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# The CoRR hypothesis for genes in organelles

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ABSTRACT

Chloroplasts and mitochondria perform energy transduction in photosynthesis and respiration. These processes can be described in physico-chemical terms with no obvious requirement for co-located genetic systems, separat from those of the rest of the cell. Accordingly, biochemists once tended to regard endosymbiosis as untestable evolutionary speculation. Lynn Sagan's seminal 1967 paper "On the Origin of Mitosing Cells" outlined the evolution of eukaryotic cells by endosymbiosis of prokaryotes. The endosymbiont hypothesis is consistent with presence of DNA in chloroplasts and mitochondria, but does not assign it a function. Biochemistry and molecular biology now show that Sagan's proposal has an explanatory reach far beyond that originally envisaged. Prokaryotic origins of photosynthetic and respiratory mechanisms are apparent in protein structural insights into energy coupling. Genome sequencing confirms the underlying, prokaryotic architecture of chloroplasts and mitochondria and illustrates the profound influence of the original mergers of their ancestors' genes and proteins with those of their host cells. Peter Mitchell's 1961 chemiosmotic hypothesis applied the concept of vectorial catalysis that underlies biological energy transduction and cell structure, function, and origins. Continuity of electrical charge separation and membrane sidedness requires compartments within compartments, together with intricate mechanisms for transport within and between them. I suggest that the reason for the persistence of distinct genetic systems within bioenergetic organelles is the selective advantage of subcellular co-location of specific genes with their gene products. Co-location for Redox Regulation – CoRR – provides for a dialogue between chemical reduction-oxidation and the action of genes encoding its protein catalysts. These genes and their protein products are in intimate contact, and cannot be isolated from each other without loss of an essential mechanism of adaptation of electron transport to change in the external environment.



## 1. A bioenergetics background

*"The speakers in this Symposium are concerned with the description of different aspects of the processes by which living organisms, as we know them to-day, came to acquire the qualitatively different properties that distinguish them from the inanimate things of their environment. I shall attempt to consider the mechanism whereby the contact between the organism and its environment is regulated, particularly in relation to the functions of the membranes that form the boundary between the organism and its environment. It will be appreciated that I cannot therefore consider the organism without its environment, and that from a formal point of view the two may be regarded as equivalent phases between which dynamic contact is maintained by the membranes that separate and link them. This circumstance serves at the outset to emphasize the fact that living organisms are distinguished, not by their momentary appearance, but by their behaviour and by their relationship to their environment."* (Mitchell, 1957).

### 1.1. Photosynthesis in isolated chloroplasts

Biochemistry begins with analysis, with taking living things apart in order to see how they work. Photosynthesis is the conversion of radiant energy into chemical potential energy, ultimately feeding and fuelling most of life on planet Earth. Photosynthesis takes place in plants, algae and some bacteria, and its study has long been based on isolation of those of their components that absorb light together with enzymes that catalyze the reactions of energy conversion (Govindjee et al., 2005). A key insight into how chlorophyll-based photosynthesis works came from grinding leaves in a sucrose medium, filtering and centrifuging the resulting purée, and isolating the green component that contains partially purified chloroplasts, still recognizable by microscopy as the subcellular organelles, each about 10 μm in diameter, seen also in sections of whole leaves. The insight came from the discovery of Robin Hill that isolated chloroplasts produce oxygen upon illumination if, and

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only if, they are in the presence of an oxidant, or electron acceptor, which becomes reduced as the reaction proceeds (Hill, 1939). The insight was that photosynthesis is light-driven electron transfer. This conclusion was consistent with independent studies of photosynthetic bacteria by van Niel in which a reductant, or electron donor, was found to be required for cell growth (Van Niel, 1954). For chloroplasts and cyanobacteria the electron donor is water, and the product of water oxidation is molecular oxygen.

Hill was interested in the role of cytochromes, already shown by Keilin to transport electrons during energy conversion, and oxygen uptake, in respiration (Keilin, 1929). Mapping the redox potentials of chloroplast cytochromes allowed Hill and Bendall (1960) to propose that two separate pigment systems cooperate, in series, to convert light energy into the transport of electrons from water to a terminal electron acceptor.

Isolated chloroplasts couple photosynthetic electron transport directly to synthesis of ATP (Arnon et al., 1954) and, if kept fully intact, can use the ATP and the reduced terminal electron acceptor, NADPH, to assimilate carbon dioxide at rates comparable to those seem in intact leaves (Walker and Hill, 1967). In short, the chloroplast itself is a complete, membrane-bound, and self-contained photosynthetic compartment.

### *1.2. Protein synthesis in isolated chloroplasts*

Using methods for isolating intact chloroplasts adapted from those of Walker, Ellis and co-workers demonstrated chloroplast protein synthesis by incorporation of radioactively labelled methionine into chloroplast polypeptides. Products of protein synthesis include the large subunit of the enzyme Rubisco (Blair and Ellis, 1973) and a “peak D” membrane protein (Eaglesham and Ellis, 1974), later identified as the D1 protein, or chloroplast *psbA* gene product, of the photochemical reaction center of one of the two pigment systems – photosystem II. Synthesis of D1 is light-dependent, and its rapid synthesis in the light is required for re-synthesis following its breakdown (Ohad et al., 1984). Clearly chloroplast DNA was coding for chloroplast proteins, synthesized on chloroplast ribosomes, and the chloroplast contains a complete, functional genetic system, albeit one that, like its complete photosynthetic system, depends also on the import of proteins encoded on chromosomes in the cell nucleus (Ellis, 1984).

### *1.3. Protein structures in vectorial electron and proton transport*

By 1967 it had become completely clear that both respiration and photosynthesis involve a chain of electron carriers including cytochromes, flavoproteins, iron-sulphur proteins and quinones, and that their electron transport is coupled to synthesis of ATP from ADP and inorganic phosphate. It was also recognised that this energetic coupling was a property of specialised biological membranes – the mitochondrial inner membrane and the chloroplast thylakoid membrane. In l961 Mitchell had put forward a radical and far-reaching proposal for the essential role of the membrane in a “chemi-osmotic” mechanism by which electron transport through the chain had a direction in space, from one side of the membrane to the other, so that protons are pumped across it (Mitchell, 1961). The ATPase reaction was likewise obligatorily linked to proton translocation, thus a transmembrane gradient of pH and electrical charge served as the intermediate between exergonic electron transfer and endergonic synthesis of ATP.

Direct evidence for Mitchell’s idea of chemiosmotic coupling came from experiments with isolated chloroplasts (Jagendorf and Uribe, 1966; Junge, 1970; Telfer and Evans, 1972) and mitochondria (Mitchell and Moyle, 1965), and from reconstitution in liposomes of coupling between beef heart mitochondrial ATP synthase powered by bacteriorhodopsin from the archean *Halobacterium halobium* (Racker and Stoeckenius, 1974). For redox-coupled proton translocation, the structure of a bacterial reaction centre from X-ray crystallography (Deisenhofer et al., 1985) confirmed the assumption of vectorial, transmembrane electron transfer, and there could remain little doubt that chloroplast and cyanobacterial photosynthetic reaction centres worked in essentially the same way. In particular, the L and M subunits of the bacterial reaction centre are structurally and functionally close relatives of the chloroplast-synthesised D1 and D2 proteins, pointing to the origin of photosystem II from a type II bacterial reaction centre (Nitschke and Rutherford, 1991; Rutherford, 1989). The structure of a mitochondrial ATP synthase (Abrahams et al., 1994) provided a common mechanism for all proton-coupled ATP synthesis and hydrolysis, whether the coupling membrane is in bacteria, archaea, or eukaryotes (Junge and Nelson, 2015).

Following Mitchell’s Nobel Prize in 1978 (Mitchell, 1978), and roughly contemporary with the first complete mitochondrial genome sequence (Anderson et al., 1981), major discoveries in mitochondria and oxidative phosphorylation were detailed in a comprehensive review (Ernster and Schatz, 1981), which stated (with references as now given herein): *“Altmann (1890) was first to recognise the ubiquitous occurrence of these structures. He called them ‘bioblasts’ and concluded that they were ‘elementary organisms’ living inside cells and carrying out vital functions. Altmann would have been greatly satisfied by knowing that his idea of the symbiotic origin of mitochondria would be revived several decades later, based on similarities between mitochondria and bacteria (Margulis, 1970)”.*

In retrospect, and given the central importance of membranes in bioenergetics, it is remarkable that the endosymbiont hypothesis took so long to gain biochemical respectability.

## 2. Cytology, genetics and biochemistry – DNA in chloroplasts and mitochondria?!

Maternal, uniparental, non-Mendelian, cytoplasmic inheritance was described for plants well over a century ago (Hagemann, 2000). Uniparental inheritance implied the presence of genetic information in the plant cytoplasm, separate from its basis in chromosomes that undergo meiosis and mitosis in the plant cell nucleus (Whitehouse, 1969). Plastids, including chloroplasts, are retained in eggs but not in sperm or pollen, and seemed to be good candidates as carriers of cytoplasmic genes. Maternal inheritance as an adjunct to Mendelian inheritance occurred also in aplastidic species such as animals and fungi, though even the idea of cytoplasmic heredity in animals was long controversial (Wilson, 1925). The identification of DNA as the chemical basis of hereditary information prompted a question that might be seen, in hindsight, to be obvious: “do plastids and mitochondria carry DNA?” By the time of Lynn Sagan’s paper (Sagan, 1967) there was abundant and clear evidence that the answer was “yes” for both theses organelles.

For chloroplasts Ris and Plaut had identified DNA in *Chlamydomonas* (Ris and Plaut, 1962). Mitochondria had been suggested to contain DNA from electron microscopy by Nass and Nass (1963) with biochemical evidence following quickly from experiments with yeast (Schatz et al., 1964) and *Neurospora* (Luck and Reich, 1964). By the time of Lynn Sagan’s paper (Sagan, 1967) extrachromosomal genetic elements were known to play role in organelle inheritance and development (Roodyn and Wilkie, 1968). It perhaps took Lynn Sagan’s cytological and evolutionary perspective to persuade sceptical biochemists that there is a simple evolutionary explanation for these phenomena: both plastids and mitochondria are descended directly, and independently of the rest of the cell, from once free-living bacteria. The completed DNA sequences of human mitochondria (Anderson et al., 1981) and of chloroplasts from tobacco (Shinozaki et al., 1986) and the liverwort *Marchantia polymorpha* (Ohyama et al., 1986) revealed that their genes en-

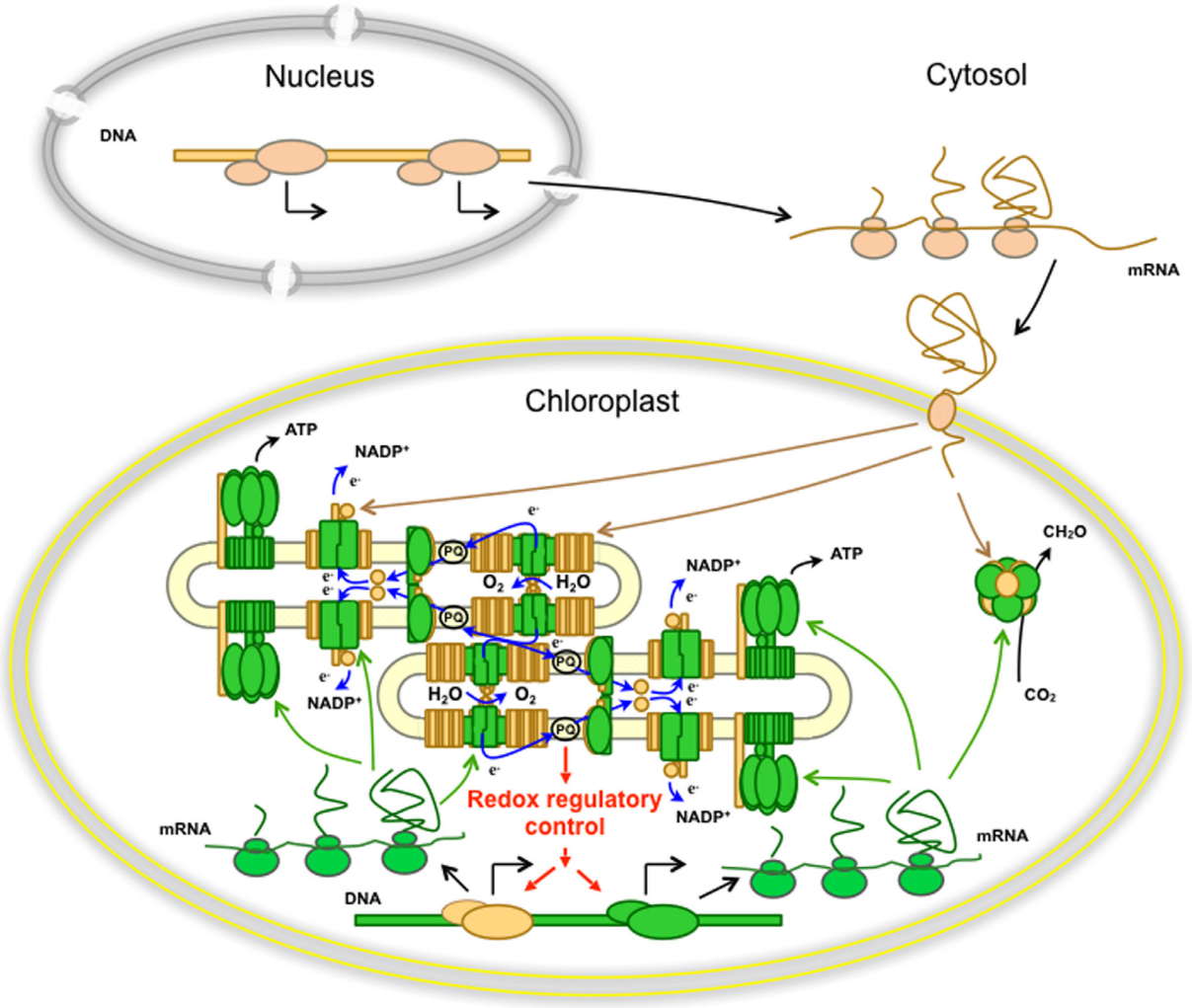


**Fig. 1.** An outline of pathways of protein synthesis and photosynthetic electron transport in one chloroplast. Co-location of chloroplast genes with their gene products. Polypeptides and protein subunits synthesised from chloroplast DNA are depicted in the colour green, as are chloroplast DNA, mRNA, and ribosomes. Polypeptides and protein subunits synthesised in the cytosol from nuclear DNA are depicted in light brown. Nuclear-encoded subunits of chloroplast proteins are imported into the chloroplast as precursors. These are processed and incorporated into protein complexes that also contain subunits encoded in chloroplast DNA. Redox regulatory control (red arrows) of chloroplast DNA transcription serves to adjust the relative quantities of electron carriers and thus the composition of photosynthetic electron transport chains (blue arrows) in thylakoid membranes, with an effect on redox state of electron (e–) carriers that is itself a signal to which chloroplast transcription responds. PQ is the electron carrier plastoquinone. Chloroplast photosystem II is in appressed thylakoid domains. Originating from $H_2O$, electrons transfer through the chain to chloroplast photosystem I in unappressed thylakoid domainas, and from there to the terminal electron acceptor, $NADP^+$. Subunits encoded in both the nucleus and the chloroplast are also present in ATP synthase in unappressed membrane domains and in the $CO_2$-fixing enzyme Rubisco in the chloroplast stroma. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

coded primarily protein components of their bioenergetic systems, especially membrane-intrinsic proteins catalysing electron transfer and proton translocation. The subset of mitochondrial and chloroplast ribosomal proteins that are also encoded in organelle DNA are likely be the result of convergent evolution and selection for a preferred site of synthesis of primary proteins of oxidative and photophosphorylation (Maier et al., 2013). At a time when many biochemists cared little for evolutionary speculation, other fields of research had thus brought an evolutionary question into focus. It was a question that could not be ignored. Colour coding membrane protein subunits for gene location (Allen, 2003a; Allen et al., 2011; Race et al., 1999) reveals a pattern that can hardly be coincidence (Fig. 1). Why have organelles chosen to retain genes for especially interesting proteins? Of all the proteins in an organelle, why does organelle DNA encode those central to biological energy transduction?

*"We find no defining structural feature that is common to all chloroplast gene products. Instead, conserved patterns of gene location are consistent with photosynthetic redox chemistry exerting gene regulatory control over its own rate-limiting steps. Chloroplast DNA carries genes whose expression is placed under this control."* (Allen et al., 2011)

## 3. Why any gene at all remains in an organelle

Photosynthesis and respiration are energy transduction by means of vectorial electron transport. Proton-pumping electron transport is catalysed by membrane-intrinsic protein complexes of chloroplasts, mitochondria and bacteria. In eukaryotes each proton-motive electron transfer complex contains a conserved and bacterially-derived core of primary subunits catalyzing proton translocation together with accessory, "supernumerary" or peripheral subunits, usually of less well-defined function – recent examples are respiratory complex I (Zhu et al., 2016) and chloroplast photosystem II (Wei et al., 2016). At its most basic, membrane bioenergetics is done by proteins and interactions

that first arose in bacteria and that have changed hardly at all by incorporation, via endosymbiosis, into eukaryotic sub-cellular organelles. If the hardware of electrochemical energy transduction is so little changed between free-living bacteria and their intracellular descendants, why has it been necessary for chloroplasts and mitochondria to retain the genes that encode it?

Redox state governs gene expression in prokaryotes (Allen, 1993a). It is proposed that genes found within chloroplasts and mitochondria are the subsets of endosymbiont genes whose protein products have always exerted direct redox regulatory control over expression of their own genes, while this control requires that the genes subject to regulation remain, with their protein products, within a single membrane-bound compartment (Allen, 1993b). This hypothesis rests firmly on the assumption of an endosymbiotic origin and was put forward explicitly for both chloroplasts and mitochondria (Allen, 1993b) and separately for chloroplasts alone (Allen, 1993c). The term *CoRR* (also *CORR*) was introduced (Allen, 2003a, b), standing for Co-location (of gene and gene product) for Redox Regulation of gene expression. As a result of this coupling between redox state and gene expression, changes in the physical environment of the cell induce direct compensatory effects on the composition of photosynthetic and respiratory electron transport chains (Allen, 2015). Fig. 1 outlines the two gene locations, nuclear and plastidic, for membrane proteins in the case of chloroplast photosynthesis. For the cell, and organism, altered redox state of primary electron carriers is a signal to which the organelle genome has no choice but to respond, and to which nuclear gene expression may then follow to give coordinated assembly of multi-subunit protein complexes. Direct experimental support for the CoRR hypothesis comes mainly from biochemical experiments with isolated chloroplasts and mitochondria, and will now be outlined.

*"It is therefore proposed that photosynthesis is under two-component regulation, with membrane phosphoproteins functioning as redox sensors, communicating the redox state of the photosynthetic electron transport chain to the redox effector of the transcriptional apparatus. By this reasoning the phenomenon of state 1-2 transitions can be viewed as just the post-translational arm of an integrated control system serving to maintain energetic homeostasis in phototrophic organisms."* (Allen, 1992).

## 4. Experimental evidence bearing on the CoRR hypothesis

### *4.1. Selection of proteins for synthesis within isolated chloroplasts and mitochondria*

Isolated chloroplasts from pea synthesize specific polypeptides in a light-dependent reaction that is inhibited by inhibitors of electron transport. The pattern of products of protein synthesis depends on the presence or absence of inhibitors and redox reagents in the incubation medium (Allen et al., 1995). Dithiothreitol, a mild reductant, supports synthesis of chloroplast-encoded proteins with a broad range of molecular masses. Ascorbate and dithionite both support polypeptide synthesis, but each gives a distinct pattern of labeled polypeptides, indicating qualitative differences between the subsets of polypeptides synthesized during the incubation. These qualitative differences are seen in both the thylakoid membrane fraction and the soluble chloroplast stroma (Allen et al., 1995).

$^{35}$S-methionine incorporation into polypeptides synthesized in isolated pea leaf mitochondria gives a set of labeled products that is specific for the redox reagent included in the incubation medium (Allen et al., 1995). Corresponding effects of respiratory chain electron donors and inhibitors suggest that redox state of respiratory electron carriers plays a role in determining the pattern of protein synthesis. Effects of site-specific electron transport inhibitors suggest that a primary site of redox control of mitochondrial protein synthesis is succinate dehydrogenase, respiratory complex II (Galvis et al., 1998)

It was concluded that *"our results are consistent with the hypothesis that the primary function of organelle genomes is the encoding in situ of proteins whose synthesis is thereby able to respond rapidly to changes in redox potential"* (Allen et al., 1995).

### *4.2. Redox regulatory control of chloroplast DNA transcription*

Redox regulatory control of transcription of chloroplast genes was reported in mustard seedlings (Pfannschmidt et al., 1999a, b) by measurement of both mRNA quantity and the initial rate of its accumulation in run-on transcription. Changes in mRNA were found to be initiated non-invasively, perturbing redox state by use of light of wavelengths selecting partially for either photosystem I or photosystem II, and also by addition to isolated chloroplasts of site-specific chemical inhibitors of photosynthetic electron transport.

Two genes of particular interest were *psbA*, encoding the D1 reaction center apoprotein of photosystem II, and *psaAB*, encoding two corresponding reaction center apoproteins of photosystem I. When experimental conditions were changed to make photosystem I rate-limiting for photosynthesis, *psaAB* was induced while *psbA* was repressed. Conversely, if photosystem II was made rate-limiting, then *psaAB* was repressed and *psbA* was induced. These responses are intelligible as initial steps taken by the chloroplast itself to rectify an imbalance in the ratio, or stoichiometry, of the reaction centres of photosystem I and photosystem II, and they signify a re-balancing of the two photosystems by redox control of chloroplast DNA transcription (Pfannschmidt et al., 1999a). The accompanying changes in the ratio of photosystem I to photosystem II were confirmed by absorption spectroscopy (Pfannschmidt et al., 1999b). The rate of chloroplast run-on transcription changed rapidly following the change in wavelength of light, with a 25–30% decrease in *psaAB* rate, for example, being detected only minutes after switching from light favouring photosystem II to light favouring photosystem I.

Nine chloroplast genes were investigated by Pfannschmidt et al. (1999b) with *rbcL* transcription being affected in the same way, and in the same direction, as *psbA*, though with a smaller amplitude of redox response. RbcL, also identified as a product of chloroplast protein synthesis (Blair and Ellis, 1973), is the large subunit of the Rubisco, the enzyme catalyzing the primary carboxylation step of the Benson-Calvin cycle. RbcL is a membrane-extrinsic protein subunit and has a very slow turnover, in complete contrast to D1/psbA.

The functional significance of chloroplasts keeping genomes is thus conservation of an essential mechanism for adjustment of the stoichiometry of photosystem I and II. The chloroplast genome permits the photosynthetic apparatus to continue to compensate for redox imbalance and inefficiency that would otherwise result from changes in the physical environment – in this case, spectral composition (wavelength) of light. The results of Pfannschmidt et al. on chloroplast transcription support the CoRR hypothesis in a direct way, and it is difficult to envisage an alternative explanation. Pfannschmidt et al. concluded that *"…such rapid and direct regulatory coupling may depend upon the genes concerned being present in the same intracellular compartment as the electron transport chain that regulates their expression, and upon the persistence there of prokaryotically derived, redox signal-transduction pathways to provide the means of control"* (Pfannschmidt et al., 1999a).

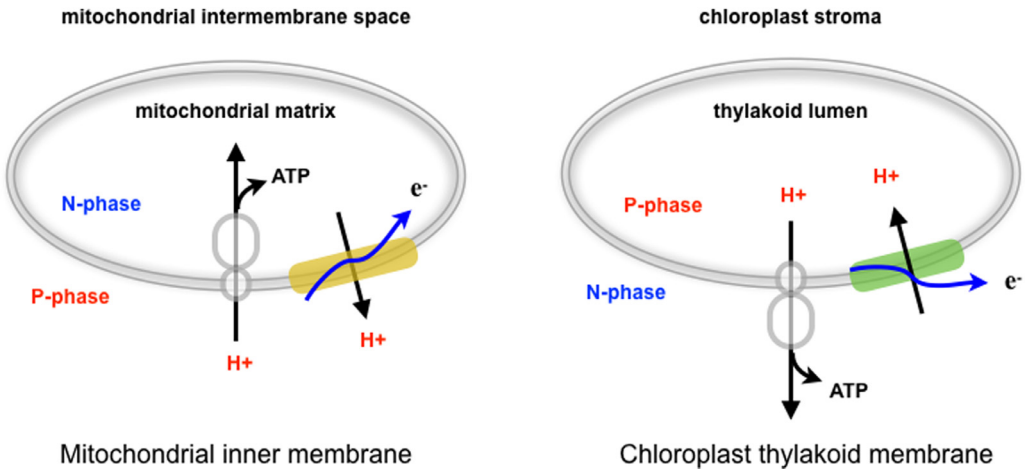


**Fig. 2.** The direction of electron transport-drive proton translocation in isolated mitochondrial inner membranes (left) and chloroplast thylakoids (right).

### 4.3. Persistence of bacterial redox sensing in chloroplasts

Chloroplast Sensor Kinase (CSK) is a protein imported into chloroplasts (Puthiyaveetil et al., 2008). The amino acid sequence of CSK contains motifs characteristic of a bacterial sensor histidine kinase (Puthiyaveetil and Allen, 2009; Puthiyaveetil et al., 2013; Puthiyaveetil et al., 2008).

In *Arabidopsis*, inactivation of the gene for CSK by T-DNA insertion results in a change in the response of *psaA* transcription to a switch between light selective for photosystem I and photosystem II. In the experiments of Pfannschmidt et al. (1999a, b) with mustard seedlings, a switch from photosystem II to photosystem I resulted in a decrease in the abundance of *psaA* transcript; an effect contributing to a decrease in the stoichiometry of photosystem I to photosystem II. In *Arabidopsis*, the same response was observed in the wild-type, control experiment, while inactivation of CSK in two independent T-DNA-insertion lines was accompanied by an increase in *psaA* transcription (Puthiyaveetil et al., 2008). This behavior of CSK knock-out mutants implicates CSK in redox regulatory control of chloroplast transcription. Chloroplast Sensor Kinase is a redox sensor and, as such, is a clear and specific prediction of the CoRR hypothesis as first put forward in 1993 (Allen, 1993b).

*"CSK therefore provides a redox regulatory mechanism that couples photosynthesis to gene expression. This mechanism is inherited directly from the cyanobacterial ancestor of chloroplasts, is intrinsic to chloroplasts, and is targeted to chloroplast genes."* (Puthiyaveetil et al., 2008).

### 4.4. Mitochondria

Incorporation of radiolabeled UTP into RNA synthesized in purified potato mitochondria responds to redox conditions, and potentiometric redox titration gives an estimated redox mid-point potential of $+270$ mV, $n = 1$, which corresponds to the value for the Rieske iron-sulfur center of the cytochrome $b$-$c_1$ complex, the site of oxidation of ubiquinone in the proton-motive Q-cycle (Pearson and Wilson, 1997; Wilson et al., 1996). Electron transport inhibitors affect transcription of a range of genes in *Arabidopsis* mitochondrial DNA (Zubo et al., 2014). In addition, and as previously described, incorporation of radiolabelled methionine into polypeptides of isolated plant mitochondria shows that redox reagents, respiratory chain donors and acceptors, and electron transport inhibitors each have specific effects on protein synthesis (Allen et al., 1995; Galvis et al., 1998).

Gene expression in the context of the CoRR hypothesis seems to have received less experimental, direct attention for mitochondria than for chloroplasts. Nevertheless, organelles related to mitochondria provide support for the CoRR hypothesis since organelles that have lost their bioenergetic function have also lost their genomes (van der Giezen, 2009). Mitosomes are relict mitochondria of unicellular parasites and can be counted as evidence for CoRR since loss of aerobic oxidative phosphorylation is accompanied by loss of mitochondrial DNA (Allen et al., 2007; de Paula et al., 2011). It is respiratory electron transport that requires the retention of mitochondrial genes.

Of every possible combination of the 65 protein-coding genes known and annotated for over 2000 mitochondrial genomes sequenced by 2016, only 74 distinct combinations are seen to occur in nature (Johnston and Williams, 2016). Calculation of probabilities for each gene's loss and replacement suggests that the genes retained in mitochondria DNA encode core subunits of proteins of the respiratory electron transport chain (Johnston and Williams, 2016). These proteins have the strongest predicted binding to neighbouring proteins, suggesting that regulation of their relative quantities determines the rate of assembly of whole respiratory chain complexes. Thus genes appear to have remained in mitochondrial DNA in order that their expression can be regulated in adjustment of the composition of the respiratory chain (Johnston and Williams, 2016).

## 5. Cells – the topology and anisotropy of energy-transducing membranes

As the endosymbiont hypothesis gained supporting evidence {Gray, 1982 #80; Margulis, 1981 #52; Sagan, 1967 #21}, so did Mitchell's chemiosmotic hypothesis (Boyer et al., 1977; Mitchell, 2011). The requirement for an anisotropic coupling membrane provides a formal connection, or interdependency, between these two revolutionary ideas. In prokaryotes the coupling membrane is the cell membrane. If chloroplasts and mitochondria originated from free-living prokaryotes then their coupling membranes must have originated as cell membranes. Despite their being buried now inside eukaryotic cells, the mitochondrial inner membrane and chloroplast thylakoid membrane remain, in relation to the input of free energy, "*membranes that form the boundary between the organism and its environment*" (Mitchell, 1957). Experiments on isolated chloroplasts and mitochondria and prompted by the chemiosmotic hypothesis revealed a surprising property of proton translocation driven by electron transport: as outlined in Fig. 2, chloroplasts pump protons in while mitochondria pump protons out. How, then, could these descendants of endosymbionts have

continued to make use of the same proton motive force as an intermediate between electron transport and ATP synthesis?

The archetype of an energy-transducing biological membrane has an inside and an outside, and the direction of proton pumping is outwards. The proton has unit positive charge, therefore the effect of electron transport is to establish and maintain a transmembrane electrical potential difference, the outside positive. An underlying unity in bioenergetics is easily appreciated if the "outside" is termed the "P" (for positive) phase and the "inside" the "N" (for negative) phase. The aqueous P-phase is the outside, or, periplasm, in prokaryotes. In mitochondria the P-phase is the intermembrane space, which must correspond to the periplasm of the ancestral endosymbiont. In the special case of chloroplast thylakoids the P-phase is the thylakoid lumen. The thylakoid lumen is viewed in microscopy as lying inside the chloroplast, while in evolutionary, endosymbiotic terms as well as in chemiosmotic terms it lies outside the cell, being continuous with the periplasm of the photosynthetic bacterium that was the chloroplast precursor. Fig. 3 illustrates the chloroplast thylakoid "*at the boundary between the organism and its environment*".

The picture of a conserved anisotropy of an outward-facing P-phase and an inward-facing N-phase is consistent with the inward direction of proton pumping in submitochondrial particles prepared by mechanical fractionation of intact mitochondria. It is also consistent with protons being pumped into isolated membrane vesicles, once termed "chromatophores", from photosynthetic bacteria.

Vectorial electron and proton transport serve to emphasise the primacy of the cell as the smallest unit that can be said to be alive, and to underline the cell's irreplaceable role in evolution and the origin of life. In 1967 Sagan reviewed contemporary suggestions that the earliest metabolism begin with scalar, atmospheric photochemistry (Sagan, 1967). Today it seems more plausible to consider that the earliest metabolism was vectorial, occurred within cells, and derived free energy from a proton motive force established by geochemistry and convection near the surface of our cooling planet (Martin and Russell, 2003; Martin and Russell, 2007; Russell, 2006; Russell and Hall, 1997). A point of agreement between bioenergetics and endosymbiosis may then be reached – the information required to make an energy-harnessing device (Sousa et al., 2013) must be accessed selectively when the external environment changes (Allen, 2010). This information and the means of its selective implementation are transmitted in cell division, and always held in the N-phase (Fig. 3). Since the origin of cells, redox regulation of the expression of genetic information has remained an essential component (Allen, 2010) of the "*mechanism whereby the contact between the organism and its environment is regulated*" (Mitchell, 1957). Genomes and genetic systems enclosed within bioenergetic membranes ensure contact, at the boundary between organism and environment, between energy transduction and information processing in regulation, synthesis, and assembly.

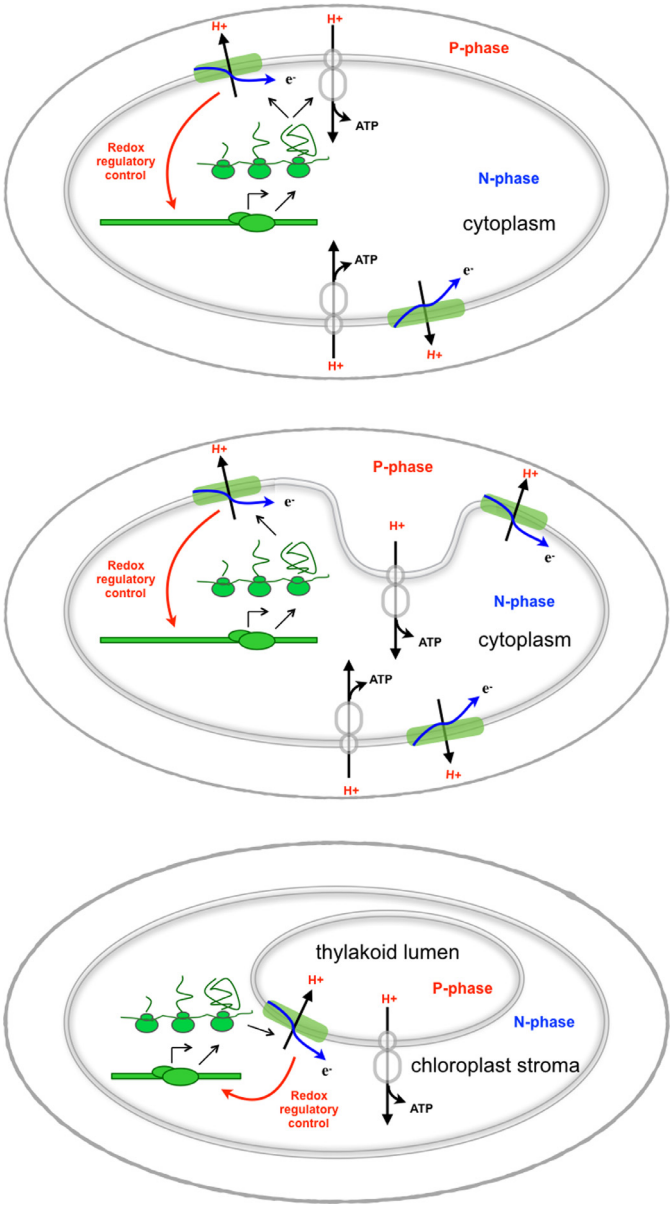


**Fig. 3.** The direction of electron transport-driven proton translocation in relation to the genetic systems of (i) mitochondria and bacteria (top); (ii) photosynthetic bacteria, including cyanobacteria (middle); (iii) chloroplasts (bottom) where the thylakoid membrane has been inverted and sealed off from the cell membrane from which it evolved, thus enclosing the thylakoid lumen (the P-phase) and separating it from the chloroplasts stroma (the N-phase), which is homologous with the bacterial cytoplasm. A schematic bioenergetic-genetic system of DNA, RNA, ribosomes and polypeptides is depicted in green, as in Fig. 1, and is located in the N-phase in all cases. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

## 6. A reflection and tribute

The value of theoretical biology is that it may provide a context within which living processes can be understood. Biochemical experiments serve to test hypotheses. While no single experiment has been attempted *in vitro*, or is easily imagined, to disprove the endosymbiont hypothesis, it is today impossible to understand the origin and internal compartmentalization of eukaryotic cells without assuming that chloroplasts and mitochondria have evolved from once free-living bacteria. The CoRR hypothesis is a biochemist's proposed solution to the problem of why bioenergetic organelles retain genomes and genetic systems from their endosymbiont ancestors. Organelle genomes are not "just there" as passive relics of an early stage in evolution, but retain a function – and natural selection still operates to determine which location, organelle or nucleus, is optimal for each and every gene.

For half a century, Lynn Sagan's paper (Sagan, 1967) has remained an insightful guide to the origin of eukaryotic cells (Margulis, 1970; Margulis, 1981). This remarkable and provocative paper (Sagan, 1967) has been an inspiration to many. *"On the Origin of Mitosing Cells"* also formed the background to experimental and biochemical approaches designed to answer the question (Allen, 1993b, 1996) "Why, then, do chloroplasts and mitochondria still carry DNA?"

**Fig. 4.** Photograph taken after lunch on 11th February 2009 in Mile End, London, following the research seminar "Earliest Evolution of Earth: Origin of Nucleated Cells" given by Lynn Margulis. Pictured, from left to right: Jeffrey Duckett (The Natural History Museum); Vivian Moses (King's College London); James McAllister (University of Massachusetts, Amherst); Philip John (University of Reading); Martin Brasier (University of Oxford); Lynn Margulis (University of Massachusetts, Amherst); John Allen (Queen Mary University of London); Carol Allen (Queen Mary University of London); Brenda Thake (Queen Mary University of London).

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