# Molecular mechanism of osmoregulation in the halotolerant green alga *Dunaliella*

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The halotolerant green alga *Dunaliella* grows in media containing 0.5 - 5 M NaCl by regulating its cellular concentration of glycerol. We have shown that $Ca^{2+}$-dependent protein kinase (CDPK) which requires only $Ca^{2+}$ as the activator, involes in the process of osmoregulation after hypoosmotic shock in *Dunaliella tertiolecta*. We have also discovered protein kinases which were activated in response to hypo- and hyperosmotic shocks.

In the present study, a cDNA coding CDPK was cloned from expession cDNA library by using an anti-*Dunaliella* CDPK antibody and its whole nucleotide sequence was determined. On the other hand, amino acid sequences of 3 polypeptides produced by V8 proteinase digestion of the purified CDPK were determined. These amino acid sequences coincided with the respective parts of the amino acid sequence deduced from the nucleotide sequence. The deduced amino acid sequence contained 11 protein kinase domains at N-terminal region, calmodulin-like domain with 4 $Ca^{2+}$ binding sites at C-terminal region, and a pseudosubstrate domain between the kinase domains and the calmodulin-like domain, indicating that rhis is a typical CDPK.

Using an in-gel protein kinase assay, protein kinases activated in response to osmotic shock in *Dunaliella* cells were analysed. Protein kinases with molecular mass of 40-kDa were activated by both hypoosmotic (0.5 to 0.2 M NaCl) and hypersomotic (0.5 to 1.1 M NaCl) shocks. The hypoosmotic shock responsive kinase (<u>l</u>ow osmotic pressure-<u>a</u>ctivated <u>p</u>rotein kinase; LAP kinase) was transiently activated with a peak 1-2 min after the shock, while the hyperosmotic shock responsive kinase (<u>h</u>igh osmotic pressure-<u>a</u>ctivated <u>p</u>rotein kinase; HAP kinase) was rapidly activarted within 20 sec and remained in the activated state over 10 min. LAP kinase phosphorylated myelin basic protein (MBP) and histone but not casein. On the other hand, HAP kinase phosphorylated casein and histone but not MBP. Activation of both kinases were suppressed by a protein kinase inhibitor K-252a. The activated kinases were deactivated by the treatment of alkaline phosphatase. These results suggest the presence of protein kinase cascades transducing osmotic signals in *Dunaliella* cells. CDPK is one of candidates for the upstream regulator of LAP kinase. In tobacco suspension culture cells, hypoosmotic shock induced a transient increase in cytosolic $Ca^{2+}$, and subsequent activation of LAP kinase-like kinase.