

## **The moss *Physcomitrella patens* chloroplast *rpoA* gene is present in the nuclear genome**

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Chloroplasts have their own transcriptional apparatus and most chloroplast genes are transcribed by a eubacterial-type plastid-encoded RNA polymerase (PEP). The core subunits of the PEP are encoded by *rpoA*, *rpoB*, *rpoC1*, and *rpoC2* on the chloroplast genome. We have determined the entire nucleotide sequence of the moss *Physcomitrella patens* chloroplast DNA and found absence of *rpoA* in the chloroplast genome. This strongly suggests that chloroplast *rpoA* gene is relocated in the nuclear genome in *P. patens*. Therefore, we initially searched *rpoA* homologs in the *P. patens* EST database (The *Physcomitrella* EST Programme: <http://www.moss.leeds.ac.uk>) and found an EST clone containing the 3' portion of *rpoA* sequence. Based on the EST sequence, we have carried out 5'-rapid amplification of cDNA ends. The cDNA clone obtained encodes a putative protein of 450 amino acid residues with 45% amino acid identity with tobacco chloroplast-encoded RpoA and 29% identity with *Escherichia coli* counterpart. Therefore, we tentatively designated this protein PpRpoA. PpRpoA contains an N-terminal extension that possibly functions as a chloroplast-targeting signal. To determine the cellular localization of PpRpoA, DNA encoding the first 94 residues containing the putative transit peptide was ligated to the coding sequence of synthetic GFP, and the resultant plasmid was introduced into the *P. patens* protonematal protoplasts. In cells expressing the PpRpoA-GFP fusion protein, green fluorescence was observed in the chloroplasts. This clearly demonstrates that PpRpoA localizes to the chloroplasts. This is the first example of the existence of nuclear-encoded chloroplast RpoA in plants and algae.