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Review

Assembly and Repair of Photosystem II in *Chlamydomonas reinhardtii*

Himanshu S. Mehra[1,2], Xiaozhuo Wang[1,2], Brandon P. Russell[1], Nidhi Kulkarni[1], Nicholas Ferrari[1], Brent Larson[1], and David J. Vinyard[1]*

[1] Department of Biological Sciences, Louisiana State University, Baton Rouge, LA USA
[2] These authors contributed equally.
* Correspondence: dvinyard@lsu.edu; Tel.: +1 (225) 578-0958

Abstract: Oxygenic photosynthetic organisms use Photosystem II (PSII) to oxidize water and reduce plastoquinone. Here, we review the mechanisms by which PSII is assembled and turned over in the model green alga *Chlamydomonas reinhardtii*. This species has been used to make key discoveries in PSII research due to its metabolic flexibility and amenability to genetic approaches. PSII subunits originate from both nuclear and chloroplastic gene products in *Chlamydomonas*. Nuclear-encoded PSII subunits are transported into the chloroplast and chloroplast-encoded PSII subunits are translated in a coordinated mechanism. Active PSII dimers are built from discrete reaction center complexes in a process facilitated by assembly factors. Phosphorylation of core subunits affects supercomplex formation and localization within the thylakoid network. Proteolysis primarily targets the D1 subunit, which when replaced, allows PSII to be reactivated and completes a repair cycle. While PSII has been extensively studied using *Chlamydomonas* as a model species, important questions remain about its assembly and repair and are presented here.

Keywords: *Chlamydomonas reinhardtii*, Photosystem II, assembly, repair

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1. Introduction

In oxygenic photosynthesis, visible light is used to energize electrons stripped from water. Simultaneously, protons are pumped across a membrane generating proton motive force. The final products, NADPH and ATP, are used for cellular functions including the fixation of CO_2 in the Calvin-Benson Cycle (reviewed in (Blankenship 2021)).

Photosystem II (PSII) is the first component of the photosynthetic electron transport chain and acts as a water-plastoquinone (PQ) oxidoreductase (see (Shevela et al. 2021; Redding and Santabarbara 2023)). PSII is a large membrane-bound complex consisting of approximately 20 unique protein subunits (Shen 2015). Within the PSII core, the P_{680} primary chlorophyll-*a* donor undergoes charge separation upon photoexcitation. On the donor side of PSII, the hole in the ground state of P_{680}^+ is filled by a redox-active tyrosine, Y_Z, which is in turn reduced by the Mn_4CaO_5 oxygen-evolving complex (OEC). Following four one-electron oxidation events, the OEC catalyzes the formation of O_2 from two molecules of water. On the acceptor side of PSII, the excited electron in P_{680}* is transferred to a pheophytin, a primary PQ acceptor, Q_A, and then a secondary PQ acceptor, Q_B (reviewed in (Lubitz et al. 2019; Vinyard and Brudvig 2017)).

PSII serves as a model system for multiple scientific fields. Protein biochemists study PSII to learn how membrane protein complexes assemble and function (see (Nickelsen and Rengstl 2013; Johnson and Pakrasi 2022)). Bioinorganic chemists have been intrigued by the multiple metal cofactors in PSII including the OEC (see (Young et

al. 2015)), cytochrome(s) (see (Chiu and Chu 2022)), and a non-heme iron (see (Müh and Zouni 2013)). Biophysical chemists and biophysicists use PSII to study visible to chemical energy conversion and exciton and electron transfer reactions (see (Sirohiwal and Pantazis 2023)). Molecular biologists have used differentially expressed PSII subunits to learn about bacterial and plastidal gene regulation (see (Allen 2015)) and have used PSII to study protein turnover mechanisms (see (Su et al. 2023)). Evolutionary biologists analyze the changes (or lack of changes) in PSII subunits from cyanobacteria to plants (see (Oliver et al. 2023)). Geologists recognize the role in PSII as the sole biological source of O_2 from water oxidation (see (Fischer et al. 2016)). PSII's ability to efficiently perform this reaction using only light as an energy input inspires synthetic chemists, materials chemists, and engineers to attempt to replicate this activity (see (Moore and Brudvig 2011)). This incomplete list illustrates the significant interest in PSII and its application to multiple research areas.

PSII and other photosynthetic complexes are generally conserved from cyanobacteria to algae to plants, and researchers in this field use all three groups as model species. For example, many biophysical studies of PSII have used membrane preparations from market spinach (Berthold et al. 1981; Greife et al. 2023; Mino and Asada 2022; Wang et al. 2020). The cyanobacterium *Synechocystis* sp. PCC 6803 has been widely used to study mutations in PSII subunits (Debus 2008; Nixon et al. 2010; Williams 1988; Ghosh et al. 2019; Avramov et al. 2020; Russell and Vinyard 2024). This mesophilic species is naturally transformable and has highly efficient homologous recombination making it a very practical genetic system (Ikeuchi and Tabata 2001; Vermaas 1996). Detergent-solubilized PSII core complexes from thermophilic cyanobacteria such as *Thermosynechococcus vulcanus* have been used for multiple influential structural studies (Umena et al. 2011; Guskov et al. 2009; Ferreira et al. 2004) and biophysical studies (Kato and Noguchi 2022; Noguchi 2015; Sugiura and Boussac 2014; Sugiura and Inoue 1999). While all this work is valuable, the structures (Nelson and Junge 2015) and assembly and repair mechanisms (Nickelsen and Rengstl 2013) of PSII are not identical between oxygenic phototrophs.

Over the past seventy years, the model green alga *Chlamydomonas reinhardtii* (hereafter *Chlamydomonas*) has been used to elucidate many discoveries in photosynthesis and other fields (see (Goodenough 2023; Dupuis and Merchant 2023)). As a unicellular eukaryote, *Chlamydomonas* is a practical model species for studying chloroplast biology. First, this species can grow photoautotrophically, mixotrophically, or heterotrophically. This trait allows *Chlamydomonas* to be studied with genetic mutations in photosynthesis genes that would be otherwise lethal. In addition, *Chlamydomonas* can reproduce asexually or sexually. In the research laboratory, cultures are typically maintained vegetatively. However, sexual reproduction facilitates genetic approaches. Because of these features, *Chlamydomonas* has been and is used to discover key components and mechanisms of PSII function, assembly, and repair.

Here, we review research studies that have used *Chlamydomonas* to gain insights into PSII. We aim to highlight the influential role of this organism in multiple scientific fields that all use PSII as a model system. We compile PSII-specific data to facilitate future studies and bring attention to areas where more research is needed.

2. Discussion

2.1. Architecture of the Chlamydomonas chloroplast

In algae and plants, photosynthesis occurs in the chloroplast. *Chlamydomonas* cells develop one cup-shaped chloroplast occupying almost half of the volume of the cell (Sager and Palade 1957; Gaffal et al. 1995) (Figure 1). The light-dependent photosynthetic reactions are localized to a network of membranes called thylakoids. These thylakoids are organized in appressed regions (regularly spaced stacks termed grana in land plants) and non-appressed regions. In *Chlamydomonas*, active dimeric PSII

complexes are organized in appressed thylakoids, and Photosystem I and ATP synthase are localized to non-appressed regions (Vallon et al. 1986; Wietrzynski et al. 2020).

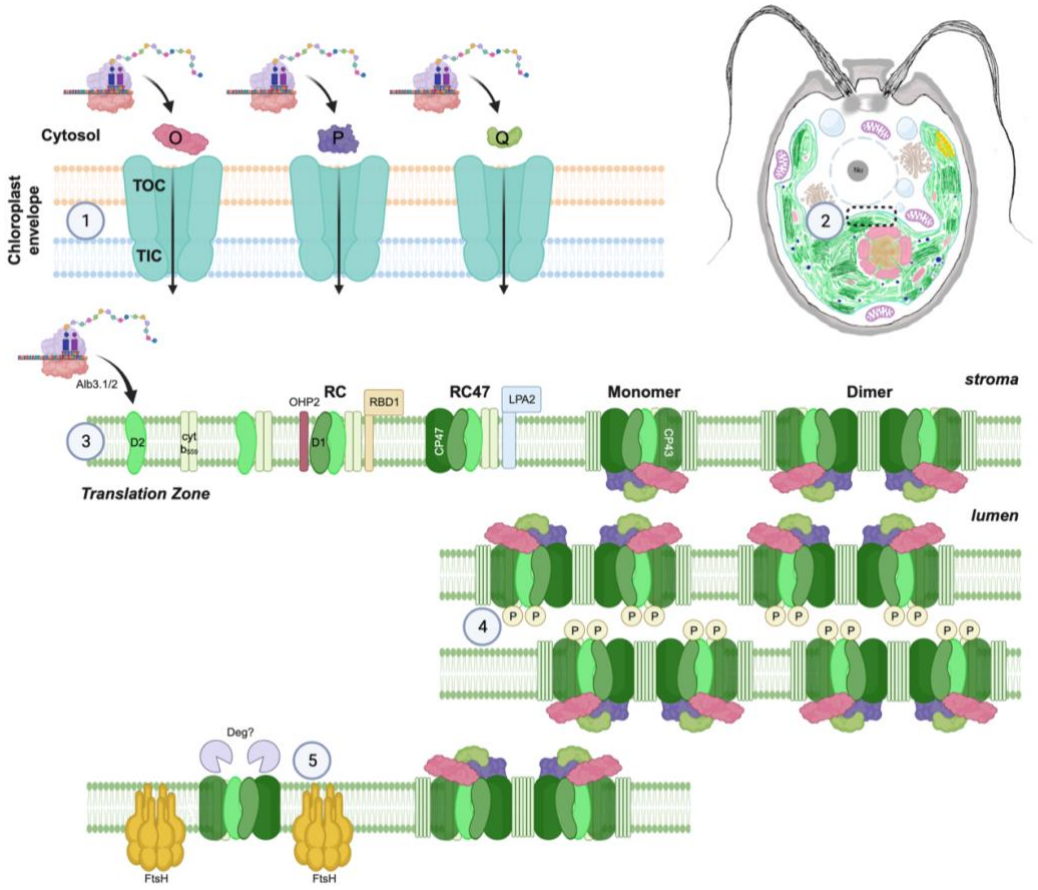

Figure 1. An overview of PSII assembly and organization in *Chlamydomonas*. ① Nuclear-encoded PSII subunits, including PsbO, PsbP, and PsbQ, are translated in the cytosol and imported into the chloroplast using the TOC TIC system. ② These complexes are spatially aligned with the chloroplast translation zone near the pyrenoid. ③ PSII subunits assemble in an organized pathway from reaction center intermediates to mature dimers. ④ Active and phosphorylated PSII dimers are enriched in appressed domains of the thylakoid membranes. ⑤ PSII assembly and degradation occur in non-appressed domains of the thylakoid membranes. Additional details and references are provided in the text.

The chloroplast emerged from the endosymbiosis of a cyanobacterium. Over the course of evolution, most of the genetic information from the original cyanobacterial genome was transferred to the host nuclear genome. In *Chlamydomonas*, only 72 unique protein-encoding genes are retained in the chloroplast genome (Maul et al. 2002). As discussed below, most PSII subunits are chloroplast encoded. The synthesis of chloroplast-encoded photosystem proteins is concentrated at the Translation or T-zone, which is located near the pyrenoid (Uniacke and Zerges 2007, 2009; Sun et al. 2019) (Figure 1). Here, newly synthesized proteins are inserted directly into the membrane (Zhang et al. 1999), which is facilitated by a complex containing Alb3.1 and Alb3.2 (Gohre et al. 2006; Ossenbühl et al. 2004). The *Chlamydomonas* chloroplast is a complex organelle with multiple sub-organellular structures (Wang et al. 2023b).

Nuclear-encoded chloroplast proteins (including *Chlamydomonas* PSII subunits PsbO, PsbP, PsbQ (Delepelaire 1984), PsbW (Bishop et al. 2003), and PsbX (Sheng et al.

2019) (Figure 2)), are translated in the cytosol (Westhoff et al. 1985), targeted to the chloroplast (Bruce 2000), and then imported across the outer and inner chloroplast membranes using energy from ATP hydrolysis (Theg et al. 1989). Chloroplast protein transport is facilitated by the translocon of the outer membrane (TOC) and translocon of the inner membrane (TIC) protein complexes (Shi and Theg 2013; Ramundo et al. 2020; Liu et al. 2023; Jin et al. 2022) (Figure 1). Interestingly, the TOC and TIC complexes spatially align with the T-zone suggesting a highly coordinated system of protein import and translation in the *Chlamydomonas* chloroplast (Schottkowski et al. 2012; Sun et al. 2021; Willmund et al. 2023).

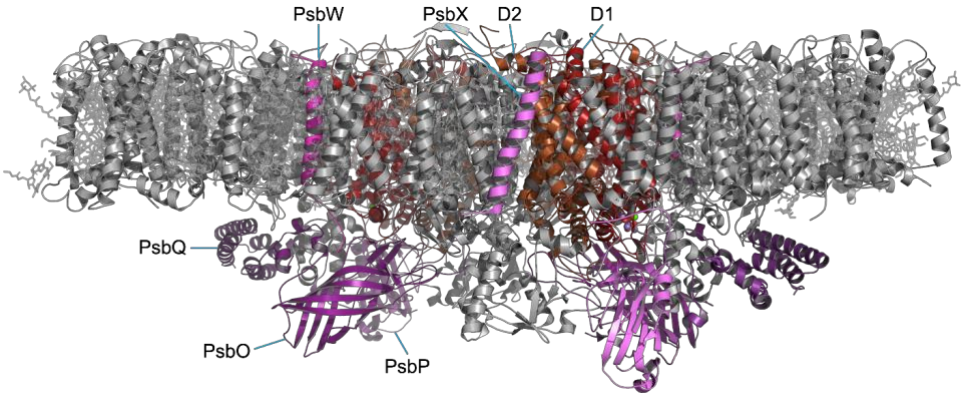

Figure 2. The PSII C_2S_2 complex from *Chlamydomonas* from (PDB ID 6KAC) (Sheng et al. 2019). Nuclear-encoded PSII subunits are shown in shades of purple.

2.2. Transcription of PSII Subunits in the Chloroplast

The chloroplast genome encodes 16 of the 21 PSII subunits in *Chlamydomonas* (Gallaher et al. 2018) (see Figure 2). The most prevalent chloroplast transcript is *psbA* which encodes the PSII core subunit D1 (Erickson et al. 1984; Bedbrook et al. 1978). However, little of this *psbA* mRNA is associated with ribosomes (Minai et al. 2006) indicating tight control of translation initiation. The second most prevalent chloroplast transcript is *psbD* which encodes the D2 core subunit and is found at levels approximately 5-fold less than *psbA* (Gallaher et al. 2018).

The PSII cytochrome b_{559} subunit is encoded by two genes, *psbE* and *psbF*. As described below, cytochrome b_{559} plays crucial roles in PSII translation control and assembly. In cyanobacteria (Pakrasi et al. 1988), some algae (Cantrell and Bryant 1988; Cushman et al. 1988), and plants (Herrmann et al. 1984), these genes are organized as part of a *psbEFLJ* operon. Surprisingly, in *Chlamydomonas*, *psbE* and *psbF* are separated and have reverse orientations (Mor et al. 1995; Alizadeh et al. 1994). *psbF* and *psbL* remain associated and are co-transcribed (Mor et al. 1995). *psbJ* is co-transcribed as part of a cluster that includes *psbD* (Liu et al. 1989; Cavaiuolo et al. 2017).

In *Arabidopsis*, a mitochondrial transcription termination factor, mTERF5, controls the transcription of the *psbEFLJ* operon (Ding et al. 2019). This regulatory mechanism is likely not conserved in *Chlamydomonas* given the differences in gene organization. Why *Chlamydomonas* evolved this unique cytochrome b_{559} expression system is not understood.

2.3. Translation of PSII Subunits in the Chloroplast – Control by Epistasy of Synthesis

The de novo synthesis of specific PSII subunits is regulated by the presence or absence of other PSII subunits through a mechanism termed Control by Epistasy of Synthesis (CES) (reviewed in (Wollman et al. 1999)). Here, we discuss CES in *Chlamdyomonas* PSII assembly, but this mechanism is more general. In *Chlamydomonas*,

CES has been observed in the assembly of cytochrome b_6f, ATP synthase, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), and PSI. It is also involved in the synthesis of Rubisco in plants and Cox1 in yeast mitochondria (see (Choquet and Wollman 2023)).

In *Chlamydomonas*, the presence of the D2 subunit is required for D1 translation (Erickson et al. 1986; de Vitry et al. 1989). Subsequently, the presence of the D1 subunit is required for CP47 (*psbB*) translation (de Vitry et al. 1989). This regulation is based on negative regulation of each unassembled polypeptide interacting with its own mRNA in the 5′ untranslated region (UTR) (Minai et al. 2006).

Regulation of cytochrome b_{559} translation in *Chlamydomonas* is more complicated. In a Δ*psbE* strain, the translation of D1, D2, CP43, and CP47 was not observed or was strongly inhibited (Morais et al. 1998). As discussed below, cytochrome b_{559} is a component of early PSII assembly intermediates. The phenotypes observed in Δ*psbE* may reflect true CES and/or a defect in the assembly pathway.

2.4. Translation of PSII Subunits in the Chloroplast – Regulatory Elements

In the chloroplast, regulation occurs mostly at the translational level and requires both cis- and trans-regulating elements (see (Bohne and Nickelsen 2023)). All characterized cis-regulatory elements are in the 5′ UTR of the genes (Mayfield et al. 1987b; Nickelsen et al. 1994; Rochaix et al. 1989). Trans-regulating elements (translational activators) are crucial components of PSII core subunits' translational control and are summarized in Table 1.

Table 1. Trans-regulatory elements of PSII subunit translation in *Chlamydomonas*.

PSII Subunit Affected	Translation Factor	Mechanism	References
psbA (D1)	RB47	Binds to A-rich region in the *psbA* 5′ UTR; required for D1 synthesis	(Danon and Mayfield 1991; Yohn et al. 1998a; Yohn et al. 1998b)
	RB60	Protein disulfide isomerase that redox regulates RB47	(Danon and Mayfield 1991; Kim and Mayfield 1997)
	TBA1	Oxidoreductase that facilitates binding of RB47 to *psbA* transcript	(Somanchi et al. 2005)
	RB55	Observed to bind *psbA* mRNA but not characterized	(Barnes et al. 2004; Yohn et al. 1996)
	RBP63	Binds to *psbA* 5′ UTR; essential for D1 synthesis; subunit of chloroplast pyruvate dehydrogenase complex that becomes a translational regulator upon acetylation	(Ossenbühl et al. 2002; Bohne et al. 2013; Neusius et al. 2022)
	CrHCF173	Homolog of *Arabidopsis* HCF173; affects D1 accumulation	(Kafri et al. 2023)
psbD (D2)	NAC1	Promotes *psbD* translation at a step that is likely after initiation	(Kuchka et al. 1988; Rattanachaikunsopon et al. 1999)
	AC115		
	NAC2	Promotes *psbD* stability by binding to its 5′ UTR	(Kuchka et al. 1989; Nickelsen et al. 1994)

	RBP40 (RB38)	Binds to U-rich region of *psbD* 5′ UTR; forms a complex with NAC2 to control *psbD* mRNA stability and initiation; also observed to bind *psbA* mRNA although this interaction may not be specific	(Ossenbühl and Nickelsen 2000; Barnes et al. 2004; Schwarz et al. 2007)
psbB (CP47)	Mbb1	Promotes *psbB* mRNA stability by interacting with its 5′ UTR; also affects *psbH* mRNA maturation	(Monod et al. 1992; Vaistij et al. 2000)
psbC (CP43)	TBC1	Facilitates *psbC* translation by binding to its 5′ UTR	(Rochaix et al. 1989; Zerges and Rochaix 1994; Zerges 1997)
	TBC2		
	TBC3		
	MBCI	Stabilizes *psbC* mRNA	(Cavaiuolo et al. 2017)

Clearly, translational control of chloroplast encoded PSII subunits is extensive. Table 1 is very likely incomplete, and more research is needed in this area. While trans-regulatory elements are discussed here for *Chlamydomonas*, similar regulation is present in plants (see (Zoschke and Bock 2018)). In contrast to the chloroplast, cyanobacteria generally control PSII expression at the transcriptional level (see (Wilde and Hihara 2016)).

2.5. Assembly of Protein Subunits and Cofactors

PSII subunits form discrete subcomplexes before assembling into monomeric then dimeric reaction centers (illustrated in Figure 1). First, D2 binds cytochrome b_{559} subunits PsbE and PsbF. D1 is then translated and binds with other subunits. The binding of the D1 subcomplex to D2-cytochrome b_{559} leads to the formation of the first reaction center (RC) complex. In *Chlamydomonas*, One-Helix Protein 2 (OHP2) stabilizes D1 during its translation by promoting chlorophyll association (Wang et al. 2023a). Another assembly factor, RBD1, promotes D1 stabilization (Calderon et al. 2023; Calderon et al. 2013) and is also involved in the delivery and/or reduction of the non-heme iron ion near the stromal surface of PSII (García-Cerdán et al. 2019). Next, CP47 is translated and binds to create the RC47 complex. The binding of CP43, facilitated by the assembly factor LPA2 (Spaniol et al. 2022; Cecchin et al. 2021), low molecular weight subunits, and extrinsic subunits forms monomeric PSII.

After the PSII core is assembled, the OEC is assembled from Mn^{2+}, Ca^{2+}, and water in a stepwise process termed photo-assembly (reviewed in (Oliver et al. 2022)). Here, metal ions and water molecules bind to the apo-OEC PSII protein. Light-driven oxidation events lead to higher valent Mn ions and the OEC cluster spontaneously assembles *in situ*. This process has mostly been studied in plants and cyanobacteria, but some groups have conducted experiments on *Chlamydomonas* whole cells (Rova et al. 1996) or isolated membranes (Vinyard et al. 2016). The kinetics and efficiency of OEC photo-assembly are similar among these organisms suggesting a conserved mechanism (Rova et al. 1996; Vinyard et al. 2016).

The binding of the extrinsic subunits PsbO, PsbP, and PsbQ occurs late in the assembly process. (Note that the terms OEE1, OEE2, and OEE3, respectively, have also been used for these proteins (de Vitry et al. 1989; Mayfield et al. 1987a; Mayfield et al. 1987b).) PsbO is required for oxygen evolution and photoautotrophic growth in *Chlamydomonas* and acts by stabilizing the OEC (Mayfield et al. 1987a). This subunit is conserved in cyanobacteria, algae, and plants (Popelkova and Yocum 2011). PsbP and PsbQ enhance oxygen evolution activity by promoting the binding of Ca^{2+} in the OEC and chloride near the OEC (Rova et al. 1994). These subunits are conserved in algae and plants (Enami et al. 2008). Mature PSII in cyanobacteria contains PsbO, PsbU, PsbV, and

PsbQ (Gisriel et al. 2022; Umena et al. 2011). The cyanobacterial PsbP homolog is an assembly intermediate (Knoppová et al. 2016) and not a component of the mature complex.

PSII reaction centers form supercomplexes with light-harvesting complex (LHC) antenna proteins (see (Redding and Santabarbara 2023)) (Figure 2). In *Chlamydomonas*, the major complexes mature as trimers and are encoded by a family of nine genes (LHCBM1-9) with high sequence homology (Minagawa and Takahashi 2004). In addition, CP26 and CP29 subunits associate to control the linkage between the PSII core and LHC antenna proteins. Single and double mutants of CP26 and CP29 show impaired photosynthesis and photoprotection (Cazzaniga et al. 2020). The PSII subunit PsbZ (also known as Ycf9) is also involved in supercomplex assembly, particularly under stress conditions (Swiatek et al. 2001).

2.6. PSII Phosphorylation and Dephosphorylation

In the chloroplast but not in cyanobacteria, phosphorylation of PSII subunits and associated antennae plays a role in supercomplex formation and PSII migration within the thylakoid network. These properties affect both protein complex turnover and state transitions (Delosme et al. 1996) (reviewed in (Tikkanen and Aro 2012)). In plants, the STN8 kinase phosphorylates PSII core subunits including D1 (Vainonen et al. 2005; Bonardi et al. 2005) which can be dephosphorylated by the PBCP phosphatase (Samol et al. 2012). In plants, the STN7 kinase phosphorylates LHCs (Bellafiore et al. 2005) (and to a lesser extent, PSII core subunits (Tikkanen et al. 2008)) which can be dephosphorylated by the PPH1/TAP38 phosphatase (Shapiguzov et al. 2010; Pribil et al. 2010).

The situation in *Chlamydomonas* is more complicated. The *Chlamydomonas* PSII subunits CP43, D2, and PsbH, but not D1, undergo phosphorylation at their N-termini (de Vitry et al. 1991). The *Chlamydomonas* ortholog of STN8, STL1, has not been fully characterized but is likely to be the PSII core kinase. The phosphorylation of PSII subunits is independent of state transitions, and the specific trigger remains unknown (Lemeille et al. 2010). *Chlamydomonas* LHCs are phosphorylated by the STT7 kinase (Depège et al. 2003). Unlike plants which have distinct phosphatases for PSII subunits and LHCs, the *Chlamydomonas* phosphatases CrPPH1 and CrPBCP can dephosphorylate both PSII and LHC (Cariti et al. 2020).

We note that PSII phosphorylation and dephosphorylation mechanisms increase in specificity over evolutionary time. These processes are absent in cyanobacteria. Algae use specialized kinases but redundant phosphatases. Plants use specialized kinases and phosphatases.

2.7. Proteolysis of the D1 Subunit

PSII in *Chlamydomonas* is undergoing frequent damage and repair. When PSII is isolated from *Chlamydomonas* cultures grown under optimal conditions, the observed manganese content is lower than expected suggesting that up to 20% of centers are in damaged or assembly states (Terentyev et al. 2019; Terentyev et al. 2020). The PSII D1 subunit is most prone to oxidative damage and is rapidly turned over. In *Chlamydomonas* under saturating light conditions, the half-life of D1 is as short as 20 minutes (Reisman and Ohad 1986). While D1 repair is costly in terms of ATP equivalents (Murata and Nishiyama 2018), replacing only this single subunit is more efficient than degrading and reassembling the entire PSII reaction center. D2 turns over at a slightly slower, but significantly rapid rate under high light conditions (Schuster et al. 1988).

The D1 degradation process is well understood in plants where soluble DEG proteases clip loops and the FtsH proteases degrade the resulting fragments. *Arabidopsis* DEG2 clips a stromal D1 loop to generate ~23 kDa and ~10 kDa fragments (Haussuhl et al. 2001), although D1 is still degraded in the absence of this protease (Huesgen et al. 2006). DEG1 clips a lumenal loop or loops to generate ~16 kDa and ~5 kDa fragments (Kapri-Pardes et al. 2007). DEG5 and DEG8 form a complex and DEG8 clips a lumenal

loop to generate ~16 kDa and ~18 kDa fragments (Sun et al. 2007). In cyanobacteria, DEG proteases are not required for D1 degradation, but do protect cells from heat and light stresses (Barker et al. 2006)

FtsH is essential for D1 degradation in *Chlamydomonas*. In a *Chlamydomonas* FtsH mutant strain exposed to light, D1 fragments of ~23 kDa, ~16 kDa, and ~6 kDa accumulate (Figure 3) (Malnoë et al. 2014). However, it is not known which proteases are responsible for these fragments. The *Chlamydomonas* genome encodes 12 DEG proteases with predicted active protease domains and up to seven are predicted to be localized to the chloroplast (Schroda and de Vitry 2023). DEG1C (Theis et al. 2019) and DEG9 (unpublished) are active proteases localized to the chloroplast stroma but are not involved in PSII repair or biogenesis. DEG8 and DEG5 are colocalized to the pyrenoid tubules (thylakoids) (Wang et al. 2023b) but have not been further characterized. The identity of the specific protease(s) involved in processing D1 before FtsH remains unknown.

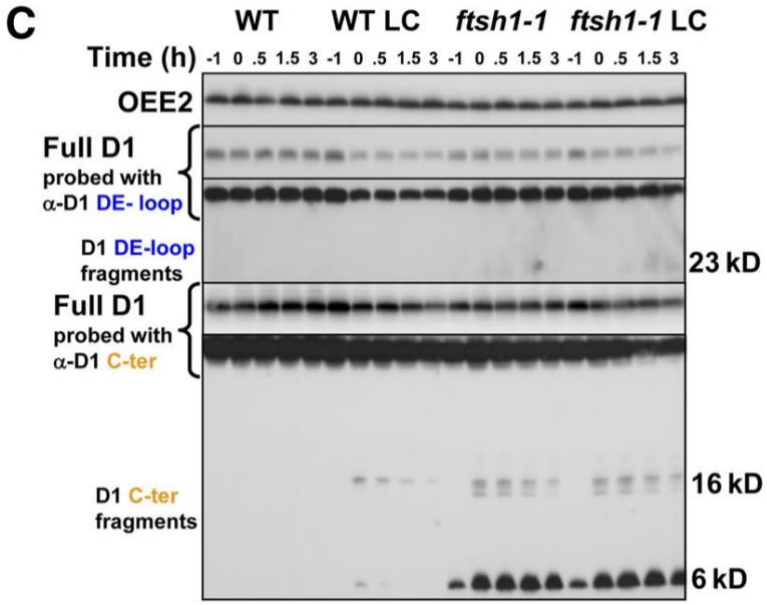

Figure 3. Discrete D1 fragments accumulate in *Chlamydomonas* in an FtsH mutant (Malnoë et al. 2014). Note the appearance of ~23, 16-20 kDa, and 6-10 kDa fragments in the lower panels. (WT: wild type; LC: chloroplast translation inhibitors lincomycin and chloramphenicol added; *ftsh1-1*: mutant with defective FtsH protease due to a FtsH1-R420C mutation; OEE2: loading control probing PsbP.) Reproduced by permission of Oxford University Press.

Intriguingly, a 23 kDa D1 fragment is also accumulated in a *Chlamydomonas* double mutant of FtsH and RBD1 in darkness. In this situation, D1 degradation is not the result of photodamage and instead may be induced by a conformational change from the lack of RBD1 (Calderon et al. 2023).

2.8. PSII Repair

Following D1 damage, CP43 dissociates from the PSII reaction center, which makes D1 more accessible to proteolysis (de Vitry et al. 1989). A new D1 polypeptide is synthesized and inserted, and CP43 rebinds (reviewed in (Theis and Schroda 2016)). This process occurs in non-appressed regions of the thylakoids. In *Chlamydomonas*, the factor TEF30 facilitates D1 insertion and/or CP43 binding during repair of monomeric PSII (Muranaka et al. 2015). Another factor, REP27 (homologous to LPA1 in plants), is also involved in D1 insertion during PSII repair (Park et al. 2007; Dewez et al. 2009).

3. Conclusions

As shown here, *Chlamydomonas* has remained an important tool for studying PSII from the 1980s to the present. The studies reviewed here have provided deeper insights into biochemical and evolutionary processes:

- PSII assembly in *Chlamydomonas* provides an excellent model system for the evolution and interplay between nuclear and organellar genomes.
- The CES mechanism well studied in PSII assembly in *Chlamydomonas* is applicable to multiple protein complexes in the chloroplast and other systems.
- Analogously, the extensive translational control of PSII subunits in the *Chlamydomonas* chloroplast has revealed gene regulation strategies.
- The PSII phosphorylation, dephosphorylation, and degradation pathways in *Chlamydomonas* show intermediate mechanisms between cyanobacteria and plants thus providing insights into evolution of photosynthetic organisms.

4. Remaining Questions

While *Chlamydomonas* has clearly contributed much to our understanding of PSII assembly and repair, key questions remain unanswered. These include:

1. What are the molecular mechanisms that allow chloroplast protein import and chloroplast protein synthesis to be coordinated?
2. Why are the genes that encode cytochrome b_{559} separated in the *Chlamydomonas* chloroplast genome?
3. What is the full suite of regulatory elements that control translation of PSII subunits in the chloroplast?
4. What are the specific triggers for PSII core subunit phosphorylation and dephosphorylation?
5. Which protease(s) degrades D1 into fragments before FtsH processing?

With these questions and others, *Chlamydomonas* will continue to be a practical and powerful system for PSII research.

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