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Review

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Dynamics of bioenergetic microcompartments

Abstract: The vast majority of life on earth is dependent on harvesting electrochemical potentials over membranes for the synthesis of ATP. Generation of membrane potential often relies on electron transport through membrane protein complexes, which vary among the bioenergetic membranes found in living organisms. In order to maximize the efficient harvesting of the electrochemical potential, energy loss must be minimized, and this is achieved partly by restricting certain events to specific microcompartments, on bioenergetic membranes. In this review we will describe the characteristics of the energy-converting supramolecular structures involved in oxidative phosphorylation in mitochondria and bacteria, and photophosphorylation. Efficient function of electron transfer pathways requires regulation of electron flow, and we will also discuss how this is partly achieved through dynamic re-compartmentation of the membrane complexes into different supercomplexes. In addition to supercomplexes, the supramolecular structure of the membrane, and in particular the role of water layers on the surface of the membrane in the prevention of wasteful proton escape (and therefore energy loss), is discussed in detail. In summary, the restriction of energetic processes to specific microcompartments on bioenergetic membranes minimizes energy loss, and dynamic rearrangement of these structures allows for regulation.

Keywords: bacterial bioenergetics; chloroplast; microcompartment; mitochondria; OXPHOS; photosynthetic electron transfer; proton motive force; supercomplex.

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Introduction

This review is devoted to the role of microcompartmentation in the cellular energy-converting systems. A microcompartment is defined as a dynamic, sub-organellar functional unit, to which proteins are recruited and released in response to the demands of the cell (see Holthuis and Ungermann, 2013, for a more general definition). In this review we will discuss how microcompartmentation occurs on two levels in bioenergetic systems. Firstly, we define structurally and functionally distinct areas of the membrane, such as the grana and stroma of chloroplast thylakoids, the cristae and inner boundary membrane of mitochondria and internal vesicles of bacteria as separate structural microcompartments. Secondly, we will review evidence that the various protein complexes, conferring functionality on bioenergetic membranes, are capable of interacting with each other, and binding or releasing various subunits in a regulated fashion. We will refer to such transient changes in assembly and localization as 'dynamic microcompartmentation'.

Abundant data from biochemical studies on bioenergetic membranes, including many early studies, have indicated the existence of such microcompartments, sometimes referred to as 'respirasomes' or 'oxysomes' in respiratory electron transfer chains (Chance et al., 1963; Schägger and Pfeiffer, 2000). While other models, such as free diffusion, remain valid in many situations, in recent years new techniques have lent further experimental weight to this principle, and here we review examples from bacteria, mitochondria and chloroplasts. The examples given are not exhaustive, and for brevity many excellent studies are not mentioned. Bioenergetic microcompartments have given variable names depending on the perspectives of the investigator (see Table 1) and in this review we retain the descriptions given in the original research. Evidence from several different techniques converges to support the existence of bioenergetic microcompartments, and in this review data have been collated from works using many techniques, including electron microscopy to perform immunogold labeling and tomography, blue and clear native-PAGE and the increasing use of high resolution imaging (see Table 2). While all of these

Term	Complex	Organism/Organelle	Reference
Oxysomes		Mitochondria	Chance et al. (1963)
Elementary particle	Electron chain	Mitochondria	Fernandez-Moran et al. (1964)
Respirasomes, supercomplexes	CI and CIII	Mitochondria	Dudkina et al. (2005); Schägger and Pfeiffer (2000)
	CI, CIII and CIV	Bacteria (*P. denitrificans*)	Stroh et al. (2004)
	Iron-oxidizing, oxygen-reducing supercomplex	Bacteria (*A. ferrooxidans*)	Castelle et al. (2008)
	Sulfide oxidase, oxygen reductase	Bacteria (*A. aeolicus*)	Prunetti et al. (2010)
Supercomplexes	Protein complexes associated with chlorophyll	Bacteria, chloroplasts	Schägger (2002) Gregory et al. (1982)
	Cytochrome bc_1 complex and cytochrome oxidase	Bacteria (*Bacillus* PS3)	Sone et al. (1987)
	Photosynthetic reaction centers, cytochrome c_2, and bc_1 complex	Purple bacteria	Joliot et al. (1989)
Supracomplex	ATP synthase, Respiratory chain	Mitochondria	Arnold et al. (1998)
Supramolecular complex, organization, structures	ATP synthase, Respiratory chain (especially rows of CI and F_0F_1 dimers)	Mitochondria	Davies et al. (2011); Krause et al. (2004)
	Thylakoid architecture with respect to PSI and PSII particles	Chloroplasts	Staehelin (1975)
	Photosynthetic reaction center, cytochrome c_2 and bc_1 complex	Purple bacteria (*R. sphaeroides*)	Verméglio and Joliot (2002)
	Respiratory chain	Bacteria (*E. coli*)	Sousa et al. (2011)
Plastic or functional respirasome	CI,CIII and CIV	Mammal mitochondria	Acin-Perez et al. (2008)
Intercomplex association	CIII and CIV	Bacteria (*M. smegmatis*)	Megehee et al. (2006)
Zipper-like arrangement	ATP synthase	Mitochondria (*Paramecium*)	Allen et al. (1989)

Table 1 Terminology alignment.

techniques have drawbacks and advantages, together they provide a growing body of evidence for dynamic microcompartments in bioenergetic membranes.

The general advantage of microcompartmentation, as discussed in the other reviews in this volume, is in facilitating the diffusion of substrates, in localizing interconnected cellular processes in a particular part of the cell and in enhancing opportunities of integrative regulation. All these factors are also beneficial for energy-converting processes, as discussed below. Moreover, the microcompartmentation of energy conversion is special because it also serves to prevent energy losses. Indeed, the energy, as gained by a cell, can be easily and irreversibly lost, if not processed promptly and properly. Energy losses from bioenergetic membranes can proceed on several levels. Firstly, electrons can be lost from the electron transfer (ET) chains as a result of interaction with inappropriate organic or inorganic electron acceptors, oxygen being the first in line. Interaction with oxygen leads not only to the well-known formation of the potentially damaging reactive oxygen species (ROS), but also to the accompanying energetic short-circuit. Hence, electron carrier efficiency should benefit from recruitment into microcompartments, where the bypass ET reactions are minimized because of a tight and proper packing of the interacting ET complexes (see the detailed discussion in later sections).

Secondly, in the specific case of (bacterio)chlorophyll-based photosynthetic systems, the energy of the captured light quanta could be lost (as heat) if not promptly delivered to the photochemical reaction centers (RC) where it could be transiently stored as the energy of separated electric charges. For this reason, photosynthesis is performed by highly organized protein-chlorophyll complexes, which could be fairly categorized as

Method	Reference	Problems, restrictions	Analysis of /by	Organism
BN/CN-PAGE after membrane protein isolation with detergents	Arnold et al. (1998); Schägger and Pfeiffer (2000)	Detergent dependent, mild isolation technique	Supercomplexes by molecular weight analysis, Western blot, 2-D PAGE, MS	Yeast F_0F_1
	Danielsson et al. (2006); Heinemeyer et al. (2004); Twachtmann et al. (2012)		Supercomplexes by molecular weight analysis, Western blot, 2-D PAGE, MS	Spinach chloroplasts, Arabidopsis chloroplasts
	Sousa et al. (2011)		Respiratory chain by BN-PAGE, activity MS, heme staining, spectroscopy	Bacteria (*E. coli*)
Chemical cross-linking of solubilized protein complexes	Zhang and Scheller (2004b) Andersen et al. (1992)	Limited specificity in impure preparations	Cross-linking to stabilize weak interactions, SDS-PAGE, immunoblotting	Arabidopsis chloroplasts, barley chloroplasts
	Tanaka et al. (1996)		CIII and CIV by SDS-PAGE, immunoblotting	Bacteria (*Bacillus* PS3)
FRET	Gavin et al. (2005)			Yeast F_0F_1
Immuno-precipitation after solubilization (pull down)	Sone et al. (1987)	Detergent dependent, mild isolation technique	CIII and CIV by heme content, SDS-PAGE, gel filtration	Bacteria (*Bacillus* PS3)
Ion-exchange chromatography after solubilization	Sone et al. (1987)	Detergent dependent, mild isolation technique	CIII and CIV by heme content, SDS-PAGE, gel filtration	Bacteria (*Bacillus* PS3)
	Prunetti et al. (2010)		Sulfide oxidase-oxygen reductase supercomplex by in-gel activity, MS, immunoblotting, spectroscopy	Bacteria (*A. aeolicus*)
	Stroh et al. (2004)		CI:CIII:CIV=1:4:4 supercomplex by 2-D BN/SDS-PAGE, gel filtration, spectroscopy, immunoblotting	Bacteria (*P. denitrificans*)
Tagged subunit, solubilization and pull-down assay	Iwai et al. (2008)	Detergent dependent, mild isolation technique	Supercomplexes by immunoblotting, MS to identify changes	Chlamydomonas chloroplasts
	Niebisch and Bott (2003)		CIII and CIV by spectroscopy, activity, SDS-PAGE, immunoblotting	Bacteria (*C. glutamicum*)
Scanning EM	Fernandez-Moran et al. (1964)	No biochemical identification	Repetitive structures	Beef heart mitochondria

(Table 2 continued)

Method	Reference	Problems, restrictions	Analysis of /by	Organism
EM with immunogold labeling	Allred and Staehelin (1985)	Co-localization only, no interaction can be detected	Microcompartmentation and co-localization of different complexes	Spinach chloroplasts
	Sherman et al. (1994)			*Synechococcus* sp.
	Vallon et al. (1991)			Maize chloroplasts
In situ scanning force microscopy	Kaftan et al. (2002)	Co-localization only, no interaction can be detected. Limited biochemical identification	Co-localization of complexes at specific membrane domains Changes in super-complex composition	Lettuce chloroplasts
Live cell epifluorescence microscopy or confocal microscopy	Johnson et al. (2004)	Microscopical conditions comparable to growth conditions	Clustered distribution of ATP synthase or CII in discrete membrane domains	Bacteria (*B. subtilis*)
	Liu et al. (2012)		Redox-regulated redistribution of CI (low/moderate light)	Cyanobacteria (*Synechococcus elongatus* PCC 7942)
Difference spectroscopy (photo-oxidation of cytochromes and reaction center primary donor)	Joliot et al. (1989)		Photosynthetic reaction centers, cytochrome c_2 and cytochrome bc_1 complex	Purple bacteria (*Rhodobacter* sp., *Rhodospirillum* sp.)
Separation of membrane subfractions Photosystem activity measurements	Anderson and Melis (1983)	Restricted to systems with physically variable membrane sub-compartments	Microcompartmentation and co-localization of different complexes	Spinach chloroplasts
Separation of plasma membrane subfractions by sucrose density centrifugation	Rexroth et al. (2011)		Microcompartmentation and co-localization of bioenergetic complexes by MS (lipid and proteome)	Cyanobacterium (*Gloeobacter violaceus*)
Single particle analysis by cryo EM after mild solubilization with detergent	Althoff et al. (2011)	Detergent dependent, mild isolation technique	Supercomplexes	Bovine mitochondria
	Nield et al. (2000)			Spinach chloroplasts
Reconstitution of isolated complexes	Liu et al. (2007)	*In vitro*, biochemical activity studies	CIV and F_0F_1 by saturation transfer electron paramagnetic resonance (STEPR) and differential scanning calorimetry	Bacteria (*B. pseudofirmus* OF4)
Knock-out of *CRD1* (cardiolipin synthase); mutagenesis of lysine cluster	Wenz et al. (2009)	Detergent dependent, mild isolation technique	CIII and CIV by 2-D BN/SDS-PAGE after mild solubilization	Yeast mitochondria

Table 2 Methods used to prove supercomplex assembly.

microcompartments. These complexes are exemplified by chlorosome-RC systems of green phototrophic bacteria (Frigaard and Bryant, 2004; Pedersen et al., 2010), proteobacterial systems where the RC is surrounded by rings of small chlorophyll-containing antenna proteins (Cogdell et al., 2006; Sturgis et al., 2009), phycobilisome-utilizing photosystems of cyanobacteria (Shi et al., 2011), and plant systems where the two photosystems form complexes with partly mobile, chlorophyll-containing light-harvesting proteins (see section on photosynthetic microcompartments and references therein).

Last but not least, the energy could be easily lost during the generation of the membrane proton potential (the transmembrane difference of electrochemical potential of proton, $\Delta\tilde{\mu}_{H^+}$ (Mitchell, 1966). The mechanisms that are used by the cell to prevent the futile dissipation of the proton potential and the role of microcompartmentalization in prevention of these losses are discussed below.

Prevention of futile proton escape by compartmentalization

Ion-motive force as cellular energy intermediate

The proton motive force (PMF) is the source of energy for a variety of cellular processes in most bacteria, mitochondria, and chloroplasts. PMF is related to $\Delta\tilde{\mu}_{H^+}$ as

$$PMF=\Delta\tilde{\mu}_{H^+}/F=\Delta\psi\text{-}2.3RT(pH_{in}\text{-}pH_{out})/F=\Delta\psi\text{-}2.3RT\,\Delta pH/F \quad (1)$$

where $\Delta\psi$ is the transmembrane difference of the electric potential, and the membrane topology corresponds to that of a prokaryotic cell where H^+ ions are pumped outwards (see Skulachev, 1988; Cramer and Knaff, 1990). In organelles with more complex topology, such as mitochondria and chloroplasts, 'in' corresponds to the side of the membrane that is negatively charged (matrix side in mitochondria and stromal side in chloroplasts), while 'out' corresponds to the membrane side that is charged positively (luminal side in chloroplasts and the side that faces the intramembrane space in mitochondria). A typical H^+ cycle includes the generation of PMF by primary transport systems (H^+ pumps), which can utilize the energy of chemical and redox reactions, as well as the energy of light. The discharge of proton potential, as catalyzed by specific enzymes, is coupled primarily to the synthesis of ATP, but also to solute transport, motility, reverse electron transport, etc. (Skulachev, 1988; Cramer and Knaff, 1990). This ion cycling within the membrane is the essence of membrane bioenergetics.

Certain bacteria and archaea use sodium ions instead of protons in energy conversion systems (Skulachev, 1988; Cramer and Knaff, 1990). Analogous to the H^+ cycle, a Na^+ cycle includes generators of sodium-motive force (SMF), in other words primary Na^+ pumps, and SMF consumers, such as the Na^+-translocating membrane ATP synthase, Na^+-dependent membrane transporters for nutrient uptake and/or a Na^+-dependent flagellar motor for motility (Skulachev, 1988; Dimroth, 1997; Hase et al., 2001; Mulkidjanian et al., 2008a). While some Na^+-specific pumps do not have homologues among the H^+ specific pumps and vice versa, all the membrane ATP synthases are homologous, irrespective of whether they translocate protons or sodium ions (Mulkidjanian et al., 2008b). The membrane ATP synthases use a unique rotary mechanism, where subsequent translocation of several ions by a rotating ring of 8–15 small, ion-binding membrane subunits within the membrane part of the enzyme leads to energy storage by elastic deformation of the cytoplasmic part of the enzyme; this stored energy periodically discharges to drive ATP synthesis at the enzyme catalytic sites, so that the full rotation of the ion-carrying ring in the membrane results in the formation of three ATP molecules in the protruding cytoplasmic part of the enzyme (Cherepanov et al., 1999).

Phylogenomic analysis has shown that the ancient form of the rotary ATP synthase translocated sodium ions (Cherepanov et al., 1999; Mulkidjanian et al., 2008b, 2009). To explain this result, it has been suggested that the common ancestor of the rotary ATPases emerged as an ATP-driven sodium outward pump, presumably in marine prokaryotes, supposedly at the stage of the last universal cellular ancestor (LUCA). The translation system of all organisms needs a high K^+/Na^+ ratio for function (Mulkidjanian et al., 2012), and thus marine organisms, both modern and primordial, should continuously pump sodium ions (which leak through the cell membrane) out of the cell. As argued elsewhere (Mulkidjanian et al., 2009), the ancestral ATPase may have shared the ability to pump Na^+ ions with other Na^+-pumps, such as the Na^+-transporting pyrophosphatase (Luoto et al., 2011) and Na^+-transporting decarboxylase (Dimroth, 1997; Dimroth and von Ballmoos, 2008).

However, unlike these other Na^+ pumps, because of its rotating scaffold the common ancestor of the rotary ATP synthases would be able to translocate Na^+ ions in both directions, so that at high external salinity the reversal of the rotation could result in Na^+-driven synthesis of ATP. This represents the beginning of membrane bioenergetics: together with the ancient Na^+ pumps, the Na^+-driven

ATP synthase was capable of completing the first, sodium-dependent bioenergetic cycle in a cell membrane (Mulkidjanian et al., 2009). The emergence of a rotary ATP synthase was a major breakthrough, as this rotary enzyme can accumulate the energy of several sequentially translocated protons or sodium ions to yield one ATP molecule. The main advantage of this membrane bioenergetics, as compared to the substrate level phosphorylation, is the possibility to accumulate small energy quanta and to use them for ATP synthesis (Pascal and Boiteau, 2011). The membrane ATP synthase functions as an 'antenna' for small energy portions of 10–20 kJ/mol available in the cell (R. Pascal, pers comm). Such small amounts of energy are readily obtained from co-translocation of anionic metabolic products and Na^+ ions out of the cell.

As argued elsewhere (Mulkidjanian et al., 2008b, 2009; Dibrova et al., 2010), the transition to the proton-translocating ATP synthase could have happened independently in archaea and bacteria, being driven (1) by the emergence of more sophisticated proton-tight membranes, and (2) by the opportunity of direct coupling between ET and proton transfer (PT) in primary proton pumps such as cytochrome bc_1 complexes and oxidases, where quinones and water are used as substrates.

Role of microcompartmentalization in preventing proton escape from the surface of coupling membranes

The transition to proton-dependent energetics, with all its advantages, also brought a problem. As long as cells pumped sodium ions out and thrived in high-sodium ion environments, the danger of diffusion of the out-pumped sodium ions away from the membrane – instead of entering the ATP synthase – was negligible. In any case, the external concentration of sodium ions would remain higher than within the cell. In the case of proton-dependent bioenergetics, the situation is quite different. Here proton concentrations outside and inside of a prokaryotic cell are usually similar, so that the PMF (calculated by applying Eq. 1), is contributed mostly by the $\Delta\psi$ term and might be insufficient to drive ATP synthesis. The case of alkaliphilic bacteria that thrive at high pH is particularly dramatic; here, the calculated PMF can drop almost to zero. Hence, in the case of proton energetics, it is crucially important that the protons ejected by primary generators of $\Delta\tilde{\mu}_{H^+}$ rapidly enter the nearest ATP synthase and do not escape into the surrounding medium (Williams, 1978; Kell, 1979). Cells use several solutions to overcome this problem. One solution is to increase the numbers of ion-binding subunits in the rotating ring; this trick seems to be particularly useful when ATP synthesis and not the ATP hydrolysis is the primary function of the rotary enzyme. This is the case of autotrophic organisms; not surprisingly the highest number of the ion-binding subunits is therefore found in the rotating rings of photosynthetic organisms, cyanobacteria and plants, with the maximum of 15 in the case of *Spirulina platensis* (Krah et al., 2010; Pogoryelov et al., 2012). Hence, the ATP synthase of *S. platensis* is capable of efficiently synthesizing ATP even at the PMF of ~150 mV.

Another solution is to invoke the so-called local coupling between generators and consumers of the PMF (Williams, 1978; Kell, 1979; Dilley et al., 1987; Hicks et al., 2010). As argued by several authors (see, Kell, 1979; and references therein), a precondition of a local coupling could be the absence of a fast equilibrium between the protons ejected at the surface and the bulk water phase. In this case, the effective concentration of protons on the external surface of proton-expelling prokaryotic cells might be higher than that in the bulk, and protons would be hindered in their attempts to escape into the bulk water phase. It has been shown that there is a kinetic barrier for protons at the surface of coupling membranes, both in archaea (Drachev et al., 1984; Heberle et al., 1994) and in bacteria (Jones and Jackson, 1989; Gopta et al., 1999). Furthermore, in experiments with archaeal membranes it has been shown that, whereas PT to the water-dissolved pH-indicator pyranine takes about 1 ms, PT along the membrane surface proceeds much faster (Heberle et al., 1994). The combination of both these effects creates a situation where the protons, ejected by bacterial cells, predominantly spread along the membrane surface and do not escape into the bulk. It has been argued, that the kinetic barrier at the membrane surface has an electrostatic origin, possibly owing to the layering of water at the electrically charged membrane surface (Cherepanov et al., 2003, 2004; Cherepanov, 2004); a water layering on the surface of biological membranes was confirmed experimentally (Higgins et al., 2006). As argued elsewhere (Mulkidjanian et al., 2006), both the interfacial potential barrier and the ability of protons to diffuse promptly along the membrane are caused by the high density of negatively charged acidic groups (see Figure 1). Thus, *in vivo* the driving force behind ATP synthesis can be defined as surface-to-surface PMF:

$$PMF^S = \Delta\psi - 2.3RT(pH^S_{in} - pH^S_{out})/F = \Delta\psi - 2.3RT\,\Delta pH^S/F \cdot \quad (2)$$

Another way to localize proton cycling is to bring the producers and consumers of $\Delta\tilde{\mu}_{H^+}$ close to each other. Examples of such super complexes are considered in the later sections on bacteria, mitochondria and

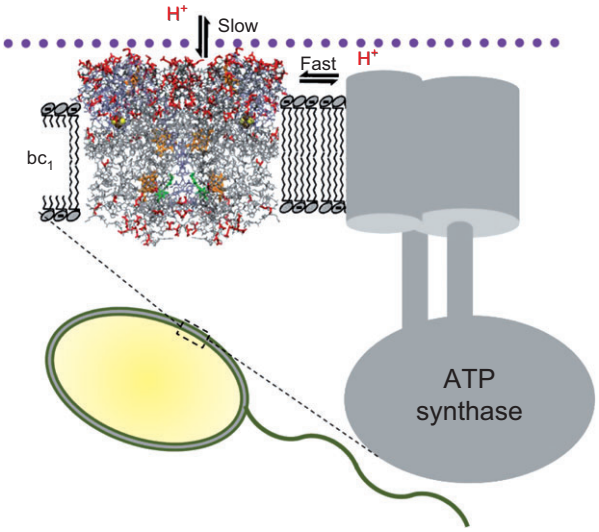

Figure 1 Proton translocation along the periplasmic surface of a bacterial coupling membrane from a generator of proton potential (cytochrome bc_1 complex) to the ATP synthase (see the text for details).
The electrostatic barrier for ions is shown by violet dots. The Asp and Glu residues, which form a proton-conducting network at the periplasmic surface of a bacterial cytochrome bc_1 complex, are shown in red. The heme cofactors are shown in orange, the iron–sulfur clusters as yellow-brown balls, and the ubiquinone molecules are shown in green. The crystal structure of the cytochrome bc_1 complex of *Rhodobacter sphaeroides* (PDB entry 2QJY) was used (Esser et al., 2008). This Figure was produced using the VMD software package (Humphrey et al., 1996).

photosynthetic organisms. In summary, while considering energy-converting supercomplexes as functional microcompartments, one should also include the adjusting water layers at the membrane surface; these layers are separated from the bulk water phase by an electrostatic barrier and, hence, compartmentalized.

At least in some lineages, prokaryotes further decreased proton losses by utilizing membrane invaginations. These invaginations bring portions of the external membrane surface into the prokaryotic cell. The emergence of such intracellular structures happened, apparently, in different lineages (Lengeler et al., 1999; Williams, 2001) and led in particular to the formation of thylakoids in cyanobacteria (as discussed later in the article) and the intracellular vesicular structures in purple bacteria, which can even be fully detached from the cytoplasmic membrane (Sturgis et al., 2009; Tucker et al., 2010). Because of the evolutionary relatedness of purple bacteria to mitochondria (Andersson et al., 1998), the latter structures might have paved the way to the generation of mitochondrial cristae (see section on mitochondrial microcompartments). If invaginations are present, protons are ejected not into the external 'Pacific Ocean', but into membraneous sacks; in this storage space, protons can dwell until they are used (Williams, 2001). It is noteworthy that the usage of membrane invaginations adds to the mechanisms of local coupling, but does not replace them. For example, the mitochondrial energy-converting enzymes still cluster into supercomplexes, as is described in detail later in this article. It has been shown that succinate oxidation by mitochondria induces an interfacial pH decrease of 0.5–0.9 pH units with no simultaneous pH changes in the bulk (Xiong et al., 2010). The importance of elevated surface proton concentration to ATP synthesis by mitochondria follows also from the extremely low number of ion-carrying subunits per mitochondrial ATP synthase – only 8 (Watt et al., 2010), which means that the synthesis of one ATP molecule is driven by translocation of only 2.7 protons. As the values of $\Delta\psi$ and ΔpH (bulk) in mitochondria are known (<200 mV and <1 pH unit), respectively (Skulachev, 1988; Cramer and Knaff, 1990), efficient ATP synthesis in mitochondria can only proceed if the pH at the membrane surface is not in equilibrium with the bulk pH under steady state conditions.

Hence, one can speak of the co-evolution of membrane bioenergetics and bioenergetic microcompartments. The latter were not crucial at the stage of primitive and robust sodium-driven bioenergetics, but become vital in preventing energy losses upon the transition to the more efficient – but more vulnerable – proton-dependent bioenergetics.

Compartmentalization in multifunctional bacterial membranes

Bacterial cytoplasmic membranes are multifunctional, because all membrane-bound enzyme activities of the cell are present within the cytoplasmic membrane, including transport processes of all kinds, chemotaxis/motility, proteins necessary for cell division and cell wall biosynthesis, as well as oxidative phosphorylation (OXPHOS). Furthermore, a characteristic of prokaryotes is their extreme respiratory flexibility. Most bacteria have the ability to use inducible alternative respiratory pathways in response to the environmental growth conditions, exploiting a large variety of electron donors as well as terminal electron acceptors other than molecular oxygen. To enable such multifunctionality, the current model predicts that – while some proteins are freely and, therefore randomly, distributed in the membrane – membrane proteins are at least partly organized in dynamic microcompartments, and that specific mechanisms are required to allow this organization (Rudner and Losick, 2010). This has been

clearly demonstrated in several cases, for example for the chemoreceptor proteins located at the cell poles or the cell division machinery located at the mid-cell. In addition, the use of fluorescently labeled lipids and lipophilic dyes has confirmed a laterally heterogeneous distribution of phospholipid molecules in the bacterial membrane producing specific environments for certain membrane proteins (Matsumoto et al., 2006), which can be seen as a possible mechanism involved in microcompartmentalization of proteins. Until now, there is only limited evidence for the presence of microcompartments in bacterial membranes, but it is a rapidly developing field, and, therefore, this part of the review will focus on examples confirming a heterogeneous distribution of membrane proteins and the influence of the membrane phospholipid composition.

Oxidative phosphorylation in *Escherichia coli*

The OXPHOS system in the inner mitochondrial membrane with its five membrane protein complexes (complex I–V; see section on mitochondrial microcompartments) is relatively inflexible, but very efficient, as would be expected for an organelle specialized to provide the cell with energy. In contrast, prokaryotic respiratory chains are located within the plasma membrane, and to generate the PMF necessary for ATP synthesis, the cell exploits branched respiratory chains in order to respond to environmental changes with great metabolic and regulatory flexibility (Unden and Bongaerts, 1997). As an example, the aerobic respiratory chain of *Escherichia coli*, a facultative anaerobe, can function by using up to five different NADH dehydrogenases (DH) including: NDH-1 (comparable to mitochondrial complex I), NDH-2, WrbA, YhdH, and QOR at the electron donor site, and up to three quinol oxidases: bo_3, *bd*-I, and *bd*-II at the electron acceptor site, and the genes for these enzymes are differently expressed in response to the oxygen content of the culture medium and the growth phase of the cells (Bekker et al., 2009; Borisov et al., 2011). In addition, other dehydrogenases, such as succinate-DH (complex II), lactate-DH, formate-DH and glycerol-1-phosphate-DH also participate in the process (Sousa et al., 2011). Furthermore, under anaerobic conditions, a completely different set of dehydrogenases and reductases is expressed (Unden and Bongaerts, 1997). Nevertheless, despite this extraordinary respiratory flexibility, the F_0F_1-ATP synthase, which uses the energy of the PMF maintained by the respiratory pathways to generate ATP, is a unique, membrane-integrated enzyme present as only a single version.

Supramolecular organization of bacterial OXPHOS complexes

The assembly of respiratory chain complexes into supercomplexes or supramolecular assemblies has previously been demonstrated in the mitochondria of several eukaryotes, and recently, two protein factors necessary for supercomplex formation and stability have been identified (summarized by Shoubridge, 2012). Furthermore, the organization of OXPHOS enzyme complexes into supramolecular assemblies, so-called respirasomes, in bioenergetic membrane domains has been also identified in all prokaryotic cell types, bacteria as well as archaea. In most cases, a supramolecular association of complex III (cytochrome bc_1 complex) and IV (cytochrome oxidase) (numbering as for mitochondria or *Paracoccus denitrificans*) to form a quinol oxidase supercomplex has been described, for example in *Bacillus stearothermophilus* PS3 (Sone et al., 1987), *Aquifex aeolicus* (Gao et al., 2012), *P. denitrificans* (Berry and Trumpower, 1985), *Corynebacterium glutamicum* (Niebisch and Bott, 2003), *Mycobacterium smegmatis* (Megehee et al., 2006), and *Bradyrhizobium japonicum* (Keefe and Maier, 1993). In *Sulfolobus acidocaldarius* sp. strain 7, the terminal oxidase is a functional fusion of complex III and IV (SoxABCD complex) as indicated by purification as well as the operon organization of the genes involved. Whereas SoxB and SoxA are related to subunits I and II of cytochrome *c* oxidase, SoxC shows homology to the cytochrome *b* component of the mitochondrial bc_1 complex and the chloroplast b_6f complex. In addition, a Rieske-type iron-sulfur protein typical for bc_1 complexes is a functional component of the purified enzyme complex (Lübben et al., 1994; Iwasaki et al., 1995a,b). Furthermore, supramolecular assemblies, constituting complete respirasomes, have also been described. For example in *P. denitrificans* – a member of the α-proteobacteria, which are regarded as the likely ancestors of present day mitochondria in the context of endosymbiotic theory – a supramolecular assembly has been reported of complexes I, III, and IV combined in a stoichiometry of 1:4:4, comparable to that characterized in mitochondria (Stroh et al., 2004). From membranes of *E. coli*, a supercomplex composed of formate-DH (fdo) and the two quinol oxidases bo_3 and *bd*-II has been isolated in addition to an independent NDH-1/NDH-2 heterooligomer (Sousa et al., 2011). Furthermore, specialized respirasomes are observed in *Aquifex aeolicus* comprising sulfide oxidase oxygen reductase (Prunetti et al., 2010) and in *Acidithiobacillus ferrooxidans* comprising iron oxidase and oxygen reductase. The latter forms a supercomplex

spanning the inner and the outer membrane as well as the periplasm (Castelle et al., 2008). In photosynthetic bacteria, such as *Rhodobacter sphaeroides* or *R. capsulatus*, the photosynthetic apparatus exhibits a supramolecular organization, consisting of two RC partially surrounded by light-harvesting complex I, connected to one cytochrome bc_1 complex by ubiquinone in the lipid phase and to one cytochrome *c* (Lavergne and Joliot, 1991; Verméglio and Joliot, 2002). It has been suggested that formation of supercomplexes or supramolecular assemblies could bring several functional advantages to the cell, such as substrate channeling, optimization of ET, protein stabilization and controlling ROS production (Genova et al., 2008; Shoubridge, 2012), and it is striking that all supercomplexes described so far involve enzyme complexes participating in aerobic respiratory chains.

The OXPHOS system suggests a direct association of the ATP synthase (F_0F_1) with respiratory supercomplexes to directly exploit the local enrichment of protons for the synthesis of ATP as described in the Introduction (Lenn et al., 2008a). For *Bacillus pseudofirmus* OF4 a close interaction between cytochrome caa_3 oxidase and ATP synthase has been demonstrated (Liu et al., 2007) and fluorescence microscopy of living *B. subtilis* cells revealed a heterogeneous distribution, in highly mobile patches, of succinate-DH and the F_0F_1, which are partially overlapping, but absent from the mid-cell region at the onset of cell division (Johnson et al., 2004; Meredith et al., 2008). Such a heterogeneous, highly dynamic arrangement has also been observed in *E. coli* membranes with fluorescently labeled quinol oxidase *bd*-I (Lenn et al., 2008b) as well as fluorescently labeled ATP synthase (Figure 2A). In an independent set of biochemical experiments on *E. coli* F_0F_1, we recently observed the formation of oligomers composed of up to four F_0F_1 complexes in membrane vesicles. Cysteine substitutions within the hydrophilic loops of the membrane-integrated F_0 subunit *a*, (which did not impact on F_0F_1 complex function), were used in experiments with zero-length intermolecular cross-linking, and detected binding to other ATP synthase complexes, confirming a clustered distribution (G. Deckers-Hebestreit et al., unpublished results). Furthermore, in cells of the cyanobacterium *Synechococcus elongatus* grown under low light, NAD(P)H-DH (NDH-1) as well as succinate-DH are concentrated in discrete patches of 100–300 nm in size in the thylakoid membranes (Figure 2B), whereas exposure to moderate light leads to a redistribution such that the complexes move out of these zones

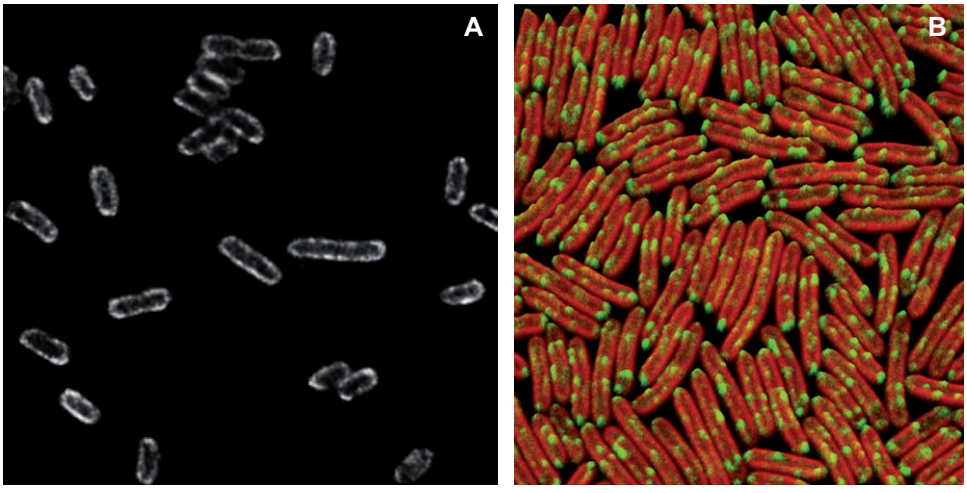

Figure 2 Clustered distribution of (A) *E. coli* ATP synthase (F_0F_1) and (B) NADH-dehydrogenase of *Synecchococcus elongatus* PCC7942 in the membrane.
(A) Epifluorescence image of *E. coli* K-12 cells producing chromosomally encoded ATP synthase complexes with EGFP fused to the membrane-integrated F_0-*a* subunit present in monomeric form in F_0F_1 (AtpB-EGFP). *atpB-egfp* (Düser et al., 2008) was transferred into the chromosome of an Δ*atpIBEF* strain combining the red recombinase system with growth on minimal medium with succinate as the sole carbon and energy source for selection. Cells were grown aerobically to logarithmic phase in minimal medium with 0.5% glycerol (Ballhausen et al., 2009), adhered to agarose (dissolved in minimal medium)-coated slides and imaged on a Leica DM5500B microscope (Solms, Germany) equipped with a HCX Plan-Apochromat 100× objective (NA: 1,46) and a SPOT Pursuit cooled CCD camera. Image courtesy of Patrick Bischoff (Universität Osnabrück, Germany). (B) Confocal fluorescence image of cells of the cyanobacterium *Synechococcus elongatus* PCC7942. Chlorophyll fluorescence is in red, representing the location of the photosynthetic complexes within the membranes, and the fluorescence from NdhM-GFP is in green, showing the clustered distribution of NADH-dehydrogenase in distinct, localized thylakoid membrane zones induced when the plastoquinone pool is predominantly oxidized as described by Liu et al. (2012). Image courtesy of Conrad W. Mullineaux (Queen Mary, University of London, UK).

and become evenly distributed in the membrane (Liu et al., 2012). The use of ET inhibitors revealed that the redistribution of the enzyme complexes is triggered by changes in the redox state of the electron carrier close to plastocyanin. These results demonstrate, for the first time in bacterial membranes, that transient interactions between membrane protein complexes allow the formation of microcompartments. This underlines the redistribution of respiratory complexes as obviously a physiological mechanism to regulate the pathways of electron flow within the membrane. In summary, the data reviewed support the presence of specialized bioenergetic microcompartments in bacterial cytoplasmic membranes, and, although interesting insights were gained in recent years, many questions concerning their generation/stabilization are still unanswered.

Influence of phospholipids on microcompartmentation

The bacterial cytoplasmic membrane has been shown to be a highly dynamic structure with defined lipid microdomains (Lopez and Kolter, 2010) supporting a mosaic character, as in eukaryotic cells (Matsumoto et al., 2006; Mileykovskaya and Dowhan, 2009). Typically, the inner membrane of *E. coli*, which is often used as a model, consists of roughly 70–75% phosphatidylethanolamine (PE), 20–25% phosphatidylglycerol (PG), and 5–10% cardiolipin (CL) (Raetz, 1978), with only three different fatty acid chains, C16:0, C16:1, and C18:1 (Cronan and Gelmann, 1975), which are not uniformly distributed. CL-enriched membrane domains are predominantly located at the cell poles and the septal regions during cell division in rod-shaped bacteria, like *E. coli* (Mileykovskaya and Dowhan, 2000), *B. subtilis* (Kawai et al., 2004) or *Pseudomonas putida* (Bernal et al., 2007) and segregation of PE from PG has been observed in membranes of living *E. coli* cells (Vanounou et al., 2003). Moreover, the osmosensory transporter ProP and the mechanosensitive channel MscS have been shown to concentrate at the cell poles at frequencies strictly correlated to the content of CL present in the *E. coli* membrane (Romantsov et al., 2010). The conical shape of CL has been considered to be an essential parameter relating to its specific enrichment at negatively curved regions in the inner leaflet of bacterial membranes (Huang and Ramamurthi, 2010; Renner and Weibel, 2011). Furthermore, osmotic pinning of the membrane to the cell wall has been proposed to naturally produce lipid microphase separation (Mukhopadhyay et al., 2008) and it has been shown that the integrity of the peptidoglycan forming the cell wall is required to maintain clusters of high-curvature lipids in *B. subtilis* (Muchová et al., 2011). Furthermore, the localization of proteins at specific positions in the bacterial cell membrane requires energy and the membrane potential has been shown to modulate the distribution of morphogenetic proteins in *B. subtilis*, such as the cell division proteins MinD and FtsA, as well as the bacterial cytoskeletal protein MreB (Strahl and Hamoen, 2010).

In *E. coli*, anionic lipids and especially CL are known to influence the topogenesis of membrane proteins (Dowhan and Bogdanov, 2009) and to reconstitute the activity of several purified respiratory complexes, such as NDH-1, lactate-DH, succinate-DH and quinol oxidase bo_3. In addition, the presence of a tightly bound CL molecule has been observed in X-ray structures of formate-DH, succinate-DH, and nitrate reductase A, indicating a fine tuning of these enzymes within the substrate binding site by CL (Arias-Cartin et al., 2012). Also, analyzing a number of crystal structures of cytochrome *c* oxidases, as well as comparing the cytochrome bc_1 complex of respiratory membranes and the corresponding cytochrome b_6f complex of photosynthetic membranes of prokaryotic and eukaryotic cell types, revealed a significant conservation of lipid-binding sites, although the specific identity of the lipids bound varies. All results together imply that bound lipids and particularly CL have an important role in assembly, subunit interaction, function, and structure of enzyme complexes (Palsdottir and Hunte, 2004; Qin et al., 2007; Hasan et al., 2011). Nevertheless, recent data on the cytochrome *c* oxidase of *R. sphaeroides* revealed a striking resilience of the enzyme to drastic changes in its lipid environment, as the cytochrome *c* oxidase produced in CL-depleted cells is fully active in structure and function. Although the lipid profile of the membrane and the lipids bound to the oxidase changed, the requirements for CL were thought to be compensated by the presence of PG, because of its high degree of structural similarity to CL with respect to negative charge and hydrogen bonding capacity (Zhang et al., 2011a,b). Moreover, in mitochondria, CL is also required for supramolecular assembly of ATP synthase complexes (Acehan et al., 2011) as well as for respiratory supercomplex formation and stabilization (Pfeiffer et al., 2003). CL stabilizes the respiratory supercomplex formation between cytochrome bc_1 complex and cytochrome *c* oxidase by neutralizing the charges of lysine residues in the vicinity of their presumed interaction domain. Mutagenesis of these lysine residues in the bc_1 complex (K288, K289, K296 in yeast mitochondria) to leucine, converted the large supercomplexes consisting of dimeric bc_1 and two copies of cytochrome *c* oxidase to smaller supercomplexes and individual complexes in the triple mutant (Wenz et al., 2009). Furthermore, in CL-free

yeast cells (CL is found exclusively in the inner mitochondrial membrane in eukaryotic cells) no supercomplex formation can be observed in the wild type, but this can be restored by removal of the positive charges by substitution of at least two of the lysine residues to leucine (Wenz et al., 2009).

The lipid packing of the membrane seems to be essential for the distribution of membrane proteins and the interaction of their transmembrane helices with the surrounding lipids. Computer simulations and optical microscopy have clearly revealed that lipid packing drives the segregation of transmembrane helices into the highly-disordered domains of heterogeneous model membranes (Schäfer et al., 2011). A comparable picture could be observed using a thermosensitive *E. coli fabA* mutant, in which the production of unsaturated fatty acids is strongly decreased at elevated temperatures (30°C vs. 37°C). A decrease in the amount of unsaturated fatty acids in the lipid core of living *E. coli* cells changed the distribution of the F_0F_1 complexes severely, yielding patches rich in ATP synthases separated from enzyme-free regions, although the changes have no effect on the functional activity of the membrane-integrated F_0F_1 complexes. Specific labeling of ordered/disordered membrane domains with fluorescent dyes revealed a clear phase separation of the lipids and an enrichment of F_0F_1 in the highly-disordered membrane domains (G. Deckers-Hebestreit et al., unpublished results). In summary, the head groups of the phospholipids with their charge and high hydrogen bonding capacity, as well as the fatty acids responsible for the lipid packing in the membrane, play a key role in the distribution of enzyme complexes within the membrane.

Dynamic microcompartments in mitochondria

Structural microcompartmentation and functional heterogeneity

Mitochondria are the main suppliers of cellular energy in non-green higher eukaryotic organisms. These organelles display variable morphologies, from sausage-like to long tubular networks, which are dynamically controlled by organelle fusion and fission (Bereiter-Hahn and Voth, 1994; Yaffe, 1999; Jensen et al., 2000; Frank et al., 2001; Legros et al., 2002; Shaw and Nunnari, 2002). Because of their evolutionary origin, mitochondria possess two membranes, the outer mitochondrial membrane (OM) and the inner mitochondrial membrane (IM). These membranes separate the mitochondria into different compartments: the OM, the intermembrane space (IMS), the IM, and the matrix (Figure 3A). Multiple tubular or disc-like intrusions into the matrix – known as cristae – enlarge the surface of the IM and generate additional microcompartments: the basem membrane of cristae (CM), the intra-cristal space and the inner boundary membrane (IBM) facing the OM (Parsons, 1963; Chance, 1965). The membrane compartments are distinct in their lipid (OM, IM) and protein composition, which defines their functionality (Stoeckenius, 1966). The high protein to lipid ratio (75%:25%; w/w) (Crane and Sun, 1972) reflects the relevance of the IM as a functional microcompartment. Roughly one-third of total mitochondrial protein and 56% of intrinsic proteins in cristae are the protein complexes of the respiratory chain and the mitochondrial F_1F_0 ATP synthase, also known as the oxidative phosphorylation (OXPHOS) system (Blair et al., 1963; Harmon et al., 1974). The ATP is produced in a coupled reaction: the stepwise oxidation of NADH/H^+ and $FADH_2$ by O_2 is combined with the translocation of protons from the matrix microcompartment into the intermembrane space, generating a ΔpH across the IM. The resulting PMF (Mitchell, 1961, 1966) finally drives the ATP synthase activity. The five protein complexes involved in this reaction chain are NADH:ubichinone oxidoreductase (complex I), succinate dehydrogenase (complex II), cytochrome bc_1 complex (complex III), cytochrome *c* oxidase (complex IV) and the F_0F_1-ATP synthase (complex V). The combined results of biochemical studies with electron microscopy (Fernandez-Moran et al., 1964; Gilkerson et al., 2003), and more recently, the probing of OXPHOS complexes by antibodies in EM (Vogel et al., 2006; Wilkens et al., 2012) strongly support the assertion that the cristae membranes are the principle sites of phosphorylation (Figure 3C), although OXPHOS complexes are also found in the IBM microcompartment to a lesser extent. A recent model suggests that sequestration of protons in the intracristae space enables a kind of source-sink mechanism for optimal usage of the proton gradient by the ATP synthase (Strauss et al., 2008).

Tomograms of mitochondria revealed that cristae form disc-like structures but only have one to few connections of narrowed tubular shape to the IBM part of the IMM (Mannella et al., 2001). The diameter of these cristae junctions is roughly 14 nm. Whether single mitochondria can be heterogeneous in their PMF is a controversial and well-discussed subject, but not conclusively proven. Intensity and distribution patterns of JC-1 and DASPMI showed zones of different membrane potential (Smiley et al., 1991; Bereiter-Hahn and Voeth, 1998), and were

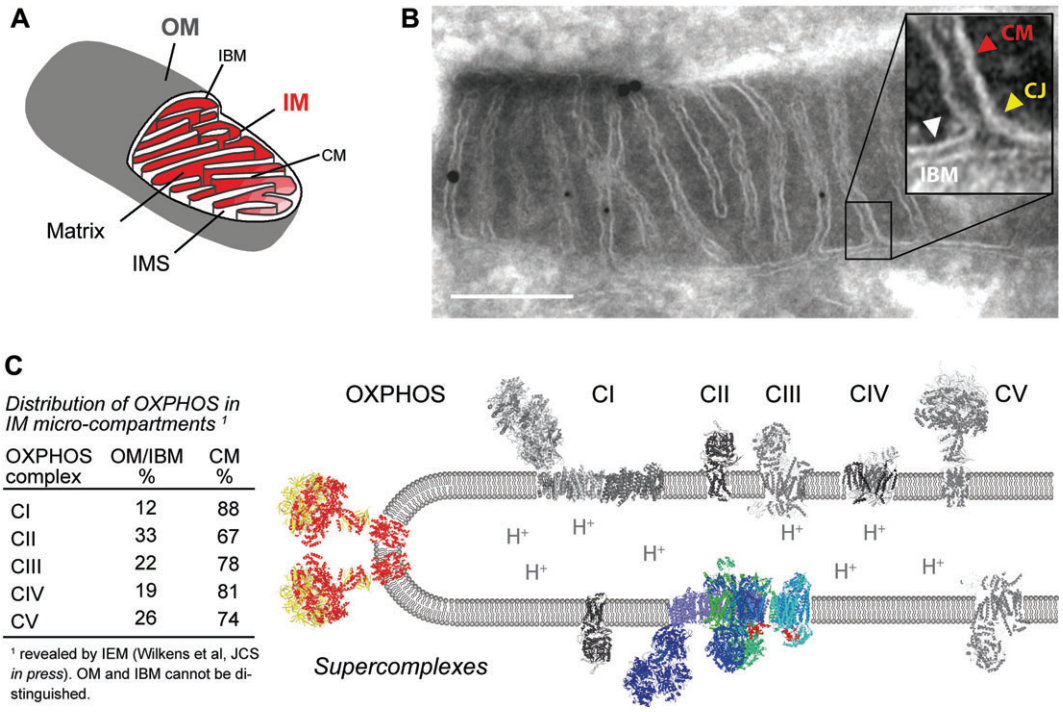

Figure 3 Mitochondrial microcompartments in terms of structure and composition.
(A) Schematic view of a mitochondrion with its main compartments (OM, outer membrane; IM, inner membrane; IMS, intermembrane space). (B) Electron micrograph from a cryo cut mitochondrion with antibody probing of OXPHOS complexes. The localization in the cristae membrane is obvious. Inset: detailed view of the two IM microcompartments CM (cristae membranes) and IBM connected by the CJ. Scale bar: 150 nm. (C) Localization of OXPHOS complexes and supercomplexes in the CM (Wilkens et al., 2012). OXPHOS structures from (Yoshikawa et al., 1998; Sun et al., 2005; Rees et al., 2009; Efremov et al., 2010; Watt et al., 2010; Althoff et al., 2011). IEM image courtesy of Verena Wilkens (Universität Osnabrück, Germany).

related to a heterogeneous mitochondrial ultrastructure such as different patterns of cristae membranes (Figure 4A) and/or with higher and lower content of functional OXPHOS complexes. The maintenance of local differences in membrane potential ensures that (1) a high local resistance and (2) a low proton/ion flux along the IM compared to the local proton transport exists. Data obtained with mitoplasts substantiate surface ΔpH^S as the primary energy source of PMF for mitochondrial ATP (Bhagawati et al., 2010). Lateral ion/proton migration is fast along

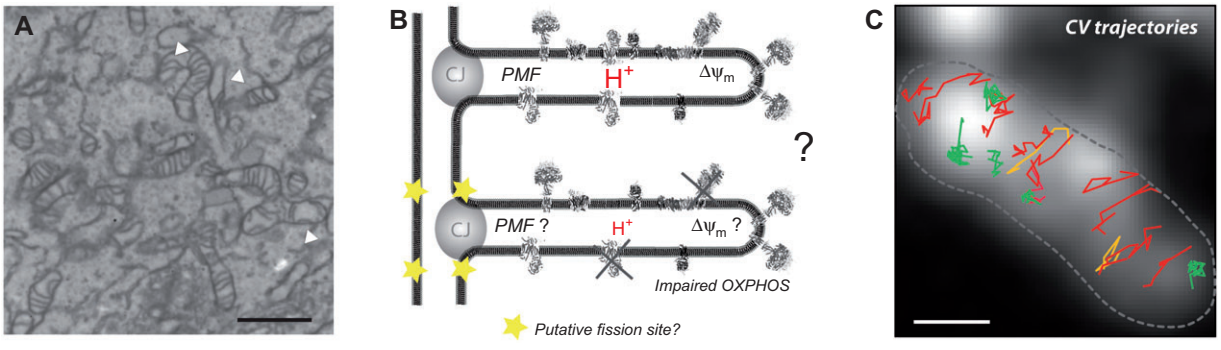

Figure 4 Possible heterogeneity of ultrastructure, function and dynamics within single mitochondria.
Arrangement of CM membranes in mammalian HeLa cells and diffusion of membrane proteins within these. (A) Mitochondria in HeLa cells with regular and irregular cristae patterns. (B) Hypothetic model for the development of local differences in proton concentration. Precondition is the existence of self-contained cristae separated by CJs from the IMS. Yellow stars indicate putative fission sites to separate damaged parts. (C) Trajectory map of single F_0F_1-ATP synthase molecules in normal mitochondria showing restricted mobility in the CM. Red and orange trajectory maps depict trajectories in the CM, green are confined immobile molecules. Scale bars: 2000 nm (A) and 500 nm (C). EM images courtesy of Verena Wilkens (Universität Osnabrück, Germany).

membrane surfaces (Heberle et al., 1994), but in complex systems like the mitochondrial and thylakoid membranes with integrated and associated proteins this might be a too simple a model, neglecting the influence of basic and acid protein surfaces on trapping and releasing protons/ions.

A further – yet hypothetical – model is that cristae junction (CJ) constitute a diffusion barrier between intracristal space and the section of the intermembrane space between IBM and OM (Figure 4B). If so, this would allow the existence of adjacent cristae with different [H^+]. How this would influence the local and general electrochemical membrane potential and PMF is not clear, however (Figure 4B). Recently, a large protein complex localized next to or at the CJ was found by several groups in independent studies. This MINOS/MitOS/MICOS (Hoppins et al., 2011; Alkhaja et al., 2012) complex – or at least particular proteins of it (Rabl et al., 2009) – likely plays a role in CJ formation. Also OPA1, a small dynamin involved in IM fusion, is probably located next to the CJ. Whether these proteins constitute a diffusion barrier for solutes of the intracristal space remains to be experimentally proven. A putative sealing of individual intracristal microcompartments is also important for a compartmentalization of cytochrome *c*. Cytochrome is the mobile electron carrier between respiratory complex III and complex IV. After intrinsic apoptotic stimuli, it is released from mitochondria setting on a deathly signaling cascade. The mechanism of cytochrome *c* (and other proteins) release from mitochondria is not conclusively clarified (Scorrano, 2009). One model favors that remodeling of the CJ is a prerequisite for the ejection from the intracristal space, while a different model suggests that cytochrome *c* diffuses freely in the entire IMS and is set free without prerequisite structural rearrangements (Sowers and Hackenbrock, 1981; Manor IV et al., 2006). In addition, putative heterogeneity of individual mitochondria is also important in the context of mitochondrial quality control. Ongoing fusion and fission of mitochondria is a prerequisite for the elimination of damaged mitochondria by mitophagy. After a fission event, daughter mitochondria frequently show different polarization states in terms of membrane potential (Twig et al., 2008). It is not elucidated, though, whether fission specifically occurs at functionally impaired sites to separate these parts from the rest of the mitochondrion (Figure 4B). The determination of membrane potential changes by dyes such as JC-1, DASPMI or TMR might bear some problems, though, as fluorescence intensity changes or emission shifts depend on the local accumulation of the dye. With the new generation of ratiometric and (thus intensity-independent) pH- and redox-sensitive fluorescent proteins (Meyer et al., 2007; Poburko et al., 2011) specifically targeted to the distinct mitochondrial microcompartments, the problem can now be addressed more accurately on the single mitochondrial level, helping to elucidate the link between ultrastructure and function.

Dynamics of mitochondrial ultrastructure

Functional changes are often linked to ultrastructural changes (Paumard et al., 2002; Zick et al., 2009; You et al., 2010) and it is known that the matrix condensation state, as well as the arrangement of cristae, depends on the metabolic state of the mitochondria. Hackenbrock first observed condensed and orthodox configurations of mitochondria, which were later correlated with respiratory states and energy loading (Hackenbrock, 1966; Mitin and Beketova, 1972). Under experimental conditions, such as injection of ADP, P_i and ATP into cells, or laser-induced depletion of PMF and subsequent recovery, this transformation could be artificially induced (Bereiter-Hahn et al., 1983). A comprehensive review on the intimate relationship between mitochondrial form and function is found here (Benard and Rossignol, 2008). Besides OPA1, and Fcj1/mitofilin as part of a larger cristae organizing complex, MINOS/MitOS/MICOS, ATP synthase is also a strong determinant of cristae shape, and recently it was shown that LACTB, a serin-protein through polymerization promotes intramitochondrial membrane organization and microcompartmentation (Polianskyte et al., 2009). Clearly, larger protein assemblies in the IMM and obviously also in the intracristal space contribute to CM formation and maintenance, and it is likely that they play a role as intramitochondrial scaffolds for microcompartmentation.

Supramolecular organization of OXPHOS in mitochondrial membranes

The question of whether single OXPHOS complexes are organized in supramolecular assemblies – and under which circumstances this occurs – is a long lasting debate, starting shortly after the discovery of the respiratory subunits on mitochondrial membranes (Hackenbrock, 1966). The random collision model, proposing freely diffusing respiratory complexes, has a counterpart in the solid state model. 'Oxysomes' (Chance et al., 1963), later termed respiratory supercomplexes, were first postulated to occur from biochemical studies, and their existence was supported by electron microscopy data that showed regularly arranged high molecular weight particles in the IM

(Fernandez-Moran, 1962; Fernandez-Moran et al., 1964). In the last decade, respiratory supercomplexes consisting of complex I, III and IV (Schägger and Pfeiffer, 2000; Eubel et al., 2004; Dudkina et al., 2005; Schäfer et al., 2006; Wittig et al., 2006; Althoff et al., 2011) have been isolated under mild solubilization conditions. Their analysis revealed diverse compositions, mostly CI, CIII and CIV, and also diverse stoichiometry. The plasticity model suggests that the assembly of OXPHOS into supercomplexes is dynamic (Acin-Perez et al., 2008), which finds support from observations that supercomplex abundance and composition also varies with age (Dencher et al., 2007). While arrays of ATP synthase are easily seen in TEM and EM, the identification of supercomplexes *in situ* still awaits verification. Dimerization of ATP synthase is often accompanied by oligomerization and row-like assembly in cristae membranes (Davies et al., 2011) and this is thought to be one of the main determining factors for CM microcompartment formation. Recent observations also suggest this F_0F_1 dominated compartmentation for bacteria (see earlier description of bacterial microcompartments). This dimerization of the ATP synthase in yeast is governed by subunits g and e (Arnold et al., 1998), while subunit b might play a role in the stabilization of dimer rows (Gavin et al., 2005). For mammalian mitochondria, it was proposed that ATP synthase dimerization is membrane potential dependent and controlled by the inhibitory factor IF1 of F_0F_1-ATP synthase (Campanella et al., 2008). Apparently, the role of ATP synthase in scaffolding is directly linked to its activity, but whether it also influences the organization of the respiratory part, still has to be revealed. Possible advantages that have been suggested for supercomplex formation include substrate channeling, higher efficiency, and reduction of ROS production, which as discussed is a significant threat to the cell. Despite many indications, our knowledge of causative and regulatory factors of mitochondrial supercomplex formation is still rudimentary.

Mobility of OXPHOS proteins in mitochondria

A precondition for the dynamic assembly/disassembly of OXPHOS complexes into supercomplexes is their mobility in the IM microcompartments. Fluorescence recovery studies after photobleaching of fluorescence-labeled OXPHOS could indeed show a diffusion of OXPHOS complexes in the IM (Sukhorukov et al., 2010), but of anomalous diffusion. However, to date the resolution of this method is too low to distinguish detailed events. Recent developments in superresolution techniques have allowed the tracking and localization of mitochondrial membrane complexes, such as OXPHOS in the IMM. In this case, individual trajectories of single F_0F_1 ATP synthase complexes could be visualized, and showed slow but clear diffusion in the cristae microcompartment (Figure 4C). In contrast, succinate dehydrogenase was definitively more mobile and also displayed a noticeable quota of molecules localized in the IBM (Appelhans et al., 2012). Succinate dehydrogenase (SDH) participates at the OXPHOS as well as the citrate cycle metabolism. Since SDH is not involved in proton translocation, it follows that it need not necessarily be constrained to the CM. Trespassing of OXPHOS between CM and IBM was rather rare, underlining the concept of spatial separation of IM microcompartments (Wilkens et al., 2012). From these new data, it has become clear that OXPHOS complexes are mobile, albeit predominantly restricted to the CM. This mobility is a prerequisite for the random collision model earlier discussed. The next step in investigating OXPHOS complex mobility will be to identify OPXHOS supercomplexes *in situ* and determine their mobility. Through recent progress in life cell superresolution techniques these spatio-temporal organization dynamics of, and within, microcompartments are now becoming visible.

Microcompartmentation in photosynthetic membranes

Photosynthetic electron transport

In photosynthetic organisms, pigment excitation by light is used to drive photosynthetic electron transfer (PET), generating PMF for use in photosynthetic phosphorylation (PHOTPHOS). Because energy input (light intensity) is beyond the control of the cell, efficient PET requires rapid and dynamic changes in macromolecular complex assembly. PMF generation must be maximized at low light intensities, but excess excitation of chlorophyll-containing complexes results in damaging ROS, and if PMF generation exceeds ATPase capacity, high proton concentrations inhibit protein function. This fundamental problem is partly solved by the separation of specific proteins and protein complexes between distinct membrane microcompartments, and their redistribution in response to energetic input and demand.

Spatial separation of PET complexes

In chloroplasts, the energetic thylakoid membrane is divided into sheet-like stromal lamellae, and assemblies

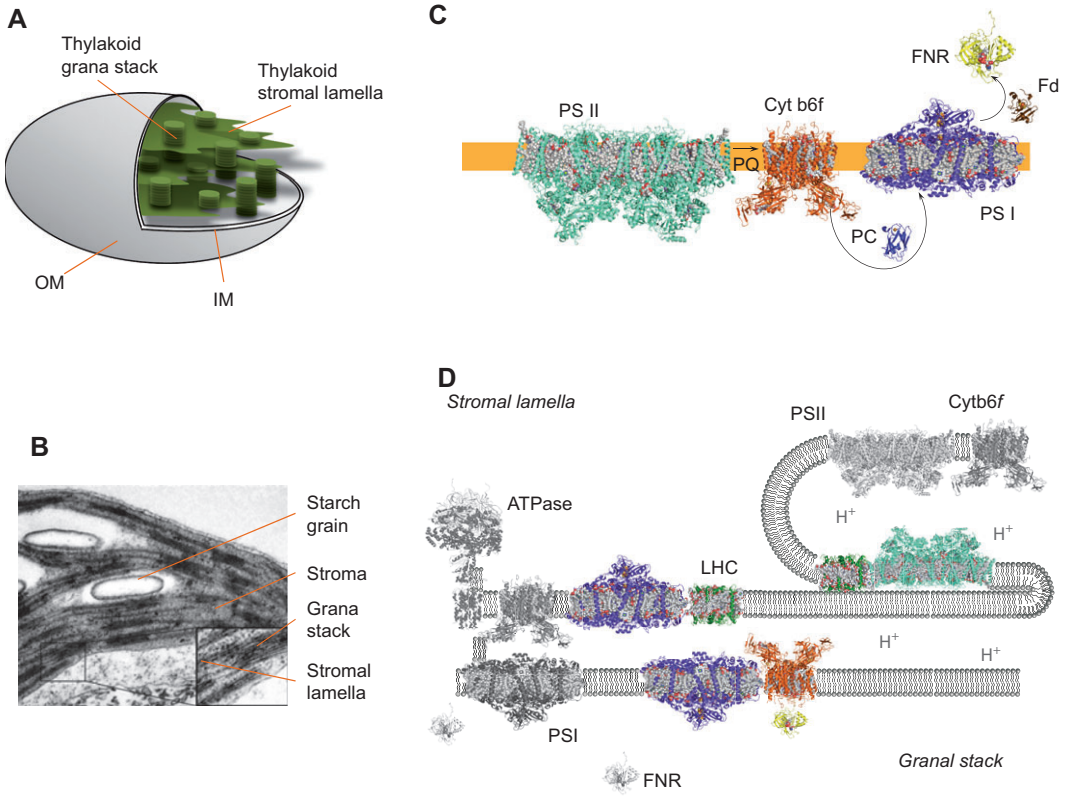

Figure 5 (A) Cartoon of chloroplast structure showing outer membrane (OM), inner membrane (IM) and the thylakoid structures. (B) Electron micrograph of chloroplast internal structure, showing starch grains and the thylakoid structures; the inset shows a magnification of stromal lamellae and granal stacks. (C) The pathway of linear photosynthetic electron transport: water is split by photoactivation of photosystem II (PSII) and the resulting electrons transported through the membrane by plastoquinone (PQ) to the cytochrome b_6f complex (Cyt b_6f). Plastocyanin (PC) then carries the electrons to photosystem I (PSI), which donates them to ferredoxin (Fd) on photoactivation. Finally, Fd transfers these electrons to Fd:$NADP^+$ reductase (FNR) for the reduction of $NADP^+$. (D) Microcompartmentation of PET complexes. PSI and ATPase are restricted to the stromal lamellae, while active PSII is only found in the granal stacks. Cyt b_6f diffuses freely between the two microcompartments, while FNR is both soluble and membrane-bound. Depending on environmental conditions, light-harvesting complex II (LHCII) may either supercomplex with PSII or PSI, supercomplexes can form between PSI and Cyt b_6f, and FNR may be solubilized or associate with different protein complexes.

of disc-like structures, known as granal stacks (Figure 5A, B). These regions are connected, enclosing a single thylakoid lumen (Shimoni et al., 2005), in which protons are accumulated during PET. Analogous to the mitochondrial IM (see earlier description), these internal membrane structures are in themselves dynamic, and 'unstacking' of grana has been shown to be induced under several conditions, including high light, when it is proposed to aid repair of damaged PSII complexes (Khatoon et al., 2009). Cyanobacteria are the endosymbiont ancestors of the chloroplast, and also contain an internal network of thylakoid membranes. Although cyanobacteria lack grana, in many species the thylakoid comprises distinct concentric shells, which merge with each other and with the cytoplasmic membrane (Nierzwicki-Bauer et al., 1983) forming a single, interconnected lumen (Nevo et al., 2007). The inner and outer layers of the thylakoid therefore represent different microcompartments in most cyanobacteria.

In classical PET, photoactivation of photosystem I (PSI) and photosystem II (PSII) allows the generation of PMF, with electron flow consecutively through the membrane complexes: PSII, the cytochrome b_6f complex (Cyt b_6f), and then PSI (Figure 5C). Plastoquinone (PQ), and the protein plastocyanin transfer electrons between PSII and the Cyt b_6f, and between Cyt b_6f and PSI, respectively, to connect the chain. The final acceptor at PSI is ferredoxin (Fd), which transfers these electrons to the Fd:NADP(H) reductase (FNR) enzyme, resulting in the photoproduction of NADPH. In PET, PMF is generated principally through the splitting of water at PSII, and by shuttling of protons across the membrane by ET between PQ and Cyt b_6f, in a Q cycle. In addition to the ATP synthesized by the generation of PMF, NADPH is also an important energetic

product of PET, and is consumed in many pathways, including carbon fixation. The rate of excitation of both photosystems is greatly enhanced by their association in super-complexes with light-harvesting complexes (LHCII at PSII and LHCI at PSI), which transfer the energy from absorbed photons to the RC of the photosystems by resonance energy transfer.

The membrane protein complexes involved in PET are not evenly distributed throughout the thylakoid membrane (Figure 5D). PSI is enriched at the stromal lamellae, while active PSII is found mainly in the appressed regions of the granal stacks (Anderson and Melis, 1983; Danielsson et al., 2006). Like PSI, the ATP synthase complex is also excluded from the appressed regions of the granal stacks, while the Cyt b_6f complex is evenly distributed between the different regions (Allred and Staehelin, 1985, 1986). It has been hypothesized that separation of the photosystems may be related to their kinetic differences: PSI pigments absorb at a longer wavelength and the complex turns over at a higher rate than PSII, meaning that if the complexes were close together PSI might drain energy away from PSII (Trissl and Wilhelm, 1993). However, the separate microcompartmentation of different PET components has implications for the efficiency of the classical, linear photosynthetic electron flow. It is generally accepted that *in vivo* PQ cannot diffuse over long distances between PSII and Cyt b_6f (Lavergne and Joliot, 1991), meaning that PSI complexes in the stromal lamellae must rely on diffusion of reduced PQ from the appressed regions. Perhaps as a consequence of this, it has been reported that PSI RC are aggregated around the granal margins, close to the source of reduced PQ (Kaftan et al., 2002), implicating the border regions between grana lamellae as the location of intense electron transport activity.

Cyanobacteria conduct PET using the same membrane complexes as chloroplasts, but in addition their membranes necessarily contain the components of OXPHOS. Distribution of all these complexes has been shown to vary, with OXPHOS complexes restricted to the outer envelope, the ATPase and PSI complexes mainly in the outer thylakoid layer, and the PSII and Cyt b_6f evenly distributed throughout the outer and inner thylakoid layers (Sherman et al., 1994). Formation of supercomplexes in cyanobacteria by these components has not been investigated, but based on data for bacteria (see earlier description of bacterial microcompartments) it is reasonable to assume that this occurs. It should be noted that not all cyanobacteria have thylakoids arranged in concentric layers, but it has recently been shown that *Synechocystis*, a cyanobacteria with an alternative, radial thylakoid structure, also contains differentiated regions of the thylakoid, either rich in PSI or PSII (Agarwal et al., 2012).

Dynamic redistribution of PET components in response to the environment

The spatial separation of different processes in the ET chain between the granal stacks and stromal lamellae helps to regulate the PET chain. To accomplish this regulation, several of the PET membrane complexes are dynamically redistributed between different microcompartments. Several of these redistribution events are now very well understood, while others are just emerging. Here we will review three specific examples of dynamic changes in PET component microcompartmentation in chloroplasts. Although little data is published for cyanobacteria, uneven distribution of the photosynthetic complexes has been reported (Sherman et al., 1994) and it has been suggested that dynamic movement of protein complexes between the outer and inner thylakoid layers might also enable modulation of PET (Nevo et al., 2007).

Changes in microcompartmentation of LHCII: state transitions

The best understood regulatory event in chloroplast thylakoids concerns redistribution of the LHCII complex in order to balance excitation between PSI and PSII, in a process called a state transition. Readers are directed to recent excellent reviews for a more detailed description (Lemeille and Rochaix, 2010; Pesaresi et al., 2011). If a plant is shaded by other leaves, the remaining light available to it will be red-enriched, while as water depth increases, less red light is available to algae. PSII absorbs light at slightly higher wavelengths (680 nm) than PSI (700 nm), and this means that, as the balance of red to blue light shifts with changing conditions, the ratio of excitation between PSI:PSII is unbalanced.

When PSII is more active than PSI, internal components of the PET chain build up in a reduced state, including PQ. The increase in the reduction state of PQ activates a kinase (Allen et al., 1981) on docking at the Q_o site on Cyt b_6f (Vener et al., 1997). A specific kinase (Stt7 in Chlamydomonas and Stn7 in Arabidopsis) is associated with the Cyt b_6f (Lemeille et al., 2009) and its activity causes phosphorylation of LHCII (Fleischmann et al., 1999; Bellafiore et al., 2005). On phosphorylation, LHCII dissociates from the PSII-LHCII supercomplex (Iwai et al., 2008) and

migrates from the grana to the stromal lamellae, where it can associate with PSI, in PSI-LHCI-LHCII supercomplexes (Zhang and Scheller, 2004a). Based on these data, phosphorylation is thought to change the relative affinity of LHCII for interaction with PSII and PSI. PSI-LHCI-LHCII super-complexes are considered to exist predominantly at the margins of the grana, rather than throughout the lamellae, because of the specific increase in PSI fluorescence here on state transition (Tikkanen et al., 2008).

If the balance of excitation between the photosystems changes, and PSI excitation increases relative to PSII, causing oxidation of internal PET components, the state transition is reversed and the LHCII complex returns to the granal stacks and associates with PSII. This happens upon dephosphorylation of LHCII, which is catalyzed by the TAP38/PPH1 phosphatase (Pribil et al., 2010; Shapiguzov et al., 2010). No information is yet available about regulation of TAP38/PPH1, but stt7/stn7 is deactivated when the internal PET components become oxidized, including and PQ at the Q_o binding site of Cyt b_6f. In summary, to ensure maximum PET efficiency, under conditions where PSII is preferentially excited, LHCII disengages from the PSII complex in the appressed grana, and dynamically changes its microcompartmentation, to associate with PSI. This has the dual effect of decreasing excitation at PSII, while increasing the light-harvesting capacity at PSI. When PSI excitation exceeds that at PSII, the process is reversed, and LHCII is dephosphorylated before migrating back to the appressed grana to reassociate with PSII.

Changes in microcompartmentation of Cyt b_6f

State transitions are very dramatic in algae, with up to 80% of LHCII reported to move (Delosme et al., 1996). By contrast, only around 10–20% of LHCII has been reported to migrate in higher plant chloroplasts (Vallon et al., 1991). However, the same study reported a surprising change in distribution of Cyt b_6f. Normally Cyt b_6f appears evenly distributed between grana stacks and stromal lamellae, but under state transition-inducing conditions, 30–40% of Cyt b_6f was reorganized, causing accumulation in the stromal lamellae (Vallon et al., 1991). This change in location is relevant to state transitions, as the activation of Stn7/Stt7 depends on PQ interaction with Cyt b_6f, but it has other implications for regulation of PET. In addition to the linear chain of PET described above, where electrons flow from water to reduce $NADP^+$, alternative pathways can also generate PMF. In such cyclic electron transport Cyt b_6f and PSI act independently of PSII, and electrons are returned to PQ by Fd or NADPH, generating a proton gradient with no net reduction of soluble components (Arnon et al., 1963; Joliot and Joliot, 2006). PC transfers electrons from Cyt b_6f to PSI, but the means of electron return from PSI to Cyt b_6f remains controversial. A unique heme group close to the stromal side of the membrane, not involved in electron transport during the Q cycle, has been identified (Kurisu et al., 2003) indicating the flow of electrons back into the PET chain may be through the Cyt b_6f. And it is known that the Pgr5 and PgrL1 proteins are involved in the switch between linear and cyclic electron transport (Munekage et al., 2002; DalCorso et al., 2008). A specific Fd:PQ reductase has been proposed to catalyze the ET reaction, but never identified, and because FNR has been shown to donate electrons to quinones (Bojko et al., 2003) and interact with Cyt b_6f, PgrL1 and a complex of Cyt b_6f, PgRL1 and PSI (Zhang et al., 2001; DalCorso et al., 2008; Iwai et al., 2010), it has been suggested that FNR might be a Fd:PQ reductase under specific conditions. State transitions result in strong upregulation of cyclic electron flow in algae, where in addition to PSI:PSII imbalance they are induced by low ATP, to enable rapid generation of PMF (Bulté et al., 1990), although there is no evidence for this in higher plants. The identification of PSI-Cyt b_6f supercomplexes suggests that the redistribution of Cyt b_6f on state transition might be due to 'trapping' of freely diffusing Cyt b_6f by PSI in the stromal lamellae, rather than targeted movement (Nevo et al., 2007). Irrespective of complex formation, the increase in co-localization of PSI and Cyt b_6f would seem to favor the induction of cyclic, rather than linear PET.

Changes in microcompartmentation of FNR

FNR is the final thylakoid associated enzyme in linear PET, and there is evidence it may also function in cyclic electron flow (Zhang et al., 2001; Bojko et al., 2003; DalCorso et al., 2008; Iwai et al., 2010). It has been shown that in cyanobacteria and chloroplasts, the enzyme is present as both soluble and membrane-bound forms (Hanke et al., 2005; Okutani et al., 2005; Thomas et al., 2006), and it has been localized at several different membrane complexes, including PSI and Cyt b_6f (Andersen et al., 1992; Zhang et al., 2001). Additionally, two specific FNR binding proteins localized at the thylakoid have recently been identified, and they vary in microcompartmentation. Tic62 is localized on the stromal lamellae, while TROL is described at the stromal lamellae, grana margins (Benz et al., 2009, 2010; Juric et al., 2009). While FNR bound to Tic62 has no photosynthetic function, FNR localized at TROL is necessary for optimal PET (Juric et al., 2009).

The localization at different membrane complexes, and abundance of the soluble enzyme, indicate that FNR recruitment may provide a mechanism for regulating PET. Indeed, it has been shown that the affinity of FNR for the Tic62 binding protein is dependent on pH, and that interaction is disrupted at the basic pH expected when PMF is generated (Alte et al., 2010). Moreover, dissociation of FNR from both Tic62 and TROL has been reported at high light (Benz et al., 2009). These observations prompted the suggestion that soluble, rather than membrane-bound FNR has the major catalytic activity in PET (Benz et al., 2010), but this contradicts reports that membrane-bound FNR is more catalytically active (Forti and Bracale, 1984), and that TROL mutants have disrupted PET (Juric et al., 2009). Moreover, in cyanobacteria FNR is bound to the thylakoid by a phycobilisome linker protein (Thomas et al., 2006), and under non-photosynthetic conditions alternative splicing results in a truncated, soluble enzyme that functions in heterotrophic, rather than photosynthetic, metabolism (Korn et al., 2009). In either case, the release of FNR from Tic62 on the stromal lamellae, in order to function as a soluble enzyme, or to allow association with other PET complexes, such as the TROL at the grana margins, shows that dynamic re-microcompartmentation of FNR plays a role in PET. Maize contains three different FNR iso-proteins that vary in respect to membrane association: FNR1 is exclusively membrane-bound, FNR2 is both membrane-bound and soluble, and FNR3 is completely soluble in maize chloroplasts (Okutani et al., 2005). When genes for these proteins were expressed in Arabidopsis, the FNR3 protein remained mostly soluble, while the FNR1 and FNR2 differentially interacted with TROL and Tic62 complexes respectively (Twachtmann et al., 2012). During the transition between dark and light, the PSI:PSII excitation ratio was higher in plants enriched in TROL FNR, but not in plants enriched at Tic62, further supporting a role for dynamic FNR relocation in PET regulation. Finally, the N-terminal structure of the FNR enzyme is critical for regulating its membrane association (Moolna and Bowsher, 2010), and the specificity of its interaction with different membrane complexes (Twachtmann et al., 2012).

Outlook

In this review we have described how the restriction of various processes to specific areas on bioenergetic membranes is fundamental to their efficient function, both in preventing energy loss and formation of harmful ROS. This basic principle of microcompartmentation is conserved from the early origins of energy-converting membranes, through the interchangeable nature of electron acceptors and donors in bacteria membranes, to the highly complex organized structures that have evolved for OXPHOS and PHOTPHOS in mitochondria and chloroplasts. We have established that in order to achieve efficient PMF formation and utilization, supramolecular organization is necessary, not only in terms of spatial organization of membrane complexes and formation of supercomplexes, but also in the water layers around membranes, and the lipid composition of the membranes themselves. There is abundant evidence for microcompartmentation in all bioenergetic membranes, and a growing body of data that support dynamic changes in response to the energetic demands placed upon a cell. The challenge in the future will be to investigate these dynamic changes and interactions, their regulatory control and their physiological impact. In order to solve these questions, the use of superresolution techniques, to monitor changes in protein complex location and supercomplex assembly in real time will increasingly prove to be an invaluable tool.

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References

Acehan, D., Malhotra, A., Xu, Y., Ren, M., Stokes, D.L., and Schlame, M. (2011). Cardiolipin affects the supramolecular organization of ATP synthase in mitochondria. Biophys. J. *100*, 2184–2192.

Acin-Perez, R., Fernandez-Silva, P., Peleato, M.L., Perez-Martos, A., and Enriquez, J.A. (2008). Respiratory active mitochondrial supercomplexes. Mol. Cell *32*, 529–539.

Agarwal, R., Maralihalli, G., Sudarsan, V., Choudhury, S.D., Vatsa, R.K., Pal, H., Melzer, M., and Sainis, J.K. (2012). Differential distribution of pigment-protein complexes in the thylakoid membranes of Synechocystis 6803. J. Bioenerg. Biomembr. *44*, 399–409.

Alkhaja, A.K., Jans, D.C., Nikolov, M., Vukotic, M., Lytovchenko, O., Ludewig, F., Schliebs, W., Riedel, D., Urlaub, H., Jakobs, S.,

et al. (2012). MINOS1 is a conserved component of mitofilin complexes and required for mitochondrial function and cristae organization. Mol. Biol. Cell *23*, 247–257.

Allen, J.F., Bennett, J., Steinback, K.E., and Arntzen, C.J. (1981). Chloroplast protein phosphorylation couples plastoquinone redox state to distribution of excitation energy between photosystems. Nature *291*, 25–29.

Allen, R.D., Schroeder, C.C., and Fok, A.K. (1989). An investigation of mitochondrial inner membranes by rapid-freeze deep-etch techniques. J. Cell. Biol. *108*, 2233–2240.

Allred, D.R. and Staehelin, L.A. (1985). Lateral distribution of the cytochrome b(6)/f and coupling factor ATP synthetase complexes of chloroplast thylakoid membranes. Plant Physiol. *78*, 199–202.

Allred, D.R. and Staehelin, L.A. (1986). Spatial organization of the cytochrome b_6/f complex within chloroplast thylakoid membranes. Biochim. Biophys. Acta *849*, 94–103.

Alte, F., Stengel, A., Benz, J.P., Petersen, E., Soll, J., Groll, M., and Bolter, B. (2010). Ferredoxin: NADPH oxidoreductase is recruited to thylakoids by binding to a polyproline type II helix in a pH-dependent manner. Proc. Natl. Acad. Sci. USA *107*, 19260–19265.

Althoff, T., Mills, D.J., Popot, J.L., and Kühlbrandt, W. (2011). Arrangement of electron transport chain components in bovine mitochondrial supercomplex I1III2IV1. EMBO J. *30*, 4652–4664.

Andersen, B., Scheller, H.V., and Moller, B.L. (1992). The PSI-E subunit of photosystem I binds ferredoxin:NADP+ oxidoreductase. FEBS Lett. *311*, 169–173.

Anderson, J.M. and Melis, A. (1983). Localization of different photosystems in separate regions of chloroplast membranes. Proc. Natl. Acad. Sci. USA *80*, 745–749.

Andersson, S.G., Zomorodipour, A., Andersson, J.O., Sicheritz-Pontén, T., Alsmark, U.C., and Podowski, R.M. (1998). The genome sequence of *Rickettsia prowazekii* and the origin of mitochondria. Nature *396*, 133–140.

Appelhans, T., Richter, C.P., Wilkens, V., Hess, S.T., Piehler, J., and Busch, K.B. (2012). Nanoscale organization of mitochondrial microcompartments revealed by combining tracking and localization microscopy. Nano Lett. *12*, 610–616.

Arias-Cartin, R., Grimaldi, S., Arnoux, P., Guigliarelli, B., and Magalon, A. (2012). Cardiolipin binding in bacterial respiratory complexes: structural and functional implications. Biochim. Biophys. Acta, *1817*, 1937–1949.

Arnold, I., Pfeiffer, K., Neupert, W., Stuart, R.A., and Schägger, H. (1998). Yeast mitochondrial F_1F_0-ATP synthase exists as a dimer: identification of three dimer-specific subunits. EMBO J. *17*, 7170–7178.

Arnon, D.I., Tagawa, K., and Tsujimoto, H.Y. (1963). Role of ferredoxin in the energy conversion process of photosynthesis. Science *140*, 378.

Ballhausen, B., Altendorf, K., and Deckers-Hebestreit, G. (2009). Constant c_{10} ring stoichiometry in the *Escherichia coli* ATP synthase analyzed by cross-linking. J. Bacteriol. *191*, 2400–2404.

Bekker, M., de Vries, S., Ter Beek, A., Hellingwerf, K.J., and Teixeira de Mattos, M.J. (2009). Respiration of *Escherichia coli* can be fully uncoupled via the nonelectrogenic terminal cytochrome *bd*-II oxidase. J. Bacteriol. *191*, 5510–5517.

Bellafiore, S., Barneche, F., Peltier, G., and Rochaix, J.D. (2005). State transitions and light adaptation require chloroplast thylakoid protein kinase STN7. Nature *433*, 892–895.

Benard, G. and Rossignol, R. (2008). Ultrastructure of the mitochondrion and its bearing on function and bioenergetics. Antioxid. Redox Signal. *10*, 1313–1342.

Benz, J.P., Stengel, A., Lintala, M., Lee, Y.H., Weber, A., Philippar, K., Gugel, I.L., Kaieda, S., Ikegami, T., Mulo, et al. (2009). Arabidopsis Tic62 and ferredoxin-NADP(H) oxidoreductase form light-regulated complexes that are integrated into the chloroplast redox poise. Plant Cell *21*, 3965–3983.

Benz, J.P., Lintala, M., Soll, J., Mulo, P., and Bolter, B. (2010). A new concept for ferredoxin-NADP(H) oxidoreductase binding to plant thylakoids. Trends Plant Sci. *15*, 608–613.

Bereiter-Hahn, J. and Voth, M. (1994). Dynamics of mitochondria in living cells: shape changes, dislocations, fusion, and fission of mitochondria. Microsc. Res. Tech. *27*, 198–219.

Bereiter-Hahn, J. and Voeth, M. (1998). Do single mitochondria contain zones with different membrane potential? Exp. Biol. (online) *3*, 1–13.

Bereiter-Hahn, J., Voeth, M., and Tillmann, U. (1983). Mitochondrial response to micro-iontophoretic injection of ADP and ATP in primary cultured cells. Eur. J. Cell. Biol. *Suppl. 2*, 5.

Bernal, P., Muñoz-Rojas, J., Hurtado, A., Ramos, J.L., and Segura, A. (2007). A *Pseudomonas putida* cardiolipin synthesis mutant exhibits increased sensitivity to drugs related to transport functionality. Environ. Microbiol. *9*, 1135–1145.

Berry, E.A. and Trumpower, B.L. (1985). Isolation of ubiquinol oxidase from *Paracoccus denitrificans* and resolution into cytochrome bc_1 and cytochrome c-aa_3 complexes. J. Biol. Chem. *260*, 2458–2467.

Bhagawati, M., Lata, S., Tampe, R., and Piehler, J. (2010). Native laser lithography of His-tagged proteins by uncaging of multivalent chelators. J. Am. Chem. Soc. *132*, 5932–5933.

Blair, P.V., Oda, T., and Green, D.E. (1963). Studies on the electron transfer system. Liv. isolation of the unit of electron transfer. Biochemistry *2*, 756–764.

Bojko, M., Kruk, J., and Wieckowski, S. (2003). Plastoquinones are effectively reduced by ferredoxin:NADP+ oxidoreductase in the presence of sodium cholate micelles. Significance for cyclic electron transport and chlororespiration. Phytochemistry *64*, 1055–1060.

Borisov, V.B., Murali, R., Verkhovskaya, M.L., Bloch, D.A., Han, H., Gennis, R.B., and Verkhovsky, M.I. (2011). Aerobic respiratory chain of *Escherichia coli* is not allowed to work in fully uncoupled mode. Proc. Natl. Acad. Sci. USA *108*, 17320–17324.

Bulté, L., Gans, P., Rebéillé, F., and Wollman, F.A. (1990). ATP control on state transitions in vivo in *Chlamydomonas reinhardtii*. Biochim. Biophys. Acta *1020*, 72–80.

Campanella, M., Casswell, E., Chong, S., Farah, Z., Wieckowski, M.R., Abramov, A.Y., Tinker, A., and Duchen, M.R. (2008). Regulation of mitochondrial structure and function by the F_1F_0-ATPase inhibitor protein, IF1. Cell Metab. *8*, 13–25.

Castelle, C., Guiral, M., Malarte, G., Ledgham, F., Leroy, G., Brugna, M., and Giudici-Orticoni, M.T. (2008). A new iron-oxidizing/O_2-reducing supercomplex spanning both inner and outer membranes, isolated from the extreme acidophile *Acidithiobacillus ferrooxidans*. J. Biol. Chem. *283*, 25803–25811.

Chance, B. (1965). Reaction of oxygen with the respiratory chain in cells and tissues. J. Gen. Physiol. *49*, Suppl., 163–195.

Chance, B., Estabrook, R.W., and Lee, C.P. (1963). Electron Transport in the Oxysome. Science *140*, 379–380.

Cherepanov, D.A. (2004). Force oscillations and dielectric overscreening of interfacial water. Phys. Rev. Lett. *93*, 266104.

Cherepanov, D.A., Mulkidjanian, A.Y., and Junge, W. (1999). Transient accumulation of elastic energy in proton translocating ATP synthase. FEBS Lett. *449*, 1–6.

Cherepanov, D.A., Feniouk, B.A., Junge, W., and Mulkidjanian, A.Y. (2003). Low dielectric permittivity of water at the membrane interface: effect on the energy coupling mechanism in biological membranes. Biophys. J. *85*, 1307–1316.

Cherepanov, D.A., Junge, W., and Mulkidjanian, A.Y. (2004). Proton transfer dynamics at the membrane/water interface: dependence on the fixed and mobile pH buffers, on the size and form of membrane particles, and on the interfacial potential barrier. Biophys. J. *86*, 665–680.

Cogdell, R.J., Gall, A., and Köhler, J. (2006). The architecture and function of the light-harvesting apparatus of purple bacteria: from single molecules to in vivo membranes. Quart. Rev. Biophys. *39*, 227–324.

Cramer, W.A. and Knaff, D.B. (1990). Energy transduction in biological membranes: a textbook of bioenergetics. (Berlin, Germany: Springer-Verlag).

Crane, F.L. and Sun, F.F. eds. (1972). Energy and coupled electron transfer in biological systems, (New York: Dekker).

Cronan, J.E., Jr. and Gelmann, E.P. (1975). Physical properties of membrane lipids: biological relevance and regulation. Bacteriol. Rev. *39*, 232–256.

DalCorso, G., Pesaresi, P., Masiero, S., Aseeva, E., Schunemann, D., Finazzi, G., Joliot, P., Barbato, R., and Leister, D. (2008). A complex containing PGRL1 and PGR5 is involved in the switch between linear and cyclic electron flow in Arabidopsis. Cell *132*, 273–285.

Danielsson, R., Suorsa, M., Paakkarinen, V., Albertsson, P.A., Styring, S., Aro, E.M., and Mamedov, F. (2006). Dimeric and monomeric organization of photosystem II. Distribution of five distinct complexes in the different domains of the thylakoid membrane. J. Biol. Chem. *281*, 14241–14249.

Davies, K.M., Strauss, M., Daum, B., Kief, J.H., Osiewacz, H.D., Rycovska, A., Zickermann, V., and Kühlbrandt, W. (2011). Macromolecular organization of ATP synthase and complex I in whole mitochondria. Proc. Natl. Acad. Sci. USA *108*, 14121–14126.

Delosme, R., Olive, J., and Wollman, F. (1996). Changes in light energy distribution upon state transitions: an in vivo photoacoustic study of the wild type and photosynthesis mutants from Chlamydomonas reinhardtii. Biochim. Biophys. Acta *1273*, 150–158.

Dencher, N.A., Frenzel, M., Reifschneider, N.H., Sugawa, M., and Krause, F. (2007). Proteome alterations in rat mitochondria caused by aging. Ann. NY Acad. Sci. *1100*, 291–298.

Dibrova, D.V., Galperin, M.Y., and Mulkidjanian, A.Y. (2010). Characterization of the N-ATPase, a distinct, laterally transferred Na^+-translocating form of the bacterial F-type membrane ATPase. Bioinformatics *26*, 1473–1476.

Dilley, R.A., Theg, S.M., and Beard, W.A. (1987). Membrane-proton interactions in chloroplast bioenergetics: localized proton domains. Ann. Rev. Plant Physiol. *38*, 347–389.

Dimroth, P. (1997). Primary sodium ion translocating enzymes. Biochim. Biophys. Acta *1318*, 11–51.

Dimroth, P. and von Ballmoos, C. (2008). ATP synthesis by decarboxylation phosphorylation. Results Probl. Cell Differ. *45*, 153–184.

Dowhan, W. and Bogdanov, M. (2009). Lipid-dependent membrane protein topogenesis. Annu. Rev. Biochem. *78*, 515–540.

Drachev, L.A., Kaulen, A.D., and Skulachev, V.P. (1984). Correlation of photochemical cycle, H^+ release and uptake, and electric events in bacteriorhodopsin. FEBS Lett. *178*, 331–335.

Dudkina, N.V., Eubel, H., Keegstra, W., Boekema, E.J., and Braun, H.P. (2005). Structure of a mitochondrial supercomplex formed by respiratory-chain complexes I and III. Proc. Natl. Acad. Sci. USA *102*, 3225–3229.

Düser, M.G., Bi, Y., Zarrabi, N., Dunn, S.D., and Börsch, M. (2008). The proton-translocating *a* subunit of F_0F_1-ATP synthase is allocated asymmetrically to the peripheral stalk. J. Biol. Chem. *283*, 33602–33610.

Efremov, R.G., Baradaran, R., and Sazanov, L.A. (2010). The architecture of respiratory complex I. Nature *465*, 441–445.

Esser, L., Elberry, M., Zhou, F., Yu, C.A., Yu, L., and Xia, D. (2008). Inhibitor-complexed structures of the cytochrome bc_1 from the photosynthetic bacterium *Rhodobacter sphaeroides*. J. Biol. Chem. *283*, 2846–2857.

Eubel, H., Heinemeyer, J., and Braun, H.P. (2004). Identification and characterization of respirasomes in potato mitochondria. Plant Physiol. *134*, 1450–1459.

Fernandez-Moran, H. (1962). Cell-membrane ultrastructure. Low-temperature electron microsopy and X-ray diffraction studies of lipoprotein components in lamellar systems. Circulation *26*, 1039–1065.

Fernandez-Moran, H., Oda, T., Blair, P.V., and Green, D.E. (1964). A macromolecular repeating unit of mitochondrial structure and function. Correlated electron microscopic and biochemical studies of isolated mitochondria and submitochondrial particles of beef heart muscle. J. Cell Biol. *22*, 63–100.

Fleischmann, M.M., Ravanel, S., Delosme, R., Olive, J., Zito, F., Wollman, F.A., and Rochaix, J.D. (1999). Isolation and characterization of photoautotrophic mutants of Chlamydomonas reinhardtii deficient in state transition. J. Biol. Chem. *274*, 30987–30994.

Forti, G. and Bracale, M. (1984). Ferredoxin-ferredoxin NADP reductase interaction, catalytic differences between the soluble and thylakoid bound complex. FEBS Lett. *166*, 81–84.

Frank, S., Gaume, B., Bergmann-Leitner, E.S., Leitner, W.W., Robert, E.G., Catez, F., Smith, C.L., and Youle, R.J. (2001). The role of dynamin-related protein 1, a mediator of mitochondrial fission, in apoptosis. Dev. Cell *1*, 515–525.

Frigaard, N.U. and Bryant, D.A. (2004). Seeing green bacteria in a new light: genomics-enabled studies of the photosynthetic apparatus in green sulfur bacteria and filamentous anoxygenic phototrophic bacteria. Arch. Microbiol. *182*, 265–276.

Gao, Y., Meyer, B., Sokolova, L., Zwicker, K., Karas, M., Brutschy, B., Peng, G., and Michel, H. (2012). Heme-copper terminal oxidase using both cytochrome *c* and ubiquinol as electron donors. Proc. Natl. Acad. Sci. USA *109*, 3275–3280.

Gavin, P.D., Prescott, M., and Devenish, R.J. (2005). F_1F_0-ATP synthase complex interactions in vivo can occur in the absence of the dimer specific subunit e. J. Bioenerg. Biomembr. *37*, 55–66.

Genova, M.L., Baracca, A., Biondi, A., Casalena, G., Faccioli, M., Falasca, A.I., Formiggini, G., Sgarbi, G., Solaini, G., and Lenaz, G. (2008). Is supercomplex organization of the respiratory chain required for optimal electron transfer activity? Biochim. Biophys. Acta *1777*, 740–746.

Gilkerson, R.W., Selker, J.M., and Capaldi, R.A. (2003). The cristal membrane of mitochondria is the principal site of oxidative phosphorylation. FEBS Lett. *546*, 355–358.

Gopta, O.A., Cherepanov, D.A., Junge, W., and Mulkidjanian, A.Y. (1999). Proton transfer from the bulk to the bound ubiquinone Q_B of the reaction center in chromatophores of *Rhodobacter sphaeroides*: retarded conveyance by neutral water. Proc. Natl. Acad. Sci. USA *96*, 13159–13164.

Gregory, R.P., Borbely, G., Demeter, S., and Faludi-Daniel, A. (1982). Chiroptical properties of chlorophyll-protein complexes separated on Deriphat/polyacrylamide gel. Biochem. J. *202*, 25–29.

Hackenbrock, C.R. (1966). Ultrastructural bases for metabolically linked mechanical activity in mitochondria. I. Reversible ultrastructural changes with change in metabolic steady state in isolated liver mitochondria. J. Cell. Biol. *30*, 269–297.

Hanke, G.T., Okutani, S., Satomi, Y., Takao, T., Suzuki, A., and Hase, T. (2005). Multiple iso-proteins of FNR in Arabidopsis: evidence for different contributions to chloroplast function and nitrogen assimilation. Plant, Cell Environ. *28*, 1146–1157.

Harmon, H.J., Hall, J.D., and Crane, F.L. (1974). Structure of mitochondrial cristae membranes. Biochim. Biophys. Acta *344*, 119–155.

Hasan, S.S., Yamashita, E., Ryan, C.M., Whitelegge, J.P., and Cramer, W.A. (2011). Conservation of lipid functions in cytochrome *bc* complexes. J. Mol. Biol. *414*, 145–162.

Hase, C.C., Fedorova, N.D., Galperin, M.Y., and Dibrov, P.A. (2001). Sodium ion cycle in bacterial pathogens: evidence from cross-genome comparisons. Microbiol. Mol. Biol. Rev. *65*, 353–370.

Heberle, J., Riesle, J., Thiedemann, G., Oesterhelt, D., and Dencher, N.A. (1994). Proton migration alone the membrane-surface and retarded surface to bulk transfer. Nature *370*, 379–382.

Heinemeyer, J., Eubel, H., Wehmhoner, D., Jansch, L., and Braun, H.P. (2004). Proteomic approach to characterize the supramolecular organization of photosystems in higher plants. Phytochemistry *65*, 1683–1692.

Hicks, D.B., Liu, J., Fujisawa, M., and Krulwich, T.A. (2010). F_1F_0-ATP synthases of alkaliphilic bacteria: lessons from their adaptations. Biochim. Biophys. Acta *1797*, 1362–1377.

Higgins, M.J., Polcik, M., Fukuma, T., Sader, J.E., Nakayama, Y., and Jarvis, S.P. (2006). Structured water layers adjacent to biological membranes. Biophys. J. *91*, 2532–2542.

Holthuis, J.C.M. and Ungermann, C. (2013). Cellular microcompartments constitute general sub-organellar functional units in cells. Biol. Chem.*394*, 151–161.

Hoppins, S., Collins, S.R., Cassidy-Stone, A., Hummel, E., Devay, R.M., Lackner, L.L., Westermann, B., Schuldiner, M., Weissman, J.S., and Nunnari, J. (2011). A mitochondrial-focused genetic interaction map reveals a scaffold-like complex required for inner membrane organization in mitochondria. J. Cell Biol. *195*, 323–340.

Huang, K.C. and Ramamurthi, K.S. (2010). Macromolecules that prefer their membranes curvy. Mol. Microbiol. *76*, 822–832.

Humphrey, W., Dalke, A., and Schulten, K. (1996). VMD: visual molecular dynamics. J. Mol. Graphics *14*, 33–38.

Iwai, M., Takahashi, Y., and Minagawa, J. (2008). Molecular remodeling of photosystem II during state transitions in Chlamydomonas reinhardtii. Plant Cell *20*, 2177–2189.

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.

Iwasaki, T., Matsuura, K., and Oshima, T. (1995a). Resolution of the aerobic respiratory system of the thermoacidophilic archaeon, *Sulfolobus* sp. strain 7. I. The archaeal terminal oxidase supercomplex is a functional fusion of respiratory complexes III and IV with *c*-type cytochromes. J. Biol. Chem. *270*, 30881–30892.

Iwasaki, T., Wakagi, T., Isogai, Y., Iizuka, T., and Oshima, T. (1995b). Resolution of the aerobic respiratory system of the thermoacidophilic archaeon, *Sulfolobus* sp. strain 7. II. Characterization of the archaeal terminal oxidase subcomplexes and implication for the intramolecular electron transfer. J. Biol. Chem. *270*, 30893–30901.

Jensen, R.E., Hobbs, A.E., Cerveny, K.L., and Sesaki, H. (2000). Yeast mitochondrial dynamics: fusion, division, segregation, and shape. Microsc. Res. Tech. *51*, 573–583.

Johnson, A.S., van Horck, S., and Lewis, P.J. (2004). Dynamic localization of membrane proteins in *Bacillus subtilis*. Microbiol. *150*, 2815–2824.

Joliot, P. and Joliot, A. (2006). Cyclic electron flow in C3 plants. Biochim. Biophys. Acta *1757*, 362–368.

Joliot, P., Verméglio, A., and Joliot, A. (1989). Evidence for supercomplexes between reaction centers, cytochrome c_2 and cytochrome bc_1 complex in *Rhodobacter sphaeroides* whole cells. Biochim. Biophys. Acta *975*, 336–345.

Jones, M.R. and Jackson, J.B. (1989). Proton release by the quinol oxidase site of the cytochrome b/c_1 complex following single turnover flash excitation of intact-cells of *Rhodobacter capsulatus*. Biochim. Biophys. Acta *975*, 34–43.

Juric, S., Hazler-Pilepic, K., Tomasic, A., Lepedus, H., Jelicic, B., Puthiyaveetil, S., Bionda, T., Vojta, L., Allen, J.F., Schleiff, E., et al. (2009). Tethering of ferredoxin:$NADP^+$ oxidoreductase to thylakoid membranes is mediated by novel chloroplast protein TROL. Plant J. *60*, 783–794.

Kaftan, D., Brumfeld, V., Nevo, R., Scherz, A., and Reich, Z. (2002). From chloroplasts to photosystems: in situ scanning force microscopy on intact thylakoid membranes. EMBO J. *21*, 6146–6153.

Kawai, F., Shoda, M., Harashima, R., Sadiae, Y., Hara, H., and Matsumoto, K. (2004). Cardiolipin domains in *Bacillus subtilis* Marburg membranes. J. Bacteriol. *186*, 1475–1483.

Keefe, R.G. and Maier, R.J. (1993). Purification and characterization of an O_2-utilizing cytochrome-*c* oxidase complex from *Bradyrhizobium japonicum* bacteroid membranes. Biochim. Biophys. Acta *1183*, 91–104.

Kell, D.B. (1979). On the functional proton current pathway of electron transport phosphorylation. An electrodic view. Biochim. Biophys. Acta *549*, 55–99.

Khatoon, M., Inagawa, K., Pospisil, P., Yamashita, A., Yoshioka, M., Lundin, B., Horie, J., Morita, N., Jajoo, A., and Yamamoto, Y. (2009). Quality control of photosystem II: thylakoid unstacking is necessary to avoid further damage to the D1 protein and to facilitate D1 degradation under light stress in spinach thylakoids. J. Biol. Chem. *284*, 25343–25352.

Korn, A., Ajlani, G., Lagoutte, B., Gall, A., and Setif, P. (2009). Ferredoxin:NADP+ oxidoreductase association with phycocyanin modulates its properties. J. Biol. Chem. *284*, 31789–31797.

Krah, A., Pogoryelov, D., Meier, T., and Faraldo-Gómez, J.D. (2010). On the structure of the proton-binding site in the F_0 rotor of chloroplast ATP synthases. J. Mol. Biol. *395*, 20–27.

Krause, F., Reifschneider, N.H., Vocke, D., Seelert, H., Rexroth, S., and Dencher, N.A. (2004). "Respirasome"-like supercomplexes

in green leaf mitochondria of spinach. J. Biol. Chem. *279*, 48369–48375.

Kurisu, G., Zhang, H., Smith, J.L., Cramer, W.A. (2003). Structure of the cytochrome b_6f complex of oxygenic photosynthesis: tuning the cavity. Science *302*, 1009–1014.

Lavergne, J. and Joliot, P. (1991). Restricted diffusion in photosynthetic membranes. Trends Biochem. Sci. *16*, 129–134.

Legros, F., Lombes, A., Frachon, P., and Rojo, M. (2002). Mitochondrial fusion in human cells is efficient, requires the inner membrane potential, and is mediated by mitofusins. Mol. Biol. Cell *13*, 4343–4354.

Lemeille, S. and Rochaix, J.D. (2010). State transitions at the crossroad of thylakoid signalling pathways. Photosynth. Res. *106*, 33–46.

Lemeille, S., Willig, A., Depege-Fargeix, N., Delessert, C., Bassi, R., and Rochaix, J.D. (2009). Analysis of the chloroplast protein kinase Stt7 during state transitions. PLoS Biol. *7*, e1000045.

Lengeler, J.W., Drews, G., and Schlegel, H.G. (1999). Biology of the prokaryotes, (Stuttgart, Germany: Georg Thieme Verlag).

Lenn, T., Leake, M.C., and Mullineaux, C.W. (2008a). Are *Escherichia coli* OXPHOS complexes concentrated in specialized zones within the plasma membrane? Biochem. Soc. Trans. *36*, 1032–1036.

Lenn, T., Leake, M.C., and Mullineuax, C.W. (2008b). Clustering and dynamics of cytochrome *bd*-I complexes in the *Escherichia coli* plasma membrane *in vivo*. Mol. Microbiol. *70*, 1397–1407.

Liu, X., Gong, X., Hicks, D.B., Krulwich, T.A., Yu, L., and Yu, C.A. (2007). Interaction between cytochrome caa_3 and F_1F_0-ATP synthase of alkalophilic *Bacillus pseudofirmus* OF4 is demonstrated by saturation transfer electron paramagnetic resonance and differential scanning calorimetry assays. Biochemistry *46*, 306–313.

Liu, L.N., Bryan, S.J., Huang, F., Yu, J., Nixon, P.J., Roch, P.R., and Mullineaux, C.W. (2012). Control of electron transport routes through redox-regulated redistribution of respiratory complexes. Proc. Natl. Acad. Sci. USA *109*, 11431–11436.

Lopez, D. and Kolter, R. (2010). Functional microdomains in bacterial membranes. Genes Dev. *24*, 1893–1902.

Lübben, M., Arnaud, S., Castresana, J., Warne, A., Albracht, S.P.J., and Saraste, M. (1994). A second terminal oxidase in *Sulfolobus acidocaldarius*. Eur. J. Biochem. *224*, 151–159.

Luoto, H.H., Belogurov, G.A., Baykov, A.A., Lahti, R., and Malinen, A.M. (2011). Na^+-translocating membrane pyrophosphatases are widespread in the microbial world and evolutionarily precede H^+-translocating pyrophosphatases. J. Biol. Chem. *286*, 21633–21642.

Mannella, C.A., Pfeiffer, D.R., Bradshaw, P.C., Moraru, II, Slepchenko, B., Loew, L.M., Hsieh, C.E., Buttle, K., and Marko, M. (2001). Topology of the mitochondrial inner membrane: dynamics and bioenergetic implications. IUBMB Life *52*, 93–100.

Manor IV, J.C., Frey, T., Baljon, A., Nulton, J.M.J., and Salamon, P. (2006). Membrane Remodelling and Diffusion of Cytochrome C from a geometrically idealized mitochondrial crista. 2006 International conference on bioinformatics and computational biology.

Matsumoto, K., Kusaka, J., Nishibori, A., and Hara, H. (2006). Lipid domains in bacterial membranes. Mol. Microbiol. *61*, 1110–1117.

Megehee, J.A., Hosler, J.P., and Lundrigan, M.D. (2006). Evidence for a cytochrome $bcc\text{-}aa_3$ interaction in the respiratory chain of *Mycobacterium smegmatis*. Microbiol. *152*, 823–829.

Meredith, D.H., Plank, M., and Lewis, P.J. (2008). Different patterns of integral membrane protein localization during cell division in *Bacillus subtilis*. Microbiol. *154*, 64–71.

Meyer, A.J., Brach, T., Marty, L., Kreye, S., Rouhier, N., Jacquot, J.P., and Hell, R. (2007). Redox-sensitive GFP in Arabidopsis thaliana is a quantitative biosensor for the redox potential of the cellular glutathione redox buffer. Plant J. *52*, 973–986.

Mileykovskaya, E. and Dowhan, W. (2000). Visualization of phospholipid domains in *Escherichia coli* by using the cardiolipin-specific fluorescent dye 10-*N*-nonyl-acridine ornage. J. Bacteriol. *182*, 1172–1175.

Mileykovskaya, E. and Dowhan, W. (2009). Cardiolipin membrane domains in prokaryotes and eukaryotes. Biochim. Biophys. Acta *1788*, 2084–2091.

Mitchell, P. (1961). Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. Nature *191*, 144–148.

Mitchell, P. (1966). Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. Biol. Rev. Camb. Philos. Soc. *41*, 445–502.

Mitin, K.S. and Beketova, T.P. (1972). [Correlation between the functional state and electron microscopic morphology of mitochondria]. Biull. Eksp. Biol. Med. *13*, 117–121.

Moolna, A. and Bowsher, C.G. (2010). The physiological importance of photosynthetic ferredoxin NADP+ oxidoreductase (FNR) isoforms in wheat. J. Exp. Bot. *61*, 2669–2681.

Muchová, K., Wilkinson, A.J., and Barák, I. (2011). Changes of lipid domains in *Bacillus subtilis* cells with disrupted cell wall peptidoglycan. FEMS Microbiol. Lett. *325*, 92–98.

Mukhopadhyay, R., Huang, K.C., and Wingreen, N.S. (2008). Lipid localization in bacterial cells through curvature-mediated microphase separation. Biophys. J. *95*, 1034–1049.

Mulkidjanian, A.Y., Heberle, J., and Cherepanov, D.A. (2006). Protons @ interfaces: implications for biological energy conversion. Biochim. Biophys. Acta *1757*, 913–930.

Mulkidjanian, A.Y., Dibrov, P., and Galperin, M.Y. (2008a). The past and present of sodium energetics: may the sodium-motive force be with you. Biochim. Biophys. Acta *1777*, 985–992.

Mulkidjanian, A.Y., Galperin, M.Y., Makarova, K.S., Wolf, Y.I., and Koonin, E.V. (2008b). Evolutionary primacy of sodium bioenergetics. Biol. Direct *3*, 13.

Mulkidjanian, A.Y., Galperin, M.Y., and Koonin, E.V. (2009). Co-evolution of primordial membranes and membrane proteins. Trends Biochem. Sci. *34*, 206–215.

Mulkidjanian, A.Y., Bychkov, A.Y., Dibrova, D.V., Galperin, M.Y., and Koonin, E.V. (2012). Origin of first cells at terrestrial, anoxic geothermal fields. Proc. Natl. Acad. Sci. USA *109*, E821–830.

Munekage, Y., Hojo, M., Meurer, J., Endo, T., Tasaka, M., and Shikanai, T. (2002). PGR5 is involved in cyclic electron flow around photosystem I and is essential for photoprotection in Arabidopsis. Cell *110*, 361–371.

Nevo, R., Charuvi, D., Shimoni, E., Schwarz, R., Kaplan, A., Ohad, I., and Reich, Z. (2007). Thylakoid membrane perforations and connectivity enable intracellular traffic in cyanobacteria. EMBO J. *26*, 1467–1473.

Niebisch, A. and Bott, M. (2003). Purification of a cytochrome $bc_1\text{-}aa_3$ supercomplex with quinol oxidase activity from

Corynebacterium glutamicum. Identification of a fourth subunit of cytochrome aa_3 oxidase and mutational analysis of diheme cytochrome c_1. J. Biol. Chem. *278*, 4339–4346.

Nield, J., Orlova, E.V., Morris, E.P., Gowen, B., van Heel, M., and Barber, J. (2000). 3D map of the plant photosystem II supercomplex obtained by cryoelectron microscopy and single particle analysis. Nat. Struct. Biol. *7*, 44–47.

Nierzwicki-Bauer, S.A., Balkwill, D.L., and Stevens, S.E., Jr. (1983). Three-dimensional ultrastructure of a unicellular cyanobacterium. J. Cell Biol. *97*, 713–722.

Okutani, S., Hanke, G.T., Satomi, Y., Takao, T., Kurisu, G., Suzuki, A., and Hase, T. (2005). Three maize leaf ferredoxin:NADPH oxidoreductases vary in subchloroplast location, expression, and interaction with ferredoxin. Plant Physiol. *139*, 1451–1459.

Palsdottir, H. and Hunte, C. (2004). Lipids in membrane protein structures. Biochim. Biophys. Acta *1666*, 2–18.

Parsons, D.F. (1963). Mitochondrial structure: two types of subunits on negatively stained mitochondrial membranes. Science *140*, 985–987.

Pascal, R. and Boiteau, L. (2011). Energy flows, metabolism and translation. Philos. Trans. R. Soc. Lond. B Biol. Sci. *366*, 2949–2958.

Paumard, P., Vaillier, J., Coulary, B., Schaeffer, J., Soubannier, V., Mueller, D.M., Brethes, D., di Rago, J.P., and Velours, J. (2002). The ATP synthase is involved in generating mitochondrial cristae morphology. EMBO J. *21*, 221–230.

Pedersen, M.O., Linnanto, J., Frigaard, N.U., Nielsen, N.C., and Miller, M. (2010). A model of the protein-pigment baseplate complex in chlorosomes of photosynthetic green bacteria. Photosynth. Res. *104*, 233–243.

Pesaresi, P., Pribil, M., Wunder, T., and Leister, D. (2011). Dynamics of reversible protein phosphorylation in thylakoids of flowering plants: the roles of STN7, STN8 and TAP38. Biochim. Biophys. Acta *1807*, 887–896.

Pfeiffer, K., Gohil, V., Stuart, R.A., Hunte, C., Brandt, U., Greenberg, M.L., and Schägger, H. (2003). Cardiolipin stabilizes respiratory chain supercomplexes. J. Biol. Chem. *278*, 52873–52880.

Poburko, D., Santo-Domingo, J., and Demaurex, N. (2011). Dynamic regulation of the mitochondrial proton gradient during cytosolic calcium elevations. J. Biol. Chem. *286*, 11672–11684.

Pogoryelov, D., Klyszejko, A.L., Krasnoselska, G.O., Heller, E.M., Leone, V., Langer, J.D., Vonck, J., Müller, D.J., Faraldo-Gómez, J.D., and Meier, T. (2012). Engineering rotor ring stoichiometries in the ATP synthase. Proc. Natl. Acad. Sci. USA *109*, E1599–1608.

Polianskyte, Z., Peitsaro, N., Dapkunas, A., Liobikas, J., Soliymani, R., Lalowski, M., Speer, O., Seitsonen, J., Butcher, S., Cereghetti, et al. (2009). LACTB is a filament-forming protein localized in mitochondria. Proc. Natl. Acad. Sci. USA *106*, 18960–18965.

Pribil, M., Pesaresi, P., Hertle, A., Barbato, R., and Leister, D. (2010). Role of plastid protein phosphatase TAP38 in LHCII dephosphorylation and thylakoid electron flow. PLoS Biol. *8*, e1000288.

Prunetti, L., Infossi, P., Brugna, M., Ebel, C., Giudici-Orticoni, M.T., and Guiral, M. (2010). New functional sulphide oxidase-oxygen reductase supercomplex in the membrane of the hyperthermophilic bacterium *Aquifex aeolicus*. J. Biol. Chem. *285*, 41815–41826.

Qin, L., Sharpe, M.A., Garavito, R.M., and Ferguson-Miller, S. (2007). Conserved lipid-binding sites in membrane proteins: a focus on cytochrome *c* oxidase. Curr. Op. Struct. Biol. *17*, 444–450.

Rabl, R., Soubannier, V., Scholz, R., Vogel, F., Mendl, N., Vasiljev-Neumeyer, A., Korner, C., Jagasia, R., Keil, T., Baumeister, W., et al. (2009). Formation of cristae and crista junctions in mitochondria depends on antagonism between Fcj1 and Su e/g. J. Cell Biol. *185*, 1047–1063.

Raetz, C.R. (1978). Enzymology, genetics, and regulation of membrane phospholipid synthesis in *Escherichia coli*. Microbiol. Rev. *42*, 614–659.

Rees, D.M., Leslie, A.G., and Walker, J.E. (2009). The structure of the membrane extrinsic region of bovine ATP synthase. Proc. Natl. Acad. Sci. USA *106*, 21597–21601.

Renner, L.D. and Weibel, D.B. (2011). Cardiolipin microdomains localize to negatively curved regions of *Escherichia coli* membranes. Proc. Natl. Acad. Sci. USA *108*, 6264–6269.

Rexroth, S., Mullineaux, C.W., Ellinger, D., Sendtko, E., Rögner, M., and Koenig, F. (2011). The plasma membrane of the cyanobacterium Gloeobacter violaceus contains segregated bioenergetics domains. Plant Cell *23*, 2379–2390.

Romantsov, T., Battle, A.R., Hendel, J.L., Martinac, B., and Wood, J.M. (2010). Protein localization in *Escherichia coli* cells: comparison of the cytoplasmic membrane proteins ProP, LacY, ProW, AqpZ, MscS, and MscL. J. Bacteriol. *192*, 912–924.

Rudner, D.Z. and Losick, R. (2010). Protein subcellular localization in bacteria. Cold Spring Harb. Perspect. Biol. *2*, a000307.

Schäfer, E., Seelert, H., Reifschneider, N.H., Krause, F., Dencher, N.A., and Vonck, J. (2006). Architecture of active mammalian respiratory chain supercomplexes. J. Biol. Chem. *281*, 15370–15375.

Schäfer, L.V., de Jong, D.H., Holt, A., Rzepiela, A.J., de Vries, A.H., Poolman, B., Killian, J.A., and Marrink, S.J. (2011). Lipid packing drives the segregation of transmembrane helices into disordered lipid domains in model membranes. Proc. Natl. Acad. Sci. USA *108*, 1343–1348.

Schägger, H. (2002). Respiratory chain supercomplexes of mitochondria and bacteria. Biochim. Biophys. Acta *1555*, 154–159.

Schägger, H. and Pfeiffer, K. (2000). Supercomplexes in the respiratory chains of yeast and mammalian mitochondria. EMBO J. *19*, 1777–1783.

Scorrano, L. (2009). Opening the doors to cytochrome c: changes in mitochondrial shape and apoptosis. Int. J. Biochem. Cell Biol. *41*, 1875–1883.

Shapiguzov, A., Ingelsson, B., Samol, I., Andres, C., Kessler, F., Rochaix, J.D., Vener, A.V., and Goldschmidt-Clermont, M. (2010). The PPH1 phosphatase is specifically involved in LHCII dephosphorylation and state transitions in Arabidopsis. Proc. Natl. Acad. Sci. USA *107*, 4782–4787.

Shaw, J.M. and Nunnari, J. (2002). Mitochondrial dynamics and division in budding yeast. Trends Cell Biol. *12*, 178–184.

Sherman, D.M., Troyan, T.A., and Sherman, L.A. (1994). Localization of Membrane Proteins in the Cyanobacterium Synechococcus sp. PCC7942 (Radial Asymmetry in the Photosynthetic Complexes). Plant Physiol. *106*, 251–262.

Shi, F., Qin, S., and Wang, Y.C. (2011). The coevolution of phycobilisomes: molecular structure adapting to functional evolution. Comp. Funct. Genomics *2011*, 230236.

Shimoni, E., Rav-Hon, O., Ohad, I., Brumfeld, V., and Reich, Z. (2005). Three-dimensional organization of higher-plant

chloroplast thylakoid membranes revealed by electron tomography. Plant Cell *17*, 2580–2586.

Shoubridge, E.A. (2012). Supersizimg the mitochondrial respiratory chain. Cell. Metab. *15*, 271–272.

Skulachev, V.P. (1988). Membrane Bioenergetics, (Berlin, Germany: Springer-Verlag).

Smiley, S.T., Reers, M., Mottola-Hartshorn, C., Lin, M., Chen, A., Smith, T.W., Steele, G.D., Jr., and Chen, L.B. (1991). Intracellular heterogeneity in mitochondrial membrane potentials revealed by a J-aggregate-forming lipophilic cation JC-1. Proc. Natl. Acad. Sci. USA *88*, 3671–3675.

Sone, N., Sekimachi, M., and Kutoh, E. (1987). Identification and properties of a quinol oxidase super-complex composed of a bc_1 complex and cytochrome oxidase in the thermophilic bacterium PS3. J. Biol. Chem. *262*, 15386–15391.

Sousa, P.M.F., Silva, S.T.N., Hood, B.L., Charro, N., Carita, J.N., Vaz, F., Penque, D., Conrads, T.P., and Melo, A.M.P. (2011). Supramolecular organizations in the aerobic respiratory chain *of Escherichia coli*. Biochimie *93*, 418–425.

Sowers, A.E. and Hackenbrock, C.R. (1981). Rate of lateral diffusion of intramembrane particles:measurement by electrophoretic displacement and rerandomization. Proc. Natl. Acad. Sci. USA *78*, 6246–6250.

Staehelin, L.A. (1975). Chloroplast membrane structure. Intramembranous particles of different sizes make contact in stacked membrane regions. Biochim. Biophys. Acta *408*, 1–11.

Stoeckenius, W. (1966). Morphological observations on mitochondria and related structures. Ann. NY Acad. Sci. *137*, 641–642.

Strahl, H. and Hamoen, L.W. (2010). Membrane potential is important for bacterial cell division. Proc. Natl. Acad. Sci. USA *107*, 12281–12286.

Strauss, M., Hofhaus, G., Schroder, R.R., and Kühlbrandt, W. (2008). Dimer ribbons of ATP synthase shape the inner mitochondrial membrane. EMBO J. *27*, 1154–1160.

Stroh, A., Anderka, O., Pfeiffer, K., Yagi, T., Finel, M., Ludwig, B., and Schägger, H. (2004). Assembly of respiratory complexes I, III, and IV into NADH oxidase supercomplex stabilizes complex I in *Paracoccus denitrificans*. J. Biol. Chem. *279*, 5000–5007.

Sturgis, J.N., Tucker, J.D., Olsen, J.D., Hunter, C.N., and Niederman, R.A. (2009). Atomic force microscopy studies of native photosynthetic membranes. Biochemistry *48*, 3679–3698.

Sukhorukov, V.M., Dikov, D., Busch, K., Strecker, V., Wittig, I., and Bereiter-Hahn, J. (2010). Determination of protein mobility in mitochondrial membranes of living cells. Biochim. Biophys. Acta *1798*, 2022–2032.

Sun, F., Huo, X., Zhai, Y., Wang, A., Xu, J., Su, D., Bartlam, M., and Rao, Z. (2005). Crystal structure of mitochondrial respiratory membrane protein complex II. Cell *121*, 1043–1057.

Tanaka, T., Inoue, M., Sakamoto, J., and Sone, N. (1996). Intra- and inter-complex cross-linking of subunits in the quinol oxidase super-complex from thermophilic *Bacillus* PS3. J. Biochem. *119*, 482–486.

Thomas, J.C., Ughy, B., Lagoutte, B., and Ajlani, G. (2006). A second isoform of the ferredoxin:NADP oxidoreductase generated by an in-frame initiation of translation. Proc. Natl. Acad. Sci. USA *103*, 18368–18373.

Tikkanen, M., Nurmi, M., Suorsa, M., Danielsson, R., Mamedov, F., Styring, S., and Aro, E.M. (2008). Phosphorylation-dependent regulation of excitation energy distribution between the two photosystems in higher plants. Biochim. Biophys. Acta *1777*, 425–432.

Trissl, H.W. and Wilhelm, C. (1993). Why do thylakoid membranes from higher plants form grana stacks? Trends Biochem. Sci. *18*, 415–419.

Tucker, J.D., Siebert, C.A., Escalante, M., Adams, P.G., Olsen, J.D., Otto, C., Stokes, D.L., and Hunter, C.N. (2010). Mol. Microbiol. *76*, 833–847.

Twachtmann, M., Altmann, B., Muraki, N., Voss, I., Okutani, S., Kurisu, G., Hase, T., and Hanke, G.T. (2012). N-terminal structure of maize ferredoxin: NADP+ reductase determines recruitment into different thylakoid membrane complexes. Plant Cell *24*, 2979–2991.

Twig, G., Hyde, B., and Shirihai, O.S. (2008). Mitochondrial fusion, fission and autophagy as a quality control axis: the bioenergetic view. Biochim. Biophys. Acta *1777*, 1092–1097.

Unden, G. and Bongaerts, J. (1997). Alternative respiratory pathways of *Escherichia coli*: energetics and transcriptional regulation in response to electron acceptors. Biochim. Biophys. Acta *1320*, 217–234.

Vallon, O., Bulte, L., Dainese, P., Olive, J., Bassi, R., and Wollman, F.A. (1991). Lateral redistribution of cytochrome b6/f complexes along thylakoid membranes upon state transitions. Proc. Natl. Acad. Sci. USA *88*, 8262–8266.

Vanounou, S., Parola, A.H., and Fishov, I. (2003). Phosphatidylethanolamine and phosphatidylglycerol are segregated into different domains in bacterial membrane. A study with pyrene-labeled phospholipids. Mol. Microbiol. *49*, 1067–1079.

Vener, A.V., van Kan, P.J., Rich, P.R., Ohad, I., and Andersson, B. (1997). Plastoquinol at the quinol oxidation site of reduced cytochrome bf mediates signal transduction between light and protein phosphorylation: thylakoid protein kinase deactivation by a single-turnover flash. Proc. Natl. Acad. Sci. USA *94*, 1585–1590.

Verméglio, A. and Joliot, P. (2002). Supramolecular organization of the photosynthetic chain in anoxygenic bacteria. Biochim. Biophys. Acta *1555*, 60–64.

Vogel, F., Bornhovd, C., Neupert, W., and Reichert, A.S. (2006). Dynamic subcompartmentalization of the mitochondrial inner membrane. J. Cell Biol. *175*, 237–247.

Watt, I.N., Montgomery, M.G., Runswick, M.J., Leslie, A.G., and Walker, J.E. (2010). Bioenergetic cost of making an adenosine triphosphate molecule in animal mitochondria. Proc. Natl. Acad. Sci. USA *107*, 16823–16827.

Wenz, T., Hielscher, R., Hellwig, P., Schägger, H., Richers, S., and Hunte, C. (2009). Role of phospholipids in respiratory cytochrome bc_1 complex catalysis and supercomplex formation. Biochim. Biophys. Acta *1787*, 609–616.

Wilkens, V., Kohl, W., and Busch, K.B. (2012). Restricted diffusion of OXPHOS complexes in dynamic mitochondria delays their exchange between cristae and engenders a transitory mosaic distribution J. Cell Sci. in press.

Williams, R.J. (1978). The multifarious couplings of energy transduction. Biochim. Biophys. Acta *505*, 1–44.

Williams, R.J. (2001). The structures of organelles and reticula: localised bioenergetics and metabolism. Chembiochem. *2*, 637–641.

Wittig, I., Carrozzo, R., Santorelli, F.M., and Schägger, H. (2006). Supercomplexes and subcomplexes of mitochondrial oxidative phosphorylation. Biochim. Biophys. Acta *1757*, 1066–1072.

Xiong, J.W., Zhu, L., Jiao, X., and Liu, S.S. (2010). Evidence for ΔpH surface component (ΔpH^S) of proton motive force in ATP synthesis of mitochondria. Biochim. Biophys. Acta *1800*, 213–222.

Yaffe, M.P. (1999). Dynamic mitochondria. Nat. Cell Biol. *1*, E149–150.

Yoshikawa, S., Shinzawa-Itoh, K., and Tsukihara, T. (1998). Crystal structure of bovine heart cytochrome c oxidase at 2.8 A resolution. J. Bioenerg. Biomembr. *30*, 7–14.

You, C., Wilmes, S., Beutel, O., Lochte, S., Podoplelowa, Y., Roder, F., Richter, C., Seine, T., Schaible, D., Uze, G., et al. (2010). Self-controlled monofunctionalization of quantum dots for multiplexed protein tracking in live cells. Angew. Chemie *49*, 4108–4112.

Zhang, S. and Scheller, H.V. (2004a). Light-harvesting complex II binds to several small subunits of photosystem I. J. Biol. Chem. *279*, 3180–3187.

Zhang, S. and Scheller, H.V. (2004b). Light-harvesting complex II binds to several small subunits of photosystem I. J. Biol. Chem. *279*, 3180–3187.

Zhang, H., Whitelegge, J.P., and Cramer, W.A. (2001). Ferredoxin:NADP+ oxidoreductase is a subunit of the chloroplast cytochrome b6f complex. J. Biol. Chem. *276*, 38159–38165.

Zhang, X., Tamot, B., Hiser, C., Reid, G.E., Benning, C., and Ferguson-Miller, S. (2011a). Cardiolipin deficiency in *Rhodobacter sphaeroides* alters the lipid profile of membranes and of crystallized cytochrome oxidase, but structure and function are maintained. Biochemistry *50*, 3879–3890.

Zhang, X., Hiser, C., Tamot, B., Benning, C., Reid, G.E., and Ferguson-Miller, S.M. (2011b). Combined genetic and metabolic manipulation of lipids in *Rhodobacter sphaeroides* reveals non-phospholipid substitutions in fully active cytochrome *c* oxidase. Biochemistry *50*, 3891–3902.

Zick, M., Rabl, R., and Reichert, A.S. (2009). Cristae formation-linking ultrastructure and function of mitochondria. Biochim. Biophys. Acta *1793*, 5–19.

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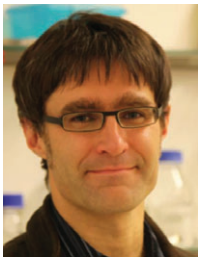

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