Responses of the green alga *Chlamydomonas reinhardtii* to excess light

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Abstract
The use of the green alga *Chlamydomonas reinhardtii* in photosynthetic research has been growing in the last decades. The availability of the genome and the extensive 'toolkit' have provided a lot of knowledge of the biosynthetic (regulatory) pathways. This article tries to provide an overview of the different sensing and responding mechanisms involved in high light acclimation of *Chlamydomonas*.

Introduction
Capturing the energy from sunlight is an idea that is becoming more and more relevant with the decrease in oil reserves and the higher demands for clean energy. Nature is already harvesting the suns energy for billions of years, having a efficiency that is far higher then current human technology can achieve. The light capturing process of photosynthesis has been studied for many years now, and the increasing knowledge of the process may help us in our search for renewable energy sources.
The chloroplast is the organelle where photosynthesis takes place. In the chloroplast, the thyalkoid membrane is separating lumen and stroma and the large proteincomplexes that drive photosynthesis are imbedded in it. These large protein complexes are the pigment binding photosystems (PS) I and II that harvest the light, the Cytochrome b_6f complex important in electron transport and the ATP synthetase that uses the photosynthetically created proton gradient to make ATP.
Phototrophs encounter environmental conditions like CO_2 limitation, sulpher or iron depletion and excess light absorption that need cellular acclimation. In the case of excess absorption of light energy, different ways in which the cell experiences stress are possible. When there is over excitation of the chlorophylls (chl) in antenna systems, these chl may get into a triplet state. These triplet states are themselves not harmful to the cell, but they can lead to the formation of singlet excited oxygen ($^1O_2$) that is very reactive and damages mainly phospholipids and the D1 reaction center of the PSII supercomplex where it's created by charge recombination. Damage to D1 'closes' the reaction center making them inactive, this process is called photoinhibition or qI.
At PSI, other reactive oxygen species (ROS) can be made when the cell runs out of NADP+ as electron acceptor. Other electron acceptors may then accept the electrons at PSI, creating O2-, OH* radical and H_2O_2. As will be explained later, ROS are involve in a process called state transition, in which the light harvesting complex (LHC) of PSII is dissociating from PSII and reattaching to PSI. This redistribution of the antenna is providing a way for phototrophs to balance the light harvesting of PSI and II in different light conditions.
The plant *Arabidopsis thalia* is a very important model organism to study photosynthesis, but in the last decades the unicellular green alga *Chlamydomonas reinhardtti* has become more and more popular in photosynthetic research (see Rochaix 2002 for a review). Perhaps the most important advantage of *Chlamydomonas* is the possibility to grow the alga in the absence of light, making it possible to study photosynthetic deficient mutants that can not be studied in plants. (Dent et al 2001)
Since the (partial) unraveling of the genome of *Chlamydomonas*, powerful methods to examine gene expression levels have been made, see for instance Elrad and Grossman 2003 (review) or Eberhard et al 2006 on the production of an oligonucleotide array. Data from this

kind of experiments have proven very valuable in the understanding of the photosynthetic process and the different regulatory pathways involved with it.
In this review we will discuss the different acclimation responses of *Chlamydomonas* to environmental stress, focussing on the different chloroplast/thylakoid associated genomic responses to High Light (HL) conditions.

Sensing High Light conditions
In order to adapt to HL stress conditions, *Chlamydomonas* needs to be able to sense these conditions. This can be done directly by proteins that are photosensitive by have photosensitive compounds bound to them, or via the indirect affects caused by the excess light absorption (singlet oxygen production, hydrogen peroxide, pH changes and the redox state of the plastoquinone pool). The sensing mechanisms are specific for different molecular conditions, but the regulatory pathways on which they act may overlap so different signals can lead to similar responses. The opposite is also true, one sensing mechanism, for instance the redox state, is used for several signal transduction pathways. These complicated interactions are necessary for an optimal photoacclimation of *Chlamydomonas*.

Direct light sensing
The most direct way of acting on different light conditions is by sensing the light using photosensitive proteins. Proteins that was proved to be photosensitive are phototrophins (PHOT's), PHOT1 is a flavin binding photoreceptor that is sensitive to blue light. When *Chlamydomonas* strains with RNAi to PHOT1 are transferred from the dark to very low light, GSAT (involved in chl biosynthesis), PDS(involved in carotenoid biosynthesis) and Lhcb gene transcripts that are rising in WT strains, don't show this response in the inhibitory strains. (Im et al 2006, for an overview of chl and carotenoid biosynthesis pathways see Lohr et al 2005). This means that PHOT1 is in some way regulating GSAT, PDS and LHC gene expression. In other experiments GSAT and PDS showed the highest response of genes involved in chlorophyll and carotenoid biosynthesis when *Chlamydomonas* cultures where transferred from dark to low light conditions. (Lohr et al 2005) Since the genes that are found most differently expressed by the switch to low light are either at the beginning of, or directly after 'crossroads' in the chl biosynthesis pathway (UROD, CHLI, CHLH, PSY), these genes may be more important in the regulation of chl biosynthesis than the others. It is tempting to speculate that both GSAT and PDS, that show the most pronounced responses, play a key role in the regulation of the chl and carotenoid biosynthesis, and that PHOT is the sensory protein that synchronizes chl, carotenoid and Lhc biosynthesis. However there are also other signals inducing/repressing these gene expressions that will be discussed later, it would be interesting to see if these signals have similar and/or related ways of influencing these different biosynthetic pathways.
Biosynthesis of chl and carotenoid pigments appears to be strongly regulated. Regulation that is needed to prevent the accumulation of intermediate compounds that are light sensitive and are therefore potentially dangerous. It is also known that intermediates of the chl biosynthesis pathway are involved in signal transduction. The intermediates Mg-protoporphyrin IX and Mg-protoporphyrin IX monomethyl ester are known to induce the transcription of HSP70, that encodes chaperone protein. (Kropat et al 2000)
Induction of HSP70 was specifically induced by these intermediates, as external addition of either compound showed similar induction of HSP70 comparing to the induction with light. However the accumulation of these intermediates in itself is not enough to induce this signal. *Chlamydomonas* cultures that measured accumulation of the porhyrine, but where kept in the dark showed no induction of HSP70. This means that light is needed to induce the expression response of HSP70.

pH triggered reactions
When the HL conditions causes an overflow of electrons in the electron transport chain, balance in the ETC's is lost. This very quickly results in a change of the pH value (of both lumen and stromal side of the thylakoid membrane). This shift in pH can be sensed by several proteins. One of these proteins is a violaxanthin de-epoxidase (VDE). The lowering of the pH triggers conformational changes in VDE , that leads to activation of the enzyme. Via two de-epoxidation steps VDE can convert the carotenoid pigment violaxanthin into zeaxanthin. Zeaxanthin has been shown to improve the process of non photochemical quenching (NPQ). By incorporation of zeaxanthin instead of violaxanthin, light harvesting antenna complexes are more efficient in releasing the excess energy of excited chlorophylls in a non radiating, non-damaging way.
pH dependent reactions are also proposed for LHC proteins. In *Arabidopsis thalia* the PsbS gene encodes a protein that is sensing the lumen pH and is involved in the pH dependent regulation of energy quenching (qE). However no PsbS could be found in *Chlamydomonas,* and endogenously added PsbS is not associating with the thylakoid membrane. (Bonente et al 2008)

Redox state induced signaling
When the rate of charge separation at PSII exceeds the rate at which the plastoquinone (PQ) pool is oxydised, the PQ pool will get over reduced. The redox state of PQ or that of the binding site in the Cyt b_6f complex can therefore act as a sensing mechanism for excessive excitation of PSII. It has been shown by Teramoto and colleagues that Lhcb gene expressions are regulated by redox signalling. The repression in high light of all the genes encoding PSII major and minor antenna were found similar. This repression was also found by Durnford et al (2003), they observed that the transcript reduction of PSII antenna complex genes, thatwas maximal with 65-80% reduction after 1-2 hours illumination with HL, recovered after about 8 hours. Interestingly they noted the recovering of transcript levels of Lhcb was before there where significant reductions of the protein levels. This is evidence that the long term acclimation of these genes is mainly regulated at the translational level.
When addeding 2-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), that is preventing the reduction of PQ by PSII thereby mimicking low illumination of PSII, mRNA levels recovered but stayed significantly lower then the normal level. (Teramoto et al 2002) Teramoto concludes from this that there is another pathway repressing Lhcb genes that doesn't involve the redox state. As discussed above, there is indeed at least one other pathway that regulates Lhcb gene expression, in which PHOT1 seems to play a crucial role. This shows that the effects of DCMU, sometimes referred to as mimicking low light condition, should be carefully addressed, making the distinction between low light conditions and the redox state of PQ.

Besides the genes encoding the antenna proteins, redox state is also involved in the regulation by thylakoid associated kinases (TAKs). One of these kinases is the Stt7 seronine threonine kinase, that was identified by Depège et al (2003) using fluorescence screening for mutants that don't show a fluorescence decrease when inducing state transition, indicating that they are unable to perform state transitions.
The activation of seronine threonine kinase Stt7 is redox state regulated, upon activation of Stt7, by overreduction of the PQ pool, the LHCII's get phosphorylated. This leads to state transition from state 1, in which the LHC trimeric antenna is attaching to PSII, to state 2 where this antenna is associated with PSI. The Stt7 gene of *Chlamydomonas* is homologous to the STN7 and STN 8 gene of *Arabidopsis thalia*. STN7 phosphorylates antenna protein

whereas STN8 is related to phosphorylation of PSII core proteins. (reviewed in Li et al 2009) Stt7 phosphorylates specific residues of several LHC genes, but additional phosphorylation of these proteins that are independent of Stt7 are recently discovered. (Lemeille et al 2010) Together with their observation that Stt7 is involved in phosphorylation of other thylakoid associated kinases this indicates that kinase dependent state transition is more complexly regulated than previously assumed. Additional research on thylakoid associated kinases and phosphorylation of the proteins in both photosystems is necessary to get a more complete understanding of state transitions.

Reactive Oxygen Species and their roles in photoacclimation

Because reactive oxygen species can cause serious damage to the organism, their existence in the chloroplast (for some also have functions in peroxisomes) are clear signals of stressed conditions in the photosynthetic pathway. Therefore ROS provoke strong acclimation processes in the organism to regain balance in photosynthesis.

(i) $^1O_2$

Singlet oxygen produced by excess light absorption in PSII, specifically induces the upregulation of the glutathione peroxidase homologous gene (GPXH) (Fischer et al 2006). GPXH is an enzyme that is catalyzing the reduction of hydrogen peroxide and other organic peroxides, that can be induced by $^1O_2$. Fischer (2007) also showed that the upregulation of this gene was caused by two independent effect. At first, singlet oxygen induces an transcriptional upregulation of Gpxh. The later, secondary effect that boost GPXH production is assumed to be a stabilization of the RNA transcript.

Besides GPXH that has the most pronounced response to singlet oxygen, only a few other genes also show different expression levels as a response to singlet oxygen (Ledford et al 2007). Among these was a glutathion S-transferase (GSTS1). Ledford showed that overexpression of GPXH or GSTS1 is enough to enhance resistance to singlet oxygen.

It is still unclear how $^1O_2$ might induce GPXH and GSTS1. It is generally believed that $^1O_2$ doesn't diffuse far from where it was made. However Fischer et al (2007) discusses that $^1O_2$ might diffuse further into stroma, cytoplasm or even the nucleus. Therefore the search for the $^1O_2$ sensing mechanism needs to be expanded to regions much further from the thylakoid membrane. Fischer found indications that $^1O_2$ sensing is indeed further away from the thylakoid membrane than expected, as same amounts of $^1O_2$ produced in the cell by either Rose Bengal or illumination with HL gave different inductions of GPXH gene expression. Because cells treated with the Rose Bengal, that stimulates $^1O_2$ production in the aqueous phase, show higher inductions of GPXH, it is more likely that the sensory mechanism responsible for this $^1O_2$ mediated response is found outside the thylakoid membrane.

Because $^1O_2$ damages the D1 reaction center of PSII, expression of the gene encoding this protein is also induced by $^1O_2$. There have been speculations that photoinhibition is a way for the cell to induce quicker responses to the condition. Because the reaction center of PSII is damaged, and the D1 protein has to be synthesized again, the overall amount of photosynthetic activity is lowered. This of course leads to changes in the pH and redox state, that can then also induce regulatory pathways. By 'sacrificing' the D1 reaction center, cells may prevent more and/or more serious damage to other cellular components. The fact that D1 needs active degradation is supporting this theory. (reviewed in Eberhard et al 2008)

(ii) H_2O_2

Not much is known about the effects of hydrogen peroxide on gene regulation in *Chlamydomonas*. But H_2O_2 seems to play an important role in the downregulation of LHCB transcripts. (Mckim and Durnford (2006) Shao and colleagues (2008) propose that *Chlamydomonas* maintains a basic level of H_2O_2 in order to quickly induce responses to ROS when needed. This is done by repression of ROS detoxifying enzymes. Repression of these

enzymes must be strictly regulated, as they are needed when H_2O_2 levels are growing. TRX, that is redox regulated but not by the PQ pool's redox state, is a good candidate to regulate this repression because TRX itself is regulated by the electron transport chain, a direct link to the source of ROS is thereby provided. (Shao et al 2008).
In plants peroxide is related to upregulation of genes that are also induced in many other stress conditions. In *Arabidopsis* H_2O_2 specific induction of the APX1 and APX2 genes is found on top of the redox regulated induction. (Li et al 2009)

Environmental condition dependant sensitivity
It is clear that there are many different responses to HL conditions that enable *Chlamydomonas* to adequately respond to the stress it encounters. The environmental conditions play a significant role in the way *Chlamydomonas* responds to HL. The influence of CO_2 concentration and the presence of acetate as an energy source have been studied by Fischer et al (2006). *Chlamydomonas* cultures where tested in high or low concentrations of CO_2, or in the presence of acetate in the medium (TAP medium). The amount of photodamage by HL illumination was similar for all cultures, however the cellular response was very different. The stress responses, especially $^1O_2$ responses, in cultures with high CO_2 or acetate are much higher then in cultures in CO_2 limiting conditions.

Low CO_2 concentration and high light conditions cause a similar problem for *Chlamydomomas*, HL gives overexcitation and low CO_2 concentrations prevents utilization of energy. Though in both cases *Chlamydomonas* is absorbing to much light, the physiological response is different (Iwai et al 2007). HL mainly causes responses to increase the quenching capacity, while low CO_2 causes state transition. This indicates that sensing of these conditions happens independent from each other and their regulatory pathways do not 'crosstalk'.

Transition of the LCHII antenna is far more abundant in *Chlamydomonas* than in plants, as ~80% of the trimeric antenna is mobile while this is only ~20% in plants. Explanations for this difference might be found in CO_2 concentrations (Im et al 2009, rev.). Because there exist independent ways of sensing and responding to low CO_2 and excess light conditions these pathways may have evolved differently in *Chlamydomonas* and *Arabidopsis*. Because plants are in a fixed place, CO_2 concentrations for *Arabidopsis* are probably more constant than for *Chlamydomonas*. Therefore the need to adapt to different CO_2 concentrations is less for *Arabidopsis*, which led to a lower responses, as seen by the lower amount of state transition. For light intensity the opposite might be true, because *Chlamydomonas* is able to swim away from excess light using is flagella, its quenching response is less than that of plants.

Translational regulation of Lhcb genes
As described above there are different pathways regulation Lhc genes expression levels. It is intressting to note that these pathways act on all the Lhcb genes simultaneously. In this way the stoichiometry of the involved proteins stays the same. Because it was also shown that some processes are mainly regulated on the translational instead of transcriptional level, it will be worthwhile investigating if the there is coordinated translational regulation of the Lhcb mRNA's. Such an experiment was already performed by Mckin and Dunford (2006) where they followed polysome profiles in vivo. They found a specific decline of lhcb translation in high light conditions that was maximal at ~2 hours and recovered ~8 hours after shifting cultures from the dark to HL. This repression is most likely caused by NAB1, that binds to the mRNA of LHCBM, preventing translation initiation by blocking the ribosome. (Mussgnug et al 2005) In a comparative proteomics study by Forster et al (2006), significant changes in NAB1 protein levels where found in very high light resistant mutants under different

conditions. The binding of NAB1 to mRNA encoding LHC proteins is consistent with the theory that acclimation to high light in *Chlamydomonas* is happening at the translational level. NAB1 apparently plays a major role in this acclimation response, further research and on how, and to what extend NAB1 is responsible for acclimation to high light will provide us with a deaper understanding of the acclimation processes in *Chlamydomonas*.

Conclusion

Although the developments in transcriptomics and proteomics have provided us with more information on the regulatory pathways involved in photosynthesis, many gaps in our knowledge of these pathways need to be filled. One of the issues that remains somewhat mysterious in photosynthetic adaptation processes is the signaling from chloroplast to nucleus. There is still very little known on how stress signals in the chloroplast are transported over the chloroplast and nuclear membrane to deliver the signal to alter gene expression. Reason for this may be that most acclimation processes in the chloroplast are mediated on the translational level, therefore signals inducing transcriptional responses may not differ significantly from non stressed situations and stay undetected. This difficulty concerning signaling across the membrane is perhaps also the reason that dangerous compounds like $^1O_2$ and H_2O_2 are used for signaling, because they can diffuse across the membrane rather easy.

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