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DISSERTATION SUMMARIES

The role of the orange carotenoid protein (OCP) and PsbU subunit in photoinhibition of Photosystem II in cyanobacteria

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Excess light is harmful for photosynthetic organisms. In *Synechocystis* 6803, high intensities of blue-green light induce Photosystem II fluorescence quenching which is specifically associated with a photoprotective phycobilisome related energy-dissipation mechanism. A soluble orange carotenoid protein (OCP), of previously unknown function, plays an essential role in this process (Wilson et al. 2006). In the absence of the OCP, the non-photochemical quenching (NPQ) induced by strong blue-green light in *Synechocystis* PCC 6803 cells is completely inhibited and the cells are more sensitive to high light intensities. In iron-starved *Synechocystis* 6803 cells a larger OCP-phycobilisome-related NPQ is observed in association with a higher concentration of OCP (Wilson et al. 2007). Highly conserved homologues of the OCP are found in almost all genomes of all cyanobacteria for which genomic data is available with the exception of *Synechoccocus elongatus*, *Thermosynechoccocus elongatus* and the *Prochlorococci*.

The existence of a blue-green light induced NPQ mechanism was tested in iron-containing and iron-depleted cells of *A. maxima* strain, containing OCP genes, and *T. elongatus* and *S. elongatus* PCC 7942 strains lacking the entire OCP gene. The kinetics of fluorescence changes was monitored by a modulated PAM (pulse-amplitude-modulated) fluorometer. Exposure of low blue light–adapted cells to high blue-green light intensities induced a quenching of the Fm' in OCP containing *A. maxima* strain. In contrast, illumination of OCP lacking *S. elongatus* and *T. elongatus* by strong blue-green light did not induce any decrease of Fm' indicating that in the absence of whole OCP gene this kind of photoprotective quenching cannot be induced. We tested possible relationship between the iron starvation and the blue-green light–induced NPQ.

Results obtained from fluorescence traces of 10 days iron-starved cells of *A. maxima* demonstrates increase of fluorescence quenching in compare with iron containing cells. This increase, like in a case of *Synechocystis* cells, can be related to larger accumulation of OCP under iron starvation. In contrast, exposure of prolonged iron starved *T. elongatus* and *S. elongatus* PCC 7942 to high intensities of light didn't induce any NPQ. Iron starvation induces the synthesis of the "chlorophyll-binding-iron-stressed induced protein", IsiA in all strains. The presence of IsiA causes a blue-shift in the room temperature Chl *a* absorbance peak (680-673 nm). Under iron stress conditions both chlorophyll and phycobilisome level decreased in all cells. According to the absorbance spectra iron starved *T. elongatus* and *S. elongatus* PCC 7942 cells show different reorganisation of the pigments compared to OCP containing *A.maxima and Synechocystis* cells: phycobiliprotein content decreased faster than chlorophyll content. Increase at 685 nm in fluorescence spectra generated at 77K by 430 nm in iron-starved *A. maxima* cells was attributed to the accumulation of uncoupled phycobilisomes with high fluorescence emission as previously shown in iron-starved *Synechocystis* cells. In contrast, in *T elongatus* and *S. elongatus* PCC 7942 cells the 685 nm emission (and 660 nm emission) decreased with the time instead of increase like in *A.maxima* cells. Thus, in *T elongatus* and *S. elongatus* PCC 7942 cells phycobiliprotein content decreased faster than chlorophyll content. This is probably essential for the longer survival of the cells in the absence of the photoprotective blue-green light induced NPQ mechanism (Boulay et al. 2008).

Photosystem II contains trans-membrane protein subunits and extrinsic proteins associated with the lumenal side. While great progress has been made in the understanding of the function of the water splitting apparatus, the role of the smaller subunits is still not well defined. Studies of deletion mutants demonstrated the importance of these proteins in stabilising the Mn cluster. PsbU is one of the extrinsic proteins which form the oxygen-evolving complex of PS II in cyanobacteria (Burnap et al. 1992). In this study we investigated the function of PsbU using a deletion mutant of the *psbU* gene in *S. elongatus* PCC 7942 (Balint et al. 2006). Flash-induced chlorophyll fluorescence measurements were used to monitor forward electron transfer at the acceptor side of PSII, as well as charge recombination processes of the reduced acceptors with oxidized donor side components. While forward electron transfer was similar between the wild type and the PsbU strains, a marked difference in the rate of back electron transfer in the presence of electron transfer inhibitor DCMU was observed in the mutant.

The overall fluorescence relaxation kinetics in PsbU mutant in the presence of DCMU resulted in a slow phase of 1.2 s and 82% amplitude whereas in mutant the corresponding values were 4.8 s, 64%. Moreover, thermoluminescence measurements have demonstrated that in the presence and absence of DCMU, the TL intensity in mutant cells was significantly increased, and this was accompanied by the shift of the peak position to higher temperatures for both the Q- and B-bands. Also the high light induced loss of oxygen-evolving activity in the presence and absence of an inhibitor of protein synthesis lyncomicin was monitored in the PsbU and wild type cells. When the high light illumination was performed in the presence of the protein synthesis inhibitor lincomycin the activity loss was significantly higher in mutant cells than in wild type. On the basis of these results, we conclude that PsbU is crutial for the stable architecture of the PSII.

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The expression of ABCC4 and ABCG2 xenobiotic transporters during keratinocyte proliferation/differentiation and in psoriasis

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Xenobiotic transporters are members of the ATP binding cassette (ABC) superfamily of proteins, responsible for the energy dependent transport of a broad range of chemically and structurally different compounds thus provide chemoresistance for various tumors. However, they also play a very important role in maintaining the chemical barrier function of organs such as brain, liver and gut (see for review Leslie et al. 2004). The human epidermis is one of the largest physical and biochemical barrier of the body. There have been only a few studies conducted regarding xenobiotic transporter expression in normal human keratinocytes and in human skin (Baron et al. 2001; Kielar et al. 2003).

We aimed to study the expression of eight xenobiotic transporters: ABCB1, ABCC1-6 and ABCG2 in in vitro models of keratinocyte differentiation. Terminal differentiation of normal human keratinocytes was promoted by increasing Ca^{2+} concentration. Validation of the differentiation model was achieved by the detection of proliferation markers Ki67 and integrin alpha 5 and differentiation markers keratin 1 and involucrin. The chemical-free synchronization of the immortalized keratinocyte cell line, HaCaT was used as another model (Pivarcsi et al. 2001), in which contact inhibiton and serum starving forces the cells into a highly differentiated quiescent state. Releasing HaCaT keratinocytes from cell quiescence by passaging and serum re-addition initiate redifferentiation and the cells start to proliferate synchronously.

Among the transporter genes tested ABCC4 and ABCG2 showed a proliferation associated expression in both *in vitro* models. ABCC4 and ABCG2 were highly expressed in undifferentiated, proliferating keratinocytes and their mRNA levels decreased in parallel with differentiation. ABCC4 and ABCG2 transporter protein levels also showed a decrease in differentiating keratinocytes, as revealed by Western blot and immunocytochemistry. Similarly, induction of ABCC4 and ABCG2 mRNAs and proteins were observed in synchronized HaCaT keratinocytes after release from cell quiescence, which further supported that ABCC4 and ABCG2 transporters have a possible function in proliferating keratinocytes.

ABCC4 protein was overexpressed in the basal layers of psoriatic lesional epidermis, supporting our *in vitro* results, while in keratinocytes of normal and non-involved skin it was expressed at very low levels. ABCC4 transporter may contribute to the pathogenesis of psoriasis since antiapoptotic/proliferation related cyclic nucleotides and important inflammatory mediators are ABCC4 substrates. ABCG2 transporter was expressed in normal and psoriatic non-involved epidermis and its expression was restricted to basal layer keratinocytes. Increased levels of ABCG2 protein was detected in psoriatic lesions, however its highest level of expression was observed in keratinocytes in the abnormally differentiating granular layer. It is known that human epidermis is a constitutively hypoxic tissue, which is more pronounced in psoriatic lesions. Hypoxia induces the generation of harmful porphyrins that are substrates of ABCG2, thus ABCG2 may protect keratinocytes from the accumulation of these compounds. The upregulation of ABCC4 and ABCG2 xenobiotic transporters in psoriatic lesions could significantly modulate drug distribution and effectiveness in the skin.

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