Steady, ready, go
– proteomics of etioplast inner membranes reveals a high readiness for light

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Abstract: Light is essential for development of photosynthetically active chloroplasts. In the absence of light proplastids develop into etioplasts which are readily transformed into chloroplasts upon illumination. The etioplast inner membranes (EPIMs) differ significantly from those of chloroplasts regarding composition and structure. EPIMs consist of two laterally separated membrane systems, namely the three-dimensional lattice of tubular membranes, prolamellar bodies (PLBs), and the flat membranes of prothylakoids (PTs) which radiate from the PLBs. PLBs and PTs offer heterogeneity in lipid, pigment and protein composition. This thesis reports on novel proteomic studies of EPIMs and analyses of the light-dependent key enzyme in the chlorophyll biosynthesis, NADPH:protochlorophyllide oxidoreductase (POR).

POR, which constitutes at least 90% of the protein content of PLBs, is known to be important for the formation of the PLB membrane structure. Light activates the POR-mediated reduction of protochlorophyllide into chlorophyllide. This event is the starting point for the dispersal of PLBs and thus the whole rebuilding of the plastid inner membranes during etioplast to chloroplast transition. POR is firmly attached to the membrane and transmembrane helix predictions show that POR is a plausible integral transmembrane protein.

Proteomic analyses were performed on EPIMs isolated from well-defined sections of dark-grown wheat (*Triticum aestivum*) leaves. Proteins of EPIMs or subfractionated PLBs and PTs were separated and identified by mass spectrometry analyses. The proteome of PLBs and PTs reveals a far more complex protein composition than previously suggested. In total, 111 proteins were identified in PLBs and PTs. The proteins represent diverse functions such as pigment biosynthesis, photosynthesis and protein degradation. The majority of the identified proteins are directly or indirectly connected to photosynthesis, thus suggesting that PLBs and PTs are well prepared for construction of the photosynthetic apparatus. The spatial separation of certain proteins between PLBs and PTs suggests that photosystem formation is initiated in the PTs. EPIMs contain numerous proteins involved in protection against excess light. Etioplasts are steady in darkness, ready for light and well prepared to go for a fast onset of photosynthesis.

Keywords: chloroplast, etioplast, NADPH:protochlorophyllide oxidoreductase, prolamellar body, proteomics, prothylakoid, transmembrane, *Triticum aestivum*, wheat

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Till Daniel,
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This thesis is based on the following papers, which are referred to by their Roman numerals.

(**I**) Blomqvist LA, Ryberg M, Sundqvist C (2006) Proteomic analysis of the etioplast inner membranes of wheat (*Triticum aestivium*) by two-dimensional electrophoresis and mass spectrometry. Physiol Plant 128, 368-381*

(**II**) Blomqvist LA, Ryberg M, Sundqvist C (2008) Proteomic analysis of highly purified prolamellar bodies reveals their significance in chloroplast development. Photosynth Res 96, 37-50*

(**III**) Blomqvist LA, Ryberg M, Sundqvist C, Aronsson H (2009) The proteomes of prolamellar bodies and prothylakoids are well prepared for a fast onset of photosynthesis. (Submitted)

(**IV**) Blomqvist LA, Töpel M, Ryberg M, Aronsson H (2009) NADPH:protochlorophyllide oxidoreductase (POR) is a plausible integral membrane protein. (Manuscript)

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Abbreviations

1-D	one-dimensional
2-D	two-dimensional
3-D	three-dimensional
a.a.	amino acid(s)
DGDG	digalactosyl diacylglycerol
EPIM	etioplast inner membrane
ESI	electrospray ionization
FTICR	Fourier transform-ion cyclotron resonance
IMAC	immobilized metal affinity chromatography
MALDI	matrix-assisted laser desorption/ionization
MGDG	monogalactosyl diacylglycerol
MOAC	metal oxide affinity chromatography
MS	mass spectrometry
MS/MS	tandem mass spectrometry
LC	liquid chromatography
Ndh	NAD(P)H:plastoquinone oxidoreductase
Pchlide	protochlorophyllide
PLB	prolamellar body
POR	NADPH:protochlorophyllide oxidoreductase
PT	prothylakoid
SDR	short-chain dehydrogenase
TM	transmembrane
ToF	time-of-flight
Trp	tryptophan
Q	quadrapole