Uri Pick

Mark Itsko
Adriana Katz
Michal Ortal-Schwarz
Yakov Paz
Tal Versano
Meira Weiss
Sigalit Zchut

Molecular basis of salinity tolerance in the halotolerant alga *Dunaliella*

Department of Biological Chemistry

Tel. 972 8 934 2732 **Fax.** 972 8 934 4118
E-mail: bcpick@weizmann.ac.il

Dunaliella is a unicellular green alga that is unique in its ability to adapt to some of the harshest environments known on earth. Most *Dunaliella* species tolerate extreme salinities, *D. acidophila* grows optimally at pH 0-1, *D. bardawil* tolerates exceptionally high irradiance and *D. antarctica* thrives at subzero temperatures.

Our laboratory focuses on understanding the molecular mechanisms that enable *Dunaliella* to adapt to extreme environments, particularly high salinity. Our aims are: to identify proteins associated with halotolerance and to clone their corresponding genes, to clarify the structural basis enabling proteins/enzymes to function at high salt concentrations and to utilize genes from *Dunaliella* to generate transgenic plants with enhanced salinity tolerance.

Salt-induced proteins

We have identified two major plasma membrane proteins which were induced by high salt in *D. salina.* The proteins are homologous to eucaryotic carbonic anhydrase and to human transferrin, the first transferrin identified in plant systems. Functional analyses showed that the transferrin-like protein mediates uptake of Fe^{3+} ions whereas the carbonic anhydrase is involved in CO_2 acquisition. The results established that iron and CO_2 acquisition are major rate limiting steps for survival in hypersaline environments. Both proteins share unique functional and structural features: they are resistant to high salt and function in low salt as well as in high salt, and both are acidic proteins and possess internal repeats (2 or 3). A comprehensive differential analysis of salt-induced proteins in *Dunaliella* is carried out by 2D gels combined with biotin tagging, mass spectroscopic analysis and sequencing (M. Ortal- Schwarz, T. Versano, collaboration with A. Zamir).

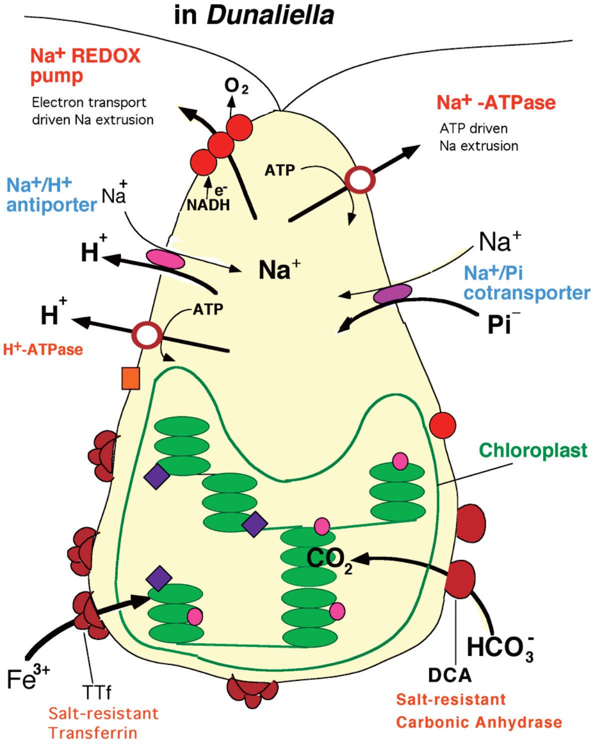
Na^+ elimination

Dunaliella has an exceptional ability to eliminate Na^+ ions, a detrimental factor for survival in hypersaline solutions. We have identified a novel redox-driven Na^+ pump, responsible for Na^+ elimination in *Dunaliella* (A. Katz). We have shown that *Dunaliella* utilizes the large electrochemical Na^+ gradient (G Na^+=180-220 mV) also for energization of phosphate and sulfate uptake via Na^+/Pi and Na^+/SO4 symporters (M. Weiss). These results suggested that *Dunaliella* recycles Na^+ ions across the plasma membrane as the main ion in transport processes (see scheme).

Cold-acclimation

Following acclimation at 7°C, *Dunaliella* salina cells aquire resistance to freezing down to- 40°C. We have shown that the primary target of freezing damage in the photosynthetic system is PS-II and that it is protected by cold-acclimation. Cold-acclimation is associated with increased desaturation level of fatty acids in the plasma and thylakoid membranes. A glycine-rich RNA binding protein which is induced at low temperature was identified and cloned (S. Zchut and M. Weiss).

Ion transporters involved in salt-resistance in *Dunaliella*



Iron acquisition

Iron availability is a major limiting factor in global biomass productivity in aquatic environments. We have cloned the first known algal Fe transporter (algal transferrin) and demonstrated that it functions in uptake of Fe^{3+} ions. Recently we cloned two additional proteins which are induced under iron deprivation: a different plasma membrane transferrin and a unique chlorophyll a/b binding protein. These studies indicate that *Dunaliella* copes with iron deprivation by overexpression of iron transporters and by extensive modification of the photosynthetic system (M.Ortal-Schwarz, T. Versano).

Selected Publications

Fisher, M., Gokhman, I., Pick, U. and Zamir (1997) A structurally-novel transferrin-like protein accumulates in the plasma membrane of the unicellular green alga *Dunaliella* salina grown in high salinities. J. Biol. Chem. 272, 1565-1570.

Chitlaru, E., Seger, R. and Pick, U. (1997) Activation of a 74 kDa plasma membrane protein kinase by hyperosmotic shocks in the halotolerant alga *Dunaliella* salina. J. Plant Physiol. 151, 429-436.

Holland, N., Evron, Y., Jansen, M.A.K., Edelman, M. and Pick, U. (1997) Involvement of thylakoid overenergization in tentoxin-induced chlorosis in Nicotiana spp. Plant Physiol. 114, 887-892.

Evron, Y. and Pick, U. (1997) Modification of sulfhydryl groups in the g subunit of chloroplast coupling factor 1 affects the proton slip through the the ATP synthase. Plant Physiol. 115, 1549-1555.

Holland, N.Belkind, H., Holland, D., Pick, U., and Edelman, M. (1998) Stress-responsive accumulation of plastid chaperonin 60 during seedling development. Plant J. 13, 311-316.

Fisher, M., Zamir, A. and Pick, U. (1998) A transferrin-like protein mediates Fe^{3+} uptake in the halotolerant alga *Dunaliella*. J. Biol. Chem. 273, 17553-17558.

Pick, U. (1998), *Dunaliella*- a model extremophilic alga. Israel J. Plant Sci. 46, 131-140.

Pick, U. (1999) *Dunaliella* acidophila- a most extreme acidopholic alga. In: Enigmatic microorganisms in extreme environments (J. Seckbach Ed.) Kluwer Academic Press, Dordrecht/The Netherlands, pp. 465-478.

Banet, G., Pick, U. Malkin, S. and Zamir, U. (1999) Differential responses to different light spectral ranges of violaxanthin de-epoxidation and accumulation of Cbr, an algal homolog of plant early light inducible proteins, in two strains of *Dunaliella*. Plant. Physiol. Biochem. 37, 875-879.

Banet, G., Pick, U. and Zamir, U. (2000) Light-harvesting complex II pigments and proteins in association with Cbr, a homolog of higher-plant early light inducible proteins in the unicellular alga *Dunaliella*. Planta 210, 947-955.

Katz, A. and Pick U. (2001) Plasma membrane electron transport coupled to Na^+ extrusion in the halotolerant alga *Dunaliella* . Biochim. Biophys. Acta, 1504, 423-431.

Weiss, M., Haimovitch, G. and Pick, U. (2001) Phosphate and sulfate uptake in the halotolerant alga *Dunaliella* are driven by Na^+-symport mechanism. J. Plant Physiol. (in press).

Zchut, S., Keren, N., Ohad, I. And Pick, U. (2001) Cold-acclimation protects photosystem II against freezing damage in the halotolerant alga *Dunaliella* salina. J. Phycol. (in press).

Pick, U. (2001) Adaptation of the halotolerant alga *Dunaliella* to high salinity. In: Salinity, environment, plants, molecules. Lauchli, A. and Luttge, U. eds., Kluwer Acad. (in press).

Acknowledgements

Incumbent of the Charles and Louise Gartner Professorial Chair.

Supported by The Avron-Minerva Center of Photosynthesis, The Israel Research Foundation of Science and Humanities, The Ministry of Industry and Commerce (MAGNET program), and Nikken-Sohonsha Corp., Gifu, Japan.