ETH zürich

ETH Library

Dose-dependent effects of 1O2 in chloroplasts are determined by its timing and localization of production

Journal Article

Author(s):
Wang, Liangsheng; Apel, Klaus

Publication date:
2019-01-01

Permanent link:
https://doi.org/10.3929/ethz-b-000313826

Rights / license:
In Copyright - Non-Commercial Use Permitted

Originally published in:
Journal of Experimental Botany 70(1), https://doi.org/10.1093/jxb/ery343

This page was generated automatically upon download from the ETH Zurich Research Collection.
For more information, please consult the Terms of use.

Journal of Experimental Botany, Vol. 70, No. 1 pp. 29–40, 2019
doi:10.1093/jxb/ery343 Advance Access publication 29 September 2018

REVIEW PAPER

Dose-dependent effects of $^1O_2$ in chloroplasts are determined by its timing and localization of production

Liangsheng Wang[1,2] **and Klaus Apel**[1,3]

[1] Boyce Thompson Institute, Ithaca, NY 14853-1801, USA
[2] Plant Molecular Biology (Botany), Department Biology I, Ludwig-Maximilians-Universität München, Planegg-Martinsried, Germany
[3] Institute of Molecular Plant Biology, Swiss Federal Institute of Technology (ETH), Universitätstr. 2, 8092 Zürich, Switzerland

Correspondence: wangliangsheng08@gmail.com

Received 3 June 2018; Editorial decision 24 September 2018; Accepted 25 September 2018

Editor: Christine Raines, University of Essex, UK

Abstract

In plants, highly reactive singlet oxygen ($^1O_2$) is known to inhibit photosynthesis and to damage the cell as a cytotoxin. However, more recent studies have also proposed $^1O_2$ as a signal. In plants under stress, not only $^1O_2$ but also other reactive oxygen species (ROS) are generated simultaneously, thus making it difficult to link a particular response to the release of $^1O_2$ and establish a signaling role for this ROS. This obstacle has been overcome by the identification of conditional mutants of *Arabidopsis thaliana* that selectively generate $^1O_2$ and trigger various $^1O_2$-mediated responses. In chloroplasts of these mutants, chlorophyll or its biosynthetic intermediates may act as a photosensitizer and generate $^1O_2$. These $^1O_2$-mediated responses are not only dependent on the dosage of $^1O_2$ but also are determined by the timing and suborganellar localization of its production. This spatial- and temporal-dependent variability of $^1O_2$-mediated responses emphasizes the importance of $^1O_2$ as a highly versatile and short-lived signal that acts throughout the life cycle of a plant.

Keywords: Chloroplast, photosynthesis, reactive oxygen species, signaling, singlet oxygen, stress

Introduction

Plants performing oxygenic photosynthesis must cope with photo-oxidative stresses throughout their life cycle. They utilize light as a primary energy source and synthesize and accumulate large amounts of chlorophyll (Chl) in chloroplasts to absorb light energy. If this light energy cannot be dissipated through photosynthetic electron transport, the excited Chl may turn into a potent photosensitizer that generates highly reactive singlet oxygen ($^1O_2$) (Apel and Hirt, 2004; Krieger-Liszkay *et al.*, 2008; Foyer and Noctor, 2009; Triantaphylidès and Havaux, 2009). Plants have evolved various protection mechanisms to alleviate photodynamic damage caused by $^1O_2$ (Foyer and Noctor, 2009; Li *et al.*, 2009; Dogra *et al.*, 2018). However, if this balance is disturbed when plants are subjected to environmental stresses, $^1O_2$ is generated. For a long time, $^1O_2$ had been recognized as detrimental to plants because of its oxidative damage effects, but more recent findings also suggest $^1O_2$ as a signal (Li *et al.*, 2009; Triantaphylidès and Havaux, 2009; Fischer *et al.*, 2013; Laloi and Havaux, 2015; Noctor and Foyer, 2016; Foyer *et al.*, 2017). Another emerging concept is that all types of oxidative modification/damage are involved in signaling (Foyer *et al.*, 2017).

For various reasons it has been difficult to assess and verify such a signaling capacity of $^1O_2$. (i) Besides $^1O_2$, there are other chemically distinct reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide ($O_2{\cdot}^-$), and hydroxyl radical (·OH), whose concentrations in plants under stress

© The Author(s) 2018. Published by Oxford University Press on behalf of the Society for Experimental Biology. All rights reserved.
For permissions, please email: journals.permissions@oup.com

Downloaded from https://academic.oup.com/jxb/article/70/1/29/5110609 by ETH Zürich user on 17 October 2023

increase almost simultaneously with those of $^1O_2$, thus making it difficult to define the biological effects of $^1O_2$ and to link it to a particular cellular response (Apel and Hirt, 2004; Foyer and Noctor, 2009; Noctor *et al.*, 2018). (ii) $^1O_2$ may be formed at different intracellular and intraorganellar sites, and its biological impact may vary depending on where it is generated (Apel and Hirt, 2004; Noctor and Foyer, 2016). (iii) $^1O_2$ interacts with proteins, lipids, carbohydrates, and nucleic acids (Sies and Menck, 1992; Davies, 2003; Triantaphylidès *et al.*, 2008) and in this way may irreversibly inactivate and/or destroy the target (Elstner, 1982). It is not known yet how such a highly reactive molecule may initiate signaling rather than causing oxidative damage. (iv) Plants are exposed to diverse environmental conditions whose impact may rapidly change. (v) Even though the activity of $^1O_2$ can be studied under steady-state conditions, it is difficult to separate primary from secondary effects of $^1O_2$. To overcome these obstacles, one needs to induce the production of $^1O_2$ non-invasively (Apel and Hirt, 2004).

In the meantime, experimental approaches have been developed that overcome most of these obstacles. Using these approaches, $^1O_2$ has been recently demonstrated to act as a highly versatile signal that induces a wide range of stress responses throughout the life cycle of a plant (Fischer *et al.*, 2013; Laloi and Havaux, 2015; Foyer *et al.*, 2017; Noctor *et al.*, 2018).

Formation of singlet oxygen

Ground state triplet molecular oxygen ($^3O_2$) may be converted to the highly reactive $^1O_2$ either metabolically or photochemically (Halliwell and Gutteridge, 1999; Apel and Hirt, 2004; Krieger-Liszkay *et al.*, 2008; Triantaphylidès and Havaux, 2009; Fischer *et al.*, 2013; Laloi and Havaux, 2015). Knowledge about the former reaction is still scarce (Kanofsky and Axelrod, 1986; Steinbeck *et al.*, 1992; Khan and Kasha, 1994), whereas photochemical formation of $^1O_2$ has been the subject of intense research (Krieger-Liszkay *et al.*, 2008; Foyer and Noctor, 2009; Triantaphylidès and Havaux, 2009; Fischer *et al.*, 2013; Laloi and Havaux, 2015). Energy transfer by an excited photosensitizer reverses the spin direction of one of the two outermost valence electrons of triplet oxygen that occupy separate orbitals with parallel spins, and allows the pairing of these electrons (Fig. 1). This spin reversal and pairing of electrons dramatically enhances the reactivity of oxygen (Fig. 1). In plants, $^1O_2$ is primarily generated in chloroplasts due to the photosensitizing activity of tetrapyrroles. The syntheses of these porphyrins share a common pathway up to the formation of protoporphyrin IX (ProtoIX), when metals are inserted (Tanaka and Tanaka, 2007). Afterwards, the pathway diverges into two major branches, with the Fe branch being directed to the synthesis of hemes and phycobilins, and the Mg branch leading to the formation of Chls. These porphyrins have the potential to act as photosensitizers and transfer the excitation energy directly to ground state triplet oxygen, leading to the formation of singlet oxygen (Fig. 1) (Apel and Hirt, 2004; Krieger-Liszkay *et al.*, 2008; Triantaphylidès and Havaux, 2009).

Chl, heme, and phycobilin are usually bound to proteins and, in this state, may use various quenching mechanisms to dissipate excess absorbed light energy. Thus, formation of $^1O_2$ by these porphyrins is usually compromised under non-stressful conditions. Their biosynthetic intermediates, however, occur mostly in a free form and are potentially much more destructive when illuminated (Mochizuki *et al.*, 2010). To avoid extensive photo-oxidative damage by these intermediates, plants prevent their accumulation by strictly controlling tetrapyrrole biosynthesis and catabolism. One important element of this regulation is the negative feedback inhibition of the first step of tetrapyrrole biosynthesis, formation of δ-aminolevulinic acid (ALA), by two effecter molecules, the FLU protein and heme, that both interact with different parts of Glu-tRNA reductase (Vothknecht *et al.*, 1998; Meskauskiene *et al.*, 2001; Goslings *et al.*, 2004; Levicán *et al.*, 2007), the first enzyme committed to tetrapyrrole synthesis (Vothknecht *et al.*, 1998; Meskauskiene *et al.*, 2001; Cornah *et al.*, 2003). In the dark, the Mg branch leads only to the formation of protochlorophyllide (Pchlide), the immediate precursor of chlorophyllide (Chlide). The subsequent step from Pchlide to Chlide requires light (Griffiths, 1978; Apel *et al.*, 1980). Once a critical level of Pchlide has been reached in the dark, ALA synthesis slows down and accumulation of Pchlide stops. Only after re-exposure to light, when Pchlide is photoreduced to Chlide, does Chl biosynthesis resume. Pchlide has been implicated in activating the FLU-dependent suppression of ALA formation, thereby allowing the Mg branch to regulate the initial step of tetrapyrrole biosynthesis, whereas the Fe branch controls this step via heme (Vothknecht *et al.*, 1998; Goslings *et al.*, 2004).

When plants are exposed to severe environmental stresses that interfere with photosynthetic electron transport, $^1O_2$ production may also be initiated in chloroplasts by the photosensitizing

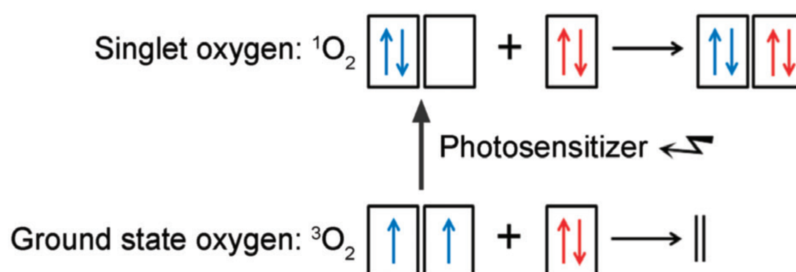

Fig. 1. Formation of singlet oxygen ($^1O_2$). The biradical $^3O_2$ has two unpaired electrons with parallel spins. To oxidize a non-radical molecule, $^3O_2$ needs to react with a partner that provides a pair of electrons with parallel spins. Pairs of electrons usually have opposite spins and thus restrict the ability of $^3O_2$ to react with other molecules. Energy transfer from an excited photosensitizer (e.g. ^{3}Chl) transforms $^3O_2$ into $^1O_2$ by reversing the spin direction of one of the two unpaired electrons and allowing their pairing. (This figure is available in color at *JXB* online.)

Downloaded from https://academic.oup.com/jxb/article/70/1/29/5110609 by ETH Zürich user on 17 October 2023

activity of protein-bound Chl (Krieger-Liszkay *et al.*, 2008; Li *et al.*, 2009; Triantaphylidès and Havaux, 2009; Fischer *et al.*, 2013; Laloi and Havaux, 2015). Upon light absorption, Chl changes from a ground state to the singlet excited state, ^{1}Chl. The fate of the excitation energy in this Chl may vary. Usually the absorbed light energy is transferred from Chl of the light-harvesting antenna complexes to the reaction center (RC) Chl where it drives photosynthetic electron transport. However, under high light, excessive excitation energy can also be dissipated as heat when the excited light-harvesting Chl interacts with carotenoids, or it can decay via formation of the excited triplet state of Chl (^{3}Chl) (Krieger-Liszkay *et al.*, 2008; Li *et al.*, 2009). ^{3}Chl has a longer life time than ^{1}Chl (Krieger-Liszkay, 2005), thereby allowing its excitation energy to be transferred to ground-state $^3O_2$ that produces $^1O_2$ if no efficient quenchers are close enough to compete for this energy (Krieger-Liszkay *et al.*, 2008). In the antenna, the ^{3}Chl is in the close vicinity of various carotenoids that are able to quench ^{3}Chl directly, and generation of $^1O_2$ is usually suppressed. In contrast, in the RC of PSII the special Chl, P680, is not in close contact with carotenoids, and the β-carotene associated with the RC of PSII is unable to quench the excitation energy of ^{3}P680; thus, generation of $^1O_2$ is favored (Trebst, 1999; Umena *et al.*, 2011) (Fig. 2A). Even though most of Chl is found in the antenna, $^1O_2$ is mainly produced in the RC of PSII (Krieger-Liszkay *et al.*, 2008). Whenever the electron acceptor of PSII remains reduced and is unable to accept electrons originating from excited P680 (e.g. under high light, low temperature, or drought conditions) (Fig. 2A), the excited triplet state of P680 may then act as a photosensitizer and produces $^1O_2$. Under the stress conditions, ^{3}Chl can also be generated in the PSII RC by charge recombination reactions (back-flow of electron transfer and charge separation reactions) and acts as a photosensitizer that produces $^1O_2$ (Krieger-Liszkay *et al.*, 2008). This $^1O_2$ is believed to interact primarily with its nearest target, the D1 protein of PSII RC that binds the P680 Chl, thereby inactivating this protein and inhibiting PSII (Keren *et al.*, 1997; Szilárd *et al.*, 2005; Ohad *et al.*, 2011; Vass, 2012; Kale *et al.*, 2017) (Fig. 2A).

Signaling versus damaging: the role of singlet oxygen

For a long time, photo-oxidative damage of plant cells had been considered to be the only biological effect of $^1O_2$ production (Elstner, 1982; Davies, 2003; Krieger-Liszkay *et al.*, 2008). Thus, it came as a surprise when $^1O_2$ was also shown to act as a signal that triggered specific nuclear gene expression changes and greatly impacted the phenotype of the affected plant (Kim *et al.*, 2008; Krieger-Liszkay *et al.*, 2008; Triantaphylidès and Havaux, 2009; Fischer *et al.*, 2013; Laloi and Havaux, 2015; Wang and Apel, 2016; Foyer *et al.*, 2017).

A signaling role for $^1O_2$ was first demonstrated in the conditional *fluorescent* (*flu*) mutant of Arabidopsis (op den Camp *et al.*, 2003; Wagner *et al.*, 2004). FLU acts as a negative regulator of tetrapyrrole biosynthesis, and *flu* seedlings lacking this regulator are unable to restrict the accumulation of the Chl precursor Pchlide in the dark. When these seedlings are transferred to the light, they rapidly bleach and die due to the photosensitizing activity of excess amounts of free Pchlide (Meskauskiene *et al.*, 2001; op den Camp *et al.*, 2003) (Fig. 3A–D). In the *flu* mutant, the amount of $^1O_2$ generated is proportional to the amount of Pchlide and thus to the duration of dark treatment (Laloi and Havaux, 2015). When grown under continuous

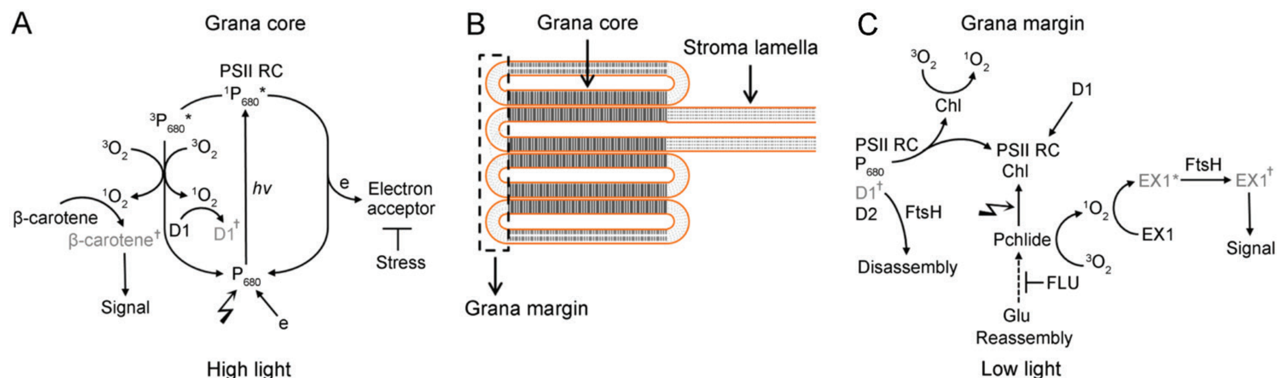

Fig. 2. Schematic diagrams that illustrate how the PSII reaction center (RC) either in its active state in the grana core (A, B) or during its repair in grana margins (B, C) is thought to generate $^1O_2$. (A) Light energy absorbed by PSII is trapped within the PSII RC whenever the electron acceptor of PSII remains reduced under various stress conditions and is unable to accept electrons which originated from the excited P680 Chl of the RC. This favors the transformation of the short-lived singlet state P680 into the more stable triplet state P680 that may then act as a photosensitizer and generate $^1O_2$. This $^1O_2$ is believed to interact primarily with its nearest target, the D1 protein of the PSII RC that binds the P680 Chl. At higher concentrations, such as under high light stress, $^1O_2$ may also interact with β-carotene that is associated with PSII. Some of the oxidative breakdown products of β-carotene may act as a signal. (B) Schematic diagram showing the different regions of thylakoid membranes. Active PSII is localized in the grana core region, whereas the repair of damaged PSII takes place within the grana margin. (C) During the repair of PSII, the damaged D1 protein is degraded and replaced by newly synthesized D1 polypeptides. During the reassembly of active PSII, the insertion of D1 and Chl needs to be strictly co-ordinated to avoid photo-oxidative damage caused by the photosensitizing activity of unbound Chl. The Chl may be derived from the damaged PSII and/or is synthesized *de novo* within the grana margin region. A perturbation of this reconstitution is expected to allow a transient accumulation of either free Chl or its precursors (e.g. Pchlide or ProtoIX) that may generate $^1O_2$. PSII within the grana margin co-localizes with the FtsH protease, EX1, FLU, and enzymes of Chl synthesis. The onset of $^1O_2$-mediated and EX1-dependent signaling strictly depends on an FtsH-dependent decline of the EX1 protein. (This figure is available in color at *JXB* online.)

Downloaded from https://academic.oup.com/jxb/article/70/1/29/5110609 by ETH Zürich user on 17 October 2023

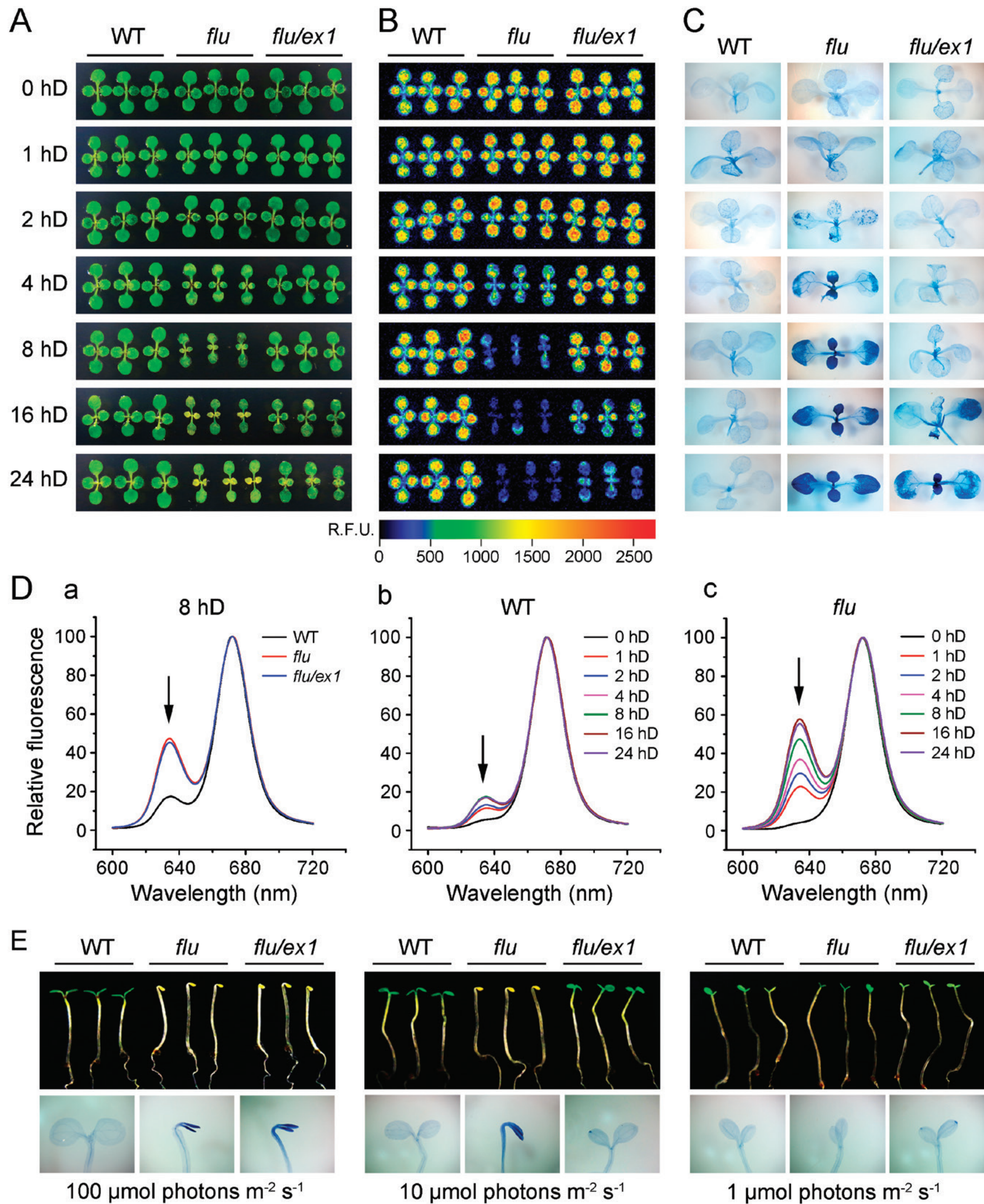

Fig. 3. Dose-dependent $^1O_2$-mediated responses of the *flu* mutant. Various concentrations of $^1O_2$ were reached by either changing the duration of the dark treatment (A–D) or using different light intensities during illumination of etiolated seedlings (E). Seven-day-old light-grown seedlings of the wild type (WT), *flu*, and *flu/ex1* were grown for 7 d under continuous light (90 μmol photons m^{-2} s^{-1}) at 22 °C in soil, and put in the dark for different periods of time (A–D) and re-exposed to light for 24 h (A–C). With increasing duration of the dark period, the amount of the photosensitizer Pchlide (indicated by arrows) increased (D). Up to an 8 h dark period $^1O_2$-mediated growth inhibition (A), photoinhibition of PSII as revealed by transient Chl fluorescence changes (B), and cell death as shown by trypan blue staining (C) in *flu* were abrogated in *flu/ex1* seedlings, suggesting that these responses of the *flu* mutant were triggered by EX1-dependent signaling. The EX1 dependency of these responses was gradually lost in *flu* plants that were kept in the dark for 16 h or 24 h. R.F.U., relative fluorescence unit. (E) Four-day-old etiolated seedlings grown on 1/2 Murashige and Skoog (MS) (0.8% agar, 0.5% sucrose) were exposed for 2 d to different light intensities as indicated at the bottom of the figure. $^1O_2$-mediated cell death was revealed by trypan blue staining. At the highest light intensity, *flu* but also *flu/ex1* seedlings initiated a cell death response, whereas at an intermediate light intensity this response was abrogated in *flu/ex1* but not in *flu*. At a very low light intensity, none of these seedlings showed a cell death response, and *flu* and *flu/ex1* seedlings looked similar to green wild-type control seedlings. Images of transient fluorescence were taken with a FluorCam 800MF system (Photon Systems Instruments) following manuals provided by the manufacturer. Trypan blue staining of dead cells and determination of Pchlide was performed as described by op den Camp *et al.* (2003).

Downloaded from https://academic.oup.com/jxb/article/70/1/29/5110609 by ETH Zürich user on 17 October 2023

light, the *flu* mutant looks exactly like the wild-type plant as Pchlide is immediately photoreduced to Chlide. These properties make the *flu* mutant an ideal tool for generation of $^1O_2$ and study of $^1O_2$-induced stress responses. In the *flu* mutant, shortly (~30 min) after the release of $^1O_2$, the chloroplast starts to lose its integrity (the chloroplast becomes leaky as the stromal protein appears in the cytosol), and afterwards rupture of the central vacuole occurs followed by final collapse of the cell (Kim *et al.*, 2012). However, all these drastic phenotypic changes disappear in the *flu/ex1* double mutant although it overaccumulates Pchlide in the dark and generates a similar amount of $^1O_2$ to that generated in the parental *flu* line during re-illumination (Wagner *et al.*, 2004; Kim *et al.*, 2012) (Fig. 3). In addition to EX1, an EX1-like protein, dubbed EX2, was identified that, unlike EX1, did not impact the $^1O_2$-mediated cell death and growth inhibition responses of the *flu* mutant but greatly affected $^1O_2$-mediated nuclear gene expression changes (Lee *et al.*, 2007).

Many genes are up-regulated in response to the release of $^1O_2$ in the *flu* mutant (op den Camp *et al.*, 2003; Gadjev *et al.*, 2006; Dogra *et al.*, 2017). The genes that are up-regulated prior to chloroplast leakage are directly affected by the release of $^1O_2$. In contrast, the genes whose expression changed after chloroplast leakage are only indirectly affected as the loss of chloroplast integrity seems to enhance the photosensitizing activity of membrane-bound Chl and to amplify drastically the production of $^1O_2$ that subsequently causes massive non-enzymatic lipid peroxidation and photo-oxidative damage (Kim and Apel, 2013). For most $^1O_2$-responsive genes, the up-regulation is first seen shortly after the loss of chloroplast integrity (Kim and Apel, 2013). A large number of these genes have been annotated as being under hormonal control (Baruah *et al.*, 2009*a*). As the concentrations of phytohormones such as jasmonic acid (JA), ethylene, and salicylic acid (SA) increase soon after the onset of $^1O_2$ production (op den Camp *et al.*, 2003; Danon *et al.*, 2005), the enhanced expression of the majority of $^1O_2$-responsive genes seems to be triggered by these phytohormones (Kim and Apel, 2013). Nevertheless, the $^1O_2$-mediated transcriptional changes rely on the presence of EX1/EX2 protein. Without these proteins, all subsequent $^1O_2$ signaling-mediated stress responses of the *flu* mutant are abolished (Wagner *et al.*, 2004; Kim *et al.*, 2012).

EX1-dependent signaling operates only in response to rather minor increase in $^1O_2$ production, when light-grown *flu* plants are transferred to the dark for up to 8 h, and are re-exposed to light (Wagner *et al.*, 2004; Wang *et al.*, 2016) (Fig. 3A–D). If the duration of the dark treatment is prolonged to 16 h or longer, *flu* seedlings accumulate more Pchlide and, upon re-illumination, generate higher amount of $^1O_2$. Under these latter conditions, $^1O_2$-mediated responses become EX independent (Fig. 3), thus re-emphasizing the fact that EX-dependent signaling in *flu* mutants occurs only at low, non-toxic concentrations of $^1O_2$. Because of its high reactivity and short half-life, primary reactions of $^1O_2$ that initiate signaling should be localized near the site of $^1O_2$ formation. Thus, in the case of EX1-dependent signaling, $^1O_2$ should be generated close to where EX1 is localized.

EX1 is present in thylakoid membranes and is restricted to the non-appressed margins of grana stacks (Wang *et al.*, 2016) (Fig. 2B, C). Grana margins play an important role during repair of damaged PSII (Khatoon *et al.*, 2009; Puthiyaveetil *et al.*, 2014; Yoshioka-Nishimura *et al.*, 2014). Active PSII is present in the core of tightly compressed grana membranes (Aro *et al.*, 1993). Following inactivation, PSII needs to be translocated to the grana margins where it is disassembled and the damaged D1 and—to a lesser extent—also the D2 protein of the PSII RC are cleaved by the FtsH protease (Fig. 2C) (Lindahl *et al.*, 2000; Bailey *et al.*, 2002; Dogra *et al.*, 2018). During the reassembly of active PSII, these proteins are replaced by D1 and D2 polypeptides synthesized on membrane-bound ribosomes attached to non-appressed stroma thylakoids (Mullet *et al.*, 1990). In grana margins, EX1 forms part of a larger physical unit that contains PSII, the FtsH protease, the NADPH-Pchlide oxidoreductases (PORs) B and C that catalyze the light-dependent reduction of Pchlide to Chlide in light-grown plants, and two elongation factors (Wang *et al.*, 2016). This localization of EX1 suggests that $^1O_2$ is produced close to where the disassembly and reassembly of PSII take place. Most probably this $^1O_2$ is generated by the photosensitizing activity of Chl or some of its intermediates that are used for the reconstitution of PSII (Fig. 2C). Turnover of Chl in light-adapted plants is confined to the core of PSII (Feierabend and Dehne, 1996), suggesting that newly synthesized Chl in green plants is primarily used for the assembly of active PSII. Enzymes of Chl biosynthesis and the FLU protein are highly enriched in grana margins and co-localize with the site of PSII repair (Wang *et al.*, 2016). The recycling and *de novo* synthesis of Chl must be tightly controlled and synchronized with the synthesis of the PSII RC proteins D1 and D2 to minimize the disruptive effect of unbound, photoreactive Chl and its intermediates (Mullet *et al.*, 1990; Müller and Eichacker, 1999). A slight disturbance of this co-ordinated assembly process is likely to enhance the level of unbound Chl or its intermediates and to generate $^1O_2$ (Fig. 2C). As shown by the example of the *flu* mutant, this $^1O_2$ may trigger EX-dependent signaling (Wagner *et al.*, 2004; Kim *et al.*, 2012; Wang *et al.*, 2016). In the *flu* mutant, the spatial distribution of the photosensitizer does not exactly match that of the EX1 protein. Whereas EX1 is confined to grana margins, Pchlide is evenly distributed in margin and core regions of grana stacks and is also found in stroma lamellae (Wang *et al.*, 2016). This difference in the distribution of photosensitizer and EX1 re-emphasizes the notion that the amounts of $^1O_2$ generated in the *flu* mutant after an 8 h dark–light shift are too low to cause apparent photo-oxidative damage to a plant. As the biological activity of $^1O_2$ depends on the presence of EX1, this protein seems to act as a sensor of $^1O_2$ that amplifies its potential signaling effect.

With the onset of $^1O_2$-mediated signaling in the *flu* mutant, there is a rapid decline of EX1 that depends on the FtsH protease (Wang *et al.*, 2016; Dogra *et al.*, 2017). Generation of $^1O_2$ without a decline of EX1 is not sufficient to trigger $^1O_2$ signaling (Fig. 2C). As FtsH also cleaves the two PSII RC proteins D1 and D2 (Lindahl *et al.*, 2000; Bailey *et al.*, 2002; Kato *et al.*, 2009), EX1-dependent signaling seems not only spatially but

Downloaded from https://academic.oup.com/jxb/article/70/1/29/5110609 by ETH Zürich user on 17 October 2023

also functionally linked to the repair of PSII (Fig. 2C). The simultaneous onset of $^1O_2$ production and decline of EX1 indicates that EX1 by interacting with $^1O_2$ becomes susceptible to proteolytic attack by FtsH. It seems conceivable that EX1 acts as a negative regulator that needs to be removed by proteolytic cleavage to activate the $^1O_2$-dependent signaling pathway. However, experimental evidence does not support such a notion. In *flu/ex1* plants, $^1O_2$-mediated signaling is only active after EX1 is expressed in the complemented mutant line (Wang *et al.*, 2016; Dogra *et al.*, 2017). At present, it is not known how FtsH modifies EX1 and whether proteolytic breakdown products of EX1 interact with downstream signaling components within the plastid or outside in the extraplastidic cytoplasm or in the nucleus.

Both cytotoxic and signaling effects of $^1O_2$ can result in plant cell death, and which of the two effects prevails depends on the amount of $^1O_2$. When the amount of $^1O_2$ increases only slightly or moderately, its signaling effects prevails. In contrast, cytotoxic effects become dominant if the amount of $^1O_2$ increases drastically. The loss of chloroplast integrity and rupture of the central vacuole can be mediated by signaling effects of $^1O_2$ (Kim *et al.*, 2012; Woodson *et al.*, 2015). It is likely that these processes can also be induced by the cytotoxic effects of $^1O_2$, but evidence is needed. To distinguish the signaling and cytotoxic effects of $^1O_2$, two hallmarks are generally used: the impact of EX1 mutation and the prevalence of either enzymatic or non-enzymatic lipid peroxidation (Przybyla *et al.*, 2008). If the $^1O_2$-induced stress responses can be suppressed by EX1 mutation, and/or the enzymatic but not non-enzymatic lipid peroxidation occurs predominantly, these responses are generally attributed to signaling effects of $^1O_2$, and otherwise to its cytotoxic effects. 13-HOT and 13-HOD are representatives of enzymatic lipid peroxidation, while 10-HOT, 10-HOD, 12-HOD, and 15-HOD are typical products of non-enzymatic lipid peroxidation (Przybyla *et al.*, 2008). The etiolated *flu* and *flu/ex1* seedlings accumulate ~3 times more Pchlide compared with seedlings that are grown under continuous light and treated with 8 h darkness. Thus, upon illumination, the etiolated *flu* and *flu/ex1* seedlings generate a much higher amount of $^1O_2$ that exceeds the threshold concentration of $^1O_2$ which induces EX1-dependent signaling. In the etiolated seedlings of both *flu* and *flu/ex1*, non-enzymatic lipid peroxidation prevails and the EX1 mutation is unable to suppress the cell death response. In contrast, in 8 h dark-treated *flu* seedlings, lipid peroxidation occurs almost exclusively enzymatically, and both the lipid peroxidation and the cell death response can be suppressed by EX1 mutation (Przybyla *et al.*, 2008).

Different roles of $^1O_2$: quality control versus induction of cell death

Once a signaling role for $^1O_2$ had been established in the *flu* mutant, interest in studying the biological activity of $^1O_2$ markedly increased. Other experimental systems have now also been established that non-invasively induce generation of $^1O_2$. One of them is the *ferrochelatase2* (*fc2*) mutant of Arabidopsis. In the *fc2* mutant, the photosensitizing activity of the tetrapyrrole intermediate, ProtoIX, generates $^1O_2$ (Woodson *et al.*, 2015). Similarly, down-regulation of a tobacco ferrochelatase by RNAi increases the level of ProtoIX that generates $^1O_2$ and leads to formation of necrotic leaves in the transformed tobacco (Papenbrock *et al.*, 2001). Moreover, when plants are treated with peroxidizing herbicides that inhibit Protox, the enzyme that catalyzes the oxidization of Protogen to ProtoIX, they start to overaccumulate ProtoIX and show severe photo-oxidative damage (Sandmann and Boger, 1988; Becerril and Duke, 1989; Watanabe *et al.*, 1998).

Studies of the *fc2* mutant have implicated the release of $^1O_2$ with activation of a ubiquitin-dependent quality control pathway that has been proposed to remove damaged chloroplasts selectively from plant cells (Woodson *et al.*, 2015). The *fc2* mutant of Arabidopsis lacks one of two ferrochelatases that catalyze formation of protoheme by inserting Fe^{2+} into ProtoIX. While *fc2* mutant plants grown under continuous light turn green like the wild type, they become pale and form abnormally small leaves under an 8 h light/16 h dark regime (Woodson *et al.*, 2015). This phenotypic change had been attributed to the generation of $^1O_2$ by the photosensitizing activity of ProtoIX (Scharfenberg *et al.*, 2015; Woodson *et al.*, 2015). Upon transfer to dark, the *fc2* mutant starts to overaccumulate ProtoIX and after 50 min of darkness reaches an ~10-fold higher maximum than the wild type, but that declines afterwards over the next 30 min. The concentration of ProtoIX at the end of the 16 h dark period has not been reported and thus it is not known whether generation of $^1O_2$ in *fc2* mutants grown under an 8 h light/16 h dark cycle is indeed caused by increased levels of ProtoIX. In addition, the transformed tobacco leaves with reduced expression of a tobacco ferrochelatase accumulate ProtoIX under light but not in the dark (Papenbrock *et al.*, 2001). Thus, it is necessary to determine the ProtoIX concentration of the *fc2* mutant at the end of the dark period and at selected time points during the light period. In etiolated *fc2* seedlings, ProtoIX reaches the same low level as in wild-type control plants (Scharfenberg *et al.*, 2015), whereas the concentration of Pchlide is higher than in the wild type but not as high as in similarly treated *flu* plants (Scharfenberg *et al.*, 2015; Woodson *et al.*, 2015). Thus, as pointed out by Scharfenberg *et al.* (2015), the *fc2* mutant displays a weak *flu* phenotype when grown in the dark, suggesting that generation of $^1O_2$ in this mutant is due to the photosensitizing activity of Pchlide. This interpretation is supported by the fact that a $^1O_2$-mediated loss of chloroplast integrity and a subsequent collapse of the affected cell appears not only in the *flu* mutant but also in the *fc2* mutant (Kim *et al.*, 2012; Woodson *et al.*, 2015). Among second-site mutations of *fc2* that restore the ability to green when grown under a dark/light regime, the *PUB4* gene encoding a E3 ubiquitin ligase has been identified (Woodson *et al.*, 2015). When grown under non-permissive dark/light conditions, the *fc2/pub4* double mutant generates $^1O_2$ and its chloroplasts appear stressed with a distorted membrane system, but unlike chloroplasts of the parental *fc2* line they remain intact and are not degraded. Thus, ubiquitination of chloroplast proteins seems to be an important step during $^1O_2$-mediated dismantling and subsequent degradation of

Downloaded from https://academic.oup.com/jxb/article/70/1/29/5110609 by ETH Zürich user on 17 October 2023

chloroplasts. Whether in *fc2* mutants this ubiquitin-dependent breakdown of chloroplasts signifies activation of a quality control pathway that is mediated by $^1O_2$ is difficult to judge. As the release of $^1O_2$ in *flu* and *fc2* mutants induces chloroplast leakage followed by the disruption of the central vacuole and the collapse of the cell (Kim *et al.*, 2012; Woodson *et al.*, 2015), it sounds impossible to identify a quality control pathway that selectively removes individual damaged chloroplasts in a collapsed cell. However, in *fc2* plants grown under continuous light that are not expected to overaccumulate ProtoIX and Pchlide, evidence for the operation of a ubiquitin-dependent quality control pathway has been obtained (Woodson *et al.*, 2015). These plants show, relative to wild-type plants, a reduced growth and slightly impaired photosynthetic electron transport (Scharfenberg *et al.*, 2015). These differences between the mutant and wild type may explain why in *fc2* the number of damaged chloroplasts is higher than in the wild type. In the *fc2/pub4* double mutant, the number of damaged chloroplasts returns to the wild-type level, suggesting that ubiquitination is indeed involved in recognizing damaged chloroplasts and subsequently allowing their degradation to proceed. However, so far it remains unclear whether activation of this pathway and/or damage of chloroplasts in these plants are caused by $^1O_2$.

Even though *fc2* resembles *flu*, the biological effects of $^1O_2$ in these two mutants show a remarkable difference. In *flu*, $^1O_2$-mediated phenotypic changes depend on EX1 and are completely abrogated in the *flu/ex1* double mutant. Whereas in *fc2*, $^1O_2$-induced changes are not affected by the absence of EX1 (Woodson *et al.*, 2015). At the moment, it is difficult to explain this difference. Since *fc2* mutants accumulate less Pchlide in the dark than *flu* mutants (Scharfenberg *et al.*, 2015), they are not expected to release a higher amount of $^1O_2$ that surpasses the signaling capacity of EX1, as shown in Fig. 3. It is not known whether FC2 is localized in the grana margin region close to EX1. As the intraorganellar location of $^1O_2$ formation is expected to influence the biological effects of $^1O_2$, the release of $^1O_2$ at a different site away from EX1 may explain why activation of $^1O_2$-mediated responses in the *fc2* mutant does not depend on EX1.

$^1O_2$-induced cell death under severe high light stress

The third experimental system used to induce generation of $^1O_2$ non-invasively and to study its signaling activity is the Chl *b*-deficient *chlorina1* (*ch1*) mutant of Arabidopsis that is devoid of PSII antenna complexes (Dall'Osto *et al.*, 2010; Ramel *et al.*, 2013; Shumbe *et al.*, 2016). Without these antenna complexes, the mutant lacks light-scavenging capacity and is highly sensitive to high light. In contrast to wild-type controls, *ch1* plants exposed to a combination of high light (1000 μmol photons m^{-2} s^{-1}) and low temperature (10 °C) suffer from severe photoinhibition of PSII and photo-oxidative damage due to an enhanced production of $^1O_2$ in the RC of PSII (Ramel *et al.*, 2013). The excitation energy trapped within the PSII RC favors the transformation of the short-lived singlet state of P680 into the more stable triplet state that allows the excited Chl to act as a photosensitizer that generates $^1O_2$ (Fig. 2A). $^1O_2$ oxidizes various chloroplast membrane constituents such as proteins (Davies, 2003), polyunsaturated fatty acids (Przybyla *et al.*, 2008; Triantaphylidès *et al.*, 2008), and carotenoids (Ramel *et al.*, 2012*a*), causing photo-oxidative damage that ultimately may lead to the collapse of cells. This cell death response of the *ch1* mutant to high light stress is not exclusively due to the toxicity of $^1O_2$ but also involves genetically controlled stress-responsive components that are activated by the release of $^1O_2$, and depends on the *OXIDATIVE SIGNAL INDUCIBLE 1* (*OXI1*) Ser/Thr kinase (Shumbe *et al.*, 2016; Foyer *et al.*, 2017; Dogra *et al.*, 2018). Expression of the *OXI1* gene in the high-light-stressed *ch1* mutant is strongly induced. Inactivation of *OXI1* reduces the extent of photo-oxidative damage and cell death in the high-light-stressed *ch1/oxi1* double mutant even though these plants generate similar amounts of $^1O_2$ to those generated by the parental *ch1* mutant (Shumbe *et al.*, 2016). *OXI1* had been shown earlier to be involved in H_2O_2-mediated signaling that controlled root hair growth and plant–pathogen interactions (Rentel *et al.*, 2004). As *OXI1*-dependent responses triggered by H_2O_2 differ from those induced by $^1O_2$, both ROS seem to activate different signaling pathways that converge on OXI1 but otherwise operate via distinct mechanisms and lead to different physiological responses of the affected plants.

At first glance, the $^1O_2$-mediated cell death responses of *ch1* and *flu* seem to be very similar. In both mutants, the release of $^1O_2$ not only leads to cell death but also affects similar sets of $^1O_2$-responsive nuclear genes, and these responses occur independently of H_2O_2-dependent signaling. However, initiation of the high-light-induced cell death in *ch1* results primarily from photo-oxidative damage, whereas in *flu* the cell death response is under genetic control and is triggered by a rapid but minor increase of $^1O_2$ that is too low to damage the cell directly (Kim *et al.*, 2012; Shumbe *et al.*, 2016; Wang *et al.*, 2016). In *ch1*, the $^1O_2$ level rises more gradually, with a production lasting as long as the light stress is maintained and the PSII RCs are still intact. During this period, increases of various hormones such as JA and ethylene form an integral part of the cell death-inducing mechanism (Shumbe *et al.*, 2016). Also in *flu*, $^1O_2$-mediated hormone changes have been reported (op den Camp *et al.*, 2003; Danon *et al.*, 2005; Przybyla *et al.*, 2008). However, in *flu*, the enhanced hormone production and expression changes of most of the $^1O_2$-responsive genes occur later than the $^1O_2$-mediated loss of chloroplast integrity (Kim *et al.*, 2012). Hence, these $^1O_2$-responsive genes seem to be only indirectly affected by $^1O_2$ and are probably activated during the loss of cellular integrity by hormones such as JA and ethylene (Kim and Apel, 2013). Generation of $^1O_2$ in *ch1* has been reported to occur within grana stacks of PSII (Laloi and Havaux, 2015), whereas in *flu* $^1O_2$ formation that induces cell death responses takes place within grana margins close to the repair site of damaged PSII (Wang *et al.*, 2016). In *flu*, the $^1O_2$-mediated rapid loss of chloroplast integrity and the subsequent cell death strictly depend on the two EX proteins that are localized within the grana margins, whereas in *ch1* the high-light-induced cell death response is not affected by the absence of these proteins (Shumbe *et al.*, 2016). Thus, even though the cell death

Downloaded from https://academic.oup.com/jxb/article/70/1/29/5110609 by ETH Zürich user on 17 October 2023

responses of *flu* and *ch1* are both triggered by $^1O_2$, they are controlled by different mechanisms.

Under moderate light stress (400 μmol photons m^{-2} s^{-1}/20 °C), $^1O_2$ induces stress acclimation in *ch1* that attenuates the $^1O_2$-mediated cell death response during a subsequent severe high light stress (Shumbe *et al.*, 2016). $^1O_2$-mediated signaling under moderate light stress seems different from *OXI1*-dependent signaling in high-light-treated plants. Under severe light stress, the $^1O_2$-mediated cell death is preceded by an enhanced expression of the *OXI1* gene, whereas under moderate light stress expression of this gene is suppressed. At the same time, an enhanced expression of acclimation-specific genes under moderate light contrasts with the down-regulation of other $^1O_2$-responsive genes that are activated under high light stress (Shumbe *et al.*, 2016). Some of these latter genes are known to control the biosynthesis of JA. In high-light-treated *ch1* plants, up-regulation of these genes correlates with high levels of JA, whereas in pre-acclimated *ch1* plants this JA accumulation during high light treatment is suppressed (Shumbe *et al.*, 2016). Exogenously applied JA restores the high light stress-induced cell death response in pre-acclimated *ch1* plants. On the other hand, a genetic block of JA biosynthesis significantly reduces the extent of photo-oxidative damage and cell death during high light stress in a JA-deficient *delayed-dehiscence2* (*dde2*)/*ch1* double mutant (Ramel *et al.*, 2013). Collectively, these results suggest that JA plays a dual role by promoting a cell death response and suppressing the effect of stress acclimation in high-light-treated *ch1* mutant plants.

$^1O_2$-mediated dose-dependent and spatially resolved responses

So far, different $^1O_2$-mediated reactions of plants have been attributed to different dose-dependent effects of $^1O_2$. In high-light-treated *ch1* plants, $^1O_2$-mediated signaling at higher $^1O_2$ concentrations contributes to the cell death response and induces an up-regulation of *OXI1* gene expression, whereas under moderate light stress a lower level of $^1O_2$ correlates with stress acclimation, suppression of *OXI1* gene expression, and reduced photodamage (Shumbe *et al.*, 2016). In light-grown *flu* seedlings transferred to the dark for up to 8 h and re-exposed to light, a lower amount of $^1O_2$ activates EX1-dependent signaling and triggers a cell death response without obvious photo-oxidative damage (Kim *et al.*, 2012; Wang *et al.*, 2016). An extension of the duration of the dark period in the *flu* mutant enhances the accumulation of the photosensitizer Pchlide that upon re-illumination generates a higher amount of $^1O_2$ and causes photo-oxidative damage (Fig. 3A–D). Under these latter conditions, the toxicity of $^1O_2$ prevails and superimposes the EX1-dependent signaling induced by $^1O_2$ (Fig. 3). It might be interesting to test the behavior of EX1-overproducing plants under these latter conditions. During illumination, the amounts of $^1O_2$ produced in etiolated *flu* and *flu/ex1* are also affected by light intensities. Under a higher light intensity (100 μmol photons m^{-2} s^{-1}), a higher amount of $^1O_2$ is produced. As a result, the cytotoxic effect of $^1O_2$ prevails and both *flu* and *flu/ex1* seedlings initiate a cell death response. However, under a low light intensity (10 μmol photons m^{-2} s^{-1}), only a moderate amount of $^1O_2$ is generated; the cell death response is abrogated in *flu/ex1* but not in *flu*. At a very low light intensity (1 μmol photons m^{-2} s^{-1}), the amount of $^1O_2$ produced is too low to lead to a cell death response even in *flu* (Fig. 3E).

Since in *flu* a cell death response is triggered by a minor transient increase of $^1O_2$ whereas in *ch1* a cell death response is seen under a higher $^1O_2$ production, $^1O_2$-mediated cell death responses do not seem to be only dose dependent. In *flu* and *ch1*, $^1O_2$ is generated at different sites within the chloroplast, suggesting that the spatial distribution of $^1O_2$ production also influences the reaction of the plant. Because of its high reactivity and short half-life (~4 μs in water), the primary reaction of $^1O_2$ is restricted to a small suborganellar area adjacent to the site of $^1O_2$ generation (Redmond and Kochevar, 2006; Laloi and Havaux, 2015). Spatially resolved $^1O_2$-initiated responses have been proposed to depend on a spatial resolution of the photosensitizer distribution (Redmond and Kochevar, 2006). However, in the case of the EX1-mediated cell death, this response of the *flu* mutant strictly depends on the presence of EX1 within grana margins and not on the distribution of the photosensitizer Pchlide (Wang *et al.*, 2016). EX1 seems to operate as a sensor of minor increases of $^1O_2$ concentrations that amplifies the potential biological activity of $^1O_2$ and reveals its signaling capacity. Thus, it is the location of EX1 but not the spatial distribution of the photosensitizer that defines the site where the primary photo-oxidation by $^1O_2$ and initiation of $^1O_2$-mediated and EX1-dependent signaling occur (Wang *et al.*, 2016).

With the onset of $^1O_2$ formation in the *flu* mutant, there is a rapid decline of the EX1 protein in grana margins that depends on the metalloprotease FtsH (Wang *et al.*, 2016). Hence, the proteolytically modified EX1 or its breakdown products may be expected to be directly involved in $^1O_2$-mediated signaling and to interact with downstream signaling components. In *ch1*, $^1O_2$ formation in grana stacks has been implicated in damaging active PSII and inhibiting photosynthesis (Shumbe *et al.*, 2016). Under these severe high light stress conditions, $^1O_2$ generates non-enzymatically a wide range of oxidation products, some of which may disseminate within the cell and act as a second messenger that triggers stress responses. For instance, oxidation of lipids has been shown to produce multiple reactive derivatives with strong electrophilic properties (Farmer and Mueller, 2013) that may activate redox-sensitive transcription factors. Oxidation of β-carotene by $^1O_2$ in the PSII RC generates various oxidative breakdown products, some of which, such as β-cyclocitral (Ramel *et al.*, 2012a, b) and dihydroactinidolide (Shumbe *et al.*, 2014), are biologically active (Fig. 2A). Intriguingly, these carotenoid oxidation products do not trigger cell death but induce stress defense responses and acclimation.

Spatially resolved responses may be triggered not only by $^1O_2$ generated in grana margins and grana stacks. There are also other sites within the chloroplast that upon stress accumulate tetrapyrroles and release $^1O_2$. The chloroplast envelope contains Pchlide (Pineau *et al.*, 1986). In cotyledons, the import of PORA into plastids depends on Pchlide that interacts with the PORA precursor polypeptide during its uptake (Kim and Apel, 2004). In the *outer plastid envelope protein16-1* (*oep16-1*) mutant, this import of PORA is impaired and free Pchlide

Downloaded from https://academic.oup.com/jxb/article/70/1/29/5110609 by ETH Zürich user on 17 October 2023

starts to accumulate (Samol *et al.*, 2011). Upon light exposure, it generates $^1O_2$ and induces a cell death response that differs from that of the *flu* mutant (Samol *et al.*, 2011). As mentioned above, the *fc2* mutant displays a weak *flu* phenotype when grown under non-permissive dark/light conditions and induces a cell death response that unlike *flu* is not affected by the absence of EX1 (Woodson *et al.*, 2015). As the intraorganellar location of $^1O_2$ formation is expected to influence the biological effects of $^1O_2$, the release of $^1O_2$ at a site other than the grana margin could explain why the $^1O_2$-mediated cell death responses in the *oep16-1* and *fc2* mutants differ from the cell death response of the *flu* mutant.

In addition to the location and the extent of $^1O_2$ formation, the developmental stage at which $^1O_2$ is produced also influences the specificity of $^1O_2$-mediated responses. A development-dependent specification of $^1O_2$-mediated responses was first documented in the *flu* mutant. At the seedling stage, the release of $^1O_2$ induced a rapid bleaching of *flu* seedlings (op den Camp *et al.*, 2003), in more mature *flu* plants the release of $^1O_2$ triggered lesion formation in leaves (Wang *et al.*, 2016), whereas in plants ready to bolt generation of $^1O_2$ led to an immediate cessation of growth (op den Camp *et al.*, 2003; Przybyla *et al.*, 2008). At all three developmental stages, $^1O_2$-mediated responses were dependent on EX1 and were abolished in the *flu/ex1* double mutant (Wagner *et al.*, 2004; Wang *et al.*, 2016). In Arabidopsis wild-type plants, $^1O_2$-mediated and EX-dependent signaling was shown also to be active during late embryogenesis prior to the onset of seed dormancy and to affect plastid development after seed germination (Kim *et al.*, 2009). Etioplasts and chloroplasts of seedlings are derived from undifferentiated progenitors in embryos named proplastids that descend from maternal plastids (Possingham, 1980). During the transition from the morphogenic phase with rapid cell division to the maturation phase of embryogenesis, proplastids may either differentiate into functional photoheterotrophic chloroplasts or remain undifferentiated (Vicente-Carbajosa and Carbonero, 2005). With the onset of seed desiccation, thylakoid membranes of these photoheterotrophic chloroplasts disintegrate and release their Chl. At the same time, $^1O_2$-mediated and EX-dependent signaling is initiated that pre-determines the fate of plastid differentiation by recruiting absisic acid that later on acts as a positive regulator of plastid formation in etiolated and light-grown seedlings. In wild-type seedlings lacking EX1 and EX2, chloroplast development in cotyledons is severely impaired. This is reflected in a reduced Chl and protein content and a much smaller size of chloroplasts in cotyledons of *ex1/ex2* seedlings that resemble undifferentiated proplastids (Kim *et al.*, 2009).

Outlook

As the biological effects of $^1O_2$ are influenced not only by the location and extent of $^1O_2$ production within chloroplasts but also change during plant development, $^1O_2$ is expected to act as a highly versatile and short-lived signal throughout the life cycle of a plant and to give rise to a surprising variety of different signaling pathways. So far little is known about what distinguishes these different signaling pathways from each other. In the *flu* mutant, second-site genetic screens aimed at identifying constituents involved in $^1O_2$-mediated signaling have only led to the discovery of the plastid-localized EX1 protein but failed to identify other signaling components (Wagner *et al.*, 2004; Baruah *et al.*, 2009*a*; Meskauskiene *et al.*, 2009). In the *fc2* mutant, similar second-site mutant screens have led to the identification of the E3 ubiquitin ligase that is required for the breakdown of chloroplasts but is unlikely to take part in cell death-inducing signaling (Woodson *et al.*, 2015). In *ch1*, OXI1 acts as an enhancer of high-light-induced cell death rather than being an obligatory component of a $^1O_2$-dependent cell death-inducing signaling pathway (Shumbe *et al.*, 2016). These results suggest that the proposed $^1O_2$-dependent signals are not transferred to the nucleus via a single linear signaling pathway that can easily be blocked genetically but rather through a more complex signaling network that is difficult to analyze by introducing only single gene mutations.

Unlike the *flu*, *fc2*, and *ch1* mutant plants, in wild-type plants $^1O_2$-mediated signaling does not operate alone but interacts with other signaling pathways that converge with $^1O_2$-dependent signaling and delay or modify some of the $^1O_2$-mediated responses seen in the three mutant lines (Baruah *et al.*, 2009*b*; Kim and Apel, 2013). For instance, perturbations of cellular homeostasis prior to $^1O_2$ production confer an enhanced stress resistance by activating acclimation that suppresses $^1O_2$-mediated cell death responses without blocking $^1O_2$-mediated expression changes of $^1O_2$-responsive genes (Baruah *et al.*, 2009*b*; Coll *et al.*, 2009; Meskauskiene *et al.*, 2009; Šimková *et al.*, 2012). $^1O_2$ itself may also modify consequences of higher $^1O_2$ concentrations through an autoregulatory feedback control that induces acclimation (Ledford *et al.*, 2007; Kim and Apel, 2013; Shumbe *et al.*, 2016). Finally, hydrogen peroxide may antagonize $^1O_2$-mediated signaling. Overexpression of a thylakoid-bound ascorbate peroxidase (tAPX, an H_2O_2 scavenger) in the *flu* mutant increases the expression of most of the $^1O_2$-induced genes and enhances the $^1O_2$-mediated cell death and growth inhibition phenotypes compared with the *flu* parental line (Laloi *et al.*, 2007). Other recent examples of $^1O_2$-mediated signaling in wild-type plants have indicated that it is not only restricted to light stress, but may also occur during wounding, pathogen attack, senescence, and drought stress (Mur *et al.*, 2010; Vellosillo *et al.*, 2010; Alboresi *et al.*, 2011; Nomura *et al.*, 2012; González-Pérez *et al.*, 2011; Gutiérrez *et al.*, 2014; Mor *et al.*, 2014; Uberegui *et al.*, 2015). $^1O_2$ that triggers these responses may not only be generated photochemically in chloroplasts, but in some cases has been suggested to be formed metabolically in the absence of light and to emanate also from other subcellular compartments (Mor *et al.*, 2014; Noctor and Foyer, 2016; Foyer *et al.*, 2017). Collectively, these data emphasize the complexity of signaling events that must be dissected before the biological significance of $^1O_2$-mediated signaling in wild-type plants can be fully understood.

Acknowledgements

During the revision of this manuscript, my excellent supervisor, and dear friend, Professor Klaus Apel (18 November 1942 to 30 June 2017) left us forever. Professor Apel was a pioneer of photosynthesis research and an authority on plant molecular genetics. He pioneered research on singlet oxygen and proved that besides its cytotoxicity singlet oxygen could

Downloaded from https://academic.oup.com/jxb/article/70/1/29/5110609 by ETH Zürich user on 17 October 2023

also be a signal. This work was supported by the National Institutes of Health Grant R01-GM085036. I thank Dr Tatjana Kleine and Dr Belén Naranjo for careful reading and advice on modifying this manuscript. I apologize to those authors whose manuscripts are not cited in this review due to limited space.

References

Alboresi A, Dall'Osto L, Aprile A, Carillo P, Roncaglia E, Cattivelli L, Bassi R. 2011. Reactive oxygen species and transcript analysis upon excess light treatment in wild-type *Arabidopsis thaliana* vs a photosensitive mutant lacking zeaxanthin and lutein. BMC Plant Biology **11,** 62.

Apel K, Hirt H. 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annual Review of Plant Biology **55,** 373–399.

Apel K, Santel HJ, Redlinger TE, Falk H. 1980. The protochlorophyllide holochrome of barley (*Hordeum vulgare* L.). Isolation and characterization of the NADPH:protochlorophyllide oxidoreductase. European Journal of Biochemistry **111,** 251–258.

Aro EM, Virgin I, Andersson B. 1993. Photoinhibition of photosystem II. Inactivation, protein damage and turnover. Biochimica et Biophysica Acta **1143,** 113–134.

Bailey S, Thompson E, Nixon PJ, Horton P, Mullineaux CW, Robinson C, Mann NH. 2002. A critical role for the Var2 FtsH homologue of *Arabidopsis thaliana* in the photosystem II repair cycle in vivo. Journal of Biological Chemistry **277,** 2006–2011.

Baruah A, Simková K, Apel K, Laloi C. 2009*a*. Arabidopsis mutants reveal multiple singlet oxygen signaling pathways involved in stress response and development. Plant Molecular Biology **70,** 547–563.

Baruah A, Simková K, Hincha DK, Apel K, Laloi C. 2009*b*. Modulation of O-mediated retrograde signaling by the PLEIOTROPIC RESPONSE LOCUS 1 (PRL1) protein, a central integrator of stress and energy signaling. The Plant Journal **60,** 22–32.

Becerril JM, Duke SO. 1989. Protoporphyrin IX content correlates with activity of photobleaching herbicides. Plant Physiology **90,** 1175–1181.

Coll NS, Danon A, Meurer J, Cho WK, Apel K. 2009. Characterization of soldat8, a suppressor of singlet oxygen-induced cell death in Arabidopsis seedlings. Plant & Cell Physiology **50,** 707–718.

Cornah JE, Terry MJ, Smith AG. 2003. Green or red: what stops the traffic in the tetrapyrrole pathway? Trends in Plant Science **8,** 224–230.

Dall'Osto L, Cazzaniga S, Havaux M, Bassi R. 2010. Enhanced photoprotection by protein-bound vs free xanthophyll pools: a comparative analysis of chlorophyll b and xanthophyll biosynthesis mutants. Molecular Plant **3,** 576–593.

Danon A, Miersch O, Felix G, Camp RG, Apel K. 2005. Concurrent activation of cell death-regulating signaling pathways by singlet oxygen in *Arabidopsis thaliana*. The Plant Journal **41,** 68–80.

Davies MJ. 2003. Singlet oxygen-mediated damage to proteins and its consequences. Biochemical and Biophysical Research Communications **305,** 761–770.

Dogra V, Duan J, Lee KP, Lv S, Liu R, Kim C. 2017. FtsH2-dependent proteolysis of EXECUTER1 is essential in mediating singlet oxygen-triggered retrograde signaling in *Arabidopsis thaliana*. Frontiers in Plant Science **8,** 1145.

Dogra V, Rochaix JD, Kim C. 2018. Singlet oxygen-triggered chloroplast-to-nucleus retrograde signalling pathways: an emerging perspective. Plant, Cell & Environment **41,** 1727–1738.

Elstner EF. 1982. Oxygen activation and oxygen-toxicity. Annual Review of Plant Physiology and Plant Molecular Biology **33,** 73–96.

Farmer EE, Mueller MJ. 2013. ROS-mediated lipid peroxidation and RES-activated signaling. Annual Review of Plant Biology **64,** 429–450.

Feierabend J, Dehne S. 1996. Fate of the porphyrin cofactors during the light-dependent turnover of catalase and of the photosystem II reaction-center protein D1 in mature rye leaves. Planta **198,** 413–422.

Fischer BB, Hideg É, Krieger-Liszkay A. 2013. Production, detection, and signaling of singlet oxygen in photosynthetic organisms. Antioxidants & Redox Signaling **18,** 2145–2162.

Foyer CH, Noctor G. 2009. Redox regulation in photosynthetic organisms: signaling, acclimation, and practical implications. Antioxidants & Redox Signaling **11,** 861–905.

Foyer CH, Ruban AV, Noctor G. 2017. Viewing oxidative stress through the lens of oxidative signalling rather than damage. Biochemical Journal **474,** 877–883.

Gadjev I, Vanderauwera S, Gechev TS, Laloi C, Minkov IN, Shulaev V, Apel K, Inzé D, Mittler R, Van Breusegem F. 2006. Transcriptomic footprints disclose specificity of reactive oxygen species signaling in Arabidopsis. Plant Physiology **141,** 436–445.

González-Pérez S, Gutiérrez J, García-García F, Osuna D, Dopazo J, Lorenzo Ó, Revuelta JL, Arellano JB. 2011. Early transcriptional defense responses in Arabidopsis cell suspension culture under high-light conditions. Plant Physiology **156,** 1439–1456.

Goslings D, Meskauskiene R, Kim C, Lee KP, Nater M, Apel K. 2004. Concurrent interactions of heme and FLU with Glu tRNA reductase (HEMA1), the target of metabolic feedback inhibition of tetrapyrrole biosynthesis, in dark- and light-grown Arabidopsis plants. The Plant Journal **40,** 957–967.

Griffiths WT. 1978. Reconstitution of chlorophyllide formation by isolated etioplast membranes. Biochemical Journal **174,** 681–692.

Gutiérrez J, González-Pérez S, García-García F, Daly CT, Lorenzo O, Revuelta JL, McCabe PF, Arellano JB. 2014. Programmed cell death activated by Rose Bengal in *Arabidopsis thaliana* cell suspension cultures requires functional chloroplasts. Journal of Experimental Botany **65,** 3081–3095.

Halliwell B, Gutteridge JMC. 1999. Free radicals in biology and medicine. New York: Oxford University Press.

Kale R, Hebert AE, Frankel LK, Sallans L, Bricker TM, Pospisil P. 2017. Amino acid oxidation of the D1 and D2 proteins by oxygen radicals during photoinhibition of photosystem II. Proceedings of the National Academy of Sciences, USA **114,** 2988–2993.

Kanofsky JR, Axelrod B. 1986. Singlet oxygen production by soybean lipoxygenase isozymes. Journal of Biological Chemistry **261,** 1099–1104.

Kato Y, Miura E, Ido K, Ifuku K, Sakamoto W. 2009. The variegated mutants lacking chloroplastic FtsHs are defective in D1 degradation and accumulate reactive oxygen species. Plant Physiology **151,** 1790–1801.

Keren N, Berg A, VanKan PJM, Levanon H, Ohad I. 1997. Mechanism of photosystem II photoinactivation and D1 protein degradation at low light: the role of back electron flow. Proceedings of the National Academy of Sciences, USA **94,** 1579–1584.

Khan AU, Kasha M. 1994. Singlet molecular-oxygen in the Haber–Weiss reaction. Proceedings of the National Academy of Sciences, USA **91,** 12365–12367.

Khatoon M, Inagawa K, Pospísil P, *et al.* 2009. Quality control of photosystem II: thylakoid unstacking is necessary to avoid further damage to the D1 protein and to facilitate D1 degradation under light stress in spinach thylakoids. Journal of Biological Chemistry **284,** 25343–25352.

Kim C, Apel K. 2004. Substrate-dependent and organ-specific chloroplast protein import in planta. The Plant Cell **16,** 88–98.

Kim C, Apel K. 2013. Singlet oxygen-mediated signaling in plants: moving from flu to wild type reveals an increasing complexity. Photosynthesis Research **116,** 455–464.

Kim C, Lee KP, Baruah A, Nater M, Gobel C, Feussner I, Apel K. 2009. $^1O_2$-mediated retrograde signaling during late embryogenesis predetermines plastid differentiation in seedlings by recruiting abscisic acid. Proceedings of the National Academy of Sciences, USA **106,** 9920–9924.

Kim C, Meskauskiene R, Apel K, Laloi C. 2008. No single way to understand singlet oxygen signalling in plants. EMBO Reports **9,** 435–439.

Kim C, Meskauskiene R, Zhang S, Lee KP, Lakshmanan Ashok M, Blajecka K, Herrfurth C, Feussner I, Apel K. 2012. Chloroplasts of Arabidopsis are the source and a primary target of a plant-specific programmed cell death signaling pathway. The Plant Cell **24,** 3026–3039.

Krieger-Liszkay A. 2005. Singlet oxygen production in photosynthesis. Journal of Experimental Botany **56,** 337–346.

Krieger-Liszkay A, Fufezan C, Trebst A. 2008. Singlet oxygen production in photosystem II and related protection mechanism. Photosynthesis Research **98,** 551–564.

Laloi C, Havaux M. 2015. Key players of singlet oxygen-induced cell death in plants. Frontiers in Plant Science **6,** 39.

Laloi C, Stachowiak M, Pers-Kamczyc E, Warzych E, Murgia I, Apel K. 2007. Cross-talk between singlet oxygen- and hydrogen peroxide-dependent signaling of stress responses in *Arabidopsis thaliana*. Proceedings of the National Academy of Sciences, USA **104,** 672–677.

Downloaded from https://academic.oup.com/jxb/article/70/1/29/5110609 by ETH Zürich user on 17 October 2023

Ledford HK, Chin BL, Niyogi KK. 2007. Acclimation to singlet oxygen stress in *Chlamydomonas reinhardtii*. Eukaryotic Cell **6,** 919–930.

Lee KP, Kim C, Landgraf F, Apel K. 2007. EXECUTER1- and EXECUTER2-dependent transfer of stress-related signals from the plastid to the nucleus of *Arabidopsis thaliana*. Proceedings of the National Academy of Sciences, USA **104,** 10270–10275.

Levicán G, Katz A, de Armas M, Núñez H, Orellana O. 2007. Regulation of a glutamyl-tRNA synthetase by the heme status. Proceedings of the National Academy of Sciences, USA **104,** 3135–3140.

Li Z, Wakao S, Fischer BB, Niyogi KK. 2009. Sensing and responding to excess light. Annual Review of Plant Biology **60,** 239–260.

Lindahl M, Spetea C, Hundal T, Oppenheim AB, Adam Z, Andersson B. 2000. The thylakoid FtsH protease plays a role in the light-induced turnover of the photosystem II D1 protein. The Plant Cell **12,** 419–431.

Meskauskiene R, Nater M, Goslings D, Kessler F, den Camp RO, Apel K. 2001. FLU: a negative regulator of chlorophyll biosynthesis in *Arabidopsis thaliana*. Proceedings of the National Academy of Sciences, USA **98,** 12826–12831.

Meskauskiene R, Würsch M, Laloi C, Vidi PA, Coll NS, Kessler F, Baruah A, Kim C, Apel K. 2009. A mutation in the Arabidopsis mTERF-related plastid protein SOLDAT10 activates retrograde signaling and suppresses $^1O_2$-induced cell death. The Plant Journal **60,** 399–410.

Mochizuki N, Tanaka R, Grimm B, Masuda T, Moulin M, Smith AG, Tanaka A, Terry MJ. 2010. The cell biology of tetrapyrroles: a life and death struggle. Trends in Plant Science **15,** 488–498.

Mor A, Koh E, Weiner L, Rosenwasser S, Sibony-Benyamini H, Fluhr R. 2014. Singlet oxygen signatures are detected independent of light or chloroplasts in response to multiple stresses. Plant Physiology **165,** 249–261.

Müller B, Eichacker LA. 1999. Assembly of the D1 precursor in monomeric photosystem II reaction center precomplexes precedes chlorophyll a-triggered accumulation of reaction center II in barley etioplasts. The Plant Cell **11,** 2365–2377.

Mullet JE, Klein PG, Klein RR. 1990. Chlorophyll regulates accumulation of the plastid-encoded chlorophyll apoproteins CP43 and D1 by increasing apoprotein stability. Proceedings of the National Academy of Sciences, USA **87,** 4038–4042.

Mur LA, Aubry S, Mondhe M, *et al.* 2010. Accumulation of chlorophyll catabolites photosensitizes the hypersensitive response elicited by *Pseudomonas syringae* in Arabidopsis. New Phytologist **188,** 161–174.

Noctor G, Foyer CH. 2016. Intracellular redox compartmentation and ROS-related communication in regulation and signaling. Plant Physiology **171,** 1581–1592.

Noctor G, Reichheld JP, Foyer CH. 2018. ROS-related redox regulation and signaling in plants. Seminars in Cell & Developmental Biology **80,** 3–12.

Nomura H, Komori T, Uemura S, *et al.* 2012. Chloroplast-mediated activation of plant immune signalling in Arabidopsis. Nature Communications **3,** 926.

Ohad I, Berg A, Berkowicz SM, Kaplan A, Keren N. 2011. Photoinactivation of photosystem II: is there more than one way to skin a cat? Physiologia Plantarum **142,** 79–86.

op den Camp RG, Przybyla D, Ochsenbein C, *et al.***.**2003. Rapid induction of distinct stress responses after the release of singlet oxygen in Arabidopsis. The Plant Cell **15,** 2320–2332.

Papenbrock J, Mishra S, Mock HP, Kruse E, Schmidt EK, Petersmann A, Braun HP, Grimm B. 2001. Impaired expression of the plastidic ferrochelatase by antisense RNA synthesis leads to a necrotic phenotype of transformed tobacco plants. The Plant Journal **28,** 41–50.

Pineau B, Dubertret G, Joyard J, Douce R. 1986. Fluorescence properties of the envelope membranes from spinach chloroplasts. Detection of protochlorophyllide. Journal of Biological Chemistry **261,** 9210–9215.

Possingham JV. 1980. Plastid replication and development in the life cycle of higher plants. Annual Review of Plant Physiology and Plant Molecular Biology **31,** 113–129.

Przybyla D, Göbel C, Imboden A, Hamberg M, Feussner I, Apel K. 2008. Enzymatic, but not non-enzymatic, $^1O_2$-mediated peroxidation of polyunsaturated fatty acids forms part of the EXECUTER1-dependent stress response program in the flu mutant of *Arabidopsis thaliana*. The Plant Journal **54,** 236–248.

Puthiyaveetil S, Tsabari O, Lowry T, Lenhert S, Lewis RR, Reich Z, Kirchhoff H. 2014. Compartmentalization of the protein repair machinery in photosynthetic membranes. Proceedings of the National Academy of Sciences, USA **111,** 15839–15844.

Ramel F, Birtic S, Cuiné S, Triantaphylidès C, Ravanat JL, Havaux M. 2012*a*. Chemical quenching of singlet oxygen by carotenoids in plants. Plant Physiology **158,** 1267–1278.

Ramel F, Birtic S, Ginies C, Soubigou-Taconnat L, Triantaphylides C, Havaux M. 2012*b*. Carotenoid oxidation products are stress signals that mediate gene responses to singlet oxygen in plants. Proceedings of the National Academy of Sciences, USA **109,** 5535–5540.

Ramel F, Ksas B, Akkari E, Mialoundama AS, Monnet F, Krieger-Liszkay A, Ravanat JL, Mueller MJ, Bouvier F, Havaux M. 2013. Light-induced acclimation of the Arabidopsis chlorina1 mutant to singlet oxygen. The Plant Cell **25,** 1445–1462.

Redmond RW, Kochevar IE. 2006. Spatially resolved cellular responses to singlet oxygen. Photochemistry and Photobiology **82,** 1178–1186.

Rentel MC, Lecourieux D, Ouaked F, *et al.* 2004. OXI1 kinase is necessary for oxidative burst-mediated signalling in Arabidopsis. Nature **427,** 858–861.

Samol I, Buhr F, Springer A, Pollmann S, Lahroussi A, Rossig C, von Wettstein D, Reinbothe C, Reinbothe S. 2011. Implication of the oep16-1 mutation in a flu-independent, singlet oxygen-regulated cell death pathway in *Arabidopsis thaliana*. Plant & Cell Physiology **52,** 84–95.

Sandmann G, Boger P. 1988. Accumulation of protoporphyrin-IX in the presence of peroxidizing herbicides. Zeitschrift für Naturforschung C **43,** 699–704.

Scharfenberg M, Mittermayr L, Von Roepenack-Lahaye E, Schlicke H, Grimm B, Leister D, Kleine T. 2015. Functional characterization of the two ferrochelatases in *Arabidopsis thaliana*. Plant, Cell & Environment **38,** 280–298.

Shumbe L, Bott R, Havaux M. 2014. Dihydroactinidiolide, a high light-induced β-carotene derivative that can regulate gene expression and photoacclimation in Arabidopsis. Molecular Plant **7,** 1248–1251.

Shumbe L, Chevalier A, Legeret B, Taconnat L, Monnet F, Havaux M. 2016. Singlet oxygen-induced cell death in Arabidopsis under high-light stress is controlled by OXI1 kinase. Plant Physiology **170,** 1757–1771.

Sies H, Menck CF. 1992. Singlet oxygen induced DNA damage. Mutation Research **275,** 367–375.

Šimková K, Kim C, Gacek K, Baruah A, Laloi C, Apel K. 2012. The chloroplast division mutant caa33 of *Arabidopsis thaliana* reveals the crucial impact of chloroplast homeostasis on stress acclimation and retrograde plastid-to-nucleus signaling. The Plant Journal **69,** 701–712.

Steinbeck MJ, Khan AU, Karnovsky MJ. 1992. Intracellular singlet oxygen generation by phagocytosing neutrophils in response to particles coated with a chemical trap. Journal of Biological Chemistry **267,** 13425–13433.

Szilárd A, Sass L, Hideg E, Vass I. 2005. Photoinactivation of photosystem II by flashing light. Photosynthesis Research **84,** 15–20.

Tanaka R, Tanaka A. 2007. Tetrapyrrole biosynthesis in higher plants. Annual Review of Plant Biology **58,** 321–346.

Trebst A. 1999. Singlet oxygen in photosynthesis. In: Dornisch ADaK, ed. Different pathways through life: biochemical aspects of plant biology and medicine. Munich, Germany: Lincom Europa, 125–142.

Triantaphylidès C, Havaux M. 2009. Singlet oxygen in plants: production, detoxification and signaling. Trends in Plant Science **14,** 219–228.

Triantaphylidès C, Krischke M, Hoeberichts FA, Ksas B, Gresser G, Havaux M, Van Breusegem F, Mueller MJ. 2008. Singlet oxygen is the major reactive oxygen species involved in photooxidative damage to plants. Plant Physiology **148,** 960–968.

Uberegui E, Hall M, Lorenzo Ó, Schröder WP, Balsera M. 2015. An Arabidopsis soluble chloroplast proteomic analysis reveals the participation of the executer pathway in response to increased light conditions. Journal of Experimental Botany **66,** 2067–2077.

Umena Y, Kawakami K, Shen JR, Kamiya N. 2011. Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9 Å. Nature **473,** 55–60.

Vass I. 2012. Molecular mechanisms of photodamage in the photosystem II complex. Biochimica et Biophysica Acta **1817,** 209–217.

Downloaded from https://academic.oup.com/jxb/article/70/1/29/5110609 by ETH Zürich user on 17 October 2023

Vellosillo T, Vicente J, Kulasekaran S, Hamberg M, Castresana C. 2010. Emerging complexity in reactive oxygen species production and signaling during the response of plants to pathogens. Plant Physiology **154,** 444–448.

Vicente-Carbajosa J, Carbonero P. 2005. Seed maturation: developing an intrusive phase to accomplish a quiescent state. International Journal of Developmental Biology **49,** 645–651.

Vothknecht UC, Kannangara CG, von Wettstein D. 1998. Barley glutamyl tRNAGlu reductase: mutations affecting haem inhibition and enzyme activity. Phytochemistry **47,** 513–519.

Wagner D, Przybyla D, Op den Camp R, *et al.* 2004. The genetic basis of singlet oxygen-induced stress responses of *Arabidopsis thaliana*. Science **306,** 1183–1185.

Wang L, Apel K. 2016. Singlet oxygen in higher plants. In: Santi Nonell CF, ed. Singlet oxygen: applications in biosciences and nanosciences. London: Royal Society of Chemistry.

Wang L, Kim C, Xu X, Piskurewicz U, Dogra V, Singh S, Mahler H, Apel K. 2016. Singlet oxygen- and EXECUTER1-mediated signaling is initiated in grana margins and depends on the protease FtsH2. Proceedings of the National Academy of Sciences, USA **113,** E3792–E3800.

Watanabe N, Che FS, Iwano M, Takayama S, Nakano T, Yoshida S, Isogai A. 1998. Molecular characterization of photomixotrophic tobacco cells resistant to protoporphyrinogen oxidase-inhibiting herbicides. Plant Physiology **118,** 751–758.

Woodson JD, Joens MS, Sinson AB, Gilkerson J, Salomé PA, Weigel D, Fitzpatrick JA, Chory J. 2015. Ubiquitin facilitates a quality-control pathway that removes damaged chloroplasts. Science **350,** 450–454.

Yoshioka-Nishimura M, Nanba D, Takaki T, Ohba C, Tsumura N, Morita N, Sakamoto H, Murata K, Yamamoto Y. 2014. Quality control of photosystem II: direct imaging of the changes in the thylakoid structure and distribution of FtsH proteases in spinach chloroplasts under light stress. Plant & Cell Physiology **55,** 1255–1265.

Downloaded from https://academic.oup.com/jxb/article/70/1/29/5110609 by ETH Zürich user on 17 October 2023