Chapter 5
Mitochondria, Hydrogenosomes and Mitosomes in Relation to the CoRR Hypothesis for Genome Function and Evolution

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5.1 Introduction

Mitochondria and chloroplasts are energy-converting organelles in the cytoplasm of eukaryotic cells. Chloroplasts perform photosynthesis; the capture and conversion of the energy of sunlight. Mitochondria perform respiration; the release of this stored energy when work is done. Mitochondria and chloroplasts also contain small, specialised genetic systems to make a few of their own proteins. Both the genetic and the energy-converting machineries of mitochondria and chloroplasts are descended, with little modification, from those of the free-living bacteria that these organelles once were. Today, almost all genes for proteins of chloroplasts and mitochondria are found on chromosomes in the nuclei of eukaryotic cells. There they code for protein precursors that are made in the cytosol for import into these two bioenergetic organelles, there to be trimmed down into their mature, functional forms. So why are any characters at all still inherited through the cytoplasm? Why do just a few genes remain steadfastly within chloroplasts and mitochondria as vestiges of ancestral, bacterial DNA?

In 1925, the American cytologist Edmund B. Wilson wrote as follows in "The cell in development and heredity," third edition, (Wilson 1925).

> . . .much interest has been aroused in recent years by cytological studies on the mitochondria and chondriosomes, cytoplasmic structural elements now widely believed to play an important part in chemical activities of cells and also in differentiation; by some

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> authors, accordingly (Benda, Meves) they have been regarded as representing a mechanism of "cytoplasmic heredity" comparable in importance with that represented by the chromosomes. This view, still very far from substantiation, remains a subject of controversy and must be taken with proper scepticism; but in spite of its doubtful status it should be kept clearly in view in all cytological discussions of these problems.

Today, there can be little doubt that mitochondria, as they are universally now known, indeed play a pivotal role in biochemistry, development, cytoplasmic inheritance and evolution (Lane 2005). In particular, the evolutionary origin of mitochondria from endosymbiotic bacteria is widely accepted (Allen et al. 2007; Gray et al. 1999). Until the discovery of mitochondrial DNA (mtDNA), however, pioneer scientists in endosymbiosis were disregarded and their conclusions displaced by other theories. A predominant theory at the time was that all structural elements of the eukaryotic cell evolved sequentially, in one lineage. Also, it was generally assumed that mitochondria are synthesised de novo as differentiated compartments within a wholly autogenous eukaryotic cell, as reviewed critically by Margulis (1970, 1981). In 1905, a Russian scientist named Constantin Mereschkowsky published a theory describing our contemporary concept of endosymbiosis (Martin and Kowallik 1999; Mereschkowsky 1905). Mereschkowsky made prodigious assumptions for his time, such as that chloroplasts, which he termed *chromatophores* (colour bearers), and autotrophy descend from cyanobacteria (then known as unicellular algae), and that nuclei have originated from an invasion of a small "micrococcus" into a larger heterotrophic amoeba-like host cell. Chloroplasts are coloured and thus conspicuous by light microscopy as intracellular organelles in plant and algal cells. Mitochondria, also termed "chondriosomes," were less well characterised and little was known of their function. Priority for the proposal of an endosymbiotic origin of mitochondria may be unclear (Martin 2007), but Ivan Wallin (1923) questioned the interpretation of previous experiments that appeared to support the nuclear-cytosolic origin of mitochondria. Wallin's experiments provided evidence in support of his assertion of a bacterial origin of this organelle (Martin 2007; Wallin 1923). The scientific community generally seems to have remained sceptical until the pioneering synthesis in 1967 by Lynn Sagan (later adopting the name Lynn Margulis) who reviewed observations supporting the endosymbiotic origin of mitochondria and chloroplasts (Sagan 1967). A milestone, this work provided an alternative to "direct filiation" (Margulis 1970) by reviewing properties that mitochondria and chloroplasts have in common with modern bacteria, such as 70 S ribosomes, circular DNA and reproduction by binary fission. Undoubtedly, this period was a scientific revolution and the beginning of a new paradigm in organelle biology.

Following evidence that mitochondria contained DNA (Luck and Reich 1964; Nass and Nass 1963), the first mitochondrial genomes were sequenced (Anderson et al. 1981). Initial analysis of mtDNA sequence and expression found unmistakable evidence that contemporary mitochondria were once free-living bacteria (Gray and Doolittle 1982). Furthermore, it has also been agreed that mitochondria are closely related to the contemporary free-living α-proteobacteria (Yang et al. 1985). However, even though studies on mtDNA have significantly increased our understanding on the evolutionary aspects of this organelle, many other questions have

arisen within and alongside the endosymbiosis theory. Which α-proteobacterial species is the closest candidate to the proposed mitochondrial ancestor? What was the evolutionary driving force for this endosymbiotic event to happen and to be maintained over billions of years? Even though the answers to these questions remain uncertain, several compelling studies have formulated assumptions worth describing.

Studies in the 1990s led to the suggestion that the α-proteobacterial order Rickettsiales contains most characteristics shared with the putative mitochondrial ancestor (Gray et al. 1998). An "Ox-Tox" model (Kurland and Andersson 2000), whereby the proto-mitochondrion served to quench oxygen free radicals fitted nicely with the strict aerobic nature of *Rickettsia* species. The main thrust of this theory was that the acquisition of oxygen tolerance, at a time when atmospheric oxygen concentration was rising due to photosynthetic activity, was the most valuable advantage for a strict anaerobic host cell to acquire from an aerobic symbiotic organism (such as *Rickettsia prowazekii*). Additionally, the "Ox-Tox" theory propounds the importance of the origin of an ADP/ATP mitochondrial translocator, which made the exchange of energetic currencies between the symbiont and the host cell possible. *Rickettsia*'s ADP/ATP translocator, however, is unrelated to the mitochondrial one (Winkler and Neuhaus 1999), partly undermining the theory that *Rickettsia* is related to the mitochondrial endosymbiont. A large-scale analysis to assess the contribution of the mitochondrial endosymbiont to eukaryote nuclear genomes indicates the massive effect of endosymbiotic gene transfer on overall eukaryotic evolution (Esser et al. 2004). This work also indicated the likelihood of other, more biochemically versatile, α-proteobacteria being good candidates for the mitochondrial endosymbiont. However, exactly pinpointing the mitochondrial endosymbiont to an extant α-proteobacterium is complicated by the dynamic nature of prokaryotic genomes due to lateral gene transfer and gene loss (Esser et al. 2007). Nonetheless, biochemically more versatile α-proteobacteria such as *Rhodobacter* do seem more often come to the fore in more intense studies (Atteia et al. 2009).

Recently, a hypothesis has been put forward elucidating the role of bioenergetics in the prokaryote to eukaryote transition. Mitochondria play an indispensable role in this hypothesis (Lane and Martin 2010). According to this hypothesis, the ATP produced by mitochondrial oxidative phosphorylation has provided the energy necessary for the expression of an immensely larger number of genes than would have been possible without such a powerhouse. The endosymbiotic event that resulted in the establishment of the mitochondrion was therefore a crucial event for the evolution of the eukaryotes as a whole. The opposite, which the evolution of a complex eukaryote enabled the endosymbiotic event that lead to the establishment of the mitochondrion, is untenable according to the rules of bioenergetics. In addition, there is currently no evidence that mitochondria-free eukaryotes ever existed. Supposedly "primitive" eukaryotes that would be devoid of mitochondria (Cavalier-Smith 1983) have been shown to be secondarily derived. Eukaryotes such as microsporidia, *Giardia*, *Trichomonas* and *Entamoeba* were put forward as related to the putative host to the mitochondrial endosymbiont due to their simple cell structures. Initial molecular phylogenies indeed placed these organisms (except

Entamoeba) at the base of the eukaryotes (Sogin et al. 1989; Vossbrinck et al. 1987). Subsequent work showed that these early phylogenetic reconstructions were fraught with methodological artefacts such as long-branch attraction (Brinkmann et al. 2005; Embley and Hirt 1998) and the true relationships within the eukaryotes are currently not certain (Simpson and Roger 2004). More importantly, the assumption that these eukaryotes were devoid of mitochondria proved to be unfounded. They were, however, devoid of "classic" 2 μm oval cristate mitochondria as their mitochondria turned out to be very small non-descript vesicles. The notion that these "primitive" eukaryotes were not that primitive after all became apparent when genes encoding typical mitochondrial proteins, such as chaperonins, were found in these organisms (Clark and Roger 1995; Horner et al. 1996; Roger et al. 1996). For *Entamoeba*, antibodies raised against these proteins localised in a punctuate pattern throughout the cytoplasm suggestive of an organellar localisation (Mai et al. 1999; Tovar et al. 1999). In addition, immunogold electron microscopy clearly labelled small organelles that had two membranes for *Giardia* and microsporidia (Tovar et al. 2003; Williams et al. 2002). The presence of two surrounding membranes is a defining feature of organelles of endosymbiotic origin (Henze and Martin 2003). These organelles of *Entamoeba*, *Giardia* and microsporidia were termed mitosomes. Subsequent genome projects and large-scale proteomics attempts to elucidate the nature of these elusive mitochondria have not been able to provide much information about the role these organelles play. A common feature seems to be the production of iron–sulphur clusters as in other mitochondria (Lill and Mühlenhoff 2005). Mitosomes do not seem to play a role in ATP production and are devoid of components of the mitochondrial electron transport chain. No organellar genome has been detected, either directly (León-Avila and Tovar 2004) or indirectly from the genome projects for these organisms (Clark et al. 2007; Loftus et al. 2005; Morrison et al. 2007). In the case of the trichomonads, the situation was slightly different as an unusual organelle was known to be present for quite some time (Cerkasovová et al. 1973; Lindmark and Müller 1973). This hydrogenosome had been shown to play a role in cellular energetics but unusually produced molecular hydrogen as a metabolic end-product (Müller 1993). Despite some initial claims (Cerkasovová et al. 1976), no organellar genome could be detected in hydrogenosomes (Turner and Müller 1983). Several mitochondrial proteins do, however, localise to these organelles (Bui et al. 1996; Horner et al. 1996; Lahti et al. 1992, 1994) and are targeted there by means of cleavable mitochondrial-like targeting signals (Bradley et al. 1997). More recently, many more variations of hydrogenosomes and mitosomes have been discovered (see for an overview van der Giezen 2009). Relevant for this chapter are the hydrogenosomes from *Nyctotherus ovalis* (Boxma et al. 2005) and *Blastocystis* (Stechmann et al. 2008). Both these organisms contain hydrogenosomes that are less derived than other hydrogenosomes. These organelles are able to take up active dyes such as Rhodamine123 or MitoTracker, which require an electrochemical gradient across the mitochondrial membrane to be actively taken up. This suggests that a proton-pumping activity would be present in these organelles and indeed, molecular evidence has been found suggesting that both organisms contain parts of Complex

I in their hydrogenosomes. Both *N. ovalis* and *Blastocystis* have been shown to contain a hydrogenosomal genome (Boxma et al. 2005; de Graaf et al. 2011; Pérez-Brocal and Clark 2008; Stechmann et al. 2008; Wawrzyniak et al. 2008). The presence of hydrogenosomes and mitosomes as part of the mitochondrial family of organelles indicates a clear spread from simple metabolic organelles such as mitosomes that have lost their organellar genomes to classic textbook aerobic mitochondria. These novel organelles do also fit with the above-mentioned bioenergetic theory of eukaryotic origins (Lane and Martin 2010; Martin and Müller 1998). However, despite numerous attempts to determine the nature of the mitochondrial ancestor and the evolutionary driving force behind endosymbiosis, this topic is still open to debate.

Since high-throughput DNA sequencing became available, and several mitochondrial genomes were sequenced, it has become obvious how variable mitochondrial genome size and gene content is among different eukaryotes (Burger et al. 2003; Lukeš et al. 2002; Martin and Müller 1998). This is the case not only between distantly related organisms but also between closely related species (see, for example, Pérez-Brocal et al. 2010). From here, another intriguing question emerges: Why is mitochondrial genome size and gene content so variable among species?

At present, it is clear that mitochondria possess their own genome which has been derived from an endosymbiotic bacterial ancestor. Many mitochondrial genomes have been sequenced by now. In addition, many α-proteobacterial genomes have been sequenced as well. Comparative genomic analysis clearly shows that modern mitochondrial genomes are severely reduced compared to those from α-proteobacteria. This has been caused by gene loss, but most importantly, because the endosymbiont's genes have been functionally transferred to the nucleus over time (Adams and Palmer 2003; Race et al. 1999; Timmis et al. 2004). This process was named endosymbiotic gene transfer (Martin et al. 2001) and is a special case of lateral (or horizontal) gene transfer. As this was not an instantaneous event but something that happened over time, each and every lineage has transferred and lost genes at his or her own pace. As a result of this, a large variety of mitochondrial and chloroplast genome sizes and genome contents can be found. An interesting example is the causative agent of malaria *Plasmodium falciparum,* which is known to possess one of the smallest mitochondrial genomes with only 5,967 base pairs (bp). This very small genome, nonetheless, still contains three core genes encoding the cytochrome *b* and cytochrome oxidase subunits I and III of the respiratory electron transport chain (Omori et al. 2007). On the other hand, the gene-rich mitochondrial genome of *Reclinomonas americana* is comprised of 69,034 bp and 67 protein-coding genes (Lang et al. 1997). Although there are plenty of notable studies unveiling the mechanisms and forces driving the lateral gene transfer to happen, this chapter aims to canvass the other side of the coin: Why is a small subset of genes always kept in the organellar genome of contemporary eukaryotes?

There are several hypotheses attempting to explain the selective pressure that maintains genomes in mitochondria and chloroplasts. The hydrophobicity

hypothesis suggests that certain organellar genes encode hydrophobic proteins which may be problematic for cellular targeting systems (see, for example von Heijne 1986). Moreover, it also suggests that hydrophobic proteins may be mistargeted to the endoplasmic reticulum (von Heijne and Segrest 1987). Support for this hypothesis comes from the observation that coxI and cob genes are present in every mitochondrial genome sequenced so far (but not on hydrogenosomal genomes from *Blastocystis* and *N. ovalis*), and the respective proteins encoded by these genes are classified as typically hydrophobic peptides (Claros et al. 1995). Moreover, experimental analysis has shown that cytosolic synthesised apocytochrome b in yeast is not properly imported into mitochondria (Daley et al. 2002). In plants, a similar experiment reported that the in vitro synthesised COX2 protein is unable to be imported into soybean mitochondria, unless one of the transmembrane domains is removed and a few critical amino acid changes are made (Daley et al. 2002). Although these data have upheld the hydrophobicity hypothesis for some genes, this hypothesis remains unable to explain various other cases. For example, using the recently published structure of *Thermus thermophilus* NADH ubiquinone oxireductase (Efremov et al. 2010) as template, we built homology models for *Arabidopsis thaliana* and *Caenorhabditis elegans* (see Fig. 5.1). From this figure, it becomes obvious that the hydrophobicity hypothesis would predict that for both species the genes encoding some of these hydrophobic proteins need to be mitochondrially located and that some can be transferred to the nucleus. The hydrophilic nad7 and nad9 subunits have indeed been transferred to the nucleus

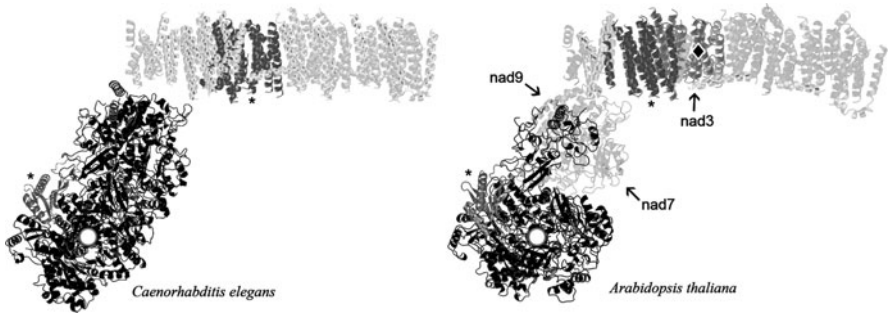

Fig. 5.1 Respiratory complex I structure of the bacterium *Thermus thermophillus* coded according to homologous gene locations in *Arabidopsis thaliana* and *Caenorhabdidis elegans*. The subunits indicated by a *white dot* are nuclear encoded, those in *light grey* are mitochondrial encoded and the subunit indicated by a *filled diamond* is present on both genomes. Subunits where no similarity at the amino acid level is found between *T. thermophillus* and the other two species are indicated by an *asterisk*. Nad 7 and nad 9 are hydrophilic polypeptides that would be encoded in the nucleus according to the hydrophobicity hypothesis. In fact they are mitochondrially encoded in *A. thaliana*, which is consistent with the CoRR hypothesis since these subunits contain the site of ubiquinone reduction by the iron–sulphur centre N2 and the initial site of chemical, redox-driven vectorial proton translocation (Efremov et al. 2010) that initiates conformationally driven proton translocation in the hydrophobic, membrane-intrinsic domain. Nad 3 is a polypeptide with a sequence of amino acid residues predicted by the nucleotide sequence of both a nuclear and a mitochondrial gene in *A. thaliana*

in *C. elegans*. However, both genes are still present on the mitochondrial genome for *A. thaliana* while there would be no hydrophobicity barrier for them to be transferred to the nucleus as well. Another problem for the hydrophobicity hypothesis is the *nad3* gene, which encodes a transmembrane H^+ pump subunit – a highly hydrophobic peptide. Intriguingly, *nad3* has two identical copies in *A. thaliana*, one located in its nuclear genome, possibly as an unexpressed and recent transformation, and the other in the mitochondrial genome. The earlier mentioned mitochondrial ADP/ATP translocator is another problem for the hydrophobicity hypothesis as these carrier proteins are almost completely buried in the mitochondrial inner membrane and are very hydrophobic but are, nonetheless, always nuclear encoded. It might be argued that these are eukaryotic inventions and would therefore never have been mitochondrially encoded in the first place. This is true but, nonetheless, the cell has no problem targeting this highly hydrophobic protein containing six membrane spanning domains to the correct cellular compartment.

Another theory seeking to explain the presence of organellar genomes states that some organellar genes have an idiosyncratic codon usage, which would preclude their nuclear expression, therefore locking them into the organelles (Doolittle 1998). In contrast, it has been shown in tobacco that the chloroplast gene encoding the large subunit of the Rubisco (rbcL) can be expressed in the nuclear genome of tobacco if relocated (Kanevski and Maliga 1994), therefore exposing a drawback on the idiosyncratic codon usage hypothesis.

Based on previous studies that had shown that gene expression was controlled by the redox state in bacteria, a hypothesis was published by one of us (Allen 1993a). This hypothesis suggests that mitochondria and chloroplasts have kept some specific genes in their genomes in order to enable an in situ redox regulation of their expression. These specific genes are thought to encode either the respiratory core subunits in the mitochondria, or the photosynthetic apparatus core subunits in the chloroplasts. In other words, if they were relocated to the nucleus, transcriptional regulation of these genes by the organellar redox state would not be possible. This hypothesis was named the CoRR hypothesis, which stands for Co-location for Redox Regulation (Allen 2003a, b). According to this hypothesis, there are two main players participating in the regulation of redox-driven gene expression: a redox sensor and a redox response regulator. The redox sensor is thought to be an electron-carrier that initiates control of gene expression upon oxidation or reduction. The redox response regulator, on the other hand, is proposed to be a DNA-binding protein that modifies gene expression as a result of the action of the redox sensor (Fig. 5.2).

Reactive oxygen species (ROS) result from excitation or incomplete reduction of molecular oxygen, and are unwelcome harmful by-products of normal cellular metabolism in aerobic organisms (Chance et al. 1979; Chen et al. 2003). In plants, mitochondrial and chloroplast electron transport is the major generator of ROS (Møller and Sweetlove 2010). It is extremely important for the plant cell to keep the ROS levels under control to avoid cell damage (Jo et al. 2001) as this can eventually lead to the initiation of programmed cell death (PCD) (Gechev et al. 2006). The CoRR hypothesis suggests that by regulating the individual expression of genes

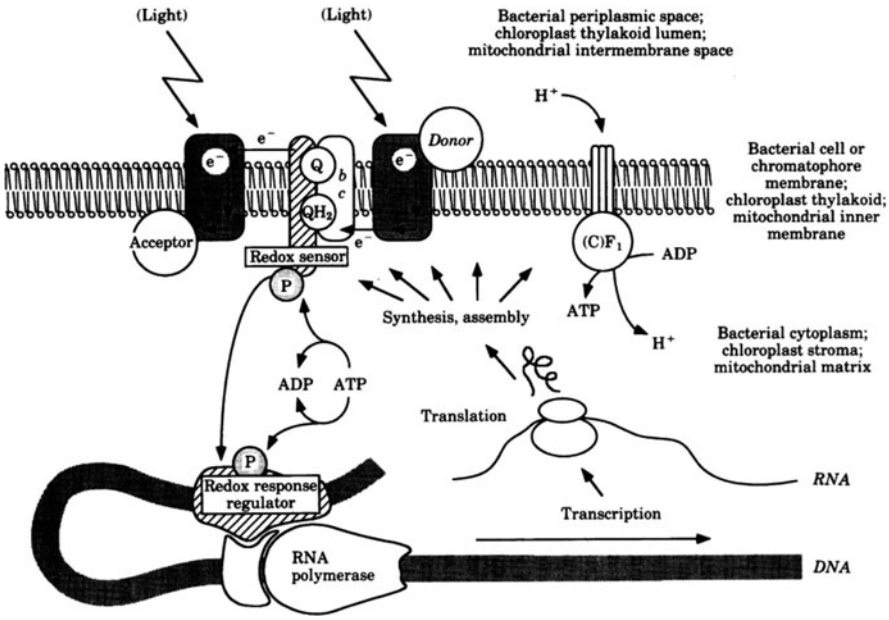

Fig. 5.2 Two-component redox regulation of transcription in bacteria, chloroplasts and mitochondria (after Allen 1993a). Vectorial electron and proton transfer exerts regulatory control over expression of genes encoding proteins directly involved in, or affecting, redox poise. This regulatory coupling requires co-location of such genes with their gene products. *CoRR* – *Co*-location for *R*edox *R*egulation – predicts that this regulatory coupling operated continuously before, during, and after the transition from prokaryote to eukaryotic organelle

encoding core subunits of the electron transport chain in mitochondria, and the photosystem I and II core subunits in chloroplasts, ROS levels can be controlled.

Light quality and quantity are known to influence the plastoquinone pool, and hence the redox state of chloroplasts (Allen et al. 1995b). Indeed, it has been shown that changes in the redox status of the plastoquinone pool by variation of light quality do control the rate of transcription of genes encoding reaction-centre apoproteins of photosystem I and II (Pfannschmidt et al. 1999). It was predicted that a redox sensor protein would control this switch. In 2008, this sensor protein was identified in *Arabidopsis thaliana* (Puthiyaveetil et al. 2008). It was termed chloroplast sensor kinase or CSK. Phylogenetic analysis shows that the plant CSK shares common ancestry with cyanobacterial histidine kinases, suggesting that photosynthetic CoRR regulation has been present since chloroplasts were still free-living cyanobacteria. Furthermore, recent studies on *A. thaliana* CSK have also indicated that specific cysteine residues are well conserved between cyanobacteria and higher plants. These are thought to be crucial for sensing the redox state of the chloroplast plastoquinone pool (Ibrahim 2009; Puthiyaveetil et al. 2010). Thiol-based regulatory switches involving cysteine residues are known to play central roles in cellular responses to oxidative stress (Paget and Buttner 2003).

For example, *Escherichia coli* ArcB is a sensor kinase that contains redox-active cysteine residues, and, upon changes in redox states of the quinone and menaquinone pools, regulates the transcription of aerobic genes (Bekker et al. 2010).

Few relevant studies have been carried out unravelling the role of the mitochondrial redox state on the regulation of organellar gene expression. One such study describes the incorporation of ^{35}S-methionine into newly synthesised mitochondrial proteins in relation to the redox status of the electron transport chain (ETC) ubiquinone pool (Allen et al. 1995a). By the use of inhibitors of specific sites of the ETC, it was reported that protein synthesis was precluded by inhibitors of ubiquinone reduction, but not by inhibitors of ubiquinol oxidation. Furthermore, it was found that electron transport through succinate:ubiquinone oxidoreductase (Complex II) was specifically required for protein synthesis, strongly suggesting that a subunit of complex II, or a component closely associated with this complex, is involved in a regulatory system that couples electron transport to protein synthesis (Escobar Galvis et al. 1998). Another study using *Solanum tuberosum* (potato) mitochondria investigated the role of a variety of electron transport inhibitors on organellar RNA synthesis. It was found that the redox state of the Rieske iron–sulphur protein was the major determinant of organellar RNA synthesis (Wilson et al. 1996). RNA synthesis was positively affected by inhibitors that act on the substrate side of the Rieske iron–sulphur protein. These inhibitors cause oxidation on the oxygen side of their site of action. On the other hand, if inhibitors were used that reduce the substrate site, then RNA synthesis was decreased. Redox regulation of plant mitochondrial glutamate dehydrogenase (Tarasenko et al. 2009) and DNA topoisomerase (Konstantinov et al. 2001) has also been reported. It has been suggested that this plays a role in coupling respiratory electron transport with mitochondrial gene expression.

In *Arabidopsis thaliana*, a large family of cysteine-rich receptor-like kinases (CRKs) have been described (Wrzaczek et al. 2010). Although only a few of these have been functionally characterised, it has been suggested that CRKs play an important role in the regulation of pathogen defence and programmed cell death, which are mainly driven by changes in ROS levels. Moreover, Wrzaczek et al. have also shown that several CRK mutants altered in hormone biosynthesis or signalling showed changes in basal and O_3-induced transcriptional responses (2010). In addition, a thorough survey of the *A. thaliana* mitochondrial proteome detected the presence of a nuclear encoded CRK protein among other kinases (Heazlewood et al. 2004). Much like CSK in *A. thaliana* and ArcB in *E. coli*, the presence of a cysteine-rich kinase such as CRK in the mitochondrial proteome strongly suggests that it might be involved in redox sensing activity and perhaps gene expression regulation of mitochondrial genes. Others studies also suggest the presence of a CoRR-like regulatory system in plant mitochondria. Transcription of mitochondrial genes in animals, fungi and plants relies on the T3/T7 phage-type RNA polymerases (RPOT). Two types of RPOTs are found in Eudicotyledonous plants. Whereas both types are nuclear encoded, one (RPOTm) is exclusively targeted to the mitochondria while the other (RPOTmp) is targeted to both mitochondria and chloroplasts. Transcriptional profiling of *A. thaliana* RPOTmp mutants has

indicated that RPOTmp is able to transcribe a small subset of genes of the mitochondrial genome. These include *nad2* and *nad6*, which encode Complex I subunits, and *coxI*, which encodes a subunit of Complex IV (Kuhn et al. 2009). In contrast, the *A. thaliana* RPOTm mutant shows a lethal phenotype indicating that RPOTm is responsible for the transcription of essential mitochondrial biogenesis and maintenance genes. It is intriguing that the only ETC complexes regulated by RPOTmp in mitochondria are complexes I and IV, which are two antagonistic redox protein complexes. In addition to these mitochondrial studies, chloroplast transcriptional regulation has been studied in *A. thaliana*. Here, the synthesis of a protein called NIP (NEP Interacting Protein) is triggered by light, subsequently activating the RPOTmp in chloroplasts (Azevedo et al. 2008). It has been shown that there are two nuclear genes encoding NIPs in *A. thaliana*. One is targeted to chloroplasts while computer algorithms predict that the other is targeted to mitochondria. Interestingly, the fact that NIP protein synthesis is up-regulated under illumination implies that NIP proteins might arise due to necessity of transcriptional regulation upon redox changes in the organelles. No further studies have been carried out to study the possible interactions between the putative mitochondrial NIP and RPOTmp in mitochondria.

The CoRR hypothesis for the function and evolutionary persistence of organellar genetic systems predicts that organelles homologous with mitochondria, such as hydrogenosomes and mitosomes, would lose their genomes when the redox and proton-motive machinery of oxidative phosphorylation are lost. In these cases, there would be no requirement for direct, local control of gene expression. It is therefore gratifying to see that mitosomes, organelles which have lost their complete electron transport chains, do not contain organellar genomes. In addition, those hydrogenosomes that have kept the ability to maintain an active proton-motive force across their organellar membranes, have indeed kept an organellar genome. Organisms such as *N. ovalis* (Boxma et al. 2005) and *Blastocystis* (Stechmann et al. 2009) offer additional support for the CoRR hypothesis and it is interesting to note that their unusual organelles, and mitosomes as a whole, were not known when CoRR was first put forward.

When taken together with the hydrogen hypothesis for the first eukaryote (Martin and Müller 1998), CoRR makes it possible to understand the distribution of cytoplasmic genomes among the full range of mitochondrial organelles, including hydrogenosomes and mitosomes, of eukaryotic cells.

A focus of interest for future research will be identification of the predicted mitochondrial sensor kinase (MSK) and mitochondrial response regulator (MRR) (Fig. 5.2). Alternatively, mitochondrial redox signalling might take the form of a single-component, iron–sulphur protein based signalling mechanism involving a mitochondrial repressor (MRP) or activator protein (MAP) (Allen 1993b).

There is now no doubt that mitochondria are agents of cytoplasmic inheritance. Wilson's words of caution (Wilson 1925) may now apply to a proposed explanation, since the CoRR hypothesis "*. . .remains a subject of controversy and must be taken with proper skepticism. . .*". Amongst proposed explanations of the function and significance of organellar genomes, we suggest that CoRR "*. . .should be kept clearly*

in view in all cytological discussions of these problems". Co-location for Redox Regulation (CoRR) is consistent with available evidence and remains testable in making clear predictions concerning the nature and distribution of redox regulatory systems controlling cytoplasmic gene expression in all eukaryotic cells.

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