Ada Zamir

Malkit Azachi
Umesh Bageshwar
Irena Gokhman
Lakshmanane Premkumar
Tatayana Savchenko

Molecular adaptations underlying the outstanding salt tolerance of the alga *Dunaliella*

Department of Biological Chemistry

Tel. 972 8 934 3831 **Fax.** 972 8 934 4118
E-mail: ada.zamir@weizmann.ac.il

Green microalgae of the genus *Dunaliella* are capable of proliferating in a very broad range of salinities, from 0.1 M to near saturation of NaCl. The algae maintain a relatively low internal ionic concentration and achieve osmotic balancing by intracellular accumulation of glycerol. Our studies aim to uncover additional mechanisms and molecular adaptations enabling the outstanding salt tolerance of *Dunaliella*, the organism and its components.

Possible involvement of fatty acid elongation in the salt tolerance of *D. salina*

A salt-inducible gene from *D. salina* encoded a protein homologous to higher plants beta-ketoacyl-CoA synthases (Kcs), enzymes catalyzing the first and rate-limiting step of fatty acid elongation. Kcs activity, localized to a microsomal fraction of *D. salina*, was enhanced in parallel to kcs mRNA in cells transferred from low to high salinity. The possible function of Kcs was revealed in lipid analyses showing that microsomes from cells grown in 3.5 M NaCl contained a considerably higher ratio of C18 to C16 fatty acids compared to cells grown in 0.5 M salt. These results suggest that modification of microsomal membranes by Kcs may be required to optimize their function in the glycerol-rich intracellular environment existing in high salinities.

The outstanding salt tolerance of plasma membrane carbonic anhydrases in *D.salina*

A 60 kDa protein, Dca, consisting of an internally-repeated alpha-type carbonic anhydrase, gradually accumulates in *D. salina* cells with rising salinity or bicarbonate depletion. Localized to the plasma membrane and extracellularly-exposed, Dca is likely to function in maintaining CO_2 availability in face of the gradual decline in CO_2 solubility with rising salinity. Hence, unlike other alpha-type carbonic anhydrases, Dca is expected to exhibit outstanding salt tolerance. Analyses of homogenously-purified Dca indicated that Dca not only withstood, but was activated by a variety of salts. Comparison of different cations and anions permitted to differentiate between stimulatory and inhibitory ion

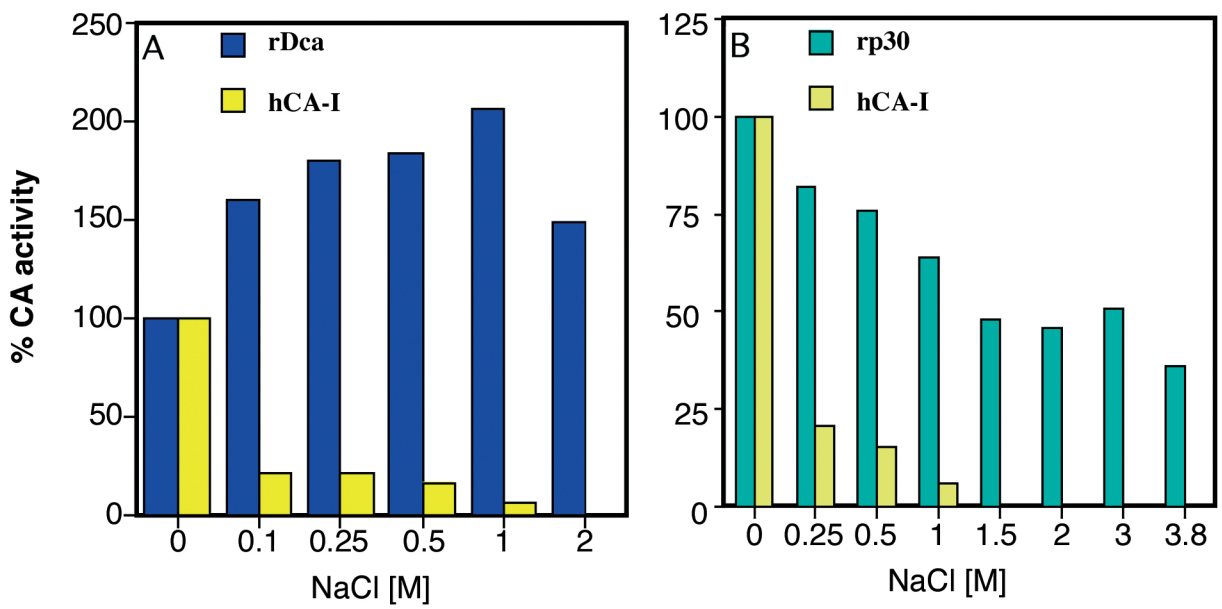

Fig. 1 *Salt tolerance of carbonic anhydrases from Dunaliella. Recombinant Dca (A) and p30 (B) were assayed for carbonic anhydrase activity together with the mesophilic human CAI*

effects. A contribution of the internal duplication to the activity and salt responses was deduced from the comparison of Dca with in vivo or recombinant truncated forms. An additional, non-duplicated, plasma membrane alpha-type carbonic anhydrase, p30, showed homology to each of the Dca repeats and exhibited salt tolerance. These results raise the possibility that the evolution of salt tolerant proteins, distinct from mesophilic or halophilic proteins, plays a role in the unique salt tolerance of *Dunaliella*.

Structural studies of halotolerant proteins

In order to elucidate the structural principles that govern the salt tolerance of the *D. salina* carbonic anhydrases, we undertook to determine their crystal structure (together with Joel Sussman, Dept. of Structural Biology). High levels of recombinant Dca and p30 were synthesized in E. coli and p30 has been crystallized to $P2_1$ space group. These crystals diffract beyond 1.8 A°. As carbonic anhydrase contains Zn, the multi wavelength anomalous dispersion (MAD) method is used to obtain the phase information .

Differential screening of salt-regulated *Dunaliella* genes by fluorescent differential display (FDD)

A screen for genes differentially expressed under high salinity is based on quantitative RT-PCR of mRNA populations from low and high salt grown cells, using as primers fluorescent oligo(dT), with A,C or G anchors, and a random primer of known sequence. We have now critically established all the conditions for obtaining fully reproducible, highly informative data. The results show both salt-induced and salt-repressed genes and adds a large number of genes potentially involved in salt tolerance of *Dunaliella*.

Selected Publications

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