Energy transduction anchors genes in organelles

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Summary

The work of mitochondria and chloroplasts is energy transduction in respiration and photosynthesis. The physico-chemical mechanisms of bioenergetics do not directly involve genes and heredity, and furthermore, redox chemistry is intrinsically mutagenic. Thus the small, functional genomes of mitochondria and chloroplasts are an oddity. Although extensively sequenced and catalogued, cytoplasmic genomes are still not explained. Genomic lethargy is not the answer. Some genes linger from the bacterial ancestors of these organelles, true, but most have left, and new ones arrive. There is a mounting case for a massive and indiscriminate intracellular gene transfer between organelles and the cell nucleus, with the frequency of relocation being comparable to that of mutation. Nevertheless, a few organellar proteins, all working at the core of bioenergetics, always seem to keep the genes encoding them close at hand. Stability amid flux suggests the invisible hand of selection. Selection for what? There are clues, and the beginnings of experimental support, for the theory that expression of mitochondrial and chloroplast genes is regulated by the function of their gene products. For safe and efficient energy transduction, genes in organelles are in the right place at the right time. ***BioEssays*** **27:426–435, 2005.**

Introduction: useful work

Mitochondria and chloroplasts are intracellular, cytoplasmic, membrane-bound organelles of eukaryotic cells. They are easily isolated and, for decades, have been the objects of extensive biochemical and biophysical research. Most of this research is directed at understanding the mechanisms by which mitochondria and chloroplasts store, convert and provide energy.[1] There are three fundamentals in the bioenergetics of mitochondria and chloroplasts. One is synthesis of ATP from ADP and inorganic phosphate, an energy-requiring process. A second is electron transfer, which, when it runs spontaneously, yields chemical free energy. The third fundamental is the mechanism by which the energy released by electron transfer is used as work, notably as the input required for ATP synthesis. If we can attach the word "function" to these organelles, energy transduction is the most-important job they do, and seems to be mostly what they are for.

Our third fundamental, coupling between electron transfer and ATP synthesis, proceeds by a process that is now seen to be a universal property of living cells, the chemiosmotic mechanism first put forward by Peter Mitchell.[2] The latest descriptions of the structures of components of this mechanism, at atomic resolution, are all consistent with the chemiosmotic theory (for reviews see Refs. 1,3–5). This theory elevates the status of biological membranes from selective boundaries to active participants in the process of energy conversion. Electron carriers are bound to proteins that are typically plugged through membranes, and their transfer of electrons is unidirectional, or anisotropic, taking place only from the outside of the membrane to the inside. Inward movement of electrons is obligatorily linked to outward movement of protons, yielding an alkaline interior aqueous phase relative to an acidic exterior. The transmembrane gradient of proton concentration thus formed, with both electrochemical and osmotic components, is the universal link between electron transport and ATP synthesis.

The only primary feature that distinguishes mitochondria from chloroplasts is the form of energy used to lower the electrochemical potential of the electrons in the first place. This energy permits the electrons to run downhill though the chain of carriers.

In mitochondria, the electrons come in already at low electrochemical potentials, as the reduced form of co-enzymes such as NADH, these being released from stepwise oxidation of various chemical constituents of food. Carbohydrates, lipids and proteins are all broken down in the cytosol, in the steps of primary metabolism, and the usual end-product entering the mitochondrion is pyruvate. Within the aqueous inner compartment, or matrix, of most mitochondria, the cycle of interconversions of the tricarboxylic acid cycle, also know as the Krebs cycle, oxidises pyruvate completely, yielding carbon dioxide and water. Water is the product of the concerted dumping of the electrons, by the inner-membrane-bound respiratory chain, onto oxygen, which is the final electron sink. The whole process is aerobic respiration; ATP is the product of oxidative phosphorylation.[6]

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DOI 10.1002/bies.20194
Published online in Wiley InterScience (www.interscience.wiley.com).

In contrast, in chloroplasts, the electrons are first pumped to a low electrochemical potential at photochemical reaction centres. Reaction centres are points in the electron transfer chain where transmembrane movement of electrons transects with migration of quanta of absorbed light energy between chlorophyll molecules.[7] The energy for photosynthetic electron transfer, and thus for subsequent ATP synthesis, comes from light. Photosynthetic reaction centres are truly the interface of the entire biosphere with its energy supply. The whole process of light-driven electron transfer, its storage and conversion, is photosynthesis,[8] and the ATP produced is the product of photosynthetic phosphorylation.[9] Of course, there has to be an ultimate source and destination for the electrons of photosynthesis, too. In plants and cyanobacteria, water is the source, and oxygen is liberated in a reaction that is, formally at least, the reverse of its consumption in aerobic respiration. The sink for electrons in photosynthesis in chloroplasts is $NADP^+$, whose reduced form, NADPH, then works together with ATP to drive assimilation of atmospheric carbon dioxide, with subsequent synthesis of precisely the carbohydrates, proteins and lipids that animals opportunistically utilise as food.

The three fundamentals of vectorial electron transfer, ATP synthesis, and chemiosmotic coupling are not really eukaryotic processes at all: they are, as it were, on loan. Bacteria themselves do all this, and more. Different prokaryotes elaborate and utilise these core reactions in various ways so that, for example, respiration can dump electrons onto a variety of inorganic oxidants, not just oxygen, and electrons for photosynthesis can be sourced from a variety of donors, not just water. Eukaryotic cells have co-opted bacteria and woven them intricately into their own energetics, to do mostly just one kind of respiration and photosynthesis, though interesting exceptions can be found.[10–12] But before eukaryotes, there was clearly a fully functional, anaerobic biosphere that predated the global oxygen cycle on which most life now depends.

Our three fundamentals may even have been in place before the origin of cells. An exciting recent proposal is that the gradient of proton concentration between inside and outside, so profoundly linking electron and phosphate transfer at the epicentre of metabolism, was first established by purely geochemical processes. Convection currents through the Earth's crust may have established an alkaline, reducing, interior for inorganic iron-sulphide vesicles whose external environment was acidic and oxidising. Such vesicles, whose formation is still observed today, are thought to have acted as 3-D templates, acting as both incubators for chemical interconversions, and as moulds for accretion of membranes that inherited the parent vesicles' proton gradient.[13] The membrane vesicles subsequently became able to maintain and utilize their proton gradient independently of their inorganic moulds, by means of vectorial metabolism. This change from chemiconvective to chemiosmotic energy transduction may have been the basis for the first appearance of cells[13,14] and provides a radically new viewpoint for multiple origins of life on Earth, with bacterial and archaeal (archaebacterial) cells arising from specific chemistries in different inorganic moulds.[14]

Cytoplasmic and Mendelian inheritance: parallel paths of vertical descent?

Before the endosymbiont theory for the evolutionary origin of mitochondria and chloroplasts,[15] it seemed possible that their DNA, RNA and the rest of the apparatus for organellar protein synthesis was a sort of offshoot, or cytoplasmic outpost, of the genetic system of the eukaryotic cell's nucleus and cytoplasm. According to this view, mitochondria and chloroplasts might arise de novo at some stage in cell development. Here the long-established, classically genetical phenomenon of cytoplasmic, non-Mendelian inheritance was the chief argument against new synthesis of cytoplasmic genetic systems from nuclear precursors. With acceptance of the endosymbiotic origin of mitochondria and chloroplasts from bacteria, once free-living, it seemed beyond reasonable doubt that their quasi-autonomy was a reflection of their retention of genes and gene expression from their bacterial precursors: organelles do not arise de novo, but come only from pre-existing organelles. Classical, Darwinian descent with modification meant that each single line of ancestry should be traceable back ultimately to one or more endosymbionts. Only accumulated differences, the result of natural selection, would set a chloroplast apart from a cyanobacterium, or a mitochondrion from a free-living, bacterial chemotroph.

Linear, vertical descent with adaptive radiation from a single, ancestral set of characters is still a broadly plausible point of view[16] for chloroplasts and mitochondria. Thus different chloroplasts, for example, have lost different genes, or groups of genes, from the "complete" set carried by their single ancestor. This ancestor is assumed, with good reason, to have resembled a modern cyanobacterium, and comparative genomics can even distinguish between modern cyanobacterial species with respect to their similarity to the single ancestor.[17] Martin et al. state "keeping in mind that lateral gene transfer between free-living prokaryotes occurs to a great extent, our data suggest that, relative to the other two cyanobacteria studied here, *Nostoc*'s overall complement of genes is more similar to that which the ancestor of plastids possessed".[17] One curious feature of this finding is that the ancestor of all chloroplasts would then have been capable of fixing of atmospheric nitrogen, a valuable trait now completely absent from chloroplasts, and, indeed, from eukaryotic cells generally.

For mitochondria, it is less easy to discern a single, convincing, modern archetype. When its genome was first sequenced completely, the obligate, intracellular human

parasite *Rickettsia prowazekii*, the causative agent of typhus, was suggested as a candidate,[18] but convergent evolution shaped by similar selective forces can also account for the similarity between the complement of genes of *Rickettsia* and the even smaller sample in mitochondria. From both biochemical[19] and genomic[20] points of view a proteobacterium resembling *Paracoccus denitrificans*, or a purple non-sulphur photosynthetic bacterium, is a more likely mitochondrial precursor. Nevertheless, the conventional position on the first mitochondrion is that it brought to the party the much higher yield of ATP from aerobic respiration, obediently exporting ATP to the fermenting, anaerobic hosts. The hosts in turn supplied the proto-mitochondrion with plentiful respiratory substrates that they gained from phagocytosis, a fairly surprising faculty given their sluggish energetics: fermentation is not a promising strategy for a predator. A radical, recent proposal for the origin of mitochondria circumvents some of these problems.

This proposal, from William Martin and Miklos Muller[21] is that the original symbiont was an anaerobe, using protons as an electron acceptor for anaerobic respiration, producing molecular hydrogen. According to this ''Hydrogen hypothesis for the first eukaryote'', the hydrogen was then used as a substrate for the autotrophic, CO_2-fixing mode of nutrition of the methanogenic, archaebacterial host cell, the latter being the evolutionary precursor of the eukaryotic nucleus and cytosol.[21] The reduced organic compounds so produced were returned to the protomitochondria where they were used as respiratory substrates. In the hydrogen hypothesis, the benefit to both partners in the syntrophic association is immediately clear: hydrogen is traded for reduced carbon compounds. Thus the archaebacterial host coincidentally acquired a respiratory chain and oxidative phosphorylation with its symbiont, but this was an unforeseen, long-term, return on its investment: the immediate gain was a guaranteed supply of hydrogen. In turn, the eubacterial symbiont gained the twin benefits of a supply of respiratory substrates from its autotrophic (CO_2-fixing) host, and a sink for its respiratory waste product, hydrogen.

In all these scenarios, the common feature of subsequent evolution of eukaryotic cells is a parallel descent of the nuclear (host) genetic system with the one or two genetic systems in the cytoplasm (endosymbiont, then organelle). This orthodox view, of course, sees subsequent modification as arising from natural selection of chance variation, with the interplay of the two or three genomes itself being susceptible to further adaptation and refinement. One common consensus picture of this refinement is that the more ''advanced'' eukaryotes have smaller organellar genomes. This picture carries an implication that there is a direction in evolution: ''All available data suggest that the ultimate aim of genome restructuring in the plant cell, as in the eukaryotic cell in general, is the elimination of genome compartmentation while retaining physiological compartmentation''.[22,23] This viewpoint depends partly on the assumption that loss of genes from organelles and their incremental acquisition by the nucleus is infrequent, progressive, and generally confers an advantage when compared with retention of each gene in the organelle; more ''primitive'' eukaryotes have larger organellar genomes. For the first steps in the evolution of cytoplasmic genetic systems, this may be, in a sense, correct: what distinguishes an intracellular symbiont, or parasite, from an organelle is not whether and how many genes have been lost, but whether any gene's function has been replaced by that of a gene in the nucleus,[24] making a precursor for import to replace the original gene product. Besides the suspect notion of present-day organisms presenting a chronicle of evolutionary progress, there seems to be no evidence, or mechanism, for concerted and on-going nuclear take-over. In particular, recent genome sequencing projects and direct experiments on naturally occurring allotopic transformation agree on a new and important factor to take into consideration: lateral gene transfer from organelle to nucleus is commonplace, and enormously more frequent than previously thought.

Adventitious acquisition and lateral gene transfer: prefabricated sources of variation

In plants, individual nuclear genes have been known for some time to have originated from mitochondria[25] and chloroplasts.[26] Until recently it was not known how much exogenous DNA can be incorporated, how frequently such transposition events occur, or what the mechanism of transformation might be.

The whole nuclear genome sequence of the flowering plant *Arabidopsis thaliana* revealed a surprising and almost intact *Arabidopsis* mitochondrial genome of 367 kb, copied to chromosome 2.[27,28] There is no evidence for a function for this adventitious whole-genome transfer, and so the very high sequence similarity (>99%) between the mitochondrial genome and its nuclear copy argues for the transposition having been a recent event. Frequency is not deduced from a sample of one, and the rice genome fairly quickly produced confirmation of wholesale import of genes into the nucleus. Thus the incorporation of whole or part of a cytoplasmic genome into the nucleus is neither an aberration of *Arabidopsis*, nor an improbable and specific failure of quality control. Rice chromosome 10 contains a small (33 kb) and recent fragment of the rice chloroplast genome, together with a larger and almost complete chloroplast-derived sequence of 131 kb[29] Already, the case for gene relocation to the nucleus as a freakish event, with a frequency so low as to be a limiting factor in cell evolution, seems impossible. One could paraphrase Oscar Wilde: to lose one cytoplasmic genome to the nucleus may be unfortunate; to lose two begins to look like carelessness.

Recent experimental estimates of the frequency of intracellular gene relocation confirm that the process occurs

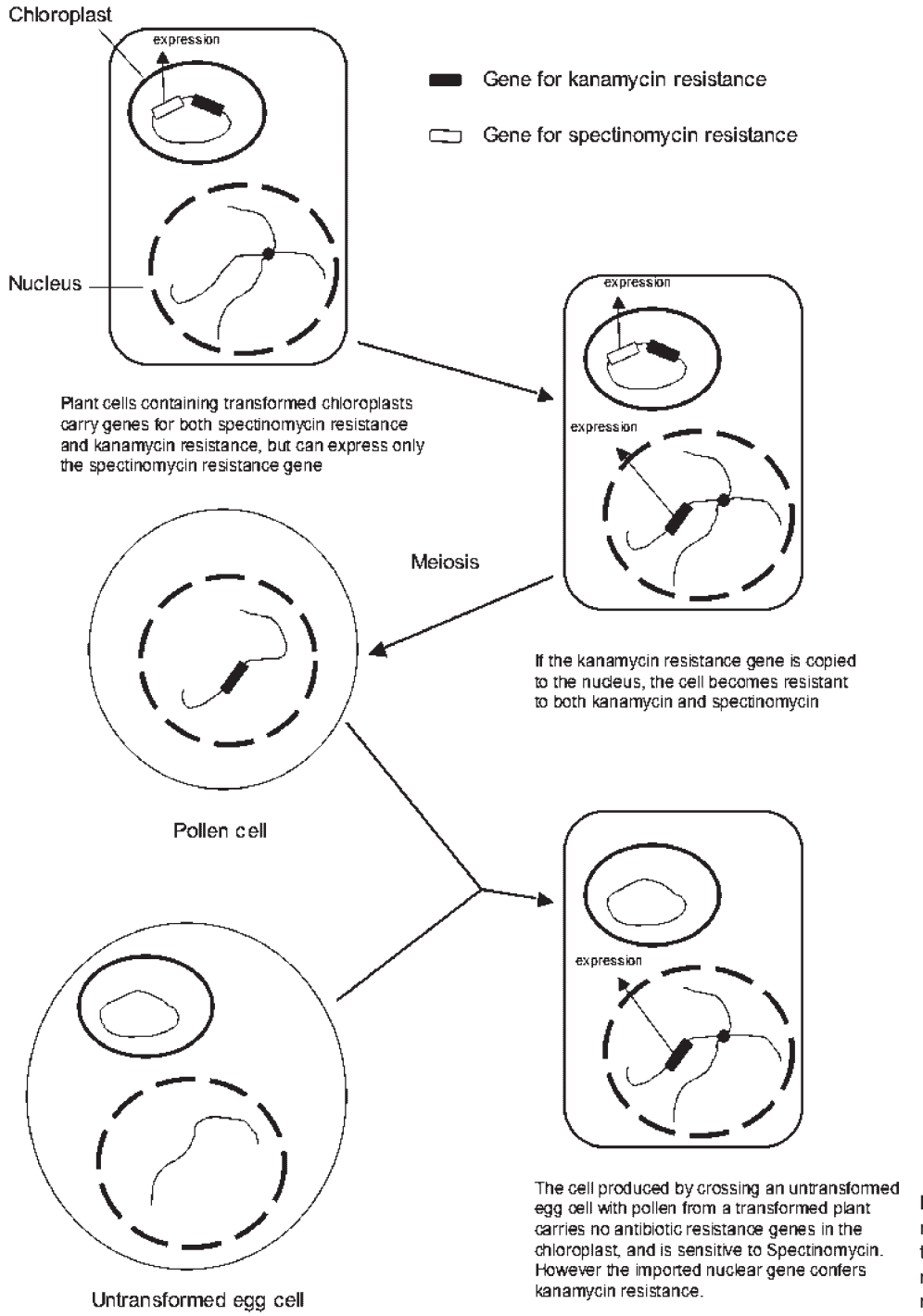


Figure 1. Any gene can be relocated. Horizontal gene transfer from chloroplast to nucleus in tobacco, as demonstrated by Huang et al.[30]

with a hugely higher frequency than even its most ardent advocates would have guessed. Huang et al.[30,31] and Stegemann et al.[32] used similar approaches but different detailed procedures to demonstrate natural transfer of chloroplast DNA to the nucleus in tobacco. Fig. 1 outlines the experiment of Huang et al.[30] Taking a gene for neomycin phosphotransferase, an enzyme whose activity confers resistance to the antibiotic kanamycin, Huang et al. transformed tobacco chloroplast DNA. However, their kanamycin-resistance gene contained an intron that can be removed by RNA splicing only when the gene is expressed in the nucleus. Furthermore, the chloroplast-located but silent kanamycin-

resistance gene was linked to an active gene for aminoglycoside-3′-adenyltransferase, whose activity confers resistance to a different antibiotic, spectinomycin, thereby allowing identification and selection of tobacco chloroplast transformants. Using pollen (haploid, male gametophytes) from the tobacco with transformed chloroplasts, and egg cells from non-transformed plants, Huang et al.[30] found that kanamycin resistance cropped up in progeny of sexual crosses, even though chloroplast genomes and characters, like mitochondrial ones, are uniparentally, and maternally, transmitted in tobacco—the basis of non-Mendelian inheritance. Furthermore, the kanamycin-resistant phenotype could not have resulted from a freak paternal transmission of chloroplast DNA through the pollen, for two reasons: first, the gene required splicing and was silent in the chloroplast; second, the kanamycin-resistant phenotype segregated with Mendelian behaviour in subsequent crosses, could only have segregated during meiosis, and was therefore carried on a nuclear chromosome. Thus the gene for neomycin phosphotransferase had moved, by some natural process, from the chloroplast to the cell nucleus. Huang et al. estimate the frequency of the transposition event for just this active marker gene as one in 16,000 pollen grains. The frequency of chloroplast DNA relocations generally is clearly likely to be higher. The results of Stegemann et al.[32] who did not use the extra safeguard of a marker gene itself unable to be expressed in the chloroplast, are nevertheless consistent with those of Huang et al.[30]

In this context, one in 16,000 is actually a huge number of chloroplast-to-nucleus transpositions per gene per generation. If we assume that the incoming gene is inserted at random places in the nuclear genome, simply extrapolation allows a rough estimate of the probability of any nuclear gene receiving a gene imported from the chloroplast. If there are 25,000 nuclear genes in Arabidopsis,[17,33] then the chance of any one receiving the chloroplast marker in the experiment of Huang et al.[30] is one in $(1.6 \times 10^4) \times (2.5 \times 10^4)$, that is, 4×10^{-8}. This frequency, on its own, is approaching mutation frequency. If we remember that the kanamycin-resistance marker gene was just one of, say, 100 in the chloroplast genome,[33] then the frequency of any chloroplast gene arriving at one randomly chosen nuclear gene will be a hundren times greater, that is, between 10^{-6} and 10^{-5} per nuclear gene per generation. This frequency is, if anything, higher than normal mutation frequency, and amounts to an informational onslaught.[34] If we may extrapolate to eukaryotes generally, the chance variation upon which natural selection acts is clearly generated not just by mutation, but also by gene translocation from organelles, which comprise an internal reservoir of diversity.[35]

Further studies with higher plants show that genes may also move between mitochondria and chloroplasts. The phenomenon of "promiscuous DNA", that is, sequences shared between mitochondria, chloroplasts and the nucleus, was first demonstrated by Stern and Lonsdale.[36] A recent paper by Cummings et al.[37] provides a phylogenetic analysis suggesting that rbcL, the gene for the large subunit of ribulose-1,5- bisphosphate carboxylase-oxygenase, has moved from the chloroplast to the mitochondrial genome and been retained there at least five times in the evolution of flowering plants. Nuclear genes of mitochondrial and plastid origin—"numts" and "nupts"—are evident in eukaryotic genome sequences, and used for phylogenetic analysis.[38,39]

Vertical descent, the foundation of the "Tree of Life"[40] needs serious qualification for prokaryotes generally, where lateral gene transfer, a combination of infection and heredity, is clearly a widespread phenomenon.[41] As an alternative to the "Tree", a "Ring of Life" is recently proposed.[20] It is clear that the captive, subcellular prokaryotes, which we call mitochondria and chloroplasts, also blur the distinction of identity through genetic continuity, both between and within genomes, compartments, cells, and organisms. If this is the case, and especially if a nuclear location for genes is in some definable way an evolutionary advance, why, after a few billion years, has this vigorous intracellular free market not eliminated mitochondrial and chloroplast genomes completely? What are they for?

Co-location for redox regulation (CORR)

In all mitochondria and chloroplasts, each organelle's genetic system contains DNA, RNA and all the components necessary for synthesis, in situ, of any of the limited number of proteins encoded in the organellar DNA. Nuclearly encoded components are always required for the operation of an organellar genetic system. Nevertheless, mitochondria and chloroplasts may be described as *quasi-autonomous* since they perform synthesis of some of their own components, even, transiently, after isolation in vitro. The proteins which are synthesised internally, and which are not part of the genetic system itself, are a subset of proteins with related functions in electron transport and closely related events in photosynthesis in chloroplasts, or respiration in mitochondria. A central core of this subset of proteins contains proteins that are universally organelle-encoded. These include precisely the membrane-spanning, primary components in which inwardly directed electron transfer is coupled to outwardly directed proton translocation. Organelle-encoded proteins are the generators of the proton motive force that couples electron transport to synthesis of ATP. Thus, in eukaryotes, genes for apoproteins of photochemical reaction centres of photosynthesis are always located in chloroplasts themselves. In mitochondria that have respiratory chains, two primary, proton-motive components, cytochrome *b* and cytochrome *c* oxidase, are always mitochondrially encoded.

The pattern of distribution of genes between cytoplasmic organelles and the nucleus is somewhat variable between phylogenetic groups, but incorporates this constant feature:

bioenergetic organelles contain, replicate and express genes for apoproteins of components carrying out primary, coupled, vectorial electron and proton transfer. Co-location of these genes with their gene products is consistent with a functional hypothesis, systematically put forward in two articles,[42,43] and more recently evaluated in the light of competing hypotheses and available evidence.[44–46] The CORR hypothesis, where "CORR" stands for "**CO**-location for **R**edox **R**egulation", envisages regulatory coupling between electron transfer (redox chemistry) and gene expression as the reason for the retention of genomes in organelles. It may be stated as follows.

"Chloroplasts and mitochondria contain genes whose expression is required to be under the direct, regulatory control of the redox state of their gene products or of electron carriers with which their gene products interact".

The transition from endosymbiotic cyanobacterium to chloroplast, as envisaged by CORR, is illustrated in Fig. 2. The general CORR hypothesis is based on ten principles, or assumptions, as listed in Box 1. Some principles are orthodox, some are novel, and all are open to testing by observation and experiment. Specific predictions that have recently been borne out include redox regulation of chloroplast reaction centre gene transcription[47,48] and chloroplast and mitochondrial protein synthesis.[49] If chloroplasts and mitochondria are viewed as intracellular, specialised bacteria, the mechanisms of redox control of gene expression are not likely to differ markedly from those already described for free-living bacteria.[50,51]

Conclusion: Trade-offs of gene location: centralisation versus responsibility

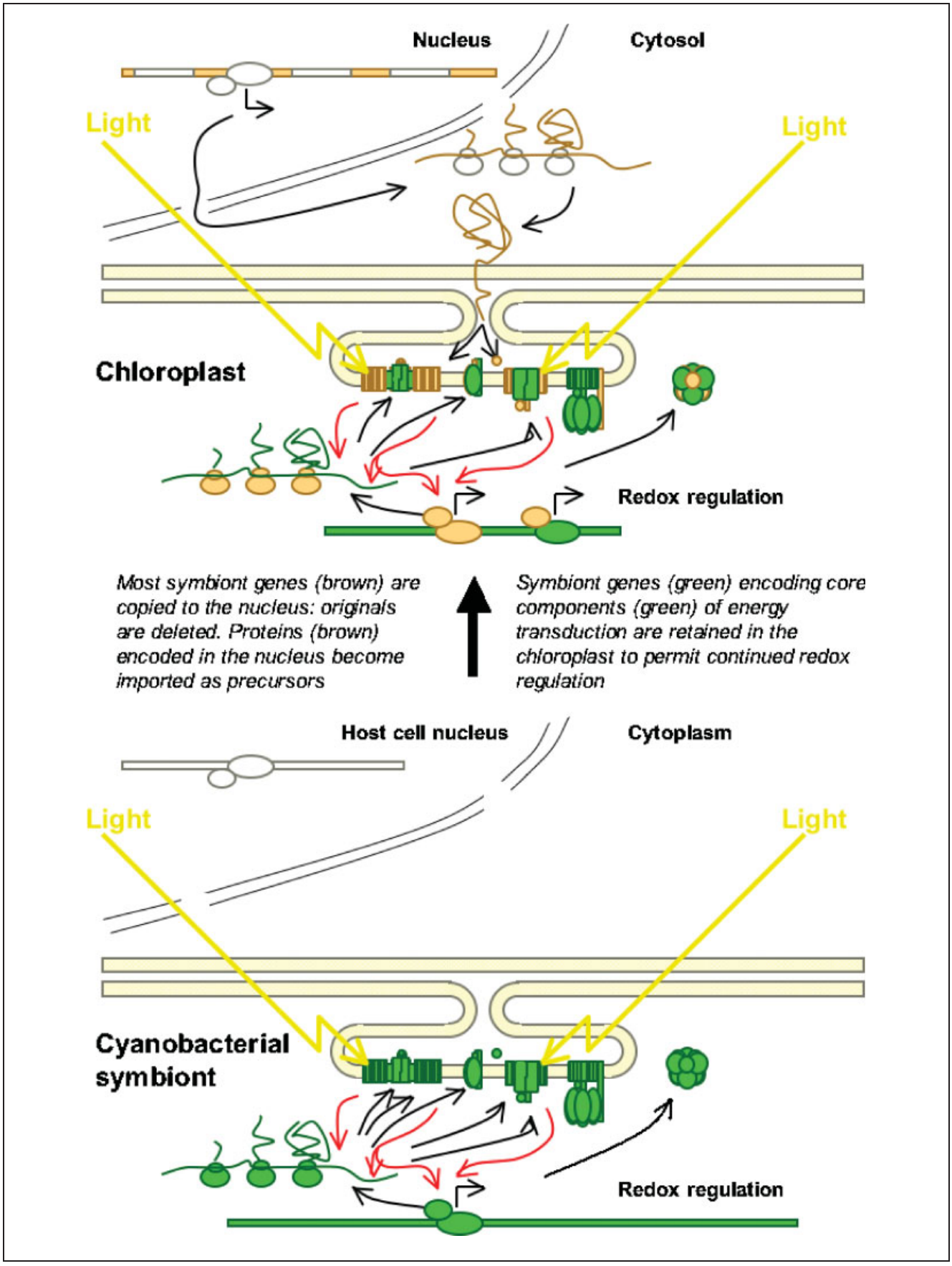
Light for photosynthesis and oxygen activity for respiration are life-sustaining environmental inputs that even complex, multicellular organisms have only a limited capacity to seek out or evade. As regards the acquisition by eukaryotes of energy from light and from the high electrochemical potential of oxygen, the bioenergetic membranes of chloroplasts (the thylakoid) and mitochondria (the inner membrane) stand at the interface of the cell with its energy supply, at its boundary with the changing but unyielding external world.

For example, when light changes in quality or quantity, photosynthetic reactions centres are the first to know, and it seems to make intuitive sense for redox state of electron carriers to be a decisive regulatory signal. The hypothesis discussed here could be paraphrased by stating that the chloroplast genetic system exists to process information about changes in the physical environment, and to provide an appropriate response. If this is the case, the signal leaving the chloroplast to provide input to control nuclear gene expression[52] may not be a direct redox signal, but a signal of the way in which the chloroplast is processing the primary signal, and what has been the response. Clearly the same argument applies for the mitochondrion's response to changing oxygen concentration. The cytochrome oxidase of the mitochondrial inner membrane uses oxygen as the terminal electron sink for respiration, and oxygen tension is a factor to which the whole organism must very quickly be able to tune its metabolism and pattern of development.

Common pictures of signal transduction by cells commence with an environmental factor interacting in some way with the cell membrane, and perhaps terminating with regulatory control of one or more nuclear genes. To understand the function of cytoplasmic genomes, we perhaps need to recall that the most important signal of all from an energetic point of view is the way in which electron transport is continuously coupled to a variable supply of energy, starving the organism if too scarce, tearing and destroying its fabric if too plentiful. It seems reasonable that the endosymbiont precursors of mitochondria and chloroplasts had precisely such mechanisms in place; they seem to be universal in modern bacteria. There may be advantages in a nuclear location for many genes for any of a number of reasons.[45,53] However, the rapid response that flows from close coupling between primary electron transfer and gene expression is most unlikely to have been a process that could be temporarily put on hold, or even one where there is much room for negotiation with a centralised system of coordinated control.

If chloroplast and mitochondrial genomes exist to respond to crises and opportunities sensed first, and dealt with first, by their gene products, an optimal strategy might be for the state-of-play in individual organelles to be an input to coordinated, nuclear control of gene expression, but never for genetic responsibility to be abdicated completely. Processing life-or-death signals requires autonomy, local authority and unconditional powers of rapid response. Despite the perspective of genomic conflict and the possible benefits of nuclear take-over, the tendency of the nucleus towards centralisation may, in fact, be selectively disadvantageous. To lapse into anthropomorphism; a wise organelle might indeed keep the nucleus posted, in the hope that the complex decision-making structure located there might eventually come up with something to help respond to change. But filing applications through approved channels is not appropriate emergency action.

Perhaps the need for genes in mitochondria and chloroplasts is effectively illustrated by analogy to human organisations, delocalised or centralised, and on whatever scale we choose. Close to the action is the best place for surveillance and rapid response. Organellar genes are near the cutting edge and are the primary, energetic, environmental interface of the eukaryotic cell. That is where gene products do more than interact with other gene products. They face the real world. That is where useful work is done, and where real responsibility resides.


Nucleus
Cytosol
Light
Light
Chloroplast
Redox regulation
Most symbiont genes (brown) are copied to the nucleus: originals are deleted. Proteins (brown) encoded in the nucleus become imported as precursors
Symbiont genes (green) encoding core components (green) of energy transduction are retained in the chloroplast to permit continued redox regulation
Host cell nucleus
Cytoplasm
Light
Light
Cyanobacterial symbiont
Redox regulation

Box 1

The CORR hypothesis for the function of genes in chloroplasts and mitochondria, and the principles upon which it is based. "CORR" stands for "**CO**-location for **R**edox **R**egulation",[45,46] and is a more clearly defined extension of a proposal explicitly put forward in 1993.[42,43] The principles are designated "O" for "orthodox", "N" for "novel", and "G" for "gaining ground".

1. *Bioenergetic organelles descend from free-living bacteria*. O.
2. *Gene transfer between the symbiont or organelle and the nucleus is a frequent occurrence, and not selective for particular genes*. G. As discussed above. In fact the problem now is not "can genes move to the nucleus?" but "why are there any left?"
3. *There is no barrier to the successful import of any precursor protein, nor to its processing and assembly into a functional, mature form*. N. Most competing hypotheses envisage barriers to import, notably of hydrophobic proteins, as the reason for retention of organellar genes, but there are too many counter-examples for this rule to hold.[34,35] As single example of an unimportable precursor protein would falsify this proposal.
4. *Direct redox control of expression of certain genes was present in the bacterial progenitors of chloroplasts and mitochondria, and was vital for cell function both before and after the transition from bacterium to organelle*. G. Redox control of bacterial gene expression is now well documented.[50,51]
5. *For each gene under redox control, it is selectively advantageous for that gene to be retained and expressed only within the organelle*. N. Redox control of organellar gene expression has now been demonstrated convincingly, at least for chloroplasts.[47,48]
6. *For each bacterial gene that survives and which is not under redox control, it is selectively advantageous for that gene to be relocated to the nucleus and expressed only in the nucleus and cytosol*. O. Without the qualification "*which is not under redox control*", this seems to be a conventional view.
7. *For any species, the distribution of genes between organelle and nucleus is the result of selective forces which continue to operate*. N. Other scenarios envisage distribution of genes as a legacy of infrequent and temporally remote events, summarised as "the frozen accident".[45]
8. *Those genes for which redox control is always vital to cell function have gene products involved in, or closely connected with, primary electron transfer. These genes are always contained within the organelle*. G. There are a few possible counter-examples to this rule, but nothing decisively disproves it. The predictions make it an promising line of attack on the hypothesis as a whole.
9. *Genes whose products contribute to the organelle genetic system itself, or whose products are associated with secondary events in energy transduction, may be contained in the organelle in one group of organisms, but not in another, depending on the physiology and biochemistry of photosynthesis and respiration in the species concerned*. O. This is a weak principle, in the sense that it is not easy to see which observations it forbids.
10. *Components of the redox-signalling pathway are themselves not involved in primary electron transfer, and so their genes have been relocated to the nucleus*. N. This principle might be wrong without damage to the whole hypothesis, provided retained regulatory components are themselves under redox control, providing an amplification or increased gain of the primary redox signal.

Figure 2. Any gene can be relocated (Fig. 1), but not all genes can function in the nucleus: the CORR hypothesis applied to chloroplasts. The lower figure shows the ancestral chloroplast as a cyanobacterium apparently unaware that it has just been engulfed. The upper figure shows the chloroplast that is its eventual fate, in the plant cell. In both cyanobacterium and chloroplast, light is absorbed by chlorophyll molecules, some of which then undergo photo-oxidation, at photochemical reaction centres. There, the primary, photochemical reactions initiate electron transport between redox-active components of the internal, thylakoid membrane, pumping protons for ATP synthesis and eventual assimilation of carbon dioxide. Unlike that of the cyanobacterium (lower figure), the chloroplast's thylakoid membrane (upper figure) is chimerical: some of its proteins are synthesised on chloroplast ribosomes from information encoded in chloroplast DNA; other chloroplast thylakoid proteins are imported from the cytosol, where they are made, as precursors, on ribosomes that translate mRNA made in the plant cell nucleus. Most genes (green) in encoding proteins (green) in the endosymbiotic, cyanobacterial precursor (lower figure) became lost. Of those that remain, most became relocated to the nucleus of the host cell, or to the plant cell nucleus (upper figure). The relocated genes (brown) are transcribed, and translated, on cytosolic ribosomes, to give precursor proteins (brown) that are imported into the chloroplast. These precursors are processed into the mature proteins (brown) that are homologous with, and functionally indistinguishable from, the corresponding proteins in cyanobacteria (lower figure). However, in the plant cell (upper figure), the original location is maintained for genes (green) whose protein products (green) carry out primary bioenergetic processes connected with vectorial electron and proton transfer, and whose stoichiometries, relative to each other, must therefore be adjusted by means of redox regulatory control. According to CORR, redox regulation is the function of the location of chloroplast genes in the same cellular compartment as their gene products. Figure adapted from Allen JF. 2003. Philos Trans R London Series B-Biol Sci 358:19–38.

Acknowledgments

JFA hold a Royal Society-Wolfson Merit Research Award, and thanks The Swedish Research Council (VR) for support. SP and CAA thank the Lawski Fund for postgraduate stipends.

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