

# Survival at **Extreme Salinity** and **Iron Deficiency**

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## Abstract

*Dunaliella* is a unicellular green alga that can tolerate high salinity and iron deprivation, two major stresses for photosynthetic organisms. Our laboratory's aims are: (i) to identify proteins associated with stress resistance and to clone their corresponding genes, (ii) to clarify the structural basis enabling proteins/enzymes to function at high salt concentrations, (iii) to utilize genes from *Dunaliella* to generate transgenic plants with enhanced salinity tolerance.

A differential proteomic analysis revealed that salt stress upregulates in *Dunaliella* key enzymes in photosynthetic carbon assimilation and energy production. We developed procedures for tagging and resolution of plasma membrane proteins that enabled to identify salt-regulated proteins in *Dunaliella* plasma membranes. Surface-exposed plasma membrane proteins that are acidic and consist of two or three repeating units, properties believed to be associated with salt resistance.

*Dunaliella salina* adapts to iron deficiency by up-regulation Fe uptake and by remodeling of their photosynthetic system. Four major proteins, including two transferrins and a multicopper ferroxidase are induced at limiting iron in the plasma membrane. These proteins assemble to create an iron-binding complex at the plasma membrane surface, which binds, occludes and internalizes large amounts of  $\text{Fe}^{3+}$  ions. A novel chlorophyll *a/b* binding protein is induced in photosystem I (PS-I) leading to enlargement of PS-I antenna and rebalancing of the energy distribution between PS-I and PS-II.

The findings reveal how organisms can adapt to extreme salinity or iron deprivation and provide potential molecular elements for enhancing salinity tolerance in crop plants.

## Introduction

***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 and will survive even in The Dead Sea, *D. acidophila* has adapted to the most acidic habitats on earth and grows optimally at pH 0-1, *D. bardawil* tolerates exceptionally high irradiance and *D. antarctica* thrives at subzero temperatures and is resistant to freezing. The special interest in *Dunaliella* is more than a scientific curiosity: *D. bardawil* is the richest known source of natural beta-carotene and is cultured in NBT Eilat for commercial production of this important chemical. *Dunaliella* is also a native pool for stress-related genes that may be utilized for generation of robust crop plants to enhance agricultural productivity.

## Specific topics

### Salt-resistance proteins

A comprehensive proteomic analysis is carried out in collaboration with a leading proteomic group in MPI, in Dresden, Germany. The aim is to obtain a large-scale identification of salt-induced proteins in *Dunaliella* to clarify their function in salt resistance and to express selected genes in crop plants with the aim to enhance their salinity tolerance (A. Katz).

In a recent analysis of soluble salt-induced proteins, we have shown that *Dunaliella* responds to salt stress by upregulation of major metabolic pathways associated with photosynthetic carbon assimilation and energy utilization (Liska et al., 2004).

Our main effort is to identify the changes in the plasma membrane proteome under salt stress. We developed procedures for tagging plasma membrane proteins, their resolution on Blue-Native gels, followed by mass spectrometry analysis and sequence homology searches. Salt-induced proteins in the plasma membrane include a unique

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transferrin mediating uptake of  $\text{Fe}^{3+}$  ions, two salt-resistant carbonic anhydrases (studied by A. Zamir), a redox-driven  $\text{Na}^+$  pump and a nitrate and ammonium transporters. The results established that iron and  $\text{CO}_2$  acquisition are major rate limiting steps for survival in hypersaline environments.

Genes of selected salt-induced proteins, such as a salt and acid-resistant  $\text{H}^+$ -ATPase, have been introduced into crop plants for generation of salt-resistant crops as part of a MAGNET program with plant seed and tissue-culture companies (M. Weiss).

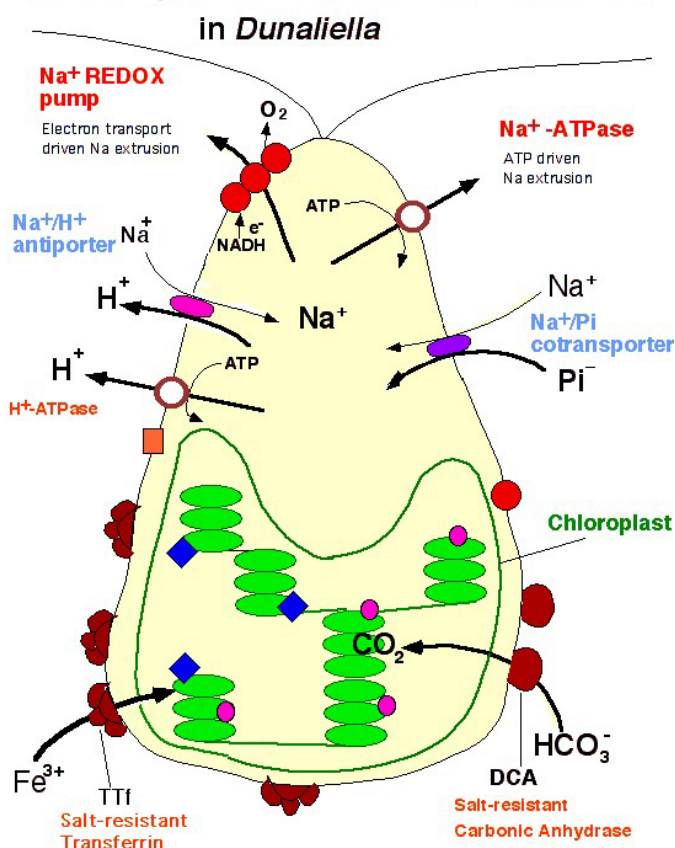
### $\text{Na}^+$ transport

Elimination of salt is detrimental for survival at hypersaline solutions. *Dunaliella* has an exceptional ability to eliminate  $\text{Na}^+$  ions and to maintain low internal  $\text{Na}^+$  concentrations (10–20mM) even at external  $\text{NaCl}$  of 3–4M. We found that *D. salina* possesses three distinct  **$\text{Na}^+$  transporters** in its plasma membranes: A  $\text{Na}^+/\text{H}^+$  antiporter and a redox-driven  $\text{Na}^+$  pump, which functions in pH homeostasis and in  $\text{Na}^+$  elimination, respectively (A. Katz). *Dunaliella* utilizes the electrochemical  $\text{Na}^+$  gradient also for energization of phosphate and sulfate uptake via  $\text{Na}^+/\text{Pi}$  and  $\text{Na}^+/\text{SO}_4$  symporters (M. Weiss). *Dunaliella* differs in this respect from most plants and algae in that it recycles  $\text{Na}^+$  ions, rather than protons across the plasma membrane as the main ion in transport processes. (See scheme below).

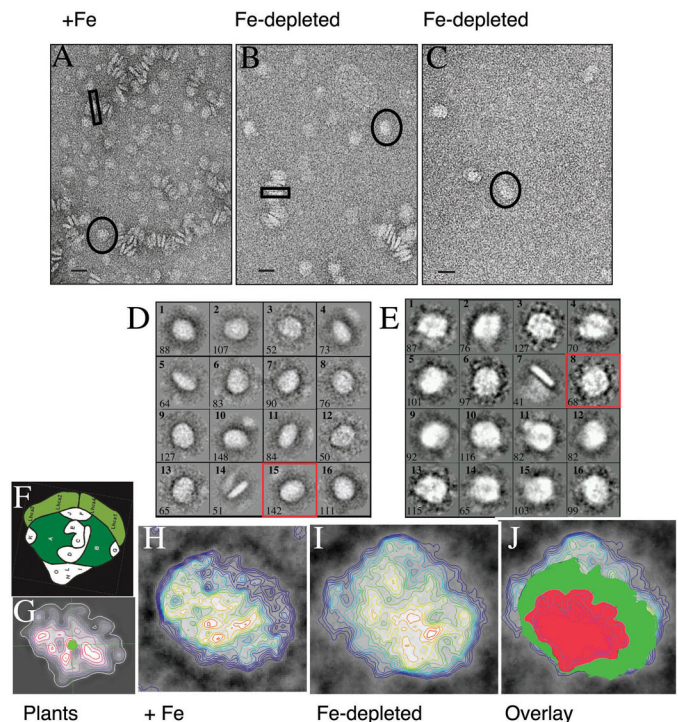
### Iron acquisition

Iron availability is recognized as a major limiting factor for proliferation of photosynthetic organisms. We have shown that *Dunaliella salina* utilizes special mechanisms to overcome the reduced bioavailability of iron in hypersaline solutions: (i) **Transferrin mediated  $\text{Fe}^{3+}$  binding and internalization:** *D. salina* over-expresses 4 major plasma membrane proteins

### Ion transporters involved in salt-resistance



## ENLARGED PS-I UNITS IN IRON-DEPRIVED DUNALIELLA SALINA



**Fig. 2** Electron micrography images of PS-I single particles from *D. salina*. Photosystem I units were purified from thylakoid membranes of control (A,D) or from iron-deficient cells (B,C,E). H–J: comparison of density maps of plant PS-I (red), control *D. salina* (green) and iron-deficient *D. salina* (magenta). (From Varsano, T., Wolf, S.G. and Pick, U., 2006).

under Fe deprivation: two different transferrins, a multi-copper ferroxidase and an unknown 130 kDa protein. These proteins form an iron-binding complex at the cell surface which enables binding and occlusion of  $10^6$ – $10^7$   $\text{Fe}^{3+}$  ions per cell, acting as a high affinity trap for iron. The bound iron is subsequently internalized into acidic vacuoles (J. Paz). This confers *Dunaliella* a high competitive potential for survival at limiting Fe. (ii) **Remodeling of PS-I:** We found that the photosynthetic system of *Dunaliella* is being remodeled under iron limitation: we identified and cloned a novel chlorophyll *a/b* binding protein, that is induced in photosystem I (PS-I) under Fe deprivation, leading to enlargement of the light harvesting antenna of PS-I (Fig 2). These studies demonstrated a new strategy to cope with iron deprivation that may be common to other photosynthetic organisms (T. Varsano, S. G. Wolf).

### Salt-resistant proteins are acidic and contain internal repeats.

Six surface-exposed proteins associated with stress resistance have been cloned from *D. salina* by A. Zamir's and our groups. They include two transferrins, two carbonic anhydrases, a multi-copper ferroxidase and one unknown protein. All six proteins are acidic compared to mesophilic counterparts (pI 4.0–4.5) and five contain 2 or 3 internal repeats, indicating that they originated from one ancestral gene. Four of these proteins, the transferrin TTf, two carbonic anhydrases and the multi-copper ferroxidase, DFox, have been functionally characterized and found to be salt-resistant. These findings suggest that an acidic surface charge and internal duplications are associated with salt resistance and have been selected by evolution as a means of adaptation of proteins for survival in hyper-saline environments.

## Selected Publications

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