

## T9: Analysis of KNOX Class1 genes in *Physcomitrella patens*

Tomoaki Nishiyama<sup>1</sup>, Keiko Sakakibara<sup>1,2</sup>, and Mitsuyasu Hasebe<sup>1,2</sup>

<sup>1</sup> National Institute for Basic Biology, Okazaki, 444-8585 Japan

<sup>2</sup> Department of Molecular Biomechanics, The Graduate University for Advanced Studies, Okazaki, 444-8585 Japan

E-mail: tomoaki@nibb.ac.jp

Vascular plants have sporophyte (diploid) dominant life cycle and produce shoots in the sporophyte generation. Mosses have gametophyte (haploid) dominant life cycle and their sporophytes produce a single sporangium. The shoots of the moss gametophyte and the shoots of vascular plant sporophyte are considered to be non-homologous structures that have evolved independently. Members of KNOX class1 play an important role in the shoot formation in vascular plants. By analyzing KNOX class1 genes in the moss *Physcomitrella patens*, we aimed to reveal whether KNOX class1 genes function in shoot of different origin or sporophyte generation without shoot.

We cloned a KNOX class1 gene in *P. patens* by RT-PCR (Sakakibara *et al.*, MOSS1998) and named it *PpKNOX1* (*PpKNI*). *P. patens* with GUS reporter gene inserted at the end of *PpKNI* coding sequence showed GUS activity in egg cells and young sporophytes, but not in the gametophyte shoots (Sakakibara *et al.*, MOSS2001). *Arabidopsis thaliana* expressing *PpKNI* cDNA under the control of cauliflower mosaic virus 35S promoter showed lobed leaves, which is similar to those reported in *A. thaliana* expressing other KNOX class1 genes under the control of the same promoter. These data suggest that *PpKNI* functions in meristem formation and maintenance of the sporophyte, which do not form shoot but exists transiently to form seta and sporangium. However, *PpKNI* disruptants formed fairly normal sporophytes.

Champagne and Ashton (2001) reported two KNOX class1 genes, *MKN2* and *MKN4*, and a 267 bp fragment (*MKN5*) that is likely a part of a KNOX class1 gene. Of these, *MKN2* was identical to *PpKNI*. The two new genes *MKN4* and *MKN5* may have redundant function to *PpKNI*, which cause weak phenotype of the *PpKNI* disruptants. Therefore, we decided to produce double and triple disruptants with *MKN4* and *MKN5*. We cloned flanking regions of *MKN5* by the TAIL-PCR method and a 6.8-kb region was obtained and sequenced. Phylogenetic analysis based on this sequence showed that *PpKNI/MKN2* and *MKN5* are very closely related and *MKN4* diverged earlier. We are now producing *PpKNI MKN5* double disruptant lines and will produce triple and other double disruptants.

Connie E. M. Champagne and Neil W. Ashton. 2001. Ancestry of KNOX genes revealed by bryophyte (*Physcomitrella patens*) homologs. *New Phytologist* 150: 23 – 36.