then subtracted. The cortical slide of the raw z-stack where F-actin is visulalized in top view was captured, duplicated, thresholded and with background-minimizing (like described above) a slide with cortical F-actin was created that was then added to the z-projection of this channel (tool: image calculator). Channel colors were adjusted: 505–550 nm false-colored yellow and 560 nm LP false-colored blue, and images merged. Stacks were created from singule images and prior creation of the montages, stacks were aligned and canvas sizes were adjusted (mostly to 200 × 310 pixel).

3. Results

3.1. Desiccation effects on physiology

Desiccation of *K. crenluatum* at (~84% RH) caused a decrease of YII from 0.54 ± 0.03 in the first 141 ± 15 min to 0 within 15 ± 4 min. (n = 32), suggesting complete inhibition of PS II. Upon rehydration, YII values recovered immediately and increased steadily towards the initial YII values at ~95% RH. After 3 s rehydration, YII had recovered to about one third of the initial YII (n = 5), 10 min rehydration enabled to recover to 63 ± 11% (n = 24), after 25 min rehydration 79 ± 12% of the initial value were reached (n = 14). Fig. 1 shows a representative curve of one experiment (n = 4).

3.2. F-actin reorganization

In control cells, prior to treatment, Atto-phalloidin staining showed an intricate cortical network of F-actin (Fig. 2A, K) that rapidly disappeared upon desiccation (YII = 0 for 20 min) and was replaced by dot-like structures (Fig. 2B, G). The cells remained desiccated for 20 min. While after 3 s rehydration, YII values had already increased by about one third of the initial value, this period was not sufficient for a recovery of the F-actin cytoskeleton (Fig. 2C, H). However, 10 min after rehydration beginning of the reassembly of F-actin was observed (Fig. 2D, I), 25 min after rehydration the F-actin network had a similar appearance with long filaments when compared to control cells (Fig. 2E, J). Fig. 2F–J shows the F-actin cytoskelton (505–550 nm) and Fig. 2K–O shows the chlorophyll autofluorescence (LP 560 nm) of the respective treatments.

4. Discussion

In the present study, we have shown dehydration effects on the F-actin cytoskeleton in *K. crenulatum* exposed to ~84% RH. As a measure for physiological activity, YII values were recorded for the