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Regulation of the generation of reactive oxygen species during photosynthetic electron transport

Anja Krieger-Liszkay[1,*] and Ginga Shimakawa[2]

[1]Université Paris-Saclay, Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, 91198 Gif-sur-Yvette cedex, France

[2]Department of Bioscience, School of Biological and Environmental Sciences, Kwansei-Gakuin University, 2-1 Gakuen, Sanda, Hyogo 669-1337, Japan

*Corresponding author: phone: +33 1 6908 1803; E-mail: anja.krieger-liszkay@cea.fr

Abstract:

Light capture by chlorophylls and photosynthetic electron transport bury the risk of the generation of reactive oxygen species (ROS) including singlet oxygen, superoxide anion radicals and hydrogen peroxide. Rapid changes in light intensity, electron fluxes and accumulation of strong oxidants and reductants increase ROS production. Superoxide is mainly generated at the level of photosystem I while photosystem II is the main source of singlet oxygen. ROS can induce oxidative damage of the photosynthetic apparatus, however, ROS are also important to tune processes inside the chloroplast and participate in retrograde signalling regulating the expression of genes involved in acclimation responses. Under most physiological conditions light harvesting and photosynthetic electron transport are regulated to keep the level of ROS at a non-destructive level. Photosystem II is most prone to photoinhibition but can be quickly repaired while photosystem I is protected in most cases. The size of the transmembrane proton gradient is central for the onset of mechanisms that protect against photoinhibition. The proton gradient allows dissipation of excess energy as heat in the antenna systems and it regulates electron transport. pH-dependent slowing down of electron donation to photosystem I protects it against ROS generation and damage. Cyclic electron transfer and photoreduction of oxygen contribute to the size of the proton gradient. The yield of singlet oxygen production in photosystem II is regulated by changes in the midpoint potential of its primary quinone acceptor. In addition, numerous antioxidants inside the photosystems, the antenna and the thylakoid membrane quench or scavenge ROS.

Introduction

Living in an oxygen-rich atmosphere carries the risk of oxidative damage of lipids, proteins and nucleic acids. Beside oxidative damage, reactive oxygen species (ROS) are also important as signaling molecules triggering the expression of responsive genes, and they play a crucial role in developmental

processes, in plant – microbe interactions and the defense against pathogen attack [1,2]. Oxygen, a triplet in its ground state, is a strong oxidizing agent, however its reactivity is limited by spin pairing and reorientation. By contrast, its singlet form, $^1O_2$, and the three intermediate redox states (superoxide, peroxide and hydroxyl radical) between O_2 and its fully reduced form H_2O are much more reactive with the highest reactivity for $^1O_2$ and the hydroxyl radical ($^{\bullet}OH$). ROS are generated by specialized enzymes like for example NADPH oxidases to fight pathogen attack [3], and ROS generation occurs as side reactions in electron transport chains like the respiratory chain [4] and in oxygen photosynthetic organisms in the photosynthetic electron transport chain. Furthermore, photosynthetic organisms have their own special oxidative stress burden like light-induced generation of excited states of the chlorophylls that can lead to $^1O_2$ generation.

Superoxide generation by photosystem I

Superoxide anion radicals ($O_2^{\bullet-}$) are generated by the reduction of O_2 at the three main complexes of the photosynthetic electron transport chain (Fig. 1), photosystem II (PSII) [5], the cytochrome b_6f complex (cytb_6f) [6], photosystem I (PSI) and at the plastoquinone (PQ) pool [7]. The acceptor side of PSI is the most important site of $O_2^{\bullet-}$ generation in the so-called Mehler reaction (Fig. 2). The Mehler reaction is per se not deleterious for PSI when it occurs at the level of the terminal acceptors F_AF_B since $O_2^{\bullet-}$ generated at the stroma site of PSI is detoxified efficiently. $O_2^{\bullet-}$ is first reduced to H_2O_2 by superoxide dismutase (SOD) or spontaneously and H_2O_2 is decomposed by ascorbate peroxidase, peroxiredoxins, and glutathione peroxidase [8]. However, the situation is different when $O_2^{\bullet-}$ is generated inside the PSI reaction center. Since the midpoint redox potential of the $O_2/O_2^{\bullet-}$ pair in thylakoid membranes is assumed to be much more negative (around -550 mV) [9], both, phylloquinones and [4Fe-4S] clusters are suggested as thermodynamically plausible sites of O_2 reduction within PSI. Recently, Kozuleva et al. (2021) reported for isolated PSI that O_2 photoreduction is observed in the absence of the [4Fe-4S] clusters and that it is enhanced with the longer lifetime of phylloquinone, suggesting that the A-branch phylloquinone is the site of O_2 reduction in isolated PSI (Fig. 2) [10]. Because of low membrane permeability of $O_2^{\bullet-}$ (2.1×10^{-6} cm s^{-1}) [11], $O_2^{\bullet-}$ generated within the protein complex or at the interface protein complex/thylakoid membrane is assumed to oxidatively attack PSI before it diffuses out of the membrane and is disproportionated enzymatically by SOD. Alternatively, $O_2^{\bullet-}$ is non-enzymatically disproportionated to H_2O_2 that reacts with the reduced [4Fe-4S] centers generating $^{\bullet}OH$ when electron transport is strongly limited at the acceptor side of PSI [12,13]. In this way, $^{\bullet}OH$, being more reactive than $O_2^{\bullet-}$, can be generated in PSI.

66 **Singlet oxygen production in photosystem II**

67 When excited chlorophyll in its triplet state (^{3}Chl) reacts with molecular oxygen ($^3O_2$) the very
68 reactive $^1O_2$ is generated. In the Chl containing antenna systems, ^{3}Chl may be generated from a singlet
69 excited chlorophyll (^{1}Chl). However, in an intact antenna system, little $^1O_2$ is generated because
70 carotenoids, including xanthophylls, are in close contact with Chl [14]. Carotenoids quench efficiently
71 ^{3}Chl and protect thereby against $^1O_2$ production. In the reaction centre of PSII, ^{3}Chl is generated by
72 charge recombination of the primary radical pair ($P680^+$ Ph^-), with pheophytin (Ph) being the primary
73 electron acceptor and P680 the primary chlorophyll electron donor (Fig. 2). When light absorption
74 exceeds the capacity of photosynthetic electron transport, the quinone acceptors of PSII (Q_A, Q_B and
75 the PQ pool) become reduced and the probability of $^1O_2$ generation increases. The midpoint potential
76 of Q_A determines whether charge recombination proceeds via the dangerous route of repopulation of
77 the primary radical pair ($P680^+$ Phe^-) generating with a certain probability ^{3}P680 and subsequently $^1O_2$
78 or via the safe charge recombination directly into the ground state of P680 without producing $^1O_2$ (for
79 details see [15-17]). The yield of $^1O_2$ formation is lowered when Q_A adopts the high potential form by
80 shifting the midpoint redox potential of Q_A to a more positive value. PSII with high potential Q_A has
81 been found in PSII with an inactive water-splitting complex when either the obligatory cofactor calcium
82 was lacking or when the Mn_4CaO_5 cluster had been removed [18,19]. Recently, using Fourier
83 Transformed Infrared (FTIR) spectroscopy instead of chlorophyll fluorescence as a measure for the
84 reduction state of Q_A, more negative potentials for the Em (Q_A/Q_A^-) have been reported, and hardly
85 any change in the Em (Q_A/Q_A^-) upon Mn depletion has been observed [20]. However, it has been shown
86 previously that PSII with an inactivated water-splitting complex generates much less $^1O_2$ despite charge
87 separation similar to active PSII [21,22], supporting the Em (Q_A/Q_A^-) values determined by chlorophyll
88 fluorescence. Further work is needed to show whether chlorophyll fluorescence or FTIR spectroscopy
89 measure the reduction state of Q_A more reliably. The Em (Q_A/Q_A^-) is also shifted towards a more
90 positive value in the absence of the ligand bicarbonate at the non-heme iron at the acceptor side of
91 PSII. This shift, although much smaller than the one found upon inactivation of the water-splitting
92 complex, decreases also significantly $^1O_2$ production [15]. A cyanobacterial assembly state of PSII has
93 been recently characterized [23]. Binding of the assembly factor Psb28 induces a large conformational
94 change at the PSII acceptor side, which distort the binding pocket of Q_B and replace the bicarbonate
95 ligand of non-heme iron with glutamate. The yield of $^1O_2$ generation is lowered in this assembly state
96 showing that the replacement of bicarbonate by glutamate protects the nascent PSII.

97 PSII centres with high potential Q_A have been observed under different physiological
98 conditions *in vivo*; (i) in green algae prior to photoactivation, i.e., the light-dependent assembly of the
99 Mn_4CaO_5 cluster [17], and (ii) in plant leaves of under high light [24] and drought [25]. Small carboxylic

100 acids generated in the photorespiratory pathway may also modify the midpoint potential of Q_A by
101 replacing bicarbonate as has been shown for the *A. thaliana* photorespiration mutant *hpr1-1* that
102 accumulates glycolate [26]. Such regulation may become physiologically relevant when the CO_2
103 concentration is temporarily low and the glycolate concentration is high inside the chloroplast. A
104 slowdown of forward electron transport at the acceptor side of PSII has been reported to occur under
105 moderate drought stress, a condition favouring photorespiration compared to CO_2 assimilation [25].
106 This observation may reflect replacement of bicarbonate by glycolate in a wild-type plant. Reversible
107 inactivation of PSII by glycolate thus represents a way how a photorespiratory metabolite can protect
108 plants from $^1O_2$ in high light, at elevated temperature and drought conditions when photorespiration
109 is more important.
110
111 **Photoinhibition in photosystem I and II**
112 ROS generated in photosystems in the light can provoke oxidative damage of the protein
113 complexes leading to an inactivation of the photosystems, a process known as photoinhibition.
114 Photoinhibition of PSII is easily observed in intact cells and leaves, when the damage exceeds the repair
115 of damaged PSII. The molecular mechanism of PSII photoinhibition is discussed controversially in the
116 literature. $^1O_2$ is a plausible cause for the damage of the D1 protein [27], but also $O_2^{\bullet-}$ and $^{\bullet}OH$
117 generated at the acceptor side of PSII [28]. These radicals have been shown to oxidise specific amino
118 acid residues of the D1 protein [29], while the specific amino acids attacked by $^1O_2$ have still to be
119 identified. In addition, photoinhibition can also take place at the donor side of PSII inhibiting the
120 Mn_4CaO_5 cluster, thereby limiting electron donation to $TyrZ^+$ and $P680^+$ [30]. Upon illumination with
121 UV light, Mn in the Mn_4CaO_5 cluster is reduced and the cluster falls apart, resulting in donor-side-
122 induced photoinhibition [31]. Damaged D1 protein is rapidly replaced by newly synthesized D1 protein
123 in the so-called PSII repair cycle [32]. Not only active PSII but also the repair of damaged PSII is sensitive
124 to ROS because the translation factor EF-Tu is easily inhibited by oxidation of a cysteine residue [33].
125 Inactivation of PSII suppresses the electron transport to PSI, protecting it by alleviating the $O_2^{\bullet-}$
126 generation in PSI. Therefore, photoinhibition of PSII is often regarded as a predetermined breaking
127 point to protect the rest of the photosynthetic apparatus against photo-induced oxidative damage.
128 As described above, $O_2^{\bullet-}$ and $^{\bullet}OH$ are the candidates to cause the inactivation of PSI. Based on
129 electron spin resonance spectroscopy in damaged PSI, [4Fe-4S] clusters are the most plausible
130 damaged sites by ROS [34,35]. Damage of the acceptor side of PSI is often observed as a decrease in
131 the total amount of P700 (the reaction centre chlorophyll in PSI) that is kept oxidized in saturating
132 light. Additionally, the inactivation of PSI is almost linearly correlated with the decrease in the rate of
133 photosynthetic CO_2 assimilation in leaves [36]. Because damaged PSI takes several days or weeks to be

134 replaced [37,38], PSI photoinhibition can be a lethal event for photosynthetic organisms. It should be
135 noted that except for extreme environmental conditions, including artificial fluctuating light [39] and
136 chilling stress [40], and for specific mutants [41,42], PSI photoinhibition is hardly observed *in vivo*,
137 which suggests that $O_2^{\bullet-}$ is efficiently scavenged before PSI is damaged or that $O_2^{\bullet-}$ generation is very
138 low.
139
140 **Scavenging $O_2^{\bullet-}$ and $^1O_2$ by antioxidants**
141 Prenyllipids like tocochromanols (tocopherols, especially α-Toc, tocotrienols and
142 plastochromanol-8), plastoquinone-9 (PQ/PQH_2) and carotenoids (carotenes and xanthophylls) are
143 lipid-soluble antioxidants in the chloroplasts, which can quench or scavenge ROS [43]. α-Toc and PQH_2
144 efficiently scavenge $O_2^{\bullet-}$ and lipid radicals, and, like carotenoids, also quench and scavenge $^1O_2$. The
145 level of the antioxidants α-Toc and PQH_2 increases under environmental conditions that promote
146 oxidative stress. Carotenoid levels and their composition also change in response to changes in
147 environmental conditions [44]. Carotenoids are essential quenchers of ^{3}Chl and $^1O_2$ in the antenna
148 systems of the photosystems and β-carotene can scavenge $^1O_2$ inside the PSII reaction centre while its
149 position is too far to quench directly the triplet state ^{3}P680. β-carotene protects also PSI against $^1O_2$
150 generation as has been shown in isolated PSI complexes from a mutant with a lower β-carotene
151 content [45]. It has also been recently proposed that α-Toc is localized near Ph_{D1} and the nonheme
152 iron, protecting amino acids of the D1 protein against oxidative modifications [46]. In a recent study,
153 the antioxidant activities of α-Toc, PQ/PQH_2, zeaxanthin in excess light stress were analysed in
154 *Arabidopsis thaliana* wild-type and in mutants affected in the individual antioxidants. It was shown
155 that $^1O_2$ is first quenched by α-Toc. When it is consumed, zeaxanthin and PQ take this role [47].
156 Cyanobacteria contain a special photoactive soluble carotenoid protein, the orange carotenoid protein
157 that is able to quench efficiently $^1O_2$ in addition to its role in thermal dissipation of excess energy by
158 the cyanobacterial antenna, the phycobilisome [48,49]. The water-soluble chlorophyll protein WSCP
159 has also $^1O_2$ quenching properties, although it does not contain a carotenoid [50]. The physiological
160 role of WSCP is far from being understood, and it is only present in some species of the Brassicaceae,
161 Chenopodiaceae, Amaranthaceae and Polygonaceae.
162
163 **Regulatory mechanisms to suppress ROS generation**
164 Photosynthetic organisms employ a large number of regulatory mechanisms for electron
165 transport and light utilisation to suppress ROS generation in response to short-term environmental
166 changes in addition to the ROS scavenging systems described above. When light is sufficient,
167 photosynthetic electron transport is usually limited by the CO_2 content inside the leaves. This is the

168 case at high light intensities when the plant is under optimal conditions. In abiotic stress conditions
169 like drought, the stomata are closed and the CO_2 concentration in the cells and chloroplasts drops,
170 thereby limiting CO_2 assimilation already at light intensities that are non-saturating in the absence of
171 such stress. In low CO_2, photorespiration is crucial to keep the electron transport chain oxidized
172 thereby protecting against photoinhibition [51]. When photosynthetic organisms are exposed to
173 excess light and the electron transport chain is highly reduced, the potential risk of photosynthetic
174 electron transport to generate ROS increases unless regulatory mechanisms start to function. Such
175 regulation mechanisms comprise i) slowing down electron transport at the acceptor side of PSII and at
176 the cytb_6f complex, ii) dissipating excess energy as heat, and iii) additional electron transport routes
177 via specialized proteins or directly to oxygen.

178 The most important control over photosynthetic electron transport is exerted by the proton
179 motive force, composed of the proton gradient (ΔpH) and the electrical gradient (ΔΨ), which is created
180 during electron transport in thylakoid membranes. Protons are liberated at the donor side of PSII
181 during water oxidation and they are pumped across the thylakoid membrane during the electron
182 transport in the cytb_6f complex. The ΔpH provides the driving force for ATP synthesis by the CF_0CF_1-
183 ATP synthase. The pH inside the thylakoid lumen is important for the protection of both PSI and PSII
184 against ROS damage by the so-called photosynthetic control and by initiating quenching mechanisms
185 in the antenna that allow dissipation of excess energy as heat (qE, the pH-dependent component of
186 non-photochemical quenching). When the ΔpH becomes larger than needed for ATP synthesis,
187 photosynthetic control sets in. Photosynthetic control is defined as the pH-dependent decrease of the
188 activity of the cyt b_6f complex [52-54]. The consequence is a limitation of electron donation to PSI,
189 leading to an accumulation of $P700^+$ that alleviates $O_2^{\bullet-}$ production, resulting in the protection of PSI
190 against ROS damage [39]. The lumen pH in Arabidopsis leaves under ambient CO_2 was estimated to
191 range from approximately pH 7.5 to 6.5 under weak and saturating light, respectively [55]. When net
192 ATP synthesis is zero, the pH in the lumen can decrease as low as pH 5.2 [53], a pH at which the water-
193 splitting activity starts to be slowed down [56]. Below pH 5.5, Ca^{2+}, an obligatory co-factor of the water-
194 splitting complex is reversibly removed, evoking a shift of Q_A to the high potential form [19,57] and
195 protecting PSII against $^1O_2$ generation (see above).

196 Moderate low pH values in the lumen allow, beside photosynthetic control, regulation at the
197 antenna level via qE. In photosynthetic eukaryotes, qE is triggered by ΔpH and commonly requires
198 three components: (1) de-epoxidation of xanthophylls, (2) conformational change in the major LHCII
199 and (3) unique proteins associated with LHCII, including PsbS in land plants, LHCSR in green algae and
200 basal land plants, and LhcX in diatoms [58-60]. Although the detailed mechanism of qE is still
201 controversial and different among organisms, ΔpH is known to function in activating the de-epoxidase

202 and/or the protonation of these unique proteins. Therefore, the capability of qE to dissipate excess
203 energy in the PSII antenna system and to prevent $^1O_2$ generation in the PSII reaction centre depends
204 on the size of ΔpH. In Arabidopsis, the pH value for zeaxanthin accumulation and PsbS protonation was
205 estimated to be about 6.8 [55].

206 Alternative electron transport pathways like the Mehler reaction and cyclic electron flow help
207 to protect the photosynthetic apparatus against damage by ROS. They do not lead to the production
208 of NADPH but theoretically contribute to the ΔpH and permit the synthesis of extra ATP. Cyclic electron
209 transport around PSI is defined as the electron transport from ferredoxin to PQ. There are two
210 pathways proposed for cyclic electron flow: one that is insensitive to the inhibitor Antimycin A and
211 involving the chloroplast NAD(P)H dehydrogenase (NDH) complex using ferredoxin instead of NAD(P)H
212 as electron donor; and the other Antimycin-A-sensitive one that involves the Cyt b_6f complex and the
213 elusive proton gradient regulation complex PGR5/PGRL1. However the exact pathway of the second
214 one is still a matter of debate [61]. The pathway via the NDH complex, a homologue of the complex I
215 of the mitochondrial respiratory chain, is probably pumping extra protons into the thylakoid lumen
216 [62] to support chlororespiration and contributes to the photosynthetic control. It should be noted
217 that the *in vivo* activity of cyclic electron flow has not yet been clarified because of the technical
218 difficulty to evaluate it separately from other electron transport pathways. In green algae and
219 cyanobacteria, O_2 photoreduction to water, catalyzed by the flavodiiron enzymes, represents a
220 significant electron transport activity (the optimal rate is almost comparable to the photosynthetic O_2
221 evolution rate) and contributes to the ΔpH, alleviating ROS damage in PSI [63-66]. The electron flux *via*
222 flavodiiron proteins is smaller but its physiological roles are still conserved in basal land plants, and
223 finally lost in angiosperms in the evolutionary history [67,68]. The *in vivo* electron flux *via* the Mehler
224 reaction (i.e., the non-enzymatic reduction of O_2 to $O_2^{\bullet-}$ in PSI) is smaller than that *via* flavodiiron
225 proteins. Nevertheless, even in the absence of the flavodiiron proteins, the reduction of O_2 to $O_2^{\bullet-}$
226 contributes to the ΔpH, and it is enhanced under certain physiological conditions like for example in
227 plants grown under short photoperiod [69].

228 Alternative electron transport routes are in competition with each other, and their activity is
229 controlled by the redox state of the chloroplast stroma *via* the thioredoxin system. It has been recently
230 shown that cyclic electron transport is controlled by thioredoxin *m* and by the NADPH-dependent
231 thioredoxin system of the chloroplast NTRC [70,71]. In this context it is interesting to mention that
232 thioredoxin *m* forms a complex with PGRL1 [72,73] that itself contains redox active cysteine residues
233 [74]. The formation of a complex between reduced thioredoxin *m* and PGRL1 may inhibit cyclic electron
234 flow by preventing the supercomplex formation required for cyclic electron flow. Possibly, O_2

235 photoreduction at PSI is activated by the thioredoxin system in a competitive manner with cyclic
236 electron flow.

237 In addition to cyclic electron flow and O_2 photoreduction at PSI, O_2 can also be reduced by the
238 plastid terminal oxidase (PTOX) that uses PQH_2 as electron donor [75]. The electron flux *via* PTOX is
239 usually much lower than that of CO_2 assimilation *in vivo*, but it is up-regulated in response to abiotic
240 stress at the expression and redox levels of the protein [76,77]. Its membrane association depends on
241 the ΔpH [78]. Thereby the enzyme may have only access to its substrate in the presence of a high ΔpH.
242 Such a regulation mechanisms avoids the competition of PTOX with linear electron flow under
243 conditions that are favorable for CO_2 assimilation. PTOX is possibly functioning as a safety valve at the
244 acceptor side of PSII to regulate ROS generation in both PSI and PSII.

245

246 **Conclusions and further research directions**

247 Much work has been done to identify the sites of ROS production in the different
248 photosynthetic complexes. However, most work on the exact sites of ROS production has been
249 performed using isolated PSII and PSI complexes. It is likely that ROS production and its control differ
250 depending on the production sites and on the organization of supercomplexes. For example,
251 differences in ROS production are likely to occur in the different assembly states of the photosystems,
252 as has been shown for $^1O_2$ in cyanobacterial PSII assembly state [23]. A detailed study on ROS
253 formation of different photosystem complex compositions would be of interest. During the repair cycle
254 of PSII, it is also likely that the yield of ROS is different in monomeric PSII compared to dimeric PSII and
255 upon the removal of the cofactors from damaged monomeric PSII. Furthermore, the yield of ROS
256 production and the stability of the ROS may depend on the site of the thylakoid membrane where ROS
257 generation takes place. It has been shown recently that the site of $^1O_2$ production is important for $^1O_2$
258 signalling through flu/executer pathway [79,80].

259 A feedback reaction of $^1O_2$ generation in PSII by photorespiratory metabolites is a new concept
260 that links photosynthetic electron transport with primary metabolism. Further research is needed to
261 show whether under photorespiratory conditions like drought or increased temperature the glycolate
262 concentration inside the chloroplast can get high enough to exchange with the bicarbonate at the
263 acceptor side of PSII in wild-type plants.

264 Beside the exact sites of ROS production, further work is required to understand the regulation
265 of alternative electron transport pathways that lower the yield of ROS production. First work has been
266 done to identify sites of thioredoxin-dependent regulation of cyclic flow. However, redox regulation of
267 the Mehler reaction and the flavodiiron proteins remains an open question. The whole complexity of
268 the interplay of the different components of the thioredoxin system, like the individual thioredoxins,

NTRC, peroxiredoxins and other players like H_2O_2 has also still to be unravelled. Redox regulation of PTOX has just been described very recently.

The relationship of controlling ROS production in photosynthetic electron transport with ROS cell signalling should be further studied. Super-resolution microscopy techniques using specific fluorescent dyes or the expression of ROS-sensitive fluorescent proteins may shine in future new light on the distribution of ROS production inside a single chloroplast, while standard confocal microscopy may allow differences in ROS production amongst individual chloroplasts. A combination of these techniques with single cell transcriptomic techniques may help to further understand ROS signalling networks.

Perspectives

- ROS generated in photosynthetic electron transport have important roles in signalling and specific reactions, but their level has to be controlled since to high ROS levels provoke oxidative damage. The detailed understanding of the mechanism of ROS production and regulation mechanisms limiting ROS formation is a prerequisite to understand acclimation to abiotic stress.

- The molecular mechanism of ROS generation at the photosystems is mainly understood on the basis of experiments with isolated thylakoid membranes and photosystems. Several players of regulatory mechanisms have been identified at the molecular level which control ROS production, but their importance *in vivo* and their interactions in responses to environmental fluctuations are far from being understood.

- Continuous technical improvement of specific spin traps, ROS specific fluorescence dyes, expression of fluorescent protein-based biosensors and super-resolution microscopy will allow in future quantitative analyses of the production rates and sites of the individual ROS species *in vivo* with spatial information and unprecedented sensitivity. Future quantitative analyses of the interactions or competitions between the different regulation mechanisms of photosynthetic electron transport will allow a comprehensive understanding of ROS levels inside the chloroplast.

Conflict of interests

The authors declare no conflict of interest.

Author contributions

A.K.L. and G.S. wrote the article.

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642 **Figures**

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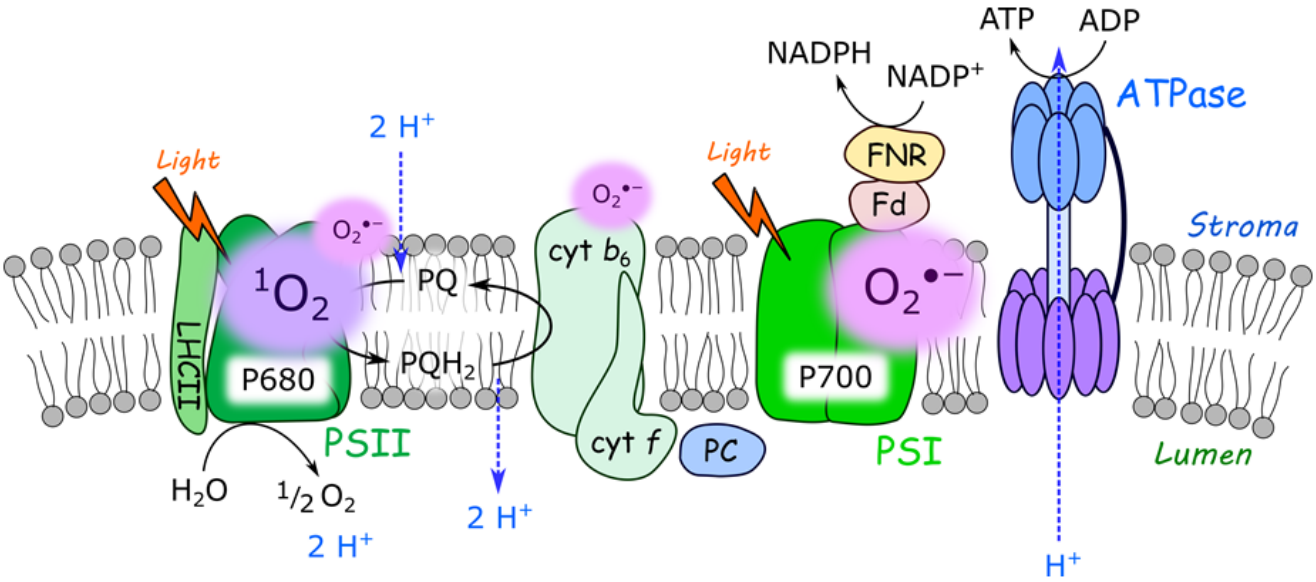

Figure 1. Photosynthetic electron transport in thylakoid membranes.
The reaction centre chlorophyll dimers P680 and P700, respectively in photosystem (PS) II and I, are photo-oxidised by light, resulting in photosynthetic electron transport from H_2O to $NADP^+$ through the interchain components, including plastoquinone (PQ), cytochrome b_6f complex (cytb_6f) and plastocyanin (PC). In the acceptor side of PSI, ferredoxin-$NADP^+$ oxidoreductase (FNR) donates electrons from ferredoxin (Fd) to $NADP^+$. The different concentration of H^+ across thylakoid membranes is the motive force for ATP production by ATP synthase (ATPase). Singlet oxygen ($^1O_2$) can be mainly generated at the light-harvesting complex (LHCII) and the reaction centre of PSII. Superoxide anion radical ($O_2^{\bullet-}$) can be mainly generated at the acceptor side of PSI, and also in PSII and cytb_6f.

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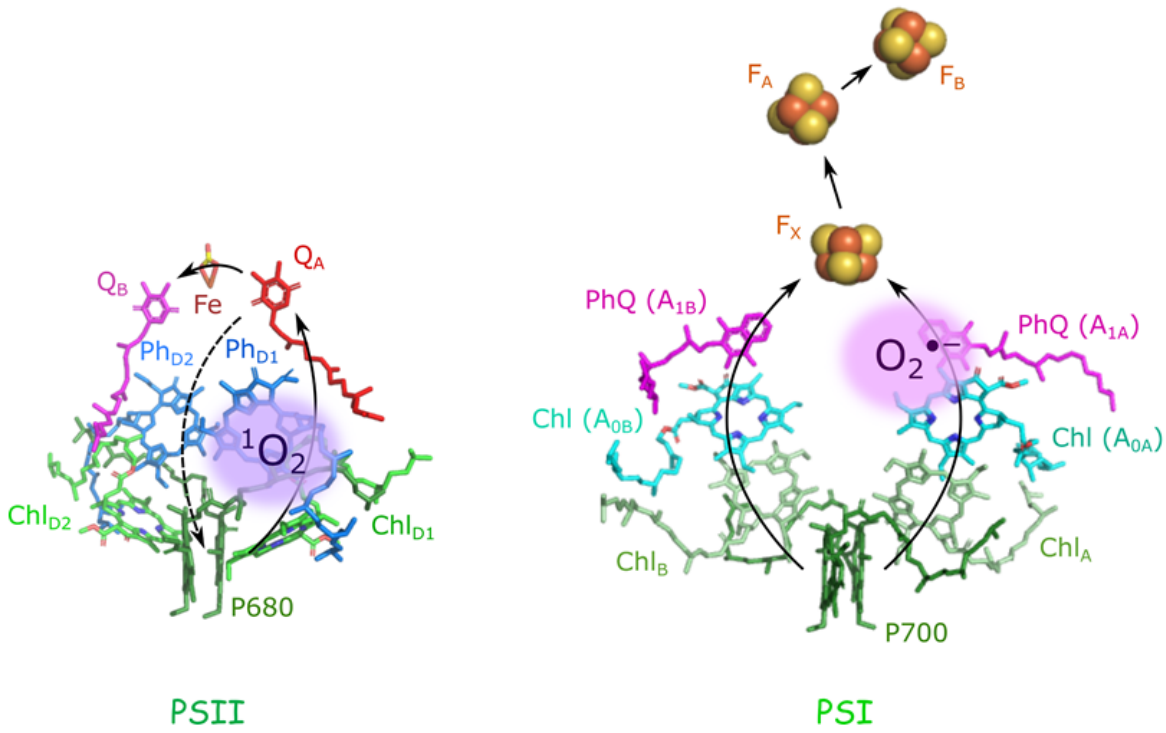

Figure 2. Generation of reactive oxygen species in the reaction centres of photosystems.
Whereas in photosystem (PS) II, the primary electron transport reaction occurs in the side of D1 protein, in PSI two branches function for the electron transport in PsaA and PsaB proteins. Singlet oxygen ($^1O_2$) is generated by the triplet state of P680 produced by the charge recombination of $P680^+$ and Ph_{D1}^-. Superoxide anion radical ($O_2^{\bullet -}$) is generated by the Mehler reaction in the A-branch phylloquinone (A_{1A}). Abbreviations are as follows: Chl, chlorophyll; Ph, pheophytin; Q, quinone; PhQ, phylloquinone; F, [4Fe-4S] cluster.