Evolution and functional diversification of *groESL* paralogs in *C. fritschii* PCC 6912

Gene duplication is an ongoing process in genome evolution and has a major impact on the gain of new protein functions. In most eubacteria the GroESL chaperonin is encoded by a single-copy bicistronic operon that includes the *groES* and *groEL* genes. A phylogenetic analysis showed that the GroESL chaperonin genes were duplicated at least once during cyanobacterial evolution. The chaperonin GroEL and its co-factor GroES promote protein folding in an ATP-dependent manner and is known to have a huge influence on the adaptation to divers stress conditions. Here we study the evolutionary and functional diversification of groEL paralogs within Stigonematalean cyanobacteria that encode for two copies of a groESL bicistronic operon (*groESL1, groESL2*) and one monocistronic paralog of groEL (*groEL3*), using the multicellular, heterocyst forming cyanobacterium *Chlorogloeopsis fritschii* PCC 6912 as a model. A comparison of gene expression under various stress conditions showed diverse expression pattern of the paralogous *groEL* under one specific condition. Also, transcriptional GFP-fusion experiments revealed a different expression localization of the paralogs during diazotrophic conditions. Furthermore, to evaluate the GroEL-GroES specificity, a bacterial-two-hybrid system was employed to investigate the protein-protein interactions between the chaperonin subunits *in vivo*. The observed differential ability for chaperonin assembly indicated structural differences between the paralogs that could also influence the chaperonin activity. A complementation assay in a *groESL* deficient *Escherichia coli* strain additionally showed that the function of the three GroEL paralogs is not redundant. In conclusion, this study showed that the evolutionary consequences of *groEL* duplication is unrelated to dosage effects and provided evidence for sub- and neofunctionalization of the paralogous genes.