

P9: Two Chloroplast Hsp70s in the moss *Physcomitrella patens*

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Heat shock protein (HSP) 70s are the eukaryotic homologs of the *E. coli* chaperone DnaK. Hsp70s are weak ATPases that are encoded by a multiple gene family and are located in different cell compartments. They have broad functions including involvement in gene transcription and translation, prevention of protein aggregation, and assistance in protein refolding. In addition mitochondrial Hsp70 has been shown to be involved in protein translocation across the mitochondrial membranes. The role of chloroplast stromal Hsp70 in protein import into that organelle is still under debate. We are trying to resolve this question using the moss *Physcomitrella patens* as a model system.

Homologous searching in the *P. patens* EST database with an *Arabidopsis* stromal Hsp70 gene resulted in 90 hits. The ESTs were assembled using CExpress and the assembled contigs were tested for predicted chloroplast transit peptides with ChloroP. Two partial sequences of chloroplast stromal Hsp70 candidates were obtained. We isolated, cloned and sequenced the full-length cDNAs of these two Hsp70 genes, termed *cpHsp70-1* and *cpHsp70-2*. The clones have been translated *in vitro* and the resulting precursors imported into pea chloroplasts, confirming their plastid location. Two knockout constructs containing resistance genes encoding either hygromycin-phosphotransferase or neomycin-phosphotransferase flanked by 600 – 1000 bp moss cpHsp70 DNA were transformed into moss protoplasts. Approximately 60 stable antibiotic-resistant colonies were obtained, and the identification and characterization of these transformants are in progress.