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Auxin effects on ion transport in *Chara corallina*

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ABSTRACT

The plant hormone auxin has been widely studied with regard to synthesis, transport, signaling and functions among the land plants while there is still a lack of knowledge about the possible role for auxin regulation mechanisms in algae with "plant-like" structures. Here we use the alga *Chara corallina* as a model to study aspects of auxin signaling. In this respect we measured auxin on membrane potential changes and different ion fluxes (K^+, H^+) through the plasma membrane. Results showed that auxin, mainly IAA, could hyperpolarize the membrane potential of *C. corallina* internodal cells. Ion flux measurements showed that the auxin-induced membrane potential change may be based on the change of K^+ permeability and/or channel activity rather than through the activation of proton pumps as known in land plants.

1. Introduction

The plant hormone auxin, IAA, plays an essential role in plant growth and development. With respect to the physiological aspects of this plant hormone we can distinguish the (polar) auxin transport related processes and the (cellular) signaling related processes. Compared to our knowledge of auxin action in (red, brown, green) algae species, the regulation, signaling and working mechanism of auxin is much more clear for the land plant species (Lau et al., 2009; Wright and Nemhauser, 2015; Enders and Strader, 2015; Di et al., 2015). In land plants, auxin is produced in apical parts of the plants and is transported to specific directions with the help of different auxin transporters among which the so called PIN efflux transporters (Morris, 2000; Viaene et al., 2013; Adamowski and Friml, 2015; Qin and Dong, 2015). At the cellular level, active auxin levels can also be regulated by biosynthesis, storage and conjugation with other molecules (Vernoux et al., 2010; Kramer and Ackelsberg, 2015). Research into auxin related cellular signaling showed different physiological responses of plant cells upon auxin stimulation (Berleth et al., 2004). In these responses membrane hyperpolarization, activation of the plasma membrane H^+-ATPases and potassium channels are well established (Ephritikhine et al., 1987; Felle et al., 1991; Van Duijn and Heimovaara-Dijkstra, 1994; Takahashi et al., 2012; Xu et al., 2012; Osakabe et al., 2013; Philippar et al., 2004; Christian et al., 2006).

As it was already shown in the 1950s or even earlier, that algae species naturally synthesize auxin and that the growth of most of marine/fresh water, unicellular/multicellular algae can be regulated by auxin, (Van Overbeek, 1940; Cooke et al., 2002; Tarakhovskaya et al., 2007) it is believed that auxin, as an important growth regulator, dates back to a very early stage of plant evolution. Despite these findings basic knowledge of the role, transport and cellular signaling of auxin in algae is very limited. With respect to polar auxin transport (PAT), as an unique aspect of auxin's role in plant growth and development, it is known that in higher plants the responsible auxin carriers can be divided into two groups, auxin-uptake carriers and auxin-efflux carriers. The carriers can be easily distinguished by their different sensitivities to different inhibitors such as NPA (1-*N*-naphthylphthalamic acid), a specific PAT inhibitor. In algae these auxin transport or carrier systems may be present as well (Dibb-Fuller and Morris, 1992; Boot et al., 2012; De Smet et al., 2011; Feraru et al., 2012). Some evolutionary less developed plants share similar body structures as the higher land plants, suggesting that auxin-like polar transport and gradients may play a role in the development and growth. Indeed in mosses the existence of polar auxin transport was reported (Fujita and Hasebe, 2009; Viaene et al., 2014), as well as in the cells of the multicellular alga *Chara corallina* (Boot et al., 2012). As a multicellular green alga, Chara has a differentiated plant body-like structure, which is also thought to be one of the clos-

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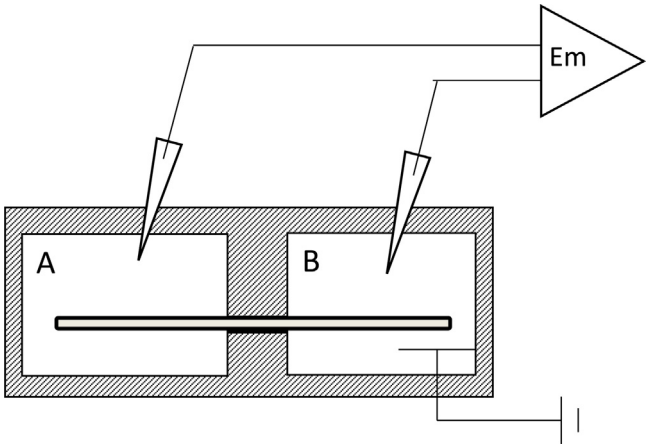

Fig. 1. Membrane potential measurement set-up according to Shimmen et al. (1976). Single internodal cells with node cells on both ends were separated from the plants and put in a chamber with two pools isolated by vaseline. Pool A was filled with APW, pool B was filled with 55 mM KCl dissolved in APW (unless stated otherwise). Electrical potential difference between the two pools is measured by insertion of an Ag/AgCl electrode in each pool connected to a current clamp amplifier.

est relatives to the land plants (Qiu and Palmer, 1999; Wodniok et al., 2011; Timme et al., 2012; Zhang and van Duijn, 2014). Understanding the role, transport and physiology of auxin in these algae may reveal the evolution of auxin signaling and its functioning in plant evolution. In addition, this may indicate to us yet unknown aspects of auxin in higher plants. It is understandable that with the development of a differentiated, multicellular plant body, a better regulated auxin transmembrane transport pathway is needed to facilitate the morphogenic-signal function of auxin. Based on the studies up to date, there are still debates on whether Chara has similar influx and/or efflux auxin transporters, and whether these transporters have similar inhibition sites binding to phototropins. Although polar auxin transport is well established for Chara cells it does not automatically imply that auxin acts as a plant hormone in these algae, that auxin signaling occurs and that it plays similar roles in development as in land plants (Lau et al., 2009; De Smet et al., 2011; Zhang and van Duijn, 2014).

To gain a better understanding of the role of auxin and auxin transport in Chara cells, we aimed to investigate the well-known membrane potential and ion transport responses of higher plant cells to stimulation with exogenous auxin in Chara internodal cells.

2. Materials and method

2.1. Algae

C. corallina was cultured indoors at room temperature in aquaria filled with artificial pond water (APW) containing 0.1 mM KCl, 0.1 mM $CaCl_2$ and 0.1 mM NaCl (pH about 6.0), and forest soil as described earlier (Berecki et al., 1999) under 8/16 light/dark conditions. Nutrients from the forest soil diffuse into the water to support growth of the algae. For the experiments fully grown algae were selected for their internodal cells to be used in measurements.

2.2. Membrane potential measurements

Changes of membrane potential were measured using the K-anesthesia method (Shimmen et al., 1976). Single internodal cells with node cells on both ends were separated from the plants and put in a chamber with two pools isolated by Vaseline with the volume of 3–4 ml each (Fig. 1). One pool (A) was filled with APW, the other one (B) was filled with 55 mM KCl dissolved in APW (unless stated otherwise). Electrical potential differences were measured by insertion of Ag/AgCl electrodes in the pools connected to an amplifier Model 750 (W.P. Instruments) in current clamp mode. Results were recorded and analyzed using the software package Clampex7 (Axon Instruments). The measurements took place inside a closed Faraday cage on a vibration free table to avoid disturbances from the environment, and to suppress the band formation ability of Chara cells, which may cause unexpected influences to the measurement, there was no extra light supplemented inside the shaded unit.

The effect of pH differences was tested with two or three levels for different concentrations of K^+ in the solution (0.1 mM, 1 mM, 10 mM and 55 mM).

Influence of three different types of auxin (IAA, 1-NAA, 2-NAA) on the cell membrane potential was tested for different concentrations (10^{-8} M, 10^{-7} M, 10^{-6} M, 10^{-5} M). IAA and the other two homologs were dissolved with ethanol at the concentration of 10^{-2} M and stored. Before use the stock was diluted with APW to final concentrations of 10^{-8} M to 10^{-5} M. To ensure that addition of auxin did not alter the pH, the pH of auxin solution was adjusted to the original APW pH. The addition of auxin was done carefully along the cell at the opposite side of the electrodes to avoid high peak concentration of auxin near the cells due to lack of dilution, as well as to avoid mechanical stimulation of the cell (Shimmen, 1997).

2.3. Ion flux measurements

Net fluxes of H^+ and K^+ were measured noninvasively using scanning ion-selective electrode technique. We used the ASET system (Automated Scanning Electrode Technique) from Science Wares Inc. The principle of this method and instrument are detailed in Jones et al. (1995), Shabala et al. (1997) and, Li et al. (2010).

Probes were fabricated from tributylchlorosilane (Fluka 90796) silanized 1.5 mm × 1.17 mm thin wall capillaries (Harvard Apparatus) pulled on a Sutter P-1000 pipette puller to give a 10 μm tip opening. Probes were backfilled with either 100 mM KCl for K^+-measurements or 15 mM NaCl, 40 mM KH_2PO_4, pH 7,0 (with NaOH) for pH measurements. The tip was filled with 150–300 μm K^+ LIX (Fluka 60031) or H^+ LIX (Fluka 95297) respectively through brief submersion of the tip in LIX held in a capillary.

In all the measurements below, the position of the probe tips was alternated with a 20 μm step between 50–70 μm from the cell surface in a perpendicular direction. At each location, the ion concentration was measured for 10 s allowing the solution to settle after the motion of the probes. The concentration difference between the two locations was directly calculated and reported as the flux. The data was recorded by ASET and further analyzed using Excel (Microsoft Corporation).

The influences of IAA, fusicoccin (FC), pH and light on the net fluxes of H^+ and K^+ were tested.

2.4. Banding formation

Band formation solution (BFS) was used to detect the acid/alkaline bands of Chara. BFS was freshly prepared by adding 0.5 mM $NaHCO_3$ and 5 mg/100 ml phenol red to APW (color range of phenol red: yellow while pH below 6.8, red while pH above 8.2). pH was adjusted to around 6.5 for a lighter background.

3. Results and discussion

3.1. Membrane potential measurements.

In higher plants membrane potential hyperpolarization is a well-established rapid response to application of auxin. In order to study the auxin responsiveness of Chara intermodal cells, the effect