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“If I claim to be a wise man,
Well, it surely means that I don't know”
-Kerry Livgren, Kansas, Carry On Wayward Son

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ABSTRACT

Photosynthetic organisms convert physical energy from the sun into chemical energy through photosynthetic light reactions catalysed by thylakoid membrane-embedded pigment-protein complexes. Four major protein complexes — Photosystem (PS) II, PSI, cytochrome (Cyt) b_6f, and ATP synthase — transform light energy into chemical energy via electron transfer and proton translocation reactions, releasing O_2 as a byproduct. The chemical energy produced in the form of NADPH and ATP is further utilized in carbon assimilation reactions, where CO_2 is metabolized into carbohydrates that serve as substrates for various metabolic processes.

Photosynthesis in plants takes place in chloroplasts, which are endosymbiotic evolutionary descendants of cyanobacteria. Chloroplasts are characterized by triple membrane system, from which the thylakoid membrane contains a unique, highly dynamic structure that is regulated according to environmental cues. Short-term changes in ambient conditions leads to adjustments in the membrane ultrastructure and its components, whereas prolonged exposure to environmental changes initiate acclimatory responses requiring alterations in the stoichiometry of photosynthetic complexes. Both, short-term regulatory mechanisms and long-term acclimatory responses are necessary for fluent photosynthesis while minimizing damage to photosynthetic apparatus. In addition to leaf tissue, also other green parts of the plant contain chloroplasts and conduct photosynthesis adapted to the specific cellular functions, thus contributing to the overall yield.

I have investigated the acclimatory and adaptive characteristics of photosynthetic machinery in the model species *Arabidopsis thaliana.* I demonstrate that light-induced dynamic regulation of the thylakoid membrane requires GNAT2, a chloroplast-located acetyltransferase, previously shown to play a crucial role in maintaining balanced excitation energy distribution between PSII and PSI. I also show that Arabidopsis stems contain high amount of PSI-NDH-complex that enhances ATP production, presumably to meet the metabolic requirements of the organ. Last, I reveal that the Cyt b_6f complex is downregulated upon daily heat stress, highlighting the dynamic nature and acclimation capacity of the photosynthetic machinery.

KEYWORDS: photosynthesis, thylakoid membrane, photosynthetic regulation, acclimation, adaptation.

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TIIVISTELMÄ

Fotosynteettiset eliöt muuttavat auringon säteilyenergian kemialliseksi energiaksi fotosynteesin valoreaktioissa. Neljä suurta tylakoidikalvoston proteiinikompleksia – fotosysteemi (PS) II ja PSI, sytokromi b_6f sekä ATP-syntaasi – muuntavat säteilyenergian kemialliseksi energiaksi elektronien ja protonien siirtoreaktioiden avulla, jolloin sivutuotteena vapautuu happea. NADPH:n ja ATP:n muodossa oleva kemiallinen energia hyödynnetään hiilensidonnassa, jossa CO_2 muunnetaan hiilihydraateiksi, joita kasvi käyttää aineenvaihduntaprosesseihinsa.

Kasveilla fotosynteesi tapahtuu kloroplasteissa, jotka endosymbioositeorian mukaan ovat kehittyneet syanobakteerista. Kloroplastin tylakoidikalvo on dynaaminen rakenne, jonka ominaisuuksia säädellään ympäristön olosuhteiden mukaan. Lyhytkestoiset muutokset kasvin ympäristössä vaikuttavat kalvoston kolmiulotteiseen rakenteeseen sekä yksittäisiin kalvoproteiineihin, kun taas pidempikestoiset muutokset vaikuttavat fotosynteettisten geenien ilmenemisaktiivisuuteen ja proteiinikompleksien määrien suhteellisten osuuksien muutokseen. Näiden säätelymekanismien avulla fotosynteesi voi toimia tehokkaasti erilaisissa ympäristöolosuhteissa. Lehtien ohella myös muut kasvin vihreät osat sisältävät kloroplasteja ja kykenevät fotosynteesiin.

Tässä väitöskirjassa olen tutkinut fotosynteesikoneiston dynaamista sopeutumista muuttuviin olosuhteisiin lituruohossa (*Arabidopsis thaliana*). Osoitan, että GNAT2-asetyylitransferaasi-entsyymi on välttämätön edellytys valon aiheuttamalle tylakoidikalvoston dynaamiselle säätelylle. Tulokseni osoittavat myös, että lituruohon varsissa on runsaasti PSI–NDH-kompleksia, joka todennäköisesti tehostaa ATP:n tuotantoa varressa. Lopuksi tarkastelen, miten toistuvat kuumajaksot vaikuttavat fotosynteettisiin proteiinikomplekseihin ja tylakoidikalvoston rakenteeseen. Sytokromi b_6f -kompleksin määrän väheneminen päivittäisessä kuumastressissä osoittaa, että fotosynteesin sopeutumiskyky mahdollistaa kasvin kasvun myös epäedullisissa ympäristöolosuhteissa.

AVAINSANAT: fotosynteesi, tylakoidikalvosto, valoreaktiot, fotosynteesin säätely akklimaatio, adaptaatio.

Table of Contents

Abbreviations

3PGA	3-phoshpoglycerate
2PGA	2-phosphoglycolate
ATP	Adenosine triphosphate
ATPase	ATP synthase
BS	Bundle sheath
C2	Dimeric PSII core
CA	Carbonic anhydrase
CAM	Crassulacean Acid Metabolism
CB cycle	Calvin-Benson cycle
CBB	Coomassie Brilliant Blue
CET	Cyclic electron transfer
Chl	Chlorophyll
CURT1	Curvature thylakoid 1A
Cyt	Cytochrome
DGDG	Digalactosyldiacylglycerol
DKN	Dual-KLAS-NIR
ECS	Electrochromic shift
Fd	Ferredoxin
Fe-S	Iron-sulphur
FNR	Fd-NADPH oxidoreductase
FRET	Fluorescence resonance energy transfer
GNAT2	GCN5-related N-acetyltransferase 2
HRP	Horseradish peroxidase
ISP	Iron–sulfur protein
JTS	Joliot-type-spectrophotometer
LCNP	Plastid lipocalin protein
LET	Linear electron transfer
LHC	Light harvesting complex
L-LHCII	Loosely bound light harvesting antenna
M	Mesophyll
MGDG	Monogalactosyldiacylglycerol

M-LHCII	Moderately bound light harvesting antenna
NADPH	Nicotinamide adenine dinucleotide phosphate
NADP-ME	NADP-dependent malic enzyme
NAD-ME	NAD-dependent malic enzyme
NDH	NADPH dehydrogenase like complex
NPQ	Non-photochemical quenching
OEC	Oxygen evolving complex
PC	Plastocyanin
PEP	Phosphoenolpyruvate
PEPCase	Phosphoenolpyruvate carboxylase
PEPCK	PEP carboxykinase
PG	Phosphatidylglycerol
PGR5	Proton gradient regulation 5
PGRL1	PGR5-like photosynthetic phenotype
Pheo	Pheophytin
PhQ	Phylloquinone
P_i	Inorganic phosphate
pmf	Proton motive force
PQ	Plastoquinone
PQH_2	Plastoquinol
PS	Photosystem
PTM	Posttranslational modification
Q	Quinol
qE	Energy-dependent quenching
qH	Plastid lipocalin (LCNP) -dependent quenching
qI	Photoinhibitory-dependent quenching
qT	State transition -dependent quenching
qZ	Zeaxanthin-dependent quenching
R5P	Ribose-5-phosphate
RC	Reaction center
ROS	Reactive oxygen species
Rubisco	Ribulose bisphosphate carboxylase/oxygenase
RuBP	Ribulose-1,5-bisphosphate
SDS	Sodium dodecyl Sulphate
S-LHCII	Strongly bound light harvesting antenna
SOQ1	Suppressor of quenching 1
STN7	State transition 7 kinase
SQDG	Sulfoquinovosyldiacylglycerol
TAP38	Thylakoid-associated phosphatase 38

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Rantala M., Ivanauskaite A., Laihonen L., Kanna S.D., Ughy B. and Mulo P. Chloroplast acetyltransferase GNAT2 is involved in the organization and dynamics of thylakoid structure. *Plant and Cell Physiology*, 2022; 63,1205-1214.

II Laihonen L., Rantala M., Ranasinghe U., Tyystjärvi E., and Mulo P. Transcriptomic and proteomic analyses of distinct Arabidopsis organs reveal high PSI-NDH complex accumulation in stems. *Physiologia plantarum*, 2024; 176, e14227.

III Laihonen L., Tomberg T., Vuorijoki L., Mulo P. and Rantala M. Daily heat stress induces accumulation of non-functional PSII-LHCII and donor-side limitation of PSI via downregulation of the Cyt b_6f complex in *Arabidopsis thaliana*. *Preprint, bioRxiv* 2025.11.06.687104.

1 Introduction

The onset of oxygenic photosynthesis in cyanobacteria led to increase in atmospheric oxygen, paving the way for life as we know it. Later during the course of evolution, ancient eukaryotic cell engulfed cyanobacterium that evolved into chloroplast. This endosymbiosis event enabled evolution of other photosynthetic organisms, algae and plants. The origin of land plants has been estimated to be between 480 and 360 Ma (Kenrick & Crane, 1997). The principle of converting energy from sunlight into chemical energy is similar in all photosynthetic organisms, but evolutionary diversification is seen especially in light harvesting systems and regulation of the photosynthetic apparatus (Iwai et al., 2024).

In photosynthesis, light energy drives the production of chemical energy in form of nicotinamide adenine dinucleotide phosphate (NADPH) and adenosine triphosphate (ATP) that are further utilized to fix atmospheric CO_2 into carbohydrates. In plants, photosynthesis takes place in chloroplast where the internal membrane system called thylakoid membrane accommodates protein complexes conducting light reactions. Four large protein complexes are responsible for light reactions: Photosystem (PS) II and PSI that bind peripheral light harvesting complexes (LHC), cytochrome (Cyt) b_6f that interconnects PSII and PSI, and ATP synthase (ATPase). Two mobile electron carriers, plastoquinone (PQ) and plastocyanin (PC), participate in transferring electrons from PSII to PSI. Carbon assimilation reactions occur in the chloroplast stroma in Calvin-Benson (CB) cycle.

Plants are constantly subjected to long- and short-term fluctuations in ambient environmental conditions. Changes in light intensity and quality, temperature and availability of water and nutrients require different acclimation strategies from plants in order to maintain photosynthetic machinery functional. Imbalance of absorbed light energy and consumption of chemical energy by downstream CB cycle is fatal to photosynthetic organisms, highlighting the importance of strict regulation of photosynthetic reactions. Dynamic changes in the thylakoid membrane ultrastructure, balancing the excitation energy between PSII and PSI, dissipation of excess excitation energy as heat and adjusting the electron and proton flux are necessary adjustments to keep photosynthetic light reactions functional under challenging and ever-changing environmental conditions.

1.1 Plastids – the multifaceted cell organelles

Plastids originate from cyanobacterium that was engulfed by a eukaryotic cell, a phenomenon known as endosymbiosis (Gould et al., 2008). Plastids are formed by a diverse group of different subtypes with each subtype containing distinct characteristics typical for the specific tissue or organ they are located (Choi et al., 2021). Meristematic and reproductive tissues of plants contain undifferentiated proplastids, which may differentiate into chloroplasts, chromoplasts or leucoplasts. Chloroplasts are the most well-known plastid types that host necessary components for photosynthesis. Chromoplasts function as a storage for carotenoids, giving them orange, red or yellow colour depending on the accumulated carotenoid type, and they are located mostly in the reproductive tissues (Waters & Pyke, 2005; Sadali et al., 2019). White-coloured leucoplasts are located in non-photosynthetic tissues and they are further divided into subgroups based on the storage compounds they contain: amyloplasts containing starch, proteinoplasts that accumulate protein bodies and elaioplasts that are enriched in hydrophobic lipids and terpenoids (Waters & Pyke, 2005; Choi et al., 2021). Remarkably, plastids can interconvert into different subtypes during plant development (Choi et al., 2021).

Chloroplasts are the only type of plastids that contain necessary components for photosynthetic reactions. Leaves are the main site for photosynthesis, but other green non-foliar tissues contain chloroplasts as well and meet the requirements for photosynthetic reactions, contributing to the overall yield (Weiss et al., 1988; Hibberd & Quick, 2002; Brazel & Ó'Maoiléidigh, 2019; Hu et al., 2019; Simkin et al., 2020). Photosynthetic reactions in non-foliar organs either utilize atmospheric CO_2 or recycle CO_2 that is released in respiratory processes (Aschan & Pfanz, 2003). The former occurs especially in green stems and flower organs (Weiss et al., 1988; Ávila et al., 2014; Hu et al., 2019; Simkin et al., 2020), whereas the latter takes place in woody tissues and fruits in which atmospheric CO_2 cannot easily penetrate due to lack or decreased number of stomata (Blanke & Lenz, 1989; Smillie, 1992; Schmidt et al., 2000). The contribution of non-foliar photosynthesis to the overall yield varies depending on the species and growth-stage (Hetherington et al., 1998; Kong et al., 2010; Hu et al., 2012; Ávila et al., 2014), but also on the environmental conditions such as abiotic stress conditions or different seasons of the year, with contribution values ranging from ~5 to 60% (Yiotis et al., 2008; Ávila et al., 2014; Simkin et al., 2020; Martínez-Peña et al., 2023; Ávila-Lovera et al., 2024).

Due to their endosymbiont origin, chloroplasts contain their own genome as well as machinery for transcription and translation. Most of the endosymbiont genes were transferred to the host nucleus, while only ca. 100 genes were retained in the chloroplasts (Sato et al., 1999). Altogether chloroplast proteome consists of approximately 3000 proteins, from which more than 90% are nuclear-encoded and

transported to chloroplast (Jarvis & López-Juez, 2013; Christian et al., 2020). Thus, most proteins involved in photosynthesis are nuclear-encoded, highlighting the importance of interdependent regulation of the nuclear and plastid genomes (Surpin et al., 2002; Pesaresi et al., 2007; Woodson & Chory, 2008; De Souza et al., 2017). Moreover, photosynthetic protein complexes are typically comprised of subunits that are encoded by both nuclear and plastid genomes, and the steady-state protein synthesis is stoichiometrically adjusted in order to ensure controlled assembly of the complexes. This is achieved by feedback regulation at the translational level, control by epistasy of synthesis, that occurs via controlling protein synthesis in assembly-dependent manner: synthesis of specific subunit controls the synthesis of another (Wostrikoff & Stern, 2007; Ghandour et al., 2025).

Feedback regulation is mediated by antero- and retrograde signaling from nucleus to chloroplast (anterograde) and from chloroplast to nucleus (retrograde). Anterograde signaling has crucial role in regulation of photomorphogenesis and thus the formation of chloroplasts from proplastids through light-dependent mechanism (Yoo et al., 2019; Mahapatra et al., 2024). Retrograde signaling usually occurs in response to environmental cues and is involved in chloroplast maintenance (De Souza et al., 2017; Mahapatra et al., 2024).

1.1.1 Chloroplast ultrastructure – architecture and composition of the triple membrane system

Chloroplast is a unique cellular organelle containing more than half of the soluble proteins in plant leaves (Kirchhoff, 2019). It is characterized by three membrane systems: the outer porous membrane enclosing the chloroplast from its cellular environment, the inner membrane enclosing the chloroplast stroma, leaving the intermembrane space between the two membranes, and the internal thylakoid membrane system. The chloroplast outer and inner membranes, i.e. the envelope, comprises of three main classes of lipids: glycerolipids sphingolipids and sterols (Reszczyńska & Hanaka, 2020), which form hydrophobic lipid bilayers. The outer membrane is permeable to ions and metabolites, while the inner membrane and the thylakoids are more strictly regulated by various transporters and channels, allowing tight regulation of the chloroplast homeostasis according to physiological requirements (Finazzi et al., 2015; Li et al., 2021).

The thylakoid membrane consists of two morphologically distinct domains called grana and stroma lamellae. Grana domains are appressed stacks that are connected by the unstacked sheet-like stroma lamellae forming a continuous membrane system throughout the chloroplast (Staehelin, 2003). The diameter of dark-acclimated grana of *Arabidopsis thaliana* (Arabidopsis) usually varies from 300 to 600 nm, the membrane thickness is ca 4.0 nm, luminal space ca 4.7 nm, and

the partition gap between adjacent grana discs ca 3.6 nm (Kirchhoff et al., 2011) (Figure 1A). The exact mechanism of grana stacking has remained elusive but the interactions of adjacent protein complexes located at the granum, namely PSII-LHCII supercomplexes, play crucial role in stacking (see review by Krysiak et al., 2025). Grana stacking will be discussed in more detail in chapter 1.3.1.

The 3D structure of thylakoid membrane has been extensively analysed with the aid of microscopical techniques, and several models have been proposed since (Austin & Staehelin, 2011; Kirchhoff, 2013; Pribil et al., 2014), with the so-called helical model being the most prevalent (Paolillo, 1970). The helical model proposes that granum stacks are helically surrounded by stroma lamellae that connect to the stacks through narrow slits. The other model known as the bifurcation or fork model suggests that grana discs within a stack are connected by bifurcations of stroma lamellar sheets resembling a fork-like structure (Arvidsson & Sundby, 1999; Shimoni et al., 2005). Recent updates to plant thylakoid membrane structure were suggested by Bussi et al. (2019) by using different electron tomography techniques. They suggest that in addition to right-handed stroma helices surrounding grana stacks, separate grana stacks are further interconnected by stroma lamellae that forms left-handed helix between the stacks (Figure 1B). The connecting domain of the right-handed stroma helices with the left-handed stroma helices are consisted of bifurcations along the interaction domain interface. The left-handed stroma lamellae helices also connect grana stacks with stroma lamellar sheets, which are not helically wound (Figure 1B). Left-handed helices interconnect grana stacks and stroma sheets above and below the sheets and allow the organization of the sheets parallel to the stacked granum and thus minimizes the surface and bending energies. This model represents combination of elements from the helical and bifurcation models. The right-handed helices around grana stacks are uniformly wound and evenly spaced, and the number of helices depends on the grana diameter rather than the number of stack layers. Grana margins consist of the curved edge of the grana stack called curvature area, and of slit junctions, defined as connecting domain, that connect the two membrane types forming continuous luminal space inside the membrane system (Daum et al., 2010; Austin & Staehelin, 2011; Trotta et al., 2025).

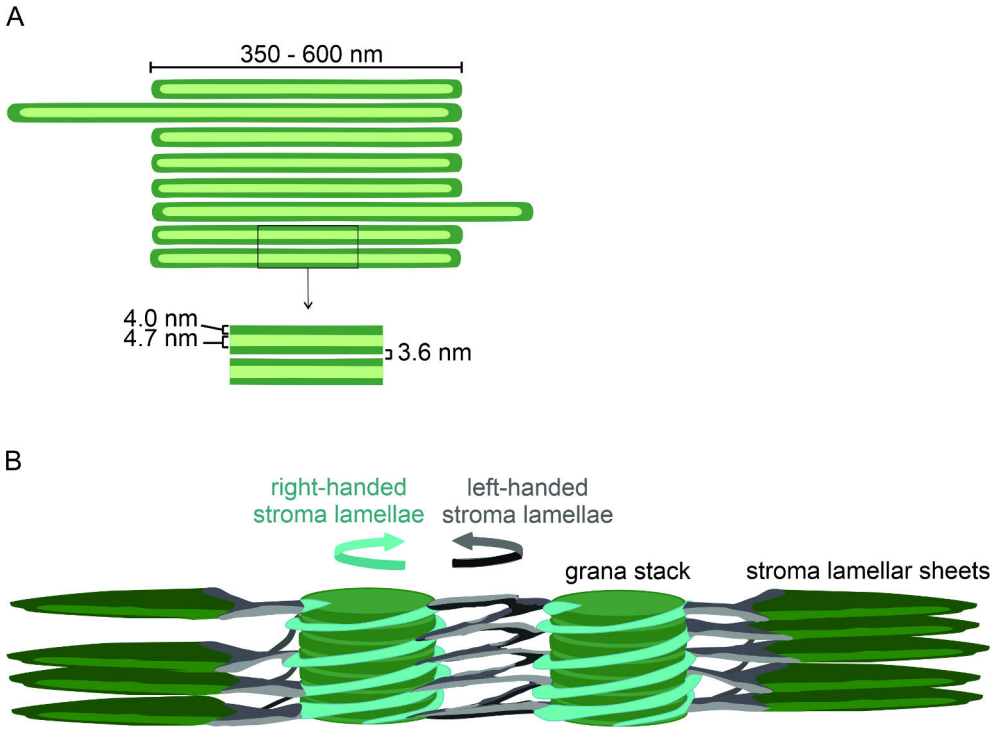

Figure 1. Scheme of thylakoid structure. A) Architecture of typical grana stack from dark-acclimated thylakoid membrane of *Arabidopsis thaliana*. Values for grana diameter, membrane thickness, luminal space and partition gap (Kirchhoff et al., 2011) are presented. B) Representative model of thylakoid membrane architecture proposed by Bussi et al. (2019). Grana stacks are surrounded by right-handed stroma lamellae helices, and the stacks are interconnected to adjacent stacks and stroma lamellar sheets via left-handed helical stroma lamellae.

The highly conserved thylakoid membrane constitutes from a high proportion of glycolipids: monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), sulfoquinovosyldiacylglycerol (SQDG) and phospholipid phosphatidylglycerol (PG). MGDG and DGDG are the most abundant lipids in the membrane (Duchêne & Siegenthaler, 2000; Boudière et al., 2014). These membrane lipids form both non-bilayer (MGDG) and bilayer (DGDG, SQDG, PG) structures (Garab et al., 2025), where proteins are embedded. Proteins occupy nearly 80% of the membrane area and are organized in the membrane domains unevenly (Kirchhoff et al., 2004) (see chapter 1.2.2).

1.2 Photosynthesis – from light driven electron transfer reactions to carbon assimilation

1.2.1 The structure of photosynthetic protein complexes in Angiosperms

The enzyme complexes that catalyse the photosynthetic light reactions consist of multiple protein subunits and cofactors. PSII contains reaction center (RC) proteins D1 and D2, that host a special redox active chlorophyll (Chl) *a* pair, P680. The minimal PSII RC complex that is capable of charge separation consists of heterodimer formed by D1 and D2, and of Cyt $_{b559}$ (Nanba & Satoh, 1987). Other components of PSII core monomer are inner light-harvesting proteins CP43 and CP47 that capture excitation energy, luminal oxygen evolving complex (OEC) that oxidizes water molecules, and several small subunits that are involved in assembly, stabilization and dimerization of PSII (Caffarri et al., 2009; Dekker & Boekema, 2005; Järvi et al., 2015; Cao et al., 2018). The PSII core is surrounded by a light harvesting antenna (LHC) comprised of Chl-binding Lhcb proteins. Plants are characterized by two Chl species, Chl *a* and Chl *b*, which differ structurally and spectrally due to oxidation of methyl group in Chl *a* to formyl in Chl *b*. From these, Chl *a* is the principal photosynthetic pigment and it is located both in core and the antenna complexes, whereas Chl *b* is mainly present in antenna (Bassi & Dainese, 1992).

Functional PSII in angiosperms consists of a dimeric core (C2) surrounded by two strongly (S-LHCII, S2) and two moderately bound (M-LHCII, M2) antennae that are attached to the C2 via minor antenna proteins Lhcb4 (CP29, hereafter Lhcb4), Lhcb5 (CP26, hereafter Lhcb5) and Lhcb6 (CP24, hereafter Lhcb6), forming C2S2M2 supercomplex (Figure 2A). The peripheral S-LHCII is formed by Lhcb1 and Lhcb2 trimers, whereas M-LHCII trimers contain Lhcb3 and Lhcb1 (Caffarri et al., 2009). Nuclear-encoded major LHCII proteins (Lhcb1-3) in plants share high sequence homology and they are composed of three membrane spanning α-helices and two luminal helices (Jansson, 1994; Barros & Kühlbrandt, 2009; Pan et al., 2020). Lhcb4 and Lhcb6 connect the peripheral M-LHCII antenna to the core, whereas Lhcb5 mediates the binding of S-LHCII. Lhcb4 stabilizes the interaction of S-LHCII with the core (Caffarri et al., 2009). Lhcb4 and Lhcb6 are essential for the C2S2M2 assembly (Kovács et al., 2006; De Bianchi et al., 2011), whereas Lhcb5 is not necessary (Vánská et al., 2025). Indeed, recent studies with *lhcb5* mutant showed that despite its absence, C2S2M2 supercomplexes without the Lhcb5 protein were detected with electron microscopy (Vánská et al., 2025). Altogether, C2S2M2 complex contains more than 60 protein subunits, over 200 Chl molecules (Su et al., 2017; Cao et al., 2018; Graça et al., 2021; Messinger et al., 2025), approximately 60

lipids and over 40 carotenoids (Graça et al., 2021). Notably, PSII antenna is dynamically adjusted depending on light conditions (see chapter 1.3.1). In some conditions, the LHCII antenna size is decreased, while it can also be increased by addition of a loosely bound (L-LHCII) trimer (Larsson et al., 1983; Wientjes et al., 2013).

Functional PSI-LHCI complex in plants is present as monomer. It consists of 18 protein subunits and nearly 200 Chls (Amunts et al., 2010; Pan et al., 2018; Wang et al., 2021). The core contains PsaA and PsaB proteins that host RC Chl P700. PsaA and PsaB form a heterodimer with 22 membrane spanning helices that accommodate most of the cofactors required for electron transfer in PSI. PsaG, PsaF, PsaK and PsaN subunits mediate the excitation energy transfer from LHCI to PSI core (Qin et al., 2015; Pan et al., 2018; Wang et al., 2021). LHCI antenna is composed of two heterodimers, Lhca1/Lhca4 and Lhca2/Lhca3, which form a belt-like structure attached to the core (Figure 2B) (Amunts et al., 2010; Qin et al., 2015; Pan et al., 2018; Wang et al., 2021; Wu et al., 2023). Depending on light conditions, pool of L-LHCII may associate to PSI through PsaH, PsaL, PsaO and PsaK subunits (Lunde et al., 2000; Zhang & Scheller, 2004; Pan et al., 2018; Wu et al., 2023). Additional LHCI proteins, Lhca5 and Lhca6, function as a docking site for the NADPH dehydrogenase like (NDH) complex (Peng et al., 2008; Kouřil et al., 2014) and are involved in cyclic electron transfer (CET) (see chapter 1.3.3). LHCI proteins contain three membrane spanning helices, one luminal helix, and so called generic LHCI motif that is characterized by sequence of hydrophobic amino acids (Jansson, 1999; Qin et al., 2015; Pan et al., 2020). Generic LHCI motif additionally includes conserved charged amino acids that maintain helices by salt bridges formed by arginine and glutamic acid (Jansson, 1999).

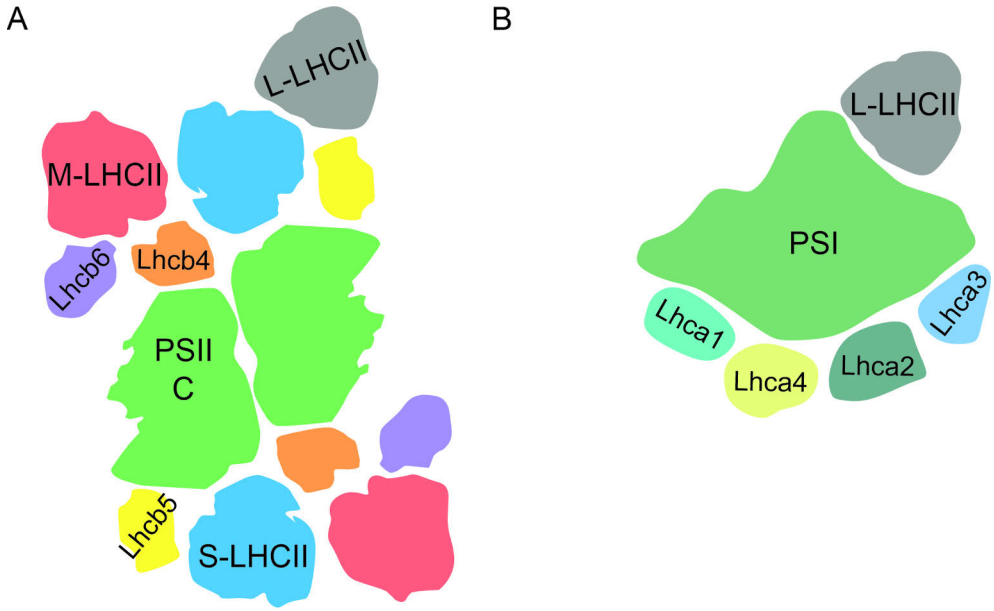

Figure 2. Top-view of the plant light harvesting antennae of photosystem (PS) II and PSI. A) External life harvesting antenna of PSII consists of LHCII trimers, which bind to the core via Lhcb4 and Lhcb6 (M-LHCII), and Lhcb5 (S-LHCII). Additional loosely bound L-LHCII is located at unknown position. B) Light harvesting antenna of PSI forms a belt-like structure composed of Lhca-proteins. L-LHCII binds to PSI in opposite site to LHCI belt.

Cyt b_6f connects PSII and PSI via PQ and PC. The functional complex of Cyt b_6f is a dimer, and each monomer consists of four large protein subunits: Cyt *f* (PetA), Cyt b_6 (PetB), Rieske iron–sulfur protein (ISP, PetC) and subunit IV (PetD), and four smaller subunits: PetG, PetL, PetM and PetN (Malone et al., 2019; Sarewicz et al., 2023). Cyt *f* and Cyt *b* are associated with redox cofactors: *c*- and *b*-type haems, respectively. Rieske protein binds iron-sulphur (2Fe-2S) cluster. Subunit IV is associated with one Chl *a* and one β-carotene, with unknown function (Malone et al., 2021). The cavity between the two monomers accommodates the substrate binding sites for plastoquinol (PQH_2) and PQ (see chapter 1.2.3).

Chloroplast ATPase consists of 26 protein subunits (Hahn et al., 2018). The subunits are divided into two parts of the complex: catalytic F_1 head where ATP synthesis occurs, and membrane-embedded F_o motor that drives the synthesis. The F_1 head is formed by three αβ heterodimers containing the catalytic sites, and by central stalk consisting of γ and ε subunits. F_o motor consists of c-ring connected to the central stalk, subunit a and peripheral stalk. The c-ring contains proton channel consisting of 14 subunits (Seelert et al., 2000), subunit a has six α helices from which helices H5 and H6 are in contact with the c-ring, and the peripheral stalk is comprised of δ, b and b′ subunits. Three different positions of the complex have been identified

(Hahn et al., 2018; Yang et al., 2020) and represent different rotary states of the complex.

1.2.2 Lateral heterogeneity, stoichiometry and turnover of the photosynthetic protein complexes

PSII and PSI are spatially separated across the thylakoid membrane network. Andersson & Anderson (1980) showed that PSII is mainly located within the grana, whereas PSI and ATPase, which cannot fit in the stromal gap, are mainly distributed to stroma lamellae. The distribution of Cyt b_6f remained elusive for a long time (Kirchhoff et al., 2017), but it is now considered to distribute evenly across the membrane (Koochak et al., 2019; Trotta et al., 2025; Wietrzynski et al., 2025). The protein components located at the grana margins consists of CURVATURE THYLAKOID 1 (CURT1) proteins, that mediate the bending of grana at the curved regions (Armbruster et al., 2013; Pribil et al., 2018; Trotta et al., 2019), and of larger protein complexes such as ATPase, Cyt b_6f and PSII repair cycle intermediates at the connecting domains (Puthiyaveetil et al., 2014; Koochak et al., 2019; Trotta et al., 2025).

The stoichiometry of photosynthetic protein complexes is regulated according to environmental cues (Schöttler & Tóth, 2014). Short-term changes in light conditions result in swift adjustments in electron and proton fluxes and light harvesting (see chapter 1.3), but long-term changes in environmental conditions, that usually take from several hours to days and weeks, trigger acclimation mechanisms that lead to stoichiometric changes in the protein complex contents. E.g. high light reduces the amount of LHCII but amount of PSII reaction centers increases, whereas PSI content is unaffected (Schöttler & Tóth, 2014). Cyt b_6f is the rate-limiting component of light reactions (Tikhonov, 2024), and its content is adjusted according to environmental conditions and plant age, and it is usually co-regulated with ATPase (Schöttler et al., 2015). Changes in redox state of photosynthetic components (i.e. PQ pool) and metabolic changes in chloroplast (i.e. accumulation of photoassimilates) mediate signals that are responsible for altering gene expression levels of photosynthetic components both in the nucleus and in the chloroplast (Pfannschmidt, 2003; Schöttler & Tóth, 2014).

PSII turnover has been studied extensively because of the susceptibility of RC protein D1 to oxidative damage. D1 can have a half-life as short as 30 min depending on conditions (Aro et al., 1993), whereas other photosynthetic protein complexes have been considered to be more stable. For example, half-life of Cyt b_6f in *C. reinhardtii* was reported to be above 21 h (Gong et al., 2001), and can be extensively longer within mature plant leaves when biogenesis rate declines (Schöttler et al., 2007). ATPase and PSI are also considered as relatively stable according to protein

turnover studies in barley (Christopher & Mullet, 1994; Nelson et al., 2014). However, Cyt b_6f and ATPase contents decrease more rapidly, within days, upon abiotic stress conditions, such as drought (Kohzuma et al., 2009), whereas in non-stressed environments this can take even weeks and is attributed mostly to leaf ontogenesis (Schöttler et al., 2015).

1.2.3 Function of photosynthetic light reactions

Both P680 and P700 are excited either directly by light, or by excitation energy funnelled by their respective LHC antenna. RC Chls excite from ground state into short-lived excited state, and rapidly lose an electron to a corresponding electron acceptor within a timescale of picoseconds. In PSII core, excited P680* donates the electron to primary electron acceptor pheophytin (Pheo). The electron is further transferred to plastoquinone Q_A, and from Q_A to Q_B. Mobile Q_B is fully reduced and protonated to PQH_2 after it has received two electrons from Q_A and two protons from chloroplast stroma. $P680^+$ is a strong oxidant and replaces the lost electron from water. Water oxidation occurs at the luminal OEC and the released electron is passed to $P680^+$ through a redox-active tyrosine (TyrZ) of D1 (Diner & Rappaport, 2002). In PSI, the excited P700* passes the electron to Chl A_0, which reduces phylloquinone (PhQ). From PhQ, electron is transferred via three Fe-S clusters (Fe_4S_4), bound to PsaC, finally to Fe-S cluster (Fe_2S_2) of ferredoxin (Fd), and ultimately to Fd-NADPH oxidoreductase (FNR) that reduces $NADP^+$ to NADPH. $P700^+$ replaces the lost electron from PC. The electron transfer in PSI is bi-directional and can occur via PsaA or PsaB. The cofactors in both reactions are identical, but there are some differences in the kinetics as shown by mutation analyses (Santabarbara et al., 2005, 2015).

Cyt b_6f functionally connects the two PSs by oxidizing PQH_2 and by reducing PC via the quinol (Q) cycle. Oxidation of PQH_2 at Cyt b_6f is considered as the rate-limiting step in the electron transfer chain (Siggel, 1976; Tikhonov, 2024). PQH_2 oxidation occurs at Rieske Q_p site where it donates two electrons. One electron passes first to Cyt *f* and then to PC. The other electron reduces first *b* haem and then *c* haem located at the Q_n site of the complex (Malone et al., 2021; Pintscher et al., 2024). Simultaneously the two protons of PQH_2 are released to lumen. After the first cycle, another PQH_2 undergoes same two electron donations while two protons are further translocated to lumen, resulting in reduced haem *c/b* pair at the Q_n site. This highly reductive haem pair reduces PQ molecule, which is produced by oxidation at the Q_p site, back to PQH_2 which can then return back to Q_p site to continue the Q cycle (Malone et al., 2021; Sarewicz et al., 2023; Pintscher et al., 2024).

Water oxidation and PQH_2 oxidation at Cyt b_6f accumulates protons in the thylakoid lumen. This results in electric potential difference and pH difference across

the membrane called proton motive force (*pmf*). This electrochemical gradient induces rotation of the central stalk of the ATPase and activates ATP synthesis. The ATP synthesis coupled to proton movement through the F_o c-ring takes place in the β subunits located in the F_1 head (Nelson & Ben-Shem, 2004; Yang et al., 2020). The central stalk rotates when the protons are pumped from lumen to stroma, and this causes a conformational change in the αβ dimers that results in synthesis of ATP molecules from ADP and inorganic phosphate (P_i) (Hahn et al., 2018). The formation of three ATP molecules requires transport of 14 protons back into the stroma yielding H^+/ATP ratio of 4.7. Electron transfer reactions and the photosynthetic protein complexes are illustrated in Figure 3. The chemical energy generated in the form of ATP, that stores high amount of energy in its phosphoryl bond, and highly reductive NADPH are mainly used CB reactions described in chapter 1.2.4.

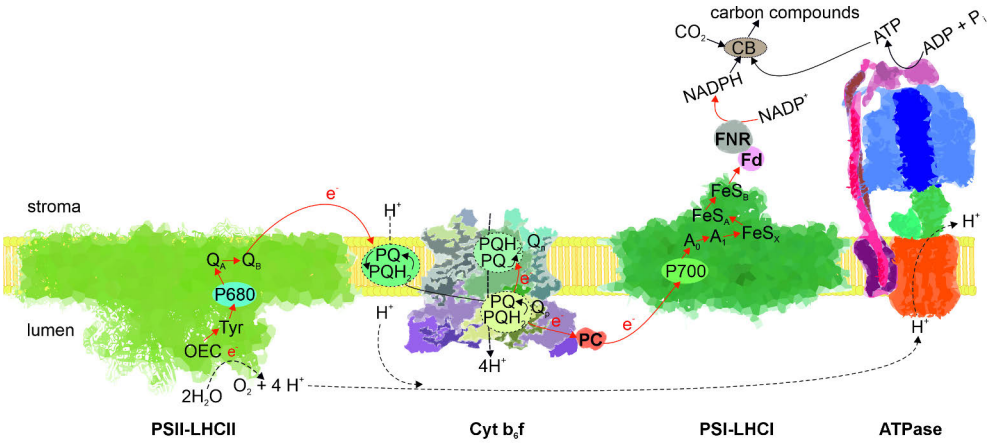

Figure 3. Illustration of photosynthetic linear electron transfer reactions. Proton (H^+) movements are presented with dashed lines and electron (e^-) movement with red lines. Details of the reactions are described in text. Abbreviations: PS, photosystem; LHC, light harvesting complex; OEC, oxygen evolving complex; Q, plastoquinone; PQH_2, plastoquinol; Cyt *b*$_6$*f*, cytochrome *b*$_6$*f*; PC, plastocyanin; A_0, chlorophyll molecule; A_1, phylloquinone; FeS, iron-sulphur-cluster; Fd, ferredoxin; FNR, Fd-NADPH oxidoreductase; ATPase, ATP synthase; P_i, inorganic phosphate; NADPH, nicotinamide adenine dinucleotide phosphate; ATP, adenosine triphosphate; CB, Calvin-Benson cycle.

1.2.4 CB cycle and carbon concentrating mechanisms

The chemical energy produced in light reactions is used to assimilate CO_2 into carbohydrates in the CB cycle. CB cycle is catalysed by enzymes located in chloroplast stroma, and it is divided into three steps: carboxylation of CO_2 into ribulose-1,5-bisphosphate (RuBP) by ribulose bisphosphate carboxylase/oxygenase (Rubisco), reduction reactions requiring ATP and NADPH produced in light

reactions, and regeneration of RuBP to continue the cycle. The reaction steps and enzymes involved have been described by Calvin, Benson, Bassham and their colleagues (Bassham et al., 1953; Bassham & Krause, 1969; Bassham, 1971).

Rubisco catalyses the first step of the pathway that involves condensation of CO_2 with RuBP into six-carbon molecule that is immediately cleaved into two 3-phosphoglycerates (3-PGA) (Quayle et al., 1954). 3-PGA undergoes reduction steps that utilize ATP and NADPH, followed by regeneration step requiring ATP that produces RuBP to repeat carbon assimilation cycle (Bassham, 1971). Three CO_2 molecules yield six 3-PGAs from which five are used in regeneration step and one is used for downstream carbon metabolism to provide substrates for diverse anabolic pathways.

Nearly two decades after discovery of Rubisco (Quayle et al., 1954), its oxygenation activity, the addition of O_2 to RuBP, was reported (Bowes, 1971; Lorimer et al., 1973). The molecular O_2 competes with CO_2 resulting in one 3-PGA and one 2-phosphoglycolate (2-PG) being produced instead of two 3-PGAs. 2-PG cannot be used in cellular metabolism so it must be recycled in order to salvage the carbon, which consumes additional energy and causes 25% loss in carbon entering the cycle (two 2-PGA molecules yield one 3-PGA and one CO_2). Conversion of 2-PGA to 3-PGA takes place in three different organelles: chloroplast, peroxisome and mitochondria, and the process consumes ATP. This process is called photorespiration. In addition to this energy-consuming side reaction, Rubisco is slow catalyst. In order to compensate the low turnover rate, chloroplasts contain significant amount of Rubisco, making it the most abundant enzyme on Earth.

Certain plant species that live in particularly challenging environments contain different kind of mechanisms that circumvents the wasteful photorespiration by concentrating carbon to the proximity of Rubisco. Carbon concentrating inhibits the oxygenation reaction of Rubisco and suppresses energy-consuming photorespiration. C4 photosynthesis and Crassulacean Acid Metabolism (CAM) are the two major ways to concentrate carbon in angiosperms. These plants increase CO_2 levels spatially and temporally, respectively (Sage, 2004; Sage et al., 2023).

C4 plants have developed specific biochemical and anatomical properties that enable them to concentrate CO_2 in proximity of Rubisco. The initial carboxylation in these plants occurs by phosphoenolpyruvate carboxylase (PEPCase) that uses HCO_3^-, converted from CO_2 by carbonic anhydrase (CA), as a substrate in carboxylation of phosphoenolpyruvate (PEP) (Hatch & Burnell, 1990). Resulting four carbon acids (malate or oxaloacetate) are transferred to proximity of Rubisco and decarboxylated, releasing CO_2 to be utilized in CB cycle, and a three-carbon compound that is regenerated into PEP to continue CO_2 concentrating (Hatch, 1987). The leaf anatomy of C4 plants differs from typical C3 plants. They possess

Kranz anatomy, consisting of two types of cells: outer layer of mesophyll (M) cells and inner layer of bundle sheath (BS) cells surrounding the vascular bundle (Hatch, 1987). PEPCase is located in the M cell layer where the initial carboxylation occurs, whereas Rubisco is in the BS cells where CB cycle takes place. The chloroplasts of BS cells are larger than the ones in M cells and they are more abundant (Sage, 2004; Lundgren et al., 2014).

1.3 Regulation of photosynthesis under dynamic environmental conditions

Plants as sessile organisms are exposed to fluctuations in environmental conditions throughout their life cycle, and photosynthetic light reactions are particularly sensitive to these changes. Imbalances in the redox state of photosynthetic complexes cause reduction of O_2 leading to formation of reactive oxygen species (ROS) that cause damage to proteins, membranes and DNA (Krieger-Liszkay & Shimakawa, 2022). Accordingly, this has resulted in plants to develop short-term regulatory mechanisms to respond to rapid changes in growth conditions.

Short-term changes in ambient conditions, e.g. light intensity, occur daily within a timescale ranging from seconds to hours, requiring rapid adjustments of the existing components of photosynthetic machinery. These short-term regulatory responses involve (i) adjustments in light harvesting to balance excitation energy received by PSII and PSI (state transitions), (ii) dissipation of excess energy as heat (non-photochemical quenching (NPQ)), (iii) regulation of the electron transfer at Cyt b_6f (photosynthetic control) and (iv) induction of alternative electron transfer routes at PSI (CET). In addition to rapid fluctuations in ambient environment, plants need to cope with long-term changes that are caused by seasonal variation. Moreover, as the ongoing climate change increases extreme weather events, plants are exposed to even more dramatic changes in ambient conditions. These long-term changes in any aspect of environmental condition (i.e. temperature, drought, nutrient availability), that occur from days to weeks, initiate acclimatory response that involves adjusting the content of photosynthetic components by synthesis and degradation of proteins (Walters, 2004; Schöttler and Tóth, 2014).

In the next chapters, photosynthetic regulatory mechanisms will be described with a focus on light reactions. Carbon metabolism is additionally regulated by modulating activity of Rubisco and other enzymes involved in CB cycle in order to balance the chemical energy supply and demand, but these mechanisms are out of the scope of this thesis.

1.3.1 Regulation of light harvesting and dynamic adjustment of grana stacking

Fluent and safe operation of the light reactions requires synchronized function of the two PSs. Even though both PSII and PSI contain their own antennae, an additional pool of L-LHCII may function as an antenna for both PSs to maintain balance upon changes in light conditions (Figure 4A) (Tikkanen et al., 2006; Galka et al., 2012; Wientjes et al., 2013). L-LHCII pool is regulated via reversible phosphorylation of threonine residues at the N-termini of Lhcb1 and Lhcb2 by STN7 kinase that is activated upon reduction of PQ pool (Vener et al., 1995, 1997; Bellafiore et al., 2005). Phosphorylated LHCII associates with PsaH/L/O/K side of PSI forming PSI-LHCII supercomplex (Lunde et al., 2000; Plöchinger et al., 2016; Pan et al., 2018) referred as state 2. Dephosphorylation of LHCII is catalysed by THYLAKOID-ASSOCIATED PHOSPHATASE 38 (TAP38)/PPH1 (Pribil et al., 2010; Shapiguzov et al., 2010) and the dephosphorylated LHCII is associated with PSII, referred as state 1. Phosphorylation status of LHCII depends on the light conditions: at lower light intensities STN7 is active (Pesaresi et al., 2011), whereas at high light intensities, STN7 is inactivated by the thioredoxin system (Rintamäki et al., 2000), which results in LHCII dephosphorylation. Since TAP38 is considered as a constitutively active enzyme, the phosphorylation state of LHCII proteins is determined by the activity of the STN7 kinase (Pribil et al., 2010; Shapiguzov et al., 2010).

The complex mechanism of grana stacking remains elusive, but the interaction of positively charged N-terminus and negatively charged stromal loops of Lhcb proteins across the stromal gap, and interaction the adjacent PSII-LHCII supercomplexes and possibly free LHCII have been suggested to drive the formation of grana stacks (Standfuss et al., 2004; Daum et al., 2010; Pribil et al., 2014; Wan et al., 2014; Albanese et al., 2020; Wietrzynski et al., 2025). Additionally, negatively charged membrane accommodates soluble ions, e.g. Mg^{2+}, which forms salt bridges with the negatively charged stromal loops of Lhcb proteins thus stabilizing stacking (Barber, 1980; Wan et al., 2014). Guardini et al. (2022) reported that mutants lacking all LHCII antenna components (Lhcb 1-6) show complete loss of the grana stacks. However, Andersson et al. (2003) reported that Arabidopsis mutants lacking Lhcb1 and Lhcb2 proteins still retained grana. Moreover, Ruban et al. (2003) reported that Arabidopsis mutants lacking Lhcb1-3 proteins still contain PSII supercomplexes to same extent as wild type (Wt) plant, but the antenna is formed by Lhcb5 trimers, although Lhcb5 is normally present as monomer. The importance of Lhcb5 protein is further supported by the result by Guardini et al. (2022) showing that mutant containing Lhcb5 alone maintained stacking up to 50% of Wt level. They also showed that expression of Lhcb2 alone maintained proportion of grana stacking, but the morphology of grana stacks was altered, characterized by much wider grana than

in Wt. The stacks, however, were broken after illumination, indicating the stabilizing role of Lhcb2 in stacking.

Lhcb1 phosphorylation has been proposed to regulate remodeling of the grana during state transitions (Pietrzykowska et al., 2014), whereas Lhcb2 phosphorylation is required for direct interaction of L-LHCII with PSI (Crepin & Caffarri, 2015; Pan et al., 2018). Phosphorylation of Lhcb2 occurs mainly in pool of L-LHCII whereas phosphorylated Lhcb1 is mostly present in the PSII-LHCII supercomplexes (Galka et al., 2012; Rantala et al., 2017), specifically in S-LHCII, since M-LHCII mostly contains Lhcb1.4 isoform that lacks phosphorylation site (Galka et al., 2012). In darkness, Lhcb1 and Lhcb2 are dephosphorylated, whereas upon exposure to light, phosphorylation of Lhcb1 and Lhcb2 result in loosening of grana stacking and migration of PSII-LHCII complexes, usually restricted to grana thylakoids, towards grana margins, thus allowing L-LHCII and PSI to interact (Kyle et al., 1983; Pietrzykowska et al., 2014; Wood et al., 2019). LHCII phosphorylation alone, not the interaction of PSI and L-LHCII, is sufficient for causing dynamic changes in grana stacking (Wood et al., 2019).

Although phosphorylation has been attributed to be the main factor driving state transitions, Koskela et al. (2018) showed that protein acetylation has a crucial role in state transitions. GCN5-related N-acetyltransferase 2 (GNAT2), which is part of the acetyltransferase family comprising chloroplast acetylation machinery (Bienvenut et al., 2020), is essential for formation of state 2, since Arabidopsis *gnat2* knockout mutant is unable to form the PSI-LHCII complex despite increased phosphorylation levels of Lhcb2 (Koskela et al., 2018). Transmission electron micrographs also showed that the *gnat2* mutant contains more tightly packed grana thylakoids compared to Wt indicating the role of acetylation in defining thylakoid membrane ultrastructure (Koskela et al., 2018). Recent study with tomato by Wang et al. (2025) showed that GNAT2 is responsible for acetylation of Lhcb2 and that GNAT2 is redox regulated, similarly to STN7 (Wu et al., 2021). Redox regulation occurs via a conserved cysteine residue in GNAT2 that is crucial for oligomerization and therefore its activity (Wang et al., 2025). In addition to Lhcb2, GNAT2 acetylates other chloroplast proteins, such as PSI subunit PsaH (Koskela et al., 2018), which mediates LHCII binding (Lunde et al., 2000).

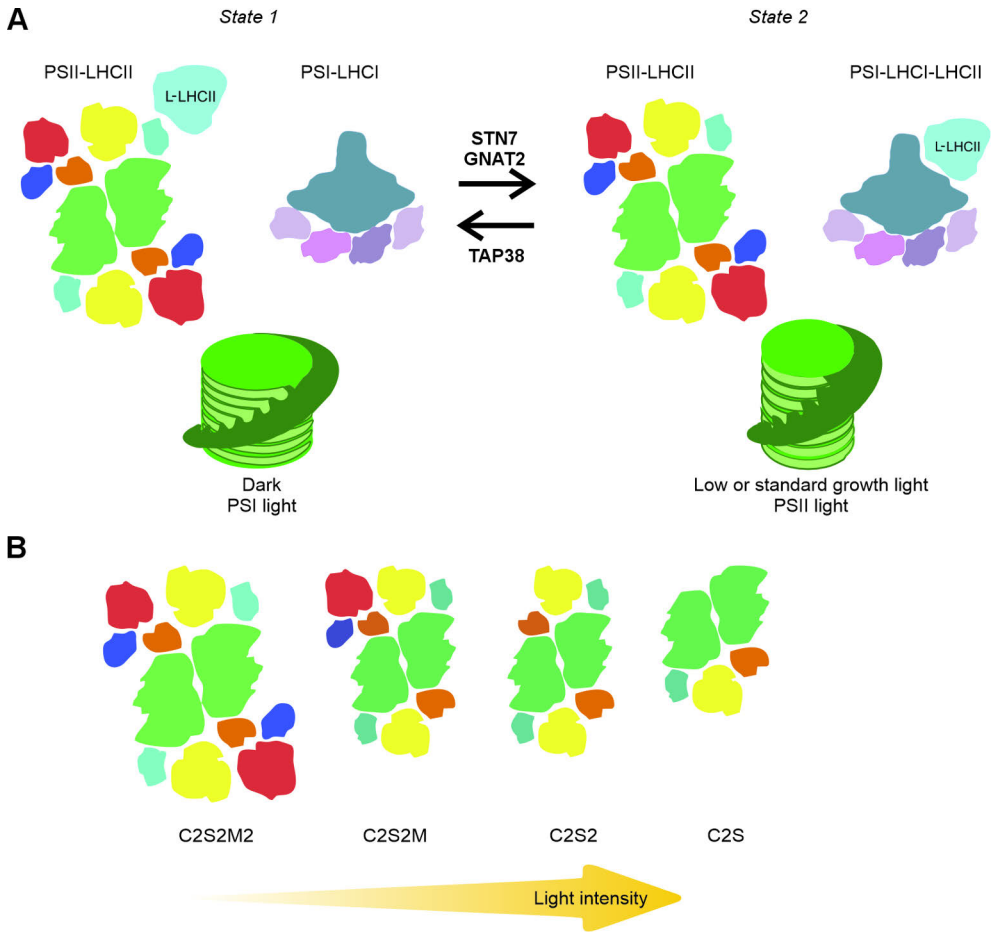

Figure 4. State transitions and modulation of PSII antenna size. A) In darkness and light conditions favouring photosystem (PS) I excitation, loosely bound light harvesting antenna (L-LHCII) is dephosphorylated and associated with PSII-LHCII. When phosphorylated, L-LHCII binds to PSI. GNAT2 acetyltransferase is required for antenna modelling through unknown mechanism. The tight packing of grana partially loosens upon shift from darkness to light. B) PSII antenna size is modulated upon changes in light intensity: under lower light intensities, larger C2S2M2 complexes are the dominant PSII-LHCII supercomplexes, whereas antenna size reduces as light intensity increases.

In addition to dynamic rearrangement of the L-LHCII antenna between the two PSs, PSII antenna size is modulated upon long-term changes in light intensity (Figure 4B) (Ballottari et al., 2007; Kouřil et al., 2013; Albanese et al., 2016). Under excess light, M-LHCII disconnects from the supercomplex making C2S2 the predominant form (Ballottari et al., 2007; Betterle et al., 2009; Albanese et al., 2016). Under prolonged high light exposure, the amount of Lhcb4.3 isoform increases over Lhcb4.1 and Lhcb4.2, accompanied by a decrease in Lhcb3 and Lhcb6, that presumably destabilizes M-LHCII association to the PSII core (Albanese et al.,

2016). This is further supported by report showing that plants containing Lhcb4.3 isoform instead of Lhcb4.1 cannot retain M-LHCII association to the core (Caferri et al., 2025).

PSII-LHCII complexes can further form larger megacomplexes that can occur either in single membrane plane or across adjacent membranes (Boekema et al., 2000; Kirchhoff et al., 2007; Daum et al., 2010; Kouřil et al., 2013; Albanese et al., 2016; Albanese et al., 2017; Nosek et al., 2017; Albanese et al., 2020; Kim et al., 2020; Kouřil et al., 2020; Shan et al., 2024; Vánská et al., 2025). Organization into arrays under lower light intensities as well as under standard growth light has been described, but array formation has been reported to be decreased under high light (Kouřil et al., 2013). Arrays comprised of C2S2 complexes are formed in the absence of Lhcb6 (De Bianchi et al., 2008), but Lhcb5 has been shown to have a key role in formation of these megacomplexes (Nosek et al., 2017; Vánská et al., 2025). Dense packing of PSII-LHCII supercomplexes into arrays may contribute to efficient energy transfer when light is limited (Tietz et al., 2015; Kim et al., 2020). However, such arrays have not been detected in intact chloroplasts, and thus they might be an artefact caused by non-native environment (Wietrzynski et al., 2025).

1.3.2 Dissipation of excess light energy as heat

When light energy exceeds the utilization capacity of the photosynthetic apparatus, NPQ dissipates excess energy as heat and safeguards photosynthetic machinery from oxidative stress. NPQ consists of several molecular mechanisms that have traditionally been separated according to their relaxation kinetics (Demmig-Adams et al., 2014). Since the relaxation time can greatly vary in nature, Malnoë (2018) proposed that the components should be separated based on the involved molecular players.

The fastest (timescale of seconds to minutes) and the major component of NPQ is energy-dependent quenching (qE) (Krause et al., 1982) that relies on protonation of glutamate residue of PsbS protein resulting from increase in ΔpH across the thylakoid membrane (Krause et al., 1982; Li et al., 2000; Liguori et al., 2019). Upon protonation, PsbS undergoes conformational change favouring its binding to LHCII (Liguori et al., 2019) that leads to energy dissipation, but the exact molecular site of quenching remains unknown (Ruban & Wilson, 2021). In addition to PsbS, induction of qE requires violaxanthin conversion to zeaxanthin (Johnson & Ruban, 2011; Sylak-Glassman et al., 2014). Zeaxanthin is also required for the second fastest NPQ component, occurring within a timescale of minutes to tens of minutes, zeaxanthin-dependent quenching (qZ) (Dall'Osto et al., 2005; Nilkens et al., 2010). In contrast to qE, qZ is independent from PsbS or ΔpH, and it involves conformational change

in the Lhcb5 protein demonstrated by a shift in the protein's isoelectric point (pI) upon accumulation of zeaxanthin (Dall'Osto et al., 2005).

Photoinhibitory-dependent quenching (qI) (Krause, 1988) is the slowest NPQ component occurring in timescale of hours or longer and is mostly comprised of inactivation and degradation of D1 resulting from photoinhibition, but the site of quenching remains unknown (Nawrocki et al., 2021). Previously, qI was attributed to all mechanisms resulting in light-induced decrease of PSII quantum yield, including photoinactivation of PSII and all uncharacterized components with slow relaxation kinetics. However, plastid lipocalin (LCNP) -dependent quenching (qH) which is sustained form of antenna quenching, that is independent of previously characterized components of NPQ, was identified by Malnoë et al. (2018). The key component of qH is the LCNP protein. Under stress conditions, LCNP is released by its negative regulator SUPPRESSOR OF QUENCHING 1 (SOQ1), and LCNP either directly forms the quenching site or induces an indirect effect by modifying the membrane environment (Malnoë et al., 2018; Amstutz et al., 2020).

Since mechanisms involved in NPQ decrease Chl fluorescence, energy re-distribution between PSII and PSI during state transitions affect the fluorescence yield, and thus have been considered as a component of NPQ. State transition -dependent component is termed qT and its contribution under saturating light has been considered to be minor (Nilkens et al., 2010). In addition to qT, chloroplast movement that leads to shading decreases Chl fluorescence and has been termed as qM component (Cazzaniga et al., 2013). However, the excess light-induced decrease in fluorescence level arises mostly from avoidance of light absorption rather than quenching.

1.3.3 Photoprotection and flux control

PSII is the most light-sensitive complex of the ETC and the damage to D1 is proportional to light intensity (Tyystjärvi & Aro, 1996). Damaged D1 is replaced by newly synthetized protein in PSII repair cycle (Aro et al., 1993). Upon damage, phosphorylated PSII core monomerizes in grana membranes (Aro, 2005) and core proteins are dephosphorylated (Koivuniemi et al., 1995) and migrated to non-appressed thylakoids. Dephosphorylated D1 is degraded by several proteases and after degradation, PSII is reassembled by co-translational insertion of newly synthesized D1 protein with the recycled core components of PSII followed by migration back to appressed grana (Järvi et al., 2015).

PSI, on the contrary, does not have a dedicated repair machinery. Once it damages, in case if electron flow from PSII is far more than the capacity of PSI, the repair process requires synthesis and assembly of the entire complex and is therefore extremely slow (Sonoike, 2011). To avoid this costly damage, PSI is protected from

over-reduction and consequent ROS formation by maintaining it more oxidized. This occurs by restricting the electron flow at Cyt *b*$_6$*f* (photosynthetic control) and by circuiting electrons back to PQ pool from PSI via CET.

Cyt *b*$_6$*f* plays key role in regulation of light reactions due to its location at the crossroad of PSII and PSI. Joliot & Johnson (2011) showed that Cyt *b*$_6$*f* is essential for protection of PSI, since dysfunctional regulation of Cyt *b*$_6$*f* showed extensive PSI photoinhibition (~70%), whereas PSII was more tolerant towards photoinhibition (~30%). Photosynthetic control is activated by acidification of lumen (Nishio & Whitmarsh, 1993; Joliot & Johnson, 2011), which leads to protonation of a histidine residue bound to Rieske Fe_2S_2 cluster preventing PQH_2 oxidation (Degen & Johnson, 2024).

CET around PSI circuits electrons from Fd back into the PQ pool. This leads to increased proton influx to the lumen resulting in ATP synthesis without production of NADPH, simultaneously increasing ΔpH (Wang et al., 2015). In angiosperms, there are two pathways for CET, one that depends on PROTON GRADIENT REGULATION 5 (PGR5) and PGR5-Like Photosynthetic Phenotype (PGRL1) proteins (Munekage et al., 2002; DalCorso et al., 2008; Hertle et al., 2013) and the other that is mediated by NDH complex (Endo et al., 1999; Joët et al., 2001; Peltier et al., 2016). The PGR5-PGRL1 pathway of CET is considered as the major CET in C3 plants (Wang et al., 2015). PGR5 and PGRL1 form a complex around PSI (Munekage et al., 2002; DalCorso et al., 2008) which accepts electrons from Fd and cycles them back to PQ pool (Hertle et al., 2013). In NDH-mediated CET, NDH-1 forms a supercomplex with PSI via Lhca5 and Lhca6 (Peng et al., 2008; Kouřil et al., 2014). Similarly to PGR5-PGRL1 -mediated CET, electrons are taken from Fd by NDH-1 complex and cycled to PQ pool (Peltier et al., 2016). Despite being known for decades, the exact molecular mechanisms of CET remain somewhat elusive (Nawrocki et al., 2019). In addition to its key role in maintaining appropriate ATP/NADPH ratio, CET is crucial when ΔpH needs to be increased in order to induce NPQ and photosynthetic control to protect PSI (Suorsa et al., 2012, 2016; Barbato et al., 2020). Intriguingly, a recent report on the interplay between NPQ and CET in diatoms showed linear increase in CET as a function of qZ (Croteau, 2024). Indeed, mutants containing increased amounts of Lhcx1, a necessary component for qZ in *P. tricornutum*, were characterized by elevated CET and qZ levels (Croteau, 2024). This further emphasizes the connection between these two regulatory mechanisms.

2 Aims of the study

During the course of evolution, the photosynthetic machinery has been evolved and adapted according to the ecological niche of the given plant species, and according to the cellular environment of the chloroplast. These adaptations ensure growth and reproduction of the plant. Under fluctuating environmental conditions, however, plants also need adjustments, i.e. acclimatory mechanisms, enabling them to regulate photosynthetic machinery according to sudden changes in ambient environmental cues. The aim of my PhD work was to reveal and understand the short- and long-term responses and regulatory mechanisms affecting thylakoid membrane architecture and organization of photosynthetic membrane-embedded protein complexes of model organism *Arabidopsis thaliana*. The specific aims were as follows:

(i) to reveal the role of GNAT2 acetyltransferase on light-dependent dynamics of thylakoid membrane

(ii) to analyse and compare the composition and organization of the thylakoid membrane protein complexes in the chloroplasts of non-foliar tissues to those of leaves

(iii) to reveal the effects of heat acclimation on thylakoid membrane and photosynthetic light reactions

3 Methodology

3.1 Plant material and growth conditions

Arabidopsis ecotype Columbia-0 (Wt, Col-0), *gnat2* (SALK_033944; Koskela et al., 2018) and *stn7* (SALK_073254; Tikkanen et al., 2006) plants were grown on a peat:vermiculite mixture (2:1) under 8 h light/16 h dark cycles (Publication I and II). Plants for publication III were grown under 12 h light/ 12 h dark cycles. All plants were grown at photosynthetic photon flux density (PPFD) of 130 µmol m^{-2} s^{-1}, + 23°C and relative humidity of 50-70% for 4-5 weeks. For publication III, heat-acclimated (HS) plants were grown in growth chamber where temperature increased daily to + 38°C for four h, at 10 am (two h after onset of light). For Publications I and III, whole rosettes were analysed, while in Publication II also non-foliar tissues (stems, flowers, siliques) were studied. Figure 5 illustrates the plant material and overall methodology used in Publications I-III.

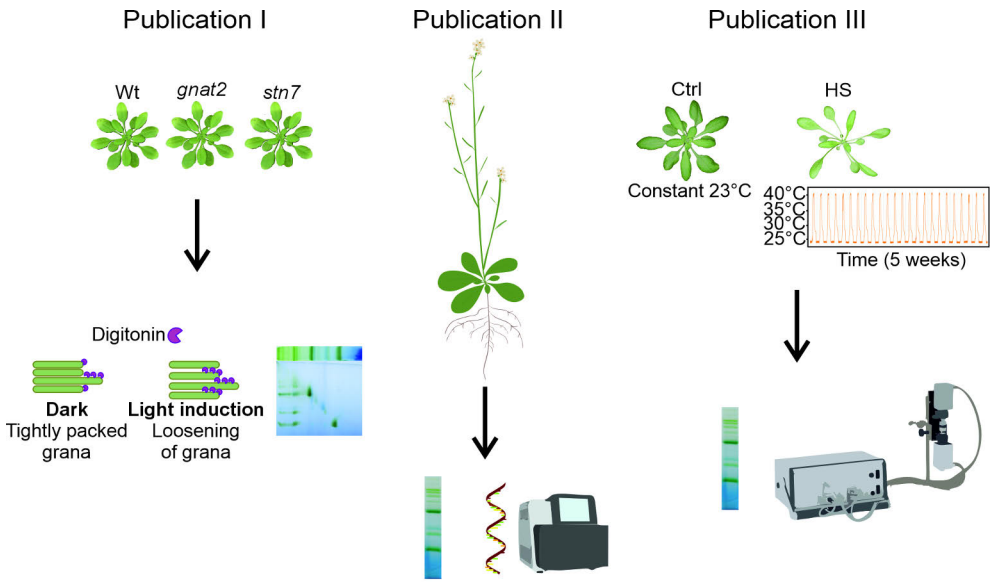

Figure 5. Plant material and main methodology used in publications I-III. Further details are described in text. Abbreviations: Wt, wild type; Ctrl, control; HS, heat-acclimated plants.

3.2 Isolation of thylakoid membranes and fractionation into stroma and grana thylakoids

Fresh plant material was used for all thylakoid isolations. Leaf suspension was washed with hypotonic buffer to break down the chloroplasts. Intact thylakoid membranes were collected and stored in isotonic buffer. Sodium fluoride was used in all used buffers to inhibit dephosphorylation of the proteins, and bovine serum albumin was used to protect protein integrity during grinding of the leaf material. Thylakoid isolation was done in dim light at 4°C to avoid pigment/protein degradation and phosphorylation of proteins. Details are described in publications I-III. The Chl concentration was determined according to Porra et al. (1989).

In publication I, thylakoid membranes were fractionated into stroma and grana thylakoids by using mild detergent digitonin. As digitonin solubilizes non-appressed membrane domains (Andersson & Anderson, 1980; Jennings et al., 1980), the size of solubilized fraction relative to the appressed insoluble fraction provides indirect information of the packing of grana. Therefore, Chl concentration from the insoluble (grana) and soluble (stroma thylakoids) fractions was determined to analyse the proportion of the solubilised and non-solubilised fractions before and after light-induction to assess dynamic changes in the membrane.

3.3 Gel-based techniques

3.3.1 Native gel electrophoresis

For all three publications, native protein complexes were analysed with Blue Native Polyacrylamide Gel Electrophoresis (BN-PAGE). Non-ionic detergents, digitonin and β-D-dodecyl maltoside (β-DDM), were used to excise protein complexes from the thylakoid membranes in their native structure (Figure 6). β-DDM solubilizes protein complexes from the entire thylakoid membrane, and digitonin selectively solubilizes the non-appressed regions of the membrane (grana margins and stroma lamellae) leaving the appressed grana unsolubilized (Andersson & Anderson, 1980; Jennings et al., 1980). When supplemented with ACA, digitonin solubilizes complexes from the entire thylakoid membrane.

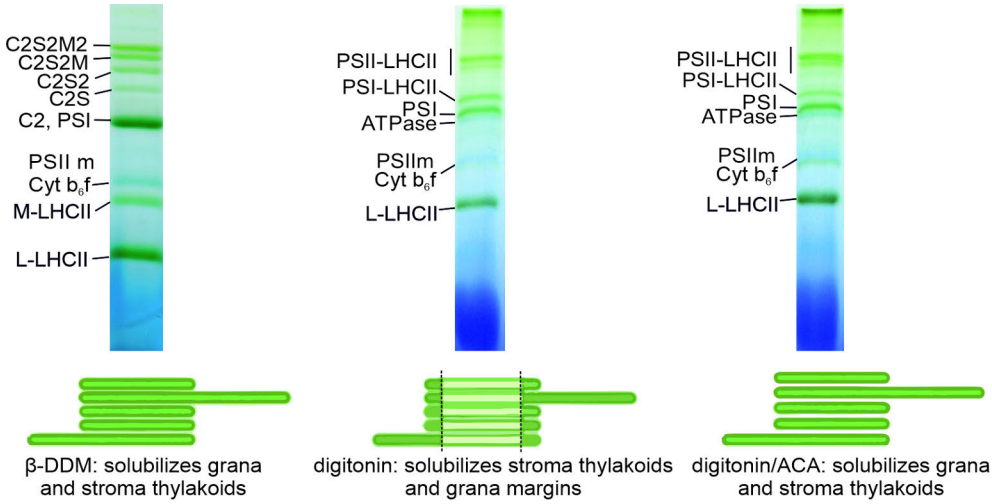

Figure 6. Different solubilization capacity of β-D-dodecyl maltoside (β-DDM) and digitonin. β-DDM solubilizes protein complexes from the appressed grana and non-appressed stroma membranes. Digitonin selectively solubilizes protein complexes from grana margins and stroma lamellae and cannot access complexes located at appressed grana stacks. When supplemented with aminocaproic acid (ACA), digitonin solubilizes complexes from entire thylakoid membrane. Protein complexes are indicated in the gel.

The solubilized thylakoid protein complexes were supplemented with Coomassie G-250 giving them a negative charge and then separated with BN-PAGE. By using BN-PAGE, protein complexes remain in their native and functional form and migrate according to their molecular mass until they reach size-dependent-pore-size limit.

In publication I, 2-dimensional (2D) BN-BN-PAGE (Rantala et al., 2017) was used to analyse different LHCII-pools. In publication II, distinct protein subunits from the complexes were further resolved with sodium dodecyl sulphate (SDS)-PAGE (Järvi et al., 2011) followed by silver staining according to Blum et al. (1987) and identification of proteins was performed by mass spectrometry.

3.3.2 SDS-PAGE and immunoblotting

For Western blot analysis, thylakoid proteins were denaturated with SDS, urea and β-mercaptoethanol (modified from Laemmli, 1970). Denaturated proteins were separated with SDS-PAGE, and the proteins were transferred to PVDF-membrane for immunoblot analysis. Details of the primary antibodies used in publications I-III are listed in Table 1. Secondary antibodies conjugated with either horseradish peroxidase (HRP) or infrared fluorescence dye were used. Membrane staining was done with Coomassie brilliant blue (CBB) or Licor Revert stain to verify equal loading according to Chl-concentration.

Table 1. Antibodies used in experiments.

Antibody	Dilution used	Chlorophyll amount used	Produced by /custom made	Used in Paper
pLhcb1	1:10 000, 1% BSA	0.5 µg	Agrisera AS13 2704	I
pLhcb2	1:10 000, 1% BSA	0.5 µg	Agrisera AS13 2705	I
Lhcb1	1:2000, 1% milk	1 µg	Agrisera AS01 004	I, III
Lhcb2	1:2000, 1% milk	1 µg	Agrisera AS01 003	I
Lhcb3	1:2000, 1% milk	1 µg	Agrisera AS01 002	I
Phospho-Thr/Ser	1:6000, 1% BSA	0.5 µg	Cell Signaling Technology, 9381S	II, III
D1 DE-loop	1:8000, 1% milk	1 µg	Kettunen et al., 1996	II, III
PSAB	1:2500, 1% milk	1 µg	Agrisera AS10695	II, III
ATPB	1:5000, 1% milk	1 µg	Agrisera AS05085	II, III
Cyt f/PetA	1:1000, 1% milk	1 µg	Agrisera AS06119	II, III
LHCA5	1:3000, 1% milk	4 µg	Agrisera AS05082	II
NDHL	1:5000, 1% milk	4 µg	T. Shikanai, Shimizu et al., 2008	II
PetC	1:5000, 1% milk	1 µg	Agrisera AS08 330	III

3.4 Proteomics

Proteins from 2D-SDS-PAGE were identified by using LC-ESI-MS/MS Analysis on a nanoflow HPLC system (Easy-nLC1000, Thermo Fisher Scientific) coupled to the Q Exactive HF mass spectrometer (Thermo Fisher Scientific) equipped with a nano-electrospray ionization source (Publication II). Analyses were conducted at the Turku Proteomics Facility supported by Biocenter Finland.

3.5 Transcriptomics

For publication II, total RNA was isolated from leaves, roots, flowers and stems followed by DNase treatment and RNA quality assessment on agarose gel. PolyA+ transcriptome sequencing (RUO) was done at the Finnish Functional Genomics Center (Turku). The paired-end libraries were sequenced using the Illumina NovaSeq 6000 S1 v1.5 (Illumina). Chipster v. 4 (Kallio et al., 2011) was used for data analysis (http://chipster.csc.fi/). Reads were mapped to the Arabidopsis genome TAIR10.42 with Bowtie2 (Langmead & Salzberg, 2012). The number of alignments mapped to each gene was counted using HTseq2 (Putri et al., 2022). Differential

expression analysis was done with the DESeq2 package (Love et al., 2014) using the Chipster platform (Kallio et al., 2011).

3.6 Functional measurements of photosynthesis

3.6.1 Chl *a* fluorescence and P700 absorbance measurements with DKN

For publication III, light response curves of Chl *a* fluorescence and P700 absorbance changes were measured with DKN spectrophotometer (Heinz Walz GmbH). Chl fluorescence is a measure of re-emitted light by PSII, and measuring fluorescence changes gives information about PSII function (i.e. photochemistry and non-photochemical events) upon prevailing conditions. PSI does not show variable Chl fluorescence at room temperature, but redox changes of P700, PC and Fd cause absorbance changes in the visible and near-infrared (NIR) wavelengths (Klughammer & Schreiber, 1991, 2016). Therefore, measuring absorption changes in NIR region provides a method to assess redox alterations in PSI by using four wavelength pairs that allows discrimination of PC, P700 and Fd redox changes.

Redox changes were deconvoluted based on differential model plots (DMPs) (Klughammer & Schreiber, 2016). Normalization of PC/P700/Fd redox changes was done according to the maximal redox changes that were determined with the software's NIR Max-script. Plants were dark-acclimated 1 h prior measurements.

3.6.2 77K measurements

To assess the relative fluorescence emission from PSII (685 nm and 695 nm) and PSI (735 nm), 77 K fluorescence emission spectra were measured from thylakoids isolated from Ctrl and heat-acclimated plants (Publication III). Since at room temperature PSII emits most of the fluorescence (Govindjee, 2004), fluorescence emission spectrum at 77 K (-196°C) provides information of both PSs. At 77 K, biochemical processes contributing to fluorescence change are mostly abolished (except for light harvesting and primary photochemistry), and fluorescence emission of both PSs are distinguishable. Each spectrum was measured with QEPro spectrometer (Ocean Optics).

3.6.3 *pmf* measurements with JTS-150

To estimate the magnitude of *pmf,* changes in the electrochromic shift (ECS) signal arising from light-induced absorbance difference between 546 nm and 520 nm were recorded with JTS-150 spectrophotometer (BioLogic) (Publication III). ECS_T,

maximum amplitude of the ECS, was calculated as the difference between total ECS in light and an Y_0 value obtained from the first-order exponential fit to the decay kinetics of the ECS signal during a dark interval. The gH^+ parameter (thylakoid membrane conductivity to protons) was calculated as the inverse of the time constant of a first-order exponential fit to ECS decay kinetics during a dark interval (Cruz, 2004; Avenson et al., 2005). The vH^+ parameter (the rate of proton flux over the thylakoid membrane) corresponding to the initial slope of the decay of the ECS signal upon cessation of illumination was calculated as *pmf* x gH^+ (Cruz, 2004). Plants were dark-acclimated for 1 h prior measurement.

3.7 Circular dichroism spectroscopy

The macro-organization of the thylakoid membranes was analysed using circular dichroism (CD) spectroscopy to obtain information on the protein-pigment and pigment-pigment interactions. Spectra were measured from intact leaves and from thylakoid membrane samples using a J815 spectropolarimeter (JASCO Europe Srl) (Publication I). Spectral range of 400 – 800 nm was used and normalization was done to the absorption maxima (680 nm). Measurements were performed in Bettina Ughy's group at University of Szeged, Hungary.

4 Overview of the Results

4.1 GNAT2 is involved in regulation of thylakoid dynamics

GNAT2 is a chloroplast-located acetyltransferase recently shown to be involved in state transitions (Koskela et al., 2018; Wang et al., 2025), that was for a long time attributed solely to reversible LHCII (de)phosphorylation by STN7 and TAP38 (Bellafiore et al., 2005; Pribil et al., 2010; Shapiguzov et al., 2010). Moreover, LHCII phosphorylation has been considered not only to rearrange the thylakoid protein complexes, but also the entire thylakoid ultrastructure (Kyle et al., 1983; Rozak et al., 2002; Wood et al., 2019). Indeed, dark to light (low or moderate light) -transition leads to loosening of grana stacks and increase in the grana margin area (Wood et al., 2019).

The *gnat2* mutant was previously shown to contain more tightly packed thylakoid membranes in darkness than Wt (Koskela et al., 2018). To assess the effect of acetylation on light-induced dynamics of thylakoid membrane, thylakoid membranes from dark- and light-acclimated *gnat2*, *stn7* and Wt were fractionated with digitonin (Publication I). Digitonin is a mild nonionic detergent that exclusively solubilizes protein complexes from the non-appressed regions of the membrane leaving the tightly packed grana insolubilized (Andersson & Anderson, 1980; Jennings et al., 1980). Dark-acclimated plants contain more tightly packed grana and there is strict lateral heterogeneity among the photosynthetic protein complexes, whereas in light, loosening of grana stacks increases the proportion of non-appressed regions and slightly decreases lateral heterogeneity (Kyle et al., 1983; Chuartzman et al., 2008; Wood et al., 2019). Therefore, in light, the proportion of membrane accessible to digitonin solubilization increases, and the proportion of the solubilized complexes can be indirectly assessed by comparing the Chl content of the fraction solubilized by digitonin to the Chl content prior the treatment.

The Chl *a* + *b* content, particularly Chl *b,* in digitonin-solubilized fraction corresponding stroma thylakoids and grana margins was significantly increased upon shift from darkness to light in Wt, indicating loosening of the grana (Publication I: Figure 1B). Interestingly, in *gnat2,* the increase in Chl content in the soluble fraction upon shift from dark to light was minimal and not statistically

significant, demonstrating the lack of light-induced dynamics in the mutant (Publication I: Figure 1B). *stn7* mutant showed similar results as *gnat2*. Indeed, *gnat2* and *stn7* both lacked the light-induced dynamic loosening of grana but in contrast to *stn7, gnat2* shows no impairment in phosphorylation of Lhcb1 and Lhcb2 (Publication I: Figure 1C).

In order to see whether the lack of PSI-LHCII complex in *gnat2* is caused by restricted LHCII migration from the tightly packed grana, thylakoid membranes were artificially destacked by depleting the thylakoids of Mg^{2+}. Desalting leads to complete thylakoid linearization and randomization of the protein complexes (Murakami & Packer, 1971; Barber, 1980). If tight packing of the grana would be the sole factor preventing LHCII interacting with PSI, cation depletion would enable their interaction. Intriguingly, only minute amount of PSI-LHCII complex ("state transition" complex (Kouřil et al., 2005; Pesaresi et al., 2009)) accumulated in the *gnat2* mutant after thylakoid linearization, demonstrating that restricted grana dynamics in *gnat2* is not the primary reason for the inability to form state transitions (Publication I: Figure 4).

Since *gnat2* showed no defect in phosphorylation of Lhcb1 and Lhcb2 (Publication I: Figure 1C), the distribution of the p-Lhcb1 and p-Lhcb2 among LHCII pools (S-LHCII, M-LHCII, L-LHCII) was assessed. Distinct LHCII pools were separated by using 2D-BN-BN-PAGE: protein complexes solubilized with digitonin were separated on 1D-BN-PAGE, followed by further solubilization with β-DDM and separation on 2nd BN-PAGE to detach separate pools of LHCII from PSII (Rantala et al., 2017). In Wt and *gnat2,* p-Lhcb1 was mainly located in PSII-LHCII supercomplexes, but *gnat2* showed slightly increased Lhcb1 phosphorylation levels also in M-LHCII and L-LHCII (Publication I: Figure 3B). In Wt, p-Lhcb2 is predominantly located in the L-LHCII but intriguingly, *gnat2* showed accumulation of (p-)Lhcb2 in all pools of LHCII (Publication I: Figure 3C).

The restricted thylakoid dynamics and differences in the distribution of (p-) Lhcb2 in *gnat2* suggest there are overall differences in the composition and organization of the LHCII antenna. To further study the macro-organization of the thylakoid membranes, CD spectroscopy was used as a non-invasive technique to analyse differences between Wt, *gnat2* and *stn7* in intact leaves and isolated thylakoids (Publication I: Figure 2). The psi-type CD spectrum gives information of dipole-dipole interactions of Chls and carotenoids within PSII and LHCII embedded in thylakoid membrane, and their interactions (Lambrev & Akhtar, 2019). Plants show distinct psi-type CD bands at three wavelengths: 690 nm, 674 nm and 506 nm (Tóth et al., 2016; Lambrev & Akhtar, 2019). The (+) 690 nm psi-type CD band of Wt was significantly higher compared to *gnat2* and *stn7* in both intact leaves and isolated thylakoid membranes (Publication I: Figure 2C, D) indicating change in the composition of the PSII-LHCII supercomplexes in the grana. Isolated thylakoids showed pronounced decrease in the (+) 506 nm band of *gnat2* and *stn7* compared to Wt, as well as in the (-) 674 nm band that was the lowest in *gnat2*

(Publication I: Figure 2D). (+) 506 nm band also depends on the amount of LHCII and PSII and (-) 674 nm band has been associated with thylakoid stacking (Dobrikova et al., 2003; Lambrev & Akhtar, 2019). Alterations in these bands reflect changes in the LHCII composition (Garab & Van Amerongen, 2009; Lambrev & Akhtar, 2019) and further emphasizes the key role of phosphorylation and acetylation on macro-organization of the thylakoid membrane protein complexes.

4.2 Arabidopsis stems contain high amount of CET-related PSI-NDH complex

Regulation of photosynthesis has been mainly studied in leaves, while non-foliar tissues containing chloroplasts have gained less attention. The contribution of non-foliar photosynthesis is more pronounced upon abiotic stress conditions (Ávila et al., 2014; Hu et al., 2014; Hu et al., 2019), reflecting the adaptive responses to fine-tune photosynthetic processes at multi-organ level. Moreover, stems and petioles of C3 plants have been shown to contain characteristics of C4 photosynthesis (Hibberd & Quick, 2002) further highlighting the diversity of the photosynthetic processes within the same species depending on the tissue. Non-foliar photosynthesis has gained some attention during the recent years with most studies focusing on crop plants (Simkin et al., 2020). However, the composition of the thylakoid-embedded photosynthetic protein complexes in non-foliar organs has remained elusive.

To gain insight into the organization and composition of the photosynthetic machinery in non-foliar organs, thylakoids from leaves, stems and green siliques were analysed. Thylakoid protein complexes from the entire thylakoid membrane were solubilized with β-DDM and separated by BN-PAGE. Thereafter, individual protein subunits from the protein complexes were separated by SDS-PAGE. Indeed, stems and siliques contained all necessary components for photosynthetic light reactions (Publication II: Figure 4). Leaves and stems accumulated similar amounts of PSII-LHCII supercomplexes, while in green siliques PSII-LHCII supercomplexes were less abundant and more PSII monomers were detected (Publication II: Figure 4). Further differences in the overall accumulation of the key subunits of photosynthetic protein complexes were analysed with immunoblotting, and no differences were observed between leaves and stems. Siliques, however, contained lower levels of all protein subunits corresponding to key protein complexes (Publication II: Figure 5). Intriguingly, stems accumulated high amount of CET-related PSI-NDH complex (Publication II: Figure 4; Figure 5; Datasets S17-S21).

To inspect further differences of photosynthetic components between leaves and non-foliar organs at transcriptional level, RNASeq was performed to analyse nuclear gene expression in leaves, stems, roots and flowers containing green sepals. All non-foliar organs showed reduced transcript abundance of most of the photosynthetic

genes as compared to leaves. As expected, roots contained the least transcripts of photosynthetic genes (Publication II: Figure 3). Photosynthetic genes in flowers were more downregulated than in stems, even though both have been shown to be photosynthetically active (Brazel & Ó'Maoiléidigh, 2019). Despite high accumulation of the PSI-NDH complex in stems, genes corresponding to subunits of NDH-complex were not upregulated (Publication II: Dataset S10).

The observation that Arabidopsis stems contain high amount of PSI-NDH complex (Publication II: Figure 4) might indicate increased CET and thus high ATP/NADPH ratio in stems. As it has been reported that tobacco stems display characteristics of C4 photosynthesis (Hibberd & Quick, 2002) and that the BS cells of C4 plants possess increased CET (Takabayashi et al., 2005; Darie et al., 2006; Majeran et al., 2008; Munekage et al., 2010; Johnson, 2011; Nakamura et al., 2013; Ermakova et al., 2024), we focused on gene expression levels of C4-decarboxylases within Arabidopsis stems. Intriguingly, stems possessed increased levels of transcripts encoding C4-photosynthesis-related enzyme isoforms (Table 2; Publication II: Dataset S10). Increased ATP demand of the cell might be related to energy-consuming lignin biosynthesis reactions occurring in stems. Indeed, five genes encoding enzymes involved in the shikimate pathway that produces phenylalanine, a precursor for lignin biosynthesis (Vanholme et al., 2010; Tohge et al., 2013), were significantly upregulated in stems (Table 2; Publication II: Dataset S10).

Table 2. Selected genes of interest upregulated in stems compared to leaves. Results are presented as Log_2 fold-changes. |Log2fold-change| >1 and adjusted P-value of <0.05 were used as thresholds for significantly up- or downregulated genes. All presented genes are significantly upregulated in stems as compared to leaves. Function of the gene and related process (C4 carbon metabolism or lignin biosynthesis) are indicated in the table. (n = 4 biological replicates).

Gene	Function/Related process	Log2 fold-change (Stems vs leaves)
AT2G19900, NADP-MALIC ENZYME 1	malate decarboxylation/C4	7.12
AT4G15530, pyruvate orthophosphate dikinase	formation of phosphoenolpyruvate/C4	2.61
AT3G5326, PHENYLALANINE AMMONIA-LYASE 2	phenylalanine metabolism/ lignin synthesis	2.57
AT2G30490, CINNAMATE 4-HYDROXYLASE	transforming trans-cinnamate into p-coumarate/lignin synthesis	3.01
AT1G51680, 4-COUMARATE: COA LIGASE	produces CoA thioesters/lignin synthesis	3.73
AT2G40890, COUMARATE 3-HYDROXYLASE	formation of lignin monomers/lignin synthesis	2.58
AT5G48930, HYDROXYCINNAMOYL-COA SHIKIMATE/QUINATE HYDROXYCINNAMOYL TRANSFERASE	acyltransferase for shikimates/lignin synthesis	2.15

4.3 Plants acclimate to long-term daily heat stress by downregulating Cyt *b*$_6$*f* with the expense of photosynthetic efficiency

In addition to light, also temperature affects photosynthesis. In fact, photosynthetic reactions are considered to be among the most susceptible cell functions to elevated temperatures (Berry & Björkman, 1980; Allakhverdiev et al., 2008). High temperature inhibits carbon assimilation (Feller et al., 1998; Law & Crafts-Brandner, 1999; Crafts-Brandner & Salvucci, 2000; Rokka et al., 2001), disrupts OEC (Santarius, 1975; Enami et al., 1994), damages D1 and disconnects LHCII from the PSII core (Armond et al., 1980; Sundby et al., 1986) and linearizes thylakoid membrane (Gounaris et al., 1984). Notably, most studies have been conducted using single heat exposures with temperatures exceeding 40°C, whereas the effects of long-term treatments using physiologically relevant temperatures are less understood. Moreover, the photosynthetic acclimatory mechanisms to repeated exposures of elevated temperatures have remained unclear.

To reveal the effects and acclimatory mechanisms of recurring elevated temperature on plant growth and photosynthesis, Arabidopsis plants were exposed daily to 38°C for four hours. Heat-acclimated plants showed significant reduction in biomass compared to control plants grown at constant 23°C, and were characterized by elongated petioles and significantly reduced Chl content (Publication III: Figure 1). Moreover, hyponastic leaf movement causing upward bending of the petioles from the soil during heat period was observed (Publication III: Supplementary video).

Due to the previously reported linearization of the thylakoid membrane upon heat exposure (Gounaris et al., 1983, 1984), thylakoid membrane ultrastructure was analysed with transmission electron microscopy to see if daily elevated temperatures result in thylakoid ultrastructural changes (Publication III: Figure 2). Heat-acclimated plants showed no linearization of the thylakoid membrane (Publication III: Figure 2). Intriguingly, grana height was significantly smaller in heat-acclimated plants compared to control plants (Publication III: Figure 2C), and the number of grana per chloroplast was higher. Moreover, heat-acclimated plants contained larger plastoglobuli compared to control plants (Publication III: Figure 2A, B).

PSII has been considered as the most prone ETC component to high temperatures (Armond et al., 1980; Enami et al., 1994; Komayama et al., 2007; Mathur et al., 2011), whereas PSI has been considered to be more stable (Ivanov et al., 2017). Strikingly, heat-acclimated plants showed tight association of the LHCII antenna to PSII core, demonstrated by increased accumulation of C2S2M2 and C2S2M supercomplexes and reduced levels of detached M-LHCII and PSII monomer (Publication III: Figure 3A). However, no reduction in the overall level of D1 or Lhcb1 was observed (Publication III: Figure 3B). Additionally, no differences in the

PSI accumulation were observed between control and heat-acclimated plants (Publication III: Figure 4A, B). Regardless of the structural integrity of PSII and PSI, heat-acclimated plants showed decreased PSII and PSI photochemistry (Publication III: Figure 3C, E; Figure 4C, D).

Importantly, PSI complex was not limited by the acceptor side in heat-acclimated plants, but rather by the donor side as supported by more oxidized PC and P700 and less reduced Fd (Publication III: Figure 5A-C). In accordance with this, immunoblot and BN-PAGE revealed that Cyt b_6f levels were reduced by as much as 40% in heat-acclimated plants (Publication III: Figure 4A; Figure 5D). Additionally, ATPase was downregulated in heat-acclimated plants (Publication III: Figure 4A; Figure 5D).

Proton translocation during photosynthetic light reactions establishes an electrochemical gradient (*pmf*) across the thylakoid membrane (Avenson et al., 2005). *pmf* has a crucial role in driving ATP synthesis and in initiating photoprotective NPQ. Due to dramatic downregulation of Cyt b_6f and slight decrease of ATPase, which are the two major sites contributing to *pmf,* ECS measurements were conducted to see whether heat-acclimated plants have defects in the formation of proton gradient. Interestingly, no differences were recorded in the *pmf* of heat-acclimated and control plants, indicating that elevated temperature does not impair the ability to maintain electrochemical gradient (Publication III: Figure 5E).

Short-term heat stress induces state transitions (Mohanty et al., 2002; Nellaepalli et al., 2011), NPQ (Tang et al., 2007) and CET (Bukhov et al., 1999.; Zhang & Sharkey, 2009). Our results showed that heat-acclimated plants contained less PSI-LHCII complex (state transition complex) and decreased Lhcb1/2 phosphorylation levels as compared to control plants (Publication III: Figure 4A; 3B). Despite decreased accumulation of the state transition complex, more excitation energy was directed to PSI (Publication III: Figure 4E). Additionally, no significant differences were observed in the NPQ of heat-acclimated plants compared with control plants (Publication III: Figure 3G).

5 Discussion

Land plants are exposed to various short- and long-term fluctuations in their growth environment. Tuning excitation energy flow to PSs, and regulation of electron transfer and proton translocation are essential for photosynthetic light reactions to meet the metabolic demands of downstream carbon metabolism, while simultaneously minimizing oxidative damage to the photosynthetic apparatus. On the other hand, photosynthetic reactions in other than leaf tissues are adapted to meet the metabolic requirements of the plant at multi-organ level (Brazel & Ó'Maoiléidigh, 2019; Simkin et al., 2020).

In recent years, great progress has been made in resolving structures of photosynthetic proteins and their organization within the thylakoid membrane (Kouřil, 2025). Moreover, characterization of novel components involved in short-term regulation of photosynthesis and thylakoid membrane dynamics, e.g. GNAT2 (Koskela et al., 2018) and CURT1 (Armbruster et al., 2013; Pribil et al., 2018), have shed light to new layers of the regulatory mechanisms underlying thylakoid membrane ultrastructure and organization of photosynthetic complexes. In addition to short-term regulation, plants exhibit long-term acclimatory mechanisms involving stoichiometric adjustment of the photosynthetic complexes (Schöttler & Tóth, 2014). These adjustments occur at transcriptional and translational level (Pfannschmidt, 2003). Understanding photosynthetic acclimatory mechanisms upon diverse environmental conditions is especially crucial as climate change causes more extreme weather events such as more frequent and severe heat waves (IPCC, 2021), posing a threat to global agriculture.

In addition to leaf tissue, other green non-foliar organs are capable of photosynthesis. Development of non-foliar photosynthesis and its contribution to overall yield (Simkin et al., 2020) in diverse conditions (Ávila et al., 2014; Hu et al., 2014; Hu et al., 2019) further emphasizes the dynamic nature of photosynthetic machinery to function at various levels according to environmental cues. Non-foliar photosynthesis has gained some attention over the few decades, but the organization and composition of the photosynthetic protein complexes within the thylakoid membranes of these organs has yet not been resolved.

5.1 What are the key components regulating thylakoid membrane organization and dynamics?

Thylakoid membrane organization into appressed grana stacks and non-appressed stroma lamellae is highly cooperative process that is considered to be affected by electrostatic interaction of charged stromal loops of LHCII (Standfuss et al., 2005; Wan et al., 2014), hydrogen bonds of vertically aligned PSII-LHCII supercomplexes (Albanese et al., 2017, 2020) and van der Waals interactions between uncharged molecules (Puthiyaveetil et al., 2017). Additionally, cations between the negative stromal areas of LHCII enhance electrochemical forces between the complexes and stabilize stacking (Barber, 1980; Wan et al., 2014). Calculations by Puthiyaveetil et al. (2017) of the balance between van der Waals interactions arising from hydrocarbon chains of the membrane lipids and electrostatic repulsive forces arising from dielectric properties of proteins suggested that van der Waals forces would be sufficient for grana stacking, but this claim was challenged by Moazzami Gudarzi et al. (2021). Van der Waals attractive forces as well as electrostatic repulsive forces are not constant in the thylakoid membrane and are affected by post-translational modifications (PTMs), pigments and cations within the membrane making it difficult to address the proportion of these forces in membrane stacking.

LHCII phosphorylation by STN7 mediates excitation energy distribution between PSII and PSI (Bellafiore et al., 2005; Galka et al., 2012; Wientjes et al., 2013), but at the same time, phosphorylation adjusts the entire thylakoid ultrastructure according to environmental cues within a time range of 10 minutes (Rozak et al., 2002; Wood et al., 2019). Moreover, phosphorylation has been considered as the key player in short-term photosynthetic acclimation (Pesaresi et al., 2009). Shift from darkness to light induces phosphorylation of Lhcb1 and Lhcb2 resulting in loosening of grana stacking that increases the interaction area between the appressed and non-appressed membranes thereby likely facilitating translocation of phosphorylated L-LHCII from PSII to PSI (Kyle et al., 1983; Chuartzman et al., 2008; Pietrzykowska et al., 2014; Wood et al., 2019). The lack of state transitions (Koskela et al., 2018) and the lack of grana dynamics in *gnat2* acetyltransferase mutant, which I demonstrated in Publication I (Figure 1), challenged the view of LHCII phosphorylation being the sole determinant of state transitions and dynamic grana stacking. The light-induced loosening of the grana has previously been proposed to result from disruption of LHCII interactions by phosphorylation that introduces additional negative charge to the membrane (Barber et al., 1982). Indeed, phosphorylation occurs in the majority of Lhcb N-terminal loops (Pesaresi et al., 2011) emphasizing the importance of PTMs on thylakoid membrane regulation. It should be noted, however, that in addition to phosphorylation, acetylation affects the protein charge by neutralizing positive charges either in the N-termini of the protein

or through acetylation of internal Lys residues (Bienvenut et al., 2020). Moreover, acetylation is widespread modification in stromal N-terminal loops of LHCII proteins and is proposed to strengthen stacking in various light conditions (Albanese et al., 2020). N-terminal acetylation is considered as an irreversible modification, while Lys acetylation is reversible. Removal of the acetyl group is catalysed by deacetylases. Only one deacetylase that functions in Arabidopsis chloroplast has been characterized so far (Hartl et al., 2017). In light of the literature and the results obtained in Publication I, it seems that dynamic grana stacking is regulated by interplay between acetylation and phosphorylation.

Intriguingly, Lhcb1 and Lhcb2 phosphorylation levels are increased in the *gnat2* (Koskela et al., 2018) (Publication I: Figure 1C), which also indicates interplay of these two PTMs. This type of cross-talk is common among PTMs (Yang & Seto, 2008; Xia et al., 2022), and altering protein acetylation levels might affect various other PTMs that occur either in same or adjacent PTM site, which might be the case in *gnat2.* Moreover, it is typical for enzymes that catalyse PTMs to be regulated via PTMs themselves, that further emphasizes the complexity of PTM cross-talk (Venne et al., 2014). This aspect however requires further studies. Hyperphosphorylation however, does not seem to be the reason for more tight packing of grana in *gnat2* since hyperphosphorylated LHCII has also been reported in the *psae1-1* mutant impaired in state transitions (Pesaresi et al., 2002), but in contrast to *gnat2, psae1-1* showed destacking of thylakoid membrane in darkness (Pesaresi et al., 2002).

Since the thylakoid membranes are tightly packed in *gnat2* (Koskela et al., 2018), I tested whether the tight packing restricts the LHCII migration thereby preventing the formation of PSI-LHCII complex. However, thylakoid linearization by depleting Mg^{2+} did not restore the ability to form PSI-LHCII in *gnat2* (Publication I: Figure 4) suggesting that ability to dock LHCII to PSI does not arise from differences in grana structure. Since p-Lhcb2 in the L-LHCII trimer interacts with PsaH/L/O/I of PSI (Lunde et al., 2000; Zhang & Scheller, 2004; Plöchinger et al., 2016; Pan et al., 2018), and *gnat2* showed decreased Lhcb2 and PsaH acetylation (Koskela et al., 2018), it is possible that either Lhcb2 or PsaH acetylation is directly required for LHCII docking. Wang et al. (2025) tested this hypothesis with the tomato *gnat2* mutant by mimicking acetylation of Lhcb2. However, $Lhcb2^{K6Q}$ only partially restored state transitions suggesting that acetylation of Lys6 in Lhcb2 is not the sole prerequisite for LHCII docking, but rather (de)acetylation of several proteins involved in PSI-LHCII interactions might affect formation of the state transition complex. It is also worth keeping in mind that the chloroplast acetylation machinery comprises of eight GNAT enzymes (Bienvenut et al., 2020) that might have redundant functions, i.e. knocking out one can be compensated by another one. Further studies with *gnat* double or triple knockout mutants are required to test this hypothesis.

Other interesting observation in the *gnat2* mutant is the presence of hyperphosphorylated Lhcb1 and Lhcb2 in all three LHCII pools (S-LHCII, M-LHCII, L-LHCII) (Publication I: Figure 3). In Wt Arabidopsis, p-Lhcb2 has been mainly shown to be localized in the L-LHCII pool and is nearly absent in PSII-LHCII supercomplexes (Galka et al., 2012; Crepin & Caffarri, 2015; Rantala et al., 2017), while in *gnat2* (p-)Lhcb2 accumulates also in the M-LHCII (Publication I: Figure 3B, C). These findings, supported by the CD spectra (Publication I: Figure 2) suggest that GNAT2 affects the composition of LHCII antenna. Interestingly, Lys-acetylated proteins are distributed unevenly among LHCII pools with L-LHCII containing more acetylated Lhcb proteins than the other LHCII pools (Wu et al., 2011). Therefore, reduction of acetylation levels might hinder proper distribution of Lhcb among the LHCII pools. This could (at least partly) explain the observed physiological differences between Wt and *gnat2* (Publication I: Figure 3).

5.2 Adaptation of photosynthetic machinery in non-foliar organs to ambient environment and metabolic requirements of the plant

Non-foliar photosynthesis occurs in almost all green vegetative and reproductive tissues and affects the overall sink-source balance of the plant (Aschan & Pfanz, 2003). In studies where stems were kept in darkness, the net photosynthesis decreased (Hu et al., 2012; Sun et al., 2021). Additionally, flowers and fruits have been shown to develop in the absence of leaves, highlighting the importance of stem photosynthesis for reproductive success of plant (Ávila et al., 2014). Stem photosynthesis is more pronounced under abiotic stress conditions (Yiotis et al., 2008; Ávila-Lovera et al., 2024), and reproductive organ photosynthesis has been reported to be enhanced under water deficit (Tambussi et al., 2005; Jia et al., 2015; Zhang et al., 2016). This emphasizes the adaptive traits of plants to fine-tune its photosynthetic machinery at the entire organism level according to environmental cues. It is important to note that there are variations in the overall contribution of non-foliar photosynthesis depending not only on abiotic conditions but on species.

Previously, no studies existed on the organization and composition of the photosynthetic machinery in non-foliar tissues. In Publication II, I show that the overall composition and organization of the thylakoid protein complexes is similar in leaves, stems and siliques, proving the capability of these organs to conduct photosynthesis (Publication II: Figure 4). The most pronounced difference in the protein complex composition between the studied organs was the high accumulation of PSI-NDH complex in stems (Publication II: Figure 4; Figure 5; Datasets S17-S21). PSI-NDH complex participates in CET by cycling electrons around PSI (Johnson, 2011; Peltier et al., 2016). Electron cycling is coupled to proton

translocation into lumen resulting in generation of ATP without the concomitant production of NADPH. Increased ATP synthesis might be a source of energy for carbohydrate transportation throughout the plant, or then it is utilized for the local cellular needs. Blocking stem photosynthesis by depleting it from light reduces root and bud biomass (Saveyn et al., 2010; Kocurek et al., 2020), highlighting the importance of carbohydrate allocation to other organs.

Another interesting possibility arises from findings by Hibberd and Quick (2002). They reported that stems of C3 plants contain cells that resemble the BS cells of C4 plants (Hibberd & Quick, 2002). BS cells of C4 plants are reported to carry out CET (Takabayashi et al., 2005; Munekage et al., 2010; Nakamura et al., 2013; Ermakova et al., 2024) and to contain high amount of PSI-NDH complex (Darie et al., 2006; Majeran et al., 2008). In addition, cells within the stems of tobacco, typical C3 plant, contain the key decarboxylases required for C4 photosynthesis (Hibberd & Quick, 2002), and similar results have also been reported in wheat grain (Rangan et al., 2016), Arabidopsis petiole veins (Brown et al., 2010) and cucumber stems and petioles (Sun et al., 2021). However, these enzymes can also be involved in other metabolic processes taking place in xylem and phloem that would explain their high abundance in the stem. Transcriptomic analysis conducted for Arabidopsis stems showed significant upregulation of NADP-ME1 (Table 2; Publication II: Dataset S10), which catalyses decarboxylation of malate into pyruvate. Pyruvate, in turn, is phosphorylated to PEP in C4 photosynthesis (Figure 7). NADP-ME-type C4 photosynthetic plants have increased ATP/NADPH ratio within the BS cells since reducing power is shuttled as malate, which requires additional ATP. This observation might suggest presence of C4-like photosynthetic reactions within the stems of Arabidopsis, but further studies are required to elucidate this. Possibly, CO_2 released upon decarboxylation could be used in stem photosynthesis, and released PEP could be directed to shikimate pathway for lignin biosynthesis (Vanholme et al., 2010; Tohge et al., 2013). This is supported by experiments using radiolabelled malate, which showed that the released CO_2 was incorporated in soluble sugars (Brown et al., 2010). In addition, metabolic profiling of Arabidopsis mid-veins from Wt and *nadp-me* mutants showed differences in the abundance of tryptophan and tyrosine derived from shikimate pathway further supporting the allocation of PEP to lignin biosynthesis (Brown et al., 2010). Adaptation of plant stems to conduct increased CET according to the metabolic requirements further highlights the capability to regulate photosynthesis at multi-organ level.

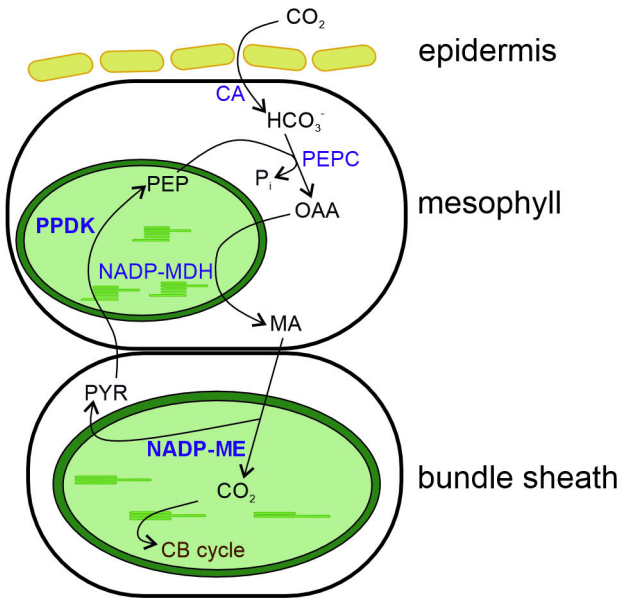

Figure 7. Schematic of C4 photosynthetic pathway (NADP-ME subtype). Enzymes (dark blue) upregulated in Arabidopsis stems are indicated in bold. Abbreviations: CA, carbonic anhydrase; PEPC, phosphoenolpyruvate carboxylase; PPDK, pyruvate/orthophosphate dikinase; NADP-MDH, NADP-dependent malate dehydrogenase; NADP-ME, NADP-dependent malic enzyme; MA, malate; PYR, pyruvate; OAA, oxaloacetate; PEP, phosphoenolpyruvate; CB cycle, Calvin-Benson cycle.

Green siliques of Arabidopsis showed decreased levels of all photosynthetic proteins compared to leaves and stems (Publication II: Figure 5), which is in line with the reported diminished photosynthetic rates of reproductive organs (Brazel & Ó'Maoiléidigh, 2019). Arabidopsis silique photosynthesis has been shown to contribute to seed yield (Zhu et al., 2018), but the contribution might be age-dependent and decrease after seeds have developed fully, as reported for alfalfa (Zhang et al., 2017). Siliques used in our experiments were fully matured, so the photosynthetic requirements of the silique photosynthesis might not be required at this developmental stage. This could in part explain reduced amount of the photosynthetic protein complexes. However, functional measurements of photosynthetic rate would be needed to assess the actual photosynthetic net rate.

The variation in the net photosynthesis of non-foliar organs upon changes in environment and fine-tuning the ATP production to cellular metabolic requirements pinpoint the adaptive nature of photosynthetic protein complexes. Understanding the mechanisms of non-foliar photosynthesis and its impact on overall yield under diverse conditions could in the future serve as one possible approach to optimize crop yields upon challenging environmental conditions.

5.3 Cyt b_6f is the key component in photosynthetic acclimation to long-term elevated temperatures

The ongoing climate change increases abiotic stress, such as heat and drought, experienced by plants. Prolonged and more severe heat waves threaten agriculture by causing severe decline in crop yields (Zhao et al., 2017). Heat affects all aspects of plant growth with photosynthesis being especially prone to increases in temperature (Berry & Björkman, 1980).

Acclimation to elevated temperatures involve adjustments at cellular and morphological level. In Publication III, I show that plants exposed daily to high temperature throughout their life cycle exhibit elongated petioles and hyponastic leaf movement during the heat period (Publication III: Figure 1A; Supplementary video 1). These morphological changes and leaf movements likely enhance plant's cooling capacity. Similar phenotype was reported in Arabidopsis that was first grown for three weeks in 20°C and then transferred to 28°C for two weeks (Crawford et al., 2012). Upward leaf movement decreases temperature as reported by Park et al. (2019), showing that the leaf cooling is correlated with the elevation angle of leaves.

In addition to morphological changes, daily exposure to high temperature leads to stoichiometric adjustments of the photosynthetic protein complexes. Surprisingly, heat-acclimated plants contained more C2S2M2 supercomplexes compared to control conditions and no differences were observed in D1 and Lhcb1 levels implying that PSII-LHCII remain structurally intact upon daily heat exposures (Publication III: Figure 3). This is in striking contrast to earlier reports showing physical disconnection of LHCII (Armond et al., 1980; Sundby et al., 1986) and degradation of D1 (Komayama et al., 2007). Notably, earlier studies focused on single exposure to high temperature. Despite structurally intact PSII-LHCII, PSII quantum yield and the number of open RCs were significantly reduced in heat-acclimated plants (Publication III: Figure 3). Thus, it seems that heat-acclimated plants accumulate non-functional PSII-LHCII complexes, indicating possible defects in PSII repair cycle. In PSII repair cycle, damaged PSII monomerizes and migrates to non-appressed membranes and the core proteins (D1, D2 and CP43) are desphosphorylated before degradation of D1 by Deg2 and FtsH proteases (Koivuniemi et al., 1995; Rintamäki et al., 1996; Järvi et al., 2015). New D1 protein is synthetized while the other PSII subunits are reused (Järvi et al., 2015). Increased temperature was reported to inhibit *de novo* synthesis of D1 in *Synechococcus* (Allakhverdiev et al., 2007), while in plants, heat-induced protein aggregation was suggested to impair D1 degradation (Komayama et al., 2007). Since heat-acclimated plants accumulated non-functional PSII-LHCII complexes and only minute amount of PSII repair cycle intermediates, PSII monomers (Aro, 2005) (Publication III: Figure 3A), it is plausible that PSII repair cycle is indeed impaired.

PSI has been reported to be relatively thermostable (Ivanov et al., 2017), and it appears to remain structurally intact in heat-acclimated plants (Publication III: Figure 4A, B). Although previous reports showed increased state transitions under heat exposure (Mohanty et al., 2002; Nellaepalli et al., 2011), heat-acclimated plants contained decreased accumulation of PSI-LHCII and decreased p-LHCII levels (Publication III: Figure 4A; Figure 3B). State transitions are considered as a short-term mechanism to balance the excitation energy distribution between the two PSs (Pesaresi et al., 2009), whereas acclimation to high temperature possibly relies more on long-term stoichiometric adjustments of photosynthetic components by down- and upregulating protein levels according to changes in chloroplast redox state (Pfannschmidt, 2003). Intriguingly, despite lower levels of PSI-LHCII complex, more excitation energy was directed to PSI (Publication III: Figure 4E). This is not explained by linearization of the thylakoid membrane that would increase spillover from PSII to PSI (Barber, 1980; Ivanov & Velitchkova, 1990), since the thylakoid membranes in heat-acclimated plants were appressed, although the grana were smaller as compared to control (Publication III: Figure 2).

Despite the structural integrity, PSI exhibits reduced photochemistry (Publication III: Figure 4C, D). Interestingly, I show that electron transfer is limited at the donor side of PSI (Publication III: Figure 5B), through dramatic downregulation of Cyt b_6f (Publication III: Figure 4A; Figure 5D). Downregulation of Cyt b_6f possibly serves as mechanism to protect PSI from excessive reduction and ROS generation with the expense of photosynthetic efficiency. In addition to decreased levels of Cyt b_6f, daily heat exposures decreased ATPase content (Publication III: Figure 4A; Figure 5D). These are the key sites controlling photosynthetic flux (Yamori et al., 2011) and they are usually co-regulated (Schöttler et al., 2015). For instance, decrease of Cyt b_6f and ATPase content was previously reported in wild watermelon under prolonged drought stress (Kohzuma et al., 2009).

Although ATPase downregulation has been shown to result in decreased proton efflux rate to the stroma leading to increased qE component of NPQ (Rott et al., 2011; Yamori et al., 2011), long-term heat exposures did not result in significant changes in NPQ (Publication III: Figure 3G). The effect of downregulated Cyt b_6f to diminished NPQ has previously observed in tobacco (Anderson et al., 1997; Schöttler et al., 2007; Yamori et al., 2011). In these studies, reduced levels of Cyt b_6f resulted in over-reduction of the PQ pool, lower ΔpH and decreased carbon assimilation rates. In contrast to previous reports, no differences in the *pmf* were observed between heat-acclimated and control plants (Publication III: Figure 5E), but the increased proton influx to thylakoid lumen in heat-acclimated plants (Publication III: Figure 5G) might indicate increased CET, which serves as a mechanism to protect PSI (Suorsa et al., 2012).

Although heat stress-associated decline in photosynthesis has been attributed mainly to result from decreased carbon assimilation (Feller et al., 1998; Law & Crafts-Brandner, 1999; Crafts-Brandner & Salvucci, 2000; Sharkey et al., 2001), there might be differences in the primary limiting factor depending on the duration and intensity of the heat exposure. Wise et al. (2004) showed that Rubisco activity was not limiting photosynthesis in field-grown cotton repeatedly exposed to heat, whereas growth chamber -grown cotton upon first heat exposure showed considerable deactivation of Rubisco, indicating differences in stress responses and acclimation. Based on biochemical and biophysical results obtained, it seems plausible that the heat-induced limitation of photosynthesis arises not only from downstream CB cycle reactions, but also from the electron transfer reactions, namely at the donor side of PSI, at least under prolonged elevated temperatures. Moreover, Cyt b_6f seems to be the key component in acclimation to chronic heat stress. Further experiments considering carbon assimilation in heat-acclimated plants are required to resolve the effects of prolonged heat exposures on Rubisco kinetics. Additionally, discriminating short-term adjustments to photosynthetic machinery and the long-term acclimatory mechanisms caused by daily heat stress is an interesting topic for further studies.

6 Conclusions

The aim of this thesis was to uncover photosynthetic acclimatory mechanisms in different environmental conditions and adaptive strategies of photosynthetic apparatus in different plant tissues in the model organism *Arabidopsis thaliana.* The focus was on three main points: i) short-term regulation of the thylakoid membrane upon light-induction, ii) characterization of the photosynthetic complexes in non-foliar organs, and iii) elucidating the acclimatory responses of photosynthetic machinery to daily heat exposures. First, my work has shown that GNAT2, a recently identified chloroplast acetyltransferase, is involved in short-term regulation of the thylakoid membrane dynamics in response to light-induction (Publication I). Indeed, absence of the GNAT2 showed loss of the light-dependent dynamic regulation of the membrane despite the presence of LHCII phosphorylation. This finding uncovers new layers into the photosynthetic regulatory mechanisms. Second, I have elucidated the adaptive nature of photosynthetic machinery at multi-organ level, since Arabidopsis stems showed high amount of PSI-NDH complex that is involved in increased production of ATP to meet the metabolic demand within the organ (Publication II). Third, I have shown that acclimation to physiologically relevant heat stress involves downregulation of Cyt b_6f complex, and that the responses to daily heat exposures differ from acute short-term heat exposures.

The exact mechanism of how acetylation by GNAT2 is required for short-term regulation of photosynthesis remains elusive and requires further studies. In addition, non-foliar photosynthesis has been previously shown to have varying contribution to the overall yield based on environmental conditions. More detailed understanding of photosynthesis and its regulation in other than leaf-tissues therefore could serve as an appealing target for improving crop yields upon abiotic stress conditions. Moreover, as the climate change increases global temperature, photosynthetic acclimatory strategies at the level of light reactions would be an essential topic to study in the future.

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