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Review

Co-existence of photosynthetic and respiratory activities in cyanobacterial thylakoid membranes☆

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ABSTRACT

The thylakoid membranes of cyanobacteria are the major sites of respiratory electron transport as well as photosynthetic light reactions. The photosynthetic and respiratory electron transport chains share some components, and their presence in the same membrane opens up the possibility for a variety of "unorthodox" electron transport routes. Many of the theoretically possible electron transport pathways have indeed been detected in particular species and circumstances. Electron transport has a crucial impact on the redox balance of the cell and therefore the pathways of electron flow in the cyanobacterial thylakoid membrane must be tightly regulated. This review summarises what is known of cyanobacterial electron transport components, their interactions and their sub-cellular location. The role of thylakoid membrane organisation in controlling electron transport pathways is discussed with respect to recent evidence that the larger-scale distribution of complexes in the membrane is important for controlling electron exchange between the photosynthetic and respiratory complexes. The distribution of complexes on scales of 100 nm or more is under physiological control, showing that larger-scale thylakoid membrane re-arrangement is a key factor in controlling the crosstalk between photosynthetic and respiratory electron transport. This article is part of a Special Issue entitled: Dynamic and ultrastructure of bioenergetic membranes and their components.

1. Introduction—comparison of thylakoid membranes in chloroplasts and cyanobacteria

Cyanobacteria and chloroplasts are closely-related and therefore it is unsurprising that their thylakoid membranes have many features in common. The thylakoid membranes form a distinct system within the cytoplasm (or the stroma in chloroplasts) with a protein composition distinct from the surrounding envelope membrane (the cytoplasmic membrane in cyanobacteria or the inner envelope membrane in chloroplasts) [1,2]. In chloroplasts there appears to be no physical connection between the two membrane systems [3]. The question of whether cyanobacterial thylakoid membranes are physically contiguous with the cytoplasmic membrane has been harder to resolve [2,3]. Studies using cryo-electron tomography reveal no obvious direct connections between the two membrane systems [4–6]. However the thylakoid membranes in several species of cyanobacteria can be seen to branch out from "thylakoid centres": rod-like bodies which are in close proximity to the thylakoid membrane [4]. The topology of chloroplast and cyanobacterial thylakoid membranes is superficially very different. Cyanobacterial thylakoids form a series of roughly parallel double membrane layers enclosing the thylakoid lumen and separating it from the cytoplasm [4–6]. The membranes are sometimes organised approximately as concentric cylinders inside the cytoplasmic membrane and surrounding the central cytoplasm containing the nucleoid [6,7]. In other cases they are less regular: in *Synechocystis* sp. PCC6803 they form a series of curved, roughly parallel sheets radiating out from the thylakoid organising centres [4,5]. In higher plant chloroplasts, the thylakoid membranes have an obvious large-scale lateral heterogeneity, with stacks of tightly-appressed membranes forming grana which are distinct in both structure and protein content from the connecting stroma lamellae [8]. The formation of grana requires interactions involving the LHCII light harvesting complex, which is not found in cyanobacteria [8]. In addition, the presence of phycobilisome light-harvesting antenna complexes on the cytoplasmic (stromal) thylakoid membrane surface prevents membrane appression both in cyanobacteria and red algal chloroplasts. However, it is intriguing that the CURT1 protein, recently shown to be required for the tight thylakoid membrane curvature at the margins of the grana, has conserved homologs in cyanobacteria

Abbreviations: ARTO, alternative respiratory terminal oxidase; CURT1, curvature thylakoid 1 protein; Cyt, cytochrome; Fd, ferredoxin; FFEM, freeze-fracture electron microscopy; Flv, flavodiiron protein; FNR, ferredoxin-NADP oxidoreductase; FRAP, Fluorescence Recovery after Photobleaching; GFP, green fluorescent protein; Hox, bidirectional Ni–Fe hydrogenase; LHCII, light-harvesting chlorophyll a/b-binding protein of photosystem II; NADP, nicotinamide adenine dinucleotide phosphate; NDH, NAD(P)H-dehydrogenase; PET, photosynthetic electron transport; pmf, proton-motive force; PQ, plastoquinone; PSI, photosystem I; PSII, photosystem II; PGR5, proton gradient regulation protein 5; PGRL1, PGR5-like protein 1; PM, plasma (cytoplasmic) membrane; PTOX, plastoquinol terminal oxidase; RET, respiratory electron transport; SDH, succinate dehydrogenase; TM, thylakoid membrane; YFP, yellow fluorescent protein

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[9]. It suggests that at least one factor controlling the ultrastructure of chloroplast thylakoid membranes could play a comparable role in cyanobacteria.

In contrast to plant chloroplasts, cyanobacterial thylakoid membranes show no obvious lateral heterogeneity in their ultrastructure, at the resolutions afforded by light microscopy and thin-section electron microscopy [7,10]. However, there is increasing evidence that specific membrane proteins can be distributed very unevenly in the membrane, as will be discussed in Section 4. Thylakoid membranes are particularly densely packed with membrane-integral proteins. This dense protein packing severely restricts the lateral diffusion of chlorophyll–proteins in chloroplast grana membranes [11], and protein mobility in cyanobacterial thylakoid membranes is also severely restricted, as exemplified by the near-immobility of photosystem II (PSII) complexes under some conditions [12,13]. Restricted protein mobility has functional consequences for any process that requires the lateral movement of proteins in the membrane, potentially including electron transport as well as mechanisms of biogenesis, regulation and repair [14]. Some consequences of restricted protein mobility with respect to electron transport and its regulation in cyanobacteria will be discussed in Sections 4–6.

In comparison to chloroplast thylakoid membranes, cyanobacterial thylakoid membranes harbour a much greater variety of electron transport complexes. Respiratory electron transport ("chlororespiration") can occur in plant thylakoid membranes, but appears to be a quantitatively minor process [15]. In plant cells, the vast majority of respiratory activity takes places in the mitochondria, segregated well away from photosynthetic electron transport in the chloroplasts. By contrast, in cyanobacteria the thylakoid membranes are the major sites of respiratory electron transport as well as photosynthesis [16,17]. The coexistence of photosynthesis and respiration in the same membrane in cyanobacteria offers possibilities for flexible and versatile electron transport pathways, but also presents special challenges for controlling the modes of electron transport according to the physiological needs of the cell. Section 2 summarises the known cyanobacterial electron transport components and the electron transport pathways that they are involved in, while Section 3 discusses the mechanisms that may control cyanobacterial electron transport. Sections 4–6 focus on the role played by thylakoid membrane heterogeneity and dynamics in cyanobacterial electron transport and its regulation.

2. Cyanobacterial electron transport pathways

2.1. Cyanobacterial electron transport complexes and their location

Table 1 provides a catalogue of the known cyanobacterial electron transport complexes, together with information on their sub-cellular location and the electron transport pathways that they are known to be involved in. Some of these electron transport pathways are illustrated schematically in Fig. 1 and discussed further below. Note that this review focuses on electron transport in unicellular cyanobacteria. In filamentous heterocyst-forming cyanobacteria such as *Anabaena*, an extra level of complexity is provided by the different modes of electron transport that predominate in the thylakoid membranes of different cell types (vegetative cells and heterocysts) [18]. This extra complexity is beyond the scope of this review.

2.2. Linear photosynthetic electron transport

The classic mode of photosynthetic electron transport involves light-powered electron extraction from water by PSII. The electrons are then passed via plastoquinone to cytochrome b_6f and then via plastocyanin or cytochrome c_6 to photosystem I (PSI) [16]. Plastocyanin and cytochrome c_6 are both located in the thylakoid lumen and they play similar roles [16]. In many cyanobacteria they are differentially expressed according to the availability of copper and iron [19]. Cytochrome c_M is a third possible electron carrier with this role in some cyanobacteria [20,21]: in *Synechocystis* it is more strongly expressed under stress conditions [20]. Electrons from the acceptor side of PSI are used to reduce $NADP^+$ to NADPH, via transfer from ferredoxin and FNR [22]. Linear photosynthetic electron transport takes place exclusively in the thylakoid membranes, the sole known exception being the atypical cyanobacterium *Gloeobacter* which lacks thylakoid membranes [23,24]. In this cyanobacterium, photosynthetic electron transport takes place in

Table 1
Catalogue of cyanobacterial electron transport components. PM: cytoplasmic (plasma) membrane; TM: thylakoid membrane; PET: photosynthetic electron transport; RET: respiratory electron transport. Locations apply to typical thylakoid membrane-containing cyanobacteria (not *Gloeobacter*). Note that not all complexes are found in all cyanobacteria.

Complex	Activity	Location(s)	Pathway(s)	Ref(s)
ARTO (alternative respiratory terminal oxidase)	Terminal quinol oxidase	PM	RET	[17]
Cytochrome *bd*	Terminal quinol oxidase	TM (+PM?)	RET	[17]
Cytochrome b_6f	PQ-cytochrome *c*/plastocyanin oxidoreductase	TM	Linear and cyclic PET, RET	[17]
Cytochrome c_6	Mobile electron carrier	Thylakoid lumen (+ periplasm?)	Linear and cyclic PET, RET	[17]
Cytochrome c_M	Mobile electron carrier	Thylakoid lumen (+ periplasm?)	Linear and cyclic PET, RET	[20,21]
Cytochrome oxidase (aa_3-type) (COX; Complex IV)	Cytochrome *c*/plastocyanin terminal oxidase	TM (+PM?)	RET, photosynthetic electron valve	[17]
Ferredoxin	Mobile electron carrier	Cytoplasm, peripheral TM association	Linear PET, cyclic PET	[22,33]
FNR	Ferredoxin-NADP oxidoreductase	Cytoplasm/TM	Linear PET, cyclic PET	[26–28,47]
Flv1/3	NADPH-oxygen oxidoreductase	Cytoplasm	Aerobic electron valve (PSI acceptor)	[43]
Flv2/4	PSII electron acceptor, final electron acceptor unknown	Cytoplasm	Electron valve (PSII acceptor)	[44]
HOX (bidirectional hydrogenase)	NAD(P)H-H^+ oxidoreductase	Cytoplasm	Anaerobic electron valve	[45]
NDH-1 (Complex I)	Quinone reductase—electron donors possibly ferredoxin and/or NADPH	TM	Cyclic PET, possibly RET	[31,33–35]
NDH-2	NAD(P)H-quinone oxidoreductase	PM (& TM?)	RET	[17]
Photosystem I	Cytochrome c_6/plastocyanin-ferredoxin oxidoreductase	TM	Linear and cyclic PET	[22,34,39]
Photosystem II	Water-quinone oxidoreductase	TM	Linear PET, water-water cycle	[37,38]
Plastocyanin	Mobile electron carrier	Thylakoid lumen	Linear and cyclic PET, RET	[17]
Plastoquinone	Mobile electron carrier	TM, PM	Linear PET, cyclic PET, RET, water–water cycle	[17]
PGR5	Ferredoxin-quinone oxidoreductase	Cytoplasm (surface of TM)	Cyclic PET	[39]
PTOX	Terminal quinol oxidase	Cytoplasm	Water–water cycle	[37,38]
Succinate dehydrogenase (Complex II)	Succinate-quinone oxidoreductase	TM	RET	[17,35]

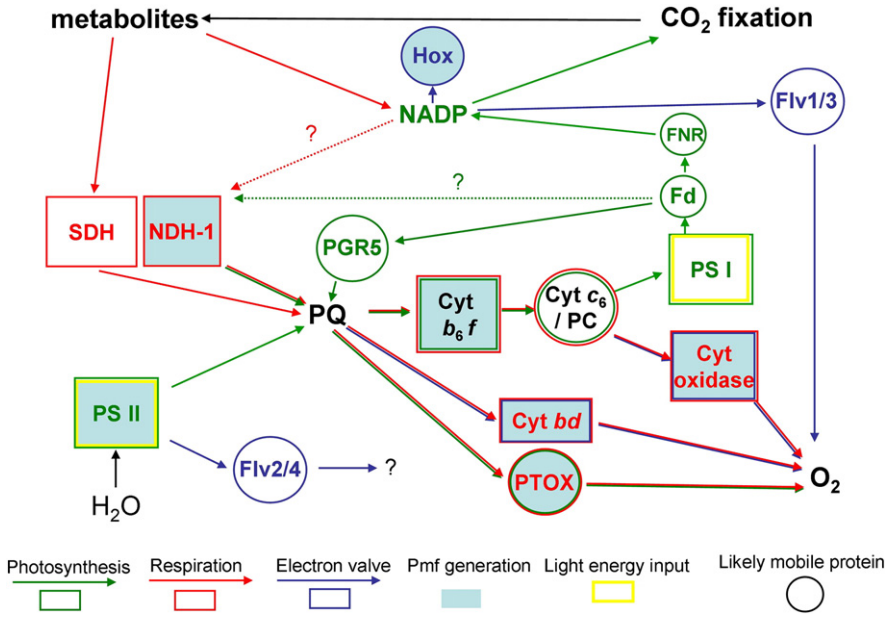

Fig. 1. Pathways of electron transport in and around cyanobacterial thylakoid membranes. Arrows indicate directions of electron flow. Proteins and protein complexes are indicated by frames: small molecules are shown without frames. Frame colour indicates involvement in photosynthesis, respiration or electron dissipation as indicated in the key. Additionally, yellow boxes indicate sites of light energy input and light blue backgrounds show sites of proton-motive force generation (by proton pumping and/or proton consumption/release on a particular side of the membrane). Cytoplasmic, lumenal or peripheral membrane proteins are likely to be rapidly mobile (therefore able to redistribute on timescales of seconds for fast switching of electron transport) and are given circular frames. Membrane-integral protein complexes are likely to show only very restricted mobility and are given square frames. For further information and references see Table 1.

the cytoplasmic membrane, whose organisation will be discussed further in Section 4.2. In all other known cyanobacteria, photosynthetic electron transport is confined to the thylakoid membrane by virtue of the fact that the photosystems are found only in this membrane. There is some evidence for the presence of partially-assembled reaction centre precursor complexes in the cytoplasmic membrane, but fully-assembled and functional complexes are found only in the thylakoids (with the exception of *Gloeobacter*) [25]. Thylakoid membrane association extends as far as FNR. This is not a membrane-intrinsic protein, but different forms of the enzyme are associated with the cytoplasmic side of the membrane via linkage to the phycobilisomes or direct association with the membrane surface [26–28]. Linear photosynthetic electron transport serves to generate a proton gradient for ATP synthesis due to proton release from water oxidation at PSII and proton translocation at the cytochrome b_6f complex (Fig. 1). In addition it generates reducing power for the cell through extraction of electrons from water. Much of this reducing power is used for CO_2 fixation (Fig. 1).

2.3. Respiratory electron transport

Respiratory electron transport takes electrons derived from the catabolism of stored metabolites and passes them to an external electron acceptor. The acceptor is usually oxygen, although many bacteria can make use of alternative electron acceptors in anoxic environments [29]. Respiration in cyanobacteria serves to maintain a transmembrane proton gradient (required to power ATP production and for some active transport) in the dark, at the expense of the oxidation of photosynthate produced in the light. A major fraction of respiratory electron transport takes place in the thylakoid membranes, where respiratory electron transport chains share some components (plastoquinone, cytochrome b_6f and plastocyanin/cytochrome *c* [16,17]) with the photosynthetic electron transport chain (Fig. 1). This gives an additional possible role for the respiratory complexes in "redox poising", that is donating or removing electrons to prevent excessive oxidation or reduction of the photosynthetic electron transport chain, and plastoquinone in particular [30]. Respiration in cyanobacteria is more diverse and less well-characterised than photosynthesis. Cyanobacteria possess several alternative electron donor complexes and oxidases (Fig. 1, Table 1), and the physiological roles of some of the complexes are still unclear [17]. In particular, controversy surrounds the role of the cyanobacterial homolog of mitochondrial Complex I (NDH-1). This has been proposed as a major input of electrons into the respiratory electron transport chain [31]. However, mutagenesis suggests that Complex II (succinate dehydrogenase, SDH) is more important for electron donation to the respiratory chain [32]. Additionally, the NDH-1 complex is present in several alternative forms with distinct physiological roles [31,33]. Furthermore, cyanobacteria lack homologs of the mitochondrial and *Escherichia coli* subunits that extract electrons from NADH (NuoEFG in *E. coli*), raising uncertainty as to the nature of the physiological electron donor(s) to NDH-1 [33]. It is plausible that cyanobacterial NDH-1 accepts electrons directly from ferredoxin reduced by PSI, consistent with a principal function in cyclic photosynthetic electron transport (see Section 2.4) rather than respiratory electron transport [34]. Both NDH-1 and SDH are detected exclusively in the thylakoid membranes [2,35], and cytochrome oxidase is probably also found mostly in the thylakoid membrane [17,36]. In addition, a terminal quinol oxidase, cytochrome *bd*, is a probable thylakoid membrane component [17] and some cyanobacteria contain another terminal quinol oxidase, PTOX, a cytoplasmic protein associated with the thylakoid surface [17,37,38] which is discussed further in Section 2.5. The most likely widespread pathways for respiratory electron transport in the thylakoid membranes are from SDH to cytochrome oxidase via plastoquinone, cytochrome b_6f and cytochrome c_6 or plastocyanin, and/or from SDH to cytochrome *bd* via plastoquinone (Fig. 1). There is also a likely complete respiratory electron transport chain in the cytoplasmic membrane, with a different set of components likely including the quinone reductase NDH-2 and the quinol oxidase ARTO [17]. This chain will not be considered further here as it has no direct interaction with the thylakoid membrane.

2.4. Cyclic photosynthetic electron transport

Cyclic photosynthetic electron transport in cyanobacteria uses PSI for light-powered cyclic electron flow, with generation of a trans-

thylakoid proton gradient for ATP synthesis. This requires electrons to be returned from the acceptor side of PSI to the cytochrome b_6f complex. Cyanobacteria seem to have at least two ways to do this (Fig. 1). One route involves the PGR5 protein, which has ferredoxin/plastoquinone oxidoreductase activity [39], while the second involves NDH-1, possibly through direct interaction with ferredoxin [34]. There is also a plausible indirect route which would involve synthesis of sugars via the Calvin cycle, followed by their breakdown via the tricarboxylic acid cycle [40] and the supply of reducing equivalents back to plastoquinone via the respiratory complexes. In contrast to linear photosynthetic electron transport, none of these forms of cyclic photosynthetic electron transport involve PSII, and they do not generate reducing power for anabolic chemistry.

2.5. The water–water cycle

Inspection of the suite of electron transport components available in the thylakoid membrane suggests the possibility of another mode of photosynthetic electron transport, this time involving PSII only. Electrons could be extracted from water by PSII and then eventually passed to a terminal oxidase which would reduce oxygen to produce water again (Fig. 1). The net result would be light-powered generation of a proton-motive force. Such a mode of electron transport appears physiologically important in some marine cyanobacteria, which use PTOX as the oxidase, with generation of a trans-thylakoid proton gradient due to proton release from water-splitting in the thylakoid lumen and proton uptake from oxygen reduction in the cytoplasm [37,38]. Here it could be advantageous in comparison to PSI-mediated cyclic electron transport if PSI biogenesis is precluded by iron-limitation [37,38,41]. Similar modes of electron transport could potentially occur under other situations in cyanobacteria lacking PTOX, since a water–water cycle was detected in a *Synechocystis* mutant lacking PSI [42].

2.6. Electron valves—Flv1–4 and the hydrogenase

Some further electron transport components appear to function primarily as "electron valves", dissipating excess electrons under conditions when the photosynthetic electron transport chain is transiently over-reduced (for example if NADPH is generated faster than it can be used in the Calvin cycle) (Fig. 1). Over-reduction of the chain could result in photodamage, so it is important to rapidly dissipate excess electrons under these conditions. The soluble cytoplasmic flavodiiron proteins Flv1–4 are oxidases involved in photoprotective dissipation of excess electrons from the photosynthetic electron transport chain: Flv1 and Flv3 form a heterocomplex that takes electrons from the acceptor side of PSI and donates them to oxygen [43], while Flv2 and Flv4 take electrons from the acceptor side of PSII before donating them to an unknown acceptor [44]. However, the respiratory terminal oxidases also seem to play a role in photoprotection by removing excess electrons from the photosynthetic electron transport chain: mutants lacking cytochrome oxidase and the thylakoid quinol oxidase cytochrome *bd* are particularly sensitive to fluctuating light [17]. The bidirectional Ni:Fe hydrogenase Hox also seems to act as an electron valve, by reducing H^+ to H_2 with electrons taken from the acceptor side of PSI [45]. Hox could serve as an electron valve under anaerobic conditions when oxygen is not available as an electron acceptor to take excess electrons from the photosynthetic electron transport chain via Flv1/3 or the respiratory oxidases.

2.7. Different modes of electron transport and their influence on the redox balance of the cell

The different modes of electron transport discussed above have different effects on the redox status of the cell. Linear photosynthetic electron transport results in the reduction of the cytoplasm through generation of NADPH with electrons extracted from water (Section 2.2). The various modes of cyclic electron transport and the water–water cycle (Sections 2.4 and 2.5) are neutral with respect to the redox status of the cytoplasm. Respiratory electron transport (Section 2.3) and the various electron valves (Section 2.6) result in net loss of reductant. Correct redox balance of the cytoplasm is crucial for cell biochemistry, so it is clearly necessary to have strict control over the numerous possible electron transport pathways in the thylakoid membrane. The different ways in which electron transport routes could be controlled are outlined in Section 3.

3. Mechanisms to control electron transport in cyanobacteria

3.1. Regulation at the level of gene expression

The most straightforward way to control pathways of electron flow is to change the proportion of the different electron transport complexes in the membrane. One might expect that expression of electron transport components is controlled by the light environment and/or the redox status of the cell, and indeed there is evidence for light and redox control of the expression of photosynthetic and respiratory complexes at the level of transcription. For example, a comprehensive study of the effects of growth under light predominantly exciting PSI and PSII showed changes in transcript levels for many components of the bioenergetic machinery, including both photosystems and the respiratory cytochrome oxidase [46]. An interesting example of an electron transport switch mediated through regulation of gene expression is the production of long and short isoforms of FNR in *Synechocystis*, mediated by alternative translational start sites [28,47]. The longer isoform is associated with the phycobilisomes, with likely effects on electron transport pathways [28]. Light and CO_2 levels influence the levels of antisense RNAs to many genes, including electron transport complexes, suggesting a role for antisense RNAs in regulation of electron transport gene expression [48]. Direct visualisation of fluorescently-tagged NDH-1 complexes in *Synechococcus* sp. PCC7942 shows that the number of complexes per cell changes with illumination conditions, on a timescale of hours [35]. This is an indication that regulation of electron transport gene expression is effective over relatively slow timescales (about 1 h and upwards). Therefore it can provide a means to adapt electron transport to long-term trends in the environment of the cells (e.g. mean light intensity or oxygen level) but it cannot be an effective means to cope with faster changes such as rapid fluctuations in light intensity.

3.2. Regulation of photosynthetic light-harvesting

One rapid way to control photosynthetic electron transport is through regulation of light-harvesting for PSI and PSII. Two mechanisms have been characterised in cyanobacteria, both effective on rapid timescales of seconds to minutes. Quenching of excitation energy at the phycobilisomes is mediated in many cyanobacteria by the Orange Carotenoid-Binding protein (OCP) whose quenching activity is activated by exposure to strong blue light [49]. Quenching by the OCP reduces energy transfer to both photosystems. Therefore this mechanism would be expected to down-regulate photosynthetic electron transport relative to respiration. State transitions rapidly regulate excitation energy transfer from phycobilisomes to PSI vs. PSII, in response to changes in the redox state of the plastoquinone pool [50]. The redox state of plastoquinone is influenced by respiratory as well as photosynthetic activity, and thus state transitions provide a feedback between the general redox status of the cell and the relative activities of the two photosystems [51]. State transitions provide rapid control over the relative activities of the two photosystems, but cyanobacteria must have additional mechanisms to regulate the numerous possible interactions between the photosynthetic and respiratory electron transport chains (Fig. 1).

3.3. Electron transport supercomplexes

There is evidence in many bioenergetic membranes for the presence of electron transport "supercomplexes": that is, a structural association between two or more individual electron transport complexes. A number of physiological functions have been ascribed to supercomplexes, ranging from acting as biogenesis platforms [52] to minimising the production of reactive oxygen species [53]. However, a key role for supercomplexes is in steering the pathways of electron transport. This has recently been demonstrated for the mitochondrial "respirasome", a supercomplex of electron transport complexes I, III and IV [54]. The respirasome appears effective in controlling the behaviour of the mobile electron carriers ubiquinone and cytochrome *c*, which both seem to be confined to dedicated pools associated with the respirasomes [54] although the confinement of cytochrome *c* remains controversial [55]. Such a mode of organisation has obvious potential for controlling other electron transport switches, such as cyclic versus linear photosynthetic electron flow. Indeed a supercomplex has been isolated from thylakoid membranes of the green alga *Chlamydomonas reinhardtii* which contains all the ingredients for a functional cyclic electron transport pathway involving PSI, cytochrome b_6f, ferredoxin, FNR and PGRL1, a membrane-integral complex shown to be essential for ferredoxin-mediated cyclic electron flow in *Arabidopsis* [56]. Importantly, the formation of the *Chlamydomonas* supercomplex is triggered by reducing conditions [57], indicating that the supercomplex is a redox-responsive electron transport switch involved in controlling the balance between linear and cyclic electron flow [56,57]. In contrast to *Chlamydomonas*, evidence for electron transport supercomplexes in cyanobacteria is sparse. Of course, this does not preclude their existence: it may simply be that the right conditions have not yet been found to isolate them. The existence of one electron transport supercomplex can be inferred in cyanobacteria, since one of the forms of FNR has been shown to be linked to the phycobilisomes via a special linker domain [26,28,47]. Phycobilisomes must be capable of association with both photosystems, since they transfer energy to PSI as well as to PSII [58]. Therefore a PSI–phycobilisome–FNR supercomplex could serve to steer electrons from the acceptor side of PSI to FNR, with possible effects on the balance of electron flow down different pathways (Fig. 1).

Given the recent emphasis on supercomplexes in the literature, it is important to note that electron transport is not always confined within supercomplexes: it can also be delocalised over large areas of the membrane by long-range diffusion of mobile electron carriers such as quinones and cytochrome *c*. Green plant chloroplasts are an obvious example, since the spatial separation between PSII in the grana and PSI in the stroma lamellae implies that linear photosynthetic electron transport takes place over extended length-scales [59]. In cyanobacteria, in contrast to chloroplasts, PSI can be found in close proximity to PSII [60], which implies that the length scale for linear photosynthetic electron transport could be short. However, the length scale of electron transport is not directly established in cyanobacteria.

3.4. Lateral segregation in the membrane

Another option for controlling pathways of electron transport in cyanobacteria would be a larger-scale lateral segregation of electron transport activities in the thylakoid membrane. The complexes involved in particular electron transport activities could be segregated into functional islands in the membrane on a scale of 100 nm or more. This would provide a way to preferentially channel the mobile electron carriers to acceptors located in close proximity in the same functional island. Switching between different modes of electron transport would then require a large-scale redistribution of complexes in the membrane. There is now evidence for one such mechanism, which will be discussed in Section 5, following a general discussion of mobility and lateral heterogeneity in cyanobacterial thylakoid membranes in Section 4.

4. Mobility and lateral heterogeneity in cyanobacterial thylakoid membranes

4.1. Protein mobility in and around the thylakoid membrane

The long-range diffusion of chlorophyll–proteins in cyanobacterial thylakoid membranes has been probed by Fluorescence Recovery after Photobleaching (FRAP) [10]. The cyanobacterium *Synechoccccus* sp. PCC7942 is a good model for such studies because of its elongated cells and regular thylakoid membrane topology [10]. FRAP studies show that PSII is virtually immobile in the membrane under normal conditions [12,13], although a proportion of PSII is mobilised after exposure to intense red light, and PSII can undergo a partial redistribution in the membrane under such conditions, on a timescale of a few minutes [61]. The IsiA chlorophyll–protein (expressed after iron-starvation) is rather more mobile, but even so, its diffusion is rather slow in comparison to protein diffusion in more typical bacterial membranes [13]. The message from these studies is that the mobility of membrane-integral complexes in cyanobacterial thylakoids is rather constrained, presumably due to supramolecular interactions and macromolecular crowding. Large-scale membrane re-organisation is possible but likely to be rather slow (on a timescale of 10s of minutes rather than seconds). By contrast, the phycobilisomes diffuse relatively freely on the cytoplasmic surface of the thylakoid membrane [12,62], indicating that diffusion of surface-associated and soluble proteins in this region could be much more rapid [14]. This has obvious implications for the rearrangement of electron transport complexes: soluble cytoplasmic complexes such as ferredoxin and Flv1–4 could redistribute very rapidly, while any membrane rearrangements involving the membrane integral complexes would be much slower (Fig. 1). There is as yet no direct data on the mobility of proteins in the cyanobacterial thylakoid lumen, although studies on the mobility of plastocyanin in green plant chloroplasts [59] and phycobilins in cryptophyte chloroplasts [63] suggest that the mobility of thylakoid lumenal proteins could be generally rather constrained. This could have implications for the length-scale of electron transport involving plastocyanin or cytochrome c_6, but as yet we have insufficient information on the question in cyanobacteria.

4.2. Evidence for spatial heterogeneity in cyanobacterial thylakoid membrane composition

Further understanding of the pathways and control of electron transport in cyanobacteria will require a much more comprehensive picture of the distribution of electron transport complexes in the thylakoid membrane. To date, the distribution of complexes in the membrane has been probed by a number of techniques. Freeze-fracture electron microscopy (FFEM) has the advantage that it reports on the layout of the membrane at relatively high resolution in flash-frozen cells which can be adapted to different physiological states. It has the drawback that only some membrane-integral protein complexes can be recognised. In many cyanobacteria, the curvature of the thylakoid membranes means that only small patches of the membrane can be visualised, so FFEM gives only a fragmentary view of the membrane layout. FFEM has been used to show that PSII is sometimes arranged in regularly spaced rows of dimers, with the packing density dependent on conditions and altered in phycobilisome mutants [64]. Negative-staining electron microscopy on partially-solubilised cyanobacterial thylakoid membranes indicated the presence of more extended PSII domains composed of multiple adjacent rows of PSII dimers [60]. If these domains occur in vivo they could result in significant spatial separation between PSII and other membrane components (including PSI and the respiratory complexes) [60].

The first evidence for a larger-scale heterogeneity in thylakoid membrane composition came from immunogold electron microscopy on cells of *Synechococcus* sp. PCC7942, which suggested a "radial asymmetry" in the distribution of the reaction centres, with PSI concentrated in

the outer thylakoid membrane layers adjacent to the cytoplasmic membrane [7]. This would imply that linear and cyclic photosynthetic electron transport have different sub-cellular locations, with linear photosynthetic electron transport occurring in the inner thylakoid membrane layers adjacent to the central cytoplasm, and PSI-dependent cyclic photosynthetic electron transport concentrated in the outer thylakoid membrane layers adjacent to the cytoplasmic membrane [7]. Fluorescence microscopy gives lower spatial resolution than electron microscopy, but is capable of revealing any spatial heterogeneity at length-scales of about 200 nm or more. It has the considerable advantages that live cells can be visualised, giving a direct window into the dynamics of the membrane, and that multiple membrane components can be monitored simultaneously. Hyperspectral fluorescence imaging was used in *Synechocystis* sp. PCC6803 to distinguish the fluorescence signatures of PSI and PSII [65]. As with the earlier immunogold work in *Synechococcus* [7], the hyperspectral study in *Synechocystis* indicated that the larger-scale sub-cellular distributions of the two photosystems are not identical [65]. However, in *Synechocystis* it appears that PSI is concentrated in the inner layers of thylakoid membrane surrounding the central cytoplasm [65]. This would suggest that these regions are the principal locations of PSI-dependent cyclic photosynthetic electron transport, while linear photosynthetic electron transport occurs in the outer thylakoid layers adjacent to the cytoplasmic membrane. This is opposite to the inference from *Synechococcus* [7], but both studies suggest that there may be larger-scale spatial separation between the two modes of electron transport. Hyperspectral imaging further suggests that the spatial segregation between the photosystems increases as an adaptive response in phycobilisome mutants [66]. Small-angle neutron scattering suggests rapid and substantial rearrangement of *Synechocystis* thylakoid membranes during light acclimation [67].

The partial segregation of the photosystems is consistent with a biochemical study showing that *Synechocystis* thylakoids can be separated into three sub-fractions with distinct reaction centre compositions [68]. Intriguingly, a combination of fluorescence microscopy with membrane fractionation indicates substantial lateral heterogeneity in the cytoplasmic membrane of *Gloeobacter violaceus*, an atypical cyanobacterium which lacks thylakoid membranes [24]. In *Gloeobacter* it appears that nearly all the photosynthetic and respiratory complexes are concentrated in highly-localised bioenergetic zones in the cytoplasmic membrane, each zone about 400 nm in diameter and in total occupying only about 6% of the membrane area [24]. The clustering of respiratory complexes in the *E. coli* cytoplasmic membrane [69] suggests that heterogeneity on length scales of 100s of nm may be the norm in bacterial membranes.

In addition to the photosystems, fluorescence microscopy in cyanobacteria can also reveal the sub-cellular distribution of non-pigmented complexes in live cells, by using genetically-encoded fluorescent protein tags. Several fluorescent tags, including GFP and YFP, have fluorescence spectra distinguishable from each other and from the photosynthetic pigments. There are some provisos in regard to the use of the technique. It is advisable to express the tagged complex from its native chromosomal locus (rather than from a different locus with a different promoter, or from a plasmid) to avoid artefacts due to over-expression. Some respiratory complexes seem to be present in very low numbers in the cell (a few hundred or fewer copies per cell [35], in contrast to photosystems which are typically present in tens or hundreds of thousands per cell [70]). Complexes present in such low numbers may not give fluorescence signals detectable above the background. It is also necessary to check that the addition of the fluorescent tag does not perturb the assembly and function of the complex: in the case of electron transport complexes this can often be done by quantitative measurement of the appropriate electron transport reaction. In *Synechococcus* sp. PCC7942, successful GFP-tagging of NDH-1 and SDH showed that both respiratory complexes are present in rather small numbers of only a few hundred complexes per cell [35]. In accordance with the current biochemical data [2], the complexes were only detectable in the thylakoid membranes [35]. Both NDH-1 and SDH proved to be very heterogeneously distributed in the thylakoids in low-light grown cells, where they formed distinct patches about 100–300 nm in diameter and containing 10s to 100s of complexes (Fig. 2) [35]. This suggests that there is a pronounced spatial separation between photosynthetic and respiratory activities, at least under some conditions.

Other minor components of the thylakoids are also heterogeneously distributed: for example GFP-tagging in *Synechocystis* shows that FtsH proteases are concentrated in distinct zones in the thylakoid, with a markedly different distribution from chlorophyll fluorescence [71]. The photosynthetic complexes are the major components of the thylakoid membrane, and at optical resolution they appear to be rather evenly spread over the whole membrane surface [13]. However, the concentration of minor membrane components and specialised activities in discrete, localised membrane zones may well be the norm.

5. Control of electron transport pathways through regulation of membrane complex distribution

Given the evidence for lateral heterogeneity in cyanobacterial thylakoids (Section 4.2) and the requirement for post-translational control of electron transport pathways (Section 3) it might be expected that there are mechanisms for control of electron transport pathways through redistribution of electron transport complexes in the membrane. The first evidence for such redistribution of complexes in the membrane came from freeze-fracture electron microscopy showing re-organisation of PSII complexes associated with state 1–state 2 transitions [64]. Evidence for larger-scale re-organisation comes from a study of the distribution of GFP-tagged NDH-1 and SDH complexes in the thylakoid membranes of *Synechococcus* sp. 7942 [35]. Both these complexes are clustered in discrete zones in the membrane in low-light grown cells (Section 4.2), but exposure of these cells to moderate light intensities leads to a post-translational redistribution of both complexes such that they become much more evenly distributed in the membrane (Fig. 2) [35]. After exposure to moderate light, the distribution of the complexes is much closer to the distribution of the photosynthetic complexes (Fig. 2), so it is plausible that electron transport interaction between NDH-1, SDH and the photosynthetic reaction centres is inhibited due to spatial segregation under low light, but enhanced after moderate light exposure [35]. Indeed, moderate light exposure results in a much higher probability of electron transfer from the respiratory complexes to PSI, indicating an enhancement of cyclic electron transfer pathways (Section 2.4) under these conditions [35]. The effects of electron transport inhibitors show that the transition is triggered by changes in the redox state of plastoquinone [35]. Thus there is a redox feedback loop that controls the prevalence of linear vs. cyclic electron flow in response to a redox cue. Reduced plastoquinone signals sufficiency in electron supply and promotes cyclic electron transport pathways, presumably through closer association of NDH-1 and SDH with PSI. The membrane reorganisation (and associated changes in electron transport pathways) occurs on a timescale of about 30–60 min. Time-lapse fluorescence images show that during this time the spots of NDH-1 remain stationary, but gradually lose intensity as complexes diffuse out of the NDH-1 clusters and spread into the bulk thylakoid membrane [35]. It is likely that the slow kinetics of the transition reflect the highly restricted mobility of thylakoid membrane-integral complexes [12,13]. A number of key questions remain to be answered about this phenomenon:

a. The signal transduction pathway that links plastoquinone redox state to redistribution of complexes in the membrane remains unknown. It is plausible that post-translational covalent modification of NDH-1 and SDH is involved, but the precise mechanism is not established.
b. More generally, we do not know the physical factors that control the lateral distribution of complexes in cyanobacterial thylakoid membranes. In green plant chloroplasts, surface charge distribution [8], specific molecular recognition [72] and physical exclusion from the

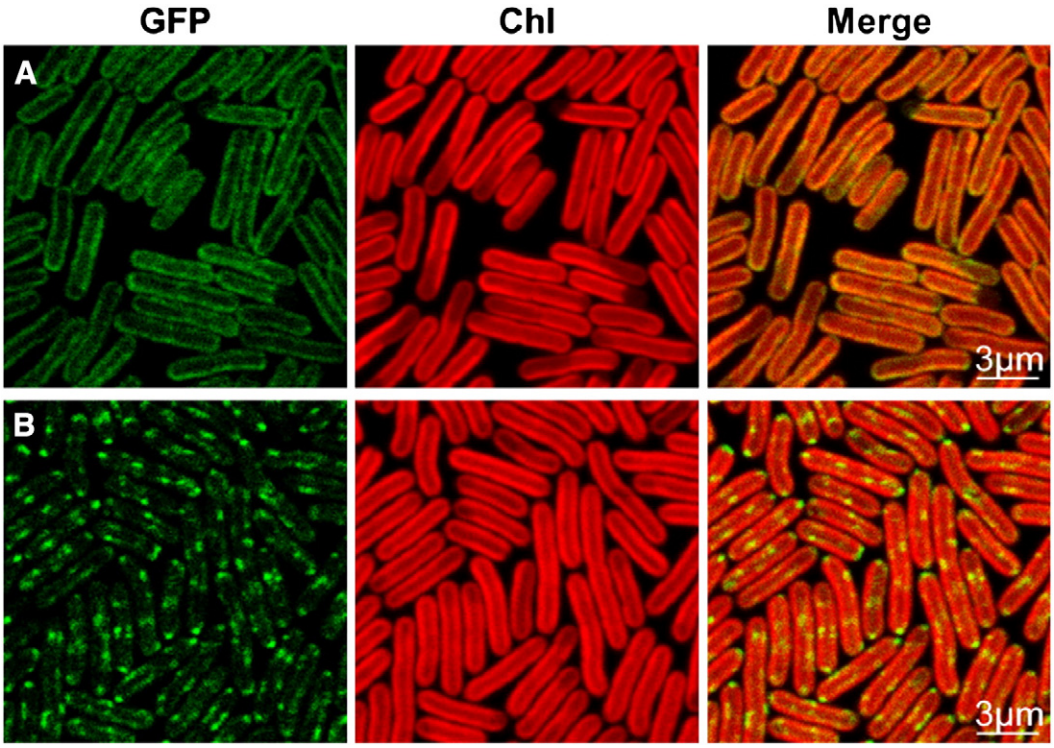

Fig. 2. Localisation of functional NDH-1 complexes in cells of *Synechococcus* sp. PCC7942 revealed by GFP-tagging of the NdhM subunit and confocal fluorescence microscopy. GFP fluorescence is shown in green. Chlorophyll fluorescence (shown in red) indicates the location of the thylakoid membranes. A. Cells grown in moderate light (60 $\mu E \cdot m^{-2} \cdot s^{-1}$) B. Cells grown in very low light (6 $\mu E \cdot m^{-2} \cdot s^{-1}$). Under these conditions NDH-1 complexes are clustered in thylakoid membrane patches with a mean diameter of 160 nm, each containing on average about 90 complexes. In this clustered state (B) the probability of electron transfer from the respiratory donors to PS I is much lower than in (A). Images obtained by Dr Lu-Ning Liu and reproduced from [35].

grana of complexes with bulky stromal domains [8] have all been proposed as factors controlling the larger-scale distribution of complexes. The first two of these factors may well be significant in cyanobacteria. The possible involvement of domains of specific lipids (analogous to the lipid rafts of eukaryotic membranes) also remains to be explored.

c. It is not yet clear whether the enhanced cyclic electron flow in *Synechococcus* is mediated by specific supercomplexes of PSI with NDH-1 and/or SDH, or whether it is simply a function of their closer proximity in the membrane. More generally, we need more information on the length-scales of electron transport in cyanobacterial thylakoid membranes: how far do the mobile electron carriers plastoquinone and cytochrome c_6/plastocyanin diffuse as they carry electrons between complexes (see Section 3.3)?
d. Our information on the multiplicity of alternative electron transport pathways in thylakoid membranes remains fragmentary until we have more information on the spatial distribution of other electron transport complexes, in particular cytochrome b_6f and the terminal oxidases cytochrome *bd*-1 and cytochrome oxidase.

6. Concluding remarks: membrane dynamics and time-scales for regulation of electron transport in cyanobacteria

As discussed in Section 3, cyanobacteria need to regulate their electron transport on several timescales, from slow responses to changes in mean conditions to rapid photoprotective responses to short-term changes in light-intensity. The restricted mobility of thylakoid membrane-integral complexes puts a significant constraint on the rate of the faster, post-translational responses [14], as is also the case in chloroplasts [73]. However, the diffusion of soluble complexes in the vicinity of the cytoplasmic surface of the thylakoid membrane appears much less constrained [14]. Therefore the fastest responses (timescales of seconds to a few minutes) are likely to involve movement only of soluble cytoplasmic and surface-associated complexes. This applies to the movement of phycobilisomes during state transitions [62], but it is also very likely to apply to the action of soluble electron valves such as Flv1–4. It is very plausible that the Flv1/3 and Flv 2/4 complexes could rapidly associate/dissociate with PSI and PSII respectively to provide short-term emergency control of electron transport [43,44]. It is interesting that the terminal oxidases cytochrome oxidase and cytochrome *bd* also seem to be important as valves for consumption of excess electron supply from PSII [17], apparently duplicating the role of Flv2/4 [44]. As cytochrome oxidase and cytochrome *bd* are membrane-integral complexes, their activation as photosynthetic electron sinks may well require a longer timescale due to the time required for the complexes to relocate in the membrane. The duplication of cyclic electron transport pathways involving NDH-1 and PGR5 (Fig. 1) may have a similar rationale. In both these instances, the pathway involving a membrane-integral complex has benefits in terms of energy conservation through pmf generation, but is likely to be activated on slower timescales due to restricted mobility of thylakoid membrane proteins (Fig. 1). Regulation of linear vs. cyclic electron flow by the redox state of plastoquinone provides a precedent for multiple mechanisms acting on different timescales: rapid regulation of light-harvesting due to mobility of phycobilisomes [62] is complemented by a slower regulation of electron transport pathways through relocation of NDH-1 and SDH [35]. The remarkable multiplicity of cyanobacterial electron transport complexes and electron transport routes (Fig. 1, Table 1) may in part reflect the need to regulate electron transport on multiple timescales.

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