Back-reactions, short-circuits, leaks and other energy wasteful reactions in biological electron transfer: Redox tuning to survive life in \( \text{O}_2 \)

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Abstract

The energy-converting redox enzymes perform productive reactions efficiently despite the involvement of high energy intermediates in their catalytic cycles. This is achieved by kinetic control: with forward reactions being faster than competing, energy-wasteful reactions. This requires appropriate cofactor spacing, driving forces and reorganizational energies. These features evolved in ancestral enzymes in a low \( \text{O}_2 \) environment. When \( \text{O}_2 \) appeared, energy-converting enzymes had to deal with its troublesome chemistry. Various protective mechanisms duly evolved that are not directly related to the enzymes’ principal redox roles. These protective mechanisms involve fine-tuning of reduction potentials, switching of pathways and the use of short circuits, back-reactions and side-paths, all of which compromise efficiency. This energetic loss is worth it since it minimises damage from reactive derivatives of \( \text{O}_2 \) and thus gives the organism a better chance of survival. We examine photosynthetic reaction centres, \( b_\text{c} \), and \( b_\text{f} \) complexes from this viewpoint. In particular, the evolution of the heterodimeric PSI from its homodimeric ancestors is explained as providing a protective back-reaction pathway. This “sacrifice-of-efficiency-for-protection” concept should be generally applicable to bioenergetic enzymes in aerobic environments.

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1. Introduction

Biological energy conversion mainly operates through membrane-spanning enzymes that build up a transmembrane electrochemical potential using the driving force provided by exergonic reactions \([1]\). As with all enzymes, those involved in energy conversion act as catalysts. Thus, even though the reaction pathways may involve a series of intermediates, completion of the enzyme cycle brings the enzyme back to its ground state. As energy-converting devices, their reaction pathways involve high-energy intermediates, which react exergonically providing the work required to drive the accumulation of the electrochemical potential (see Fig. 1).

These two basic characteristics pinpoint one of the main challenges encountered by these enzymes: how to favour the energy-productive processes over competing reactions in which the high energy intermediates decay without going through the useful energy converting step(s). In other words, to be efficient they must avoid back-reactions, short-circuits, by-passes, side-reactions, futile cycles and leaks (Fig. 1).

Owing to the thermodynamic reversibility of the forward, productive reactions, intermediates may simply decay by back-reacting to the ground state of the enzyme via the thermally-activated repopulation of higher energy states (Fig. 1). The transiently-stored energy would thus be released without any work being extracted from the overall process.

Productive energy conversion can be lost by processes other than simple back-reactions (Fig. 1). Such processes could involve charge recombination from a high energy state directly to the ground state without retracing the steps of forward electron transfer. Equally they could involve the formation of a lower energy form of the same component, such as a change in the protonation state or a secondary change in the redox state, reactions common in quinone chemistry. These may be termed “short-circuits” or “by-passes” depending on rates, routes, distances and semantics. In other cases, the lower energy intermediates may involve components different from those involved in the productive pathway. Such routes can be called “side pathways”, in some case these could fall into the category of “futile cycles”. The range of routes that lead to energy loss is broad and this is reflected by the loosely fitting terminology (Fig. 1).

An additional route of energy loss is worth mentioning specifically: the leak. In this category the main focus of attention is the 1-electron reduction of \( \text{O}_2 \) by reducing components of the electron...
transfer pathways to produce superoxide radical, O$_2^-$ (Fig. 1). Superoxide is one of the so-called “reactive oxygen species”, it is easily reduced to form even more reactive species (peroxide Superoxide is one of the so-called ''reactive oxygen species'', it to interaction with triplet state of chlorophyll.

2. Photosynthetic reaction centres: an overview

All known photosynthetic reaction centres share the common structural feature of pseudo-C$_2$ symmetry both at the level of the protein backbone and of the arrangement of the redox cofactors (Fig. 2) (see e.g. [7–11]). This reflects their evolution from a common ancestral reaction centre which was made up of a homodimer of core protein subunits [12]. Despite this basic structural similarity, two classes of reaction centres are defined based on their terminal electron acceptors. Type II reaction centres use light to drive the reduction of quinone, while Type I reaction centres use light to drive the reduction of ferredoxin (or flavodoxin).

In Type II reaction centres, there are two bound quinones, QA and QB, which act in series as electron acceptors. QA acts as a 1-electron carrier, while QB undergoes two sequential reductions and associated protonations to form the quinol, QbH$_2$ [13]. The quinol then exchanges with an oxidized quinone from the membrane. This function requires that the semiquinone, Q$_b^-$, formed on the first photochemical turnover, remains stable until a second electron arrives upon the subsequent photochemical turnover. In Type I reaction centres, the terminal electron acceptors are iron–sulfur clusters but two bound quinones are also present. In this case, the quinones are both 1-electron carriers, relaying electrons from photooxidized chlorophyll to iron–sulfur clusters (see [14] for a review).
Thus, whereas Type I reaction centres work as purely monoelectronic devices that are not coupled to protons, Type II reaction centres couple the monoelectronic photochemical charge separation to the two-electron (and two-proton) reduction of a quinone to a quinol. As will be discussed below, this essential functional difference results in a strong mechanistic constraint that has shaped the evolution from Type I to Type II reaction centres.

The energy wasteful reactions are a particular problem for photosynthetic reaction centres because the photochemistry gives such high energy intermediates. This problem is mainly dealt with through kinetic control; i.e. the forward reactions are faster than the back reactions. This is achieved by having cofactors appropriately spaced within the protein to allow rapid vectorial electron transfer across the membrane, separating the positive and negative charges from each other. Small energy losses occur on the forward reactions thus making back-reactions thermodynamically unfavourable. In contrast, the direct recombination reactions of the radical pairs are strongly exergonic. In this case however the standard free energy gap is so big that the reactions fall in the ‘‘Marcus inverted region’’ and are thus relatively slow [15]. In addition, as the distance increases between the two charges of the radical pair, the direct recombination electron transfer routes become slower: short-circuits decrease [16].

When highly reducing intermediates are formed they will have a tendency to react with O₂ if it is present. This is a particular problem for PSI where even the terminal acceptors are more reducing (−520 mV) than the O₂ / O₂⁻ couple (−330 mV under 10⁵ Pa of O₂ and −160 mV for O₂ in aqueous solution, with [O₂] = 1 M [21]). When very oxidizing species are formed, and this is a particular problem of Photosystem II, the adventitious oxidation of cofactors (and perhaps proteins and lipid) can occur and may propagate out from the reaction centre. Both of these can be considered as ‘‘leaks’’.

When high energy radical pairs involving chlorophyll recombine, they can form chlorophyll triplet states that can react with O₂ to form singlet oxygen, a reactive oxygen species that is much more damaging than superoxide. This problem is common to all reaction centres in aerobic environments. This can be considered a short-circuit (chlorophyll triplet formation), resulting in a leak (triplet-sensitized ¹O₂ formation, a leak of energy not of an electron), in some cases preceded by a back-reaction (e.g. P⁺ Q₅⁺ to P⁺ Phe⁺). In this case the energy loss to the leak does not drive the short-circuit, as the triplet state would decay, albeit more slowly, without reacting with oxygen. The consequences of ¹O₂ formation are presumably so negative that a range of strategies are employed to prevent this route from occurring.

3. Type I reaction centers

Photosystem I is a plastocyanin/ferredoxin photooxidoreductase (in some species and conditions the donor may be cytochrome c₅₅ and the acceptor flavodoxin) and is present in plants, algae and cyanobacteria. It is thought to have evolved from an ancestral homodimeric reaction centre that resembled those in the present day Heliobacteria and Chlorobiaceae, both of which grow in anaerobic conditions [17]. These homodimeric reaction centres are composed of two identical subunits, each bearing a redox chain capable of light-driven charge separation [18,19].

Even in the absence of a crystallographic structure, the main structural features of the Heliobacteria and Chlorobiaceae reaction centres can be deduced given the similarities to PSI. The two electron transfer branches diverge from a (bacterio)chlorophyll pair,
which is close to one side of the membrane, and converge at the level of the \( F_x \) iron sulfur cluster on the other side of the membrane \([12,20–22]\). In a homodimer, with a truly symmetric structure, both pathways are expected to function symmetrically.

### 3.1. Heterodimeric PSI: adaptive redox tuning to deal with life in \( O_2 \)

Existing PSI has evolved to have greater asymmetry, with duplication of the core reaction centre gene and separate evolution of the two resulting genes giving rise to a heterodimeric reaction centre \([17,23]\). The two near-symmetrical electron transfer branches of PSI, which have around 60% identity between the A and B subunits, thus show differences at the amino-acid side chain level resulting in several functional differences (see Figs. 2 and 3).

Under normal circumstances, when PSI undergoes photochemistry, reduced \( F_{A/B} \) is rapidly oxidized by ferredoxin or flavodoxin. Prior to the activation of the \( CO_2 \)-fixation enzymes however, the amount of oxidized ferredoxin is limited and it builds up in its reduced form. The back-reaction \( F_{A/B} \) with \( P_700^- \) is 40 ms, but under normal circumstance reduced donors are available and electron donation prevents the back-reaction. \( F_{A/B}^- \) will thus accumulate, when fully reduced, further light excitations will result in formation of \( F_x \) and the two \( A^- \) acceptors, all three of which are rather close in energy and these will back-react with \( P_700^- \) when present.

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**Fig. 3.** Scheme illustrating the dominance of the \( A^- \)-branch as a back-reaction pathway in Photosystem I. Both panels show the standard free energy levels of the radical pairs formed by charge separation in Photosystem I (estimate from \([14,24]\)) and a structural scheme of the same reactions \([8]\). The order of the forward reactions is indicated by numbered red arrows. Broken arrows show back-reactions with grey designating the disfavoured reactions. Panel A shows charge separation initiated on the \( A^- \) branch and the dominant back-reactions are shown, illustrating the idea that charge recombination occurs mainly between \( A^- \) and \( P^- \) thereby minimizing the formation of \( P \) triplet state and thence \( O_2 \). Panel B shows charge separation on the \( B^- \) branch and here too the dominant back-reactions pathway is suggested to be the non-triplet generating \( A^- \) branch as explained in the text. Note that charge separation is considered to occur between the \( Chl \) and \( A_0 \) pigments followed by rapid donation from the \( P \) chlorophylls in accordance with \([45,138]\).
These back-reactions take place at rates ranging from milliseconds to hundreds of microseconds. Again, unless the plastocyanin pool is fully oxidized, electron donation to \( P_{700} \) should be fast enough to trap the acceptors in the reduced form. Thus when the soluble electron acceptors are limited, electrons linger on the terminal electron acceptors and upon further turnovers the preceding acceptors can become reduced. Leaks and back reactions are then predicted to occur.

When the PSI electron acceptors are reduced, their very low potentials mean that \( O_2 \) can easily be reduced to \( O_2^- \). This leak occurs not only at the stromal surface but also perhaps within the membrane, presumably from the A1 semiquinones when the iron sulfur centres are reduced (see [26]). At the onset of illumination, before the enzymes for \( CO_2 \) fixation are activated, nearly all the electrons coming through the electron transfer chain end up forming \( O_2 \). In recent years \( O_2 \) has been recognised as a signalling molecule (in addition to its established reputation as a potentially damaging reactive oxygen species) and so this leak is not necessarily a bad thing. But it is something that needs to be regulated and exactly how that is done is still not clear in detail. It does seem likely that the regulation of electron donation into and out of the reaction centre in both linear and cyclic electron transfer, are all important and that redox tuning may also play a role.

One feature of PSI that may reflect redox tuning to limit \( O_2 \) reduction is that the more stable, highest potential acceptor is not the \( F_X \), the exposed terminal acceptor that interacts with ferredoxin, but rather the \( F_A \) centre which is buried inside the protein (see [27]). The more sequestered location of the reduced \( F_X \) could slow its reaction with \( O_2 \). Interestingly in the green sulphur bacterial reaction centre this \( F_X \)-type acceptor appears to be the more stable of the two iron-sulfur centres [21]. This would make sense since it lives in an anaerobic environment and thus does not need to protect itself against \( O_2 \).

The back-reactions occurring when the electron acceptor side of PSI is blocked can result in chlorophyll triplet formation and hence singlet \( O_2 \) formation. This might be one of reasons underlying the photosensitivity of PSI in mutants lacking PGR5 [28], a protein shown to promote cyclic electron flow and proposed to be involved in the formation of supercomplexes comprising all the players required for an efficient cyclic electron flow around PSI [29,30]. Indeed, such redox cycling supercomplexes, which function without the diffusion of soluble electron carriers such as ferredoxin or plastocyanin, are expected to be less susceptible to the electron acceptor-side limitations described above. Below we propose that back-reaction pathways leading to the chlorophyll triplet formation are specifically minimized by redox tuning.

Asymmetry in PSI also exists at the level of the phyloquinones (compare Fig. 3A and B). The forward electron transfer rates for the two phyloquinones, \( A_{1A} \) and \( A_{1B} \), to \( F_X \) differ by an order of magnitude (200 and 20 ns respectively) [31–34] due to differences in the reduction potential of the two phyloquinones (estimated to be \(-671\) and \(-844 mV\) respectively [35], although functional studies point to the difference being smaller [36,37]). The origins of these very low potentials and the difference in the two potentials are discussed in detail elsewhere [25], briefly it results from a combination of electrostatic effects notably those from the \( F_X \) and \( F_{Xh} \) and from the protein environment with the asymmetry arising from differential effects of the protein backbone and asymmetry in specific ionicable amino acids [35].

The mechanistic significance of the kinetic and redox asymmetry associated with the quinones is unexplained. A rate of 200 ns compared to 20 ns is not expected to have any functional significance since these rates are both much faster than the competing back-reaction \( P^- A_{1A} \) recombination occurs in 200 \( \mu \)s. However, one can predict that the back-reaction pathway from \( F_X \) will be dominated by the \( A \)-side route since \( A_{1A} \) is slightly downhill from \( F_X \), while the \( F_X \) to \( A_{1B} \) step is uphill. It seems possible that this characteristic of PSI could be beneficial under high-light conditions.

It is known that chlorophyll triplets can form in the PSI reaction centre when illuminated under reducing conditions [38] or when the secondary acceptors are removed [39]. Under physiological conditions it seems possible that PSI would encounter conditions in which electron donors and acceptors would be insufficient to prevent charge recombination within PSI, under supersaturating light intensities for example, and this may be expected to result in triplet formation. Fig. 3 illustrates how the A-side, with its high potential phyloquinone, would be favoured for the back-reaction from \( F_X \), compared to the uphill transfer from \( F_X \) to \( A_{1B} \).

On the B-side, the energy gap between \( P^- A_{1A} \) and \( P^- A_{1B} \) is smaller than the equivalent reaction on the A-side. This means the population of \( P^- A_{1B} \) from \( P^- A_{1A} \) will be rapid and hence triplet formation will be favoured. On the A-side however, the equivalent back-reaction will be slower and the triplet formation will be disfavoured provided that the \( P^- A_{1A} \) recombination reaction occurs directly to ground state with a rate that out-competes the back-reaction to \( P^- A_{1B} \). Therefore, if the back-reaction occurs mainly down the A branch, as suggested above, then triplet formation overall will be minimized (see Fig. 3). The lowered triplet yield could constitute a significant advantage. A corollary of this idea is that native PSI should show less triplet formation per back reaction than seen in the anaerobic, homodimeric reaction centres of Helobacteria and Chlorobium. In these bacteria, however, triplet formation would be less of a problem as these they grow in strictly anoxic conditions and thus singlet oxygen will not be formed.

When Ishikita et al. [35] calculated the potentials for the quinones, they found that a key amino acid responsible for the asymmetric potentials was Asp575-PSaB. This group is closer to \( A_{1A} \) than \( A_{1B} \) and it was suggested that it undergoes a deprotonation in response to the formation of the semiquinone, thereby stabilizing the semiquinone. Given the very rapid forward electron transfer in PSI however, the protonation may not have time to influence the forward reactions, and indeed site-directed mutations of this particular residue had little effect on the reoxidation rates of the phyloquinones [40]. However under conditions where charges accumulate and back-reactions occur, it seems possible that this protonation does occur. This could switch \( A_{1B} \) to an even higher potential and thus the protective mechanism suggested above would be even more effective. Intriguingly Asp575-PSaB is changed to a Asn in the unusual gene variant present in nitrogen fixing cyanobacteria and expressed in heterocysts [41]. It seems clear the high potential \( A_{1A} \) and the tuning switch that we suggested above would not be needed in PSI functioning in the anaerobic conditions encountered during nitrogen fixation. If there is a change to two-sided low-potential PSI in anaerobic conditions, we must suppose that there is an advantage in doing this. A comparison of this anaerobic PSI with normal PSI might show differences in efficiency as well as susceptibility to \( O_2 \) related damage.

Current views of the evolution of Type II reaction centres propose that the Type II reaction centres diverged from the Type I reaction centres while both were homodimeric. This is particularly compelling since the majority of features (the cofactors and their environments) that differentiate Type II RCs from Type I RCs are symmetrical over both sides of the reaction centre. These differences were therefore almost certainly present in an ancestral homodimeric Type II reaction centre [42]. The heterodimericity of PSI, should the word exist, most likely evolved relatively recently compared to the separation of Type I and Type II reaction centres. Indeed the two sides of PSI, PsA and PsB, have not diverged greatly from each other (60% identity). In the preceding text, the asymmetry existing in PSI was rationalised in terms of protection and regulation in the context of reactions with oxygen.
We therefore suggest that heterodimerisation in PSI occurred after O₂ appeared in the environment, i.e. after the evolution of water splitting PSII and probably in the same membrane as the nascent water oxidizing reactions. Based on other arguments, a similar conclusion has been arrived at independently (John Golbeck personal communication). The tuning of electron transfer needed to deal with leaks and damaging back-reactions seems to be a requirement associated with life in the presence of oxygen.

4. Type II reaction centres

4.1. No charge separation in the B-branch of Type II reaction centres: a requirement for an efficient two-electron gate

In Type II reaction centres the quinone, Q₆ is the last electron acceptor in the chain and Q₆ must be stable until another photo-chemical turnover provides the second electron required to complete its 2-electron reduction. At this stage there is no forward reaction which can compete with the back-reaction and so kinetic control is not an option to prevent the back-reaction. The simplest way to prevent the energy loss is to slow-down the backward rate. This is done by making the back-reaction, or at least one step in the back-reaction pathway, strongly uphill in energy.

The shortest route for electrons to get to P³⁺ from Q₅⁻ is via the PheoB, the pheophytin on the “non-functional” B-side of the reaction centre. The distance between Q₆ and PheoB is thought to be very large in all Type II centres (the potential of PheoB has not been determined but is considered to be more negative than that of PheoA and the potential of Q₆ is around 100 mV higher than QA see [43,44]). Thus no P³⁺ Q₅⁻ back-reaction takes place by this route. This is a major factor contributing to the long lifetime of QA. In the evolution of the Type II reaction centres, the switching-off of the B branch presumably occurred by a mutation or mutations that lowered the potential of PheoB, simultaneously switching-off the charge separation on the B-side and blocking Q₅⁻ from back-reacting through PheoB. Interestingly, it has been shown in Photosystem I that raising the potential of A₉B, which is analogous to PheoB in Photosystem II, lowers the yield of the B-branch without affecting the overall quantum yield of charge separation, suggesting that the proposed evolutionary tinkering does not impact the overall charge separation efficiency [45].

The evolution of a large energy gap between Q₆ and PheoB contributed to a more efficient reaction centre by elimination of this direct back-reaction route. Several other features of the current Type II reaction centres can be seen as greatly increasing the efficiency of reaction centre as a quinol-producing device, compared to the ancestral homodimeric quinol-producing reaction centre. These have been dealt with in detail elsewhere [42,46]. Basically a homodimeric quinone-reducing reaction centre would suffer inefficiencies associated with Pheo⁻ encountering semiquinone (which would be awaiting the second electron) or an empty site (due to quinol/quinone exchange and incomplete occupancy). The heterodimer evolved (i) a specialised QA which is always bound and only does rapid, 1-electron chemistry and (ii) a specialised Q₆ site that stabilises a semiquinone adjacent to the non-functional PheoB.

4.2. Back-reactions in Type II reaction centres: the purple bacterial reaction centres

For Q₅⁻ the first step in the back reaction is electron transfer back to QA, in this case however, these two components are not far apart in energy, the two semiquinones equilibrate (K = 20). It is on the next back-reaction step that a big energy gap exists: the QA to Pheo step requires several hundred meV [47,48]. Across the Type II reaction centres this energy gap varies, having marked effects on back-reaction rates and this has clear mechanistic significance.

There are several examples that illustrate the extent to which the lifetime of the radical pair involving QA depends on the free energy difference associated with the electron transfer from QA to the nearby PheoA. In Rhodobacter sphaeroides, the lifetime of the radical pair changes as a function of the energy gap between QA and Pheo and this has been studied by substituting different quinoines for QA and by imposing an external field [47,49,50]. When the energy gap is smaller than around 350 meV then repopulation of the P³⁺Pheo⁻ state dominates; when the energy gap is larger than that, the direct tunnelling recombination reaction dominates [47,49,50].

Variations in this energy gap and hence the back-reaction kinetics are seen in different species of purple bacteria. In R. sphaeroides or Rhodobacter capsulatus, where QA is a ubiquinone (UQ), the lifetime of QA is significantly longer than in Rhodobacter viridis, a bacteriochlorophyll b-containing species in which QA is a menaquinone (MQ) [49,51,52]. This is explained by the smaller energy barrier for the repopulation of Pheo⁻ back to QA in R. viridis due to the higher potential of the Pheo (and perhaps a contribution from the slightly lower potential of MQ as QA). The smaller energy gap here is mainly a consequence of R. viridis’s use of longer wavelength light for photosynthesis. The energy available from 960 nm light (1.292 eV) is significantly less than 870 nm light (1.425 eV) used by R. sphaeroides and yet the reaction centre bacteriochlorophyll b gives rise to a P³⁺ cation that has approximately the same oxidizing power as that from R. sphaeroides (+450 mV). This means that it has around 130 mV less reducing power on the acceptor side and this is mainly seen as a diminution in the Pheo to QA energy gap. The rapid recombination via repopulation of the P³⁺Pheo⁻ is expected to give rise to a high yield of the triplet P³⁻ (see Fig. 4).

R. sphaeroides does not suffer from this energetic squeeze and so is able to have an energy gap between QA and PheoA that is more than enough to prevent the back-reaction by that route. Instead the P³⁺ QA⁻ recombination reaction takes place via a slow reaction that involves a tunnelling process. We shall see in the following section that PSI is like R. viridis insofar as it has insufficient energy in the absorbed photon to allow it to maintain an energy gap between Ph and QA that is big enough to render the Pheo to QA step irreversible. In PSI the energy squeeze is not caused by a lower energy photon (indeed it uses the highest energy photon of all the photosynthetic reaction centres) but by the high energy requirement at the oxidising side of the reaction centre. PSII needs all the energy it can get to take electrons out of water with a reasonable over-potential.

4.3. Two back reaction pathways in PSII: redox switching

PSII contains two charge recombination pathways for P³⁺ QA⁻, one of which is comparable to that in R. viridis and the other more similar to that in R. sphaeroides [48,53]. As described in the purple bacterial reaction centre, the size of the energy gap between QA and the Pheo determines the back-reaction rate and thus the recombination route. Here however this has more important mechanistic implications and remarkably PSIIL is able to modulate the size of the energy gap and hence the yields of these pathways in order to mitigate damage and optimize function.

As described above for R. viridis, the indirect pathway in PSII takes place with the formation of the P³⁺Pheo⁻ radical pair, which then decays to the P³⁻ triplet state ([54,55] and see [56]), for a review of the bacterial case see [57]). Unlike R. viridis, however, PSIIL is far from anaerobic, indeed it makes the O₂ and its P³⁻ triplet state...
lies about 1.3 eV above the ground state, which is more than enough energy to promote the triplet to singlet oxygen transition (0.98 eV [58]). Thus the chlorophyll triplet state is likely to encounter \(^3\text{O}_2\), transfer excitation to it and generate singlet oxygen \(^1\text{O}_2\), a highly reactive and damaging species [55,59].

Most purple bacterial reaction centres, even those in which the direct route is optimised and which live in low \(\text{O}_2\) environments, still have a system for quenching \(^3\text{P}\). In this case it is only formed by \(\text{P}^+\) when it is formed. In this case it is only formed by \(\text{P}^+\) when it is formed. For each type of reaction centre the energy available in the first excited singlet state (\(\text{P}^+\)) corresponds to the photon absorbed and that is indicated at the foot of each column. Note that for the *R. sphaeroides* and *R. viridis* the term \(\text{P}^+\) represent the special pair of bacteriochlorophylls. In PSI and PSII this is more complicated. For PSI as shown in Fig 3, the excited state would be located over the component designated ChlA in panel A (or ChlB in panel B). For PSII the excitation is not thought to be localised on a single pigment at room temperature, instead is distributed over several pigments: mainly on the component ChlD1, but also partly on P680 and P700 and also to a smaller extent on ChlD2 and even the Pheos [43,46].

Each of these strategies may compromise the overall efficiency of the energy converter. Indeed, since Photosystem II is a "shallow photochemical trap" [61,62] (another consequence of being energy squeezed), raising the free energy level of the primary radical pair impacts the quantum yield [63–65]. Similarly, decreasing the free energy level of \(\text{Q}_A\) (relative to \(\text{Q}_B\) and or the quinone pool) will increase its steady state concentration, thereby impacting the overall energetic efficiency of the system [53,66]. There is thus a trade-off between the need to limit potentially harmful but, under standard conditions, rare (chlorophyll triplet generating) back-reactions and the optimization of the energy conversion yield. The latter seems to have been favoured since the relative yield of the indirect, back-reaction pathway in Photosystem II, under functional conditions is rather high and accounts for ~70% of the charge recombination process [67]. Under normal functional conditions electrons are plentiful from water splitting and these potentially damaging reactions occur infrequently. When however conditions are encountered in which back-reactions are more frequent, then this damaging route can be essentially switched off [53].

While this switching process involves kinetic control, the switch itself is through a conformational change and this can be viewed as kinetic gating. Photosystem II is fully assembled in the membrane as a photosynthetic reaction centre without its active site, the Mn\(_{6}\)O\(_5\)Ca cluster. The cluster is assembled by a process known as photoactivation. Prior to and during photoactivation, electrons are much less readily available to stabilize the oxidizing equivalent resulting from photochemistry, thus charge recombination would be expected. However when the cluster is absent, the reduction potential of \(\text{Q}_A\) is higher by 150 mV than in the functional enzyme [69]. Consequently, the free energy gap between \(\text{P}^+\text{Q}_A^+\) and \(\text{P}^+\text{Pheo}^-\) is large and the direct charge recombination pathway is favoured, while the indirect pathway, and its troublesome \(^3\text{P}\) intermediate, is avoided [53] (see Fig. 4).

It is known that the simple absence of Ca\(^{2+}\), rather than the whole cluster, is responsible for the switching effect [68,69]. It is possible that this occurs under physiological conditions, for example in the presence of high local proton concentrations [68,69] and in certain S states [70], and this would result in the same kind of redox switching. This would protect PSII should this occur but it also could be a regulatory mechanism in high light conditions.

It is assumed that the structural modifications resulting from the absence of the Mn\(_6\)Ca cluster, or indeed just the Ca\(^{2+}\), propagate over to the other side of the protein (almost 40 Å away) and induces a down-shift of the midpoint of the \(\text{Q}_A/\text{Q}_B\) couple [53,69,71]. The nature of this change is not clear however it has been suggested to be related to the presence of an H-bond from threonine (217 of D2) to the carbonyl on QA that is proximal to the non-heme iron [72]. The presence of this H-bond has been calculated to produce just such an up-shift in potential. The simple rotation of the OH group of the threonine could make or break this bond. It is not clear how the binding status of the Ca\(^{2+}\) 40 Å away would influence this rotation. Alternatively, the ionization of amino acids in the region of QA, perhaps the bicarbonate/carbonate that ligands the non-heme Fe, could also be responsible for this redox shift [46]. A well resolved crystal structure of the Mn\(_6\)Ca-depleted PSII may help understand this effect and how it is propagated across the protein.

The relationship between the redox potential of \(\text{Q}_A\) (and hence the \(\text{P}^+\text{Q}_A^-\text{P}^+\text{Pheo}^-\) energy gap) and the generation of \(^1\text{O}_2\) has been established experimentally using spin trap EPR methods. This was done in a site-directed mutant that lowered the potential of \(\text{Q}_A\) and it duly gave rise to more \(^1\text{O}_2\) [73]. Another demonstration was done using herbicides. The binding of herbicides in the \(\text{Q}_B\) site results in changes in the potential of \(\text{Q}_A\) and this again affects the
Qₘ to Pheo energy gap [74]. The yield of singlet oxygen increased when the binding of phenolic herbicides decreased the size of the energy gap due to the increased yield of the indirect pathway via the Pheo⁻⁻ giving rise to 3P formation [59]. These herbicides not only block electron transfer but they also redox tune Qₘ, favouring the back-reaction up to a high energy intermediate (P⁺ Pheo⁻⁻), this reacts by a short-circuit (charge recombination) to form a reactive state (3P) that reacts with O₂ (a leak) and this kills the plant [75].

In the Qₘ site, phenolic herbicides seem to H-bond strongly to the imidazole that ligands the non-heme iron, this effect may be relayed to the H-bonded Qₘ on the other side of the imidazole-Fe-imidazole motif leading to a weaker H-bond to the Qₘ and thus generating the lower potential [76]. In line with this, the affinity of the Qₘ pocket for phenolic herbicides depends on the redox state of Qₘ [77]. This herbicide-induced modulation of the Qₘ potential is additive to the Ca-induced effect, so clearly the chemical origin of the redox effects are different [74]. These observations also could indicate that the native occupant of the Qₘ site, PQ (its presence and absence and each of its different redox states), could influence the reduction potential of Qₘ and thus tune the forward and back reactions. This has yet to be studied.

Based on the effect of Ca⁺⁺ on the potential of Qₘ [78] and the fact that Ca⁺⁺ binding changes during the S-state cycle [70], it has been suggested that the potential of Qₘ could be tuned to suit specific properties of the S states [46]. For example a short-lived S₃TyrZ` state may have less chance of back-reacting if the Qₘ potential were increased in S₃.

4.4. Back reactions in PSII: modulating the potential of pheophytin

The standard free energy level of the PheoΔ state can be modified depending on the strength of the H-bond to the C₅Fe-O of PheoΔ from the amino acid side chain at D₁-Gln130. In nature this residue can be Gln or Glu but a range of site-directed mutants have been made and studied. The lifetime of Qₘ decreased as the H-bond was strengthened (as the Ph potential became more positive) and the corresponding increase in the lifetime of Qₘ occurred when the H-bond was weakened (as the potential of Ph became more negative) [48,65]. As expected these studies showed the correlation between the size of the standard free energy gap between PheoΔ and Qₘ and the lifetime of the semiquinone.

The impact of modulating the free energy level of the PheoΔ state is not limited to the back electron transfer rate from Qₘ⁻ but indeed the forward reactions are also affected. Site-directed mutants at the position D₁-130 position showed the rate and quantum yield of the primary charge separation were dependent on the H-bond strength, with increased rates and yields when the H-bond strength was decreased [63,64,79]. Lowering the potential of Pheo leads to lower yields of charge separation as well as slower back reactions.

In nature only the high potential form of Pheo (with the strong H-bond from D₁-Glu130) is present in plants and algae. By contrast, in cyanobacteria either Glu or Gln is found at position 130 in D₁. All known cyanobacteria possess several genes coding for the D₁ subunit, which together with D₂ constitutes the core of the reaction center. These multiple D₁ genes are differentially expressed depending on the environmental conditions [80,81]. In all known cyanobacterial sequences, the only conserved difference between the two expressed isoforms is at position D₁-130, being Glu in high light D₁ but Gln in the low light form. It is tempting to suppose that the reason for this differential gene expression is to decrease the yield of potentially damaging reactions when Photosystem II is exposed to over-saturating light. And indeed, the D₁ isoform expressed in high light does confer photo-resistance, compared to “low-light” isoforms and species [82–84]. Again, this tuning results in a kinetic control but this is obtained by substituting one isoform of the D₁ subunit by another, something that might be considered a special form of large-scale gating. However, the mechanisms underlying this photo-protection effect are not clearly understood.

The Glu at position D₁-130, which is present in all the high-light isoforms should result in a stronger H-bond to PheoΔ and thus have the following effects: (i) the potential of the PheoΔ is expected to become more positive; (ii) the quantum yield of charge separation is expected to increase because of the greater driving force for P⁺ Pheo⁻⁻ formation from P and (iii) the yield of the indirect, triplet-generating, charge-recombination pathway should increase because the energy gap between P⁺ Qₘ and P⁺ Pheo⁻⁻ is smaller. These are not obvious ploys for coping with too much light, indeed we might expect them to have exactly the opposite effect and to make matters worse. How can this be rationalized?

One possibility is that the decreased photo-sensitivity of the high-light Photosystem II isoforms stems from the combination of multiple functional effects arising from the range of amino acid changes. The recently documented changes on the electron transfer rates on the donor–side of Photosystem II might be one of these [84,85]. But this is highly unsatisfactory as an explanation: why should the Glu be selected in every high-light strain, if it did not play a positive role? Here are some suggestions.

The photosensitivity of PSII mutants bearing either a Leu, Gln or Glu at position D₁-130 has shown that photoinhibition is more pronounced when the energy level of the P⁺ PheoΔ state is high. This observation should help solve the paradox.

Vass and Cser [86] invoked the Marcus theory and pointed out that changes in the potential of PheoΔ, not only affect the thermally activated repopulation of the P⁺ Pheo⁻⁻ but also the electron transfer rate between P⁺ Qₘ⁻ and P⁺ Pheo⁻⁻. They proposed that increasing the midpotential of PheoΔ would indeed favour the thermally activated repopulation of P⁺ Pheo⁻⁻ from P⁺ Qₘ⁻ but in addition it would favour radical pair recombination from the singlet over the triplet route. This can be rationalized in terms of Marcus theory: the very large driving force for singlet recombination to the ground state (above 1.6 eV) would put the reactions into the inverted region (i.e. a decrease in driving forces accelerates the reaction), while recombination to the triplet, with a driving force around 0.2–0.3 eV, behaves conventionally (i.e. a decrease in driving force slows the reaction). Thus a positive shift in the potential of Ph (i.e. with the stronger H-bond from D1Glu-130) would decrease the driving force for P⁺ Pheo⁻⁻ recombination, accelerating singlet recombination to the ground state but slowing triplet formation. This then is positive protective effect. While this is plausible, it is not wholly satisfying since the rate effects are not expected to be large and the increased decay of P⁺ Qₘ⁻ by the dangerous, indirect back-reaction route has to be compensated for before any protective benefit can be gained by this mechanism. Below we suggest some alternatives.

The modulation of the energy gap as a method of minimizing the formation of the PSII triplet, as originally formulated by Johnson et al [53], was seen as a protection strategy for preventing P⁺ Pheo⁻⁻ formation when populated from P⁺ Qₘ⁻, i.e. to prevent electrons from returning to Pheo from Qₘ⁻ (or from Qₘ⁺ via Qₘ⁺). This is a particular risk when the enzyme is unable to provide electrons: i.e. when the water splitting function is absent (before photoassembly of the MnCa cluster) or disabled (after photodamage or when Ca⁺⁺ is lost) or when the light intensities is so low that the reduction potential of QA and thus tune the forward and back reactions, the
P\textsuperscript{−}Pheo\textsubscript{−}Q\textsubscript{A} state will still be formed [61,62]. Recombination of this state will produce triplet [61]. Now, returning to the change in the potential of Ph associated with the H-bonding Glu/Gln at D1-130, we suggest that a smaller driving force from P\textsuperscript{−}Pheo\textsuperscript{−} state (with stronger Glu 130 H-bond) may simply diminish the triplet yield and favour singlet recombination. For this to occur then, like Vass and Cser [86], we resort to the Marcus theory. In this case however, recombination is from P\textsuperscript{−}Pheo \textsuperscript{−} Q\textsubscript{A} \textsubscript{−} (rather than P\textsuperscript{−}Pheo \textsuperscript{−} Q\textsubscript{A}). Because of the electrostatic effect of the charge on Q\textsubscript{A} on Ph\textsuperscript{−}, this radical pair would be at an even higher energy, even further into the Marcus inverted region for singlet recombination to the ground state and thus its rate would be more susceptible to a small changes in the driving force. The Glu 113 H-bonded interaction to the ground state and thus its rate would be more susceptible to a small changes in the driving force. The Glu 113 H-bonded Ph would thus be an advantage in the high light strains. The difference to a small changes in the driving force. The Glu 113 H-bonded interaction to the ground state and thus its rate would be more susceptible to a small changes in the driving force. The Glu 113 H-bonded Ph would thus be an advantage in the high light strains. The difference to a small changes in the driving force.

In the potential of Ph associated with the H-bonding Glu/Gln at D1-130, we suggest that here the energy gap between P\textsuperscript{−}Q\textsubscript{A} and P\textsuperscript{−}Pheo \textsuperscript{−} Q\textsubscript{A} is greatly increased. Furthermore, the proposed Q\textsubscript{A}, electron transfer quenching of P\textsuperscript{−} will also be lost, allowing the triplet lifetime to increase by more than 2 orders of magnitude [61,89]. This would give rise to severe oxidative damage if O\textsubscript{2} were present. This has been suggested to occur under some photoinhibitory conditions [87,90]. The increase in the potential of the Pheo due to the presence of H-bonded D1Glu-130 would decrease the driving force for this second reducing step, Ph \textsuperscript{−}Q\textsubscript{A} \textsuperscript{−}2H\textsuperscript{−} \rightarrow \text{Ph}Q\textsubscript{H\textsubscript{2}}, and might be expected to slow it down. This would constitute a significant advantage for selecting of Glu-130 in high light conditions.

4.5. Side path, futile cycle and oxidative leaks in PSII

The very high potentials involved in water photolysis can result in the adventitious oxidation of nearby organic species in and around the PSII reaction centre. The longest-lived of the chlorophyll cations formed during primary charge separation, because the electrostatic influence of Q\textsubscript{A} on the energy level of P\textsuperscript{−}Pheo \textsuperscript{−} is removed, the energy gap between this state and \textsuperscript{P}\textsuperscript{−} increases and so does the quantum yield of charge separation: in short the shallow trap effect is reversed [61]. As a result of the increased quantum yield of P\textsuperscript{−}Pheo \textsuperscript{−} formation, the yield of the \textsuperscript{P}\textsuperscript{−} is greatly increased. Furthermore, the proposed Q\textsubscript{A}, electron transfer quenching of P\textsuperscript{−} will also be lost, allowing the triplet lifetime to increase by more than 2 orders of magnitude [61,89]. This would give rise to severe oxidative damage if O\textsubscript{2} were present. This has been suggested to occur under some photoinhibitory conditions [87,90]. The increase in the potential of the Pheo due to the presence of H-bonded D1Glu-130 would decrease the driving force for this second reducing step, Ph \textsuperscript{−}Q\textsubscript{A} \textsuperscript{−}2H\textsuperscript{−} \rightarrow \text{Ph}Q\textsubscript{H\textsubscript{2}}, and might be expected to slow it down. This would constitute a significant advantage for selecting of Glu-130 in high light conditions.

Electron donation from TyrZ can occur in tens of ns. Nevertheless it is predicted to occur with a very low quantum yield under normal conditions and with an increased quantum yield whenever P\textsuperscript{−} lifetime is longer (i.e. when electron donation from water is inhibited or absent). The carotenoid is a 20 Å-long cofactor and it gets within a short distance of Cyt b\textsubscript{559} and makes van der Waals contact with ChlZ\textsubscript{D2}. Given its low potential relative to the ChlZ\textsubscript{D2}, Cyt b\textsubscript{559} if reduced, donates electrons rapidly to the β-carotene cation. The oxidized Cyt b\textsubscript{559} is relatively stable but can be reduced slowly by plastoquinol [97]. This completes a futile cycle. Not much is known about the reduction of the Cyt b\textsubscript{559} except that it is slow and is blocked by the same herbicides that bind to the Q\textsubscript{A} site. Given the long distance, electron transfer is expected to take around a second to occur from the Q\textsubscript{A} site to the heme of Cyt b\textsubscript{559}. It seems possible that faster electron donation may occur under other circumstances through a less well-defined quinone site that is closer to the Cyt b\textsubscript{559} (a “Qc site”) and that also herbicide sensitive [98,99]. A third functional quinone in isolated reaction centres [100] was detected by crystallography bound in a channel close to the heme of Cyt b\textsubscript{559} [92], although electron transfer from here to the heme could be rapid, it seems somewhat unlikely that this corresponds to the earlier defined Qc site [46].

This inefficient Cyt b\textsubscript{559}-mediated cycle has been proposed to protect against oxidative damage caused by P\textsubscript{680} [101] or by Car\textsuperscript{−} (see citations in [34]). In marine plankton a much more efficient futile cycle exists under high light [102]. This may represent a souped-up version of the Cyt b\textsubscript{559} cycle described here. In order to work so much more efficiently, some kind of redox switch seems to be required. It was suggested recently [46] that this could involve a perturbation of the relative redox potentials of the core chlorophylls so that the chlorophyll cation may be distributed onto ChlD\textsubscript{2}, i.e. closer to the carotenoid. This could occur by for example the electrostatic influence of accumulated oxidising species, say TyrZ′ or at least its proton [46].

When the cytochrome b\textsubscript{559} is already oxidized, the carotenoid cation can be reduced by chlorophyll, most likely ChlZ\textsubscript{D2}, which is calculated to have a uniquely low potential [103]. This branch of the futile cycle may serve as a trap for the reaction and as a fluorescence quencher that will protect against over excitation of PSII.

It is possible that other pigments further from the reaction centre may undergo oxidation due to further oxidation of side-path components. Reports exist in the literature of multiple chlorophylls and carotenoids undergoing slow bleachings with prolonged illumination [104]. Such oxidations, should they occur under physiologically relevant conditions, may be considered as oxidative leaks. The oxidation of carotenoids in the antenna will remove their protective (triplet quenching) influence from the nearby chlorophylls and this will start a chain reaction of light-driven, triplet-mediated \textsuperscript{1}O\textsubscript{2} damage [75]. These oxidative leaks (and several other oxidative leaks occurring under other circumstances) are clearly to be avoided and the futile cycle and the up-shift in the Q\textsubscript{A} potential when water splitting is non-functional, as described above, are both useful for that.

5. The cytochrome bc\textsubscript{1}/b\textsubscript{5f}

In the Q cycle of cytochromes bc\textsubscript{1}/b\textsubscript{5f}, a reversible oxidation of quinol in the catalytic Q\textsubscript{A} site delivers one electron into the high potential c-chain and the other into the low potential b-chain, giving rise to a charge-separated state in the enzyme [105]. This reaction relies on i) the energetic coupling of the two reduction/oxidation reactions, one involving the FeS center of the c-chain, the other heme b\textsubscript{5f} of the b-chain, and ii) on the split between the midpoint potentials of the quinol/semiquinone and semiquinone/quinone redox couples illustrated in Fig. 5, estimated to be at least ~800 mV [106,107]. The electrons are then transferred from the FeS center to the heme c\textsubscript{5f} in the c-chain and from the heme b\textsubscript{5f} via the heme b\textsubscript{5f} to the second catalytic quinone site, the Q\textsubscript{A} site in the b-chain. While in mitochondria and purple bacteria the
enzyme works as a quinol:cytochrome oxidoreductase, there are examples of bacteria which rely for their growth on reverse electron flow from cytochrome c to quinone [108].

Despite a great deal of research, the mechanism of the Qo site catalysis and the way the two chains are connected are still not fully understood and remain the subject of intense debate (for recent references see for example [109–112]). The connection between the two chains has an additional level of complexity arising from the fact that the enzyme has a homodimeric structure with each monomer containing one c- and one b-chain that together form an H-shaped electron transfer system.

From a kinetic point of view, the direction of electron flow through the two coupled chains would be expected to depend exclusively on the rates of all partial reactions, including the Q/H2 exchange reaction under and from the catalytic sites, provided that all the reactions within the chains are fully reversible. The equilibrium of one reaction in the coupled chains influences the equilibrium of all other reactions. A kinetic model based on this is sufficient to explain the re-equilibration reactions occurring following a flash-induced change in redox level of quinone pool and the cytochrome c pool [113].

However, rapid reversibility (within the catalytic timescale) of all partial reactions, including the initial charge separation at the Qo site [114], raises a mechanistic problem in understanding how all partial reactions, including the initial charge separation at the cytochrome b complex, influence the equilibria of all other reactions. A kinetic model based on this is specifically indicated. As a convention the arrows start from the redox couple that provides the electron donor and points toward the redox couple that provides the electron acceptor.

must apply to quinone reduction at the Qo site, which would require both FeS and heme b to be present in the reduced form at the same time.

The second possibility is that the site favours a concerted, two-electron oxidation of the quinol that does not involve a semiquinone intermediate [114,115,119], or at least keeps its concentration extremely low. Again this would apply to the reverse reaction, quinone reduction.

Deciding between these two possibilities, as well as developing a precise understanding of the molecular mechanisms occurring awaits further studies. One interesting line of investigation has opened up recently with the reports of methods for the trapping of the semiquinone formed at the Qo site [106,107].

The short-circuit reactions are traditionally referred to as “un-wanted” because they dissipate energy and thus lower the energetic efficiency of the system. Recent studies however, indicate that their occurrence on a much longer time-scale (seconds) may have physiological relevance [120–122]. This relates to the redox conditions in which the cofactors remain in the reduced state for long periods, for example, when the Qo site is unable to accept electrons from the reduced b hemes. As discussed below, under these circumstances short-circuits might compete effectively with electrons leaking onto O2 and thus would diminish O2 formation [120,121]. Furthermore, they might even allow quinol oxidation at Qo site to remain functional at a residual, yet physiologically competent, level [122].

5.1. Competition between short-circuits and leaks of electrons

Superoxide, O2−, is formed by the cytochrome bc1 complex under certain circumstances. If, as seems likely, the reaction of SQo with oxygen is directly responsible for the generation of O2 [106,107,123], then the probability of this reaction will increase when the reduced forms of the heme b or FeS remain present long enough to prevent complete oxidation of the quinol. A similar argument applies for the reverse reaction, where complete reduction of the quinone will not be achieved if either heme b or FeS is not reduced upon arrival of the quinone in the Qo site. But SQo may also engage in competitive reactions that retain electrons within enzyme rather than reducing oxygen. As described in detail in [120,121], several of those reactions result in short-circuits (see Fig. 5).

Because semiquinone in the Qo site can be formed in two ways, either by the withdrawal of electron from QH2 by FeS (the semiforward reaction) or the reduction of Q by heme b (the semireverse reaction), both of these reaction can potentially generate O2−. Experimental analysis of O2 production in various mutants of Rb. capsulatus cytochrome bc1, combined with modelling, suggested that the dominant reaction responsible for O2− formation is electron transfer from heme b to quinone [121]. This leads to a build-up of steady state levels of SQo, which can react with O2 [121]. According to this model, when SQo is formed at a time when the head domain of the mobile FeS is away from Qo site, the FeS cluster cannot immediately react to neutralize SQo. Thus the lifetime of SQo is relatively long and the probability that SQo will reduce oxygen is therefore greater. If however the FeS cluster is close to the Qo site, when in its oxidized form it simply picks up the electron from the SQ (resulting in a short-circuit), while if it is in its reduced form it will donate an electron to SQ (completing full quinone reduction, i.e. the reverse reaction). In either case SQO is rapidly removed by internal reactions occurring within the Qo site before it can react with O2.

This type of kinetic competition between short-circuits and leaks also probably occurs in the mitochondrial system, where the electron transfer from heme b to Q has also been proposed to be responsible for generation of reactive oxygen species by
cytochrome $bc_1$ [124,125]. Such competition may conceivably work as a protective mechanism with which the living cells would minimize the deleterious formation of $O_2$ through the use of competing energy-wasting but leak-proof and safer short-circuits [120]. But on the other hand, under some conditions the $O_2$ production could occur as part of a signalling pathway, reflecting the redox state of the electron transfer chain so the leak itself, when it occurs, could be physiologically relevant [126].

5.2. Short circuits in the broken Q-cycle

We have discussed above the possibility of the back-reaction between reduced $b_1$ and quinone and how this can lead either to the reduction of the oxidized FeS by the resulting SQo, or to leaks to oxygen. As shown in Fig. 5, another possible short-circuit involving the reduced $b_1$ is the electron transfer from $b_1$ to SQo. Such a situation might occur when the oxidized FeS oxidizes the quinol yielding SQo, while $b_1$ is reduced.

Owing to the large separation between the potentials of the quinol/semiquinone and semiquinone/quinone couples (at least $\sim800 \text{ mV}$ [106,107]), the unstable SQo is both an efficient electron donor and acceptor and it is thus capable of accepting an electron from the reduced $b_1$ yielding a quinol.

The occurrence of this reaction was demonstrated recently in the $b_{1f}$ complex when the Q site was inhibited [118] or disabled, and it was found that the reduction of SQo, at the expense of $b_1$ was very slow ($\sim250 \text{ ms}$ [122]). Despite its sluggishness, the very existence of this short-circuit is important because it represents an “emergency exit” pathway which bypasses the Q-cycle. This reaction thus allows the quinol-plastocyanin oxidoreductase activation to occur and thus the entire photosynthetic chain to function, even with a non-functional Q cycle.

This short circuit mechanism may be relevant to wild-type cytochrome $bc$ and $b_{1f}$ complexes under steady-state conditions. As the proton motive force builds up in the light, the driving force for the electrogenic electron transfer from $b_1$ to $b_{1f}$ decreases, thereby increasing the steady state level of reduced $b_1$ and thus the relative yield of the short-circuit pathway.

The slow rate of electron transfer from reduced $b_1$ to SQo under these conditions is not understood. Notably it is much slower than electron transfer from SQo to oxidized $b_1$ ($\sim250 \text{ ms}$ for the former vs $3 \text{ ms}$ for the latter [122]). The slower electron transfer rate might be simply due to the standard free energy change for the electron transfer from $b_1$ to SQo being much larger than that of SQo to $b_1$, putting the latter reaction into the “inverted region” of the Marcus curve [15]. According to the current available estimates for the $\Delta G_0's$ of these reactions, however, their reorganization energies would have to be unusually low for this to occur. Alternatively, and more likely, the rate-limiting step may not be electron transfer, but limited by a change in the binding of the quinol or of SQ in the site, indeed the appropriate configuration of the site for the formation of SQo may depend on the redox state of $b_1$ (see [116,117] for a discussion of such possibilities).

5.3. Cytochrome $bc_1$ as a functional dimer and its possible role in diminishing ROS

The recent demonstration of all electron transfer paths in the $bc$ dimer provided important functional principles. In addition to the well-known electron transfers through the c- and b-chains of each monomer, a functional electron transfer bridge connecting the two monomers formed between the two hemes $b_1$ in a core of dimers was demonstrated [127,128]. With this bridge, all cofactor paths within the dimer assemble into an H-shaped electron transfer system linking the two $Q_b$ sites on one side of the membrane with the two $Q_c$ sites on the other side of the membrane. This system distributes electrons between these four catalytic sites within a timescale of the catalytic turnover (milliseconds) and acting like a molecular-scale “bus bar” with four terminals [127]. As a result, any connection between the catalytic sites on opposite sides of the membrane allows the enzyme to be catalytically competent. It remains to be seen whether and how this “bus bar” design contributes to regulation of electron flow in respiratory and photosynthetic systems. It does seem clear that the built in redundancy will allow enzymological function even if operation or mutation disables specific component branches.

An important question concerns the relative ratio of intra- versus inter- monomer pathways in the functioning dimer. Considering electrochemical properties of the cofactors and distances between them, it is expected that under the conditions of unperturbed electron flow in all parts of the dimer (i.e. when all four terminals are “fully open”) the intra-monomer electron transfer would dominate [129]. However, as the equilibrium levels change and electron flow in parts of the dimer is suppressed (i.e. when any of the energetic and/or structural conditions lead to the bus bar terminals become “partly or entirely closed”, for example where $\Delta \mu_{H_2}$ builds up), then the contribution of the inter-monomer electron transfer is expected to increase.

Another important question concerns a possible role of electron exchange between all four hemes b within a dimer to diminish leaks of electrons and generation of superoxide [114,127,130]. A general concept behind those possibilities assumes that the connection between hemes unites them with all four catalytic quinone oxidation/reduction sites in such a way that multiple unpaired electrons produced during the Q cycle can by collected and neutralized [114,127]. This means that the cross-dimer electron transfer may help in sweeping the b-chain of reduced heme b [114]. As discussed in paragraph 5.1, an increased level of reduced hemes b is associated with the increased probability of SQ and for $O_2$ formation. Thus, any means to diminish the level of reduced hemes b is expected to diminish the risk of $O_2$ formation.

Those two general questions set now the stage for further studies to clarify and define intra- and inter- monomer electron transfer in this system and its possible physiological role in regulating electron flow and guarding against unwanted ROS.

6. Overview and conclusions

The energy-converting enzymes evolved from ancestral enzymes that functioned in conditions of low $O_2$ concentration or anaerobicity. These ancestral proteins did not have to deal with the inevitable side-reactions (leaks) that occur when $O_2$ is present. The different redox tuning adaptations that we have discussed above can be rationalised in the context of avoiding side-reactions with $O_2$. The key physical values that seem to have had such an influence on this area of bioenergetics are $\sim160 \text{ mV}$ (the Em of $O_2/O_2^-$ but note this will have varied depending on the concentration of $O_2$ in the atmosphere) and $1 \text{ eV}$, the energy difference between $O_2$ triplet and its highly reactive singlet state.

Redox reactions occurring with intermediates with potentials in the region of $\sim160 \text{ mV}$ and lower face the possibility of electrons leaking out to $O_2$ and forming superoxide. This is dealt with in diverse ways, some of which, but not all, have been presented here: (i) the regulation of electron transfer to avoid the build up of reducing intermediates, e.g. cyclic electron flow around PSI, regulation of PSII; (ii) kinetic control, involving fast electron transfer steps through the most reducing states, e.g. $F_x$ and $A_1$ in PSI or $b_1$ to $b_6$ electron transfer in $bc_1/b_{1f}$; (iii) redox tuning by raising the potential of some intermediates, e.g. stabilising the $Q_b$ and $Q_c$ semiquinones, or switching from low potential menaquinone to
high potential ubiquinone and plastoquinone [131], (iv) changes in mechanisms/structures, e.g. the appearance of an additional heme in the quinone reduction site of the b$_6$f complex with respect to its b$_c$ counterpart [132–135]. Another adaptation was of course to remove O$_2$- and peroxide with specific scavenging enzymes but this was clearly a second line of defence.

When chlorophyll-containing species found themselves in the presence of O$_2$ then there was a big problem: the excited singlet and more importantly the longer-lived triplet state had more than enough energy (1.3 eV) to drive the conversion of triplet O$_2$ to its highly reactive singlet form (1 eV). Redox tuning does not help here, this is an energy question and red light is as low in energy as possible for efficient oxygenic photosynthesis. The main fix was to wheel out the carotenoids as quenchers of chlorophyll triplet states and of singlet O$_2$. However in the reaction centres, chlorophyll triplet could be formed by short circuits such as charge recombination. Here again carotenoid quenching has been employed where possible but this is clearly insufficient and many mechanisms exist where by these short-circuits are minimised. These include the following: (i) big energy gaps, when energetically possible, to prevent back reactions, (ii) redox switching to control the energy gap, e.g. the high and low potential of Q$_A$ in PSII which is related to donor side function; (iii) switching to a lower potential Ph in PSI in high light isomers of D1, (iv) a bigger energy gap for the specific back-reaction side of the reaction centre (the A-side) in PSI, (v) also perhaps the switching on and off of a futile cycle in PSI [see [46]].

In PSII, given the extreme oxidising chemistry associated with water oxidation and generation of O$_2$, there are other protective mechanisms that appear to be there to prevent over-oxidation of the cofactors and groups in their environment [see [46]]. These too may have secondary reactions associated with O$_2$ and its derivatives.

The basic requirements for electron transfer in biology have been established and a few basic rules have been defined [5,16]. What is required is proximity between cofactors and appropriate overall driving forces. Once these are in place, the system has little or no need for fine-tuning in order to promote productive electron transfer. Indeed, it has been shown that big variations in the energy levels of intermediates have little effect on the final (quantum) yield of the forward reactions [see e.g. [136,137]]. This provides great robustness to the system. Here however we have presented several cases (and there are others), where the fine-tuning of energy levels does occur. Among these there are examples where redox tuning is used to obtain totally different outcomes, while the same cofactor distances are maintained. These situations are specifically associated with circumstances in which the desired energy-useful outcome of catalysis becomes less important than saving the system from damaging reactions, particularly with O$_2$, that put the viability of the organism in danger.

Some of the key features of the bioenergetic redox enzymes can thus be understood from an evolutionary viewpoint, where enzymes that were already optimised for productive energy conversion in an anaerobic or low O$_2$ environment had to compromise efficiency in order to survive the arrival of O$_2$. Here we have dealt with only a limited number of enzymes and a limited number of their features. It seems likely that similar thinking could provide further insights not only into these complex enzymes but also into other bioenergetic redox enzymes.

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References

[1] Mitchell, P. (1961) Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. Nature 191, 144–148.
[2] Wood, P.M. (1988) The potential diagram for oxygen at pH 7. Biochem. J. 253, 287–289.
[3] Gibson, G.E., Starkov, A., Blass, J.P., Ratan, R.R. and Beal, M.F. (2010) Cause and consequence. mitochondrial dysfunction initiates and propagates neuronal dysfunction, neuronal death and behavioral abnormalities in age-associated neurodegenerative diseases. Biochim. Biophys. Acta 1802, 122–134.
[4] Finkel, T. (2011) Signal transduction by reactive oxygen species. J. Cell Biol. 194, 7–15.
[5] Moser, C.C., Keske, J.M., Warncke, K., Farid, R.S. and Dutton, P.L. (1992) Nature of biological electron transfer. Nature 355, 796–802.
[6] Moser, C.C., Chobot, S.E., Page, C.C. and Dutton, P.L. (2008) Distance metrics for heme protein electron tunneling. Biochim. Biophys. Acta 1777, 1032–1037.
[7] Deisenhofer, J., Epp, O., Miki, K., Huber, R. and Michel, H. (1985) Structure of the protein subunit in the photosynthetic reaction center of Rhodopseudomonas viridis at 3 A resolution. Nature 318, 618–624.
[8] Jordan, P., Fromme, P., Witt, H.T., Klukas, O., Saenger, W. and Krauss, N. (2001) Three-dimensional structure of cyanobacterial photosystem I and A. Nature 411, 599–617.
[9] Rhee, K.H., Morris, E.P., Barber, J. and Kühbrandt, W. (1998) Three-dimensional structure of the plant photosystem II reaction centre at 8 A resolution. Nature 396, 283–286.
[10] Zouni, A., Witt, H.T., Kern, J., Fromme, P., Krauss, N., Saenger, W. and Orth, P. (2001) Crystal structure of Photosystem II from Synecococcus elongatus at 3.8 Å resolution. Nature 409, 739–743.
[11] Ben-Shem, A., Frow, F. and Nelson, N. (2003) Crystal structure of plant photosystem I. Nature 426, 630–635.
[12] Nitschke, W. and Rutherford, A.W. (1991) Photosynthetic reaction centres: variations on a common structural theme? Trends Biochem. Sci. 16, 241–245.
[13] Vermeglio, A. and Clayton, R.K. (1977) Kinetics of electron transfer between the primary and the secondary electron acceptor in reaction centers from Rhodopseudomonas sphaeroides. Biochim. Biophys. Acta 461, 159–165.
[14] Brettel, K. (1997) Electron transfer and arrangement of the redox cofactors in Photo system I. Biochim. Biophys. Acta 1318, 322–373.
[15] Marcus, R.A. (1956) On the theory of oxidation–reduction reactions involving electron transfer. J. Chem. Phys. 24, 966–978.
[16] Moser, C.C. and Dutton, P.L. (1996) Outline of theory of protein electron transfer in: Protein electron transfer (Bendall, D.S., Ed.), pp. 1–21, BIOS Scientific Publishers, Oxford.
[17] Baymann, F., Brugna, M., Muhlenhoff, U. and Nitschke, W. (2001) Daddy, where did (PSII) come from? Biochim. Biophys. Acta 1507, 129–139.
[18] Haukaa, G., Schoedl, T., Remug, H. and Tisotis, G. (2001) The reaction center of green sulfur bacteria [C3H4]. Biochim. Biophys. Acta 1507, 260–277.
[19] Heinreckel, M. and Golbeck, J.H. (2007) Helio(bacterial) photosynthesis. Photosyn. Res. 92, 35–53.
[20] Heinreckel, M., Shen, L. and Golbeck, J.H. (2007) Identification and characterization of PsbH, the dicluster ferredoxin that harbors the terminal electron acceptors F(A) and F(B) in Heliobacterium modesticaldum. Biochemistry 46, 2530–2536.
[21] Nitschke, W., Feier, U. and Rutherford, A.W. (1990) Photosynthetic reaction center of green sulfur bacteria studied by EPR. Biochim. Biophys. CA 2834–3842.
[22] Nitschke, W., Setif, P., Liebl, U., Feier, U. and Rutherford, A.W. (1990) Reaction center photochemistry of Heliobacterium chlorum. Biochim. Biophys. CA 11079–11088.
[23] Ben-Shem, A., Frow, F. and Nelson, N. (2004) Evolution of photosystem I – from symmetry through pseudo-symmetry to asymmetry. FEBS Lett. 564, 274–280.
[24] Brettel, K. and Liebl, W. (2001) Electron transfer in photosystem I. Biochim. Biophys. Acta 1507, 100–114.
[25] Srinivasan, N. and Golbeck, J.H. (2009) Protein-cofactor interactions in bioenergetic complexes: the role of the A1A and A1B phylloquinones in Photosystem I. Biochim. Biophys. Acta 1787, 1057–1088.
[26] Mukarashin, M.M. and Ivanov, B.N. (2010) The production and scavenging of reactive oxygen species in the plastoquinone pool of chloroplast thylakoid membranes. Physiol Plant 140, 103–110.
[27] Jagnannathan, B. and Golbeck, J.H. (2009) Breaking biological symmetry in membrane proteins: the asymmetrical orientation of Psac on the pseudo-C2 symmetric Photosystem I core. Cell Mol Life Sci. 66, 1257–1270.
[28] Munekage, Y., Hojo, M., Meurer, J., Endo, T., Takeda, M. and Shikanai, T. (2002) PGRS is involved in cyclic electron flow around Photosystem I and is essential for photoprotection in Arabidopsis. Cell 110, 361–371.
[29] DalCorso, G., et al. (2008) A complex containing PGR1 and PGR5 is involved in the switching between linear and cyclic electron flow in Arabidopsis. Cell 132, 273–285.
[30] Iwai, M., Takizawa, K., Tokutsu, R., Okamuro, A., Takahashi, Y. and Minagawa, J. (2010) Isolation of the elusive supercomplex that drives cyclic electron flow in photosynthesis. Nature 464, 1210–1213.
Sebban, P. and Wraight, C.A. (1989) Heterogeneity of the P-Gopher, A., Blatt, Y., Schönfeld, M., Okamura, M.Y. and Feher, G. (1985) The Woodbury, N.W., Parson, W.W., Gunner, M.R., Prince, R.C. and Dutton, P.L. (1986) Kinetic studies on the Rappaport, F., Guergova-Kuras, M., Cohen, R.O., Golbeck, J.H., Vass, I. and Campbell, D.A. (2006) Charge recombination of photosystem II and the effect of pH and temperature. Biochim. Biophys. Acta 78, 1249–1250.

Krieger, A., Weiss, E. and Demeter, S. (1993) Low-pH-mediated Ca2+ ion release in the water-splitting system is accompanied by a shift in the midpoint redox potential of the primary quinone acceptor, Qb, in photosystem II. Biochim. Biophys. Acta 1229, 193–201.

Krieger, A. and Krieger-Liszkay, A. (2002) Kinetics and pathways of charge recombination in photosystem II. Biochemistry 41, 8518–8527.

Boussac, A., Sugiuira, M. and Rappaport, F. (2011) Probing the quinone binding site of photosystem II from Thermosynechococcus elongatus containing either Pb3A or Pb3M as the D1 protein through the binding characteristics of herbicides. Biochim. Biophys. Acta 1807, 119–129.

Krieger, A. and Krieger-Liszkay, A. (2002) Influence of herbicides on the redox properties of the primary quinone acceptor of photosystem II: relevance to photodamage and photobiology. Biochimica 37, 17339–17344.

Krieger-Liszkay, A. and Krieger, A.W. (1998) Inhibition of herbicide-induced oxidative stress in photosystem II. Trends Biochem. Sci. 30, 648–653.

Takahashi, R., Hasegawa, K., Takeko, A. and Noguchi, T. (2010) Structures and binding sites of phenolic herbicides in the Q(B) pocket of photosystem II. Biochemistry 49, 5445–5454.

Boscaro, C., Brown, W., Ouk, S. and Rappaport, F. (2011) Probing the quinone binding site of photosystem II from Thermosynechococcus elongatus containing either Pb3A or Pb3M as the D1 protein through the binding characteristics of herbicides. Biochim. Biophys. Acta 1229, 193–201.

Krieger, A. and Krieger-Liszkay, A. (2002) Influence of herbicides on the redox properties of the primary quinone acceptor of photosystem II: relevance to photodamage and photobiology. Biochimica 37, 17339–17344.

Krieger-Liszkay, A. and Krieger, A.W. (1998) Inhibition of herbicide-induced oxidative stress in photosystem II. Trends Biochem. Sci. 30, 648–653.

Takahashi, R., Hasegawa, K., Takeko, A. and Noguchi, T. (2010) Structures and binding sites of phenolic herbicides in the Q(B) pocket of photosystem II. Biochemistry 49, 5445–5454.

Boscaro, C., Brown, W., Ouk, S. and Rappaport, F. (2011) Probing the quinone binding site of photosystem II from Thermosynechococcus elongatus containing either Pb3A or Pb3M as the D1 protein through the binding characteristics of herbicides. Biochim. Biophys. Acta 1229, 193–201.

Krieger, A. and Krieger-Liszkay, A. (2002) Influence of herbicides on the redox properties of the primary quinone acceptor of photosystem II: relevance to photodamage and photobiology. Biochimica 37, 17339–17344.

Krieger-Liszkay, A. and Krieger, A.W. (1998) Inhibition of herbicide-induced oxidative stress in photosystem II. Trends Biochem. Sci. 30, 648–653.

Takahashi, R., Hasegawa, K., Takeko, A. and Noguchi, T. (2010) Structures and binding sites of phenolic herbicides in the Q(B) pocket of photosystem II. Biochemistry 49, 5445–5454.

Boscaro, C., Brown, W., Ouk, S. and Rappaport, F. (2011) Probing the quinone binding site of photosystem II from Thermosynechococcus elongatus containing either Pb3A or Pb3M as the D1 protein through the binding characteristics of herbicides. Biochim. Biophys. Acta 1229, 193–201.

Krieger, A. and Krieger-Liszkay, A. (2002) Influence of herbicides on the redox properties of the primary quinone acceptor of photosystem II: relevance to photodamage and photobiology. Biochimica 37, 17339–17344.

Krieger-Liszkay, A. and Krieger, A.W. (1998) Inhibition of herbicide-induced oxidative stress in photosystem II. Trends Biochem. Sci. 30, 648–653.

Takahashi, R., Hasegawa, K., Takeko, A. and Noguchi, T. (2010) Structures and binding sites of phenolic herbicides in the Q(B) pocket of photosystem II. Biochemistry 49, 5445–5454.

Boscaro, C., Brown, W., Ouk, S. and Rappaport, F. (2011) Probing the quinone binding site of photosystem II from Thermosynechococcus elongatus containing either Pb3A or Pb3M as the D1 protein through the binding characteristics of herbicides. Biochim. Biophys. Acta 1229, 193–201.

Krieger, A. and Krieger-Liszkay, A. (2002) Influence of herbicides on the redox properties of the primary quinone acceptor of photosystem II: relevance to photodamage and photobiology. Biochimica 37, 17339–17344.

Krieger-Liszkay, A. and Krieger, A.W. (1998) Inhibition of herbicide-induced oxidative stress in photosystem II. Trends Biochem. Sci. 30, 648–653.

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