

Commentary

Alternative electron pathways in photosynthesis: strength in numbers

Electrons supplied by photosystem I (PSI) can have various fates, serving to regenerate NADPH during linear electron flow (LEF), or entering any of several minor ‘alternative electron pathways’ (AEPs). AEPs are thought to play a role in the regulation of photosynthesis and the alleviation of PSI photoinhibition (reviewed in Allahverdiyeva *et al.*, 2015; Yamori & Shikanai, 2016; Alboresi *et al.*, 2019). Several AEPs have been described, including two modes of cyclic electron flow (CEF), one involving the NADH dehydrogenase-like (NDH) complex (NDH-CEF), the other employing the two proteins PGR5 and PGRL1 (PGR5-CEF) (Fig. 1). Pseudo-cyclic electron flow (PCEF) results in the reduction of oxygen to water – by the Mehler reaction (Mehler-

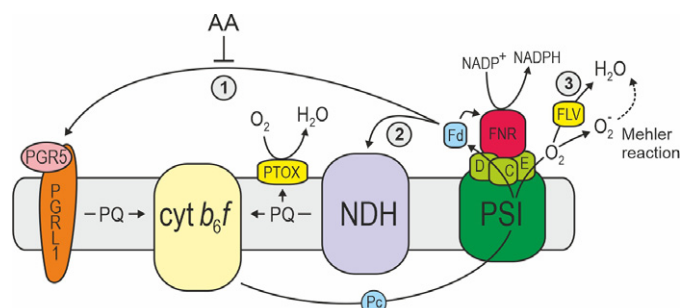


Fig. 1 A schematic representation of alternative electron pathways involving photosystem I (PSI) in plants. Linear electron flow (LEF) involves photosystem II (PSII), plastoquinone (PQ), the cytochrome b_6/f complex (cyt b_6/f) and PSI, and results in the production of ATP and NADPH. Here, the part starting from PQ and ending with the transfer of electrons from ferredoxin (Fd) to NADP⁺ catalysed by Fd-NADP⁺ reductase (FNR) is shown. Cyclic electron flow (CEF) operates via two partially redundant pathways, a major one that is PGR5/PGRL1-dependent and antimycin A (AA)-sensitive (1) and a minor one involving a NADH dehydrogenase-like (NDH) complex that is AA-insensitive (2). Fd serves as the electron transporter during LEF and CEF, but not in pseudo-cyclic electron flow (PCEF), which involves direct transfer from PSI to oxygen. Flavodiiron proteins (FLVs) mediate direct reduction of O₂ to water (3), while reactive oxygen species (ROS) are produced during the Mehler reaction, which are then scavenged by superoxide dismutase and ascorbate peroxidase, ultimately leading to the generation of water. Plastid terminal oxidase (PTOX) accepts electrons from PQ and transfers them to O₂ to produce water. Thioredoxins and hydrogenases (in some algae) also receive electrons from PSI (not shown). If PSI receives more electrons from its donor side than it can distribute on its acceptor side, electrons accumulate at the stromal side of PSI and damage its iron–sulphur clusters.

PCEF) during which reactive oxygen species (ROS) are generated and scavenged (reviewed in Leister, 2019), or by the direct reduction of oxygen to water catalysed by flavodiiron proteins (FLVs) (FLV-PCEF) (reviewed in Alboresi *et al.*, 2019) (Fig. 1). These four AEPs are not uniformly distributed across all phylogenetic groups of photosynthetic organisms, but the moss *Physcomitrella patens* harbours all of them, and is, therefore, ideally suited for investigations of their functional overlaps. In this issue of *New Phytologist*, Storti *et al.* (2020; pp. 1316–1326) have extended their previous study on AEPs in *P. patens* and combined and analysed mutations in three AEPs – the two CEF pathways and FLV-PCEF. They found that *P. patens* tolerates the simultaneous loss of both CEF pathways, but becomes severely impaired even under very low light intensities if FLV-PCEF is inactivated as well. This demonstrates that any one of the three AEPs is dispensable, but each one makes a contribution to the maintenance of PSI function even under nonstressful conditions.

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CEF does not regenerate NADPH and reinjects electrons from PSI via ferredoxin into the plastoquinone (PQ) pool, thereby bypassing photosystem II (PSII) and generating a proton gradient to synthesize ATP and to downregulate LEF at the site of PSII. In plants with C₃ photosynthesis, PGR5-CEF is the major CEF pathway (Munekage *et al.*, 2004) and NDH-CEF the minor one; loss of the latter has minimal effects on growth and acclimation (reviewed in Shikanai & Yamamoto, 2017). The two CEFs are found in all phylogenetic groups of organisms with oxygenic photosynthesis, albeit not in all families (reviewed in Alboresi *et al.*, 2019), and with some variation in protein composition. For instance, plant PGR5-CEF can be rebuilt in cyanobacteria, although cyanobacteria lack a true PGRL1 homologue (Dann & Leister, 2019). Another example is *Chlamydomonas reinhardtii*, which lacks a plant-like NDH complex and appears to utilize a special supercomplex-based CEF pathway (reviewed in Alboresi *et al.*, 2019). The question whether PGR5 and PGRL1 primarily serve as stromal electron acceptors in CEF or are modulators of LEF makes little sense semantically, because the latter results from the former. In fact, inactivation of PGR5 (or PGRL1) reduces ATP production and therefore depresses the ATP : NADPH ratio, which results in over reduction of the stroma. The relative lack of acceptors

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for electrons from PSI (PSI acceptor side limitation) in turn leads to the reduction of PSI and to photoinhibition (reviewed in Yamori & Shikanai, 2016). Additionally, lack of PGR5 results in lower levels of lumen acidification, proton motive force and nonphotochemical quenching (NPQ) (also because of high ATPase conductivity due to stromal ATP depletion), which in turn prevents downregulation of LEF activity at the site of Cyt b_6/f and PSII. Therefore, other mutations affecting the PSI oxidation state can exacerbate the phenotype of PRG5 mutants (DalCorso *et al.*, 2008). In consequence, PGR5-CEF provides both electron-sink capacity by adjusting the ATP/NADPH production ratio (acceptor-side regulation) and down-regulation of Cyt b_6/f activity for slowing down the electron flow toward PSI (donor-side regulation) (Yamamoto & Shikanai, 2019).

PCEF is also called the 'water–water cycle' because electrons generated from water by PSII are used in this pathway to reduce oxygen to water. This reaction produces a proton gradient and therefore ATP, but (in contrast to CEF) here the electrons re-form water. The Mehler-PCEF pathway is found in all oxygenic photosynthetic organisms from cyanobacteria to angiosperms, and comes into play when PSI directly oxidizes O_2 to form O_2^- , which is then converted first into hydrogen peroxide (H_2O_2) and finally to O_2 and water by superoxide dismutase and ascorbate peroxidase. While Mehler-PCEF makes a limited contribution to electron flow, FLV-PCEF represents a larger electron sink (reviewed in Alboresi *et al.*, 2019) but has been lost in angiosperms during evolution. It has been speculated that FLV-PCEF might be constitutively active, and because it is energetically less favourable than CEF, this could have set up a trade-off (e.g. under low light conditions) that facilitated FLV loss during evolution (reviewed in Alboresi *et al.*, 2019).

Storti *et al.* generated mutant lines of *P. patens* in which only one of the three more significant AEPs (i.e. both CEF pathways and FLC-PCEF) was active. Taking the reduction of the PSI : PSII ratio as a measure of PSI damage, plants with PGR5-CEF performed better than plants with NDH-CEF, which in turn performed better than plants in which only FLV-PCEF was functional. Lines deprived of all three AEPs performed worst and suffered marked PSI damage.

How do these findings relate to previous analyses done in other photosynthetic species? In fact, redundancy of AEPs has been also demonstrated by mutant analyses in the flowering plant *Arabidopsis thaliana*, which naturally lacks FLV-PCEF but has retained both types of CEF. Under steady-state photosynthesis conditions, *A. thaliana* plants lacking either of the two CEF pathways are only affected to a minor extent, if at all, in photosynthesis and growth. If both pathways are inactivated in *A. thaliana* – a situation that corresponds to that of the *P. patens* lines used by Storti *et al.* that lack all three AEPs – photosynthesis and growth are strongly affected (Munekage *et al.*, 2004). Interestingly, inactivation of the plastid terminal oxidase (PTOX) that accepts electrons from plastoquinone and transfers them to oxygen to produce water – in effect similar to the water–water cycle but occurring 'upstream' of PSI – alleviates the growth phenotype of CEF-less *A. thaliana* lines (Okegawa *et al.*, 2010), indicating that other AEPs acting upstream of the donor side of PSI also need to be considered to fully

understand the complex interplay of the different routes of thylakoid electron transport pathways.

Under fluctuating light conditions in which acclimation responses are triggered, the function of AEPs becomes crucial, as revealed by the strong phenotypic effects observed when individual AEPs are inactivated. Thus, *A. thaliana* seedlings without PGR5 die, whereas plants lacking a functional NDH complex survive (Suorsa *et al.*, 2012). Of the *P. patens* lines employed by Storti *et al.*, those with only FLV-PCEF left performed better under fluctuating light than those that retained either of the two CEF pathways, indicating that, in this moss species, FLV-PCEF is the AEP that is most relevant for avoiding acceptor-side limitation during acclimation. As in *A. thaliana*, under all other nonfluctuating light conditions the PGR5-CEF pathway emerged as the functionally most significant of the three AEPs studied in *P. patens*. Again, the activity of LEF needs to be considered when studying the impact of AEPs on acclimation, because defects in PSII or Cyt b_6/f can alleviate the strong phenotypic effects of fluctuating light on Arabidopsis plants that lack PGR5-CEF (Suorsa *et al.*, 2016; Yamamoto & Shikanai, 2019).

Mimicking the *P. patens* AEP repertoire in *A. thaliana* by expressing FLVs in this species showed that FLVs can partially replace PGR5-CEF under fluctuating light (Yamamoto *et al.*, 2016; Shikanai & Yamamoto, 2017), implying that each of these pathways is capable of protecting PSI when light levels are varied. However, FLV-PCEF cannot compete with PGR5-CEF during steady-state photosynthesis, but it can function as an efficient electron sink during the induction of photosynthesis after overnight dark adaptation (Yamamoto *et al.*, 2016). When FLVs were introduced into rice, they could substitute for PGR5- and NDH-dependent CEF without any decrease in CO_2 assimilation or biomass production (Wada *et al.*, 2018), which argues against an energy trade-off with FLV-PCEF, at least in this instance. In consequence, the studies in flowering plants, and the analysis in *P. patens* reported by Storti *et al.* in *New Phytologist*, show that AEPs display a high level of redundancy, as well as complex interactions that extend to LEF and chlororespiration (PTOX). In contrast to PSII, which is constantly subject to damage by excess light energy and repaired, PSI normally is very stable, even under very high light intensities, as long as it does not become reduced too much. But if this happens, PSI damage is largely irreversible, owing to very slow turnover and inefficient repair (reviewed in Allahverdiyeva *et al.*, 2015). Some AEPs, and certainly AEPs as a whole, are essential for protecting PSI against photodamage by avoiding acceptor-side limitation, in particular under fluctuating light conditions.

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