

T3: Overexpression screening for genes involved in asymmetric cell division during protoplast regeneration of *Physcomitrella patens*

Tomomichi Fujita¹, Yuji Hiwatashi¹, Tomoaki Nishiyama¹, Takashi Murata¹, Mitsuyasu Hasebe^{1,2}

¹ National Institute for Basic Biology, Okazaki 444-8585, Japan

² Department of Molecular Biomechanics, SOKENDAI, Okazaki, 444-8585 Japan

E-mail: tfujita@nibb.ac.jp

Asymmetric cell division is of fundamental importance in the generation of overall cellular pattern. Its molecular mechanism, however, remains largely unknown in plants. The moss *Physcomitrella patens* provides a good system to dissect this molecular mechanism, not only because its body plan is relatively simple, enabling observation at a cellular level, but also because it is the only plant in which gene targeting exhibits a high rate of success. Moreover, regeneration of the protoplasts involves many aspects of cell polarity, asymmetric cell division, and subsequent cell differentiation. Isolated protoplasts show polar outgrowth and divide asymmetrically, resulted in non-equivalent daughter cells regarding their shape and nature.

We have devised a systematic overexpression screening for genes affecting the regeneration step of protoplasts in *P. patens*. We constructed three full-length cDNA libraries from non-treated, auxin-treated, and cytokinin-treated protonemal cells of *P. patens*, then determined the sequences of more than 40,000 cDNAs from the both ends (Nishiyama *et al.* 2003). We used these clones as materials for the overexpression screening. Individual cDNAs were selected based on their sequence, subcloned under a constitutive promoter and introduced into the protoplasts of *P. patens* for transient expression. We observed phenotypes of the regenerating protoplasts under a dissecting microscope. About 6% of cDNAs caused abnormal regeneration at various degrees, some of which encode cytoskeletal proteins, signal transduction proteins, unknown proteins and novel proteins. We will examine cellular localization of the cDNA-GFP translational fusion proteins during regeneration of protoplasts.

Nishiyama *et al.* 2003, *Proceedings of the National Academy of Science* 100, 8007 - 8012)