Light and temperature induced state transitions of photosystems and its signal mechanism in *Arabidopsis thaliana*: Biochemical and biophysical characterization

A thesis submitted to the University of Hyderabad for the award of Doctor of Philosophy in Biochemistry

By

N Sreedhar
Regd. No: 06LBPH09

Department of Biochemistry
School of Life Sciences
University of Hyderabad
Hyderabad-500 046
India.

NOVEMBER, 2011

University of Hyderabad

(A Central University established in 1974 by act of parliament)

Hyderabad-500 046, INDIA

DECLARATION

I, N Sreedhar, hereby declare that this thesis entitled **"Light and temperature induced state transitions of photosystems and its signal mechanism in *Arabidopsis thaliana*: Biochemical and biophysical characterization"** submitted by me under the guidance and supervision of **Dr S Rajagopal** is an original and independent research work. I also declare that it has not been submitted previously in part or in full to this University or any other University or Institution for the award of any degree or diploma.

Date

N Sreedhar
(Regd. No: O6LBPH09)

Dr. S Rajagopal
(Research supervisor)

University of Hyderabad
(A Central University established in 1974 by act of parliament)

Hyderabad-500 046

CERTIFICATE

This is to certify that this thesis entitled **"Light and temperature induced state transitions of photosystems and its signal mechanism in *Arabidopsis thaliana*: Biochemical and biophysical characterization"** is a record of bonafide work done by **N Sreedhar**, a research scholar for Ph.D. programme in Biochemistry , Department of Biochemistry, School of Life Sciences, University of Hyderabad under my guidance and supervision. The thesis has not been submitted previously in part or in full to this or any other University or Institution for the award of any degree or diploma.

Dr. S Rajagopal
(Research Supervisor)

Head,

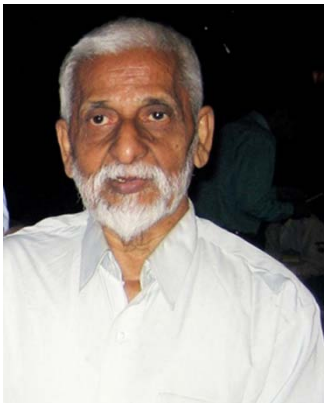
Department of Biochemistry.

Dean,

School of Life Sciences.

Dedicated to my parents and beloved Professor

Prasanna Mohanty



ACKNOWLEDGEMENTS

I owe a deep sense of gratitude towards my research supervisor **Dr. S Rajagopal** for readily accepting me as his research student and providing me the opportunity to work under his invaluable guidance. Above all and the most needed, he provided me unflinching encouragement and support in various ways. I gratefully acknowledge his support and guidance during every single obstacle that I faced during my research.

I am much indebted to **Prof. Prasanna Mohanty** for his valuable advice during scientific discussion, supervision and constructive comments on every aspects of this research. Words are not enough to show my gratitude to him for all the love and affection, which he bestowed on me. I can never thank him enough.

I would also like to record my gratitude to to **Prof. Győző Garab** and his research group Drs. Ottó Zsiros, László Kovács, Zsuzsanna Varkonyi, Szilvia Zita Toth, Milán Szabo, Gábor Steinbach, Ms Tünde Toth, for the wonderful scientific discussions and the pleasure of working together during my visit to Biological Research Center, Szeged, Hungary in 2009 and 2010. Special thanks to Prof. Zoltán Gombos for his wonderful discussions.

I sincerely thank the present and former Deans, Prof. M Ramanadam and Prof. AS Raghavendra, School of Life Sciences, for allowing me to use school facilities. I thank present and former Heads, Prof. KVA Ramaiah, and Prof. M Ramanadam, Dept. of Biochemistry for allowing me to use department facilities.

I am gratefully acknowledge my Doctoral committee members Prof. M Ramanadam and Prof. AR Reddy for their valuable suggestions and encouragements.

I express my gratitude to Prof. AS Raghavaendra, Prof. AR Reddy, Dr. M Bramhanandam, Dr. S Naresh and their research scholars for extending their lab facilities. I specially thank Prof MNV Prasad, Dept. of Plant Sciences for his support and encouragement.

I sincerely acknowledge the kind gift of wt and *stn7* from Prof. Imran Siddique (CCMB) and Dr. Dario Leister (LUM). Sincere acknowledgements also go to Prof. Reto J Strasser for providing Handy PEA instrument.

I would remember the marvellous company of my lab mates Venkat, Mahesh, Satya Bala and Mouli for their cooperation and help. My special thanks to Nageswar Rao and Tirupathi for helping me in experiments. Kind help from Dr. Sireesha on fluorescence studies also acknowledged. Special thanks to Mr. Abhay, Department of Plant Sciences for providing research plants during crucial time of my Ph.D. My sincere thanks also go to Mr. Ganesh, lab assistant for his assistance in daily lab chores.

My sincere gratitude to Dr. Sreedevi for her care and support. Lovely thanks to Abilash.

I thank faculty members, School of Life Sciences for their support and encouragement. Sincere thanks to non teaching staff for their kind help and support. Special thanks to staff of CIL, HCU for allowing me to use CD spectroscopy and Proteomic Facility, SLS for MALDI analysis.

I shall remember the marvellous company of my friends Dr. Girish, Dr. Otto, Dr. Anumod, Dr. Sunil, Dr. Sireesha, Mallikarjuna, Saikrishna, Tunde, Chandu, Aravinda, Haribabu and all the friends at HCU and RIE, Mysore for being with me all the times.

It was my pleasure to be a student of great teachers Mr. Basavanna, Mr. Baaksad, Mr. Kabir, Mrs. Lokeswari, Prof. Kulkarni (RIE, Mysore), Prof. Siddique (RIE, Mysore) and all who taught and inculcated scientific temper in me.

Funding from DST and DBT to Dr. S Rajagopal as well as DST, DBT, CSIR, DST-FIST, UOH-CREBB, UGC-SAP (for funding to Department and School) are greatly acknowledged.

The financial support from CSIR-UGC, New Delhi for **JRF** and **SRF** is gratefully acknowledged. I specially thank DST PURSE grant for allowing me to visit Beijing International photosynthesis conference held in Aug 2010.

Last but not the least it was the support, love and care of my **Parents**, **Brother**, all relatives and well wishers, throughout these years got me here without which it would have remained as an unfulfilled dream.

SREEDHAR

CONTENTS

List of Figures

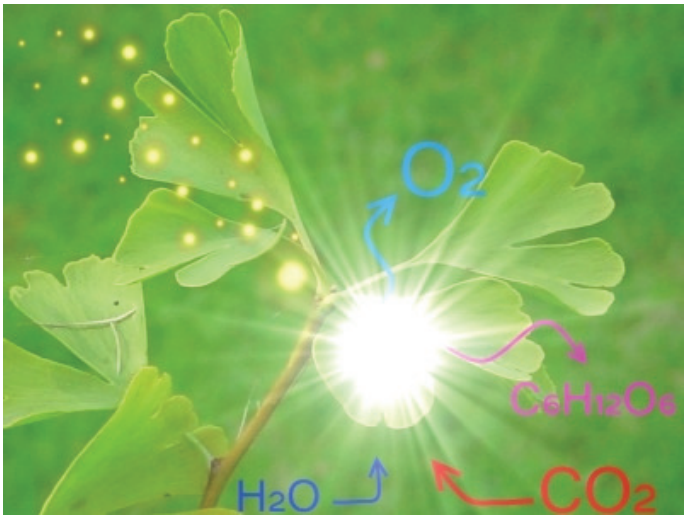
List of Tables

ABBREVIATIONS

77 K	77 degrees Kelvin = –196 degrees Celsius
BN-PAGE	blue-native polyacrylamide gel electrophoresis
CET	cyclic electron transfer around PSI
Chl	chlorophyll
CP29, CP26, CP24	minor Chl *a/b* binding proteins of LHCII
CP43, CP47	chl *a* binding proteins of PSII RC
Cyt b_6/f	cytochrome b_6/f complex
D1 and D2	reaction centre proteins of PSII
DBMIB	2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone
DCMU	3-(3,4-dichlorophenyl)-1,1-dimethylurea
DDM	n-Dodecyl β-D-Maltoside
ETC	electron transfer chain between PSII and PSI
Fd	ferredoxin
F_m	maximum fluorescence in the dark-adapted.
FNR	ferredoxin-$NADP^+$ reductase
F_o	minimum fluorescence in the dark-adapted state
F_o'	minimum fluorescence in the dark-adapted state
$F_{s'}$	steady-state fluorescence under light
F_V/F_m	ratio of variable to maximal fluorescence
HL	high light
kDa	kilo daltons
LHCII	light harvesting complex II
LL	low light
NDH	NAD(P)H dehydrogenase
NPQ	non-photochemical quenching
OEC	oxygen evolving complex

P680, P680*	reaction centre Chl of PSII and its excited singlet state
PSI	photosystem I complex
PSII	photosystem II complex
PTOX	plastid terminal oxidase
Q_A and Q_B	primary and secondary plastoquinone electron acceptor of PSII
RC	Reaction centre
SDGUC	sucrose density gradient ultracentrifugation
SDS-PAGE	sodium dodecyl sulphate-polyacrylamide gel electrophoresis
stn7	mutant lack of STN7 kinase

Introduction



Chapter 1

1. INTRODUCTION

The primary source of energy for life in our planet is obtaining from sun. The energy in sunlight is introduced into the biosphere by a process known as photosynthesis, the physico-chemical process by which plants, algae and certain bacteria convert light energy into chemical energy of organic compounds, with concomitant use of these components in the bioenergetic processes. Photosynthesis is directly related to the life and survival of all the living creatures on the earth. It is is one of the most essential biological processes on earth. By liberating oxygen and consuming carbon dioxide, it has transformed the world into the hospitable environment. Photosynthesis developed very early in the history of life. In plants, algae and cyanobacteria, the photosynthetic processes results in the fixation of carbon dioxide (CO_2) from the atmosphere that is used to synthesize carbohydrates and release of molecular oxygen to the atmosphere. Directly or indirectly, photosynthesis fills all of our food requirements and many of our needs for fiber and building materials. This being the case, scientific research into photosynthesis is vitally important. If we can understand and control the intricacies of the photosynthetic process, we can learn how to increase crop yields of food, fiber, wood, and fuel, and how to better use our lands. The energy-harvesting secrets of plants can be adapted to man-made systems which provide new, efficient ways to collect and use solar energy.

Study on photosynthesis can avoid adversely affecting processes, precipitating environmental or ecological disasters and also help to enhance production of food, fiber and energy. Understanding the natural process, which has been developed by plants over several billion years, will also allow us to use the basic chemistry and physics of photosynthesis for other purposes, such as solar energy conversion, the design of electronic circuits, and the development of medicines and drugs. Research on the photosynthesis has been performed since the

seventeenth century leading to a considerable basis of knowledge. The earliest studies of photosynthesis involve the nature of the components used and produced by plants. The application of advanced physical instrumentation combined with biochemical and molecular biology techniques, has provided a wealth of information concerning the primary reactions of photosynthesis but not much has been studied on the photosynthetic reaction centers, key steps in photosynthesis like absorption of solar energy by antenna pigments and efficient transfer of excitation energy to photochemical reaction centers .

1.1 Typical structure of chloroplast and its membrane system

All plants and green algae contain energy producing cell organelles called chloroplast. This photosynthetic unit is the organelle in which the conversion of light energy into chemical energy takes place. Chloroplast contains internal membranes, called thylakoids, which include two laterally segregated compartments, highly stacked grana membranes and unstacked stroma membranes (Fig. 1.1). The soluble part of the chloroplast is also distributed in two different compartments separated by the thylakoid membrane, that is, the thylakoid membrane holds the luminal compartment and is itself surrounded by the stroma. Thylakoid membranes contain integral membrane proteins which play an important role in light harvesting and the light-dependent reactions of photosynthesis. There are four major protein complexes in the thylakoid membrane: photosystems (PS) I and II, cytochrome b_6f complex (Cyt b_6/f), ATP synthase (Fig. 1.2). The grana stacks are enriched with PSII-LHCII complexes and grana margins and stroma membranes are enriched with PSI-light harvesting complex I (LHCI) complexes. ATP synthase is located in stroma membranes and Cyt b_6/f complex is more or less equally distributed between the grana and stroma membranes. Carbon fixation reactions take place in the soluble stroma part.

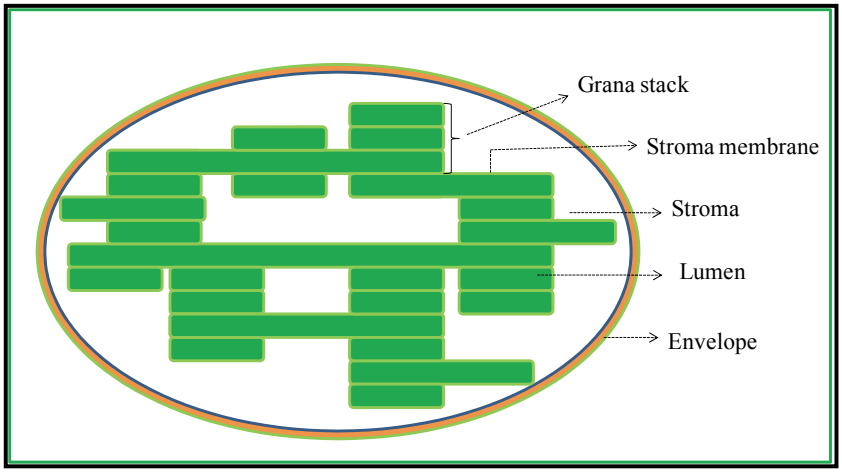


Fig. 1.1 Sketch of the chloroplast structure showing thylakoids composed of highly stacked grana membranes and unstacked stroma membranes. Light dependent reactions takes place in thylakoid membranes and carbon fixation reactions take place in the soluble stroma.

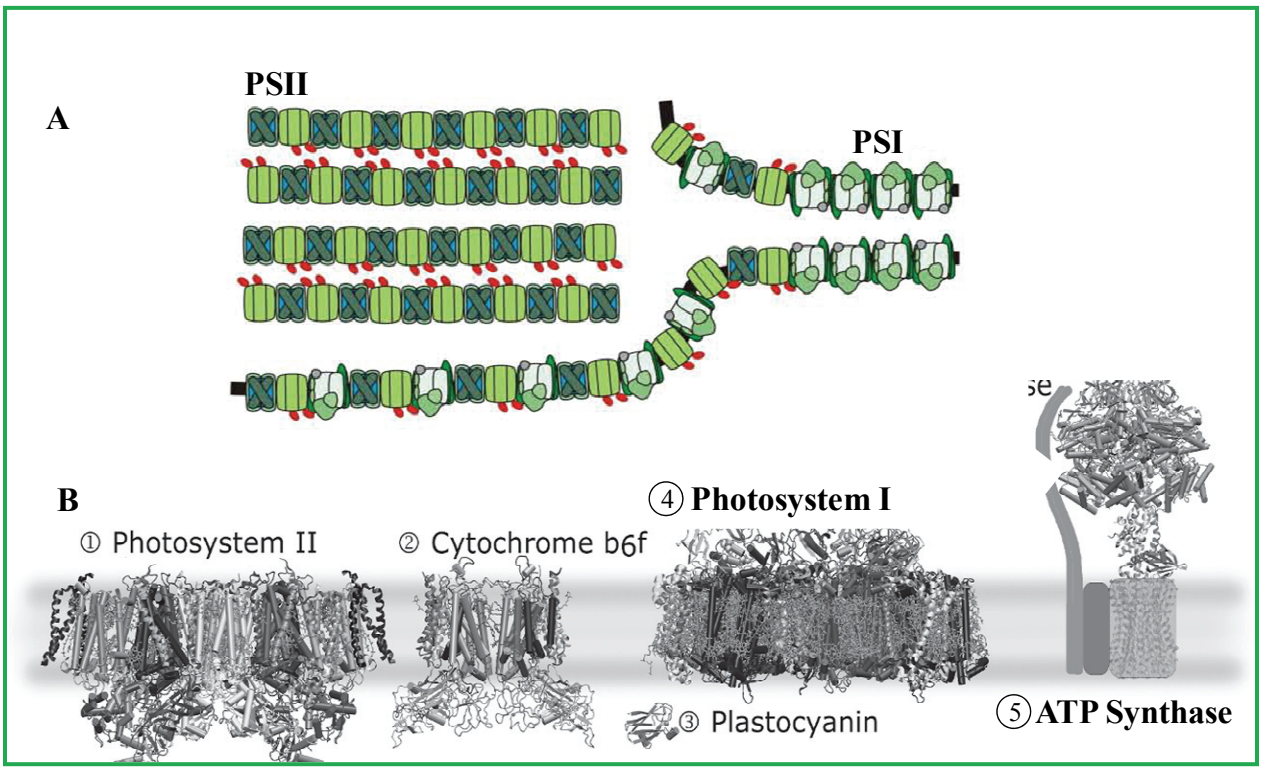


Fig. 1.2 Sketch of the thylakoid membrane composed of highly stacked grana membranes enriched with PSII-LHCII complexes and unstacked stroma membranes enriched with PSI-LHCI complexes (A). ATP synthase is located in stroma membranes and Cyt b_6/f complex is more or less equally distributed between the grana and stroma membranes. Carbon fixation reactions take place in the soluble stroma. Structures of PSII , Cyt b_6/f, PC, PSI and ATP Synthase (B). (Adapted from Fromme and Allen, 2008)

The thylakoid membranes contain PSI and PSII, which include the reaction centers for converting light energy into chemical energy.

1.2 Light harvesting and electron transport in thylakoid membranes

Fig. 1.3 illustrates the components of electron transport chain of different organisms containing either Phycobilisomes (PBS) or LHC. In cyanobacteria (Ex. *Anabaena*) and red algae (Ex. *Galdieria*), PBS acts as antennae and harvests light energy, however, in plants and green algae (*Chlamydomonas*), LHC of PSI and PSII harvests light energy (Joshua and Mullineaux, 2004; Fork and Satoh, 1986; Mullineaux and Emlyn-Jones, 2005). The light energy absorbed by the light harvesting antenna system directed to the reaction centers in order to run the actual photosynthetic reactions. Harvested light energy excites the PSII reaction center chlorophyll molecule P680, from which the electron is transferred to a PQ molecule. After receiving two electrons, the plastoquinol is released from the PSII complex and in the next step it becomes oxidized by the Cyt b_6f complex. Electrons are further transferred from Cyt b_6f to the PSI complex, which is again excited by light, and the electrons released from P700 are finally transferred to ferredoxin (Fd). Ferredoxin-$NADP^+$ oxidoreductase (FNR) oxidizes ferredoxin and concomitantly generates NADPH from $NADP^+$ (Carrillo et al., 2003).

Oxidized $P680^+$ in PSII is an extremely strong oxidant and it withdraws electrons from the manganese cluster of the oxygen evolving complex of PSII. The manganese cluster, in turn, finally withdraws the electrons from water and releases oxygen as a by-product. Water-splitting reactions and oxidation of plastoquinol create a proton gradient across the thylakoid membrane, which is released via the ATP synthase to produce ATP. Instead of directing electrons to carbon assimilation or to other reducing reactions in the stroma, PSI can cycle part of the electrons back to plastoquinol and by this mechanism it can enhance the production of ATP at the expense of

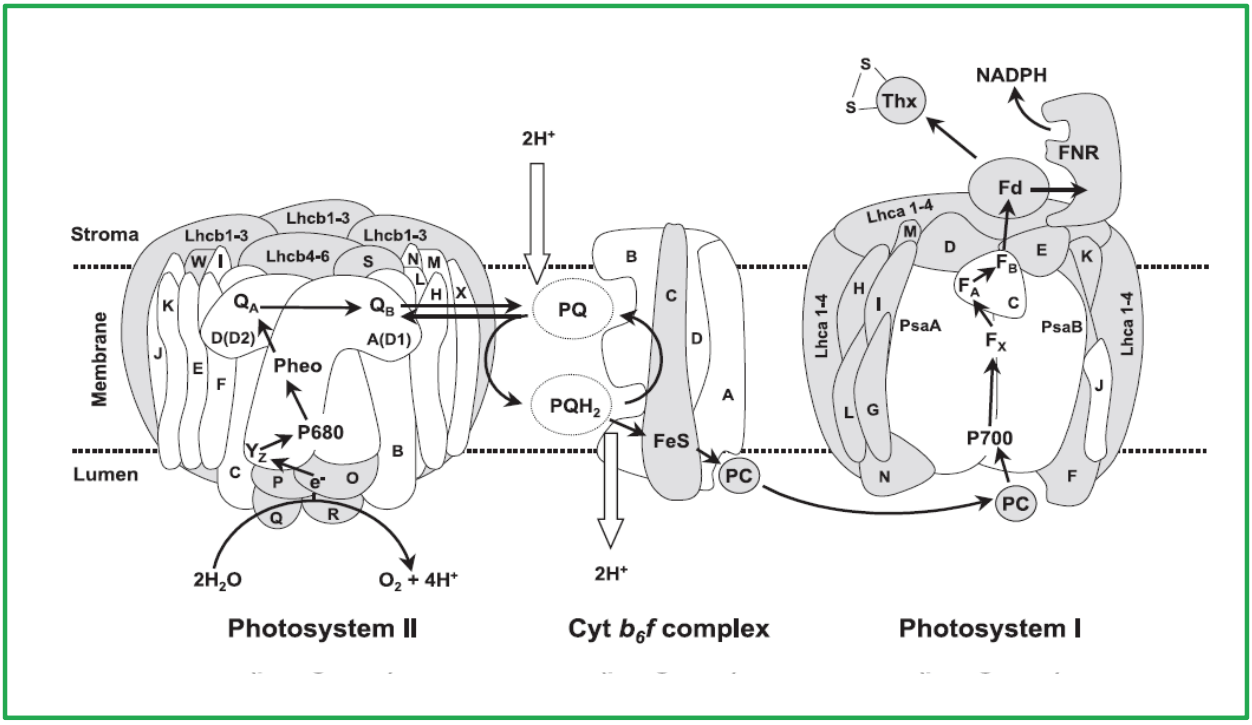


Fig. 1.3 Schematic representation of electron transport pathway from water to NAD^+ in thylakoid membranes. Transfer of excitation energy to the reaction center Chl P680 and P700 powers the entire process. Mn_4O_xCa is the manganese center, which participates in splitting of two water molecules into 4 H^+, 4 e^- and one O_2. Excited P680* has the energy of a photon that was captured by and transmitted from its light gathering antenna complex to pheophytin (chlorophyll with its Mg^{2+} replaced by two protons). $Q_{A,}$ is tightly bound immovable molecule accepts and transfers one electron at a time. Q_B, is loosely bound and movable molecule which accepts and transfers two electrons and protons at a time. Q_BH_2, reduced Q_B, shuttling 2 electrons and protons within the hydrophobic core to Cyt b_6/f. Plastocyanin is a highly mobile copper protein. P700 is the excited state of reaction center Chl of PSI transfer electron to Ao (special Chl accepts electron from PSI. A_1 is the phylloquinone (vitamin K). F_X, $F_{A,}$ F_B are the immobile iron-sulfur protein centers. F_D is ferredoxin, a mobile iron-sulfur protein participates in cyclic electron transport along with other iron sulfur centers. FNR is the enzyme ferredoxin-NADP oxidoreductase, which enables $NADP^+$ to accept two electrons and a proton. (Adapted from Wilson et al., 2006)

NADPH production, and thus uncouple the regulation of the proton gradient from the water-splitting activity of PSII. Part of the electrons can also be directed to oxygen ($O_2 \rightarrow H_2O$) via chlororespiration (Peltier and Cournac, 2002) and the Mehler reaction (Badger et al., 2000).

PSII is located mostly in the grana thylakoids, whereas PSI and ATP synthase are mostly located in the stroma thylakoids and the outer layers of grana. The Cyt b_6/f complex is distributed evenly throughout thylakoid membranes. Due to the separate location of the two photosystems in the thylakoid membrane system, mobile electron carriers are required to shuttle electrons between them. These carriers are plastoquinone (PQ) and plastocyanin. PQ shuttles electrons from PS II to the Cyt b_6/f complex, whereas plastocyanin carries electrons from the Cyt b_6/f complex to PSI (Govindjee and Govindjee et al., 1975). Together, these proteins make use of light energy to drive electron transport chains that generate a chemiosmotic potential across the thylakoid membrane and NADPH, a product of the terminal redox reaction. The ATP synthase uses the chemiosmotic potential to make ATP during photophosphorylation.

1.3 Structure and function of photosystem II

PSII is a large pigment–protein supramolecular complex embedded in the thylakoid membrane of plants, algae and cyanobacteria, which splits water into oxygen, protons and electrons during the photosynthetic process (Barber, 2003) (Fig. 1.4A-C). This complex thus provides the energy and the oxygen, which sustain all life on earth. The structure of PSII has been reported at resolutions from 3.8 to 1.9 Å (Kamiya and Shen, 2003; Guskov et al., 2009; Zouni et al., 2009; Kawakami et al., 2011). PSII is present mainly in dimeric form, each monomer consisting of at least 27–28 subunits organized in two moieties: the core complex and the antenna system (Dekker and Boekema, 2005; Caffarri et al., 2009). The core is composed of several proteins: (i) D1 and D2 containing the reaction centre P680 and all the cofactors of the electron transport

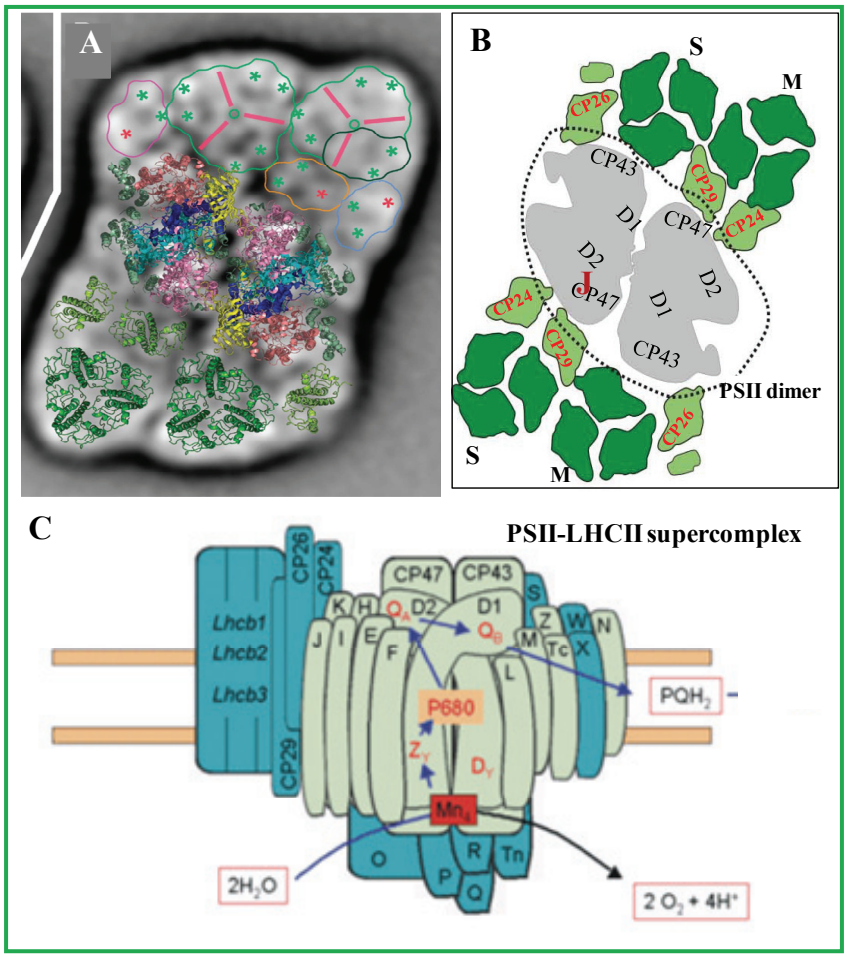


Fig. 1.4 Projection maps showing structure of megasupercomplexs composed of PSII-LHCII dimers. Schematic representation of functional PSII is composed of PSII-LHCII supercomplexes (A). Inner antennae CP26, 24, and 29 are shown in light green, close to reaction centers, are composed of monomeric Chl *a*/*b*, carotenoid binding proteins, and the outer ones (Lhcb1, Lhcb2 and Lhcb3) exist as trimers and are represented in dark green colour. Sketch of PSII megasupercomplex (dimer) with major subunits highlighted (B). Schematic representation of subunits of PSII-LHCII supercomplex (C). (Adapted from Caffarri et al., 2009).

chain; (ii) CP47 and CP43, which coordinate chlorophyll (Chl) *a* molecules and act as an inner antenna and (iii) several low molecular subunits whose role has not yet been fully understood (Shi and Schroder, 2004). The structure of the PSII core of cyanobacteria shows 35 Chl *a* molecules, 2 pheophytins and 12 molecules of β-carotene (Loll et al., 2005; Guskov et al., 2009). In higher plants, on the lumenal side of the membrane, the products of the *psbO*, *psbP* and *psbQ* genes compose the oxygen evolving complex (OEC33, 23 and 17, respectively), which participates to the stabilization of the Mn cluster required for an efficient oxygen evolution. However, the exact role and locations of these subunits has not been fully clarified yet (Roose et al., 2007). The peripheral antenna system has a primary role in light harvesting, transfer of excitation energy to the reaction centre and photosynthesis regulation through photoprotective mechanisms, which dissipate the excess energy absorbed by the system as heat under stress conditions (non-photochemical quenching) (Schmid, 2008). It is composed of six different complexes, belonging to the light-harvesting complex (Lhcb) multigenic family (Jansson, 1999), which coordinate Chl *a*, Chl *b* and xanthophylls, in different ratios. The major antenna complex, LHCII, is organized in heterotrimers composed of the products of the Lhcb1-3 genes (Caffarri et al., 2004), while the three other subunits, CP29 (Lhcb4), CP24 (Lhcb6) and CP26 (Lhcb5) are present as monomers (Dainese and Bassi, 1991). The subunits of PSII-LHCII supercomplex are listed in Table 1. The crystal structure of PSII at a resolution of 1.9Å in *Thermosynechococcus vulcanus* located all of the metal atoms of the Mn_4CaO_5 cluster, together with all of their ligands (Umena et al., 2011). It is found that five oxygen atoms served as oxo- bridges linking the five metal atoms, and that four water molecules were bound to the Mn_4CaO_5 cluster; some of them may therefore serve as substrates for dioxygen formation. We identified more than 1,300 water

Gene	Subunit	Location of the gene	Molecular mass (kDa)	Functions
PsbA	D1	C	32	RC binding protein
PsbD	D2	C	34	P680, Pheo, Q_A, Q_B, Y_Z, Y_D, Mn
PsbB	CP47	C	51	Core antennae protein
PsbC	CP43	C	43	Core antennae protein
PsbE	Cyt b	C	9	Electron transfer, Photoprotection
PsbF	559 β subunit	C	4	Electron transfer, Photoprotection
PsbH	PsbH	C	3.8	Influences acceptor side function
PsbI	PsbI	C	4.5	Protects PSII against light
PsbJ	PabJ	C	4	Binding of PsbP
PsbK	PsbK	C	3.5	Unknown function
PsbL	PsbL	C	4	Influences Q_A coordination
PsbM	PsbM	C	4	Stabilization of ET in the RC
PsbN	PsbN	C	4	Unknown function
PsbO	PsbO	N	33	Manganese stabilizing protein
PsbP	PsbP	N	23	Regulation of OEC function
PsbQ	PsbQ	N	16	Regulation of OEC function
PsbR	PsbR	N	10	Regulation of OEC function
PsbS	PsbS	N	22	May bind chlorophyll
PsbT	PsbT	C	4	Protects PSII against light
PsbW	PsbW	N	6	Stabilization of PSII dimer
Lhcb4	Lhc-IIa (CP29)	N	29	Light harvesting, state transition
Lhcb1	Lhc-IIb Lhcb1	N	27-28	Light harvesting, state transition
Lhcb2	Lhcb2	N	25-27	Light harvesting, state transition
Lhcb3	Lhcb3	N	25	Light harvesting, state transition
Lhcb5	Lhc-IIc (CP26)	N	26	Light harvesting
Lhcb6	Lhc-IId (CP24)	N	24	Light harvesting

Table 1. Subunits and its functions of PSII-LHCII supercomplex. The location of the gene in plants in the chloroplasts (C) or nucleus (N) is indicated. (Adapted from Pakrasi, 1995 and Muh et al., 2008).

molecules in each PSII monomer. Some of them formed extensive hydrogen-bonding networks that may serve as channels for protons, water or oxygen molecules.

1.4 Structure and function of Photosystem I

PSI is a large thylakoid membrane multi-subunit protein complex, which is vital for oxygenic photosynthesis (Nelson and Ben-Shem, 2002; Jolley et al., 2005; Amunts et al., 2010) (Fig. 1.5). The PSI generates the most negative redox potential in nature (-1 V), and thus largely determines the global amount of enthalpy in living systems. The crystal structure of PSI resolved at 2.5 Å from the thermophilic cyanobacterium *Synechococcus elongatus*, which have 12 protein subunits and 127 cofactors including 96 chlorophylls, 2 phylloquinones, 3 Fe_4S_4 clusters, 22 carotenoids, 4 lipids, a putative Ca^{2+} ion and 201 water molecules (Jordon et al., 2001). Cyanobacterial PSI is a trimer consisting of 36 proteins, to which 381 cofactors are non-covalently attached. The higher plant crystal structure of PSI from Pea at 4.4 Å resolution showed 12 core subunits, 4 different light-harvesting membrane proteins assembled in a half-moon shape on one side of the core, 45 transmembrane helices, 167 chlorophylls, 3 Fe–S clusters and 2 phylloquinones. In higher plants PSI mediates the light driven electron transport from plastocyanin to ferredoxin (Jensen et al., 2003). The core complex, composed of 15 subunits (PsaA to L and PsaN to P), which contains the primary donor P700 and all the cofactors of the electron transport chain as well as approximately 100 Chl *a* and 20 β-carotene molecules (Knoetzel et al., 2002). The core antenna is bound to the LHCI forming a crescent-shaped structure at the PsaF/PsaJ site of the core. The core and the antennae are coupled by gap chlorophylls (Ben-Shem et al., 2003). The external antenna composed of four polypeptides, belonging to the Lhc family and named Lhca1–4, with molecular weight between 21 and 24 kDa (Knoetzel et al., 2002).

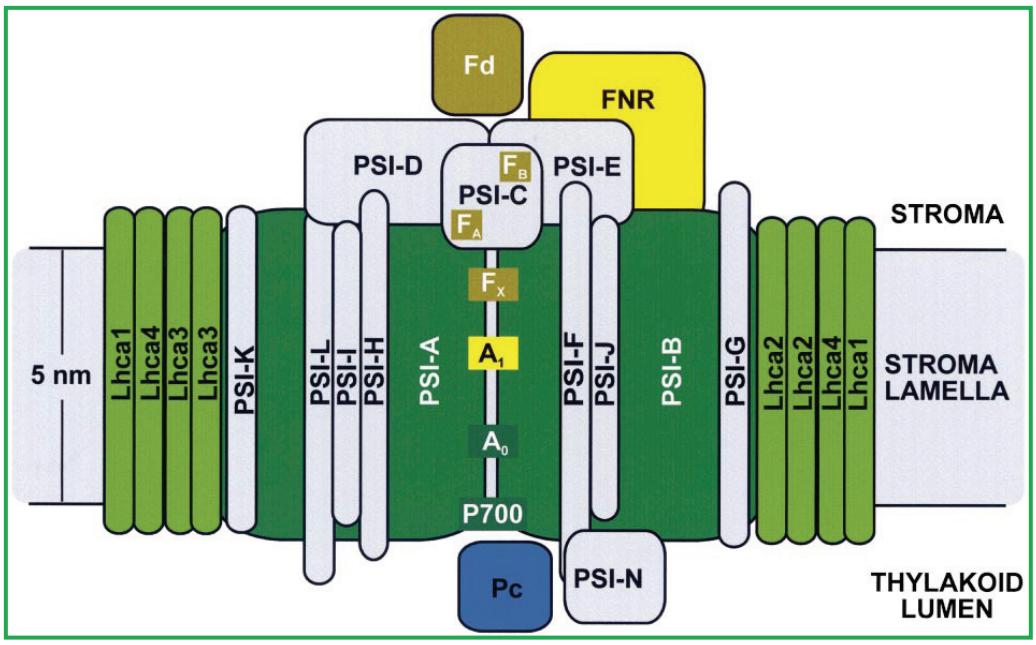


Fig. 1.5 Schematic representation of PSI-LHCI supercomplex from plants. (Adapted from Scheller et al., 2001).

However, the recent crystal structure of higher plant PSI-LHCI supercomplex of 3.3 Å showed 18 protein subunits and 193 non-covalently bound photochemical cofactors (Amunts et al., 2010). LHCI complex shows a high degree of variability in size, subunits composition and bound pigments, which is due to the large variety of different habitats of photosynthetic organisms. The LHCI proteins (Lhca1, Lhca2, Lhca3 and Lhca4) have been assumed to be present in a stoichiometric ratio close to 1:1:1:1, but the ratio may vary under certain conditions, due either mutations or changes in growth conditions. In addition, a novel type of Lhca protein, Lhca5, has recently been found to associate with PSI. The main function of LHCI is to harvest light and transfer the excitation energy to the reaction centre, where it is used for charge separation. In higher plants, there are two major LHCI sub-fractions with different protein compositions and fluorescence properties (Lam et al., 1984; Knoetzel et al., 1992). LHCI-680 and LHCI-730 has characteristic 77K fluorescence emission at 680 and 730 nm, respectively. LHCI-680 consists mainly of monomeric Lhca2 and Lhca3 and lacks the red-shifted emission, whereas LHCI-730 is strongly enriched in the Lhca1/4 heterodimer (Lam et al., 1984; Schmid et al., 1997). It was assumed that only the Lhca1/4 dimer contains 'red' Chls. However, the low temperature 680 nm fluorescence was absent from preparations containing all four Lhca complexes in their native dimeric form (Croce et al., 1998; Ihalainen et al., 2000). Furthermore, dimeric fractions enriched in Lhca1/4 or Lhca2/3 both showed a red-absorption tail with similar amplitude, indicating that red forms are also present in Lhca2/3 (Croce et al., 2002). The subunits of PSI-LHCI supercomplex is listed in Table 2. Compared to vascular plants, the LHCI complex of *C. reinhardtii* is more complex. Biochemical studies showed that it is composed of between 10 and 18 proteins (Hippler et al., 2001; Strauber et al., 2001; Yadavalli et al., 2011)

Gene and Subunit	Location of the gene	Molecular mass kDa	Cofactors	Functions
PsaA, PSI-A	C	83.2	≈ 96 Chl *a*, ≈ 22 β-carotene, P700, A_0	Light harvesting
PsaB, PSI-B	C	82.4	A_1, F_X	Charge separation Electron transport
PsaC, PSI-C	C	8.8	F_A, F_B	Electron transport
PsaD, PSI-D	N	17.6		Binding of ferredoxin Binding of PSI-C
PsaE, PSI-E	N	10.8		Binding of ferredoxin and FNR Involved in cyclic electron transport
PsaF, PSI-F	N	17.5	Chl *a* ?	Binding of Plastocyanin
			Chl *a* ?	Binding of LHCI-730
PsaG, PSI-G	N	10.8		Binding of LHCI-680
PsaH, PSI-H	N	10.2		Binding of LHCII (State transition) Stabilization of PSI-D
PsaI, PSI-I	C	4.0		Stabilization of PSI-L
PsaJ, PSI-J	C	5.0		Stabilization of PSI-F
PsaK, PSI-K	N	9.0	Chl *a* ?	Binding of LHCI-680
PsaL, PSI-L	N	18.0	Chl *a* ?	Stabilization of PSI-H
PsaM, PSI-M	C	3.3		Absent in angiosperms
PsaN, PSI-N	N	9.8		Docking of plastocyanin
Lhca1, Lhca1	N	22	≈ 10 Chl *a*, ≈ 2 Chl *b*, ≈ 3Carotenoids	Light-harvesting LHCI-730
Lhca2, Lhca2	N	23	≈ 10 Chl *a*, ≈ 2 Chl *b*, ≈ 3Carotenoids	Light-harvesting LHCI-680B
Lhca3, Lhca3	N	25	≈ 10 Chl *a*, ≈ 2 Chl *b*, ≈ 3Carotenoids	Light-harvesting LHCI-680A
Lhca4, Lhca4	N	22	≈ 10 Chl *a*, ≈ 2 Chl *b*, ≈ 3Carotenoids	Light-harvesting LHCI-730

Table 2. Subunits and its functions of PSI-LHCI supercomplex. The location of the gene in plants in the chloroplasts (C) or nucleus (N) is indicated. In algae other than green algae, more genes are located in the chloroplast or cyanelle genome. (Adapted from Scheller et al., 2001)

and with the availability of a genomic sequence it was possible to demonstrate that all nine genes encoding for LHCI proteins (*lhca1–9*) are expressed on the protein level (Stauber et al., 2003).

1.5 Cyt b_6/f

The Cyt b_6/f complex is a dimer, with each monomer composed of eight subunits (Whitelegge et al., 2002). These consist of four large subunits: a 32 kDa Cyt f with a c-type Cyt, a 25 kDa Cyt b_6 with a low- and high-potential heme group, a 19 kDa Rieske iron-sulfur protein containing a [2Fe-2S] cluster, and a 17 kDa subunit IV; along with four small subunits (3-4 kDa): PetG, PetL, PetM, and PetN. The total molecular weight is 217 kDa (Whitelegge et al., 2000). The crystal structure of Cyt b_6/f complexes from *C. reinhardtii*, *Mastigocladus laminosus*, and *Nostoc* sp. PCC 7120 have been determined (Stroebel et al., 2003; Yamashita et al., 2007; Baniulis et al., 2009). The complex is structurally similar to cytochrome bc_1. Cyt b_6 and subunit IV are homologous to Cyt b and the Rieske iron-sulfur proteins of the two complexes are homologous. However, Cyt f and Cyt c_1 are not homologous. Cyt b_6/f contains seven prosthetic groups. Four are found in both Cyt b_6/f and bc_1: the c-type heme of Cyt c_1 and f, the two b-type hemes (b_p and b_n) in bc_1 and b_6f, and the [2Fe-2S] cluster of the Rieske protein. Three unique prosthetic groups are found in Cyt b_6/f: Chl *a*, β-carotene, and heme c_n (also known as heme x) (Stroebel et al., 2003).

Cyt b_6/f complex mediates electron transfer between the PSII and PSI reaction center complexes in oxygenic photosynthesis by oxidizing the relatively low potential (Em7≈+0.1 V) lipophilic plastoquinol and reducing soluble plastocyanin or Cyt c6 (Allen, 2004; Kurisu et al., 2004; Smith et al., 2004). These electron transfer events are linked to proton translocation across the low dielectric membrane that generates a proton electrochemical potential gradient of approximately 250 mV on lumen side of thylakoid membrane. The complex occupies an

electrochemically central position in the linear electron-transport chain (LET) (Allen, 2003; Cramer et al., 2006). The plastoquinol-plastocyanin Cyt c6 oxidoreductase activity of the complex provides an electronic connection between the two photosynthetic reaction center complexes, PSI and II in which electron transfer through the complex is coupled to proton transfer from the electrochemically negative (n) to the positive (p) side of the complex. In a separate reaction, the Cyt b_6/f complex plays a central role in cyclic photophosphorylation, when $NADP^+$ is not available to accept electrons from reduced ferredoxin.This cycle results in the creation of a proton gradient by Cyt b_6/f, which can be used to drive ATP synthesis. It has also been shown that this cycle is essential for photosynthesis, in which it is proposed to help maintain the proper ratio of ATP/NADPH production for carbon fixation (Bendall and Manasse, 1995).

1.6 State transitions

The quality and quantity of electromagnetic radiation varies depending on the dynamic behavior of atmosphere and climate. Daylight, the combination of sunlight and skylight (blue sky), is the natural source for sustenance of living organisms especially oxygenic photosynthetic organisms. In general, the daylight spectral distribution peaks at different wavelengths depending on the time of the day. When the sun is low in the sky (sunrise and sunset), spectral distribution has less intensity in the blue end of the visible region and more in the red region. In the sunset spectrum, the red “dips” are prominent at any given time. The quantity of this red light reaching atmosphere is not always the same (Rechtsteiner and Ganske, 1998) and atmospheric gases play a major role in determining the quality and quantity of the electromagnetic spectrum reaching the earth.

The LHCII, which acts as a major light harvesting antenna of PSII, is enriched in Chl *b* and has less of Chl *a* pigment, most of which absorb in 670–676 nm region (van Grondelle and Novoderezhkin, 2006). The PSI antenna, on the other hand, has less number of Chl *b* molecules and it possesses a number of red Chl *a* pigments with absorption extending to 690–710 nm region. This difference dictates a different spectral design of the respective antenna systems resulting in the PSII antenna to have more blue absorption relative to the PSI. Earlier studies have shown that PSII exhibits maximal absorption in blue, red region and less absorption in far red region, whereas the PSI shows maximal absorption at red and far red region and less at blue region of electromagnetic spectrum. This differential absorption of PSI and PSII makes differential excitation leading to the imbalance in energy distribution. So, all types of plants, green algae, red algae and cyanobacteria have evolved a mechanism by which equal distribution of absorbed energy occurs between the two photosystems to ensure the optimal photosynthetic electron flow and thus, optimization of the quantum yield of photosynthesis is called state transitions.

1.6.1 Mechanism of state transition

There has a mechanism in which the absorbed energy redistributed between PSII and PSI in higher plants, green algae, red algae and cyanobacteria (Fork and Satoh, 1986, Mohanty et al., 2011). The schematic representation of state transition has been depicted in Fig. 1.6. The preferential excitation of PSII makes the PQ pool in the electron transport chain more reduced (Allen et al., 1981; Horton et al., 1981). These conditions favor the docking of PQH_2 to quinol oxidation (Qo) site of Cyt b_6/f complex (Zito et al., 1999; Mao et al., 2002), which in turn activates an enzyme, thylakoid protein kinase STN7 in higher plants and green algae (*stt7* is a ortholog of *stn7* in *Chlamydomonas*) (Pesaresi et al., 2009). Redox activated kinase STN7/STT7

phosphorylates major LHCII and it gets associates with PSI, thereby increasing the absorption cross section of PSI complex leading to state II condition. In *C. reinhardtii*, STT7 kinase associated with photosynthetic membrane complexes PSI-LHCI-LHCII and Cyt b_6/f (Takahashi et al., 2006; Lemeille et al., 2009). The state in which the major LHCII is associated with PSI-LHCI is called state II. State transitions are crucial for plant fitness under natural light conditions. The preferential excitation of PSI makes the PQ pool oxidized, which is the signal for the activity of phosphatase to dephosphorylate LHCII (Allen et al., 1981). The association of LHCII with PSII is called state I. Recently, it has been shown that thylakoid-associated phosphatase TAP38 was required for LHCII dephosphorylation, thus leading to transition from state II to state I in *A. thaliana* (Pribil et al., 2010; Pesaresi et al., 2011).The transition from state II to state I or state I to state II is termed as state transition.

In *C. reinhardtii*, three LHCII polypeptides were associated with a PSI–LHCI supercomplex in state II and identified them as two minor monomeric LHCII proteins (CP26 and CP29) and one major LHCII protein type II, or LhcbM5 (Takahashi et al., 2006). These three LHCII proteins, in addition to the major trimeric LHCII proteins, were phosphorylated upon transition to state II. There are four distinct branches for the major LHCII proteins: type I (LhcbM6, LhcbM4, LhcbM3, LhcbM8, and LhcbM9), type II (LhcbM5), type III (LhcbM2 and LhcbM7), and type IV (LhcbM1). The four types of major LHCII proteins in *C. reinhardtii* do not correspond to the three types of major LHCII proteins in higher plants, whereas the minor LHCII proteins, CP29 and CP26, are conserved (Teramoto et al., 2001). Among the three polypeptides identified as mobile LHCII components, two were minor LHCII proteins, CP29 and CP26, and the other was a major LHCII protein (type II) relatively close to the minor LHCII proteins. Intriguingly, these three proteins display the highest similarity to the LHC proteins associated

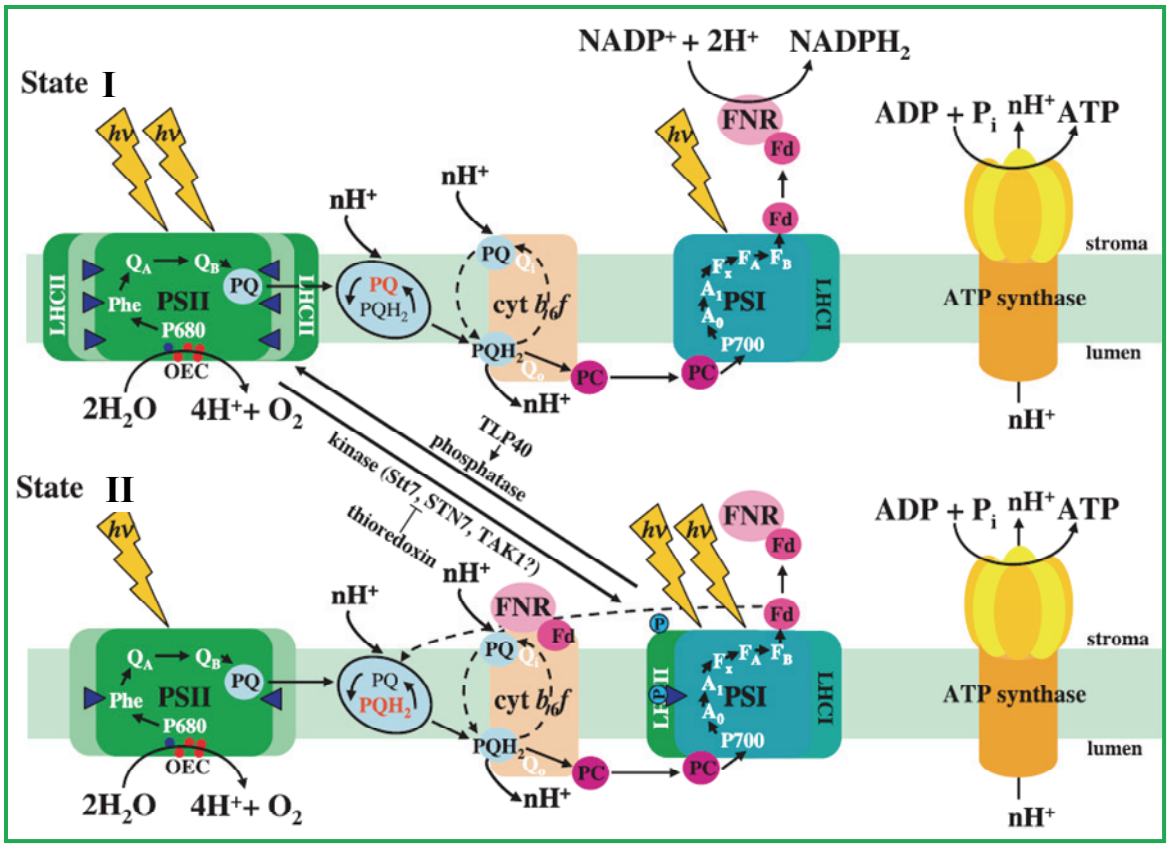


Fig. 1.6 Diagrammatic overview of the state transitions process. State I is induced by excess PSI light (light 1) and state II by excess PSII light (light 2). State I to state II transition occurs in response to illumination with excess light 2, when the PQ pool becomes over-reduced. Binding of the PQH_2 to the quinol-binding site of the Cyt b_6/f complex activates a specific thylakoidbound kinase that directly interacts with the Cyt b_6/f complex and phosphorylates the mobile LHCII antenna (dark green; P, phosphate groups). The activity of LHCII kinase is regulated by co-operative redox control, both via PQ and Cyt b_6/f, and through the thioredoxin / ferredoxin system in the stroma. Phospho-LHCII, together with the phosphorylated linker subunit CP29 (and possibly TSP9 in plants) (blue triangles), detaches from PSII and docks onto PSI to redirect absorbed excitation energy to PSI at the expense of PSII. Under the conditions of over-excitation of PSI, oxidation of the PQ pool occurs followed by de-activation of LHCII-specific kinases and dephosphorylation of mobile LHCII by redox-independent constitutively active phosphatase (although its activity may be regulated by immunophilin-like lumenal TLP40 protein). Dephosphorylated LHCII detaches from PSI and functionally couples with PSII (state II to state 1 transition), favouring energy redistribution towards PSII. In *Chlamydomonas*, a switch between linear (state I) and cyclic (state II) electron flow around PSI occurs. Observed migration of the Cyt b_6/f to the stroma lamellae in state II adaptation may promote preferential binding of ferrodoxin-NADP reductase with this complex and increase the rate of PQ reduction via the cyclic electron flow around PSI (dashed arrows), which exclusively generates ATP by driving protons across the membrane. Any metabolic depletion of the cellular ATP level would switch between both types of photosynthetic electron transport and would therefore induce state I to state II transition. (Adapted from Kargul and Barber, 2008)

with PSI (LHCI) among the LHCII proteins that are thought to be associated with PSII. In higher plants, the three minor LHCII proteins, CP24, CP26 and CP29 are associated at each side of the PSII dimer in PSII–LHCII supercomplex and lie at the interface between the PSII dimer and the LHCII trimers (Yakushevska et al., 2003; Dekker and Boekema, 2005).

It has been reported that major LHCII undergoes phosphorylation in *A. thaliana*. The LHCII occurs in a trimeric oligomerization state and consists of various combinations of three very similar proteins, encoded by the Lhcb1, Lhcb2 and Lhcb3 genes, which usually occur in a ratio of about 8:3:1 (Jansson, 1999). In addition, there are three "minor" antenna complexes, which are called Lhcb4 (CP29), Lhcb5 (CP26) and Lhcb6 (CP24) and usually occur in monomeric states. However, it is not clear that which component of LHCII undergoes phosphorylation during state transition in *A. thaliana*.

1.6.2 History of state transitions

Murata (1969a,b), using the red alga *Porphyridium cruentum,* found that pre-illumination of phycoerythrin pigment in the PBS excited at 433 nm decreased the yield of fluorescence emissions at 685 nm and 695 nm originating from PSII and increased at 715 nm emission originating from PSI. Pre-illumination of phycoerythrin excited at 533 nm yielded fluorescence emissions at 648 nm and 664 nm originating from PBS remained unchanged. Also on pre-illumination with 694 nm, preferentially excited Chl *a*, the fluorescence yields showed little change. Later, described this observation as "a control mechanism of excitation transfer between the two pigment systems" (Murata et al., 1966; Murata, 1969a; Murata, 1969b). These series of observations suggested that light induced control of excitation energy transfer, which enables the organisms to utilize absorbed light energy efficiently in oxygenic photosynthesis. A similar conclusion was arrived independently by Bonaventura and Myers (1969) using the green algae

Chlorella pyrenoidosa and monitoring both oxygen and fluorescence transients. These authors used both light induced Chl *a* fluorescence and oxygen evolution. They observed that state I light condition was characterized by a low O_2 yield and high fluorescence yield, while in state II light condition, the oxygen yield was high and fluorescence yield was low. Upon switching on far red light to drive state II to state I and then back to state II upon switch off, there was transient parallel increase in both oxygen and fluorescence yield. When the time dependent changes in fluorescence and oxygen yield in *Chlorella* were followed simultaneously, the kinetics was not always antagonistic or anti-parallel as one would expect from the notion that the two processes were always competitive. However, Bonaventura and Myers (1969) observed both anti-parallel and parallel changes in both O_2 and fluorescence yields, suggesting the shifting of states. Thus, from these two classic experiments of Murata and that of Bonaventura and Myers (Japan), the concept of energy sharing between two photosystems was pitched and the state transitions refers to change of the two systems from one adaptive low energy sharing, state I to the high energy sharing, state II and each state has its characteristic features.

As stated earlier, the PSII absorbs more of photosynthetic active radiation (PAR) from sunlight than PSI because of relatively more abundance of accessory pigments in their antennas resulting in a downhill energy transfer from PSII to PSI (Haldrup et al., 2001). The actual mechanism of energy transfer from PSII to PSI was proposed to be by two processes called *a* change (sharing of antennae) and spill over. Bennett demonstrated a light induced activation of phosphorylation of light harvesting chlorophyll protein complex by thylakoid protein kinase in algal thylakoids (Bennett, 1977, 1979). Both redox state of PQ (Allen et al., 1981) and Cyt b_6/f were involved in phosphorylating Chl *a/b* complex (Bennett et al., 1980, 1988). Either the presence of PSII inhibitor DCMU in light or the electron donation by reduced DCPIP to PSI did

not cause phosphorylation of LHCII, suggesting the involvement of Cyt b_6/f in light induced phosphorylation of LHCII antennas. These experiments brought forward the idea that the regulation of excitation energy transfer involves protein phosphorylation and migration (Bennett, 1977; Bennett et al., 1980). These conclusions were confirmed by the use of single turnover flashes that reduced PQ and activated the thylakoid kinase that phosphorylated threonine residue of LHCII (Bennett, 1977, 1979; Bennett et al, 1980). From all their studies, these authors could establish that phosphorylation/dephosphorylation of LHCII was due to thylakoid bound protein kinase/ phosphatase dependent upon illumination and/or by Mg^{2+} ions (Bennett, 1979; Bennett et al, 1980). In this model, the α change involved migration of LHCII antennae protein from PSII located in the grana region to stroma lamellae located PSI by phosphorylation and released from PSI upon dephosphorylation by the enzyme phosphatase (Pribil et al., 2010). From these landmark discoveries on state transition mechanisms, attempts were made to characterize in detail the transition from state I to state II and vice versa. It is to be noted that the uphill transfer of excitation energy would involve energy transfer from PSI to PSII, which is energetically unfavorable.

1.6.3 Role of Cyt b_6/f in state transition

Cyt b_6/f is a membrane bound complex that transfers electrons from lipophilic quinones to hydrophilic plastocyanin, and in the process, transfers proton across the thylakoid membrane and develops proton gradient, thereby controlling not only electron transfer, but also energy transduction (Kurisu et al., 2004). It is now known that this membrane protein also holds a key role in state transions (Zito et al., 1999).

It has been shown that Cyt b_6/f less mutant of *Lemna* lacks LHCII phosphorylation (Gal et al., 1987). Further, it has also been shown in *Acetabularia* that the inhibition of quinol oxidase

site of the Cyt b_6/f complex inactivated the LHCII kinase in the dark, and also in the light, or in the presence of duroquinol when the PQ pool was reduced. Inhibition of the quinone reductase site of the Cyt b_6/f complex has practically no effect in the dark and stimulates the kinase activity in the light. Gal et al (1988) from their studies with the mutant *Acetabularia* lacking Cyt b_6/f complex proposed that the activity of LHCII kinase was regulated by the redox state of a Cyt b_6/f complex component (s) that balances electron flow from PSII to PSI via PQ pool. Similar results were obtained by Bennet et al (1988) using PQ antagonist type inhibitors in pea thylakoids. Further Vener et al (1995) reported that LHCII phosphorylation could be activated in spinach thylakoids without light by low pH or by the addition of reductant in spinach thylakoids. The induction of LHCII phosphorylation by low pH or light treatment was completely inhibited by the oxidizing agent ferricyanide, suggesting that the redox state of Cyt b_6/f is the key regulator for the state transition. The same group proposed that the activation/deactivation cycle of redox controlled thylakoid protein phosphorylation was the regulating feature in state transition. The occupancy of the quinol-oxidation site of the Cyt b_6/f complex by plastoquinol, and the redox state of protein thiol groups act as elements of the signal transducing chains (Vener et al., 1998). In this line of research, Zito et al (1999) demonstrated that Qo site of Cyt b_6/f complex possessed the affinity for plastoquinol, which activated LHCII kinase. These authors used a *Chlamydomonas* mutant designated *PWYE* (amino acid sequence at the Qo site) in which the Qo site of Cyt b_6/f was unable to bind plastoquinol. Photoacoustic and fluorescence studies confirmed that Qo site of Cyt b_6/f complex indeed controls the activation of LHCII kinase.

1.7 Effect of temperature on photosystems

Temperature stress is one of the major environmental factors that limit plant growth and productivity. Among the plant physiological processes, photosynthesis is most sensitive to heat

stress, with inhibition occurring at higher-than-optimal growth temperatures (Allakhverdiev et al., 2008). At moderate heat stress, reversible inhibition of photosynthesis occurs, while at extremely high temperatures damage to the photosynthetic machinery is irreversible (Allakhverdiev et al., 2008; Mathur et al., 2011). Among the photosynthetic machinery, PSII has been shown to be most sensitive (Berry and Björkman, 1980; Lipova et al., 2010). In addition to the down-regulation of PSII, inactivation of PSII reaction centers (RC) (Bukhov et al., 1990; De Las Rivas and Barber, 1997) and electron transfer from Q_A to Q_B at the acceptor side of PSII is affected at moderately elevated temperature (Ducruet and Lemoine, 1985; Kouril et al., 2004). Further, prolonged exposure to elevated temperature may lead to the degradation of the OEC, resulting in reduced electron flow from water to the PSII reaction center (Enami et al., 1994). Conversely, it was demonstrated that moderate heat stress does not cause serious impairment of PSII function but instead inhibits PSII repair, while PSI activity is actually enhanced (Bukhov et al., 1999; Allakhverdiev et al., 2008). However, in case of chlororespiration during dark incubation of *Chlorella*, high temperature doubled the initial Chl fluorescence yield (F_o) (Chemeris et al., 2004). Elevated temperature also affects the structural and functional organization of thylakoid membranes (Cseh et al., 2000), excitation energy transfer, distribution and dissipation apart from effects on the photochemical reaction (Weis, 1985).

1.8 Objectives of the study

In this study, we have characterized mechanism of state transition using biochemical and biophysical methods in wt and *stn7* (mutant lacking STN7 kinase, inhibiting state transitions). This study was build upon the above background and quintessential to changing global environment, where continous rise in atmospheric temperature might effect state transition and inturn photosynthesis. This study highlights the structural, functional organization of PSII-LHCII

and PSI-LHCI supercomplexes under light and temperature induced state transition in wt and *stn7* mutant of *A. thaliana*. Our study also focused on the factors responsible for alterations in the mechanism of state transition at moderate temperature. Generally, the mechanism of energy distribution between PSI and PSII occurs in low light conditions, along with low light we have further studied the effects of the dark moderate temperatures on the state transitions. Inorder to achieve our major goal of study, we formulated the following objectives.

Objectives of my research

1. **Characterization of light induced state transitions- Identification of phosphorylated major LHCII and its role on grana and photosystem I organization.**
2. **Characterization of moderate temperature induced state transitions.**
3. **Unraveling the mechanism of moderate temperature triggered state transition; combined effect of moderate temperature and light on state transition.**

Experimental procedures

Chapter 2

2. Experimental procedures

2.1 Plant growth

Experimental growth conditions of *Arabidopsis thaliana* (*A. thaliana*) and its mutant *stn7*: Wild type (wt) *A. thaliana* ecotype Columbia (kind gift from Dr. Imran Siddique, CCMB, India.) and its mutant *stn7* (kind gift from Dr. Dario Leister, LMU, Munich, Germany) were grown in controlled environmental chambers for 6 weeks at 120–150 μmol photons m^{-2} s^{-1}, with 8 h light/16 h dark periods and relative humidity of 70%. All physiological and biochemical analysis were performed with rosette leaves, harvested before flowering. In *stn7* mutant, the gene responsible for expression of STN7 kinase which phosphorylates LHCII was blocked.

2.2 Induction of state transition

Wt plants were exposed for 45 min to state II light to preferentially excite PSII and state I light to preferentially excite PSI with low light (35-40 μmol photons $m^{-2}s^{-1}$). Blue wavelength filters (450-550 nm) and red filters (650-750 nm) were used to obtain state II and state I, respectively. Transmission spectra of wavelength filters were recorded (Fig. 2.1A). Schematic representation of plants adapted to either state I or state II light were shown in Fig. 2.1B. Temperature was maintained at 25 °C using a water-cooled glass chamber between the fluorescence tube and plants.

2.3 Temperature and light treatment

Mature plants were dark adapted overnight, the leaves of the same size were detached from the plant and subjected to heat treatment at 35 °C, 40 °C, and 45 °C for varying time periods from 0 to 60 min in dark. During the treatment period the detached leaves were floated in beaker containing water and kept in water bath to control the temperature. Heat-treated leaves were frozen in liquid nitrogen for further analysis. Low light (LL) (35-40 μmol photons $m^{-2}s^{-1}$) or high

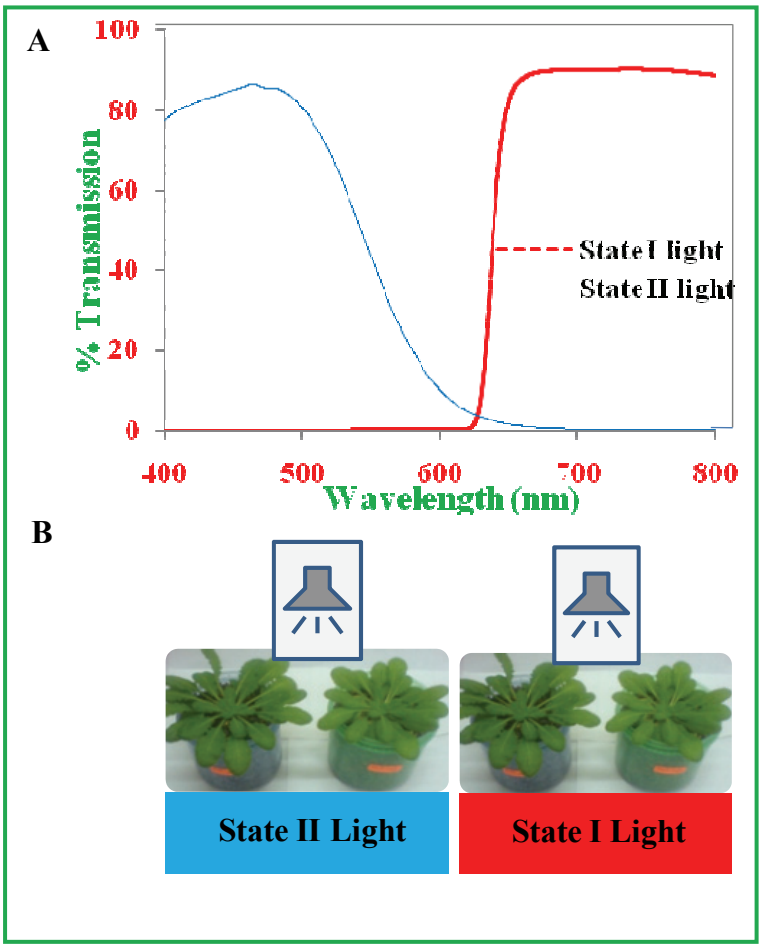


Fig. 2.1 Transmission spectra of red and blue filters for inducing state I and state II, respectively (A). A sketch of experimental design for inducing state transitions by using optical filters (B).

light (HL) (1000 μmol photons $m^{-2}s^{-1}$) with or without heat treatment were also given. 40 ^{o}C has been considered as moderate temperature for studying temperature triggered state transition.

2.4 Inhibitor treatment

A. thaliana leaves were treated with inhibitors with concentration of 10 μM DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) or 10 μM DBMIB (2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone) (both obtained from Sigma-Aldrich), for 1 h prior to temperature treatment.

Also, during temperature treatment the detached leaves were floated in an inhibitor solution for effective action. *A. thaliana* leaves were treated with antimycin or $HgCl_2$ (obtained from Sigma-Aldrich) at a concentration of 10 μM for 1 h prior to temperature and/or light treatments. Also, during the heat and/ or light exposure the detached leaves were floated in an inhibitor solution for effective action. $HgCl_2$ inhibits plastidial NAD(P)H dehydrogenase (NDH) and thereby dark reduction of PQ pool whereas antimycin inhibits cyclic electron transport (CET).

2.5 Isolation of thylakoid membranes

Isolation of thylakoid membranes from state I and state II adapted and heat treated leaves were carried out as described by Kargul et al., 2003 and Subramanyam et al., 2006 with minor modifications. Leaves were homogenized in 25 mM HEPES (pH 7.5, KOH), 0.3 M sucrose, 10 mM $MgCl_2$, 5 mM $CaCl_2$ and1mM PMSF. Leaf homogenate was filtered through four layers of cheesecloth and the filtrate was centrifuged at 200 x g for 3 min. The supernatant was again centrifuged at 5000 x g for 10 min and the pellet was resuspended in 5 mM HEPES (pH 7.5, KOH), 0.3 M sucrose, 10 mM EDTA. This suspension was centrifuged at 18,000 x g for 10 min and the pellet was resuspended in 20 mM Tricine (pH 7.5), 0.3 M sorbitol, 10 mM $MgCl_2$, 5 mM $CaCl_2$ to yield thylakoids.

2.6 Chlorophyll estimation

The Chl concentration was estimated by adding 10 µL aliquot of the sample containing Chl to 1 mL of 80% (v/v) acetone. The aliquot was vortexed and then centrifuged for 2 min at room temperature. The supernatant was collected into a fresh microfuge tube without disturbing the pellet, to take the absorbance readings.

Chlorophyll concentration was estimated by measuring absorption with a spectrophotometer at 645 and 663 nm using the following equation (Arnon, 1949).

Chlorophyll *b* (mg/mL) = $(22.9 \times A_{645}) - (4.68 \times A_{663}) \times$ dilution factor.

Chlorophyll *a* (mg/mL) = $(12.7 \times A663) - (2.69 \times A645) \times$ dilution factor.

Total chlorophyll (mg/mL) = Chl *a* + Chl *b* = $(20.21 \times A645) + (8.02 \times A663) \times$ dilution factor.

2.7 PSI-LHCI supercomplex isolation

PSI-LHCI supercomplexes were isolated according to Kargul et al (2003) by sucrose density gradient ultracentrifugation (SDGUC) with some modifications. For isolation of PSI, thylakoid membranes (1mg Chl/ml) were solubilized with n-Dodecyl β-D-Maltoside (DDM) (Sigma) at a final concentration of 0.9% (w/v); this sample was stirred slowly for 20 min at 4 ^{o}C and centrifuged at 48,400 x g. 0.2 ml supernatant was loaded on a 5 ml of 0.25-2.0 M sucrose density gradient, containing 5 mM tricine-NaOH pH 8.0, 0.5 M sucrose, 0.5 M betaine and 0.05% DDM (w/v). After centrifugation at 180,000 x g for 16 h, collected 3 fractions and were concentrated with Amicon ultra filters, finally suspended in PSI buffer (5 mM Tris (pH 8.0), 0.03% DDM, 5 mM $MgCl_2$ and 5 mM $CaCl_2$) . The F1 (LHCII), F2 (PSII-LHCI/PSI-LHCI), F3 (PSI-LHCI), thus, for our experiments we have used F3 fractions as it contains rich of PSI-LHCI supercomplexes. Densitogram was carried for all the samples (photographs of ultra tubes containing fractions) by using Gel Pro Analyser 3.1.

2.8 Chlorophyll *a* fluorescence: Emission spectra and fast transient

2.8.1 The low temperature 77K fluorescence emission

Isolated thylakoid membranes diluted to 10 µg Chl ml^{-1} were excited at 436 nm and emission spectra at 77K were measured in between 600 and 780 nm. Low temperature fluorescence emission spectra were recorded using a (Perkin Elmer, LS-55) fluorescence spectrophotometer.

2.8.2 The fast OJIP fluorescence transient measurements

Chl fluorescence fast induction curves were measured using a plant efficiency analyzer (PEA), Hansatech, King's Lynn, Norfolk, UK. The dark adapted leaves were excited by an array of three light-emitting diodes peaking at 650 nm at a photon flux density of 3000 µmol photons m^{-2} s^{-1}. The fast fluorescence transients (OJIP) were measured (Strasser and Srivastava, 1995) from control and moderate temperature-induced (40 °C) leaves of wt and *stn7*.

2.9 Absorption spectroscopy

The fractions (F1, F2 and F3) obtained after sucrose density gradient ultracentrifugation were used for measuring room temperature absorption spectra. F3 of dark control and 40 ^{o}C dark of wt and *stn7* were compared to test the binding of LHCII with PSI-LHCI supercomplex. The spectra were recorded from 400-750 nm with scan speed of 100 nm min^{-1} by using UV-3600 Shimadzu spectrophotometer.

2.10 Chlorophyll *a* fluorescence induction

A dual PAM100 (Pulse amplitude modulator) Chl fluorescence photosynthesis analyzer (Heinz Walz) was used to monitor Chl *a* fluorescence under different treatments. Actinic light (AL) illumination was provided by two arrays of 635-nm LEDs illuminating both the adaxial and abaxial surfaces of the leaf, and applied 1 ms after turning on the 460 nm measuring beam. Chl *a* fluorescence quenching measurements were carried out by applying actinic light (AL) of 450

μmol photons m^{-2} s^{-1} for 15 min and turned off later for 10 min (Kovacs et al., 2006). The maximal fluorescence in the dark-adapted state (F_m) and during the course of actinic illumination (F_m') and the subsequent dark relaxation period were determined by a 0.8-s saturating (4000 μmol photons m^{-2} s^{-1}) light pulse applied at 1-2 min intervals. NPQ was defined as F_m/F_m'-1.

Post-illumination of Chl *a* fluorescence transient measurements were measured for leaf samples by applying AL (450 μmol photons m^{-2} s^{-1}) for 10 min, later on raise in F_o' was measured for 60 sec after switching off the AL to monitor dark reduction of PQ pool (Yamazaki et al., 2004). Cyclic electron transport (CET) was also assessed by Chl *a* fluorescence quenching measurements. During the measurements of fluorescence transients, the AL (50 μmol photons m^{-2} s^{-1}) was switched on for 3 min and turned off AL later for 60 sec and switching on the FR (far red). The rise in fluorescence when the AL switched off and FR switched on corresponds to incresase in CET.

2.11 Immunoblotting analysis of thylakoid membrane protein

Tricine SDS-PAGE was carried out as described by Schägger (2006). For immunoblotting, proteins were separated by Tricine SDS-PAGE and transferred on to polyvinylidene fluoride (PVDF) membrane (Bio-Rad) using the transblot apparatus (Bio-Rad). Blots were probed with antiphospho-threonine polyclonal antibodies purchased from New England Biolabs (Cell Signaling Technologies, UK) to detect phosphorylated proteins at threonine sites. The polyclonal LHCII antibodies, i.e., Lhcb1, Lhcb2 and Lhcb3 (Agrisera, Sweden), were used for detection of major LHCII proteins. Equal Chl or proteins was loaded on each lane of SDS-PAGE gel. Protein concentration was measured according to Lowry et al (1951).

2.12 2D gel electrophoresis

To precipitate the proteins, a 25 μL aliquot of thylakoid membrane solution having Chl concentration of 1.0 μg/μL was taken. A volume of 400 μL of methanol was added to the solution and vortexed thoroughly (modified method from Hippler et al., 2001; Yadavalli et al., 2011) and to this solution, 200 μL of water-saturated chloroform was added and the solution was vortexed. To the above fraction, 200 μL of deionized water was added, and the solution vortexed and centrifuged for 5000 x g in a microcentrifuge. The protein precipitate was formed between the interphase. The upper and lower phases were carefully removed and discarded. The protein precipitate was washed with 300 μL of methanol and then with another 300 μL of 95% methanol. The pellet was air dried until methanol evaporated. The pellet was solubilized with 130 μL of rehydration buffer (7 M urea, 2 M thiourea, 80 mM DTT, 4% CHAPS (3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulfonate), 0.1% DDM, 0.01% triton X-100, 1% biolyte, 0.001% bromophenol blue) by vortexing for 2 h. The solubilized pellet solution was centrifuged at 7000 x g for 15 min at room temperature to avoid any unsolubilized proteins. To rehydrate the 7-cm immobilized pH gradient (IPG) strip, 125 μL of sample containing ~150 μg of protein was added to one side of rehydration tray placed on an even surface. The sample was left with the mineral oil for at least 14 h to rehydrate the strip. d. The IPG strip that has been rehydrated was loaded into the focusing tray by placing the gel side down and overlaying it with 1.5 ml of mineral oil and covering with the lid. The following program is followed to run the isoelectric focusing at 20 °C. Step 1: 250 V, 20 min, linear Step 2: 4,000 V, 2.30 h, linear Step 3: 4,000 V, 12,000 V-H, rapid (Hippler et al., 2001). After the isoelectric focusing is completed, the IPG strips can be run by Tricine SDS-PAGE immediately after treating with equilibration buffers. A volume of 2 mL of equilibration buffer I (375 mM tris-Hcl, pH 8.8, 6M urea, 2%SDS,

2%DTT) was added to the tray containing IPG strip and IPG strip (after IEF run) was incubated on the rocker with slow motion for 15 min. The equilibration buffer I was discarded by slanting one side and then 2 mL of equilibration buffer II (375 mM tris-Hcl, pH 8.8, 6M urea, 2%SDS, 2.5% IAA) was added and the tray incubated for 15 min on the rocker. The equilibrated strips were then run for 2nd dimension by Tricine SDS-PAGE.

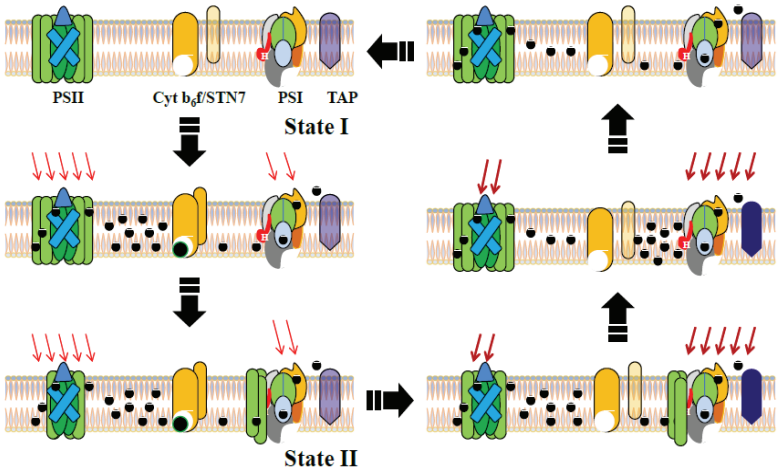
2.13 Transmission electron microscopy

State transitions induced Leaf discs of wt and *stn7* mutant were fixed with 2% glutaraldehyde diluted with 0.1 M phosphate buffer (pH = 7.2) for 3 h according to Brangeon and Mustardy, 1979 and Shimoni et al., 2005. Glutaraldehyde was washed out by overnight incubation in 0.1 M phosphate buffer. Samples were post fixed with 1% osmium-tetroxide diluted in 0.1 M phosphate buffer for 2 h. After short (~1 min) washing with 0.1 M phosphate buffer, samples were gradually dehydrated in 30, 50, 70, 90, 96 and 100% ethanol for 10-15 min at each step. The incubation step in 100% ethanol was repeated 3 times. After that, samples were incubated twice in propylene oxide for 10-15 min each time and in 1:1 ratio of propylene oxide: Araldite for overnight. Samples were transferred to pure Araldite resin and incubated for 24 h. The microfuge tubes containing the samples embedded in the resin were placed to thermo-stated chamber in order to harden the resin for 48-72 h at 56 °C. After trimming the hardened resin blocks, ultrathin sections with a thickness of 70-90 nm were cut with ultramicrotome (Leica Ultracut UCT). Sections were taken up onto 400 mesh copper grids. For contrasting the specimen, sections were stained with 2% uranyl acetate for 45 min, washed with distilled water and stained with lead citrate for 15-20 min and washed with distilled water.

All the experiments presented here were repeated more than three times independently and obtained similar results.

Characterization of light induced state transitions- Identification of phosphorylated major LHCII and its role on grana and photosystem I organization.

Chapter 3



INTRODUCTION

In oxygenic photosynthetic organisms the light energy conversion into chemical energy, involving photooxidation of water and reduction of CO_2 to carbohydrates is mediated by two interactive photochemical reactions namely PSI and PSII. The two photosystems absorb solar radiation differentially resulting in unbalanced energy distribution which triggers a mechanism in which the energy can be balanced for optimal photosynthesis, this phenomena is popularly known as state transition (Allen, 2003; Allen and Mullineaux, 2009; Wollman, 2001; Tikkanen et al., 2011). State transitions occur in natural environments, where light quality and quantity fluctuate with the time (Pesaresi et al., 2010).

In higher plants, 10-15% of the light harvesting complexes are transferred during state transitions (Allen, 1992). The preferential excitation of PSII makes the PQ pool more reduced. The binding of Q_o site of Cyt b_6/f with plastoquninol activates thylakoid kinase (STN7/STT7 kinase) that mediates phosphorylation of LHCII (Wollman and Lemaire, 1988; Vener et al., 1997; Bellafiore et al., 2005; Gal et al., 1990). The phosphorylated LHCII dissociates from PSII and associates with PSI thereby increasing absorption cross-section of PSI and distributing the energy to PSI leading to state II (Ruban and Johnson, 2009). When PSI is preferentially excited, the reduced PQ pool is converted in to oxidized PQ pool, which in turn activates an enzyme called thylakoid phosphatase TAP38/PPH1 which dephosphorylates LHCII (Pribil et al., 2010; Shapiguzova et al., 2010). The dephosphorylated LHCII unbinds from PSI and reassociates with PSII, enhancing the absorption cross-section of PSII, thereby leading to state I. Reversible lateral movement of phosphorylated LHCII from appressed grana region of thylakoids to non-appressed (stroma lamelle) region and its docking with PSI might leads to structural changes at thylakoid membrane (Danielsson et al., 2006; Iwai et al., 2008). LHCII phosphorylation and state

transitions have been extensively studied in the green alga *C. reinhardtii* (Wollman, 2001; Rochaix, 20011). In *C. reinhardtii*, the impact of state transitions on inter-photosystem energy balancing and on promoting cyclic electron flow is well established (Finazzi et al., 1999; Wollman, 2001; Eberhard et al., 2008). *stn7* is required for phosphorylation of several LHCII polypeptides (Depege et al., 2003; Bellafiore et al., 2005; Kargul et al., 2008; Pesaresi et al., 2009; Iwai et al., 2010a), thus providing further evidence that protein phosphorylation is essential for state transitions both in green algae and higher plants.

Though, it has been extensively studied in *C. reinhardtii*, state transition in *A. thaliana* is still unclear particularly LHCII phosphorylation. Some reports show that only a particular subunits of LHCII is migrating from PSII to PSI. The earlier reports shows that the entire trimer of LHCII is migrating towards PSI in state II (Kouril et al., 2005). In the present objective, we have investigated the components of LHCII which undergo phosphorylation and its relation on thylakoid membrane (Grana) organization.

3.2 RESULTS AND DISCUSSION

3.2.1 Morphological differences

We have observed the morphological differences between wt and *stn7*. There was a minor growth difference between the wt and *stn7* in all the stages from germination to reproduction. The growth of the *stn7* plants were slower than the wt, however, the mutant plants were as healthy as the wt plants (Fig. 3.1). In this chapter we have investigated the phosphorylation of major LHCII subunits, thylakoid proteomic changes by 2D analysis and thylakoid granal stacking organization by transmission electron microscopy (TEM) during state transitions.

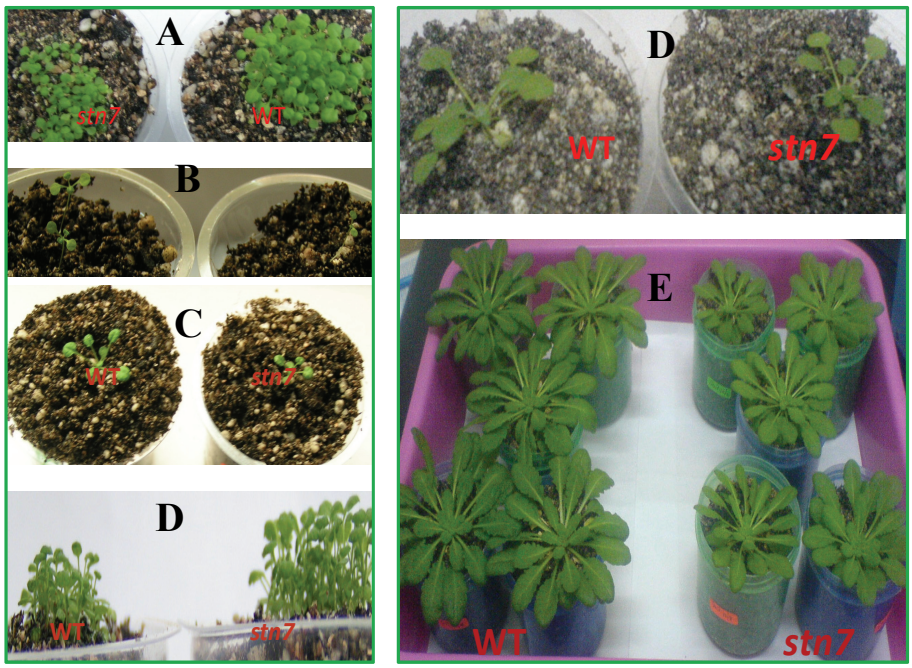


Fig. 3.1 Morphological differences at different growth stages of wt and *stn7* mutant of *A. thaliana*. 2 weeks after germination (A,B,C); 4 weeks old plants (D); 8 weeks old plants (E).

3.2.2 Analysis of state transitions

Fig. 3.2A. shows the Chl *a* slow fluorescence induction curves dissecting the changes in state I and state II light adapted conditions in wt and *stn7*. The extent of state transition was measured by using relative change in fluorescence (F_r) parameter. The F_r was calculated for wt and *stn7* plants from Chl *a* fluorescence induction curves (Fig. 3.2B). The F_r was found to be very low in *stn7* plants and high in wt plants. Thus, these results shows that, state transition mechanism is well operative in wt, however, *stn7* plants were unable to exhibit state transition since *stn7* gene is knocked out. Lunde et al (2001) reported that *psaH* mutant exhibited low Fr due to lack of state transitions showing psaH is involved in state transition. The drop in fluorescence is low when PSI light is turned on in wt, however, in *stn7* the drop is very high (Fig. 3.2A). The schematic representation of state I and state II adapted leaves were drawn in Fig. 3.2C,D.

The 77K Chl *a* fluorescence emission was also monitored for state I and state II adapted leaves in wt and *stn7* (Fig. 3.3). The earlier reports show that the red emission bands at 725 nm and 685 nm emanating from PSI and PSII, respectively in *A. thaliana* (Bellafiore et al., 2005; Nellaepalli et al., 2011). An increase in PSI (~725 nm) emission, relative to PSII (685 nm) was observed in state II adapted wt plants (Fig. 3.3A). This suggests that state transition mechanism is operating in wt leaves for energy distribution, whereas in *stn7* mutant no such significant changes in the PSI fluorescence (Fig. 3.3B), due to the absence of STN7 kinase activity. These results are in agreement with previously published data (Bellafiore et al., 2005; Kouril et al., 2005).

3.2.3 Phosphorylation of thylakoid membranes

The Tricine SDS-PAGE have shown that protein levels did not changes in 1 h illumination of plants with either state I or state II light in both wt and *stn7* (Fig 3.4A). Whereas, isolated

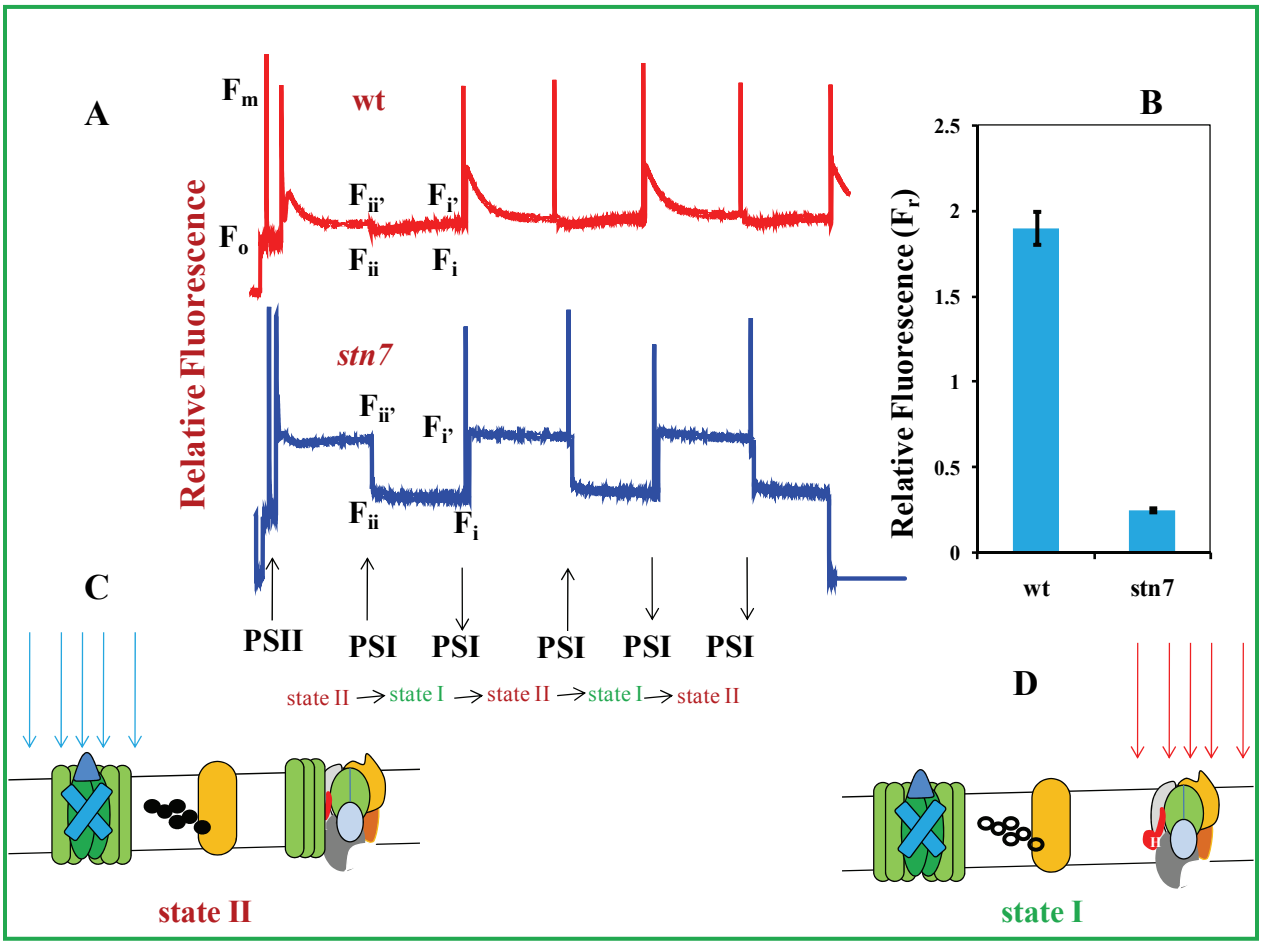


Fig. 3.2 Chl *a* fluorescence transients for analysis of state transition in wt (Red) and *stn7* (blue) (A). Leaves were dark adapted before measurement, saturating pulse was applied (for determination of F_m) for 1 sec before illuminating with either actinic light (state II light) or and with far red light (state I light). The relative change in fluorescence was calculated as $F_r=((F_{i'} - F_i)-(F_{ii'} - F_{ii}))/(F_{i'}-F_i)$, where F_i and F_{ii} designate fluorescence in the presence of PSI light in state 1 and state 2, respectively, while $F_{i'}$ and $F_{ii'}$ designate fluorescence in the absence of PSI light in state 1 and state 2, respectively (B). Schematic representation of thylakoid membranes under state I and state II light adapted condition (C,D).

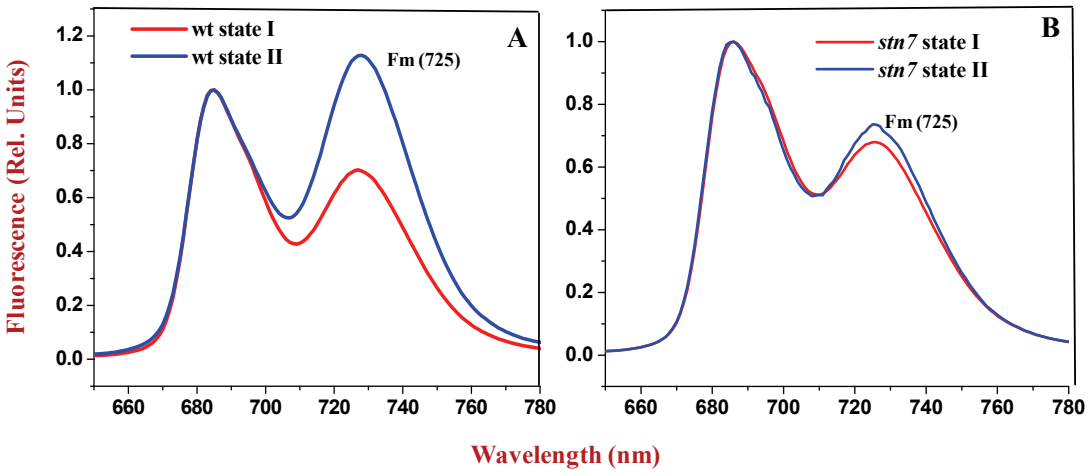


Fig. 3.3 77K fluorescence emission spectra of thylakoid membranes isolated from state I or state II adapted leaves of wt (A) and *stn7* (B). The spectra normalized at 685 nm.

thylakoids from preilluminated plants with either state I or state II light, have shown differential signals for phospho thylakoid membrane proteins when blotted with antiphospho-threonine antibody (from Cell Signaling Technology, UK) (Fig. 3.4B). We have observed three major signals which corresponds to phospho proteins of thylakoid membranes (Fig. 3.4B) i.e., CP43, D1/D2 and major LHCII. Similar pattern were observed when plants were preilluminated with low intensity light (30 μm m^{-2} s^{-1}) for different time periods and also in state I and state II adapted leaves (Tikkanen et al., 2010). In our study, LHCII underwent phosphorylation under state II and not in state I. However, CP43 and D1/D2 proteins were phosphorylated under both state I and state II light adapted conditions. At the same time *stn7* plants exposed to either state I light or state II light, did not show any signal of phosphorylated LHCII, however, signals for phosphorylated CP43 and D1/D2 proteins were observed. Earlier reports have demonstrated that apart from LHCII, PSII core proteins were also differentially phosphorylated in low-light conditions (Bonardi et al., 2005; Tikkanen et al., 2008). It has been reported that in high light treated leaves of *A. thalina,* phosphorylation of PSII proteins i.e, D1, D2, CP43, and PsbH were monitored (Vener et al., 2001). Thus, the differential phosphorylation may not be due to state transition, however, these changes might be playing a significant role in organization of reaction center either in state I or state II.

Later, the blots were also probed with Lhcb antibody, where it was exactly matching with the lowest band which was phosphorylated. Kouril et al (2005) showed from the electron micrograph that the entire trimer was migrated to PSI in *A. thaliana*, however, they did not show whether all trimeric subunits were phosphorylated. Thus, in our study, we also carried out immunoblotting analysis for determination of components of major LHCII which undergo phosphorylation.

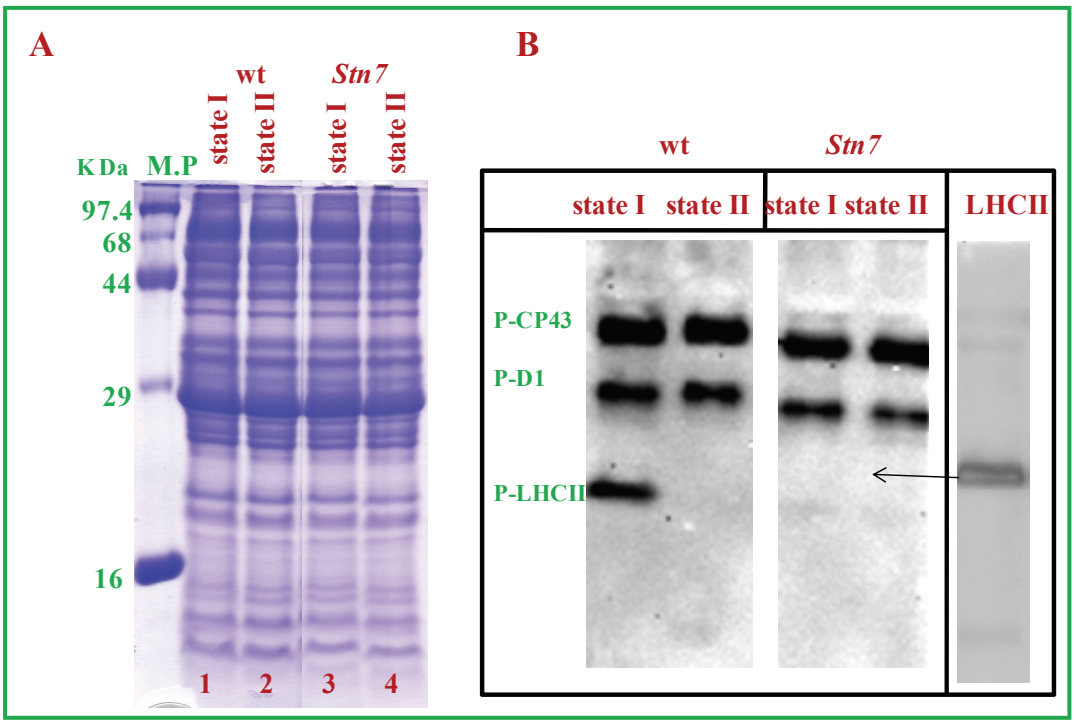


Fig. 3.4 Tricine SDS-PAGE showing protein profile of thylakoid membranes isolated from state I or state II induced wt and *stn7* plants (A). Immunoblotting of thylakoid membrane proteins probed with anti phopho threonine antibody. Thylakoid membranes isolated from state I or state II adapted leaves of wt and *stn7* plants, (B). Equal chlorophyll concentration was loaded on each lane. M.P, marker protein in kDa.

3.2.4 Phosphorylation of major LHCII

The isolated thylakoid membranes were transferred to PVDF membrane and the membranes were blotted with different major components of LHCII and antiphospho-threonine antibodies. The protein levels of Lhcb1, Lhcb2 and Lhcb3 of PSII in wt and *stn7* were found to be unaltered in state I and state II light adapted conditions (Fig. 3.5). Our study shows that Lhcb2 is the only component of major LHCII which underwent phosphorylation when the same membrane was blotted with Lhcb2 and antiphospho-threonine antibody.

3.2.5 Phosphorylation induced changes at 2D proteome level in state transition induced thylakoids

2D electrophoresis has revealed changes in the thylakoid proteins under state I and state II light exposed conditions. Thylakoids isolated from wt and *stn7* mutant plants preilluminated with either state I or state II light condition, have shown very similar pattern of 2D spots (Fig. 3.6 A-F). However, in the range of 25-27 kDa, we have observed three major spots (iso-forms) in state I preilliminated wt plants, where as in state II exposed condition, an additional spot was resolved as fourth spot (Fig. 3.6A,C and D).These isoforms are found to be Lhb2 (Fig. 3.6 inset). In *stn7* plants, three isoforms were found both in state I and state II light adapted leaves (Fig. 3.6B,E and F). 2D gels were probed with antiphospho-threonine antibody where 27 kDa protein spots were phosphorylated; however, this observation was not seen in state I. It has been reported that Lhcb2.1, Lhcb2.2, Lhcb2.4, Lhcb1.1, Lhcb1.2 and Lhcb3 were phosphorylated at threonine residues (Fristedt and Vener, 2011). Whereas, Lhcb3 did not show phosphorylation. However, the PSII light harvesting protein Lhcb3 also affects the macrostructure of PSII and the rate of state transitions in *A. thaliana* (Damkjaer et al., 2009). Recently, it was shown that the

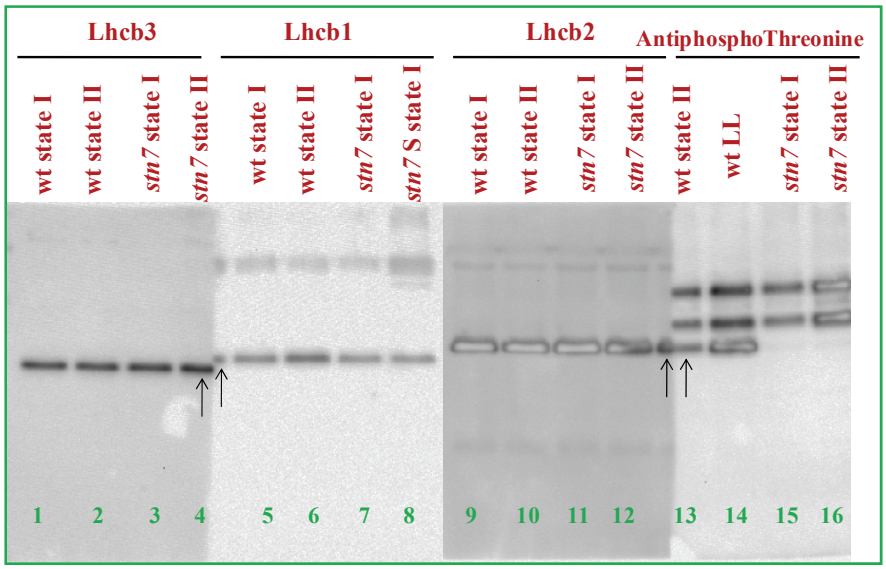


Fig. 3.5 Immunoblotting analysis of thylakoid membranes isolated from state I or state II adapted leaves probed with components of major LHCII antibodies such as Lhcb1 (lanes 1,2,3 and 4), Lhcb2 (lanes 5,6,7 and 8), Lhcb3 (lanes 9,10,11, and 12) and antiphospho-threonine (lanes 13,14,15 and 16) in wt and *stn7*. Pictures were taken with same pixel size in order to compare their molecular weight and it is clear that all major proteins of Lhcb1,2 and 3 were migrated different positions. The arrow marks indicated show differential migration of LHCII subunits and the status of phosphorylation.

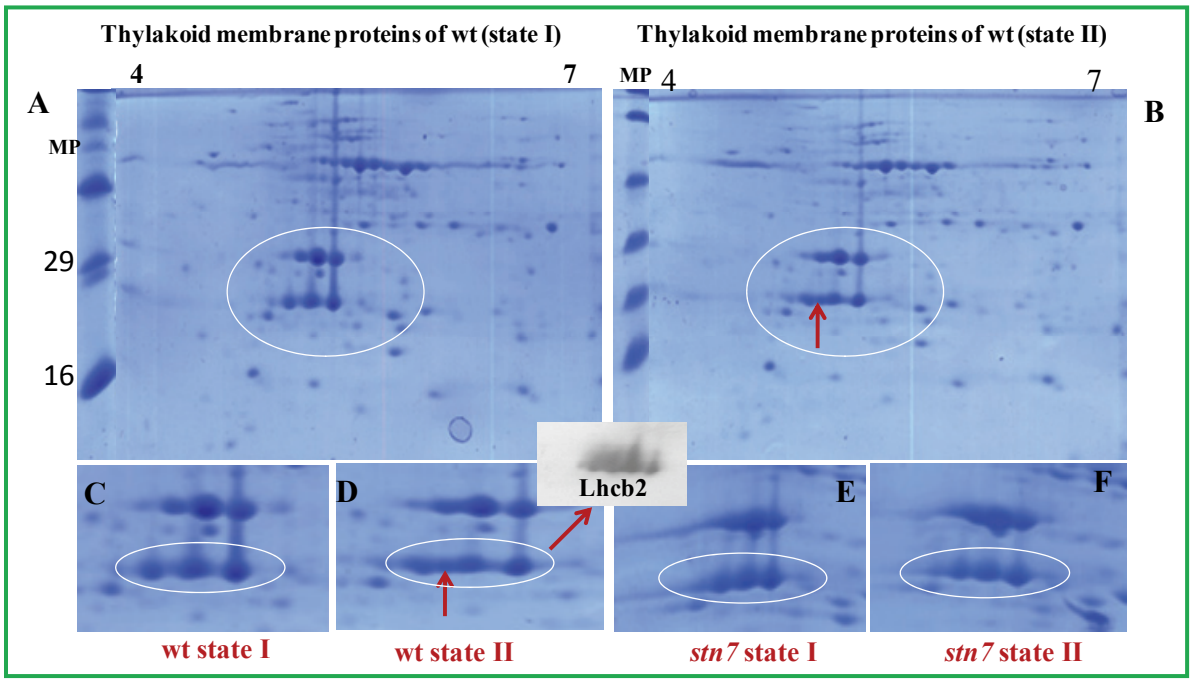


Fig. 3.6 2D gel showing (PI of 4-7) thylakoid membrane protein profile of state I or state II preilluminated leaves of wt and *stn7* (A,B). The spots in the white circle of Fig A and Fig B show the differences in state I and state II thylakoid membrane proteins. The circles in Fig A and Fig B are zoomed and represented as Fig C (state I) and Fig D (State II) of wt, respectively. Fig E and Fig F are state I and state II of *stn7*. Immunoblot of Lhcb2 containing four isoforms is represented in gray colour box. The spots corresponds to Lhcb2 isoforms in the circle of Figs C,D,E and F. In state II of wt, there was four isoforms, however, in state I of wt, state I and state II of *stn7* showed three isoforms. M.P stands for marker protein in kDa.

disassembly of PSII supercomplex in *A. thaliana* required STN7 dependent phosphorylation of CP29, which is component of minor LHCII (Fristedt and Vener, 2011).

Further, to identify the migration of LHCII from PSII to PSI, PSI-LHCI super complex was isolated.

3.2.6 Structural changes in PSI-LHCI supercomlex under state I and state II

Sucrose density gradient ultracentrifugation was carried out for isolation of PSI-LHCI supercomplex to examine the migration of LHCII which is involved in state transition to PSI supercomplex. At the end of the spin for 16 h we have observed 3 major fractions in all the samples i.e wt and *stn7* mutant and both in state I and state II light adapted conditions. Fig. 3.7A shows that the sucrose density gradient separation of PSI-LHCI supercomplexes. The upper fraction, F1, contains LHCII and PSII complexes. F2 majorly contains PSII-LHCII and F3 contain the PSI-LHCI supercomplex (Subramanyam et al., 2006). The absorption spectra of F1 have three major peaks at 673 nm and 652 nm in red region and 437 nm and 471 nm in blue region, which is the characteristic spectral feature of LHC (Fig. 3.7B). The F2 band shows two major peaks at 676 nm, 437 nm and minor peaks at 469 nm which corresponds to enriched PSII and small fraction of PSI and F3 shows major peaks at 680 nm and 438 nm and minor peaks at 469 nm and 496 nm which is the characteristic absorption spectra of PSI-LHCI supercomplex. The absorption maximum of the F1, F2 and F3 were 673 nm, 676 nm and 680 nm (Fig. 3.7B). Similar reports were observed in Haldrup, 2003 and Subramanyam et al., 2006. The densitometry analysis was made for the fractions that were obtained in Fig. 3.7A. There was no change in F2 and F3 content in state I of wt, however, F3 content was higher than F2 in state II induced wt plants (Fig. 3.7C). The increased density of F3 in state II was may be due to migrated LHCII from PSII to PSI. In *stn7*, there was no significant difference in F2 and F3 of state I and

state II due to lack of ability to undergo state transitions (Fig. 3.7C). These results clearly show that some of the PSII subunits are moving to PSI, which probably LHCII. These results have shown that LHCII proteins have migrated from PSII to PSI (Fig. 3.7C). Tricine SDS-PAGE was carried out for F3 of stateI and stateII in wt. These results have shown that LHCII is associated with PSI-LHCI supercomplex (Fig. 3.7D). By performing immunoblotting we could able to identify the major components of LHCII i.e. Lhcb1, Lhcb2 and Lhcb3 were identified in F3 of state II in wt. Also, from isolated PSI-LHCI complexes we have clearly demonstrated by using antibodies that the major subunits of LHCII i.e. Lhcb1, 2 and 3 were associated with PSI supercomplex which indicates that these subunits were migrated from PSII in state II light adapted state (Fig. 3.7E). In previous section (Fig. 3.4) showed that only Lhcb2 was phosphorylated in state II, this indicates that the phosphorylated Lhcb2 is the signal to migrate major subunits LHCII towards PSI.

Thus, our study show that phosphorylation of Lhcb2 dislocates the total major LHCII from PSII and associates with PSI-LHCI. It has been reported that, three LHCII polypeptides were associated with a PSI–LHCI supercomplex only in state II and identified them as two minor monomeric LHCII proteins (CP26 and CP29) and major LHCII protein type II, or LhcbM5 in *C. reinhardtii* (Takahashi et al., 2006). These three LHCII proteins, in addition to the major trimeric LHCII proteins were phosphorylated upon transition to state II in *C. reinhardtii.* In *A. thaliana*, in the LHCII trimer, the N-terminal threonine residues of the Lhcb1 and Lhcb2 proteins are reversibly phosphorylated (Fristedt and Vener, 2011) whereas Lhcb3 is not a phosphoprotein. Three minor Chl-binding proteins- Lhcb4 (CP29), Lhcb5 (CP26), and Lhcb6 (CP24) are monomeric, and of these proteins only Lhcb4 is a phosphoprotein in higher plants (Bergantino et al., 1995, 1998). In *C. reinhardtii*, CP26, CP29, and LhcbM5, which have been viewed as

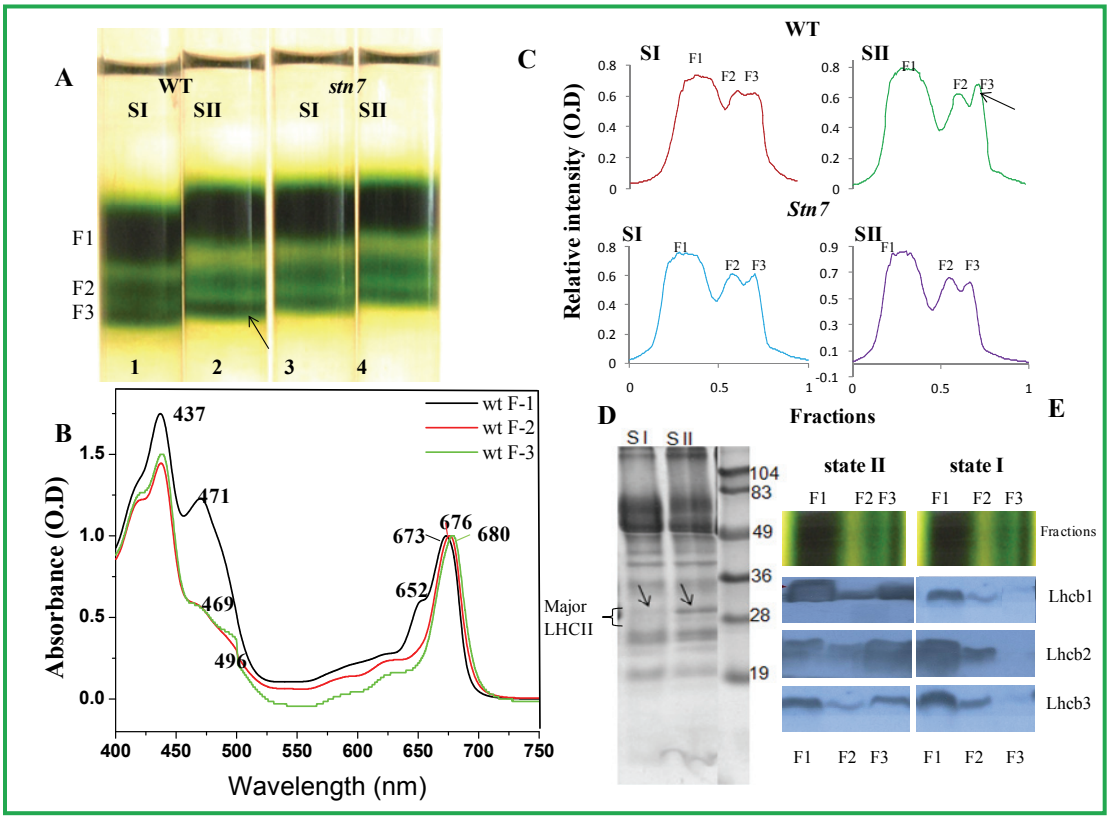


Fig. 3.7 Density gradient ultracentrifuge tubes showing different fractions (F1-F3) of state I or state II thylakoid membranes of wt and *stn7*. Leaves were pre-exposed to state I or state II light condition before isolation of thylakoid membranes (A). Absorption spectra of fractions (F1-F3) collected from Fig. 3.7A (B). Densitometry analysis of fractions obtained from Fig. 3.7A (C). Tricine SDS-PAGE of PSI-LHCI supercomplex isolated from state I or state II of wt plants (D). Immunoblot analysis of PSI-LHCI supercomplex isolated from state I or state II light conditions in wt with LHCII antibodies (Lhcb1, Lhcb2 and Lhcb3) (E).

belonging solely to the PSII complex, are now postulated to shuttle between PSI and PSII during state transitions, thereby acting as docking sites for the trimeric LHCII proteins in both PSI and PSII (Takahashi et al., 2006). The same group demonstrated that CP29, not CP26, is an essential component in state transitions and demonstrates that CP29 is crucial when mobile LHCIIs re-associate with PSI under state II conditions (Tokutsu et al., 2009).

3.2.7 Circular dichroism analysis of PSI-LHCI supercomplexes

The visible CD spectroscopy is a sensitive technique of intramolecular pigment–pigment interactions and/or pigment–protein interactions. In the Qy region, there are two negative peaks at 645, 678 nm and one positive peak at 668 nm (Fig. 3.8), similar to previous reports (Bassi and Simpson 1987; Subramanyam et al., 2006). The two major bands at 664 and 678 nm are due to Chl dimers caused by the excitonic interactions of Chl a in PSI–LHCI supercomplexes, while the negative peak at 645 nm is the characteristic of Chl *b* (Bassi and Simposn, 1987). In the soret region, the positive band peaking at 443 nm originates from Chl *a*, while the negative peak at 460 nm is characteristic of Chl *b* (Fig. 3.8). The CD signals is slightly increased at 678, 664 nm and 645 nm in state II of PSI-LHCI supercomplexes. However, no change in the CD signals in *stn7* mutant of state I or state II adapted PSI-LHCI supercomplexes. Thus, this increase in CD signal could be due to migration of PSII pigment complexes to PSI-LHCI supercomplexes. Similar results were shown in anaerobic induced state transition in *C. reinhardtii* (Subramanyam et al., 2006).

3.2.8 Phosphorylation induced changes at thylakoid membrane stacking

Transmission electron micrographic studies were carried out inordeer to observe the effects of phosphorylation of LHCII on the thylkoid membrane stackings. The electron micrograph pictures of thylakoid membranes of state I and state II adapted leaves in wt and *stn7* are depicted

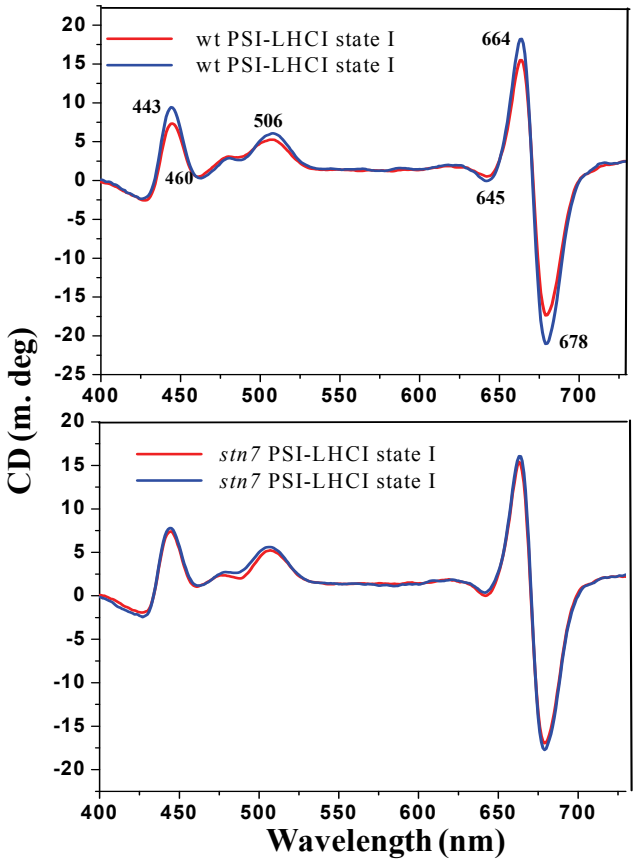


Fig. 3.8 CD analysis of PSI-LHCI supercomplex isolated from state I or state II light adapted conditions from wt and *stn7*. Leaves were pre-illuminated with either state I or state II light before isolation of PSI-LHCI supercomplex..

in Fig. 3.9. In state II, the dimension of the grana stacking have been increased in state II adapted leaves (Fig. 3.9A). Phosphorylation of LHCII leads to loosening of granal stacking for easy migration of LHCII. Whereas in case of *stn7*, the difference between granal thylakoid stacking in state I and state II was negligible. These observations made us to conclude that during state II, loosening of granal stacking makes LHCII to move from grana to stroma thylakoids. This would not happen in mutant in which phosphorylation of LHCII is arrested. Statistical analysis of granal thylakoid stacking analysis has been shown in Fig. 3.9E that the clear difference between state I and II. These observations made us to conclude that phosphorylation of major LHCII have effect on thylakoid membrane organization in state II light conditions. A similar report was observed recently and it has been suggested that reorganization of the thylakoid membranes involves fission and fusion events that occur at the interface between the appressed (granal) and nonappressed (stroma lamellar) domains (Chuartzman et al., 2008). Vertical and lateral displacements of the grana layers presumably follow these localized events, eventually leading to macroscopic rearrangements of the entire membrane network. Thus, our results support that increased granal dimension was due to macroscopic rearrangement of grana membrane which might enhance LHCII mobilization from grana to stroma lamellae. Fig. 3.10 shows the cartoon representation of phosphorylation induced changes in grana thylakoid membranes adapted to state II or state I light condition. The phosphate groups of LHCII (P-LHCII) of neighboring thylakoid grana membranes repel each other in state II adapted condition in wt. Consequently, there are numerous changes occur in thylakoid membranes which facilitate migration of LHCII from grana to stroma lamellae.

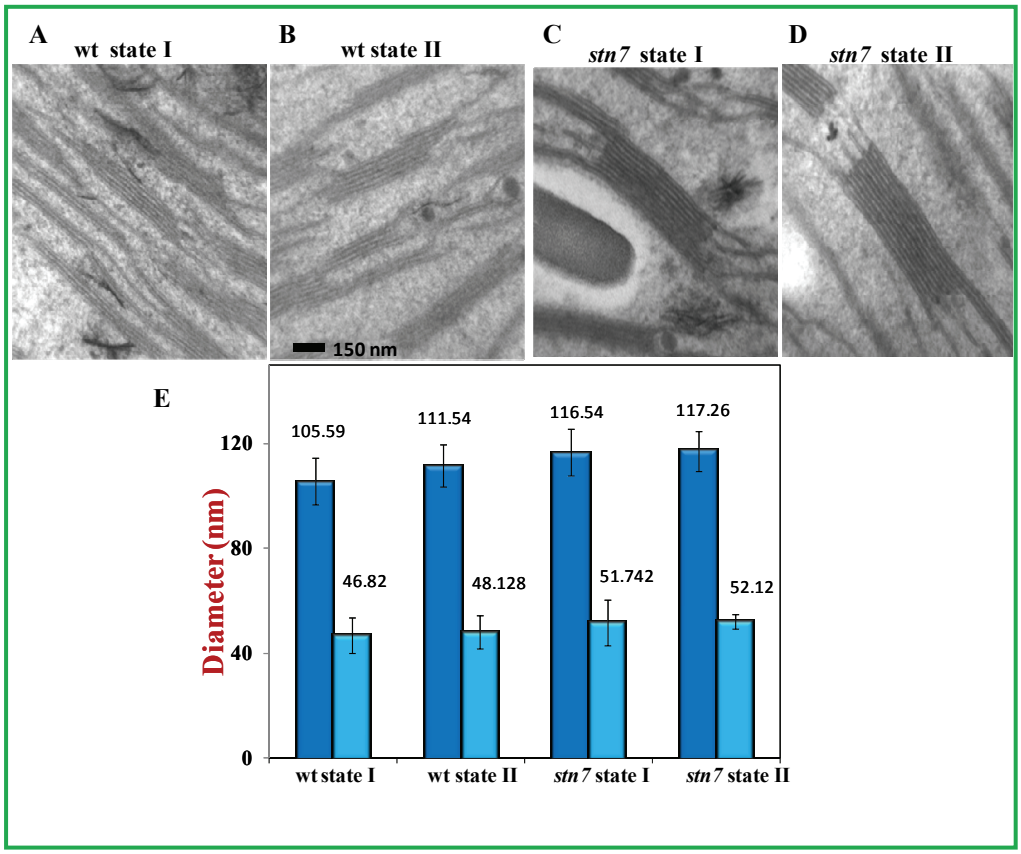


Fig. 3.9 Transmission electron micrographs of thylakoid membranes of plants pre-illuminated to state I or state II light. Granal stacks of state I adapted leaves in wt and *stn7* (A,C), granal stacks of state II adapted leaves in wt and *stn7* (B,D), statistical analysis of granal stacking (E).

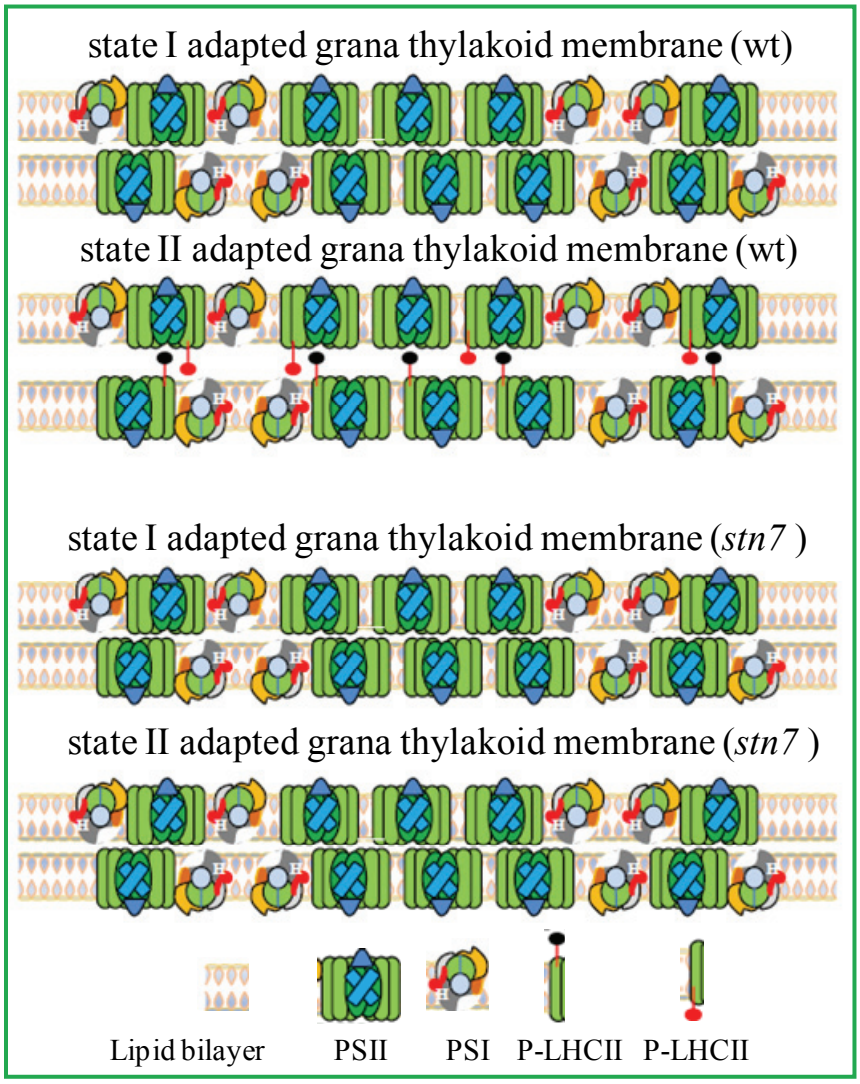


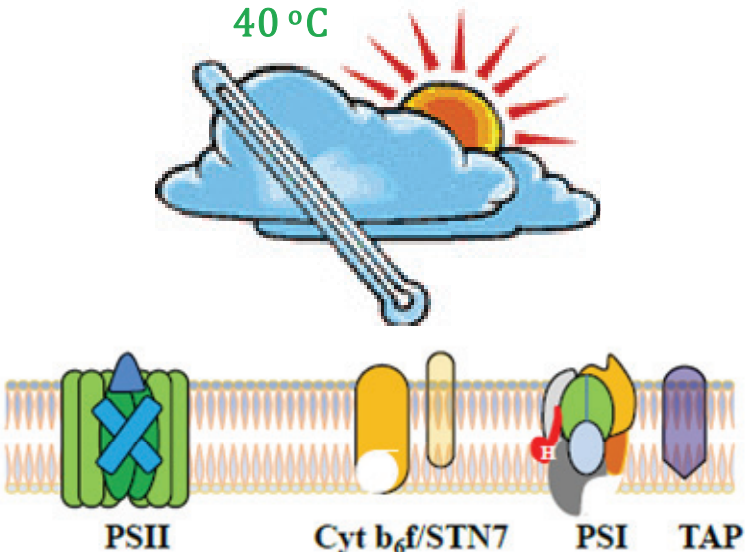
Fig. 3.10 Schematic representation, showing phosphorylation (P-LHCII) induced changes in granal stacking of state II or state I adapted conditions of wt and *stn7*. The repulsion between phosphate groups of P-LHCII present in the neighboring granal thylakoid membranes makes the grana to loosen for migration of P-LHCII from grana to stroma lamellae in wt. There was no phosphorylation of LHCII in *stn7*. Phosphate groups are shown in red and black circles of neighboring granal thylakoid membranes.

3.3 CONCLUSIONS

The mechanism of state transition is an important process that exists in oxygenic photosynthetic organisms for optimization of photosynthetic performance. LHCII is involved in energy distribution between PSII to PSI. Phosphorylation of LHCII was observed in state II adapted leaves of wt and absent in *stn7* mutant. In this study we report, Lhcb2 is one of the component of LHCII which undergo phosphorylation. LHCII was found to be associated with PSI-LHCI supercomplex in state II adapted condition. These results confirms that phosphorylation of Lhcb2 is enough for migration of major LHCII from grana enriched PSII to stroma enriched PSI supercomplex. However, in *stn7* mutant the same mechanism was found to be absent. CD spectra of PSI-LHCI have shown increased peaks at 679, 663 and 645 nm due to more pigment association of LHCII migrated from PSII. The height of the granal stacking has been increased due to repulsion of negative charge of phosphate groups of LHCII in state II adapted grana thylakoids for mobilization of LHCII from grana to stroma.

Characterization of moderate temperature induced state transitions

Chapter 4



4.1 INTRODUCTION

Elevated temperature affects the structural and functional organization of thylakoid membranes (Cseh et al., 2000), also affects excitation energy transfer, distribution and dissipation apart from effects on the photochemical reaction (Weis, 1985). The energy distribution between PSI and PSII for optimal photosynthesis has been shown to be affected by moderate to high temperature (Sane et al., 1984; Weis, 1985; Sundby and Andersson, 1985; Mohanty et al., 2002). These studies were focused on demonstrating the effects of elevated temperature on energy distribution, however, the moderate temperature on state transition mechanism remain uncharacterized. A more detailed explanation for elevated temperature induced changes on photosystems has been given in section 1.7.

In this study, attempts have been made to dissect and characterize the effect of moderate temperature stress on state transition in higher plants using *A. thaliana* and its mutant *stn7*, in which expression of *stn7* gene is blocked responsible for synthesis of STN7 kinase enzyme. Our results indicate that moderate temperature elevation under dark conditions induces phosphorylation and migration of LHCII similar to that of light-induced state transitions, suggesting that LHCII phosphorylation can also be thermally-triggered.

4.2 RESULTS AND DISCUSSION

We have designed our experiments on *A. thaliana* wt and *stn7* (expression of *stn7* gene is blocked) mutant to assess state transitions in elevated temperature (40 ^{o}C). However, we note that no significant differences were observed in the fast fluorescence transients (OJIP) upon heating from 25 ^{o}C (ambient temperature) to 35 ^{o}C . For our present study we have exposed the plants to 40 ^{o}C for a short duration (0 to 60 min) to characterize temperature-induced state

transitions and LHCII phosphorylation. All the experiments presented here were repeated more than three times independently and obtained identical results.

4.2.1 State transitions at ambient 25 ^{o}C temperature

The 77K Chl *a* fluorescence emission was monitored either in state I or in state II (Fig. 4.1). An increase in PSI (725 nm) emission, relative to PSII (685 nm) was observed in state II adapted wt plants (Fig. 4.1A). This suggests normal state transitions in wt leaves, whereas in *stn7* mutant no such significant changes in the PSI fluorescence as a result of state I/state II light adaptation were observed (Fig. 4.1A,B), due to the absence of STN7 kinase activity. These results are in agreement with previously published data (Bellafiore et al., 2005; Kouril et al., 2005).

4.2.2 Effects of elevated temperature on PSI absorption cross-section in wt and *stn7* plants

Exposure of detached leaves to 40 ^{o}C for varying time periods caused alteration in the 77K emission spectra of thylakoid membranes isolated from wt plants, but not in the *stn7* mutant sample. Upon exposure to heat treatment, wt plants showed a progressive increase in the F725 band (originating from PSI). To quantify the increase in PSI fluorescence, the spectra were normalized at 685 nm (the PSII emission peak) (Fig. 4.2A,B). The shape of the 77K spectra of *stn7* mutant at 40 ^{o}C showed similar spectra like wt and unlike in wt, the increase in duration of heat treatment did not show any progressive enhancement of PSI emission peak at 725 nm (Fig. 4.2C).

The relative ratio of the F685 (PSII) to the F725 (PSI) is a useful marker to study the extent of state transitions linked to migration of LHCII (Bonaventura and Myers, 1969). Fig. 4.2B exhibits the increase in F725/F685 ratio from 0.8 to 1.2 after heat treatment, while this ratio did not change with increase in duration of heat treatment in the *stn7* mutant (Fig. 4.2D). According to the classical state transition model, the phosphorylation of LHCII proteins causes migration

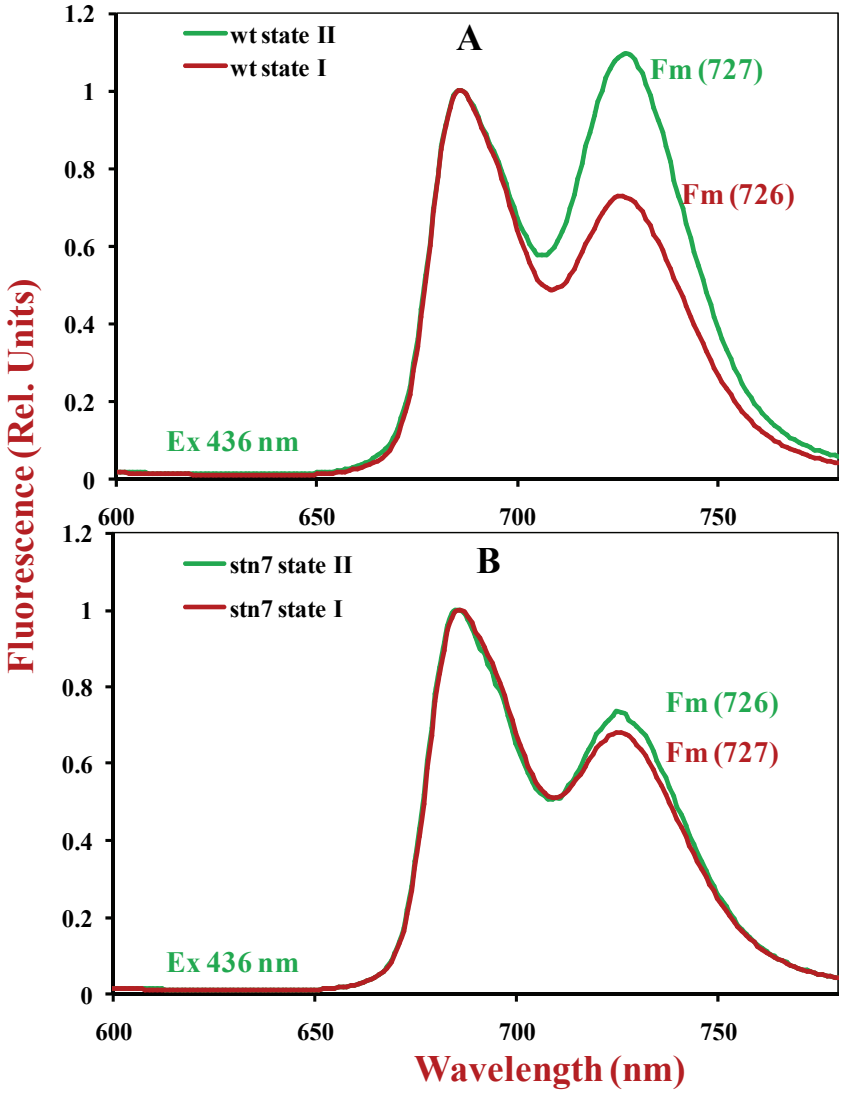


Fig. 4.1 77K chlorophyll *a* fluorescence emission spectra of thylakoid membranes isolated from *A. thaliana* leaves, preilluminated with either state I or state II light . (A) wt (B) *stn7*. Plants were dark adapted overnight before acclimating to state I or state II light. Plants were pre-illuminated with red light (λ = 660 nm) for 45 min to obtain state II and pre-illuminated with far red light (λ = ~700 nm) for 45 min. Spectra normalized at 685 nm.

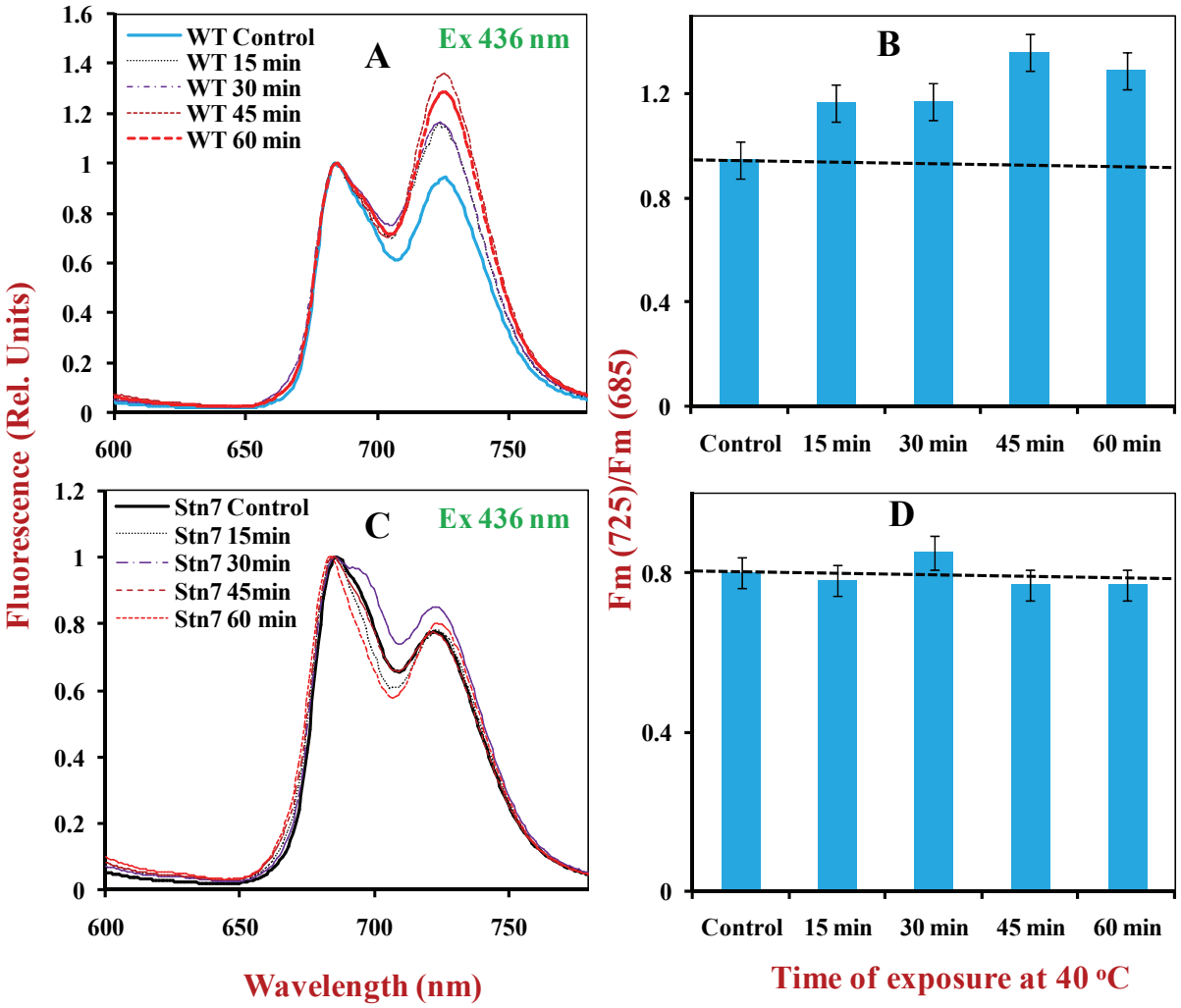


Fig. 4.2 77K chlorophyll *a* fluorescence emission spectra and F725/F680 ratio of thylakoid membranes of *A. thaliana* and its mutant *stn7* leaves treated at 40 °C for different time periods (0 to 60 min) (spectra normalized at 685 nm). A and B: wt; C and D: *stn7* mutant.

from grana region, thus, decreasing the antenna size (absorption cross section) of PSII and the phosphorylated LHCII antennae associate with PSI, enhancing long-wavelength emission at 77K. Thus, our results demonstrated that enhancement of PSI to PSII emission by heat treatment in dark conditions in wt *A. thaliana* were due to state transition-type migration of LHCII and consequent resulting change in the relative absorption cross-sections of PSI and PSII, similar to light-induced state transition events. However, these changes were not observed in the mutant *stn7.*

4.2.3 Phosphorylation of LHCII under elevated temperatures

The effects of 40 ^{o}C heat stress on the phosphorylation of thylakoid membrane proteins were investigated by immunoblotting. Fig. 4.3A shows the immunoblot of thylakoid membrane proteins isolated from leaves exposed to varying periods of temperature treatment, probed with an antiphospho-threonine antibody. Differential phosphorylation of CP43 and D1/D2 was not observed after heat treatment in both wt and *stn7* mutant at different time intervals. However, differential phosphorylation of LHCII was observed in wt. The LHCII phosphorylation signal was detected during 20, 40 and 60 min of moderate temperature treatment, and the phosphorylation signal was absent in 0 min sample. In contrast, similar heat treatment for 20, 40 and 60 min exhibited no phosphorylation of LHCII in *stn7* mutant (Fig. 4.3A). These results demonstrate the involvement of STN7 kinase in heat-induced phosphorylation of LHCII. While CP43 and D1/D2 showed minimal phosphorylation under the conditions investigated, the degree of phosphorylation of these proteins was not affected significantly by heat treatment. Earlier reports have demonstrated that apart from LHCII, PSII core proteins were also differentially phosphorylated in low light conditions (Tikkanen et al., 2006; Bonardi et al., 2005). However, under dark ambient condition also phosphorylation of CP43, D1 and D2 was observed (Tikkanen

et al., 2006; Tikkanen et al., 2008). These results indicate that the enzyme responsible for LHCII phosphorylation by heat treatment is also the same STN7 kinase.

Fig. 4.3B shows the immunoblot probed with major LHCII antibodies. The expression profile of major LHCII proteins, namely, Lhcb1, Lhcb2, Lhcb3 are almost remained identical after heat treatment for different periods of time from 0 to 60 min in both wt and *stn7* mutant. However the extent of LHCII phosphorylation was enhanced as exposure time increased in wt (Fig. 4.3A). These data indicated that the relative increase of PSI emission (Fig. 4.2A) after heat treatment was owing to phosphorylation of LHCII. As the *stn7* plants lacked the ability to phosphorylate LHCII by STN7 kinase, heat stress could not induce changes in PSI and PSII fluorescence characteristics. Recent reports suggested that, under low-light conditions, when excitation energy transfer from LHCII to reaction centers is efficient, the STN7-dependent LHCII protein phosphorylation guarantees a balanced distribution of excitation energy to both photosystems (Depege et al., 2003; Bellafiore et al., 2005; Tikkanen et al., 2010). Similarly, results of this study indicate that at moderately elevated temperature (40 ^{o}C), the STN7 kinase was found to be active, and possessed the ability to phosphorylate LHCII thereby bringing about an increase in the absorption cross section of PSI complex.

In order to determine the earliest onset of LHCII phosphorylation, wt plants were exposed to moderate temperature at 40 ^{o}C from 0 to 10 min. Interestingly, the phosphorylation of LHCII was detected within 2 min and reached maximum after 10 min heat treatment (Fig. 4.3C). Previous reports demonstrated that, under state II conditions, LHCII phosphorylation increased with increasing period of red light illumination. The half time (t½) of the activation of STN7 kinase (previously known as LHCII kinase) was estimated to be approximately 4–5 min (Vener at al., 1995; Forsberg and Allen, 2001), which is similar to the t½ for saturating LHCII

phosphorylation (Su and Shen, 2005). In this study, LHCII was phosphorylated within 2 min at 40 ^{o}C (Fig. 4.3C). This indicates that phosphorylation of LHCII is one of the initial responses to temperature stress in *A. thaliana*. The above results revealed that the signal for phosphorylation of LHCII may be perceived by STN7 kinase at the initial onset of heat exposure. In order to examine the dephosphorylation of temperature-induced phosphorylated LHCII, we cooled the temperature-treated *A. thaliana* leaves back to room temperature (25 ^{o}C). Upon cooling, dephosphorylation of LHCII (i.e. the decrease in LHCII phosphorylation) was observed, as in a transition back to state I. Unlike the regular light-induced state transition mechanism, it took a minimum of 2 h for dephosphorylation of LHCII when the heat treated leaves were brought to room temperature. We report here that the relaxation of LHCII phosphorylation was slow and was monitored up to 30 min of exposure (Fig. 4.3C), while the complete relaxation of LHCII phosphorylation required more than 2 h. Hence we conclude that temperature induced state transition in dark is also a short term adaptive change but the relaxation of LHCII phosphorylation (nothing but dephosphorylation, state I) is slower than the regular light induced state transitions linked LHCII dephosphorylation.

4.2.4 Role of redox carriers on LHCII phosphorylation

Electron transport inhibitors like DCMU and DBMIB are known to alter the redox poise of the PQ pool in light. To check the signal processing mechanism in temperature induced state transition, we used these two inhibitors and monitored the phosphorylation levels of LHCII by moderate temperature treatment. DBMIB is an inhibitor of reoxidation of PQH_2 molecules by Cyt b_6/f (Schansker et al., 2005) and DCMU blocks the PQ binding site of PSII, disallowing the electron flow from PSII to PQ pool (Papageorgiou et al., 1968; Tóth et al., 2005). It is well known that in normal state transitions, DCMU block PQ pool reduction, and consequently

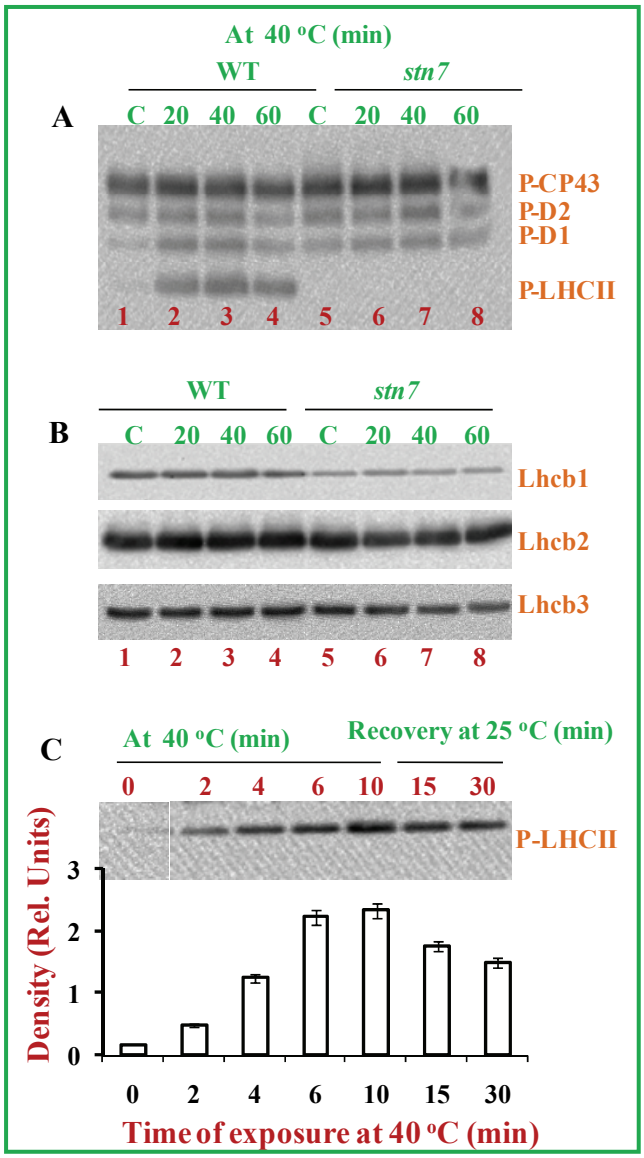


Fig. 4.3 Immunoblotting analysis of thylakoid membranes isolated from temperature-exposed *A. thaliana* leaves of wt (lanes 1-4) and *stn7* mutant (lanes 5-8) for different time periods (0 to 60 min) with antiphospho-threonine antibody (A). Immunoblot of thylakoid membrane proteins isolated from temperature exposed leaves for different time periods probed with major light harvesting complex protein antibodies (Lhcb1, Lhcb2 and Lhcb3), wt, lanes 1-4; *stn7*, lanes 5-8 (B). Immunoblot of thylakoid membrane proteins probed with antiphospho-threonine antibody (highlighted only LHCII) and its density graph, isolated from leaves exposed to short-term temperature treatment (0 to 10 min) and its recovery at 25 ^{o}C for 15 and 30 min (C).

phosphorylation of LHCII and its migration to PSI. In our experiments, we used these inhibitors to examine the effect of the PQ pool redox state and Cyt b_6/f at elevated temperatures. Using inhibitors such as DBMIB would help to examine role of Cyt b_6/f in STN7 kinase activation and determine whether STN7 kinase is being activated by another route. The LHCII phosphorylation has been observed in light and temperature induced state transitions in control samples (no inhibitor) (Fig. 4.4 Lanes 1, 2 and 7). In DCMU treated samples, the phosphorylation of LHCII is blocked in light induced state transition (Fig. 4.4A, lane 4), however, it is unable to block the phosphorylation of LHCII in temperature induced state transition (Fig. 4.4A, lane 9). These results show that reduction of PQ pool occurs non-photochemically by stromal reductants, which may trigger LHCII phosphorylation. However, in light induced state transition, DBMIB was unable to block fully the LHCII phosphorylation (Fig. 4.4A, lane 6). Also the extent of phosphorylation of LHCII in heat treated DBMIB leaves is lower when compared to heat treated samples (Fig. 4.4A, lane 11). The 77K emission spectra results with DBMIB at 40 ^{o}C (in dark) have shown no significant difference with control samples (in dark at room temperature) whereas at 40 ^{o}C, dark treated leaves have shown higher PSI fluorescence emission (Fig. 4.4B). This result shows that the involvement of Cyt b_6/f in STN7 kinase activation and subsequent phosphorylation of LHCII under temperature induced state transition. In addition, the moderate temperature treatment may enhance the chlororespiration which leads to activation of STN7 kinase and subsequently phosphorylation of LHCII. Our results are in agreement with previous reports on elevated temperature induced chlororespiration (Havaux, 1996). It is well know that, in light-induced state transitions, both the PQ pool and Cytb_6/f are known to be triggering signals for the activation of STN7/STT7 kinase (Zito et al., 1999; Lemeille et al., 2010). We also checked the infiltration of inhibitors to the target sites of the leaves by using OJIP transients (Fig.

4.4C). The disappearance of IP phase after DBMIB treatment indicates that under saturating light conditions the I phase approached the maximum fluorescence level and it reflected a signature of effective infiltration of DBMIB (Schansker et al., 2005) (Fig. 4.4C). In DCMU-treated leaves, a single induction phase is observed and the F_m reached after 2 ms (=J-step) (Tóth et al., 2005) (Fig. 4.4C). Thus, we conclude from these results that the temperature-induced signaling pathway of STN7 kinase activation is dependent on redox state of Cyt b_6/f similar to light triggered state transition mechanism.

4.2.5 Primary photochemistry and the redox poise of PQ pool under elevated temperature

Apart from LHCII phosphorylation, we also assessed the extent of moderate heat induced alterations at PSII level by using fast Chl *a* OJIP fluorescence transients in *A. thaliana*. Several studies have shown that OJIP fluorescence transients can be used to monitor heat induced photosynthetic damage in plants (Srivastava et al., 1997; Tóth et al., 2007). The OJIP fluorescence transient represents the successive reduction of acceptor pools of PSII (Govindjee, 1995). The O-J raise phase is linked to Q_A reduction (Lazar, 1999), the raise from J-I phase in the transient is due to electron transfer from Q_A^- to Q_B^- and subsequent Q_A^- to Q_B^{2-} (Strasser et al., 1995). Thus O to P raise in the fluorescence yield reflecting the variable fluorescence raise, F_v is due to reduction of the PQ pool. Fig. 4.5 exhibits the raise in fluorescence from O to P for different dark heat-treatment periods from 0 to 60 min. As the duration of heat treatment was increased, the raise in path of the fluorescence transient was altered and maximal fluorescence yield (F_{max}) at P was decreased. This result was expected because of partial inactivation of PSII by heat treatment. Furthermore, heat exposure affected both the F_o and F_m levels of the transients. Where F_o is the minimal fluorescence, when all PSII reaction centers are open (at t=0) and F_m is the maximal fluorescence, when all PSII reaction centers are closed. The F_o level

increased with the time of exposure while the F_m level decreased (Fig. 4.5 C,D), suggesting a small decrease in the photochemical yield of F_v/F_m from 0.8 to 0.74 (Fig. 4.5 E). It can be denoted analytically as: increasing F_o = ABS kF/(kP+kN) and decreasing F_m=ABS kF/kN as the temperature increases. Decrease of F_v/F_o from 1 to 0.62 and therefore an increase of kN from 1 to 1.13 and a decrease of kP from 1 to 0.69 while heating from 0 to 60 minutes. Thus, OJIP curves show clearly that the heating of 60 minutes is increasing kN for about 13% while kP is decreasing for 31 %. The increase in F_o level by the moderate heat treatment could be ascribed to back transfer of electron from the secondary Q_B^- to the stable primary acceptor Q_A of PSII (Ducruet, 1999). Similar results were reported earlier, wherein at elevated temperatures the PQ pool is reduced in dark by stromal reductants (Srivastava et al., 1997).

The minor decrease in the F_m level could also be due to decrease in PQ pool, even though the extent of changes was quite small. The dark heat treatment likely induced heterogeneity in intersystem electron transport carriers in photosynthetic electron transport chain (Bukhov and Carpentier, 2000), thus causing a change in the cellular redox poise. Fig. 4.5 shows OJIP fluorescence transients normalized at F_o level, in order to demonstrate the relative changes at the J, I and P phases. Marginal decrease of all the phases of fluorescence transient raise indicates partial decrease in electron transport from PSII to PSI during 1 h heat treatment. The above results with OJIP fluorescence transients studies have shown that the altered redox state of the PQ pool by moderate temperature elevation was noticeable, but these changes did not influence the temperature-induced state transitions (Fig. 4.2 and 4.3).

Apart from 40 ^oC, we have also monitored changes in OJIP transients of 35 ^oC and 45 ^oC treated leaves. We have found no difference in all the phases of fluorescence induction curve of

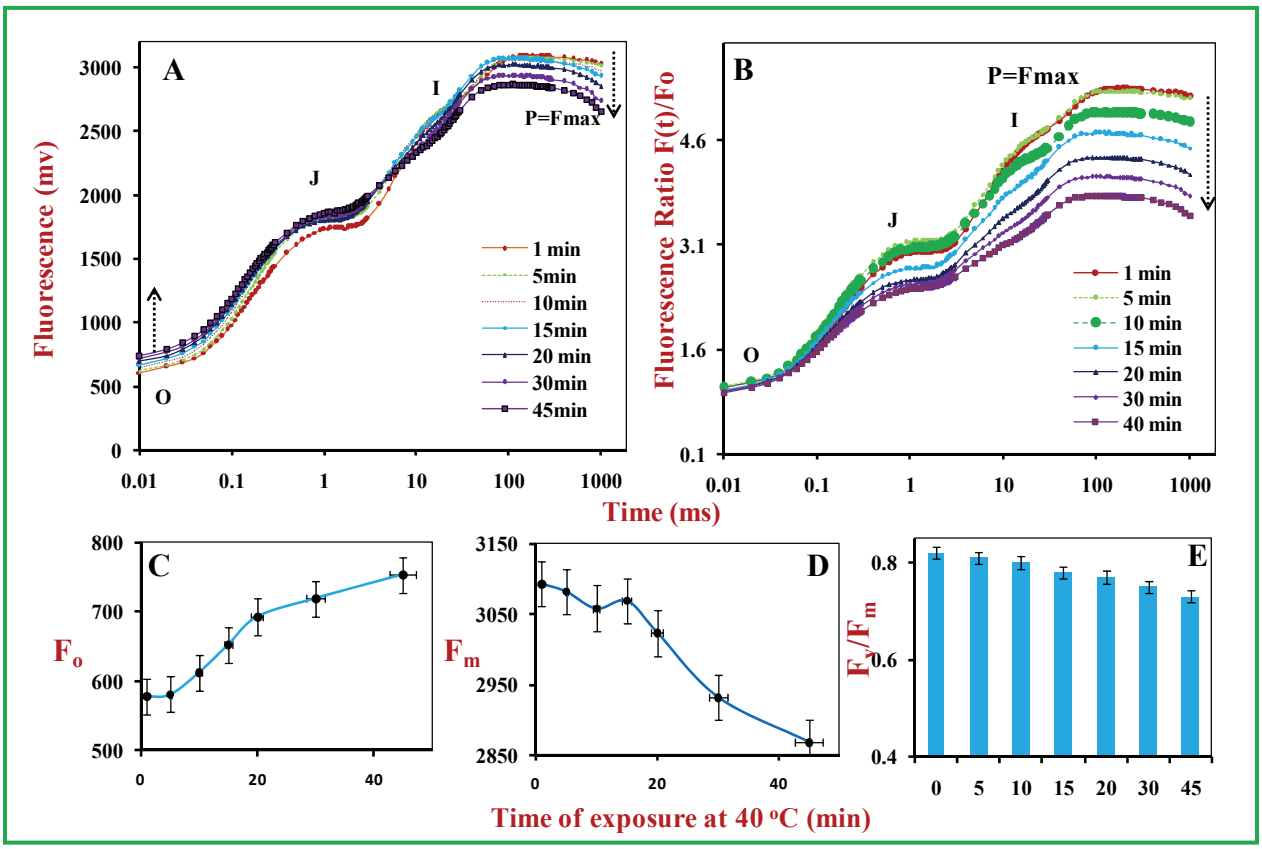


Fig. 4.5 Fast Chl *a* fluorescence transient of heat treated leaves for different time periods. Raw and normalized (F_o) curves (A,B). Spectra were recorded in dark adapted leaves using handy PEA. Fo, Fm and Fv/Fm are shown in C, D,E.

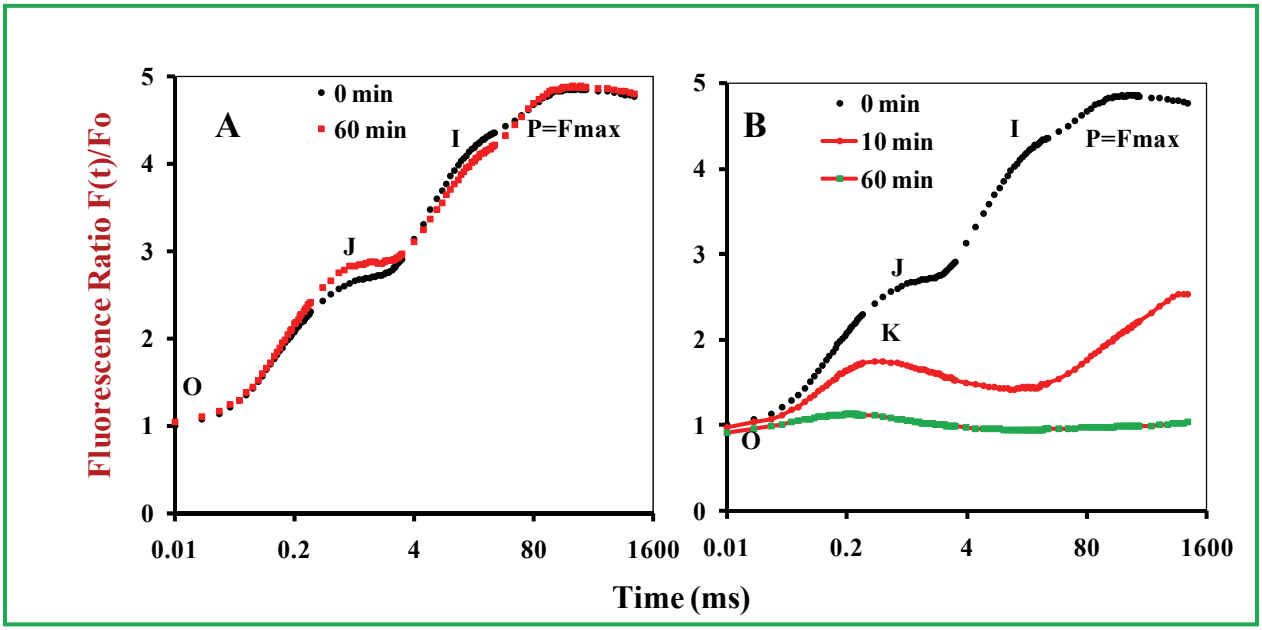


Fig. 4.6 Normalized fast Chl *a* fluorescence transients of heat treated leaves at 35 ^{o}C (A) and 45 ^{o}C (B) for different time periods (0 to 60 min).

35 ^{o}C treated leaves (Fig. 4.6 A). However, at 45 ^{o}C, after 60 min of exposure there was no appearance of OJIP phases indicating loss of photochemical function (Fig. 4.6B).

4.2.6 Proposed mechanism of state transition under elevated temperature

The redox state of the PQ pool and Cyt b_6/f constitutes the central signal for state transition mechanism (Allen, 2003). In general, docking of plastoquinol to the Qo site of the Cyt b_6/f complex activates the kinase responsible for LHCII phosphorylation (Fig. 4.7A-C). Phosphorylated LHCII dissociates from PSII and associates preferentially with PSI, which is enriched in the thylakoid stroma, thereby increasing the absorption cross section of PSI supercomplex. In higher plants and green algae, dark adaptation generally leads to oxidation of PQ pool. Thus, plants under dark conditions are considered to be in state I (Fig. 4.7A). In our experiments, leaves were kept in dark during temperature treatment, and were therefore expected to be in state I. However, we observed that the heat treatment in dark caused a transition from state I to state II. The involvement of the PQ pool and Cyt b_6/f on the temperature induced state transitions have been assessed by using DCMU or DBMIB (Fig. 4.4). The activation signal for STN7 kinase appeared to be from Cyt b_6/f, but the source of electrons was from stromal reductants. It is possible that in the dark elevated temperature, the non-photochemical reduction of PQ pool is enhanced due to chlororespiration (Fig. 4.7D and E).

4.3 CONCLUSIONS

In summary, our experimental data with *A. thaliana* point to the fact that moderately elevated temperature (40 °C) can induce phosphorylation of LHCII by STN7 kinase and subsequent migration of P-LHCII from PSII to PSI, similar to that of light-induced state transitions. This conclusion is supported by experiments using the *stn7* mutant, which does not carry out state transitions under moderately elevated temperature, similar to its inability to perform light-

induced state transitions. We propose that moderate temperature under dark condition induces state transition mechanism similar to light-induced state transitions. Under elevated temperature in dark the chlororespiration is increased, which may lead to activation of STN7 kinase. Thus, our study may facilitate in detecting the early events of abiotic stress in plants.

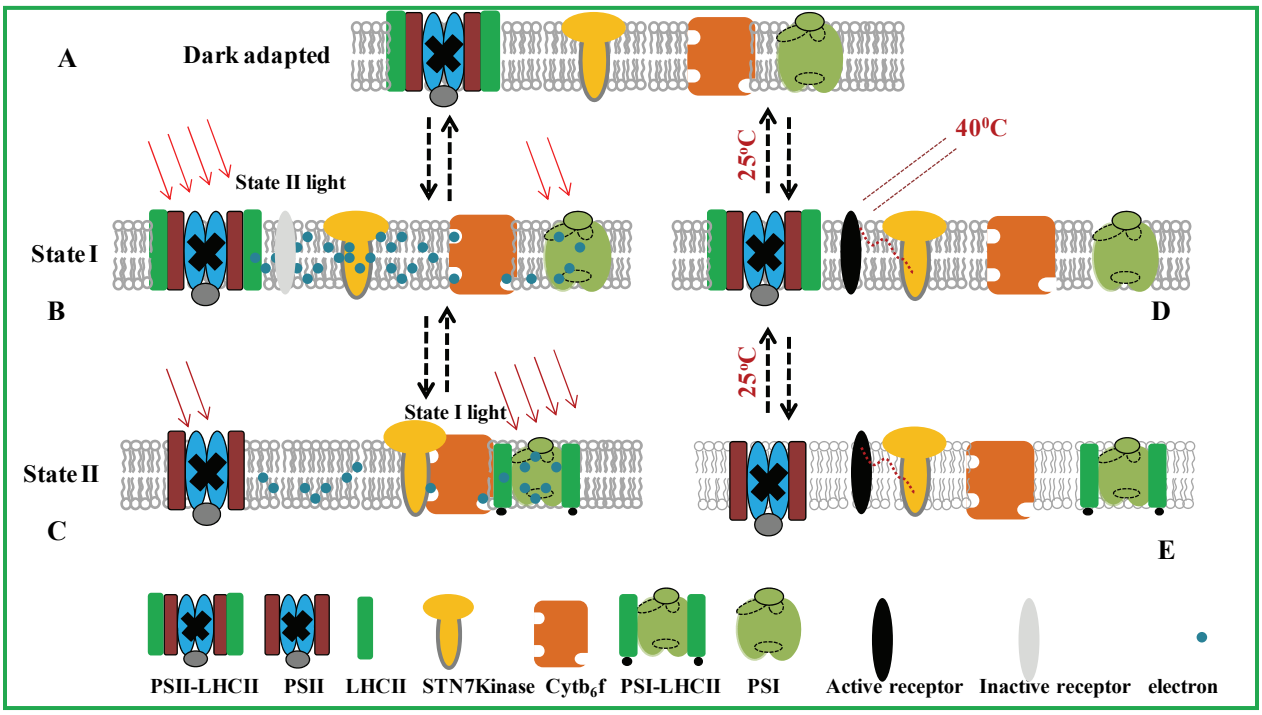
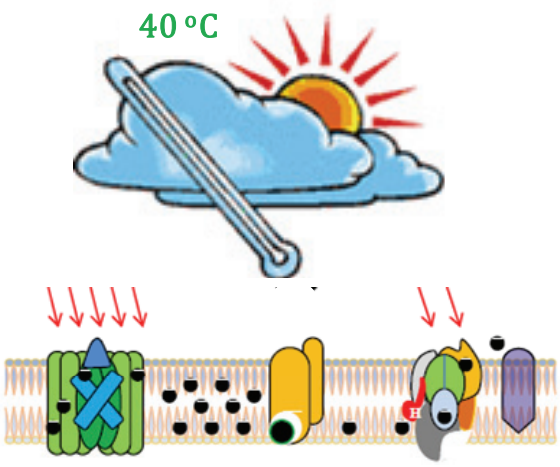


Fig. 4.7 Schematic models of light induced (A-C) and proposed model of temperature induced state transitions (D and E). Thylakoid membrane showing the PQ pool in oxidized state in dark adapted leaves (A). When PSII is preferentially excited; PSI cannot compete with PSII, leading to reduced state of PQ pool (B). The binding of plastoquinol to Cyt b_6/f activates STN7 kinase which phosphorylates LHCII, resulting in dissociation of LHCII from PSII and association with PSI (C). When the leaf exposed to elevated temperature (40 ^{o}C), electrons from stromal substrates passed to Cyt b_6/f leading to activation of STN7 kinase (D). STN7 kinase phosphorylates LHCII. The phosphorylated LHCII associated with PSI (E). In temperature induced state transition mechanism also, LHCII phosphorylation and migration may be dependent on intersystem redox carriers (PQ pool and Cyt b_6/f).

Unraveling the mechanism of moderate temperature triggered state transitions; combined effect of moderate temperature and light on state transition.

Chapter 5



5.1 INTRODUCTION

The redox state of PQ pool plays a major role in both cyclic and non-cyclic electron transport mechanisms. Apart from CET and LET, alternative electron transfer pathways have also been proposed which influence the redox state of PQ pool at the expense of stromal electron donors or acceptors. The functions for these alternative electron transport routes are to adjust the ATP/NADPH stoichiometry and to provide additional control on PSII activity (Heber and Walker, 1992). A significant contribution from alternative electron transport routes to the energetics of chloroplasts under light is questionable in healthy leaves, as the capacity of linear electron transport already exceeds that of carbon metabolism (Farquhar et al., 1980). This is not the case, however, in leaves experiencing unfavorable environmental conditions. Electron transfer from stromal reductants to O_2 through the PQ pool occurs (Garab et al., 1989; Peltier and Cournac, 2002; Bukhov and Carpentier, 2004). The existence of such pathways has been supported by the discovery of new molecular components of thylakoid membranes, including a plastidial NADH dehydrogenase complex, a plastid terminal oxidase (PTOX) and a key component of cyclic electron reactions around PSI. It has been reported that high temperature enhances chlororespiration (Havaux, 1996).

Numerous abiotic stresses weaken PSII, which is in most cases less tolerant than PSI (Baker, 1991). Consequently, the rate of linear electron transport, the main source for generation of the trans-thylakoid proton gradient, declines. This creates a deficit in energy supply for the dark enzymatic reactions in the chloroplast stroma. In addition, down-regulation of residual PSII activity mainly provided by the trans-thylakoid ΔpH (Horton et al., 1996) becomes weakened as well. The significance of alternative PSI-driven electron transport routes for the energetics and

the control of metabolic activity are expected, therefore, to be increased in leaves exposed to stressful conditions.

It should be noted that most studies on the effects of heat stress on photosynthesis have been carried out in the dark. Under natural conditions, particularly in the day time of the summer season where temperatures often rise to 30–45 ^{o}C and the light intensity can reach 1000–2000 μmol photons m^{-2} s^{-1}, thus, light and heat stresses are superimposed. Indeed, enhanced thermal injury to photosynthesis by high light is observed under such conditions. However, it is not known how heat stress under high light conditions affects PSII photochemistry and dissipation of excess light. The primary site of thermal damage to the photosynthetic function is believed to be associated with PS II. Plants have evolved a number of molecular mechanisms to cope with high temperature and to protect the photosynthetic machinery. Several faster response mechanisms to elevation of the ambient temperature have also been proposed to occur in chloroplasts.

The present study reveals the moderate temperature triggered non-photochemical reduction of the PQ pool and consequently LHCII phosphorylation which are connected to state transitions in the dark moderate temperature and in combination with either high or low light. Also, we have evaluated the LHCII phosphorylation and the factors responsible for the dark-reduction of the PQ pool under moderate temperature or in combination with light (low or high), for different time periods in *A. thaliana* and its mutant *stn7*, in which expression of *stn7* gene is blocked.

5.2 RESULTS AND DISCUSSION

In chapter 4th we have shown that moderate heat stress (40 ^{o}C) triggers phosphorylation of LHCII in the dark (Nellaepalli et al., 2011) in *A. thaliana*. However, the factors responsible for temperature induced LHCII phosphorylation and its migration are still not clear. In the present study, we have demonstrated the reduction of PQ pool and Cyt b_6/f in the dark; this is in turn

responsible for the activation of STN7 kinase and phosphorylation of LHCII in wt and *stn7* plants. We have made a study on the effect of LHCII phosphorylation when temperature and light treatments were superimposed.

5.2.1 The relation between LHCII phosphorylation and dark reduction of PQ pool under moderate temperature

It has been shown that in light induced state transitions the PQ pool and Cyt b_6/f are involved in STN7 kinase activation, leading to phosphorylation of LHCII (Zito et al., 1999). Generally, dark adaptation of plants and green algae in non-stressed conditions drives the PQ pool to be in an oxidized state and LHCII in a dephosphorylated state. However, as shown in the previous studies we have observed the phosphorylation of LHCII at moderate temperatures in dark for different time periods from 0 to 60 min in wt, whereas in mutant *stn7* no such phosphorylation was observed (Fig. 5.1). Previous studies using DBMIB have shown that in dark moderate temperature the Qo site of Cyt b_6/f is occupied by plastoquinol; this enables the activation of STN7 kinase, which leads in turn to phosphorylation of LHCII, so that temperature-induced and light-induced state transitions occur by essentially the same mechanism (Zito et al., 1999; Nellaepalli et al., 2011). In this study, we also monitored the nonphotochemical reduction of PQ pool (which is responsible for LHCII phosphorylation) by Chl *a* fluorescence induction curves using PAM fluorometer (Fig. 5.2A-F). Chl *a* fluorescence transients were recorded for 10 min when AL turned on and for 60 sec when AL turned off. Dark adapted leaves have shown minimal fluorescence (equal to F_o) when AL turned off (Fig. 5.2A). When leaves that had been heat-treated in the dark (450 μ E $m^{-2}s^{-1}$), the Chl *a* fluorescence transients after the AL was turned off showed a transient dip in fluorescence followed by a slower rise (Fig. 5.2B). The rise in Chl fluorescence when AL was turned off was referred to as F_o' and monitored for 60 sec.

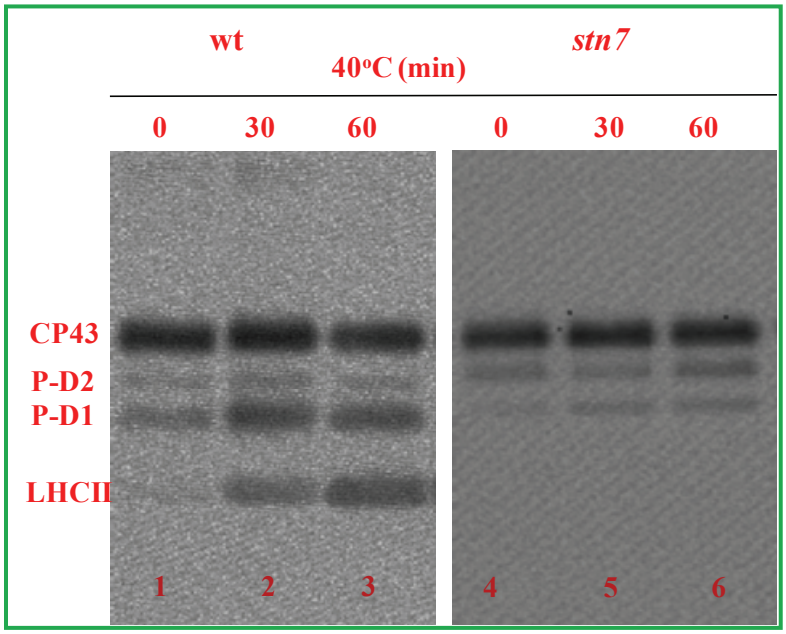


Fig. 5.1 Immunoblotting analysis of thylakoid membranes isolated from moderate temperature-exposed *A. thaliana* leaves of wt (lanes 1-3) and *stn7* mutant (lanes 4-6) for different time periods (0, 30 and 60 min) with anti-phosphothreonine antibody.

However, in control leaves that had been kept in the dark but not heat-treated, the rise in F_o' was not observed (Fig. 5.2A). When moderate heat (40 ^oC) treatment was given for 30 and 60 min, there is a increase in F_o' level in wt (Fig. 5.2B,C). A similar effect was observed in s*tn7* plants which had been moderate heat-treated for 60 minutes (Fig. 5.2D,E). The increase in F_o' can be ascribed to increased electron transfer from stromal reductants to PQ pool and Cyt b_6/f (Groom et al., 1993; Havaux, 1996). Thus, our results show both increase in F_o' and LHCII phosphorylation in wt (Fig. 5.1 and Fig. 5.2B,C). However, in *stn7* the rise in F_o' and absence of LHCII phosphorylation was observed due to lack of STN7 kinase (Fig. 5.1 and Fig. 5.2E). In wt, the increase in F_o' after 30 min of heat treatment leads to reduction of PQ pool and Cyt b_6/f, in which the Qo site is occupied by plastoquinol, that activates STN7 kinase and in turn phosphorylates LHCII. The extent of phosphorylation of LHCII has been enhanced with increasing exposure of moderate temperature as a result of enhanced reduction of the PQ pool and Cyt b_6/f. The process of non-photochemical reduction of PQ operates in both wt and mutant *stn7* at moderate temperature (Fig. 5.2). However, in *stn7* plants the phosphorylation of LHCII is arrested (Fig. 5.1). It has been reported that mercury inhibits the non-photochemical reduction of PQ by exogenous NADPH and NADH in spinach chloroplasts (Haldimann and Tsimilli-Michael, 2002). In our study, leaves pre-treated with $HgCl_2$ were exposed to 40 ^oC and displayed no increase in F_o', showing the active involvement of plastidial NAD(P)H dehydrogenase (NDH) enzyme in non-photochemical PQ pool reduction and thereby LHCII phosphorylation (Fig. 5.2F). Thus, our study showed that NDH is the key enzyme in non photochemical reduction of PQ pool at moderate temperature in both wt and mutant *stn7*.

The reduction of the PQ pool in higher plant chloroplasts is well documented as a light-driven process. In general, the PQ pool become reduced during low light illumination and

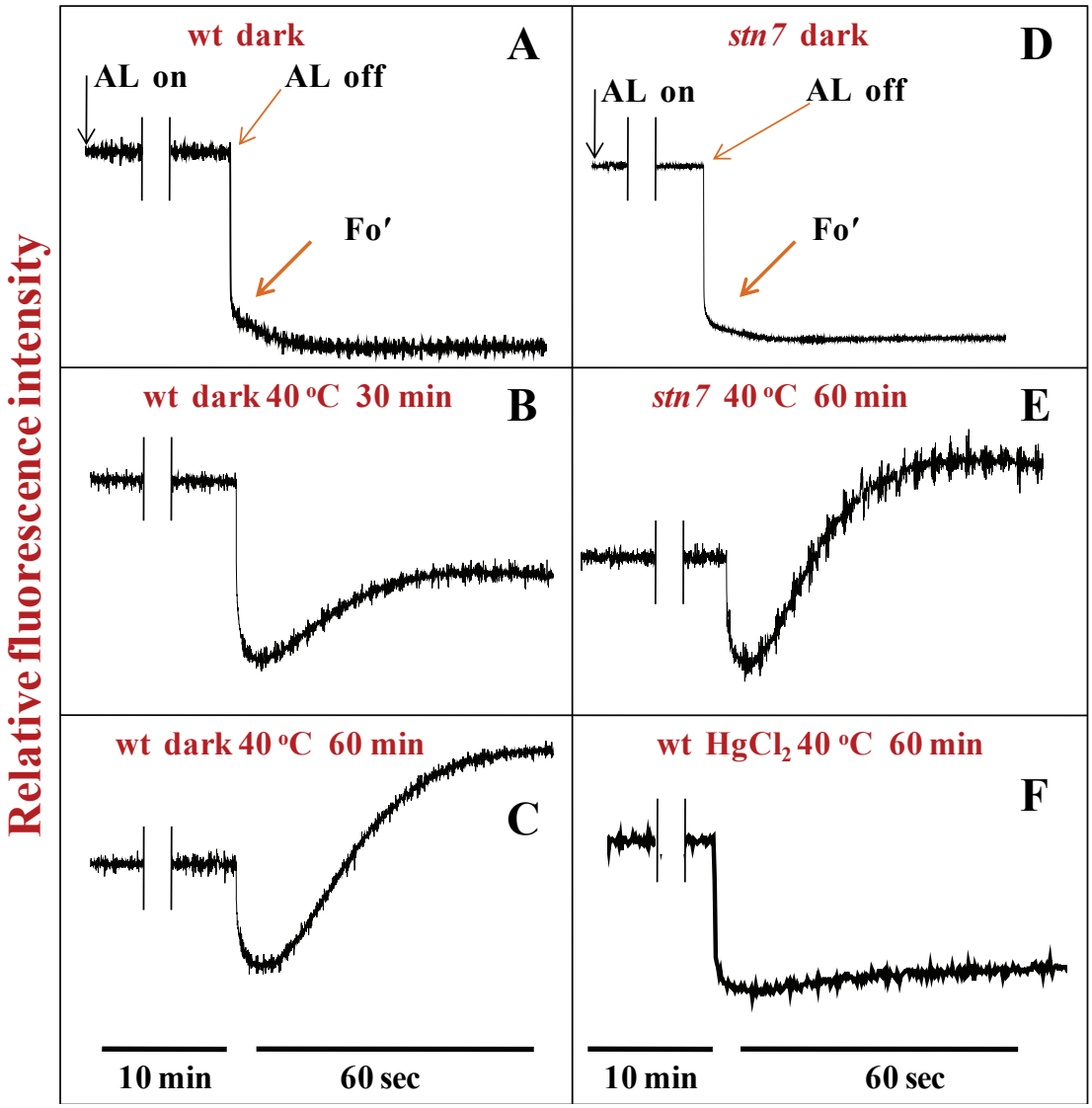


Fig. 5.2 Chl *a* fluorescence transients for determination of dark reduction of PQ pool. The AL turned off after 10 min of illumination to monitor raise in F_o' level in heat treated leaves for different time periods (from 0 to 60 min). Transients were measured for 10 min until reaching steady state fluorescence (F_s), and turned off for 60 sec to monitor the raise in F_o' level. AL was turned off and the subsequent change in Chl *a* fluorescence level was monitored. (A) wt dark adapted; (B) wt 40 °C and dark treated for 30 min; (C) wt 40 °C and dark treated for 60 min; (D) *stn7* dark adapted; (E) *stn7* 40 °C and dark treated for 60 min; (F) wt 40 °C and $HgCl_2$ treated for 60 min.

reoxidized when the leaves transferred to dark (Allen, 2003). On the other hand, there are reports showing that PQ accumulates in its reduced form following a light-to-dark transition (Groom et al., 1993; Feild et al., 1998) and also when leaves are exposed to anaerobic conditions in the dark (Harris and Heber, 1993; Farineau, 1999). It was reported that heat stress significantly enhances the dark reduction of PQ pool (Bukhov and Carpentier, 2004) and increases the trans-thylakoid proton gradient (Havaux, 1996; Bukhov et al., 2000).

Studies with mutants containing disrupted *ndh* genes have demonstrated that the plastid Ndh complex is involved in the post-illumination reduction of the PQ pool (Burrows et al., 1998; Endo et al., 1998; Kofer et al., 1998; Sazanov et al., 1998). On the other hand, there are several reports postulating that only NADPH (and not NADH) can reduce the PQ pool in the dark and that the reduction is predominantly, if not exclusively, released via an electron transport pathway involving ferredoxin-NADP+ oxidoreductase and a hitherto uncharacterized ferredoxin-PQ oxidoreductase (FQR) (Bendall and Manasse, 1995; Endo et al., 1998). Thus, from the above results it is concluded that under moderate temperature, NDH was involved in reduction of PQ pool and LHCII phosphorylation. It is well known that in light induced state transition, the absorption cross section of PSI increases by binding to LHCII. In this study, we also assessed the difference in absorption cross section of PSI-LHCI at dark optimal temperatures and dark moderate temperatures by sucrose density gradient ultracentrifugation and absorption spectroscopy.

5.2.2 Increased absorption cross-section of PSI-LHCI at dark moderate temperature

Fig. 5.3A shows the thylakoid membrane complexes separated by using sucrose density gradient ultracentrifugation from dark control and/or dark 40 ^{o}C treated wt and *stn7* plants. Three major fractions were observed (F1, F2 and F3) after sucrose density gradient ultracentrifugation. The

absorption spectra of F1 have major peaks at 673 nm and 652 nm in red region and 437 nm and 471 nm in blue region, which is the characteristic spectral feature of LHC. The F2 band shows two major peaks at 676 nm, 437 nm and minor peak at 469 nm, which corresponds to enriched PSII, and F3 shows major peaks at 679 nm and 438 nm and minor peaks at 469 nm and 496 nm which is the characteristic absorption spectra of PSI-LHCI supercomplex (Subramanyam et al., 2006, also see previous chapter Fig. 3.7B). The densitometry analyses were made for the fractions that were obtained in Fig. 5.3A. There was no difference in absorption intensity (densitometry) of F2 (PSII) and F3 (PSI) content in dark adapted wt, however, F3 (PSI) content was higher than F2 (PSI) in 40 ^{o}C treated wt plants (Fig. 5.3B). The increased density of F3 was may be due to LHCII migrated from PSII to PSI. In *stn7*, there was no significant difference in F2 (PSII) and F3 (PSI) of dark as well as 40 ^{o}C treated due to lack of ability to undergo state transitions in dark (Fig. 5.3B).

Room temperature absorption spectra were carried out for F3 of wt and *stn7*, exposed to dark control or dark 40 ^{o}C (Fig. 5.3C). In temperature treated samples there is an increase in absorption maxima at 469 nm and 496 nm compared to wt dark control samples; similar changes were not observed in *stn7* mutant samples. The increase in absorbance at 469 and 496 nm is due to Chl *b* and carotenoids of LHCII, respectively, which become bound to the PSI-LHCI supercomplex (Kargul et al., 2003). These results have shown that dark temperature treatment increases the absorption cross section of PSI by LHCII association. Similar observations have been reported for light induced state transitions (Haldrup et al., 2001).

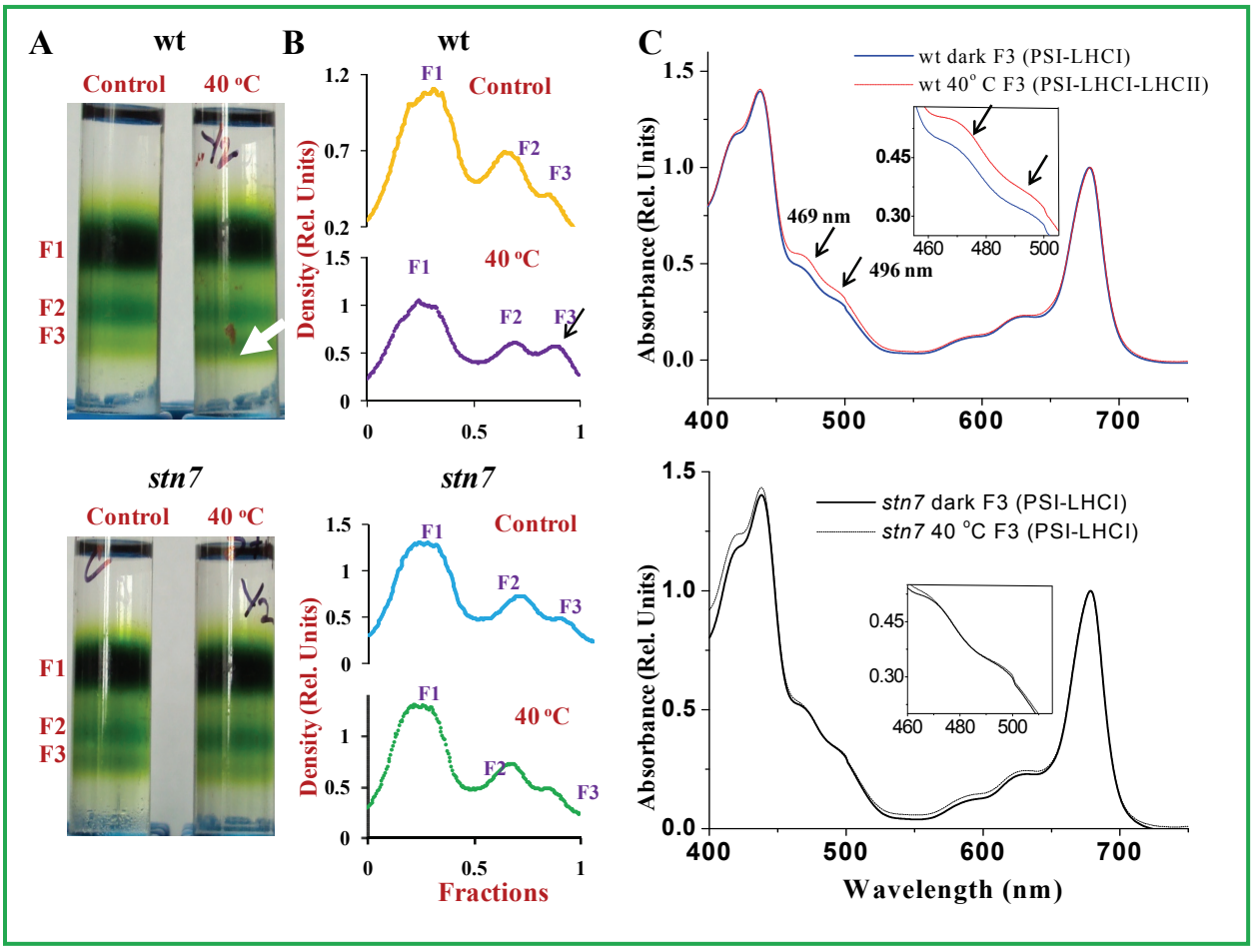


Fig. 5.3 Sucrose density gradient ultracentrifuge tubes showing 3 major fractions (F1-F3) containing different pigment protein supercomplexs in wt and *stn7* pre-exposed to 0 and 30 min of moderate temperature (40 ^{o}C) (A). Densitometry analysis of dark control and wt dark 40 ^{o}C of wt and *stn7* (B). Absorption spectra of PSI-LHCI supercomplexes (F3) of dark control and moderate temperature treated samples (40 ^{o}C) from wt and *stn7* leaves (C). The downward arrow (black) in Fig. C shows increased absorption of 469 and 496 nm peaks due to association of LHCII to PSI-LHCI supercomplex. Inset shows absorption spectra ranging from 460 to 540 nm. Spectra normalized at 678 nm. The downward arrow (white and dark) shows increased density of F3 in 40 ^{o}C treated sample.

5.2.3 Combined effect of light and moderate temperature on state transition

5.2.3.1 Phosphorylation of LHCII and other subunits of PSII

When moderate temperature is superimposed with low light, one might expect the phosphorylation level of LHCII to double. However, no such additional phosphorylation of LHCII was observed when moderate temperature was superimposed with low light or high light for 30 min (Fig. 5.4A,B). In HL treated (1000 μmol photons m^{-2} s^{-1}) samples, the hyperphosphorylation of reaction center proteins of PSII (D1/D2) and also CP43 subunits were observed, and not in LL treated samples or moderate temperature treated samples (Fig. 5.4B). Also, similar hyper-phosphorylation was noticed when HL and moderate temperature were superimposed (40 ^{o}C+HL). This shows that moderate temperature did not influence the hyper phosphoryaltion of PSII core proteins such as D1/D2 and CP43 when superimposed with high light. The earlier reports show that STN8 protein kinase is involved in phosphorylation of PSII core proteins (D1/D2 and also CP43) in higher plants and green algae (Vainonen et al., 2005, Tikkanen et al., 2008, 2010). These interesting findings have shown that STN8 kinase is not deactivated when moderate temperature was superimposed with high light. However, temperature treatment was unable to induce LHCII phosphorylation when combined with HL treatment (Fig. 5.4B). Nonetheless, when moderate temperature was superimposed with high light (40 ^{o}C+HL), the phosphorylation of LHCII as well as hyper-phosphoryaltion of D1/D2 were not observed (Fig. 5.4B). It is reported that high light can induce over-reduction of PQ pool, but still the LHCII phosphorylation was not observed because of inactivation of LHCII kinase mediated through the ferredoxin/thioredoxin system at high light intensities (Rintamaki et al., 2000). Thus, when HL superimposed with moderate temperature did not show state transition. However, when LL was superimposed with moderate temperature, still there was no

state transition. This may be due to acceleration of linear electron transport in which most of the electrons enter through the chlororespiration were used up by linear electron transport. It is clear that individually, the treatments could induce state transition, however, when superimposed with low or high light does not show state transition. Thus, it deciphers the moderate temperature in presence of light (40 ^{o}C+LL and 40 ^{o}C+HL) deactivates STN7 kinase and consequently ceases LHCII phosphorylation. In light-induced state transitions both the PQ pool and Cyt b_6/f are known to be triggering signals for the activation of STN7/STT7 kinase (Vener et al., 1997; Zito et al., 1999). Thus, moderate temperature in presence of light (both high or low) did not show the phosphorylation of LHCII, however, hyper phosphorylated D1/D2 and CP43 were observed which are not known to be involved in state transitions but could help in the macro-organization of PSII and PSI supercomplexes.

5.2.3.2 Effect of moderate temperature and low light on PSI absorption cross-section

We have also measured 77K fluorescence emission spectra to monitor the changes in PSI absorption cross section (Fig. 5.4C). We have observed increase in F726 band when samples are treated separately with either moderate temperature (40 ^{o}C) or low light. The increase in F726 band is due to increased fluorescence emission from PSI due to binding of LHCII (Bennett et al., 1980; Bellafiore et al., 2005). However, there was no increase in F726 band when moderate temperature treatment was superimposed with light (40 ^{o}C+LL), indicating that light could not induce state transitions when superimposed with moderate temperature (Fig. 5.4C). These results show that the reduced F726 was due to lack of LHCII phosphorylation when moderate temperature combined with low light. However, there was no increase in F725 in dark, LL, 40 ^{o}C and Heat +LL treated *stn7* samples (Fig. 5.4C). The absence of increase in F725 was due to lack of association of LHCII with PSI-LHCI supercomplex in *stn7* plants.

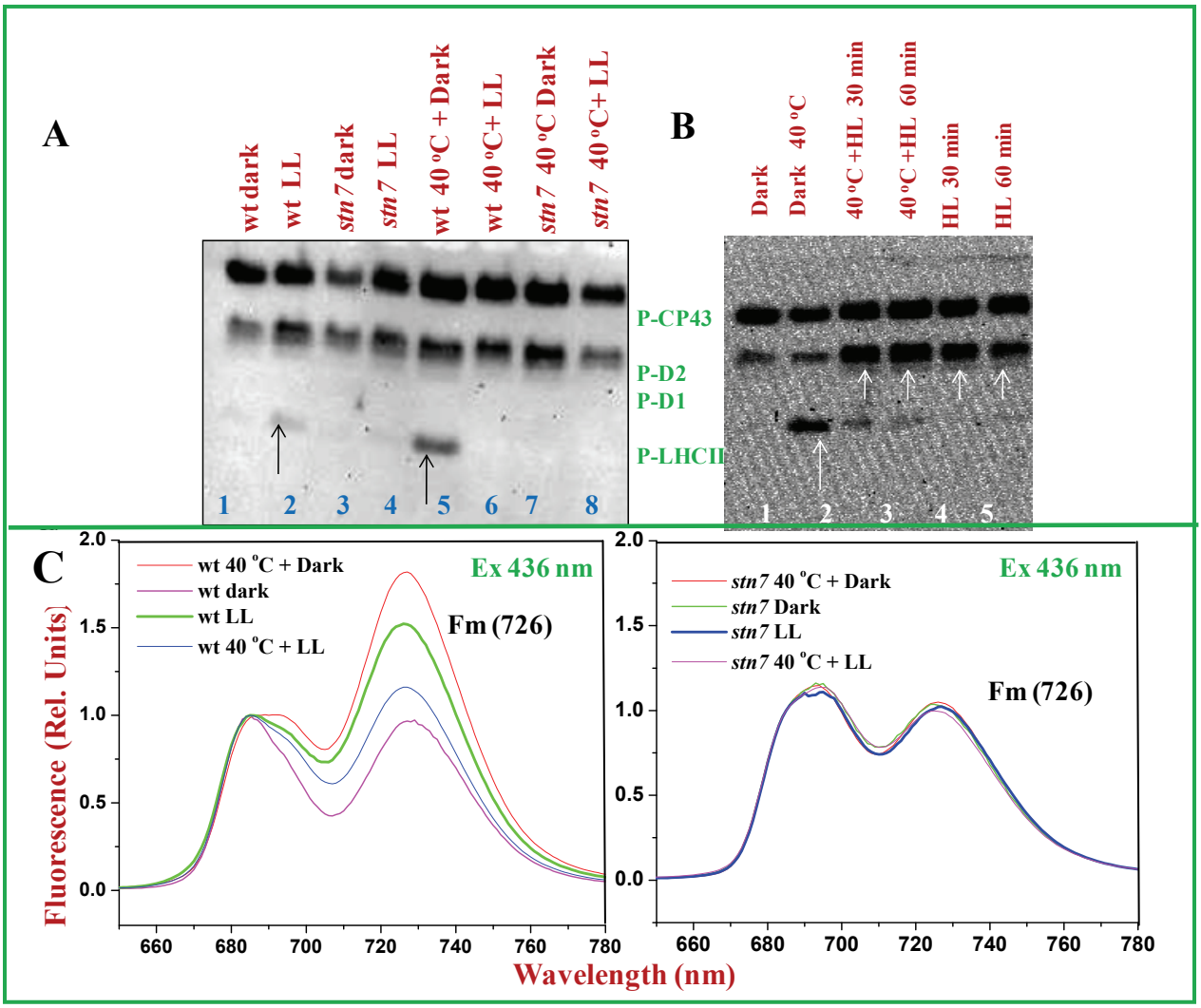


Fig. 5.4 Immunoblotting analysis of thylakoid membranes isolated from moderate temperature treated and or LL exposed (35-40 µmol m^{-2} s^{-1}) wt and *stn7* leaves (A). The duration of each treatments was 60 min. Immunoblotting analysis of thylakoid membranes isolated from moderate temperature and or HL exposed (1000 µmol m^{-2} s^{-1}) wt leaves for 0 (1ane 1), 30 (lanes 3,5) and 60 min (lanes 2, 4,6) with antiphospho-threonine (B). 77K Chl *a* fluorescence emission spectra of thylakoid membranes isolated from wt, *stn7* exposed to dark, low light (LL), 40 °C and 40 °C + LL (35-40 µmol m^{-2} s^{-1}) conditions (C). Spectra normalized at 685 nm.

5.2.3.3 Acceleration of cyclic electron transport

CET has been monitored at moderate temperature and light conditions individually and cumulatively (Fig. 5.5A). Dark and LL adapted leaves did not show any rise in fluorescence when the far red light turned on, showing the oxidized state of PQ pool (Fig. 5.5B,C). Preferential excitation of PSI by FR leads to oxidized state of PQ pool in dark and LL adapted leaves. However, if the leaf had been exposed to moderate temperature there was increase in fluorescence due to reduced state of PQ pool (Fig. 5.5D,E). The reduced PQ pool during far red light treatment in moderate temperature treated leaf was due to electron flow from PSI to PQ pool and Cyt b_6/f. The reduction of PQ pool by FR light was may be due to cyclic electron transport around PSI. Thus, these results have shown that at moderate temperature cyclic electron transport has been enhanced. However, when the temperature (40 ^{o}C) and LL given together, no PQ reduction was observed (Fig. 5.5F). It has been reported that PSI mediated electron transport triggered in heat stresses chloroplasts (Thomas et al., 1986). However, once the treatments given together (40 ^{o}C+LL) there was no cyclic electron transport in our studies. Moreover, it has been assumed that LL inhibits CET at moderate temperature. Thus from the above studies, it has been shown that LL when combined with moderate temperature did not show phosphorylation due to acceleration of non cyclic electron transport. This conclusion is further supported by experiments involving treatment with antimycin, (5μM) which inhibits CET (Thierry et al., 2001). Leaves were pre-treated with antimycin and exposed to moderate temperature (40 ^{o}C), after which cyclic electron transport was monitored. There was no increase in fluorescence when far red light was turned on, indicating that antimycin inhibits cyclic electron transport (Fig. 5.5G). It has been reported that cyclic electron flow around PSI is part of the plant abiotic stress response (Casano et al., 2000; Rumeau et al., 2007). Further, it has been

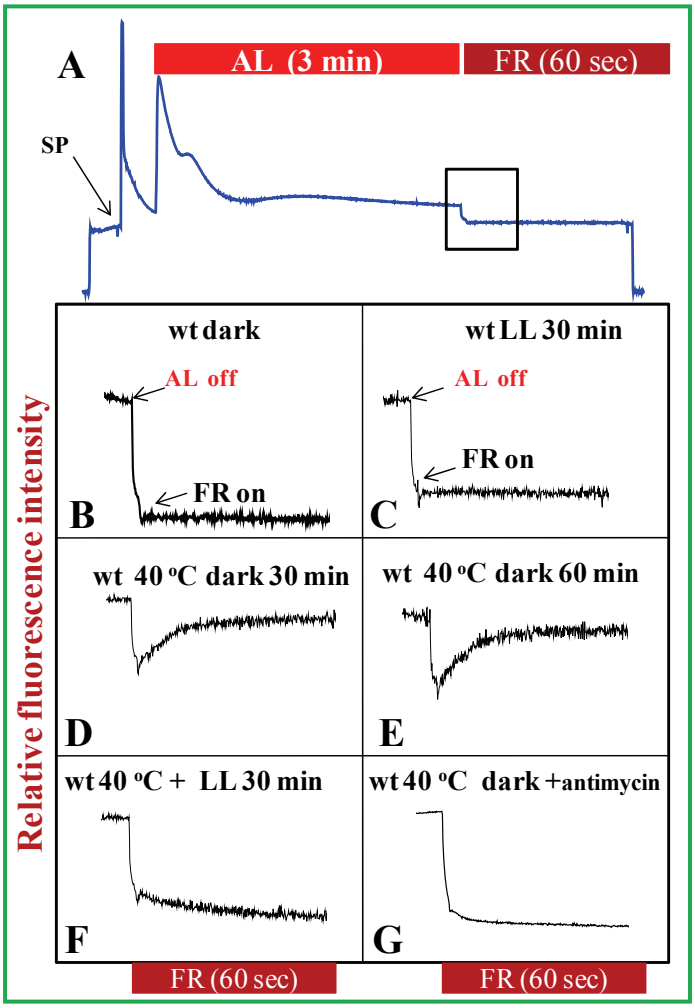


Fig. 5.5 Chl *a* fluorescence analysis of temperature and or light treated leaves to monitor cyclic electron transport. Typical Chl *a* fluorescence transient of dark adapted leaves during AL illumination (50 µmol m^{-2} s^{-1}) for 3 min and 60 sec after switching off AL and switching on the FR light (A); zoomed area of the fluorescence transient of wt dark and LL pre-treated leaves, respectively (B,C); 30 and 60 min of dark moderate temperature exposed leaves (D,E); pre-exposed to moderate temperature combined with LL condition (30 min) (F); pre treated antimycin leaf exposed to moderate temperature (G).

reported that NDH participates in cyclic electron transport. Two different pathways of cyclic electron flow have been reported around PSI in higher plant chloroplasts. One of these pathways involves ferredoxin PQ reductase (Cleland and Bendall, 1992), whereas the other involves the NDH complex (Jans et al., 2008). Based on our findings by using 10 μM $HgCl_2$ and 5μM antimycin, we report here that both ways of cyclic electron transport are accelerated at moderate temperature treatment. In *C. reinhardtii*, it has been reported that in state II (PQ pool reduced) apart from the binding of mobile LHCII to PSI also occurs association of Cyt b_6/f and FNR with PSI forming a super-supercomplex (CEF supercomplex), which is required to establish CEF between PSI and the PQ pool for generating mostly ATP (Iwai et al., 2010b; Minagawa, 2011).

5.2.3.4 Chl *a* fluorescence quenching

Fig. 5.6 displays Chl *a* fluorescence quenching after various treatments of dark, 40 ^{o}C, LL, HL, 40 ^{o}C+LL and 40 ^{o}C+HL. The increase in F_o was only observed in 40 ^{o}C and HL treated leaves and there was no such increase in LL, 40 ^{o}C+LL and 40 ^{o}C+HL treated leaves (Fig. 5.6A-F). The increase in F_o in HL treated leaves is due to photochemical over reduction of PQ pool and in 40 ^{o}C both F_o and F_o' was increased due to non photochemical reduction of PQ pool. At room temperatures light treatments (dark, LL and HL) could not induce rise in F_o' level (Fig. 5.6A,C,E). Moderate temperature (40 ^{o}C) is the triggering signal for dark reduction of PQ pool. However, when moderate temperature combined with light (40 ^{o}C+LL and 40 ^{o}C+HL), there was no dark reduction of PQ pool (Fig. 5.6D,F). Thus, light inhibits dark reduction of PQ pool at moderate temperature (40 ^{o}C). However, there are various changes apart from F_o' such as F_s (steady state fluorescence), non photochemical quenching and electron transport.

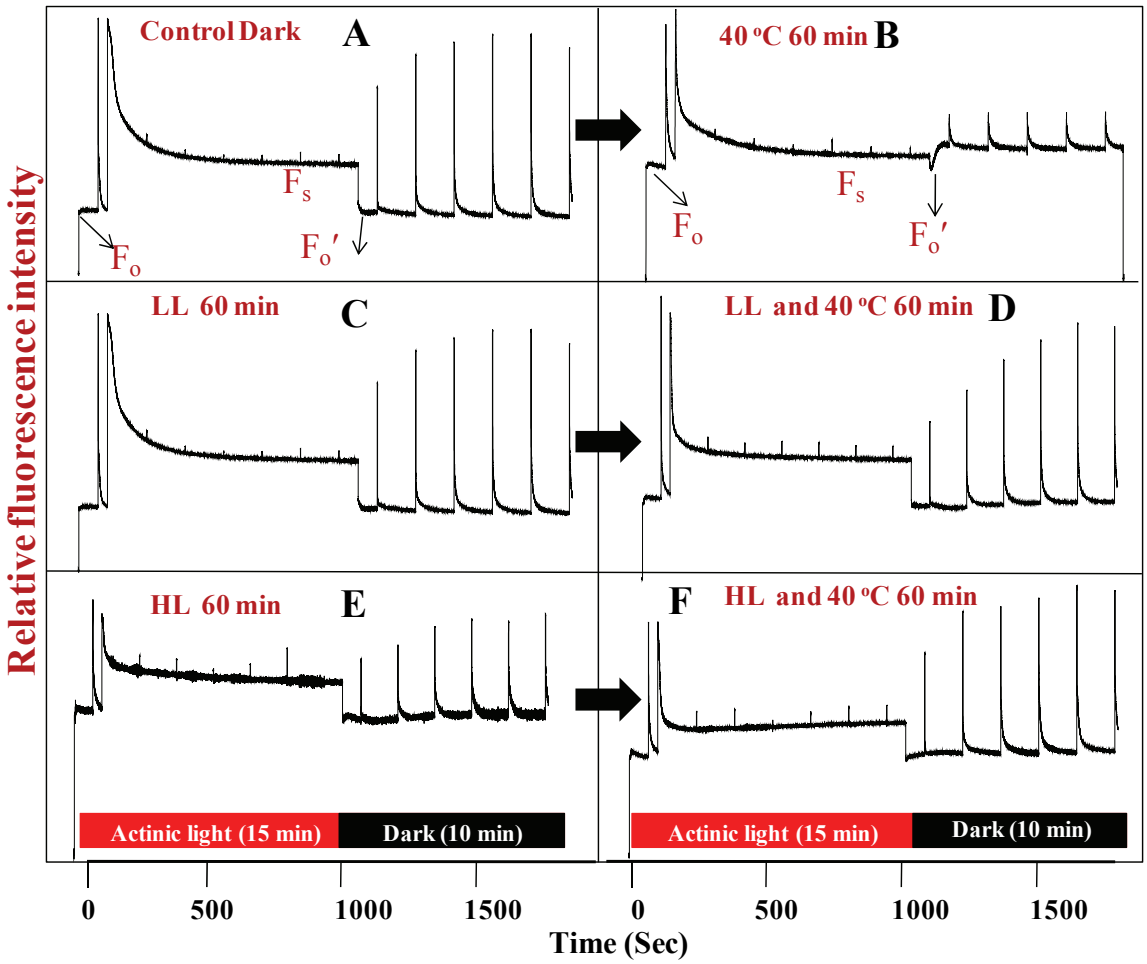


Fig. 5.6 Chl *a* fluorescence quenching for moderate temperature (40 °C), LL, HL, LL+40 °C, HL+40 °C treated leaves for different time periods in wt. (A) dark adapted; (B), moderate temperature exposed (60 min); (C), LL treated; (D), LL and moderate temperature (40 °C); (E), HL; (F), HL and moderate temperature (40 °C). Actinic light, intensity 450 µmol photons m^{-2} s^{-1}, was given for 15 min followed by a 10 min dark period. The arrow marks show shifting of plants from optimal temperatures to moderate temperature.

When moderate temperature was combined with light (40 ^{o}C+LL and 40 ^{o}C+HL), the fluorescence reached steady state as soon as the actinic light was turned on (Fig. 5.6D,F). In other conditions such as dark, 40 ^{o}C, LL, 40 ^{o}C+LL (Fig. 5.6A-D), reaching F_s was delayed.

5.2.3.5 Non photochemical quenching and electron transport

Apart from LHCII phosphorylation and dark reduction of PQ pool, we have also analyzed the effect of given treatment on electron transport (ETR) and NPQ. Actinic light was given for 10 min and for every 2 min saturating pulses were applied to determine NPQ and ETR. Non photochemical quenching has been decreased from 1.2 to 0.8 (30%) at moderate temperature from 0 to 90 min (Fig. 5.7A). LL exposed leaves exhibited similar NPQ and ETR to dark treated samples. However, when LL was combined with moderate temperature neither ETR nor NPQ was significantly altered (Fig. 5.7C,D). HL treated leaves have shown decreased NPQ to 35%, however when combined with moderate temperature the decrease in NPQ is around 90% (Fig. 5.7E), the electron transport has been decreased to 50% after 60 min of exposure (Fig. 5.7F). However, the electron transport at dark and HL treated leaves were same. But, when the HL and heat treatments given together (40 ^{o}C+HL), the electron transport decreased to 50% (Fig. 5.7F). These results show that HL could not cause cumulative effect on electron transport when treated with moderate temperature (Fig. 5.7). These results show that NPQ has been drastically reduced when moderate temperature combined with HL, however, electron transport is still existing about 50% (5.7E).

A comparative summary of changes observed during various treatments of our study was mentioned in Table 3.

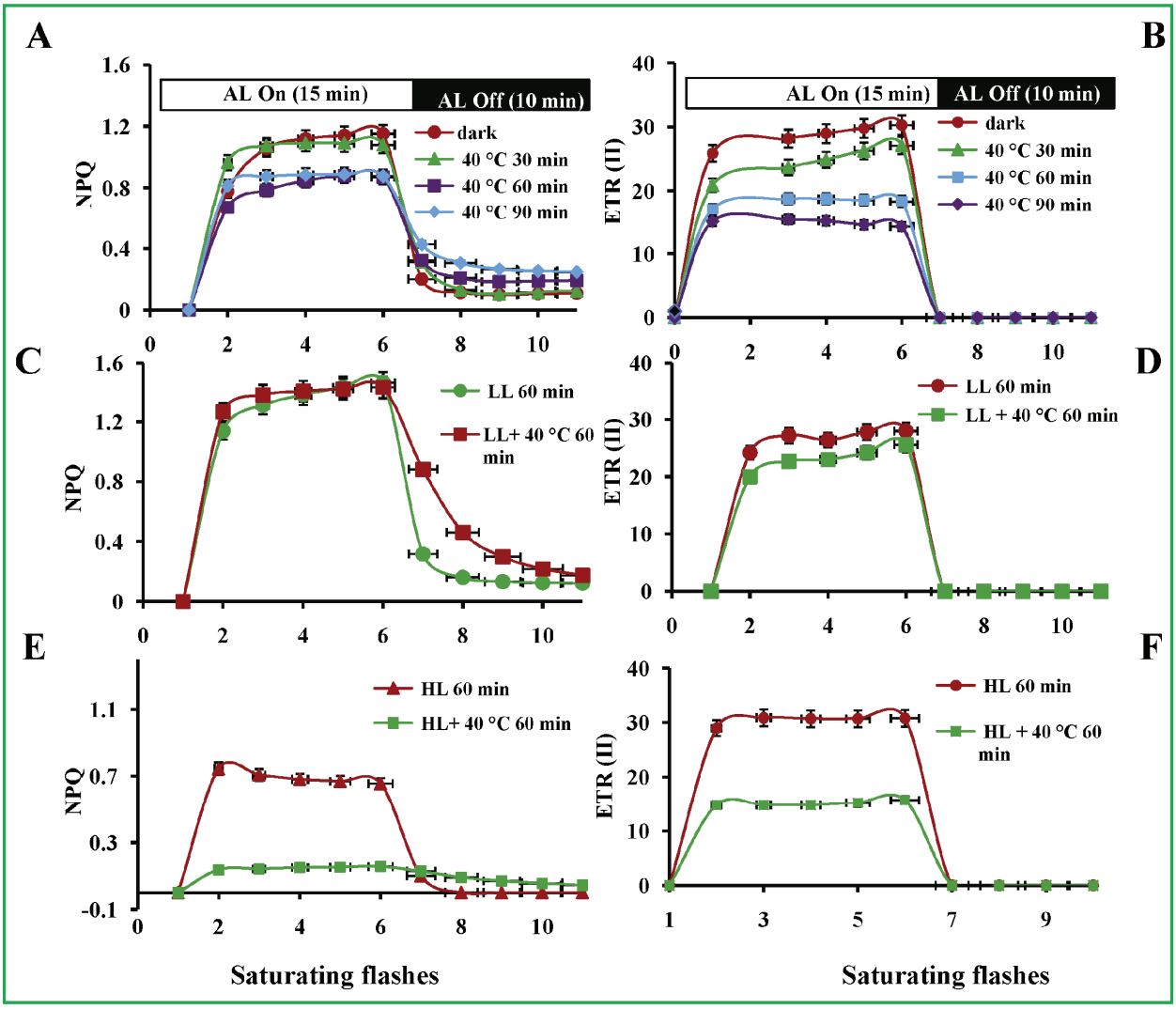


Fig. 5.7 Non photochemical quenching and electron transport (NPQ) and ETR were calculated from Fig. 5.6 in wt. Saturating pulses were applied during AL illumination and dark period for total of 20 min of measurements, were used for determination of NPQ and ETR. Dark and moderate temperature (A,B); LL and or moderate temperature (C,D); HL and or moderate temperature (E,F).

Events	Dark	LL	HL	40 °C+ Dark	40 °C +LL	40 °C +HL
LHCII phosphorylation	-	+	-	+	-	-
Increase in F725 Band	-	+	-	+	-	NS
Dark reduction of PQ pool (F_o)	-	-	-	+	-	-
F_o	-	-	+	+	-	+
CET enhanced	-	-	-	+	-	-

+, presence; - , absence; NA, Not Studied

Table 3: Comparative summary of changes observed during various treatments in wt leaves.

5.2.4 Thylakoid membrane protein expression level and effect on OEC complex

We have monitored changes in thylakoid membrane protein level at moderate temperature in wt as well as in *stn7* mutant (Fig. 5.8A). However, we have found no differences in thylakoid membrane protein levels after 0 to 120 min of heat exposure (40 ^{o}C). It has been reported that the first detectable change in structural and functional organization of PSII (PSII) appeared be at 40–50 °C (Lípová et al., 2010). In these temperatures the PSII core dimers began to dissociate into monomers, which was accompanied by a decrease in PSII photochemistry and reflected in fluorescence temperature curve as the first fluorescence increase (Lípová et al., 2010). However, there were no change at thylakoid protein level (Lhcb1, Lhcb2, Lhcb3, D1/D2 and PsaA/B) at 40 ^{o}C in wt and *stn7*.

Heat inactivation of the OEC has been studied by fast Chl *a* fluorescence transients. Fig. 5.8B exhibits the rise in fluorescence from O to P for different dark heat treatment periods from 0 to 120 min. As the duration of heat treatment was increased, the rise in path of the fluorescence transient was altered and maximal fluorescence yield (F_{max}) at P was decreased. This result was expected because of partial inactivation of PSII by heat treatment. Furthermore, heat exposure affected both the F_o and F_m levels of the transients. Where F_o is the minimal fluorescence, when all PSII reaction centers are open (at t=0) and F_m is the maximal fluorescence, when all PSII reaction centers are closed.

The disappearance of J and I steps and appearance of K peak at 300–400 μs is the evidence for inactivation of OEC complex in wt after 120 min of heat exposure (Fig. 5.8B). The K peak may result from the only stable charge separation possible when the OEC is completely destroyed, because TyrZ can provide only one electron. Similar results were obtained when photosynthetic electron transport activity in heat-treated barley leaves assessed (Tóth et al.,

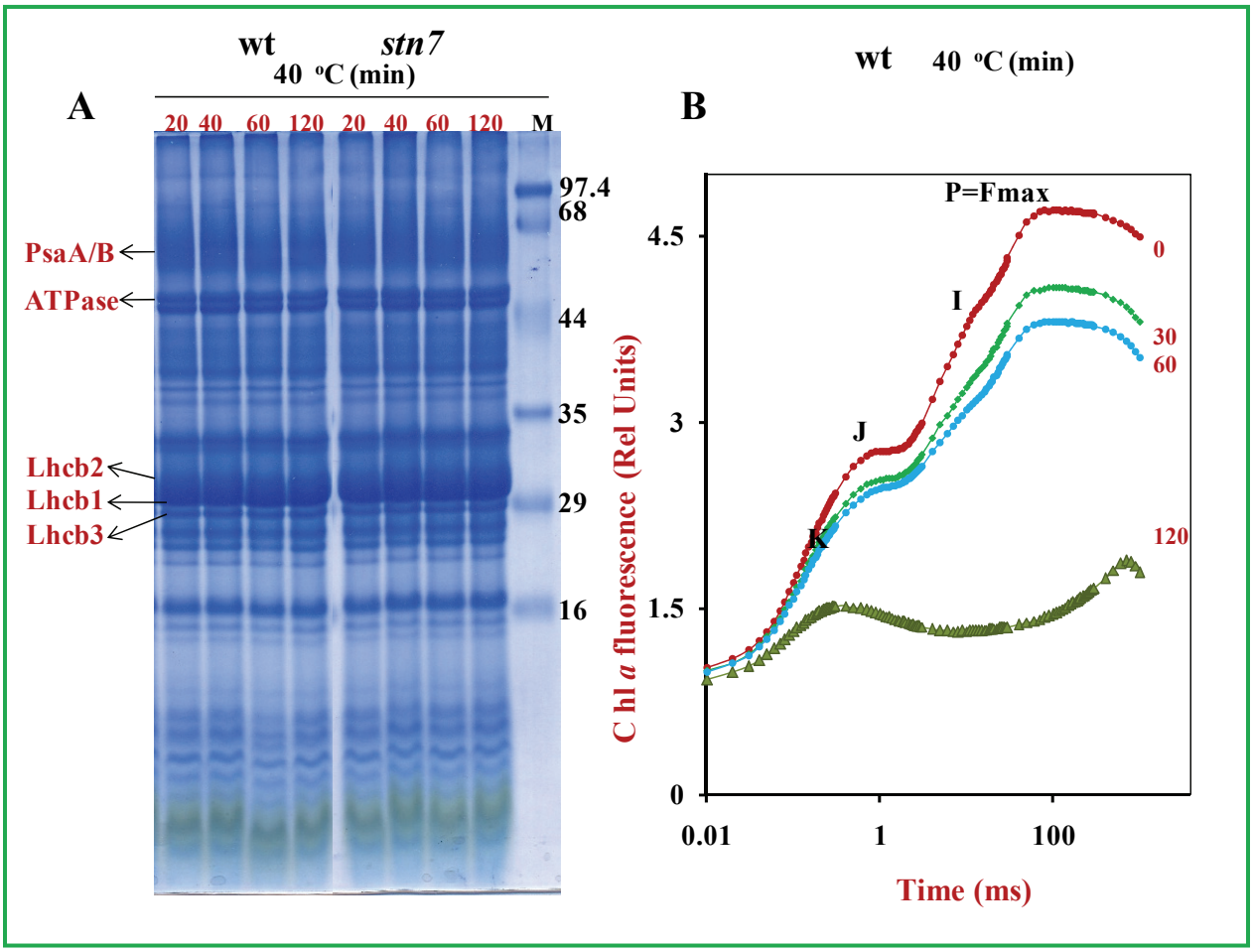


Fig. 5.8 Protein profile of thylakoid membrane proteins separated by Tricine SDS-PAGE isolated from moderate temperature exposed wt and *stn7* plants for different time periods (0 to 120 min) (A). Fast Chl *a* fluorescence transient of moderate temperature exposed leaves for different timeperiods from 0 to 120 min (B). Equal protein concentration were loaded on each lane. MP, marker protein in kDa.

2007). The dark heat treatment likely induced heterogeneity in intersystem electron transport carriers in photosynthetic electron transport chain (Bukhov and Carpentier, 2000), thus causing a change in the cellular redox poise. The above results with OJIP fluorescence transient's studies have shown that the altered redox state of the PQ pool by moderate temperature was minimal until 60 min of heat exposure, but these changes were destructive after 120 min heat treatment.

5.2.5 Proposed model of chlororespiration triggered state transition

At dark ambient temperatures in higher plants, the PQ pool remains in an oxidized state leading to state I, in which LHCII associated with PSII (Fig. 5.9A). However, dark moderate temperature enhances non-photochemical reduction of PQ pool and Cyt b_6/f by a process called chlororespiration in which NDH complex plays a major role. Cyclic electron transport was also found to be accelerated at moderate temperature. Dark reduction of PQ pool and Cyt b_6/f by stromal reductants such as NAD(P)H activates STN7 kinase, which in turn phosphorylates LHCII. The phosphorylated LHCII dissociates from PSII and re-associates with PSI (Fig. 5.9B). In LL conditions, light induced state transitions have been observed in which the PQ pool is reduced photochemically and phosphorylated LHCII migrates to PSI (Fig. 5.9C). However, when LL combined with moderate temperature, neither light induced nor temperature triggered state transition were observed, and the PQ pool remains in an oxidized state (Fig. 5.9D). In high light conditions, the PQ pool is reduced, but still no state transition is observed because of the inactivation of STN7 kinase by thioredoxin (Rintamaki et al., 2000) (Fig. 5.9E).When HL is combined with moderate temperature, no state transition are observed (Fig. 5.9F). Alternatively, when light treatment is combined with moderate temperature, there was no dark reduction of PQ pool, LHCII phosphorylation and no increase in PSI fluorescence.

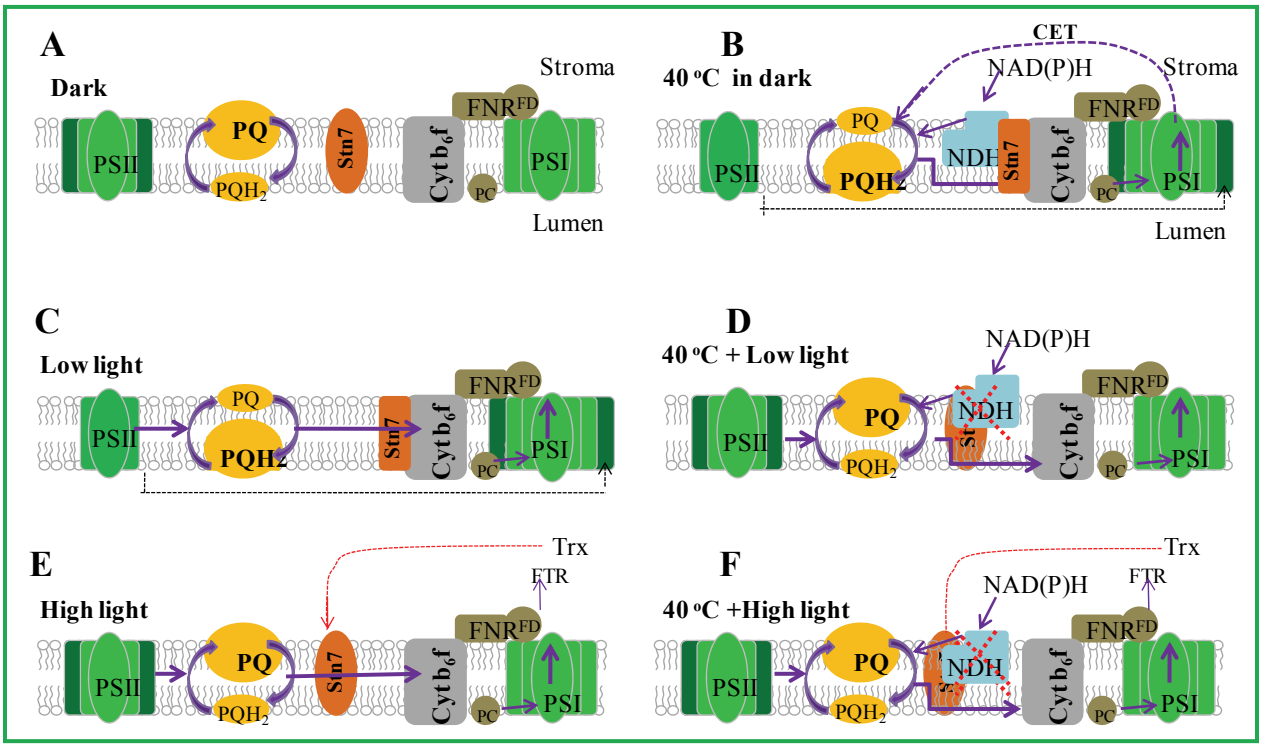


Fig. 5.9 Schematic model of temperature induced state transitions. Thylakoid membrane showing the PQ pool in oxidized state in dark adapted leaves (A). When the leaf exposed to moderate temperature (40 ^{o}C), electrons from stromal substrates passed on to Cyt b_6/f is enhanced leading to Qo site of Cyt b_6/f occupied by plasoquinol and activation of STN7 kinase by chlororespiration. STN7 kinase phosphorylates LHCII. The phosphorylated LHCII associated with PSI. In temperature induced state transition mechanism, LHCII phosphorylation and migration may also be dependent on intersystem redox carriers (PQ pool and Cyt b_6/f) like in light induced state transition mechanism (B). LL induces state transition mechanism in LHCII migrates to PSI (C). When LL combined with moderate temperature the migration of LHCII is inhibited. Its due to absence of dark reduction of PQ pool and also no enhancement of cyclic electron transport (D). HL induces photochemical reduction of PQ pool, however, thioredoxine deactivates STN7 kinase and thereby no LHCII phosphorylation (E). The same phenomena similar to HL might be taking place when both HL and moderate temperature superimposed (F). Conformational changes of STN7 kinase has been represented as rectangle (smooth corners) orange block for active form and oval orange block for inactive form.

5.3 CONCLUSIONS

At moderate temperature, LHCII phosphorylation was observed. The increase in level of LHCII phosphorylation at moderate temperature was due to reduction of PQ pool and Cyt b_6/f by stromal reductants. It has been monitored by PAM fluorometry, where increase in Fo′ was found to be increased due to non-photochemical reduction of PQ pool. Later, PSI-LHCI was isolated and found to be associated with LHCII at moderate temperature. In *stn7*, there was increase in Fo′ due to non-photochemical reduction of PQ pool, however, LHCII phosphorylation was absent due to absence of STN7 kinase. Phosphorylation of LHCII and dark reduction of PQ pool were also studied under combined effect of moderate temperature and light (low and high).

In the first objective of our study, it has been reported that LHCII phosphorylated in light adapted condition. In the second objective, it has been well shown that apart from light, moderate temperature also triggered phosphorylation of LHCII. Thus, in this study, it was expected that under combined effect of light and temperature, the phosphorylation of LHCII might be doubled. However, there was no phosphorylation of LHCII in combined effect. Acceleration of cyclic electron transport and decline of non cyclic electron transport was found to the cause for absence of LHCII phosphorylation in combined effect. Later, NPQ and ETR was also monitored and observed to be decreased when light and temperature were superimposed. OEC was damaged after 2 h of moderate temperature exposure. However, protein levels of thylakoid membranes were unaffected.

Summary and conclusions

SUMMARY

There has a mechanism (state transition) in which the absorbed energy is redistributed between PSII and PSI in higher plants, green algae, red algae and cyanobacteria (Fork and Satoh, 1986, Mohanty et al., 2011). The preferential excitation of PSII makes the PQ pool in the electron transport chain more reduced (Allen et al., 1981; Horton et al., 1981). These conditions favor the docking of PQH_2 to quinol oxidation (Qo) site of Cyt b_6/f complex (Zito et al., 1999, Mao et al., 2002), which in turn activates an enzyme, thylakoid protein kinase STN7 in higher plants and green algae (Pesaresi et al., 2009). Redox activated kinase STN7/STT7 phosphorylates major LHCII and it gets associated with PSI, thereby increasing the absorption cross section of PSI complex. In *C. reinhardtii*, STT7 kinase associated with photosynthetic membrane complexes PSI-LHCI-LHCII and Cyt b_6/f (Takahashi et al., 2006; Lemeille et al., 2009). The state in which the major LHCII is associated with PSI-LHCI is called state I. State transition are crucial for plant fitness under natural light conditions. The preferential excitation of PSI makes the PQ pool oxidized, which is the signal for the activity of phosphatase to dephosphorylate LHCII (Allen et al., 1981). The association of LHCII with PSII is called state I. Recently, it has been shown that thylakoid-associated phosphatase TAP38 was required for LHCII dephosphorylation, thus leading to transition from state II to state I in *A. thaliana* (Pribil et al., 2010).

However, it has been reported that major LHCII undergoes phosphorylation in *A. thaliana*. The LHCII occurs in a trimeric oligomerization state and consists of various combinations of three very similar proteins, encoded by the Lhcb1, Lhcb2 and Lhcb3 genes, which usually occur in a ratio of about 8:3:1 (Jansson, 1999). In addition, there are three “minor” antenna complexes, which are called Lhcb4 (CP29), Lhcb5 (CP26) and Lhcb6 (CP24) and usually occur in

monomeric states. However, it is not clear that which component of LHCII undergoes phosphorylation during state transition in *A. thaliana*.

In the first objective, we have carried out characterization of light induced state transitions- Identification of phosphorylated major LHCII and its role on grana and PSI organization in wt and *stn7* (which blocks the STN7 kinase, thus it will not perform state transitions). Due to global warming, the continuous raise in temperature may also affect the mechanism of state transition. Thus, in our 2nd and 3rd objectives, we have focused on moderate temperature triggered state transition in dark and light combinations. Also focused on the factors responsible for dark reduction of PQ pool. Generally, the mechanism of energy distribution between PSI and PSII occurs in low light conditions. However, in this study we are first time reporting that dark moderate temperature triggers LHCII phosphorylation and also its signaling mechanism of dark temperature induced state transition in wt and *stn7*.

In our study, we have induced state transitions by using blue and red filters for the expression of state II and state I, respectively in wt and mutant (*stn7*) plants of *A. thaliana*. The mechanism of state transitions have been confirmed by using PAM and 77K fluorescence emission spectroscopy. Western blotting and 2D electrophoresis were carried out for determination of phosphorylated LHCII and its isoforms, respectively. Morphologically, the difference in growth pattern of *A. thaliana* of wt and *stn7* mutant was not significant, however, *stn7* plant grow quite slowly compared to wt plants.

Isolated thylakoids from preilluminated plants with either state I or state II light, have shown signals for phospho proteins when blotted with antiphospho-threonine antibody (from Cell Signaling Technology, UK). We have observed three major signals which corresponds to phospho proteins of thylakoid membranes which corresponding to CP43, D1/D2 and LHCII.

Similar pattern was observed when plants were preilluminated with low intensity light (30 µmol photons m^{-2} s^{-1}) for different time periods (Tikkanen et al., 2010). In our study, major LHCII underwent phosphorylation particulary Lhcb2 under state II light condition and not in state I. However, CP43 and D2 proteins were phosphorylated under both state I and state II. At the same time *stn7* plants exposed to either state I light or state II light, did not show any signal for phosphorylated LHCII, but CP43 and D2 proteins underwent phosphorylation. This indicates that phosphorylation of core proteins may play role in organization of PSII. Based on our studies it is clear that Lhcb2 was one of the major antennae proteins which undergo phosphorylation during state transitions.

2D electrophoresis has revealed changes in the thylakoid membrane proteins under state I and state II light exposed conditions. Thylakoids isolated from wt and *stn7* mutant plants preilluminated with either state I or state II light, have shown very similar pattern of 2D spots. It has been reported that Lhcb2 has three isoforms. However, in the range of 25-27 kDa, we have observed three major spots in state I preilluminated wt plants (corresponds to three isoforms), where as in state II exposed light condition, an additional spot was observed as fourth spot. In *stn7* plants this was not observed. 2D gels were probed with antiphospho- threonine antibody, where 27 kDa protein spots were phosphorylated; however, this observation was not seen in state I. We presume that the resolution of 4^{th} spots in state II was due to phosphorylation of Lhcb2. Phosphorylation of one of the isoform of Lhcb2 is enough for mobilization of major LHCII from PSII to PSI. PSI-LHCI supercomplexs were isolated by sucrose density gradient ultracentrifugation and fraction composition were analyzed by Tricine PAGE in order to see the migration of LHCII to PSI. These results have shown that major LHCII is associated with PSI-LHCII. The redox status of PQ pool was analyzed by fast Chl *a* fluorescence transients. Plants

adapted to state II light have shown relatively less fluorescence in OJIP fluorescence transients. In another hand, the TEM images showed that the increase in grana stacking in state II adapted leaves were due to phosphorylation of major LHCII to facilitate its mobilization to stroma lamellae where PSI is enriched.

In the second objective, we have focused on demonstrating the effects of elevated temperature on energy distribution. Our results indicate that moderate temperature elevation under dark conditions induces phosphorylation and migration of LHCII similar to that of light-induced state transitions, suggesting that LHCII phosphorylation can also be thermally-triggered.

The effect of temperature on the photosynthetic machinery is crucial for the fundamental understanding of plant physiology and the bioengineering of heat-tolerant varieties. In the second objective of our study, *A. thaliana* was exposed to moderate (40 °C) short-term heat stress in the dark to evaluate the heat-triggered phosphorylation and migration of light harvesting complex (LHC) II in both wt and mutant lacking STN7 kinase. The 77K emission spectra revealed an increase in PSI relative to PSII emission similar to increase observed during light-induced state I to state II transitions in wt but not in *stn7* mutant. Immunoblotting results indicated that the major LHCII was phosphorylated at threonine sites under moderate temperature stress in wt plants but not in the *stn7* plants. These results supported the proposition that moderate temperature stress triggers state transitions in the dark similar to light-induced state transitions, which involve phosphorylation of LHCII by STN7 kinase. Pre-treatment of *A. thaliana* leaves with inhibitor DBMIB, altered the extent of LHCII phosphorylation and PSI fluorescence emission suggests that activation of STN7 kinase may be dependent on Cyt b_6/f under elevated temperatures in dark. Furthermore, fast Chl *a* transient of temperature-exposed leaves of wt showed a decrease in the F_v/F_m ratio due to both an increase in F_o and a decrease in Fm. In

summary, our findings indicate that a moderate temperature treatment induces state transitions in the dark resulting in the migration of phosphorylated LHCII from the grana to the stroma region.

The redox state of the PQ pool and Cyt b_6/f constitutes the central signal for state transition mechanism. In general, docking of plastoquinol to the Qo site of the Cyt b_6/f complex activates the kinase responsible for LHCII phosphorylation. Phosphorylated LHCII dissociates from PSII and associates preferentially with PSI, which is enriched in the thylakoid stroma, thereby increasing the absorption cross-section of PSI supercomplex. In higher plants and green algae, dark adaptation generally leads to oxidation of PQ pool. Thus, plants under dark conditions are considered to be in state I. In our experiments, leaves were kept in dark during temperature treatment, and were therefore expected to be in state I. However, we observed that the heat treatment in dark caused a transition from state I to state II. The involvement of the PQ pool and Cyt b_6/f on the temperature-induced state transitions has been assessed by using DCMU or DBMIB. The activation signal for STN7 kinase appeared to be from Cyt b_6/f, but the source of electrons was from stromal reductants. It is possible that in the dark elevated temperature, the non-photochemical reduction of PQ pool is enhanced due to chlororespiration. However, the above changes were not observed at 35 ^{o}C. The detailed study of signal mechanism of temperature induced state transitions was discussed in objective 3.

In the 3rd objective, *A. thaliana* was exposed to moderate temperature (40 ^{o}C) for short duration (up to 60 min) in dark and/or light to evaluate the source of electrons to the PQ pool and Cyt b_6/f, which triggered STN7 kinase activation and subsequent phosphorylation of LHCII. The PAM fluorometry results have shown that the PQ pool and Cyt b_6/f were non-photochemically reduced by a process called chlororespiration. $HgCl_2$ treatment leaves have suggested the involvement of NDH in catalyzing the non-photochemical reduction of the PQ pool. Further, far

red illumination and antimycin studies have shown that moderate temperature enhanced cyclic electron transport. Sucrose density gradient ultracentrifugation and absorption spectroscopy have shown that the increase in absorbance at 469 and 496 nm were due to Chl *b* and carotenoids of LHCII that associated with PSI-LHCI when the leaves were exposed to 30 min of dark moderate temperature in wt. The same increase was not observed in *stn7* mutant plants, which does not perform state transitions. However, immunoblotting results have shown that moderate temperature applied together with low light (35-40 μmol photons m^{-2} s^{-1}) or high light (HL) (1000 μmol photons $m^{-2}s^{-1}$) did not cause phosphorylation of LHCII, whereas temperature treatment (40 ^{o}C) caused phosphorylation of LHCII. Moreover, HL and HL superimposed with moderate temperature induced hyper phosphorylation of D1/D2 and CP43. 77K fluorescence emission spectra revealed that LL in combination with temperature did not show any increase in PSI fluorescence. These results demonstrated that the moderate temperature in combination with either low or high light (40 ^{o}C + LL and 40 ^{o}C + HL), non-cyclic electron transport may be accelerated unlike at moderate temperature alone which triggered cyclic electron trnasport. Also, it is indicated that STN7 kinase and subsequent LHCII phosphorylation is necessary in chlororespiration triggered state transitions in order to withstand abiotic stress such as moderate temperature.

CONCLUSIONS

In light induced state transitions, Lhcb2 was one of the component among major LHCII which undergo phosphorylation during state II. Further, 2D electrophoresis studies have revealed that additional isoform was appeared as Lhcb2 which may be due to phosphorylation during state II. Also, the phosphorylated Lhcb2 prompt to migrate other subunits of LHCII to PSI. For easy

mobilization of LHCII from grana to stroma lamellae the diameter of the grana has been increased and also Chl *a* dimers in PSI-LHCI increased due to association of LHCII.

This is, to our knowledge, the first demonstration (discovery) that moderate temperature (40 ^{o}C) enhances the chlororespiration (objective 2 and 3) by which PQ pool and Cyt b_6/f are reduced, which activated by STN7 kinase to phosphorylate LHCII. In this mechanism, NDH is the enzyme actively involved in the non-photochemical reduction of the PQ pool. The phosphorylated LHCII increases the relative absorption cross section of PSI-LHCI supercomplex. However, the phosphorylation of LHCII was not observed when low or high light conditions are combined with moderate temperature. Nevertheless, LL and moderate temperature could induce LHCII phosphorylation when applied individually. Cyclic electron transport and linear electron transport plays a major role in the phosphorylation of LHCII during these different treatments. The pathways of cyclic electron transport involving ferredoxin PQ reductase and NDH complex were accelerated at moderate temperature. However, LL combined with moderate temperature decreased cyclic electron transport and accelerated linear electron transport. Thus, dark reduction of PQ pool by NDH activity was one of the major factor triggered state transitions under moderate temperature and this study is important to identify the early stress signals when plants exposed to abiotic stress.

Literature cited

REFERENCES

Allakhverdiev, S.I., Kreslavski, V.D., Klimov, V.V., Los, D.A., Carpentier, R. and Mohanty, P. (2008) Heat stress: an overview of molecular responses in photosynthesis, Photosynth Res 98: 541-550.

Allen, J. (2003) Cyclic, pseudocyclic and noncyclic photophosphorylation: New links in the chain. Trends Plant Sci 8: 15–19.

Allen, J. F. (2004). Cytochrome b_6f: structure for signaling and vectorial metabolism. Trends Plant Sci 9: 130–137.

Allen, J.F, (2003) State transitions - a question of balance. Science 299, 1530-1532.

Allen, J.F., Bennett, J., Steinback, K.E. and Arntzen, C.J. (1981) Chloroplast protein phosphorylation couples PQ redox state to distribution of excitation-energy between photosystems. Nature 291: 25-29.

Allen, J.F. and Mullineaux, C, (eds) (2009) State transitions. Photosynth Res 99, 155-255.

Allen, J.F. (1992) Protein phosphorylation in regulation of photosynthesis. Biochim. Biophys. Acta 1098: 275-335.

Amunts, A., Toporik, H., Borovikova, A. and Nelson, N. (2010) Structure determination and improved model of plant photosystem I. J. Biol. Chem. 285: 3478-3486.

Arnon, D.I. (1949) Copper Enzymes in Isolated Chloroplasts. Polyphenoloxidase in Beta Vulgaris. Plant Physiol. 24: 1-15.

Badger, M.R., Von Caemmerer, S., Ruuska, S., Nakano, H., Laisk, A., Allen, J.F., Asada, K., Matthijs, H.C.P. and Griffiths, H. (2000) Electron flow to oxygen in higher plants and algae: Rates and control of direct photoreduction (Mehler reaction) and rubisco oxygenase. Phil. Trans. R. Soc. B 355: 1433-1446.

Baker, N.R. (1991) A possible role for photosystem II in environmental perturbations of photosynthesis. Physiol. Plant. 81: 563– 570.

Baniulis, D., Yamashita, E., Whitelegge, J.P., Zatsman, A.I., Hendrich, M.P., Hasan, S.S., Ryan, C.M. and Cramer, W.A. (2009) Structure-Function, Stability, and Chemical Modification of the Cyanobacterial Cytochrome b_6f Complex from *Nostoc* sp. PCC 7120. J. Biol. Chem. 284: 9861–9869.

Barber, J. (2003) Photosystem II: the engine of life. Q Rev Biophys 36: 71–89

Bassi, R. and Simpson, D. (1987) Chlorophyll–protein complexes of barley photosystem I. Eur. J. Bio. Chem. 163: 221–230.

Bellafiore, S., Barneche, F., Peltier, G. and Rochaix, **J.D.** (2005) State transitions and light adaptation require chloroplast thylakoid protein kinase STN7. Nature 433: 892-895.

Bendall, D.S. and Manasse, R.S. (1995) Cyclic photophosphorylation and electron transport. Biochim. Biophys. Acta 1129: 23–38.

Bennett, J. (1977) Phosphorylation of chloroplast membrane polypeptides. Nature 269: 344-346.

Bennett, J. (1979) Chloroplast phosphoproteins. Phosphorylation of polypeptides of the light-harvesting chlorophyll protein complex. Eur. J. Biochem. 99:133–137.

Bennett, J. (1980) Chloroplast phosphoproteins. Evidence for a thylakoid-bound phosphoprotein phosphatase. Eur. J. Biochem. 104: 85–89.

Bennett, J., Elizabeth, K. and Michel, H. (1988) Cytochrome b6f complex is required for phosphorylation of light-harvesting chlorophyll a/b complex II in chloroplast photosynthetic membranes. Eur. J. Biochem. 171: 95-100.

Bennett. J., Steinback, K.E. and Arntzen, C.J. (1980) Chloroplast phosphoproteins - regulation of excitation-energy transfer by phosphorylation of thylakoid membrane polypeptides. Proc. Natl. Acad. Sci. USA 77: 5253-5257.

Ben-Shem, A., Frolow, F. and Nelson, N. (2003) Crystal structure of plant photosystem I. Nature 426: 630–635.

Bergantino, E., Dainese, P., Cerovic, Z., Sechi, S. and Bassi, R. (1995) A post-translational modification of the photosystem II subunit CP29 protects maize from cold stress. J. Biol. Chem. 270: 8474–8481.

Bergantino, E., Sandona, D., Cugini, D. and Bassi, R. (1998) The photosystem II subunit CP29 can be phosphorylated in both C3 and C4 plants as suggested by sequence analysis. Plant Mol. Biol. 36: 11–22.

Berry, J. and Björkman, O. (1980) Photosynthetic response and adaptation to temperature in higher plants. Annu. Rev. Plant Physiol. 31: 491-543.

Bonardi, V., Pesaresi, P., Becker, T., Schleiff, E., Wagner, R., Pfannschmidt, T., Jahns, P. and Leister, D. (2005) Photosystem II core phosphorylation and photosynthetic acclimation require two different protein kinases. Nature 437: 1179-1182.

Bonaventura, C. and Myers, J. (1969) Fluorescence and oxygen evolution from *Chlorella Pyrenoidosa*. Biochim. Biophys. Acta 189: 366–383.

Brangeon, J., and Mustardy, L. (1979). The ontogenic assembly of intra-chloroplast lamellae viewed in 3-dimensions. Biol. Cell. 36**,** 71–80.

Bukhov, N. and Carpentier, R. (2004) Alternative photosystem I-driven electron transport routes: mechanisms and functions. Photosynth. Res. 82: 17–33.

Bukhov, N.G. and Carpentier, R. (2000) Heterogeneity of photosystem II reaction centers as influenced by heat treatment of barley leaves. Physiol. Plant 110: 279-285.

Bukhov, N.G., Sabat, S.C. and Mohanty, P. (1990) Analysis of chlorophyll *a* fluorescence changes in weak light in heat treated *Amaranthus* chloroplasts. Photosynth. Res. 23: 81-87.

Bukhov, N.G., Samson, G. and Carpentier. R. (2000) Non photosynthetic reduction of the intersystem electron transport chain of chloroplasts following heat stress. Steady-state rate. Photochem. Photobiol. 72**:** 351–357.

Bukhov, N.G., Wiese, C., Neimanis, S. and Heber, U. (1999) Heat sensitivity of chloroplasts and leaves: leakage of protons from thylakoids and reversible activation of cyclic electron transport. Photosynth. Res. 59: 81-93.

Burrows, P.A., Sazanov, L.A., Svab, Z., Maliga, P. and Nixon, P.J. (1998) Identification of a functional respiratory complex in chloroplasts through analysis of tobacco mutants containing disrupted plastid *ndh* genes. EMBO J. 17: 868–876.

Caffarri, S., Kouřil, R., Kereïche, S., Boekema, E.J. and Croce, R. (2009) Functional architecture of higher plant photosystem II supercomplexes. EMBO J. 28: 3052-3063.

Caffarri, S., Croce, R., Cattivelli, L. and Bassi, R. (2004) A look within LHCII: differential analysis of the Lhcbl-3 complexes building the major trimeric antenna complex of higher-plant photosynthesis. Biochemistry 43: 9467–9476.

Casano, L.M., Martin, and M., Sabater, B. (2001) Hydrogen peroxide mediates the induction of chloroplastic Ndh complex under photooxidative stress in barley. Plant Physiol. 25: 1450–1458.

Carrillo, N. and Ceccarelli, E.A. (2003) Open questions in ferredoxin-NADP+ reductase catalytic mechanism. Eur. J. Biochem. 270: 1900–15.

Chemeris, Yu. K., Shenderova, L.V., Venediktov, P.S. and Rubin, A.B. (2004) Activation of chlororespiration increases chlorophyll fluorescence yield in *Chlorella* adapted to darkness at high temperature. Biology Bulletin 31: 143-150.

Chuartzman, S.G., Nevo, R., Shimoni, E., Charuvi. D., Kiss, V., Ohad, I., Brumfeld, V. and Reicha, Z. (2008) Thylakoid Membrane Remodeling during State Transitions in *Arabidopsis*. Plant Cell 20: 1029–1039.

Cleland, R.E. and Bendall, D.S. (1992) Photosystem I cyclic electron transport: measurement of ferredoxin-PQ reductase activity. Photosynth. Res. 34: 409–418

Cramer, W. A., Zhang, H., Yan, J., Kurisu, G. and Smith, J.L. (2006) Trans-membrane traffic in the cytochrome b6 f complex. Annu. Rev. Biochem. 75: 769–790.

Croce, R., Zucchelli, G., Garlaschi, F.M. and Jennings, R.C. (1998) A thermal broadening study of the antenna chlorophylls in PSI-200, LHCI, and PSI core. Biochemistry 37: 17355–17360.

Croce, R., Morosinotto, T., Castelletti, S., Breton, J. and Bassi, R. (2002) The Lhca antenna complexes of higher plants photosystem I. Biochim. Biophy. Acta 1556: 29–40.

Cseh Z., Subramanyam, R., Tsonev, T., Bushega, M., Papp, E. and Garab, G. (2000) Thermooptic effect in chloroplast thylakoid membranes. Thermal and light stability of pigment arrays with different levels of structural complexity. Biochemistry 39: 15250-15257.

Dainese, P. and Bassi, R. (1991) Subunit stoichiometry of the chloroplast photosystem- II antenna system and aggregation state of the component chlorophyll-a/b binding proteins. J. Biol. Chem. 266: 8136–8142.

Damkjær, J.T., Kereı̈che, S., Johnson, M.P., Kovacs, L., Kiss, A.Z., Boekema, E.J., Ruban, A.V., Horton, P. and Jansson, S. (2009) The Photosystem II Light-Harvesting Protein Lhcb3 Affects the macrostructure of photosystem II and the rate of state transitions in *Arabidopsis*. Plant Cell 21: 3245–3256.

Danielsson, R., Suorsa, M., Paakkarinen, V., Albertsson, P.A., Styring, S., Aro, E.M. and Mamedov, F (2006) Dimeric and monomeric organization of photosystem II: Distribution of five distinct complexes in the different domains of the thylakoid membrane. J. Biol. Chem. 281: 14241–14249.

De Las Rivas, J. and Barber, J. (1997) Structure and thermal stability of photosystem II reaction centers studied by infrared spectroscopy, Biochemistry 36: 8897-8903.

Dekker, J.P. and Boekema, E.J. (2005) Supramolecular organization of thylakoid membrane proteins in green plants. Biochim. Biophys. Acta 1706: 12–39

Delosme, R., Olive, J. and Wollman, F.A. (1996) Changes in light energy distribution upon state transitions: An in vivo photoacoustic study of the wild type and photosynthesis mutants from *Chlamydomonas reinhardtii*. Biochim Biophys Acta 1273: 150-158.

Delosme. R., Be¨al, D. and Joliot, P. (1994) Photoacoustic detection of flash-induced charge separation in photosynthetic systems. Spectral dependence of the quantum yield. Biochim. Biophys. Acta 1185: 56-64.

Depege, N., Bellafiore, S. and Rochaix, **J.D.** (2003) Role of chloroplast protein kinase Stt7 in LHCII phosphorylation and state transition in *Chlamydomonas*. Science 299: 1572-1575.

Ducruet, J.M. (1999) Relation between the heat induced increase of F_o fluorescence and a shift in the electronic equilibrium at the acceptor side of PSII. Photosynthetica 37: 335-338.

Ducruet, J.M. and Lemoine, **Y.** (1985), Increased heat sensitivity of the photosynthetic apparatus in triazine-resistant biotypes from different plant species. Plant Cell Physiol. 26: 419-429.

Eberhard. S., Finazzi, G. and Wollman, F.A. (2008) The dynamics of photosynthesis. Annu. Rev. Genet. 42, 463–515.

Enami, I., Kitamura, M., Tomo, T., Isokawa, Y., Ohta, H. and Katoh, S. (1994) Is the primary cause of thermal inactivation of oxygen evolution in spinach PS-II membranes release of the extrinsic 33 kDa protein or of Mn?. Biochim. Biophys. Acta 1186: 52-58.

Endo, T., Shikanai, T., Sato, F. and Asada, K. (1998) NAD(P)H dehydrogenase-dependent, antimycin A-sensitive electron donation to PQ in tobacco chloroplasts. Plant Cell Physiol. 39: 1226–1231.

Farineau, J. (1999) Study of the non-photochemical dark rise in chlorophyll fluorescence in pre-illuminated leaves of various C3 and C4 plants submitted to partial anaerobiosis. Plant Physiol. Biochem. 37: 911–918.

Farquhar, G.D., von Caemmerer, S. and Berry, J.A. (1980) A biochemical model of photosynthetic CO_2 assimilation in leaves of C3 species. Planta 149: 78-90.

Field, T.S., Nedbal, L. and Ort, D.R. (1998) Nonphotochemical reduction of the PQ pool in sunflower leaves originates from chlororespiration. Plant Physiol. 116: 1209–1218.

Finazzi, G., Furia, A., Barbagallo, R.P. and Forti, G. (1999) State transitions, cyclic and linear electron transport and photophosphorylation in *Chlamydomonas reinhardtii*. Biochim Biophys Acta 1413, 117-129.

Finazzi, G., Rappaport, F., Furia, A., Fleischmann, M., Rochaix, J.D., Zito, F. and Forti, G, (2002) Involvement of state transitions in the switch between linear and cyclic electron flow in *Chlamydomonas reinhardtii*. EMBO reports 3, 280–285.

Fork, D.C. and Satoh, K. (1986) The control by state transitions of the distribution of excitation energy in photosynthesis. Annu. Rev. Plant Phys. 37: 335-361.

Forsberg, J. and Allen, J.F. (2001) Protein tyrosine phosphorylation in the transition to light state 2 of chloroplast thylakoids. Photosynth. Res. 68: 71-79.

Fristedt, R. and Vener, A.V. (2011) High light induced disassembly of photosystem II supercomplexes in *Arabidopsis* requires STN7-Dependent phosphorylation of CP29. PLoS ONE, 6: e24565.

Fromme, P. and Allen, J.P. (2008) X-ray Crystallography of Photosynthetic Proteins. Aartsma T.J and Matysik, J. (eds): Biophysical Techniques in Photosynthesis II, Springer pp. 97–124.

Gal, A., Schuster, G., Frid, D., Canaani, O., Schwieger, H.G. and Ohad, I. (1988) Role of the cytochrome b6.f complex in the redox-controlled activity of Acetabularia thylakoid protein kinase. J. Biol. Chem. 263: 7785-7791.

Gal, A., Shahak, Y., Schuster, G. and Ohad, I. (1987) Specific loss of LHCII phosphorylation in the *Lemna* mutant 1073 lacking the cytochrome b6f complex. FEBS Lett. 221: 205-210.

Gal, A., Hauska, G., Herrmann, R. and Ohad, I. (1990) Interaction between Light Harvesting Chlorophyll-a/b Protein (LHCII) Kinase and Cytochrome b_6/f Complex; In vitro control of kinase Activity. J Biol Chem 265, 19742-19749.

Garab. G., Ferenc Lajko, J., Mustardy, L. and Marton, L. (1989) Respiratory control over photosynthetic electron transport in chloroplasts of higher-plant cells: evidence for chlororespiration. Planta l79: 349-358.

Govindjee. (1995) Sixty-three years since Kautsky: chlorophyll a fluorescence. Aust. J. Plant Physiol. 22: 131–160.

Govindjee and Govindjee, R. (1975) Introduction to Photosynthesis, In: Bioenergetics of Photosynthesis, (ed. Govindjee) Academic Press, NY, pp. 1-50

Groom, Q.J., Kramer, D.M., Crofts, A. and Ort, D.R. (1993) The nonphotochemical reduction of PQ in leaves. Photosynth. Res. 36: 205–215.

Guskov, A., Kern, J., Gabdulkhakov, A., Broser, M., Zouni, A. and Saenger, W. (2009) Cyanobacterial photosystem II at 2.9-A resolution and the role of quinones, lipids, channels and chloride. Nat. Struct. Mol. Biol. 16: 334–342.

Haldimann, P. and Tsimilli-Michael, M (2002) Mercury inhibits the non-photochemical reduction of PQ by exogenous NADPH and NADH: evidence from measurements of the polyphasic chlorophyll *a* fluorescence rise in spinach chloroplasts. Photosynth. Res. 74: 37–50.

Haldrup, A., Jensen, P.E., Henrik, C.L. and Scheller, V (2001) Balance of power: a view of the mechanism of photosynthetic state transitions. Trends Plant Sci. 6: 301-305.

Harris, G.C. and Heber, U. (1993) Effects of anaerobiosis on chlorophyll fluorescence yield in spinach *(Spinaciaoleracea*) leaf discs. Plant Physiol. 101: 1169–1173.

Havaux, M. (1996) Short-term responses to photosystem I to heat stress, Induction of a PS II-independent electron transport through PSI fed by stromal components. Photosynth. Res. 47: 85-97.

Heber, U. and Walker, D. (1992) Concerning a dual function of coupled cyclic electron transport in leaves. Plant Physiol. 100: 1621–1626.

Hippler, M., Klein, J., Fink, A., Allinger, T., and Hoerth, P. (2001) Towards functional proteomics of membrane protein complexes: analysis of thylakoid membranes from *Chlamydomonas reinhardtii*. Plant J. 28; 595–606.

Horton, P., Allen, J.F., Black, M.T. and Bennett, J. (1981) Regulation of phosphorylation of chloroplast membrane polypeptides by the redox state of PQ. FEBS Lett. 125: 193-196.

Horton, P., Ruban, A.V. and Walters, R.G. (1996) Regulation of light harvesting in green plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 47: 655–884.

Ihalainen, J.A., Gobets, B., Sznee, K., Brazzoli, M., Croce, R., Bassi, R., van Grondelle, R., Korppi-Tommola, J.E.I. and Dekker, J.P. (2000) Evidence for two spectroscopically different dimers of light-harvesting complex I from green plants. Biochemistry 39: 8625–8631.

Iwai, M., Noriko, M.Y.I. and Minagawa, J. (2010a) Live-cell imaging of photosystem II antenna dissociation during state transitions. Proc. Natl Acad. Sci. USA 107: 2337-2342.

Iwai, M., Takizawa, K., Tokutsu, R., Okamuro, A., Takahashi, Y. and Minagawa J. (2010b) Isolation of the elusive supercomplex that drives cyclic electron flow in photosynthesis. Nature 464: 1210–1213.

Iwai, M., Yuichiro, T. and Minagawa J. (2008) Molecular remodeling of photosystem II during state transitions in *Chlamydomonas reinhardtii*. Plant Cell 20: 2177–2189.

Jans. F., Mignolet, E., Pierre-Alain, H., Cardol, P., Ghysels, B.,Cuiné, S., Cournac, L., Peltier, G., Remacle, C. and Franck, F. (2008) A type II NAD(P)H dehydrogenase mediates light-independent PQ reduction in the chloroplast of *Chlamydomonas*. Proc. Natl. Acad. Sci. USA 105: 20546–20551.

Jansson, S. (1999) A guide to the Lhc genes and their relatives in *Arabidopsis*. Trends Plant Sci. 4: 236–240.

Jensen, P.E., Haldrup, A., Rosgaard, L. and Scheller, H.V. (2003) Molecular dissection of photosystem I in higher plants: topology, structure and function. Physiol. Plantarum 119: 313–321.

Jolley, C., Ben-Shem, A., Nelson, N. and Fromme, P. (2005) Structure of plant photosystem I revealed by theoretical modeling. J. Biol. Chem. 280: 33627-33636.

Jordan, P., Fromme, P., Witt, H.T., Klukas, O., Saenger, W. and Krauu, N. (2001) Three-dimensional structure of cyanobacterial photosystem I at 2.5Å resolution. Nature 411: 909-917

Joshua, S. and Mullineaux, C.W. (2004) Phycobilisome diffusion is required for light-state transitions in cyanobacteria. Plant Physiol. 135: 2112–2119.

Kamiya, N. and Shen, J.-R. (2003) Crystal structure of oxygen-evolving photosystem II from *Thermosynechococcus vulcanus* at 3.7-Å resolution. Proc. Natl. Acad. Sci. USA 100: 98–103.

Kargul, J. and Barber, J. (2008) Photosynthetic acclimation: Structural reorganisation of light harvesting antenna – role of redox-dependent phosphorylation of major and minor chlorophyll *a/b* binding proteins. FEBS J. 275: 1056–1068

Kargul, J., Nield, J. and Barber, J (2003) Three-dimensional reconstruction of a light-harvesting complex I-photosystem I (LHCI-PSI) supercomplex from the green alga

Chlamydomonas reinhardtii Insights into light harvesting for PSI. J. Biol. Chem. 278: 16135-16141.

Kawakami, K., Umena, Y., Kamiya, N. and Shen, J.-R. (2011) Structure of the catalytic, inorganic core of oxygen-evolving photosystem II at 1.9 resolution. J. Photochemi. Photobiol. B 104: 9-18

Knoetzel, J., Mant, A., Haldrup, A., Jensen, P.E. and Scheller, H.V. (2002) *PSI-O, a new 10-kDa subunit of eukaryotic photosystem I.* FEBS Lett. 510: 145– 148.

Knoetzel, J., Svendsen, I. and Simpson, D.J. (1992) Identification of the Photosystem I antenna polypeptides in barley: isolation of three pigment-binding antenna complexes. Eur. J. Biochem. 206: 209–215.

Kofer, W., Koop, H.U., Wanner, G. and Steinmüller, K. (1998) Mutagenes is of the genes encoding subunits A, C, H, I, J and K of the plastid NAD(P)H-PQ-oxidoreductase in tobacco by polyethylene glycol-mediated plastome transformation. Mol. Gen. Genet. 258: 166–173.

Kouril, R., Zygadlo, A., Arteni, A.A., de Wit, C.D., Dekker, J.P., Jensen, P.E., Scheller H.V. and Boekema, E.J. (2005) Structural Characterization of a Complex of Photosystem I and Light-Harvesting Complex II of *Arabidopsis thaliana*. Biochemistry 44: 10935-10940.

Kouril, R., Lazar, D., Ilik, P., Skotnica, J., Krchnak, P. and Naus, J. (2004) High-temperature induced chlorophyll fluorescence rise in plants at 40–50°C: experimental and theoretical approach. Photosynth. Res. 81: 49-66.

Kovacs, L., Damkjaer, J., Kereiche, S., Ilioaia, C., Ruban, A.V., Boekema, E.J., Jansson, S. and Horton, P. (2006) Lack of the light-harvesting complex CP24 affects the structure and sunction of the grana membranes of higher plant chloroplasts. Plant Cell, 18, 3106–3120.

Kurisu, G., Zhang, H., Smith, J. L. and Cramer, W. A. (2004) Structure and function of the cytochrome b6f complex of oxygenic photosynthesis. Proteins, Nucleic Acids, Enzymes 49: 1265–1273.

Lam, E., Ortiz, W. and Malkin, R. (1984) Chlorophyll *a/b* proteins of photosystem I. FEBS Lett. 168**:** 10–14.

Lazar, D (1999) Chlorophyll *a* fluorescence induction. Biochim Biophys Acta 1412, 1–28.

Lemeille, S. and Rochaix, J.D. (2010) State transitions at the crossroad of thylakoid signalling pathways. Photosynth Res 106: 33-46.

Lemeille, S., Willig, A., Fargeix, D.N., Delessert, C., Bassi, R. and Rochaix, J.D. (2009) Analysis of the chloroplast protein kinase Stt7 during state transitions. PLoS Biol. 7: e1000045.

Lípová, L., Krchňák, P., Komenda, J. and Ilík, P. (2010) Heat-induced disassembly and degradation of chlorophyll-containingprotein complexes in vivo. Biochim. Biophys. Acta. 1797: 63–70.

Loll, B., Kern, J., Saenger, W., Zouni, A and Biesiadka, J. (2005) Towards complete cofactor arrangement in the 3.0 angstrom resolution structure of photosystem II. Nature 438: 1040–1044.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–75.

Lunde, C., Jensen, P.E., Haldrup, A, Knoetzel, J.and Scheller, H.V. (2000) The PSI-H subunit of photosystem I is essential for state transitions in plant photosynthesis. Nature 408: 613-615.

Mathur, S., Allakhverdiev, S.I. and Jajoo, A (2011) Analysis of high temperature stress on the dynamics of antenna size and reducing side heterogeneity of Photosystem II in wheat leaves (*Triticum aestivum*). Biochim. Biophys. Acta 1807: 22-29.

Mao, H.B., Li, G.F., Ruan, X., Wu, Q.Y., Gong, Y.D., Zhang, X.F. and Zhao, N.M. (2002) The redox state of PQ pool regulates state transitions via cytochrome b_6/f complex in *Synechocystis* sp. *PCC 6803*. FEBS Lett. 519: 82-86.

Minagawa, J. (2011) State transitions; The molecular remodeling of photosynthetic supercomplexes that controls energy flow in the chloroplast. Biochim. Biophys. Acta 1807: 897–90.

Mohanty, P., Vani, B. and Prakash, J.S.S. (2002) Elevated temperature treatment induced alteration in thylakoid membrane organization and energy distribution between the two photosystems in *Pisumsativum*. Z Naturforsch 57c: 836-842.

Mohanty, P., Nellaepalli, S., Mishra, S. and Subramanyam, R. (2011) State shifts in photosynthetic organisms: Tracking traits and techniques. In: S. Itoh, P.Mohanty, K.N. Guruprasad (Eds.), Photosynthesis – Overviews on Recent Progress and Future

Perspective, I.K. (Information & Knowledge) International Publishers, New Delhi, India (2011) pp 38–59.

Muh, F., Renger, T. and Zouni, A. (2008) Crystal structure of cyanobacterial photosystem II at 3.0 Å resolution: A closer look at the antenna system and the smallmembrane-intrinsic subunits. Plant Physiol. Biochem. 46: 238-264.

Mullineaux, C.W. and Emlyn-Jones, D. (2005) State transitions – an example of acclimation to low-light stress. J. Exp. Bot. 56: 389-393

Murata, N., Nishimura, M. and Takamiya, A. (1966) Fluorescence of chlorophyll in photosynthetic systems III. Emission bands of chlorophyll *a* and the energy transfer between two pigment systems. Biochim. Biophys. Acta; 126: 234-243.

Murata. N. (1969a) Control of excitation transfer in photosynthesis. I. Light-induced change of chlorophyll *a* fluorescence in *Porphyridium cruentum*. Biochim. Biophys. Acta 172: 242.

Murata. N. (1969b) Control of excitation transfer in photosynthesis. II. Magnesium ion-dependent distribution of excitation energy between two pigment systems in Spinach chloroplasts. Biochim. Biophys. Acta 189: 171.

Nellaepalli, S., Mekala, N.R., Zsiros, O., Mohanty, P. and Subramanyam, R. (2011) Moderate heat stress induces state transitions in *Arabidopsis thaliana*. Biochem. Biophys. Acta 1807: 1177–1184.

Nelson, N. and Ben-Shem, A. (2002) Photosystem I reaction center: Past and future. Photosynth. Res. 73: 193–206.

Pakrasi, H.B. (1995) Genetic analysis of the form and function of photosystem I and photosystem II. Annu. Rev. Genet. 29: 755-76.

Papageorgiou, G. and Govindjee. (1968) Light-induced changes in fluorescence yield of chlorophyll Alpha in vivo .I. *Anacystis Nidulans*. Biophys. J. 8: 1299-1315.

Pastenes, C. and Horton, P (1996) Effect of high temperature on photosynthesis in beans; Oxygen evolution and chlorophyll fluorescence. Plant Physiol. 112: 1245–1251.

Peltier, G. and Cournac, L (2002) Chlororespiration. Ann. Rev. Plant Biol. 53: 523–550.

Pesaresi, P., Hertle, A., Pribil, M., Kleine, T., Wagner, R., Strissel, H., Ihnatowicz, A., Bonardi, V., Scharfenberg, M., Schneider, A., Pfannschmidt, T. and Leister, D (2009) *Arabidopsis* STN7 Kinase Provides a Link between Short- and Long-Term Photosynthetic Acclimation. Plant Cell 21: 2402–2423

Pesaresi, P., Hertle, A., Pribil, M., Schneider, A., Kleine, T. and Leister, D. (2010) Optimizing photosynthesis under fluctuating light the role of the *Arabidopsis* STN7 kinase. Plant Signaling and Behavior 5: 21-25.

Pesaresi, P., Pribil, M., Wunder, T. and Leister, D. (2011) Dynamics of reversible protein phosphorylation in thylakoids of flowering plants: The roles of STN7, STN8 and TAP38. Biochim. Biophys. Acta 1807: 887–896.

Pribil, M., Pesaresi. P., Hertle. A., Barbato, R. and Leister, D. (2010) Role of plastid protein phosphatase TAP38 in LHCII dephosphorylation and thylakoid electron flow. PLoS Biol 8, e1000288.

Rechtsteiner, G.A. and Ganske, J.A. (1998) Using natural and artificial light sources to illustrate quantum mechanical concepts. The Chem. Edu. 3: 1-12.

Rintamaki, E., Martinsuo, P., Pursiheimo, S. and Aro, E.M. (2000) Cooperative regulation of light-harvesting complex II phosphorylation via the plastoquinol and ferredoxin–thioredoxin system in chloroplasts. Proc. Natl. Acad. Sci. USA 97: 11644–11649.

Rochaix, J.D. (2011) Regulation of photosynthetic electron transport. Biochim. Biophys. Acta 1807: 375-383.

Roose, J.L., Wegener, K.M. and Pakrasi, H.B. (2007) The extrinsic proteins of photosystem II. Photosynth. Res. 92: 369–387

Ruban, A.V. and Johnson, M.P. (2009) Dynamics of higher plant photosystem cross-section associated with state transitions. Photosynth. Res. 99: 173-183.

Rumeau, D., Peltier, G. and Cournac, L. (2007) Chlororespiration and cyclic electron flow around PSI during photosynthesis and plant stress response. Plant Cell Environ. 30: 1041-1051.

Sane, P.V., Desai, T.S., Tatake, V.G. and Govindjee. (1984) Heat induced reversible increase in photosystem I emission in algae, leaves and chloroplasts: Spectra, activities, and relation to state changes. Photosynthetica 18: 439-44.

Sazanov, L.A., Burrows, P.A. and Nixon, P.J. (1998) The chloroplast Ndh complex mediates the dark reduction of the PQ pool in response to heat stress in tobacco leaves. FEBS Lett. 429: 115–118

Schägger, H. (2006) Tricine–SDS-PAGE. Nature Protocols 1: 16 - 22.

Schansker, G., Toth, S.Z. and Strasser, R.J. (2005) Methylviologen and dibromothymoquinone treatments of pea leaves reveal the role of photosystem I in the Chl *a* fluorescence rise OJIP. Biochim. Biophys. Acta 1706: 250-261.

Scheller, H.V., Jensen, P.E., Haldrup, A., Lunde, C. and Knoetzel, J. (2001) Role of subunits in eukaryotic Photosystem I. Biochim. Biophys. Acta 1507: 41-60.

Schmid, V.H.R., Cammarata, K.V., Bruns, B.U. and Schmidt, G.W. (1997) In vitro reconstitution of the photosystem I light harvesting complex LHCI- 730: heterodimerization is required for antenna pigment organization. Proc. Natl Acad. Sci. USA 94: 7667–7672.

Schmid, V.H.R. (2008) Light-harvesting complexes of vascular plants. Cell Mol. Life Sci. 65: 3619–3639

Shapiguzov, A., Ingelsson, B., Samol, I., Andres, C., Kessler, F., Rochaix, J.D., Vener, A.V. and Goldschmidt-Clermont, M. (2010) The PPH1 phosphatase is specifically involved in LHCII dephosphorylation and state transitions in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* 107: 4782-4787.

Shi, L.X. and Schroder, W.P. (2004) The low molecular mass subunits of the photosynthetic supracomplex, photosystem II. Biochim Biophys Acta-Bioenerg 1608: 75–96.

Shimoni, E., Rav-Hon, O., Ohad, I., Brumfeld, V. and Reich Z. (2005) Three-Dimensional Organization of Higher-Plant Chloroplast Thylakoid Membranes Revealed by Electron Tomography. Plant Cell 17: 2580–2586.

Smith, J. L., Zhang, H., Yan, J., Kurisu, G. and Cramer, W. A. (2004) Cytochrome bc complexes: a common core of structure and function surrounded by diversity in the outlying provinces. Curr. Opin. Struct. Biol. 14: 432–439.

Srivastava, A., Guissre, B., Greppin, H. and Strasser, R.J. (1997) Regulation of antenna structure and electron transport in photosystem II of *Pisum sativum* under elevated temperature probed by the fast polyphasic chlorophyll *a* fluorescence transient: OKJIP. Biochim. Biophys. Acta 1320: 95-106.

Stauber, E.J., Fink, A., Markert, K., Kruse, O., Johanningmeier, U. and Hippler, M. (2003) Proteomics of *Chlamydomonas reinhardtii* light-harvesting proteins. Eukaryot. Cell 2: 978–994.

Stauber, E.J., Busch, A., Naumann, B., Svatos, A. and Hippler, M. (2009) Proteotypic profiling of LHCI from *Chlamydomona reinhardtii* provides new insights into structure and function of the complex. Proteomics 9: 398–408.

Strasser, R.J. and Srivastava, A. (1995) Govindjee, Polyphasic chlorophyll *a* fluorescence transients in plants and cyanobacteria. Photochem. Photobiol. 6: 32-42.

Stroebel, D., Choquet, Y., Popot, J.L. and Picot, D. (2003) An atypical haem in the cytochrome b(6)f complex. Nature 426: 413–8.

Su, J.H. and Shen, Y.K. (2005) Influence of State-2 transition on the proton motive force across the thylakoid membrane in spinach chloroplasts. Photosynth. Res. 85: 235-245.

Subramanyam, R., Jolley, C., Brune, D.C., Fromme, P. and Webber, A.N. (2006) Characterization of a novel Photosystem I–LHCI supercomplex isolated from *Chlamydomonas reinhardtii* under anaerobic (State II) conditions. FEBS Lett. 580: 233–238.

Sundby, C. and Andersson, B. (1985) Temperature-induced reversible migration along the thylakoid membrane of photosystem II regulates it association with LHC-II. FEBS Lett. 191: 24-28.

Takahashi, H., Iwai, M., Takahashi, Y. and Minagawa, J. (2006) Identification of the mobile light-harvesting complex II polypeptides for state transitions in *Chlamydomonas reinhardtii*. Proc. Natl. Acad. Sci. USA 103: 477-482.

Teramoto, H., Ono, T.-A. and Minagawa, J. (2001) Identification of Lhcb gene family encoding the light-harvesting chlorophyll-a/b proteins of photosystem II in *Chlamydomonas reinhardtii*. Plant Cell Physiol. 42: 849–856.

Thierry. J., Laurent, C., Eva, M.H., Peter, M. and Gilles, P. (2001) Increased Sensitivity of Photosynthesis to Antimycin A Induced by Inactivation of the Chloroplast *ndhB* Gene. evidence for a participation of the NADH-Dehydrogenase complex to cyclic electron flow around Photosystem I. Plant Physiol. 125: 1919–1929.

Thomas, P.G., Quinn, P.J. and Williams, W.P. (1986) The origin of photosystem I-mediated electron transport stimulation in heat-stressed chloroplasts. Planta 167, 133–139.

Tikkanen, M., Grieco, M. and Aro, E.M. (2011) Novel insights into plant light-harvesting complex II phosphorylation and state transitions. Trends Plant Sci. 16: 126-131.

Tikkanen, M., Grieco, M., Kangasjarvi, S. and Aro E.M. (2010) Thylakoid Protein Phosphorylation in higher plant chloroplasts optimizes electron transfer under fluctuating light. Plant Physiol. 152: 723-735.

Tikkanen, M., Nurmi, M., Suorsa, M., Danielsson, R., Mamedov, F., Styring, S. and Aro, **E.M.** (2008) Phosphorylation-dependent regulation of excitation energy distribution between the two photosystems in higher plants. Biochim. Biophys. Acta 1777: 425–432.

Tikkanen. M., Nurmi, M., Kangasjärvi, S. and Aro E.M. (2008) Core protein phosphorylation facilitates the repair of photodamaged photosystem II at high light. Biochim. Biophys. Acta 1777: 1432–1437.

Tikkanen, M., Piippo, M., Suorsa, M., Sirpio, S., Mulo, P., Vainonen, J., Vener, A.V., Allahverdiyeva Y. and Aro, E.M. (2006) State transitions revisited: buffering system for dynamic low light acclimation of *Arabidopsis*. Plant Mol. Biol. 62: 779-793.

Tokutsu, R., Iwai, M. and Minagawa, J. (2009) CP29, a Monomeric Light-harvesting Complex II Protein, Is Essential for State Transitions in *Chlamydomonas reinhardtii.*. J. Biol. Chem. 284: 7777–7782.

Toth, S.Z., Schansker, G. and Strasser, **R.J.** (2005) In intact leaves, the maximum fluorescence level (F_m) is independent of the redox state of the PQ pool: a DCMU inhibition study. Biochim. Biophys. Acta 1708: 275-282.

Tóth, S.Z., Schansker, G., Garab, G. and Strasser, R.J. (2007) Photosynthetic electron transport activity in heat-treated barley leaves: The role of internal alternative electron donors to photosystem II. Biochim. Biophys. Acta 1767: 295-305.

Umena, Y., Kawakami, K., Shen, J.-R. and Kamiya, N. (2011) Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9 Å. Nature 473: 55-60

Vainonen, J.P., Hansson, M. and Vener A.V. (2005) STN8 Protein kinase in *Arabidopsis thaliana* is specific in phosphorylation of photosystem II core proteins. J. Biol. Chem. 280: 33679–33686.

van Grondelle, R. and Novoderezhkin, V.I. (2006) Energy transfer in photosynthesis: experimental insights and quantitative models. Phys. Chem. Chem. Phys. 8: 793-807.

Vener, A.V., Harms, A., Sussman, M.R. and Vierstra, R.D. (2001) Mass spectrometric resolution of reversible protein phosphorylation in photosynthetic membranes of *Arabidopsis thaliana*. J. Biol. Chem. 276: 6959–6966.

Vener, A.V. and Ohad, I. (1998) Andersson B. Protein phosphorylation and redox sensing in chloroplast thylakoids. Curr. Opin. Plant Biol.1: 217-223.

Vener, A.V., van kan, P.J.M., Gal, A., Andersson, B. and Ohad, I. (1995) Activation/deactivation cycle of redox-controlled thylakoid protein phosphorylation. J. Biol. Chem. 270: 25225-25232.

Vener, A.V., van kan, P.J.M., Rich, P.R., Ohad, I. and Andersson, B. (1997) Plastoquinol at the quinol oxidation site of reduced cytochrome bf mediates signal transduction between light and protein phosphorylation: thylakoid protein kinase deactivation by a single turnover flash. Proc. Natl. Acad. Sci. USA 94: 1585-1590.

Weis, E. (1985) Light and temperature-induced changes in the distribution of excitation energy between Photosystem I and Photosystem II in spinach leaves. Biochim. Biophys. Acta 41731: 118-126.

Wilson, K.E., Ivanov, A.G., Öquist, G., Grodzinski, B., Sarhan, F. and Huner, N.P.A. (2006) Energy balance, organellar redox status, and acclimation to environmental stress. Canad. J. Bot. 84: 1355-1370.

Whitelegge, J.P., Zhang, H., Aguilera, R., Taylor, RM. and Cramer, W.A. (2002) Full subunit coverage liquid chromatography electrospray ionization mass spectrometry (LCMS+) of an oligomeric membrane protein: cytochrome b(6)f complex from spinach and the cyanobacterium Mastigocladus laminosus. Mol Cell Proteomics 1: 816–27.

Wollman, **F.A.** (2001) State transitions reveal the dynamics and flexibility of the photosynthetic apparatus. EMBO J. 20: 3623-3630.

Wollman, F.A. and Lemaire, C. (1988) Studies on kinase-controlled state transitions in Photosystem II and b6f mutants from *Chlamydomonas reinhardtii* which lack quinone-binding proteins. Biochim. Biophys. Acta 933: 85–94.

Yadavalli, V., Nellaepalli, S. and Subramanyam, R. (2011) Proteomic analysis of thylakoid membranes. Methods Mol. Biol. 684: 159-70.

Yadavalli, V., Malleda, C. and Subramanyam, R. (2011) Protein-protein interactions by molecular modeling and biochemical characterization of PSI-LHCI supercomplexes from *Chlamydomonas reinhardtii*. Molecular BioSystems, 7: 3143-3151.

Yakushevska, A. E., Keegstra, W., Boekema, E. J., Dekker, J. P., Andersson, J., Jansson, S., Ruban, A. V. and Horton, P. (2003) The Structure of Photosystem II in *Arabidopsis:* Localization of the CP26 and CP29 Antenna Complexes. Biochemistry 42: 608–613.

Yamashita, E., Zhang, H. and Cramer, W.A. (2007) Structure of the cytochrome b6f complex: quinone analogue inhibitors as ligands of heme cn. J. Mol. Biol. 370: 39–45

Yamazaki, H., Tasaka, M. and Shikanai, T. (2004) PPR motifs of thenucleus-encoded factor, PGR3, function in the selective and distinct steps of chloroplast gene expression in *Arabidopsis*. Plant J. 38: 152–163.

Zito, F., Finazzi, G., Delosme, R., Nitschke, W., Picot, D., and Wollman, F.A. (1999) The Qo site of cytochrome b_6f complexes controls the activation of the LHCII kinase. EMBO J. 18: 2961-2969.

Zouni, A., Witt, H.T., Kern, J., Fromme, P., Krauß, N., Saenger, W. and Orth, P. (2009) Crystal structure of photosystem II from Synechococcus elongatus at 3.8 Å resolution. Nature 409: 739–743.

Publications

1. **Nellaepalli**, **S**., Mekala, N.R., Zsiros, O., Mohanty, P. and Subramanyam, R. (2011) Moderate heat stress induces state transitions in *Arabidopsis thaliana.* Biochem. Biophys. Acta 1807: 1177–1184.
2. Mohanty, P., **Nellaepalli**, S., Mishra, S. and Subramanyam, R. (2011) State Shifts in Photosynthetic Organisms: Tracking Traits and Techniques In: Photosynthesis: Overviews on Recent Progress and Future Perspectives (Eds. Shigeru Itoh, Prasanna Mohanty, K.N. Guruprasad) I.K. (Information & Knowledge) International Publishers, New Delhi, India (2011) pp 38–59.
3. **Nellaepalli**, **S**., Zsiros, O., Kovács, K., Yadavalli, V, Mekala, N.R., Mohanty, P. and Subramanyam, R. (2011) State transition mechanism in *Arabidopsis thaliana*: Biophysical and proteomic studies. Proceedings in 15th International congress of photosynthesis, held in Beijing during 21-28th August (In Press)
4. Yadavalli V, **Nellaepalli**, **S**. and Subramanyam, R. (2011) Proteomic analysis of thylakoid membranes. Methods Mol. Biol. 684:159-70.
5. Subramanyam, R., Jolley, C., Thangaraj, B., **Nellaepalli**, **S**., Webber, A.N. and Fromme, P. (2010) Structural and functional changes of PSI-LHCI supercomplexes of *Chlamydomonas reinhardtii* cells grown under high salt conditions. Planta 231:913–922
6. Subramanyam, R., Goud, M.G., Sudhamalla, B., Reddeem, E., Gollapudi, A., **Nellaepalli**, **S**., Yadavalli, V., Chinnaboina, M., Amooru, D.G. (2009) Novel binding studies of human serum albumin with trans-feruloyl maslinic acid. J. Photochem,. Photobiol. B 95: 81–88
7. **Nellaepalli**, **S**., Kodru, S. and Subramanyam. R., State transition triggered by chlororespiration at elevated temperature (40°C) in *Arabidopsis thaliana.* **(Submitted to the Plant J)**
8. **Nellaepalli**, **S**., Malavath, T., Zabo, M., Garab, G., Subramanyam, R. Phosphorylation induced organization of thylakoid membrane and PSI-LHCI supercomlex in *Arabidopsis thaliana* under state transition **(Submitted to Planta)**.
9. **Nellaepalli, S**., Otto, Z., Toth, T., Yadavalli, V., Kovacs L., Garab, G., Rajagopal, S. (2011) Heat and light induced structural changes of PSI-LHCI

Supercomplexes in *Pisum sativum*: A thermo-optic mechanism (**(Submitted to PLoS ONE)**.

10. **Nellaepalli, S**., Sireesha, K., and Rajagopal, S. (2011) Effects of cold temperature (10 ^{o}C) on mechanism of state transition in *Arabidopsis thaliana* **(MS under preparation)**.

Presentations in conferences

1. **Sreedhar Nellaepalli**, Prasanna Mohanty, Subramanyam Rajagopal, Temperature induced state transition mechanism in *Arabidopsis thaliana*. International Conference on Plant Science in Post-Genomic Era, School of Life Sceinces, Sambalpur University, Orissa (2011).
2. **Sreedhar Nellaepalli**, Prasanna Mohanty, Does the State Shift also function as a multiple regulatory switch?? International Conference on Plant Science in Post-Genomic Era, School of LifeSceinces, Sambalpur University, Orissa (2011).
3. **Sreedhar Nellaepalli**, Ottó Zsiros, László Kovács,Yadavalli Venkateswarlu, Mekala Nageswara Rao, Prasanna Mohanty, Rajagopal Subramanyam, State transition mechanism in *Arabidopsis thaliana*: Biophysical and proteomic studies. 15th International congress of photosynthesis, held in Beijing during 21-28th August (2010)
4. **Sreedhar Nellaepalli**, Milan Szabo, Prasanna Mohanty, Rajagopal Subramanyam, State transition, role on structure and function of thylakoid membranes and identification of phosphorylation sites in light harvesting complex II, BARC, Mumbai (2009)
5. **Sreedhar Nellaepalli**, Gokara Mahesh, Yadavalli Venkateswarlu, Agepati S. Raghavendra, Subramanyam Rajagopal, Phosphorylation of PSII subunits and its cooperation with OJIP Chl a fluorescence in state transition mechanism of *Arabidopsis thaliana*. International conference on Photosynthesis in the Global Perspective. School of Life Sciences, DAVV, Indore, India (2008)

Biochimica et Biophysica Acta 1807 (2011) 1177–1184

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

Moderate heat stress induces state transitions in *Arabidopsis thaliana*

Sreedhar Nellaepalli [a], Nageswara Rao Mekala [a], Otto Zsiros [b], Prasanna Mohanty [c,d], Rajagopal Subramanyam [a,d,*]

[a] Department of Biochemistry, School of Life Sciences, University of Hyderabad, Hyderabad 500046, India
[b] Institute of Plant Biology, Biological Research Center, Hungarian Academy of Sciences, H-6701, Szeged, Hungary
[c] Agricultural Biotechnology, Orissa University of Agriculture and Technology, Bhubaneswar, 751003, India
[d] Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad 500046, India

ARTICLE INFO

Article history:
Received 10 March 2011
Received in revised form 17 May 2011
Accepted 18 May 2011
Available online 26 May 2011

Keywords:
Arabidopsis thaliana
Elevated temperature
Photosystems
PQ pool
State transition
STN7 kinase

ABSTRACT

The effect of temperature on the photosynthetic machinery is crucial for the fundamental understanding of plant physiology and the bioengineering of heat-tolerant varieties. In our study, *Arabidopsis thaliana* was exposed to mild (40 °C), short-term heat stress in the dark to evaluate the heat-triggered phosphorylation and migration of light harvesting complex (LHC) II in both wild-type (wt) and mutant lacking STN7 kinase. The 77 K emission spectra revealed an increase in PSI relative to PSII emission similar to increases observed in light-induced state I to state II transitions in wt but not in *stn7* mutant. Immunoblotting results indicated that the major LHCII was phosphorylated at threonine sites under heat stress in wt plants but not in the mutant. These results support the proposition that mild heat stress triggers state transitions in the dark similar to light-induced state transitions, which involve phosphorylation of LHCII by STN7 kinase. Pre-treatment of *Arabidopsis* leaves with inhibitor DBMIB, altered the extent of LHCII phosphorylation and PSI fluorescence emission suggests that activation of STN7 kinase may be dependent on Cyt b_6/f under elevated temperatures in dark. Furthermore, fast Chl *a* transient of temperature-exposed leaves of wt showed a decrease in the F_v/F_m ratio due to both an increase in F_o and a decrease in F_m. In summary, our findings indicate that a mild heat treatment (40 °C) induces state transitions in the dark resulting in the migration of phosphorylated LHCII from the grana to the stroma region.

1. Introduction

Temperature stress is one of the major environmental factors that limit plant growth and productivity. Among the plant physiological processes, photosynthesis is most sensitive to heat stress, with inhibition occurring at higher-than-optimal growth temperatures [1]. At moderate heat stress, reversible inhibition of photosynthesis occurs, while at extremely high temperatures damage to the photosynthetic machinery is irreversible [1,2]. Among the photosynthetic machinery, photosystem II (PSII) has been shown to be most sensitive [3,4]. In addition to the down-regulation of PSII, inactivation of PSII reaction centers (RC) [5,6] and electron transfer from Q_A to Q_B at the acceptor side of PSII is affected at moderately elevated temperature [7,8]. Further, prolonged exposure to elevated temperature may lead to the degradation of the oxygen evolving complex (OEC), resulting in reduced electron flow from water to the PSII reaction center [9]. Conversely, it was demonstrated that moderate heat stress does not cause serious impairment of PSII function but instead inhibits PSII repair, while PSI activity is actually enhanced [1,10]. However, in case of chlororespiration during dark incubation of *Chlorella pyrenoidosa*, high temperature doubled the initial chlorophyll fluorescence yield (F_o) [11].

Elevated temperature also affects the structural and functional organization of thylakoid membranes [12], excitation energy transfer, distribution and dissipation apart from effects on the photochemical reaction [13]. The energy distribution between PSI and PSII for optimal photosynthesis has been shown to be affected by moderate to high temperature [13–16]. These studies were focused on demonstrating the effects of elevated temperature on energy distribution; however, the moderate temperature on state transition mechanism remained uncharacterized.

The molecular mechanism of state transition in green algae and higher plants has been well elucidated [17–19]. Plants and green algae possess a unique ability to balance the differences in photochemical energy between PSI and PSII due to their differential light absorption characteristics by altering their relative absorption cross-sections [20,21]. The redox state of the intersystem PQ pool in the linear electron

Abbreviations: Cyt b_6/f, cytochrome b_6/f; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone; LHCII, light harvesting complex II; OEC, oxygen evolving complex; PEA, plant efficiency analyzer; PQ pool, plastoquinone; PS, photosystems; RC, reaction center; wt, wild-type

* Corresponding author at: Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad 500046, India. Tel.: +91 40 2313 4572; fax: +91 40 2301 0120.
E-mail address: srgsl@uohyd.ernet.in (R. Subramanyam).

0005-2728/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbabio.2011.05.016

Proceeding of the 15th International Congress on Photosynthesis
August 22-27, 2010 Beijing, China

State transition mechanism in *Arabidopsis thaliana*: Biophysical and proteomic studies

Sreedhar Nellaepalli[a], Ottó Zsiros[c], László Kovács[c],Yadavalli Venkateswarlu, Mekala Nageswara Rao[a], Prasanna Mohanty[b,d], Rajagopal Subramanyam[a,b*]

[a]Department of Biochemistry, [b]Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad, 500046 India.
[c]Institute of Plant Biology, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary.
[d]College of Agriculture, Orissa University of Agriculture and Technology (OUAT), Bhubaneswar, India.
[*]Corresponding author. Tel. No.+91-40-2313 4572; Fax No. +91-40-2301 0120; E-mail: srgsl@uohyd.ernet.in

Abstract: The redox state of plastoquinone (PQ) pool is the incipient signal in the signal transduction pathway of state transition mechanism, shifting from state I to state II and *vice versa*. The redox state of the Q_A, the primary acceptor of photosystem II (PSII) and the PQ pool are easily monitored by the OJIP fast fluorescence transients. The OJIP fast Chl *a* fluorescence transient studies revealed that in state II, there was reduction in maximal fluorescence which could be due to decreased antennae size of PSII. The same changes were not observed in *Stn7* mutant lacking thylakoid kinase which phosphorylates light harvesting complex (LHC) II. The phosphorylated LHCII is associated with PSI under state II condition. The redox state of PQ pool is signal for the kinase to phosphorylate/dephosphorylate major LHCII of PSII. The 2-D electrophoresis results showed that LHCII is resolved into 3 spots in state I. However, in state II this has been resolved into 4 spots. However, *Stn7* mutant there was no change of 2D spots in state II. The additional spot is yet to be investigated.

Key Words: 2D electrophoresis; *Arabidopsis thaliana*; Chl *a* fluorescence; photosystems; state transition

Introduction:

In oxygenic photosynthetic organisms the light conversion into chemical energy, involving photo oxidation of water and reduction of CO_2 to carbohydrates is mediated by two interactive photochemical reactions namely photosystem I (PSI) and photosystem II (PSII) (Blankenship, 2008). The two photosystems absorb solar radiation differentially resulting in unbalanced energy which requires a mechanism by which the energy can be balanced for optimal photosynthesis. This mechanism is known as state transition (Allen and Mullineaux, 2009).

The preferential excitation of PSII makes PQ pool more reduced. The reduced PQ pool tends to associate with cytochrome b6/f and activates thylakoid kinase (stn7/stt7 kinase) that mediates phosphorylation of light harvesting complex II (LHCII) (Gal et al., 1990). The phosphorylated LHCII migrates from PSII to PSI thereby increasing absorption cross-section of PSI, distributing the energy to PSI leading to state II (Murata, 1969; Bonaventura and Myers, 1969). When PSI gets preferentially excited, the reduced PQ pool starts oxidizing and if it reaches below the saturation level of oxidation of PQ pool, it in turn activates an enzyme called thylakoid phosphatase (TAP38) which dephosphorylates the phosphorylated LHCII (Pribil et al., 2010). The dephosphorylated LHCII undocks from PSI and reassociates with PSII, enhancing absorption cross-section of PSII, thereby leading to state I. The transition from state I to state II and state II to state I is termed as state transition. State transition occurs in natural environments, where light quality and quantity fluctuate with the time (Iwai et al., 2008).

the plant journal

State transition triggered by chlororespiration at moderate temperature in Arabidopsis thaliana

Journal:	*The Plant Journal*
Manuscript ID:	Draft
Manuscript Type:	Original Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Subramanyam, Rajagopal; School of Life Sciences, University of Hyderabad, Plant Sciences Kodru, Sireesha; School of Life Sciences, University of Hyderabad, Plant Science Nellaepalli, Sreedhar; School of Life Sciences, University of Hyderabad, Biochemistry
Key Words:	Arabidopsis thaliana, Chlororespiration, light harvesting complex, phosphorylation, State transitions, Photosystems

Planta (2010) 231:913–922
DOI 10.1007/s00425-009-1097-x

ORIGINAL ARTICLE

Structural and functional changes of PSI-LHCI supercomplexes of *Chlamydomonas reinhardtii* cells grown under high salt conditions

Rajagopal Subramanyam · Craig Jolley · Balakumar Thangaraj · Sreedhar Nellaepalli · Andrew N. Webber · Petra Fromme

Received: 8 November 2009 / Accepted: 23 December 2009 / Published online: 10 January 2010

Abstract The effect of high salt concentration (100 mM NaCl) on the organization of photosystem I-light harvesting complex I supercomplexes (PSI-LHCI) of *Chlamydomonas reinhardtii* was studied. The electron transfer activity was reduced by 39% in isolated PSI-LHCI supercomplexes. The visible circular dichroism (CD) spectra associated with strongly coupled chlorophyll (Chl) dimers were reduced in intensity, indicating that pigment–pigment interactions were disrupted. This data is consistent with results from fluorescence streak camera spectroscopy, which suggest that red-shifted pigments in the PSI-LHCI antenna had been lost. Denaturing gel electrophoresis and immunoblot analysis reveals that levels of the PSI reaction center proteins PsaD, PsaE and PsaF were reduced due to salt stress. PsaE is almost completely absent under high salt conditions. It is known that the membrane-extrinsic subunits PsaD and E form the ferredoxin-docking site. Our results indicate that the PSI-LHCI supercomplex is damaged by reactive oxygen species at high salt concentration, with particular impact on the ferredoxin-docking site and the PSI-LHCI interface.

Keywords *Chlamydomonas* · Excitation energy · Light harvesting complexes · PSI-LHCI supercomplexes · Photosystem I core · Reactive oxygen species · Salt stress · Superoxide dismutase

Abbreviations

CD	Circular dichroism
Chl	Chlorophyll
DDM	*n*-Dodecyl-*β*-maltoside
LHC	Light harvesting complex
MALDI-TOF	Matrix assisted laser desorption ionization time-of-flight mass spectrometry
PS	Photosystem
SOD	Superoxide dismutase

R. Subramanyam (✉) · S. Nellaepalli
Department of Biochemistry, School of Life Sciences, University of Hyderabad, Hyderabad 500046, India
e-mail: srgsl@uohyd.ernet.in

R. Subramanyam · A. N. Webber
The Center for Bioenergy and Photosynthesis, School of Life Sciences, Arizona State University, PO Box 874501, Tempe, AZ 85287, USA

R. Subramanyam · B. Thangaraj · P. Fromme
Department of Chemistry and Biochemistry, The Center for Bioenergy and Photosynthesis, Arizona State University, Tempe, AZ 85287-1604, USA

C. Jolley
Department of Physics, The Center for Bioenergy and Photosynthesis, Arizona State University, Tempe, AZ 85287-1504, USA

Present Address:
C. Jolley
Department of Chemistry and Biochemistry, Montana State University, Bozeman, MT 59717, USA

Introduction

Salinity-stress effects on plant growth often involve impairment of photosynthesis. High amounts of sodium in the soil impair cell metabolism and photosynthesis by increasing osmotic pressure (Brini et al. 2007). The molecular mechanisms responsible for the salt-induced inactivation of the photosynthetic machinery have been studied extensively, with particular focus on photosystem II (PSII), in vitro (Miyao and Murata 1983) and in vivo (Allakhverdiev et al. 1999, 2000; Demetriou et al. 2007). When PSII membrane

CHAPTER 3

State Shifts in Photosynthetic Organisms: Tracking Traits and Techniques

Prasanna Mohanty[1, 2, 3], N Sreedhar[2], Sujata Mishra[1] and Subramanyam Rajagopal[2]

Regional Plant Resource Center, Bhubaneswar, India.
Department of Biochemistry, University of Hyderabad, India.
Jawaharlal Nehru University, New Delhi, India.

ABSTRACT

State transitions, in general, refer to the short term physiological adaptation by the photosynthetic organisms to adjust to the changing light conditions in the environment and efficiently sharing the absorbed light energy by photosystem I (PS I) and photosystem II (PS II) under limiting light conditions. State transition or state shift occurs by well defined mechanism(s) in plants, algae, and cyanobacteria by one or other mechanisms, defined as spill over and/or change in absorption cross section in the proximity of PSI and PSII called α change. Besides balancing of absorbed excitation between the two photosystems, the state transition also serves as a regulatory valve between light harvest and its optimization in the assimilatory processes through redox controlled poising of cyclic and non-cyclic electron transfer. Here we discuss some of the landmarks on the discovery and the mechanisms of state transitions. The mechanism of state transition has been elucidated for green plants and cyanobacteria. Attention has been paid to the differences in light harvesting mechanism between the prokaryotic oxygenic photosynthetic organism (cyanobacteria), green plants and red algae. Some of their mutants widely used in state transition or state shift studies have been highlighted. Mutants generated in *Arabidopsis* and *Chlamydomonas* related to state transition strengthened the concepts of state transition. Many techniques played crucial role in the discovery of state transitions. Apart from chl *a* fluorescence spectroscopy, some other techniques like kinetics of oxygen evolution, photoacoustic techniques, Atomic Force Microscopy (AFM), Fluorescence Recovery After Photobleaching (FRAP), Single particle electron microscopy, and fluorescence video imaging have contributed richly to our understanding of state transition. These techniques have been briefly discussed in this review. Recent

Chapter 14

Proteomic Analysis of Thylakoid Membranes

Venkateswarlu Yadavalli, Sreedhar Nellaepalli, and Rajagopal Subramanyam

Abstract

Chlamydomonas is a model organism to study photosynthesis. Thylakoid membranes comprise several proteins belonging to photosystems I and II. In this chapter, we show the accurate proteomic measurements in thylakoid membranes. The chlorophyll-containing membrane protein complexes were precipitated using chloroform/methanol solution. These complexes were separated using two-dimensional gel electrophoresis, and the resolved spots were exercised from the gel matrix and digested with trypsin. These peptide fragments were separated by MALDI-TOF, and the isotopic masses were blasted to a MASCOT server to obtain the protein sequence. Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF). The method discussed here would be a useful method for the separation and identification of thylakoid membrane proteins.

Key words: 2D electrophoresis, *Chlamydomonas*, IEF, MALDI-TOF, Peptide fragments, Photosystems, SDS-PAGE, Thylakoid proteins, Trypsin digestion

1. Introduction

The primary reactions of oxygenic photosynthesis occur at the thylakoid membrane and are catalyzed by several multi-molecular complexes including photosystem II (PSII), photosystem I (PSI), their associated light-harvesting complexes, the cytochrome b_6f complex, and the ATP synthase. Each of these complexes consists of multiple subunits, pigments, and redox cofactors (1). *Chlamydomonas reinhardtii* is a valuable model system for determining the structure and function of polypeptides of the photosynthetic apparatus and the dynamic aspects of photosynthesis (2).

We present here an easy analytical method for the separation and identification of thylakoid proteins by proteomics tools. The three major steps in proteome analysis are (1) the separation of complex protein mixtures, (2) the characterization of the separated

Robert Carpentier (ed.), *Photosynthesis Research Protocols*, Methods in Molecular Biology, vol. 684,
DOI 10.1007/978-1-60761-925-3_14,