

Signal transduction in a protozoa, *Euglena gracilis*, under salt stress.

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Euglena gracilis Z is a protozoa, living in fresh water. When *Euglena* are transferred to stressed conditions, for example, salt or heat stress, *Euglena* synthesize and accumulate trehalose as a compatible solute to adapt environmental changes. Although the regulation of this phenomenon is not well known, we have reported that fructose-2,6-bisphosphate (Fru-2,6-P₂), which is known as a regulator of sugar to metabolism in mammals, is a key regulator in trehalose synthesis under salt stress in this organism. Here, we report that the signal transduction involved in the trehalose synthesis of *Euglena* under salt stress.

E. gracilis treated with protein kinase inhibitors, staurosporine, calphostin C, 2,5-Mec and harbimycin, were transferred to a medium containing salt and then the trehalose content in the cell was measured. Trehalose accumulation was markedly inhibited in the cells treated with staurosporine, 2,5-Mec and harbimycin. The Fru-2,6-P₂ content in the cells not treated with them in the salt stressed conditions decreased rapidly, while the content of Fru-2,6-P₂ in the cells treated with the protein kinase inhibitors decreased gradually. Furthermore, decrease of F-6-P 2-kinase activity under the salt stress was moderated in the cells treated with the protein kinase inhibitors. We also found that cyclic AMP dependent protein kinase catalytic subunit suppressed F-6-P 2-kinase activity *in vitro*. These results showed that the protein kinase cascade is involved in the signal transduction regarding the trehalose accumulation of *E. gracilis* under salt stress and that phosphorylation of serine/threonine residue regulate the F-6-P 2-kinase activity directly.

We detected tyrosine phosphorylated peptide by immunoblot technique with an anti-phosphotyrosine antibody. Several peptides were found in the extract of *E. gracilis* under salt stress and the N-terminal amino acid sequence of one of the protein was determined.