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Focus

Free-Radical-Induced Mutation vs Redox Regulation: Costs and Benefits of Genes in Organelles

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Abstract. The prokaryotic endosymbionts that became plastids and mitochondria contained genes destined for one of three fates. Genes required for free-living existence were lost. Most genes useful to the symbiosis were transferred to the nucleus of the host. Some genes, a small minority, were retained within the organelle. Here we suggest that a selective advantage of movement of genes to the nucleus is decreased mutation: plastids and mitochondria have high volume-specific rates of redox reactions, producing oxygen free radicals that chemically modify DNA. These mutations lead to synthesis of modified electron carriers that in turn generate more mutagenic free radicals—the ''vicious circle'' theory of aging. Transfer of genes to the nucleus is also advantageous in facilitating sexual recombination and DNA repair. For genes encoding certain key components of photosynthesis and respiration, direct control of gene expression by redox state of electron carriers may be required to minimize free radical production, providing a selective advantage of organelle location which outweighs that of location in the nucleus. A previous proposal for transfer of genes to the nucleus is an economy of resources in having a single genome and a single apparatus for gene expression, but this argument fails if any organellar gene is retained. A previous proposal for the retention of genes within organelles is that certain proteins are organelle-encoded because they cannot be imported, but there is now evidence against this view. Decreased free radical mutagenesis and increased sexual recombination upon transfer to the nucleus together with redox control of gene expression in organelles may now account for the slightly different gene distributions among nuclei, plastids, and mitochondria found in major eukaryote taxa. This analysis suggests a novel reason for uniparental inheritance of organelles and the evolution of anisogametic sex, and may also account for the occurrence of nitrogen fixation in symbionts rather than in nitrogen-fixing organelles.

Key words: Aging — Chloroplasts — Mitochondria — Cell evolution — Cytoplasmic genomes — Gene transfer — Redox regulation — Free radical mutagenesis — Nitrogen fixation — Endosymbiosis — Mutation frequency — Uniparental inheritance

Introduction

It is now generally accepted that plastids and mitochondria have evolved, via endosymbiosis, from free-living prokaryotes (Cavalier-Smith 1987a; Khakhina 1992; Margulis, 1981). The genes derived from these prokaryotes have suffered one of three fates, having been lost, transferred to the nucleus of the eukaryotic host, or retained within the cytoplasmic genome of the organelle.

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The genes that were completely lost coded for functions no longer performed by organelles, and their absence, among other factors, now prevents organelles from reverting to free-living cells. Some genes were lost from organelles but retained by the nucleus. Some of these genes' products now function in the cytosol. For example, the nuclear-encoded cytosolic NAD^+-glyceraldehyde-3-phosphate dehydrogenase of glycolysis and gluconeogenesis has been derived by gene transfer from the prokaryote (α-proteobacterium) that gave rise to the mitochondrion (Kersanach et al. 1994; Logan 1994). About 90% of the genes now encoding plastid or mitochondrial products were also transferred to the nucleus. The mRNAs from these genes are processed and translated on 80S ribosomes in the cytosol, and the precursor polypeptides are targeted to the organelle, imported, processed, and assembled into their functional forms. The remaining 10% or so of genes encoding organellar products have been retained within the organelle and now comprise plastid and mitochondrial genomes. Their gene products include most of the RNA and protein components of the organellar genetic apparatus itself, together with a small but rather constant subset of proteins involved in energy transduction and closely related events.

Here we review the possible reasons why natural selection has favored the transfer of some genes essential for organellar function from the (proto-)organelle to the host nucleus but has favored the retention of others. This analysis may help to explain the differences that now exist between major taxa in the genes retained by organelle genomes. The analysis may also explain why gene transfer to the nucleus has not occurred in endosymbioses (diazotrophic prokaryotes in higher plants; eukaryotic algae in invertebrates) that might otherwise have led to the establishment of nitrogen-fixing organelles.

Selective Advantages of Relocating Genes to the Nucleus

Economy of Resource Use: A Cell with Only One Genetic System Needs Fewer Genes, and Fewer Resources Devoted to Protein Synthesis

Photosynthetic eukaryotes have three genomes, each with its own gene expression system, while chemoorganotrophic eukaryotes, with few exceptions (Cavalier-Smith 1987b), have two. If all genes were consolidated into a single genome, the smaller number of genes needed to produce a single apparatus of gene expression would represent an economy of resource use. Since the same total number of other gene products and the rate at which they are produced at a given specific growth rate are unaltered by the consolidation of genes into the nucleus, it could be that any economy in the number of kinds of genes for transcription and translation is offset by the need for more copies of the genes for each function of a single genetic apparatus. Any such economy is unlikely to be more than a fraction of a percent of the energy, C, N, or P budget of the cells (Raven 1994). Furthermore, such savings could not be fully realized until all genes have been transferred from organelle genomes to the nuclear genome; the last gene remaining in an organelle would need the full suite of genes and their products associated with protein synthesis in order to be expressed. The mature chloroplast of C_3 plants is a case in point, where the complete chloroplast genetic system functions as if for the synthesis of a single polypeptide (Ellis 1981)—the rapidly turned over D_1 (psbA) protein of the reaction center of photosystem II. We therefore suggest that economy of resource use has not been a significant factor in transfer of chloroplast and mitochondrial genes to the nucleus.

Decreased Mutation: Bioenergetic Organelles Have High Volume-Based Rates of Redox Reactions, High Steady-State Concentrations of Oxygen Free Radicals, and Hence High Mutation Frequencies: Transfer of a Gene to the Nucleus Decreases Its Mutation Frequency

As a result of their higher volume-based redox activity, the rate of production of oxygen free radicals is higher in plastids and mitochondria than in the nucleus (Halliwell and Gutteridge 1989; Martin and Pallumbi 1993; Raven et al. 1994a; Raven et al. 1994b). The most dangerous (reactive) of the toxic oxygen species generated in this way is the hydroxyl radical, ·OH, while another reactive oxygen species is singlet oxygen, $^1O_2$. Singlet oxygen is produced by the rapid reaction of triplet (ground-state) molecular oxygen with chlorophyll *a* triplet states in photosynthesis. The structure of the chloroplast light-harvesting protein reveals triplet-state desensitizing carotenoids (lutein) in van der Waals contact with chlorophyll *a* (Kühlbrandt et al. 1994). Singlet oxygen is implicated in photoinhibition of photosynthesis, where protein cross-linking underlies the need for rapid breakdown and replacement of D_1 (Mishra et al. 1994). Tyrosine free radicals participate in electron transfer on the donor side of photosystem II (reviewed by Rutherford 1989). Furthermore, semiquinone radicals are obligatory intermediates in proton motive Q-cycles in both photosynthesis and respiration, and transfer of single electrons from semiquinones to oxygen generates superoxide anion radicals. Superoxide is also produced at the acceptor side of photosystem I of photosynthesis in chloroplasts (Allen and Hall 1973). Chloroplasts and mitochondria have high activities of enzymes which remove the H_2O_2 (peroxidases) and O_2^- (superoxide dismutases). Hydrogen peroxide (H_2O_2) and superoxide ($O_2^{\cdot -}$) are produced by reduction of O_2 and are the immediate precursors of ·OH (reviewed by Halliwell and Gutteridge 1989; Raven et al. 1994a; Raven et al. 1994b). Furthermore, these

organelles also have high levels of scavengers of ·OH (e.g., water-soluble ascorbate; lipid-soluble tocopherols) and of $^1O_2$ (e.g., the lipid-soluble β-carotene) (reviewed by Halliwell and Gutteridge 1989; Raven et al. 1994a,b). Superoxide production by autoxidation of ferredoxin may be mitigated by its reduction, also by ferredoxin, to hydrogen peroxide (Allen 1975). These enzymatic quenching and scavenging mechanisms do not completely remove the most toxic oxygen species, and it should be expected from work on eubacteria and on the nuclear genome of mammalian cell cultures (reviewed by Raven et al. 1994a,b) that mutation rate increases with an increase in the concentration of toxic oxygen radicals such that organellar genomes mutate with higher frequency than nuclear genomes under otherwise similar circumstances.

It has been argued that this prediction is supported by data on evolution rates of mitochondrial and nuclear genomes in mammals (Gray 1989; Martin and Palumbi 1993). Gray (1989) reviews data showing similar rates of evolution of mitochondrial and nuclear genomes in most invertebrates (including protists). Plant mitochondrial genomes evolve significantly less rapidly than does the comparable plant nuclear genome, with the chloroplast genome evolving at a rate somewhere between these two (reviewed by Gray 1989). Gray (1989) discusses a number of possible reasons for variations in genome evolution rates. A high rate of sequence divergence, as found in mammalian mitochondria relative to mammalian nuclei, could be a result of: (1) more damage (e.g., from toxic oxygen radicals) in mitochondria; (2) a more error-prone DNA replication (low fidelity of selection of nucleotides, or of editing); (3) absence of, or deficiency in, DNA repair; (4) relative lack of recombination which could otherwise eliminate mildly deleterious mutations; (5) high turnover rate for DNA; or (6) greater selection pressure. Gray (1989) favors relaxed codon recognition in accounting for the rapid evolution rate of animal mitochondrial DNA; such relaxed codon recognition does not occur in plant mitochondria.

It is important here to draw a clear distinction between mutation frequency and evolution rate, even where the latter is assessed as the number of point mutations by which two or more sequences have diverged. Since evolution is natural selection of random variation generated by mutation, a high mutation frequency may result in slow evolutionary divergence if the selection pressure maintaining the original sequences (alleles) is strong: an example, at the molecular level, of stabilizing selection; 23S ribosomal RNA, ribosomal elongation factors, and membrane domains of ATP synthases may be extreme examples, showing high conservation of sequence between disparate evolutionary groups. Equally, a low mutation frequency may nevertheless produce rapid evolutionary divergence where selection has a relatively high probability of favoring new phenotypes. Thus the greater divergence of mammalian mitochondrial sequences may be consistent with their greater mutation frequency, but may also be explained by a disparity in the magnitude and direction of selective forces acting on mitochondrial and nuclear genes. It is therefore possible to retain the hypothesis that free-radical-induced damage to DNA produces a much higher mutation frequency in all plastids and mitochondria than in nuclei.

Mitochondrial respiration has long been considered to play an important role in generation of free radicals, and free radicals have long been implicated in aging (Harman 1972). Following the recognition that mitochondria contain cytoplasmic genetic systems (reviewed by Attardi and Schatz 1988), it was suggested that mitochondrial mutation may be a consequence of respiratory free radical production (Miquel et al. 1980; Fleming et al. 1982). A ''vicious circle'' of energy loss was thus proposed as a cause of aging, whereby mitochondrial division is impaired and mitochondria become incapable of replenishment in postmitotic cells such as those of muscle (Miquel et al. 1980; Fleming et al. 1982). More recently, it has been recognized that a further consequence of mitochondrial mutation is synthesis of defective, mitochondrially encoded proteins, including electron carriers that will thus increase production of mutagenic free radicals (Allen 1996; Ozawa 1995). The arguments apply equally to chloroplasts (Allen 1996). This ''vicious circle'' of free radical mutagenesis of organelle DNA is depicted in Fig. 1. There is increasing direct evidence that damage to mitochondrial DNA indeed accrues rapidly, within the lifetime of individual animals, and plays a central role in the phenomenon of aging (Ames et al. 1993; Loft et al. 1994; Ozawa 1995; Shigenaga et al. 1994). Proximity of certain regions of chloroplast and mitochondrial DNA to the membrane-bound photosynthetic and respiratory electron transport chains should be expected to lead to mutational ''hot spots'': regions of DNA close to sites of $^1O_2$ generation and thus of especially high mutation frequency (Münscher et al. 1993).

We propose that decreased mutation stands as a strong selective reason for transferring genes from both plastids and mitochondria to the nucleus. Such transfer is depicted schematically in Fig. 2.

Increased Recombination, DNA Repair, and Biparental Inheritance: Lack of Recombination of Organelle Genomes Restricts Options for Repairing Mutation and for Maintaining Fitness in Changing Environments

Organelle genomes are generally inherited uniparentally in sexual reproduction. Organelles are generally acquired from the female parent in those cases (oogamous sexuality) in which the male and female gametes can be morphologically distinguished as sperms and eggs. However, paternal inheritance of chloroplasts and (frequently) of mitochondria occurs in coniferous gymnosperms (Stine

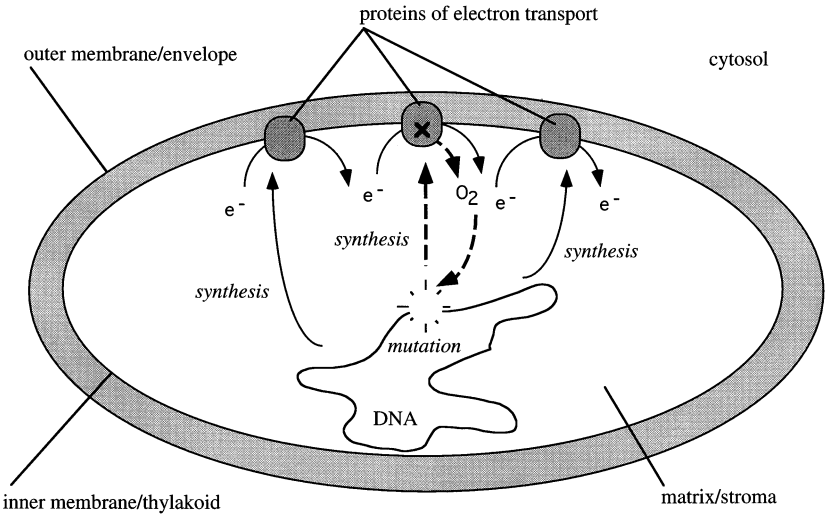

Fig. 1. The ''vicious circle'' theory of aging by free radical mutagenesis in bioenergetic organelles. In respiratory and photosynthetic electron transport (e^-) some electron transfers to oxygen produce free radicals that cause mutation. Mutation itself results in synthesis of defective electron transport proteins (x), increasing the frequency of free radical production. Both these ''incorrect'' electron transfers and mutation are thus self reinforcing (*broken lines*). The accumulated effects of the cycle will eventually extend to the nuclear–cytosolic system and have been suggested, for mitochondria, to be the cause of aging (Allen 1996; Ozawa 1995).

and Keathley 1990). Furthermore, a novel mechanism of mitochondrial inheritance occurs in the bivalve mollusc *Mytilus.* Here two different mitochondrial genomes, *F* and *M*, occur; they exhibit 10–20% sequence divergence (Hurst and Hoekstra 1994; Skibinski et al. 1994; Zouros et al. 1994). Daughters receive only the *F*-type genome, almost entirely from their mothers, because there is a very low level of *F*-type mitochondria in sperm (Skibinski et al. 1994; Zouros et al. 1994). Sons receive *M*-type mitochondria from their fathers and *F*-type mitochondria from their mothers; despite the initial numerical preponderance of the *F*-type mitochondria from the eggs the faster multiplication of the *M*-type mitochondria means that they predominate in adult males (Skibinski et al. 1994; Zouros et al. 1994). Biparental inheritance of plastids occurs in certain isogamous species of *Chlamydomonas* (Harris 1989).

Uniparental inheritance of organelle genomes has been suggested to be advantageous in preventing the spread of deleterious organelle genomes in sexually reproducing populations (Hurst and Hamilton 1992; Hurst and Hoekstra 1994; Law and Hutson 1992). If organelle genes were transferred to the nucleus, the constraints on the spread of deleterious but rapidly reproducing genes derived from the organelles would be identical to those in the nuclear genome as a whole. By their presence in the sexually inherited nuclear genome, genes originating from organelles would also be subject to such long-term advantages of recombination of repairing mutation and for maintaining and improving fitness in changing biotic and abiotic environments (Bell 1988).

An alternative proposal for the selective advantage of uniparental inheritance of organelles is that it serves to separate an independently replicating line of organelles that are thereby released from their bioenergetic function, and hence protected from free radical mutagenesis (Allen 1996). This hypothesis combines the proposal that decreased mutation is the primary advantage of relocat-

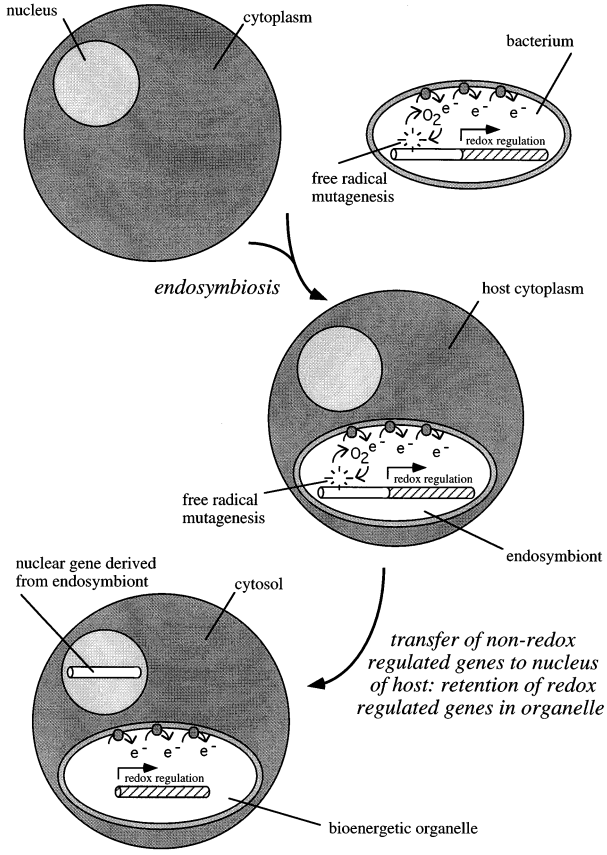

Fig. 2. Distribution of genes between nucleus and organelle following endosymbiosis. Most endosymbiont genes (symbolized by the *open rectangle*) are subject to free radical mutagenesis (see Fig. 1), which they evade upon transfer to the nucleus; this is proposed as the primary reason for nuclear acquisition of symbiont genes. In contrast, genes (symbolized by the *crosshatched rectangle*) for certain key redox components of bioenergetic organelles remain in situ, since their expression must be subject to redox regulation in order to respond directly to environmentally induced changes in electron transport (e^-). Transfer of such genes to the nucleus would uncouple redox control, and hence increase free radical production within the organelle and, through the ''vicious circle'' (Fig. 1), throughout the cell as a whole.

Table 1. Major chloroplast genes and gene products in green algae and higher plants and their functions[a]

Gene	Protein	Standard redox potential Em_7 (mv)	Substrate
	Photosystem II		
psbA	D_1 of PS II reaction center	+1,170(P^+/P) −800(P/P*)	Light
psbD	D_2 of PS II reaction center	+1,170(P^+/P) −800(P/P*)	Light
psbB, psbC	CP47 and CP43 of PSII	—	Light
	Electron transport/energy coupling		
petA	Cytochrome *f*	+350	Electrons, pmf
petB	Cytochrome b_6	−50	Electrons, pmf
atpA,B,E	ATP synthase CF_1, α,β,ϵ	—	pmf, ATP
atpF,H,I	ATP synthase CF_o I, III, IV	—	pmf, ATP
	Photosystem I		
psaA	A_1 of PS I reaction center	−1,250(P/P*) +500(P^+/P)	Light
psaB	A_2 of PS I reaction center	−1,250(P/P*) +500(P^+/P)	Light
	Respiration: cyclic electron transport/CO_2 fixation		
ndhA–F	NADH dehydrogenase subunits 1–5	−324	Electrons from PSI or respiratory substrates
rbcL	Rubisco large subunit	—	CO_2

[a] After Allen (1993b)

ing genes to the nucleus with the proposal, discussed later, that certain genes for key components of bioenergetic systems must be retained within the organelle to permit redox control of gene expression. According to this hypothesis (Allen 1996), anisogametic sex is a division of labor (Maynard Smith and Szathmáry 1995) between germ cell lines adapted to bioenergetic function (''male'') and fidelity of organelle replication (''female''). If this hypothesis is correct, uniparental mitochondrial and plastid inheritance is a consequence of free radical mutagenesis, and not in itself a reason for transfer of genes to the nucleus.

Despite this qualification, the nuclear genome has a range of options for repair and recombination of genes, the latter associated classically with independent segregation and random assortment. These together now represent a selective advantage of moving genes from organelles to the nucleus.

Selective Advantages of Retaining Genes in Organelles

Some Gene Products May Be Unimportable

Von Heijne (1986) for mitochondria and Palmer (1993) for plastids have argued that the reason for retention of certain genes in organelles is that they are simply poor candidates for successful gene transfer and so did not suffer the fate of relocation to the nucleus. However, there is no clear line of demarcation between existing plastid (Tables 1 and 3) or mitochondrial (Table 2) proteins that indicates why their genes might have different susceptibilities to transfer, nor why their gene products might be more or less successfully imported from the cytosol in precursor form. Hydrophobicity (von Heijne 1986) is clearly not the determinant. Thus the light-harvesting proteins of chlorophyll *b*-containing organisms are hydrophobic yet nuclear-encoded, and there seems to be no special problem in transporting precursors of these polypeptides across the plastid envelope membranes to the thylakoid membrane (Mullet 1988). The same is true of the light-harvesting fucoxanthin-chlorophyll *a,c* proteins of diatoms, which have to traverse four membranes rather than two en route to the thylakoid (Bhaya and Grossman 1991). Furthermore, the Rubisco large subunit is a component of a protein in the aqueous, stromal phase of the plastid but is nevertheless synthesised in situ from a plastid gene (Ellis 1981, 1984). Neither is there any clear correlation between gene location and whether the polypeptide functions as part of an oligomeric complex. Experimental studies on relocation of organelle genes suggest that it is possible to make a successfully re-imported construct for essentially any gene, and that these can arise spontaneously at reasonable frequency. A falsifying counterexample to the ''unimportability'' hypothesis is the work of Kanevski and Malaga (1994), who show that *rbcL,* encoding the large subunit of Rubisco, can be deleted from the tobacco chloroplast and placed in the nucleus, where full expression of a chloroplast-targeted precursor leads to reconstitution of autotrophic growth. *RbcL* appears to be gen-

Table 2. Major mitochondrial genes and gene products and their functions[a]

Gene	Protein	Standard redox potential Em_7 (mv)	Substrate
	Electron transport/Energy coupling		
nadA–F	Seven units of NADH dehydrogenase	−324	Respiratory substrates
coxA–C	Cytochrome c oxidase subunits I, II and III	+815	O_2
atp 6	ATP synthase F_1 subunit 6	—	pmf, ATP
cob	Cytochrome *b*	−50	Electrons, pmf

[a] After Allen (1993b)

Table 3. Major chloroplast genes and gene products in the red alga *Porphyra purpurea* (Reith and Munholland 1993) and their functions[a]

Gene	Protein	Standard redox potential Em_7 (mv)	Substrate
	Photosystem II		
psbA	D_1 of PS II reaction center	+1,170(P^+/P) −800(P/P*)	Light
psbD	D_2 of PS II reaction center	+1,170(P^+/P) −800(P/P*)	Light
psbB,C	CP47 and CP43 of PSII	—	Light
apcA,B	Allophycocyanin α, β subunits	—	Light
apcD	Allophycocyanin B α subunit	—	Light
apcE	Phycobilisome anchor subunit	—	—
cpcA,B	Phycocyanin α, β subunits	—	Light
cpeA,B	Phycoerythrin α, β subunits	—	Light
	Electron transport/Energy coupling		
petF	Ferredoxin	−420	Electrons
petG	Cytochrome b_6–*f* complex subunit V		Electrons/pmf
petJ	Cytochrome c_{553}		Electrons
atpA,B,D,E	ATP synthase CF_1 α,β,δ,ε	—	pmf, ATP
atpF,G,H,I	ATP synthase CF_o I,II,III,IV	—	pmf, ATP
	Photosystem I		
psaA	A_1 of PS I reaction center	−1,250(P/P*) +500(P^+/P)	Light
psaB	A_2 of PS I reaction center	−1,250(P/P*) +500(P^+/P)	Light
	CO_2 fixation		
rbcL	Rubisco large subunit	—	CO_2
rbcS	Rubisco small subunit	—	

[a] Em_7 values are taken from Cramer and Knaff (1990)

erally plastid-encoded (Tables 1 and 3), though a form II Rubisco, resembling that of purple nonsulphur bacteria, is encoded in the nucleus of dinoflagellates (Morse et al. 1995; Whitney et al. 1995).

Accordingly, the ''unimportability'' hypothesis is inconsistent with available data and is unlikely to be a factor in the maintenance of organelle genomes.

Redox Regulatory Control of Gene Expression

Allen has proposed that a major reason for retention of certain genes in plastids (Allen 1993a,b) and mitochondria (Allen 1993b) is a requirement for direct regulation of their expression by changes in redox state of electron carriers of photosynthesis and respiration. Plastids and mitochondria carry out electrochemistry sequestered from the rest of the cell, and their genes encode those of their proteins which operate at extreme redox potentials and which connect their electron transport chains with the physical environment (Allen 1993a,b). Certainly a common feature of organelle-encoded gene products seems to be direct participation in electron transfer or in closely-related reactions of light harvesting, energy coupling, or CO_2 assimilation (Tables 1–3).

In bacteria there are now many examples of redox regulation of gene expression (Allen 1993c). There is now direct evidence that such redox regulatory components still operate in plastids and mitochondria, providing a *raison d'être* of their small genomes as quasi-autonomous genetic outposts. Recent evidence suggests

that redox signalling mechanisms involving protein phosphorylation (Allen 1992) operate in both chloroplasts (Allen 1995; Allen et al. 1995) and mitochondria (Håkansson and Allen 1995). Different redox conditions appear to select different subsets of proteins for synthesis by isolated chloroplasts and mitochondria (Allen 1995) and to influence mitochondrial DNA and RNA synthesis (Konstantinov et al. 1995). We conclude that current evidence supports the hypothesis that organelle genomes satisfy the requirement for direct redox control of expression of genes whose products function in primary electron transport, or which mediate environmental effects on primary electron transport (Allen 1993a,b). A secondary requirement for a genetic system that operates in situ may explain the location of genes for components of organelle genetic systems themselves. Retention of redox-regulated genes and removal of others to the nucleus is depicted in Fig. 2.

Variation Among Major Taxa in the Genes Retained by Energy-Transducing Organelles

Although there are significant differences in the genes present in the mitochondrial genomes of different major taxa (Gray 1989; Newton 1988; Schuster and Brennicke 1994), it is in the plastid genome that recent work reveals major differences in genes present among higher (divisions and above) taxa (Reith and Munholland 1993). The main distinction for those higher taxa examined seems to be between the green algae and higher plants (containing chlorophyll *b*) and the red and chromophyte (i.e., chromistan, cryptophyte, dinophyte, and haptophyte) algae (Cavalier-Smith 1993; McFadden et al. 1994; Medlin et al. 1994; Moestrup 1992; Palmer 1993; Reith and Munholland 1993).

Table 3 shows the major genes related to photosynthesis which are coded in the plastid genome of *Porphyra purpurea*, the red alga whose plastid genome is known in most detail. Comparison with Table 1 for a green (chlorophyll *b*–containing) organism reveals a number of similarities and differences. Similarities include the encodement in the plastid genome of the reaction center cores of photosystem II (D_1 and D_2) and photosystem I (A_1 and A_2) as well as the light-harvesting complexes closest to the reaction centre of photosystem II (CP47 and CP43, psbB and psbC, respectively). The chloroplast genome also codes for most subunits of the coupling ATPase components CF_1 and CF_o, as well as the large subunit of Rubisco (ribulose bisphosphate carboxylase-oxygenase).

Turning to differences, the plastid genome of *Porphyra* (Table 3) codes for the polypeptides of the phycobilin light-harvesting pigment-protein complexes, while the corresponding apoproteins of the chlorophyll *a–b* light-harvesting complexes in green plants are nuclear-encoded. Furthermore, the plastid genome of *Porphyra* codes for additional subunits of ATP synthetase (δ of CF_1; II of CF_o). While it is not clear if cytochromes b_6 and *f* are plastid-encoded in *Porphyra*, it is clear that subunit V of the cytochrome b_6-*f* complex is encoded in the *Porphyra* plastid, unlike the situation in green plants. Cytochrome c_{553} is encoded in the plastid genome of *Porphyra*, although this soluble (intrathylakoid) cytochrome is nuclear-encoded in those green algae in which it occurs, as is the functionally analogous plastocyanin in green algae and higher plants (Harris 1989). Finally, among important photosynthetic components which are plastid-encoded in *Porphyra* but nuclear-encoded in green plants is ferredoxin.

A major absence from the *Porphyra* plastid genome of the redox components encoded in the plastids of green plants is that of the NAD(P)H dehydrogenase components I–IV. Recent evidence suggests a role for this redox complex in the chloroplast in cyclic electron flow (and thus photophosphorylation) as well as in chlororespiration, and suggests that cyclic electron transport occurs in all major algal groups investigated as well as in higher plants (Fork and Herbert 1993). Any involvement of NAD(P)H dehydrogenase in plastid processes such as cyclic electron flow and chlororespiration would involve plastid-expressed but nuclear-encoded NADH dehydrogenase in the red algae and, probably, the chromophytes (Reith and Munholland 1993).

In addition to these ''photosynthetic'' genes, the *Porphyra* plastid genome (Table 3) also encodes two genes producing polypeptides involved in redox reactions in plastids which potentially catalyze consumption of photoreductant by processes other than CO_2 fixation. These genes code for glutamate synthase (GOGAT) and for 3-ketoacyl ACP synthase III; in green plants these genes are nuclear-encoded.

Gene distribution between the plastid genome and the nuclear genome of the red alga *Porphyra* is probably indicative of the situation in other, less completely investigated, red algae, and in representatives of major taxa of eukaryotic photosynthetic organisms lacking chlorophyll *b*. Clearly the distribution of the phycobilin apoprotein genes is not applicable to chromophytes which lack these pigments, but the data for these taxa and also for cryptophytes for genes which are common to all O_2-evolvers shows very similar gene distributions to those found in *Porphyra* (see original data, and references and discussion in Reith and Munholland 1993; Palmer 1993; Fujiwara et al. 1993). However, the light-harvesting fucoxanthin-chlorophyll *a,c* protein of the diatom *Phaeodactylum tricornutum* is nuclear-encoded (Bhaya and Grossman 1991). This contrasts with the situation for phycobilin polypeptides in *Porphyra* and resembles that for chlorophyll *a,b* protein in green plants.

The concept of control of transcription of organellar genes by the redox state of the organelle (Allen 1993a,b)

could clearly be applied to the ''extra'' genes found in the plastid genome of organisms lacking chlorophyll *b*. This is particularly the case for those related directly or indirectly to redox reactions. However, there is the problem of explaining why there are more of these putatively redox-regulated genes in the plastids of non–chlorophyll-*b*-containing organisms than in green eukaryotic photosynthesizers. The heterogeneity of life form and habitats occupied by the non–chlorophyll-*b*-containing organisms does not permit any easy correlation of intracellular gene distribution with environment, since there is very considerable overlap in life form and habitat between the green and nongreen photosynthetic eukaryotes (see Round 1981; Raven 1984). Accordingly it is unlikely that we can appeal to environmental factors such as hyperoxia (Raven et al. 1994a,b) having less effect on the nongreen photolithotrophs, and thus reducing the selection pressure for gene transfer to the nucleus. While the Cu-Zn superoxide dismutase of higher green plants (tracheophytes, bryophytes) and charophycean green algae does not, when overexpressed, increase resistance to acute damage caused by O radicals, the Mn superoxide dismutase when expressed in the plastids of transgenic higher plants *does* increase resistance (Bowler et al. 1992; Raven et al. 1994a,b). However, there is no correlation of the absence of plastid Cu-Zn superoxide dismutase with high gene diversity of plastid DNA, since the Cu-Zn enzyme is absent from noncharophycean green algae as well as red algae, chromista, and dinophytes (Halliwell and Gutteridge 1989; Raven et al. 1994a,b).

Secondary Endosymbioses

The original processes by which eubacteria became plastids and mitochondria (Morden et al. 1992) are not, of course, the end of the possibilities for endosymbiosis. Plastids from some large-celled marine green (*Codium, Caulerpa*) and red (*Griffithsia*) algae are sequestered by herbivorous nudibranch molluscs of the green *Elysia* and *Hermea* and remain functional in the gastropods for weeks or months. Law and Lewis (1983) point out that ''symbiosis'' is a misnomer for this arrangement, since the ''symbiont'' is not genetically autonomous. The limited longevity of the plastids in the molluscs may well be related to this limited genetic autonomy which prevents ''repair'' (replacement) of any damaged proteins which are nuclear-coded. There would be more such ''irreplaceable'' components in the green than in the red algal plastid (compare Tables 1 and 3).

Ingestions of green and red algal plastids appear to have led to a stable second round of symbioses, leading to further photosynthetic eukaryotes (Bhattacharya and Medlin 1995; Moestrup 1992; Reiser 1992). These are recognized inter alia by the presence of three (dinophytes, euglenophytes) or four (chromista, cryptophytes, haptophytes, chloroarachniophytes) rather than two (rhodophytes, chlorophytes) membranes around plastids (Reiser 1992). Here the nuclear genes necessary for plastid synthesis in the red or green algae must persist in the second round of symbioses. An intermediate stage may be seen in the ''nucleomorph'' of chlororachniophytes and cryptophytes; in both cases the plastid compartment is derived from a rhoedophyte, with the pigmentation of chlororachniophytes (chlorophylls *a* plus *b*) reflecting a pigment change of the type seen in chlorophytes in primary endosymbioses of cyanobacteria, and in chloroxybacteria (prochlorophytes) in free-living cyanobacteria (Bhattacharya and Medlin 1995; Cavalier-Smith 1993). An intermediate stage in a tertiary endosymbiosis is found where a chromistan is being ingested by a dinophyte (Reiser 1992). In these cases a residuum of the nucleus of the phagocytosed eukaryote remains in a compartment containing the plastid(s), and presumably retains the genes needed for plastid function. In dinophytes, euglenophytes, chromista, and haptophytes these genes have presumably been transferred to the nucleus of the host cell.

A possibility which relates to the different gene content of plastid genomes in the green algae (and presumably their secondary symbiotic derivatives, the euglenophytes) and red algae (and their secondary symbiotic derivatives, the cryptophytes, chlororachniophytes, chromista, dinophytes, and haptophytes) is that of retention of nuclear genes for plastid function from a previous photosynthetic episode, facilitating a new plastid acquisition. This might, for example, occur in dinophytes, which appear to be in the act of acquiring chrysophycean, chromophyte plastids. The presence, in dinophytes, of nuclear genes appropriate to a chromophyte plastid function might obviate the need for nucleus-to-nucleus gene transfer of the chromophyte genes (Raven 1987). However, at the moment these fucoxanthin-containing dinophytes retain the chrysophyte nucleus (Reisser 1992).

Absence of Nuclear Gene Transfer from N_2-Fixing Symbioses in Plants

It appears that there are no ''diazoplasts'': N_2-fixing organelles (Smith and Douglas 1987; Sprent and Sprent 1990; Douglas 1994). All prokaryotes involved in N_2-fixing symbioses with eukaryotes seem to be facultatively symbiotic, retaining the capacity for independent existence. This means that all genes necessary for independent life have been retained, although it does not rule out the occurrence of duplicates of prokaryote genes in the host nucleus (Raven 1993). The absence of N_2-fixing organelles is very unlikely to be a result of mechanistic problems of transfer of genes essential for N_2 fixation by

the prokaryotes to the host nucleus, at least for the rhizobia symbiotic with legumes and *Parasponia*. This conclusion is based on the very close phylogenetic relationship between the rhizobia and the most likely ancestors of mitochondria in the α-subdivision of the proteobacteria (gram-negative eubacteria) (Logan 1994). Prokaryote-to-eukaryote gene transfer clearly occurred for the ancestors of mitochondria, so it is reasonable to assume that it might also have occurred for rhizobia. The same argument can be made for the cyanobacteria (also gram-negative eubacteria) in N_2-fixing symbioses, since their relatives which gave rise to chloroplasts clearly transferred genes to the eukaryotic partner. Similar arguments are not possible for the other eubacteria involved in diazotrophic symbioses, since *Frankia* (an actinomycete [gram-positive] eubacterium) has no close relatives which gave rise to organelles.

Raven et al. (1994a,b) suggest that N_2-fixing symbioses might be less susceptible to decreased mutation rate upon gene transfer from prokaryote to eukaryote. The argument is based on the low steady-state oxygen concentration in the prokaryotic cells. This is especially the case for rhizobia in nodules which regulate gas exchange between the environment and the N_2-fixing cells. However, it also applies to actinorhizal and cyanobacterial symbioses where regulation of oxygen level around nitrogenase relates as much to properties of the cell wall of heterocysts (cyanobacteria) and vesicles (*Frankia*) as to host processes, so the oxygen level in the non-N_2-fixing prokaryotic cells may well be higher than in heterocysts, vesicles, and rhizobia. In all cases the lower oxygen concentrations could offset the high volume-based rate of redox reactions, restricting the rate of production of toxic oxygen radicals and hence the potential for mutation. This weakens the argument for gene transfer to the host genome as a means of limiting mutation rate. Furthermore, retention of genes by the prokaryote permits regulation of their expression by the redox state of the N_2-fixing cells; such regulation is known for some genes of N_2 fixation (Allen 1993c). A further argument for retention of all genes needed for independent life of the prokaryote is that the eukaryote could ''pick and choose'' among prokaryotes from its environment in finding the most functionally appropriate genotype for a given situation (Raven 1993). This suggestion is also applicable to the symbioses of eukaryotic microalgae with invertebrates (Raven 1993). In both cases there is a potential problem with availability to the host of inoculum microorganisms in their habitat.

Smith and Douglas (1987) and Douglas (1994) suggest that symbioses between higher plants and prokaryotic N_2-fixers evolved when plants were already complex morphologically, and involved the occurrence of the prokaryotes at high densities in parts of the plant distant from the meristems, giving rise to reproductive structures. The localized occurrence of the prokaryotes relates inter alia to the protection of nitrogenase from O_2 (see above) and prevents the contribution of genes from the prokaryote which have been transferred to neighboring host nuclei to the ''germ line.'' It is significant that the restriction on transmission to the new generation of prokaryote genes, either in the prokaryote or the host nuclei, from N_2-fixing regions of the plants, does not apply to certain N_2-fixing symbionts which have retained their genetic autonomy. Thus the motile (by gliding) cyanobacterial symbionts of *Azolla* can move to the sporocarp and be transmitted on megaspores (Smith and Douglas 1987), just as some corals have transovarian transmission of their dinophyte symbionts (Raven 1993).

Conclusions

We suggest that the present distribution of genes among organellar and nuclear genomes is not so much a ''frozen accident'' as a result of selective forces favoring movement of some genes from organelles to the nucleus balanced by selective forces favoring retention of other genes in organelles. The major selective forces favoring transfer of genes to the host genome may be the higher mutation frequencies of organellar genes. These result from higher rates of generation of mutagenic oxygen free radicals (Raven et al. 1994a,b). An additional factor is the availability of sexual recombination in nuclear genomes which can repair mutation and maintain and improve fitness in changing environments. A major selective force for retention of genes in organelles is the control of their expression by the redox state of the organelle (Allen 1993a,b). Redox control within organelles of expression of genes for electron transfer components may serve to minimize the total oxygen free radical-derived mutational load on eukaryotic cells. Thus, while genes are generally selfish (Dawkins 1976), plastid and mitochondrial genes show symptoms of altruism: They pay the penalty of high mutation frequency, but their location also enables them to minimize inappropriate electrochemistry in photosynthesis and respiration, and hence to decrease effects on the whole cell of oxygen free radicals, generated as an unavoidable consequence of the bioenergetic requirement for oxygen redox chemistry. Thus we predict that any gene experimentally translocated to the nucleus (such as *rbcL* in the experiment of Kanevski and Malaga 1994) would be subject there to a *higher* mutation frequency than in the organelle, at least in natural environments, since the absence of direct redox control of its expression would lead to increased production of oxygen free radicals within the organelle, and throughout the cell as a whole. Given the requirement for organelle genomes to permit redox control of gene expression, anisogametic sex may represent a division of labor between the male and female germ lines that resolves the conflict between energetic require-

ments for gamete and gametophyte motility and fidelity of organelle replication (Allen 1996). Organelle genomes in general are thus maternally inherited.

While these trade-offs of cost and benefit may not explain all aspects of the distribution of genes between organelle and nucleus in every eukaryotic cell, they comprise a more testable hypothesis than a ''frozen accident'', and appear to be consistent with present data.

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