Overexpression screening of genes involved in asymmetric cell division in the moss *Physcomitrella patens*

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Asymmetric cell division is of fundamental importance in the generation of the overall cellular pattern. Its molecular mechanism, however, remains unknown in plants. The moss *Physcomitrella patens* will be usuful to dissect this molecular mechanism, not only because its body plan is relatively simple, making a study at a single cell level feasible, but also because it is the only plant in which gene targeting exhibits a high rate of success. Regeneration of moss protoplasts assures a good system for the study of several interesting facets such as cell polarity, asymmetric cell division, and cell differentiation. We have devised a comprehensive system to screen genes affecting the regeneration step of protoplasts in *P. patens*.
We constructed three kinds of full-length cDNA libraries based on biotinylated cap trapper method from non-treated, auxin-treated, and cytokinin-treated protonemal cells of *P. patens*, then determined the sequences from 5' and 3' ends of cDNA clones from each library. The sequence data were clustered, annotated by BLAST search and more than 50,000 ESTs were deposited in public database. Sequence analyses of the ESTs revealed that about 93% of our cDNA clones appeared to cover the complete coding region, suggesting that these will serve as a good source of full-length clones for functional analysis of genes and their products.
We used these clones as materials for overexpression screening. Individual cDNAs were selected based on their sequence, subcloned under a constitutive promoter and introduced into moss protoplasts for transient expression. We scored the phenotypes of regeneration of the protoplasts and found that some clones caused the delay of regeneration. About 1% of cDNAs exhibited aberrant cell shapes when overexpressed, some of which encode cytoskeletal proteins and unknown proteins. We will present the current status of this project.