

Cloning and analysis of glycine-rich RNA binding protein in *Physcomitrella patens*

Tsuyoshi Nomata, Yukihiro Kabeya, Naoki Sato
Department of Molecular Biology, Saitama University
Contact email: nomatuyo@mac.com

Glycine-rich RNA-binding proteins (GRP) have been identified in a number of plants and animals. Most of them have on their N-terminus RNA recognition motif (RRM), which is RNA binding domain, and on their C-terminus glycine-rich domain. It has been suggested that some may be involved in stress response, as their mRNA accumulation level was modified following exposure to cold, wounding, acute hypersensitive response, ABA treatment, salicylic acid treatment, or water stress. For example, GRP homologues in *Arabidopsis* (*AtGRP7* and *AtGRP8*) are regulated by low temperature as well as circadian clock. But the physiological function of GRP remains unknown. To analyze the role of GRP by gene disruption, we characterized the cDNAs of GRP in *Physcomitrella patens*.

Three full-length cDNA clones each encoding a putative GRP were isolated from a cDNA library prepared from polyA⁺ RNA from 7 day old protonemata of *P. patens*. They were named *PpGRP1*, *PpGRP2* and *PpGRP3*, which encode putative polypeptides of 162, 178 and 155 residues, respectively. The RRM regions of *PpGRP1* and *PpGRP2* were, respectively, 74% and 71% similar to the RRM of *AtGRP7*. Preliminary genomic sequencing suggested that the positions of three introns in *PpGRP3* is similar to those of introns in *Arabidopsis* GRP genes. *PpGRP3* had a putative transit sequence. The protein-sGFP fusions of *PpGRP1* and *PpGRP2* were targeted to the cell nucleus, while that of *PpGRP3* was targeted to mitochondria. The level of *PpGRP* transcripts after cold treatments was increased. Treatment with ABA had no significant effect on the level of *PpGRP* transcripts.