

Immunogold localization of a hsp72 homologue in bean leaf cells

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Heat shock proteins (hsps) were discovered in *Drosophila* during the 1970s. Soon it became evident that these protein-protecting proteins (chaperones) were induced also by other stress factors, moreover some of them were constitutive, i.e. constantly present in the cell, coupled to nascent or transport-competent proteins. They have a conservative structure in all eukaryotes.

In our Department of Plant Anatomy, in collaboration with Department of Plant Physiology and Molecular Plant Biology, we investigate the beneficial, stimulative effect of mild stress (eustress), and its mechanism compared to that of the damaging stress (distress). We addressed the question, whether heavy metal ions in submicromolar concentrations may induce a member of the hsp70 protein family, as it is known for millimolar concentrations in other plant species [1].

Young bean plants were sprayed with 0.1 microM Cd(NO₃)₂ solution every other day for two weeks. The treatment significantly increased the chlorophyll content and CO₂ fixation [2]. Samples were fixed from the first leaves of both treated and control plants with 3% FA – 0.4% GA in 0.1 Na-cacodylate buffer. After dehydration and embedding (Durcupan ACM), sections were cut onto Ni grids, then incubated with anti-hsp72. /The antigen had been synthesized in a bacterial system using the cloned variable sequence of inducible hsp72 gene of rat [3]. Polyclonal antibody was raised in rabbit; we used this for a three-step immunogold-labeling/. Finally the sections were contrasted according to the standard procedure.

Our results and conclusions are as follows :

- a/ Both treated and control samples were labeled similarly and to the same extent. This shows a constitutive presence of a hsp72 cognate protein in bean leaves.
- b/ The localization is found exclusively in the chloroplast, around the starch grains.
- c/ According to our hypothesis, the function of this protein is to stabilize the conformation of enzymes active in the diurnal cycle of starch synthesis and degradation. This seems to be necessary because of the „molecular crowding”, when oligoglucans are moved between fluid and solid phases at the surface of the starch grain.

References

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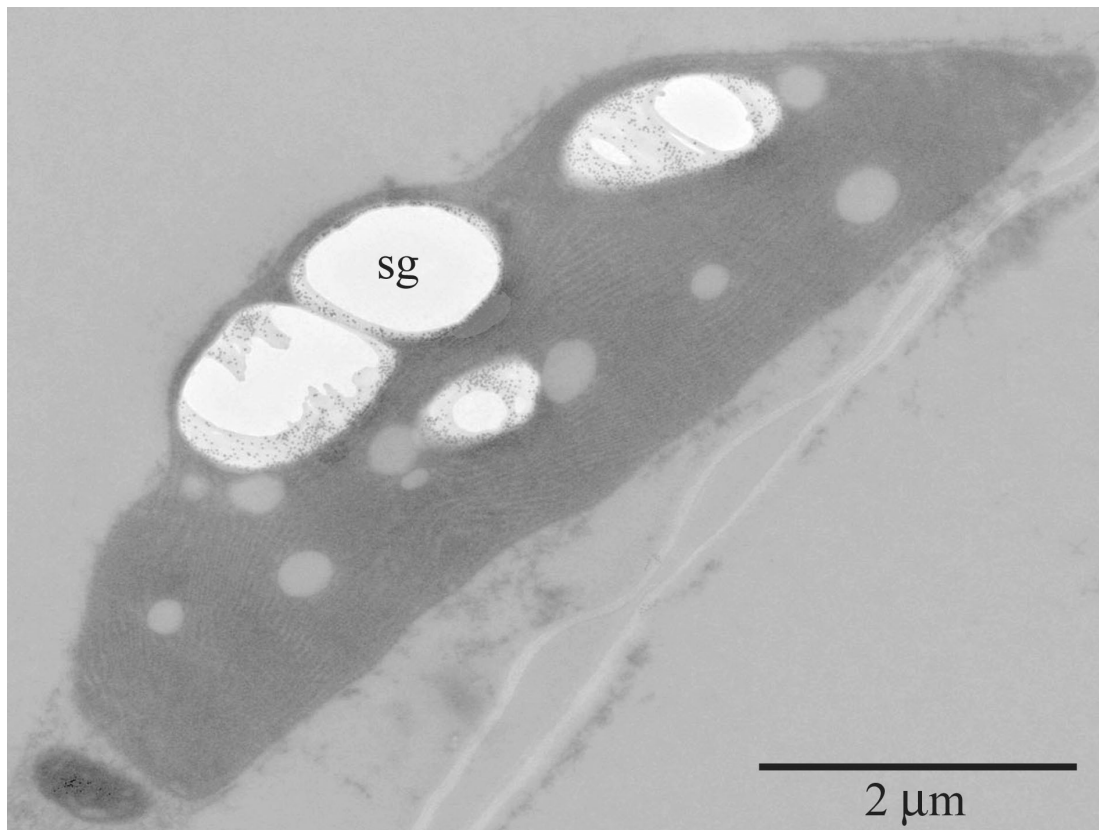


Figure 1. Hsp72-specific gold labeling is seen around starch grains (sg) in chloroplast.

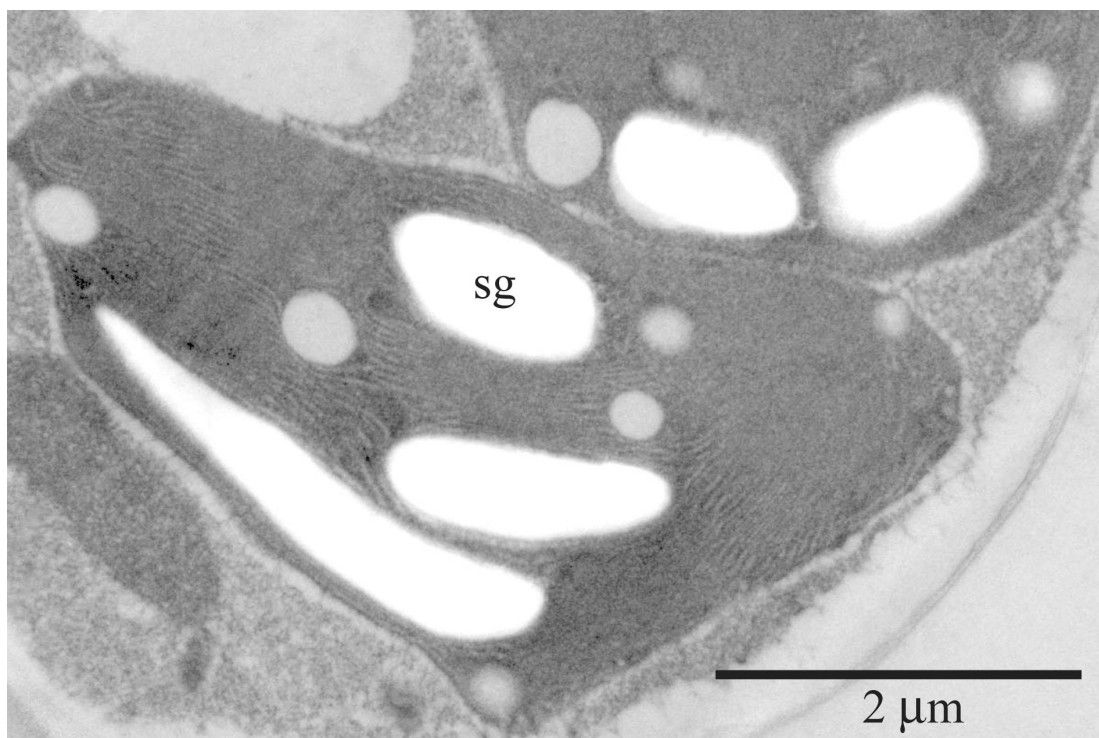


Figure 2. No labeling can be found in chloroplast without first antibody.