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Do Higher Plants and Mosses Respond to Low Temperatures Differently? — Physiological and Molecular Aspects of Responses to Cold Stress in *Physcomitrella patens*

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Bryophytes, which appeared in terrestrial regions on the earth more than 400 million years ago, are now distributed throughout the world including the lands with severe environment such as high altitude regions and Antarctica. Bryophytes that have successfully adapted to environment with cold seasons are thought to possess a mechanism for sensing and tolerating low temperatures. It is well established in higher plants that cold tolerance is acquired by exposure to non-freezing low temperatures, accompanied by gene activation by specific transcription factors and increases in levels of stress hormone abscisic acid (ABA). However, little information is available on genes and mechanisms underlying cold responses in bryophytes. We have been using the moss *Physcomitrella patens* as a model system for studies of cold responses taking advantage of its simple structure and established protocols for gene manipulations. We carried out experiments using its protonema cells to clarify their behavior under subzero temperatures and other physiological alterations associated with changes in freezing tolerance. The protonema cells cultured under normal growth conditions had low levels of freezing tolerance, with an LT_{50} value of about -2°C. When subjected to slow equilibrium freezing, the protonema cells were shrunk and deformed due to dehydration caused by growing extracellular ice crystals. Plasma membranes of the protonema cells had characteristic ultrastructures known to be associated with freezing injury, such as aparticulate domains and fracture-jump lesions. Treatment of the cells with 10^{-5} M abscisic acid (ABA) resulted in rapid increases in freezing tolerance with changes in LT_{50} from -2°C to -10°C in one day, associated with reduced injury in plasma membrane structures. In order to identify genes involved in development of freezing tolerance, we analyzed genes induced by ABA in the protonema cells. The results indicated that a large proportion of the ABA-induced genes encoded proteins with sequence similarity to higher plant proteins reported to be induced during cold acclimation. These results suggest that *P. patens* and higher plant cells share common features with respect to freezing behavior as well as genes required for development of freezing tolerance. We also found several ABA-induced genes not conserved in higher plants, which might contribute to high stress resistance of the moss cells.