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Phosphatidylglycerol is important in the assembly and function of PSII reaction center

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Phosphatidylglycerol (PG) is a ubiquitous anionic phospholipid in almost all organisms. The structural and functional roles of anionic lipids in photosynthesis have raised scientific interest for a long time. The role of PG in photosynthetic organisms has previously been studied using either biochemical or molecular genetic approaches. The recent identification of genes encoding enzymes required for the biosynthesis of PG in cyanobacteria and eukaryotic plants, and the subsequent generation of mutants defective in the biosynthesis of PG, has provided powerful molecular tools to understand the function of PG in photosynthetic organisms. The role of PG has been extensively studied in two PG-less mutant strain of *Synechocystis sp.* PCC6803: Δ*pgsA* (Hagio 2000) and Δ*cdsA* (Sato 2000). Previously it was demonstrated that PG is required for the formation and function of thylakoid membranes in cyanobacteria and plants (Wada and Murata 2007; Domonkos 2008).

In the present investigation we constructed and characterized a new PG deficient mutant of *Synechocystis sp.* PCC6803. We inactivated the *cdsA* gene in phycobiliproteinless mutant, PAL, which compensates the missing light harvesting complex by high cellular content of PSII (Ajlani 1998). The PAL/Δ*cdsA* mutant provided a unique experimental system for a more detailed study of the role of PG in PSII function/assembly. We analyzed the influence of PG depletion on the fluorescence induction, thermoluminescence, biosynthesis and assembly of PSII protein subunits. The mutant cells grew only in a medium supplemented with PG. Depletion of PG in the cells resulted (i) in an inhibition of cell growth/division, (ii) in a small change in pigment composition, (iii) in the inactivation of oxygen evolution, (iv) in a modification of the fluorescence induction curve that pointed to some damage of Q_B, but not the donor side, (v) in a modification of the TL glow curve to give only shifted Q-band which is an indicator for suppression of electron transfer between Q_A and Q_B, and it does not affect the redox levels of Q_A and S_2. Two-dimensional PAGE showed that in the absence of PG (a) PSII dimer was monomerised, and (b) the CP43 protein was detached from a major part of the PSII core complex. [35S]-methionine labeling confirmed that PG depletion did not block de novo synthesis of PSII proteins. We conclude that PG is required for the binding of CP43 within the PSII core complex (Laczko-Dobos 2008). This is in good agreement with the presence of a PG molecule localized between D1 and CP43 subunits by X-ray crystallographic structure of *Thermosynecococcus elongatus* (Guskov 2009).

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Characterization of catalase genes in *Rhizopus oryzae*

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Zygomycosis is a diverse group of mycotic diseases caused by members of the class Zygomycetes. The main risk factors are diabetic ketoacidosis; cancer and its therapy; solid organ or bone marrow transplantations; prolonged steroid use; neutropaenia; deferoxamine treatment to manage iron overload and burn injuries (Papp et al. 2008). Thermophilic members of the genus *Rhizopus*, especially *R. oryzae*, are considered as the main causative agents of zygomycoses. During the past decades, such infections have emerged in an increasing number due to the widespread use of immunosuppressive therapy, intensive cancer chemotherapeutic regimens and broad-spectrum antimicrobial agents. High mortality rates, difficulties in the diagnosis and resistance to the most widely used antifungal drugs are characteristic features of zygomycoses underlying the importance of this fungal group (Ribes et al. 2000). All these aspects indicate that development of new strategies to prevent and treat these infections is urgently needed.

The aim of our study is identification and analysis of the genetic background of the virulence of opportunistic pathogen Zygomycetes.

Generation of oxidative products by phagocytic cells is known to be one of the important host defence mechanisms directed towards the killing of invading microorganisms (Gallin at al. 1993). Catalases may provide protection against reactive oxygen species produced by neutrophile granulocytes of the human immune system (Chang et al. 1998). Neutropenia is a considerable risk factor of zygomyçoses. In this study, catalase encoding genes of *R. oryzae* have been isolated, and their functional analysis has been started.

Four possible catalase genes were found in the *R. oryzae* genome database (*Rhizopus oryzae* Sequencing Project) by similarity searches with known fungal catalases. These genes and their adjacent regions were amplified by PCR from the genomic DNA of *R. oryzae* and cloned into the vector pBluescriptII SK+ (Stratagene). To reveal their function and to investigate their possible role in the pathogenicity, deletion mutants were created in the case of each isolated genes. Four vectors suitable to create deletions in the different genes were constructed; in each vector, the *pyrG* gene of *R. oryzae* encoding orotidine-5'-monophosphate decarboxylase was placed between the 5' and 3' flanking regions of the appropriate catalase genes. To ensure double crossover gene replacement, linear fragments were cut from the plasmids and used to transform protoplasts of a uracil auxotrophic *R. oryzae* strain using the polyethylene glycol-mediated method.

Integration of the transferred DNAs into the host genome and deletion of the appropriate catalase genes was proven by PCR and Southern blot analysis. Catalase activity of the recipient strain and the four mutants constructed were *in vitro* tested. Effect of hydrogen peroxide on the fungal growth was examined on agar plates and in a microtiter plate assay. All four catalase genes proved to be functional. In all types of mutants, deletion of a catalase gene increased markedly the sensitivity of the transformants to hydrogen peroxide. The strain deficient in the gene designated as 16995 was the less susceptible to hydrogen peroxide whereas the strains deficient in the other genes proved to be more sensitive. Further gene expression studies with isolated genes are in progress and we also plan to use the constructed deletion mutants in pathogenicity tests.

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Comprehensive genetic and biochemical examination of the polyubiquitin receptors in *Drosophila melanogaster*

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The ubiquitin-proteasome system is responsible for the polyubiquitilation and selective degradation of damaged, misfolded and short-lived regulatory proteins to ensure the proper homeostasis of the eukaryotic cell. Recognition of polyubiquitinated substrates by the proteasome is a highly regulated process that requires polyubiquitin receptors (p54/Rpn10: proteasome receptor subunit; Dsk2 and Rad23: non-proteasomal receptors). The mechanism of substrate recognition and delivery to the proteasome is well known in single cell eukaryotes (e.g. yeast), but unresolved in Metazoans.

We found that the subunit composition of the regulatory particle (RC) of the *Drosophila* 26S proteasome changes in a developmentally regulated fashion (Lipinszki et. al. submitted manuscript). The concentration of the p54/Rpn10 subunit falls suddenly at the end of embryogenesis, remains low throughout the larval stages, starts to increase again in the late third instar larvae and remains high in pupae and adults. A similar developmentally regulated fluctuation could be observed in the concentrations of the Rad23 and Dsk2 extraproteasomal polyubiquitin receptors. Our *in vitro* experiments revealed that protein extracts of first or second instar larvae can selectively degrade the embryonic p54/Rpn10 subunit of the 26S proteasome and the Dsk2 and Rad23 polyubiquitin receptors; whereas all the other tested proteins remained intact. The above observations and the fact that the gene expressions of the receptors remain constant during the development suggest that a selective protease is activated during the early larval stages. We successfully purified and identified this protease. Moreover, all the three receptors carry an extended intrinsically unstructured segment within the molecule, which can be the hot spot for the regulator protease.

To follow the *in vivo* fate of subunit p54/Rpn10, transgenic *Drosophila melanogaster* lines encoding the N-terminal half (NTH), the C-terminal half (CTH) or the full-length p54/Rpn10 subunit have been established in the inducible Gal4-UAS system. The daughterless-Gal4 driven whole-body expression of the full-length subunit or its NTH did not produce any detectable phenotypic changes and the transgenic products were incorporated into the 26S proteasome. The transgene-encoded CTH was not incorporated into the 26S proteasome, caused third instar larval lethality and it was found to be multiubiquitinated. This modification, however, did not appear to be a degradation signal, the half-life of the CTH is over 48 hours. The accumulation of the CTH disturbed the developmentally regulated changes of the subunit composition of the RP and, interestingly, the emergence of the selective proteolytic activity responsible for the depletion of the polyubiquitin