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Ascorbate, as alternative electron donor to photosystem II, protects plants against photoinhibition and stimulates the photoproduction of hydrogen in green algae

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Oxygenic photosynthetic organisms produce organic materials by using light energy and water as terminal electron donor. The water splitting enzyme, *i.e.* oxygen evolving complex (OEC) is one of the most vulnerable components of the photosynthetic electron transport chain. It has been shown (Tóth et al. 2007, Biochim Biophys Acta 1767: 295-305) that if the OEC is damaged, alternative electron donors, present in large amounts, donate electrons to photosystem II (PSII) in vivo. Our studies carried out on higher plant leaves and algal cells have shown that this alternative electron donor molecule is ascorbate; the rate of electron donation depends on the ascorbate content of leaves: $t_{1/2}$ is approximately 25 ms in wild type Arabidopsis plants and about 55 ms in ascorbate-deficient mutants (Tóth et al. 2009 Plant Physiol. 149: 1568-1578).

When OEC is damaged by heat stress highly oxidising components (Tyr_Z^+ and $P680^+$) accumulate in PSII in the light, leading to the fast inactivation of PSII. We have demonstrated that under these conditions ascorbate has a protective function by providing electrons to PSII and slowing down the harmful accumulation of Tyr_Z^+ and $P680^+$ (Tóth et al. 2011 Plant Physiol. 156:382–392).

Based on these results, it was reasonable to assume that ascorbate, by replacing the water splitting enzyme and supporting the electron transport without oxygen evolution, can enhance the photoproduction of hydrogen in *Chlamydomonas reinhardtii* cells. Photoproduction of hydrogen is known to depend on the activity of PSII; however, the oxygen evolution associated with PSII activity strongly inhibits the hydrogenase. It has earlier been shown that Chlamydomonas cells are able to evolve considerable amounts of hydrogen under anaerobic conditions following their sulphur deprivation, which suppresses their PSII activity (Melis and Happe 2001; 127:740–748). Our experiments have shown that the addition of 10 mM ascorbate to sulphur-deprived cell culture accelerates significantly the linear electron transport via PSII to PSI and to the hydrogenase, leading to a three-fold increase in hydrogen production. Similar results were obtained by using diphenylcarbazide (DPC), an artificial electron donor to PSII. The stimulation of hydrogen production was sensitive to diuron and dibromothymoquinone (inhibitors of PSII and the cytochrome b_6f complex, respectively), which proves that the enhancement of the hydrogen evolution by ascorbate and DPC can indeed be accounted for by their functioning as alternative PSII electron donors.

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Characterisation of host-pathogen interaction during *Candida* infections

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Candida species are known as members of the normal human flora. However under certain circumstances these commensalist yeasts are able to transform themselves into opportunistic pathogens. *C. parapsilosis* is considered to be the second or third most common *Candida* species causing candidiasis after *C. albicans*. The response of the mammalian immune system given to the *C. albicans* is well-exemined, and based on our pervious work it is clear, that some *Candida* derived lipases play role as virulence factor. On the other hand little is known on the interaction between the immune system and other *Candida* species, like *C. parapsilosis*.

We previously showed that *C. parapsilosis* lipase knockout (LIP-) mutants were significantly deficient in their capacity to produce biofilm, to grow in lipid rich medium, and to survive in macrophages. In an attempt to understand this reduced virulence phenotype, we developed an *in vitro* model system using murine macrophage -like cell line J774.2. We examined the gene expression in J774.2 macrophages infected with wild type (WT) *C. parapsilosis* and LIP- cells. The complex response of murine macrophages to infection with *C. parapsilosis* was investigated at the level of gene expression using Agilent mouse microarray. 155 and 512 genes were identified as being differentially regulated at 3 and 8 hours post infection, respectively. Most of the upregulated genes encoded molecules that were involved in immune response and inflammation, transcription, signalling, apoptosis, cell cycle, electron transport and cell adhesion. Of particular interest were the upregulation of proinflammatory cytokines, typical of the classically activated macrophages such as TNF, IL-1 and IL-15, and also the upregulation of TNF-receptor family members such as *TNFRSF9* associated with Th1 T-helper cell responses. Additionally, the microarray data indicate significant differences between the response to *C. parapsilosis* infection and that of *C. albicans*.

Flow cytometry analysis proved, that elevated mRNA level of TNFRSF9 correlated to the elevated amount of protein on the surface of J774.2 macrophage cells upon *C. parapsilosis* WT infection. Similar pattern of TNFRSF9 (CD137) regulation could be observed in cells from whole human blood upon *C. parapsilosis* WT infection. To further examine the host pathogen interactions we established a human monocyte (THP-1) cell line infection model. THP-1 cells were infected with eight different *Candida* strains from the parapsilosis *sensu lato*

group and subjected for complete transcriptome analysis. In order to profile the transcriptome changes with the best possible resolution, we utilized the robustness and accuracy of the Next-generation Sequencing (NGS) RNA-seq technology.

To further develop our infection models we established an in vitro system using primary human mononuclear blood cells. Monocyte-derived immature and mature dendritic cells (iDCs, mDCs) as well as macrophages (MΦ) co-cultured with live WT or LIP- *C. parapsilosis* strains were studied to determine the host response. We determined that all cell types efficiently phagocytosed and killed *C. parapsilosis*, furthermore our results show that the phagocytic and fungicidal activities of both iDCs and mDCs are more potent for LIP- compared to WT yeast cells. Notably, MΦ showed eleveted fungal killing activity to LIP- cells but no increased phagocitic capacity was detectable. In addition, the LIP- *C. parapsilosis* cells induce higher gene expression and protein secretion of proinflammatory cytokines and chemokines in all cell types relative to the effect of co-culture with WT yeast cells. Our results show that both DCs and MΦ are activated by exposure to *C. parapsilosis*, as shown by increased phagocytosis, killing and proinflammatory protein secretion. Moreover, these data strongly suggest that *C. parapsilosis* derived lipase has a protective role during yeast:phagocyte interactions, since lipase production in wt yeast cells decreased the phagocytic capacity (in case of DCs) and killing efficiency of host cells and downregulated the expression of host effector molecules.

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The role of guard cell photosynthesis in biotic stress-induced stomatal closure

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Guard cells (GCs) control gas exchange and transpirational water loss of leaves by turgor-driven volume changes. Environmental and hormonal signals regulate opening and closure by activating diverse signalisation networks and membrane transporters. GCs also respond to the presence of microbes following perception of microbe-associated molecular patterns, such as a fungal elicitor chitosan (CHT). It has been shown that CHT inhibits the blue light-induced stomatal opening and can trigger stomatal closure through distinct signaling pathways and transporters. Stomatal opening and closure is related to the H^+-ATPase activity in the GC plasma membrane, as it affects the transport of osmotically active solutes. ATP for proton pumping is partly supplied from photophosphorylation in *Vicia faba* GCs (Mawson 1993). In order to investigate whether CHT affects the photosynthetic ATP production, the light-dependence of the photosynthetic electron transport rate of individual GCs was assayed. We found that when CHT was applied before sunrise, the apparent relative linear electron transport rate (ETR) remained low contrary to control. However, when CHT was sprayed on leaves by day, it only induced slight stomatal closure without a significant change of these photosynthetic parameters. CHT was shown to induce the generation of both reactive oxygen and nitrogen species in pea GCs (Srivastava et al. 2009). Using fluorescent probes we found that one hour of CHT treatment led to a significant increase in both hydrogen peroxide (H_2O_2) and nitric oxide (NO) levels of *Vicia* GC chloroplasts. H_2O_2 accumulated mainly in chloroplast stroma and nucleus, while NO was found in the cytosol and chloroplasts. We also found that the inhibitory effect of CHT on the morning photosynthesis can be mimicked by exogenously applied NO, therefore it can be hypothesized that CHT acts through the NO signaling pathway.

NO triggers the release of Ca^{2+} from intracellular stores in GCs, which leads to stomatal closure. NO may also have an indirect effect through the decrease of intracellular ATP level generated by GC chloroplasts. Earlier we showed that NO slows down electron transfer between Q_A and Q_B, and inhibits charge recombination reactions of Q_A^- with the S_2 state of the water-oxidizing complex in pea leaves (Wodala et al. 2008). The microscopy version of a pulse-amplitude-modulated chlorophyll fluorometer (PAM) combined with a rapid solution exchanger allowed us to monitor the photosynthetic activity of a GC before and after the addition and also rapid removal of NO. The concentration of NO released from GSNO under light was measured in a solution entering the recording chamber using a NO-electrode. We found that NO decreases the electron transport rate resulting in a modest acidification of the thylakoid lumen and conceivably a reduced ATP synthesis. Variable fluorescence yield (Fv) was increased immediately in a biphasic manner after the inflow of the NO-containing solution, in line with the kinetic differences in the changes of photochemical and non-photochemical quenching. The wash-out of NO resulted in a sudden decrease of Fv, which was further accelerated by bicarbonate, a competitor of NO to the binding side of the non-heme iron between Q_A and Q_B.

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