The Chara plasma membrane system : an ancestral model for plasma membrane transport in plant cells

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Summary

Multicellular giant algae *Chara* species have been widely used in physiological studies for decades. Thanks to the large cell size, and the easy accessible and well-controllable cell membrane system, research on these cells specifically targeted ion channels, cytoplasmic streaming, hormone transporting, cellular organization and so on. With the fast progress in bio-informatics and molecular biology, also for *Chara*, the need to complement this model system with more meticulous and thorough studies at the molecular level is apparent.

In our studies we started to link the physiological phenomena associated to the role of the plant hormone auxin to molecular mechanisms, impelling a more advanced and comprehensive usage of *Chara* as a model system.

Chapter 1 reviews some of the remarkable features of *Chara* within the focus on cell biology and electro-physiological studies. A model is proposed for the possible mechanism behind the *Chara* band formation phenomenon. Two key elements, auxin and plasma membrane (PM) H^+-ATPases are highlighted with regard to their known characters from the higher land plant model systems.

To be able to exploit *Chara* as a research model system beyond classical physiological studies a sustainable and standardized *Chara* culture is not only handy but also necessary. In **Chapter 2**, the experiences and lessons gained from the *Chara* laboratory culture experimentation are listed and discussed. Though in general, the culture of *Chara* in a laboratory environment is inexpensive and needs not much special care, the culture as a whole system with reliable production is quite fragile. A minor disturbance or environmental change may cause a severe damage to the whole culture system. To establish a healthy, long-lasting *Chara* laboratory culture, a high starting density turned out to be required. The right combination of soil, light and temperature is crucial for the dominance of *Chara* against the secondary algae/ fungi/ bacteria. In principle, low nutrient, low light intensity and relatively low temperature could sufficiently diminish the overgrowing of unwanted plankton and Cyanobacteria. Other than the fundamental settings, hygiene is always important when handling the culture.

In **Chapter 3**, the role and effects of auxin in the functioning and membrane transport in algae cells was studied and analyzed in comparison with auxin's role and effects in higher plants. Two parallel models based on Arabidopsis and *Chara* were built to highlight the similarities and differences, which indicates

that *Chara* cells have certain properties to provide a good model system for auxin research. The chapter lists out the unknown from the known and points out new research directions.

In **Chapter 4**, the effects and possible role of auxin in *Chara* cells was investigated. Membrane potential and ion fluxes (K^+ and H^+ in particular) of intact *Chara* internodal cells were measured upon different stimuli, e.g. light, salt, auxin and pH. Through the noninvasive ion-selective vibrating probe scanning electrode technique, the pH banding pattern of *Chara* internodal cells can be monitored, as well as the real time K^+ and H^+ ion fluxes responding to the light and ion solution change. Results show the physiological responses of *Chara* cells towards the application of exogenous auxin (mainly IAA) stimulation, such as a hyperpolarization of the membrane potential. Investigation of K^+ and H^+ ion fluxes separately, reveals that compared to K^+ ion fluxes, there is no remarkable influence on H^+ ion fluxes by the addition of IAA. Thus, the combination of data indicates that, different from the land plants, auxin may change the permeability and/or (K^+) channel activities of the *Chara* plasma membrane, but it has no strong effect on *Chara* PM H^+-ATPases.

To understand the differences in relationship between auxin and *Chara* PM H^+-ATPases, as compared to higher plant H^+-ATPases, and the possible differences between *Chara* PM H^+-ATPases and other well studied PM H^+-ATPases from higher plants, in **Chapter 5**, a potential *Chara* PM H^+-ATPase gene (*CHA1*) was isolated, sequenced and analyzed. Bio-informatics analysis showed a new pattern in the evolutionary perspective between algae and land plants. Heterologous expression of CHA1 in yeast and plant protoplast confirmed that CHA1 can reach the plasma membrane and function as a PM H^+-ATPases with an auto-inhibition domain at the C-terminal. Although, the switch mechanism of CHA1 seems different from the known plant PM H^+-ATPases, as they may not need the involvement of a 14-3-3 protein combination.

In conclusion, this study illustrates that even though the pH difference among/ along the *Chara* cell membrane may influence auxin transport and distribution, auxin itself doesn't show direct effects on the activity of *Chara* PM H^+-ATPases. *Chara* plasma membrane transport under different conditions was studied. In specific, PM H^+-ATPase, as a key player in the membrane transport system, was investigated at the molecular level. Bio-informatics and functional analysis of the isolated *Chara* PM H^+-ATPase reveals a new amino acid pattern which could be

essential for structural stabilization ATP hydrolysis and proton transport. This includes a potentially different regulation mechanism from the known plant and yeast PM H^+-ATPases, which would shed light on the PM H^+-ATPase studies within the algae group and from the evolutionary perspective. By building up a standard lab culture and new progress at the molecular level, the *Chara* cells will offer a re-newed promising platform for different fundamental and practical studies.