A novel salt resistance protein, mangrin, isolated from a mangrove plant

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"Mangrove" is a general term for salt-tolerant woody plants growing along the seashore in tropical and subtropical areas. Mangrove plants may have acquired specific genes essential for salt tolerance during the course of their evolution. Finding such genes may greatly influence agricultural productivity in the future, because salt stress is a serious factor limiting plant growth and productivity throughout the world.

To find key genes essential for salt tolerance in a mangrove plant, *Bruguiera sexangula*, functional screening was performed using *Escherichia coli* as the host organism. A transformant expressing a protein homologous to *Lycopersicon* (tomato) allene oxide cyclase (AOC) displayed enhanced salt tolerance. However, this unusual trait is not conferred by *Lycopersicon* AOC or its *Arabidopsis* homolog. Analysis of the functional region revealed a sequence of only 70 amino acids, which contains an unusual sequence that is essential for the salt-tolerant phenotype. On the basis of its unusual function, the mangrove AOC homolog is designated "mangrin". Furthermore, expression of mangrin driven by the *GAL1* promoter and the 35S cauliflower mosaic virus (CaMV) promoter in *Saccharomyces cerevisiae* and tobacco cell lines, respectively, also gave rise to enhanced salt tolerance. Mangrin transcripts increased in cultured *B. sexangula* cells in response to salt stress. We propose that mangrin plays an important role in the salt-tolerance mechanism of *B. sexangula*, and that the biosynthesis of mangrin might be an effective means of enhancing salt tolerance in higher plants.

In addition to the finding of mangrin, we also find that a transformant *E. coli* expressing a cytosolic chaperonin-containing TCP-1 alpha (CCT alpha) homolog displayed enhanced salt tolerance. Analysis in *E. coli* of the functional region revealed that a sequence of only 218 amino acids, containing the apical domain, is necessary for salt tolerance. Furthermore, this domain shows chaperone activity *in vitro*. Therefore, CCT alpha facilitates the folding of proteins without ATP or the cage-like structure, and may play an important role in stress tolerance in *B. sexangula*.

Suaeda japonica, a member of the family Chenopodiaceae, is a halophyte that grows on the shores of the Ariake Sea in Japan. This plant species can grow in the presence of 0.7 M NaCl. Therefore, we also constructed a cDNA library and undertook functional screening for genes essential for salt-tolerance mechanisms, using *Escherichia coli* as the host organism. This screening isolated a transformant with enhanced salt tolerance that expressed RelA/SpoT homolog (Sj-RSH). In bacteria, RelA/SpoT determines the level of guanosine tetraphosphate (ppGpp) and guanosine pentaphosphate (pppGpp). These unusual nucleotides were produced under starvation and other stress conditions, and altered the expression of a large number of gene products in order to adjust cellular metabolisms under the stress conditions. This physiological phenomenon is called the stringent response. (p)ppGpp has been found not only in bacteria but also in lower eukaryotes including fungi and yeasts. However, its physiological functions in eukaryotes are still an enigma. Furthermore, the role of eukaryotic RelA/SpoT homologs in salt stress resistance has not been described previously. Therefore, the physiological function of Sj-RSH was investigated in this study. Complementation analysis using the *relA* mutant of *E. coli* showed that Sj-RSH conferred the phenotype associated with (p)ppGpp synthesis. Furthermore, expression of Sj-RSH driven by the *GAL1* promoter also gave rise to enhanced salt tolerance in yeast. Northern blot analyses of the yeast transformant revealed that the transcriptional levels of stress responsive genes including *GPD1, VMA6, BMH1, HYP1,* and *HOG1* were clearly enhanced in the Sj-RSH transformant when compared with an empty vector transformant under stress-free and 1.5 M NaCl stress

conditions. These results suggest that (p)ppGpp synthesis mediated by plant RelA/SpoT homologs plays a critical role for the transcriptional induction of several stress responsive genes, directly or indirectly in yeast, and that the conserved stress-resistance system may exist in higher plants.

Based on these data, "the functional screening method" is one of a useful approach to analyze the salt-resistance mechanisms of halophytes.

References

Yamada A., Saitoh T., Mimura T., Ozeki Y. Plant Cell Physiol 903-910 (2002)

Yamada A., Sekiguchi M., Mimura T., Ozeki Y. *Plant Cell Physiol* 1043-1048 (2002)

Yamada A., Tsutsumi K., Tanimoto S., Ozeki Y. *Plant Cell Physiol* 3-9 (2003)