Control of Gene Expression by Redox Potential and the Requirement for Chloroplast and Mitochondrial Genomes

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Recent experiments with bacteria have shown that light and oxygen can control gene expression through effects on oxidation-reduction potential. The term "redox sensor" is proposed as a general term for electron carriers that initiate control of gene expression upon oxidation or reduction. The term "redox response regulator" is proposed for DNA-binding proteins that modify gene expression as a result of the action of redox sensors. Redox sensors and redox response regulators may function together in feedback control of redox potential in photosynthesis and respiration, protecting the cell from damage caused by electrochemistry operating on inappropriate electron donors and acceptors. Chloroplast and mitochondrial redox sensors and redox response regulators, themselves encoded in the nucleus, may place expression of chloroplast and mitochondrial genes under redox regulatory control. This hypothesis offers an explanation for the persistence, in evolution, of chloroplast and mitochondrial genomes, and for the constancy of the subset of chloroplast and mitochondrial proteins encoded and synthesized within the organelle.

Introduction

In photosynthesis and respiration, environmental and metabolic oxidants and reductants are linked by a sequence of intermediate electron carriers, all operating relatively close to their mid-point potentials, E_m. As a result of environmental changes which affect availability of energy and electron sources and sinks, the ratio of activities of the reduced and oxidized forms, (red)/(ox), of any electron carrier may change. This in turn affects the redox potential, E, of the electron carrier, in accordance with the Nernst equation (see, for example, Cramer & Knaff, 1990).

$$E = Em - \frac{RT}{nF} \ln \frac{[red]}{[ox]}.$$

In bacteria, control of gene expression has been shown to occur in response to changes in redox potential that are themselves caused by changes in light intensity and oxygen concentration. This control provides a mechanism for redox homeostasis, and operates by means of components with similarities to those maintaining homeostasis for a variety of other factors, including nitrogen status and osmotic potential.

In eukaryotic cells, the initial effects of environmental changes affecting redox potential occur in chloroplasts and mitochondria. Chloroplasts and mitochondria are bioenergetic organelles, of prokaryotic origin, that contain the sequestered

electrochemistry of photosynthesis and respiration. It is proposed here that pro-karyotic redox regulatory mechanisms are present in chloroplasts and mitochondria, and that the requirement for rapid and direct redox response at the genetic level is responsible for the maintenance of chloroplast and mitochondrial genomes as small but relatively constant subsets of the genes derived from the prokaryotic ancestors of chloroplasts and mitochondria. Thus bioenergetic organelle genomes encode pro-teins that function as the primary sites at which redox potential is influenced by environmental change. The components that function in redox control of chloroplast and mitochondrial gene expression may include redox sensors and redox response regulators. Such redox regulatory components, in common with the majority of chloroplast and mitochondrial proteins, are now encoded in the cell nucleus and synthesized in the cytosol as precursor proteins for import into the organelle. These and other predictions are examined.

Redox Regulation of Gene Expression

Bacterial two-component regulatory systems control gene expression by means of environmental sensors and response regulators (or effectors) (Ronson et al., 1987; Saier et al., 1990; Stock et al., 1990). The sensor, which is usually a membrane protein, becomes phosphorylated in response to the environmental signal, and its substrate, the response regulator, is a DNA-binding protein that initiates transcrip-tion of a specific gene or genes by interacting, in its phosphorylated form, with an RNA polymerase. An example of a two-component regulatory system is the Ntr system controlling nitrogen assimilation in enteric (Ninfa & Magasanik, 1986; Magasanik, 1989) and photosynthetic (Kranz et al., 1990; Tsinoremas et al., 1991) bacteria. Other factors controlling gene expression through two-component regula-tory systems include osmotic pressure, phosphate assimilation, virulence, transport, chemotaxis, motility and sporulation (Stock et al., 1989). These systems appear to share a common mechanism of transcriptional activation (Aoyama & Atsuhiro, 1990). Sensors undergo histidine phosphorylation at a conserved site, and response regulators are phosphorylated on aspartate by a phosphotransferase or kinase (Stock et al., 1989).

TWO-COMPONENT REDOX REGULATION OF THE RESPIRATORY CHAIN

There are now clear indications that redox potential exerts effects on gene expression by two-component regulation. The Arc system of *Escherichia coli* is an example: mutations in two genes, *arcA* and *arcB*, increase the anaerobic expression of a number of genes normally repressed by anoxyia and whose products (including succinate dehydrogenase and cytochrome *d* oxidase) are involved specifically in aerobic metabolism (Iuchi et al., 1989; Guest et al., 1990; Spiro & Guest, 1991; Guest, 1992). ArcB is a protein kinase which contains two membrane-spanning helices flanking a cytoplasmically exposed loop, the latter containing a histidine which is the site of autophosphorylation. Autophosphorylation occurs in response to decreased

redox potential, rather than in response to decreased oxygen concentration *per se*. This is illustrated by oxygen-like effects of alternative oxidants such as fumarate and nitrate, and by deletion of cytochromes *o* and *d* (Iuchi *et al.*, 1990), which mimics the effects of anoxyia in a manner which is reversed by mutations in *arcA* and *arcB* (Iuchi *et al.*, 1990). ArcA is a soluble protein which is phosphorylated by ArcB (Iuchi & Lin, 1988) and which contains the helix-turn-helix motif of DNA-binding proteins (Harrison, 1991). I suggest that the terms "redox sensor" and "redox response regulator" are appropriate descriptions of ArcB and ArcA, respectively.

TWO-COMPONENT REDOX REGULATION OF PHOTOSYNTHESIS

In the purple non-sulphur bacterium *Rhodobacter capsulatus*, the opposing effects of light and oxygen on synthesis and assembly of photosynthetic reaction centre and light-harvesting complexes are inhibited by mutations in a single regulatory DNA sequence (Klug *et al.*, 1991). This sequence lies in the region of the promoter of the *puf* operon, which encodes proteins of the reaction centre and the inner light harvesting complex, LH1 (Taremi & Marrs, 1990; Klug *et al.*, 1991; Sganga & Bauer, 1992). The regulatory gene product has been termed PPBP, for *puf* promoter-binding protein (Taremi & Marrs, 1990), and the gene has been termed *regA* (Sganga & Bauer, 1992). Like ArcA, RegA shows the helix-turn-helix motif of DNA-binding proteins and sequence similarities with other response regulators. RegA is therefore likely to be a redox response regulator in the sense defined here.

OTHER TWO-COMPONENT REDOX REGULATORY SYSTEMS

The *petR* gene of *R. capsulatus* is located upstream of the *fbcFBC* operon which encodes components of the cytochrome b/c_1 complex (Tokito & Daldal, 1992). Its gene product is similar to ArcA and other response regulators (Tokito & Daldal, 1992). Regulation of *sod* genes, encoding Mn-superoxide dismutase in *E. coli*, involves *arcA* and *arcB*, but also has a further two-component regulation involving *soxS* (superoxide sensor) and *soxR* (superoxide response regulator). It is possible that *soxS* senses redox potential rather than superoxide or its products (Fee, 1991). PetR and SoxR may therefore be redox response regulators in the sense defined here.

REDOX ACTIVATORS AND REPRESSORS: FNR

Other regulatory systems link gene expression to redox potential. The *E. coli* FNR protein (regulating synthesis of fumarate and nitrate reductase) is a helix-turn-helix DNA-binding protein with an amino-terminal segment containing cysteines that ligate a redox-active iron atom (Guest *et al.*, 1990; Unden *et al.*, 1990; Spiro & Guest, 1991). As in the Arc system, FNR responds to redox potential. Reduction of the iron from Fe^{III} to Fe^{II} is thought to cause a protein structural change which regulates transcription. From effects of poising the growth medium at different potentials on expression of a reporter gene for *frd* (encoding fumarate reductase), the standard mid-point potential (Em_7) of the FNR response has been estimated at $+400 \, mV$ (Unden *et al.*, 1990).

HAEM REDOX SENSORS

The oxygen sensor controlling expression of nitrogen-fixation genes in *Rhizobium meliloti* is a membrane haemoprotein with protein kinase and phosphotransferase activity, termed FixL (Gilles-Gonzalez *et al.*, 1991). FixL catalyses its own phosphorylation, and is a protein kinase for FixJ, the oxygen response regulator. Thus certain redox sensors may also be haemoproteins, haem redox sensors in general standing in relation to haem oxygen sensors as cytochromes do to haemoglobins and myoglobins.

REDOX REGULATION AT SPECIFIC POTENTIALS

I suggest that FNR-type redox control of gene expression (Guest *et al.*, 1990; Unden *et al.*, 1990; Spiro & Guest, 1991; Guest, 1992) could be termed high-potential redox control, and the proteins termed Fe-redox repressors. Two-component redox control may operate at lower redox potentials, according to the prosthetic groups concerned. Haem, quinone, flavin, and iron-sulphur centres might each serve as the redox-active groups of different redox sensors, each responsible for maintenance of the appropriate rates of synthesis of specific complexes. The terms haem-, quinone-, flavin- and iron-sulphur-redox sensors might then be appropriate, though prefixes based on Em_7 values (e.g. high, medium and low potential redox sensors, or "x mV redox sensor") would contain more information concerning function.

A general scheme for the operation of redox sensors and redox response regulators is shown in Fig. 1.

Redox Regulation and Chloroplast and Mitochondrial Genomes

Chloroplasts and mitochondria from various eukaryotic groups differ greatly in total genome size and copy number, but show a relatively constant functional coding

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Fig. 1. Two-component redox regulation of transcription. Redox sensors are redox-active components of complexes of photosynthetic and respiratory electron transport chains, located in the bioenergetic membrane (bacterial cell or chromatophore membrane; chloroplast thylakoid; mitochondrial inner membrane). Redox sensors may respond to altered redox potential by autophosphorylation, which in turn causes phosphorylation of redox response regulators. Redox response regulators are sequence-specific DNA-binding proteins whose phosphorylation is required for binding of RNA polymerase and initiation of transcription at promoters of genes whose expression is thereby made subject to control by redox potential. Redox sensors and redox response regulators are shown as diagonally cross-hatched shapes. The bioenergetic membrane is a diagrammatic composite of respiratory and photosynthetic membranes. The redox sensor shown interacting with its response regulator is a medium potential redox sensor, associated with the cytochrome *b/c* complex. Redox sensors and response regulators are shown as separate components, but the same function may be performed by redox activators or repressors, each a single polypeptide, as in FNR (Guest *et al.*, 1990; Unden *et al.*, 1990; Spiro & Guest, 1991). Two-component systems may also have sensor and response regulator domains in a single polypeptide, for example VirA/VirG of *Agrobacterium* (Stock *et al.*, 1989). Single redox sensor-response regulator polypeptides may therefore provide plants of attachment of DNA to cell membranes, thylakoids and mitochondrial inner membranes.

(Light)　　　　(Light)　　　　Bacterial periplasmic space;
　　　　　　　　　　　　　　chloroplast thylakoid lumen;
　　　　　　　　　　　　　　mitochondrial intermembrane space

H^+

Donor

e^-　　e^-

e^-　　Q　b
　　　　　　　c
Acceptor　QH$_2$

e^-

(C)F$_1$　ADP

Redox sensor

P

Synthesis, assembly

ATP

H^+

Bacterial cell or
chromatophore
membrane;
chloroplast thylakoid;
mitochondrial inner
membrane

Bacterial cytoplasm;
chloroplast stroma;
mitochondrial matrix

ADP　ATP

Translation

P

Redox response
regulator

RNA
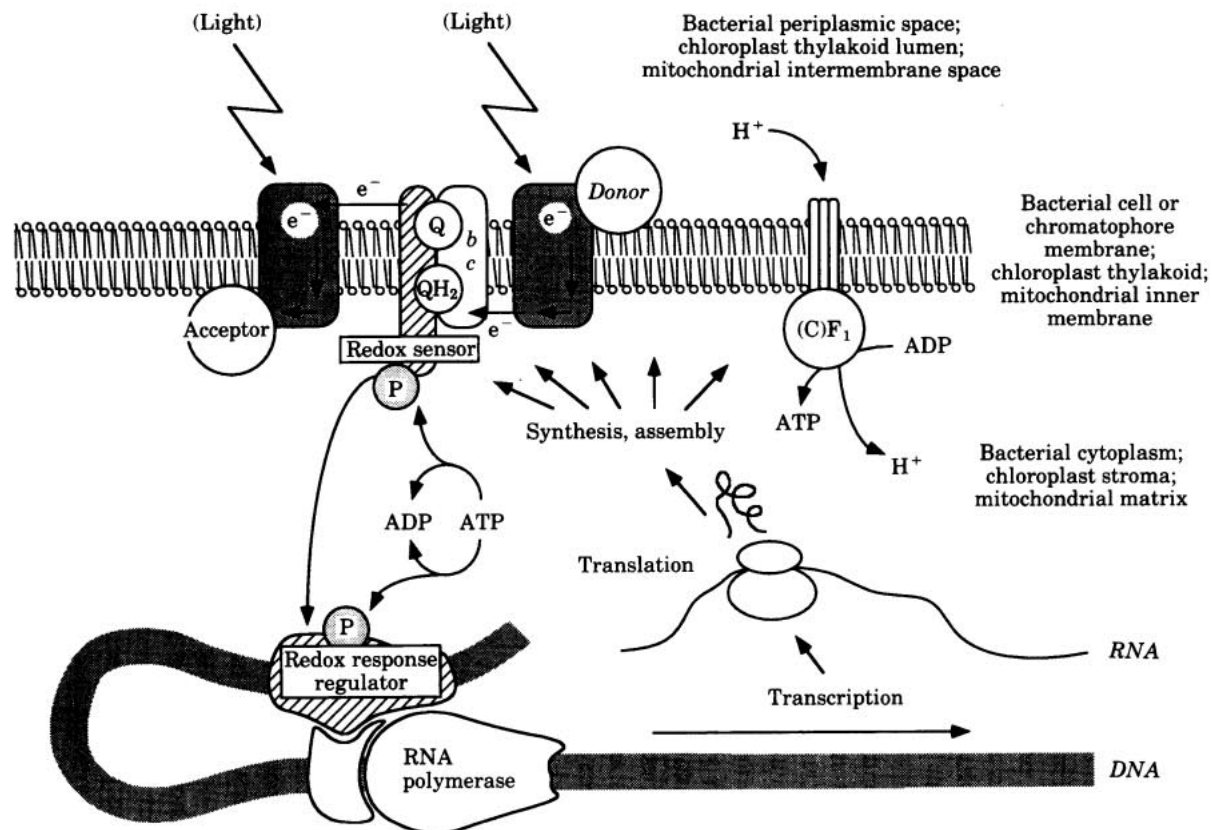polymerase

Transcription

RNA

DNA

Fig. 1.

capacity. The genes they encode comprise a small but remarkably constant subset of bioenergetic organelle proteins (Ellis, 1983; Borst *et al.*, 1984). An endosymbiotic origin for chloroplasts and mitochondria (Sagan, 1967; Whatley *et al.*, 1979; Cavalier-Smith, 1981; Margulis, 1981; Cavalier-Smith, 1987a, b) requires that the majority of symbiont genes have been transferred to the nucleus of the host (Brennicke, 1992), whence nuclear transcription and cytosolic translation of precursors for subsequent import and processing (Hartl & Neupert, 1990; Robinson, 1990; Smeekens *et al.*, 1990) provide a pathway for bioenergetic organelle biogenesis and assembly. This requirement creates two further problems, however.

The first problem can be stated as follows. Given that endosymbiont genes have been requisitioned by the nucleus, if any gene can be transferred, why not all? Restated: what do proteins encoded by bioenergetic organelle DNA have in common that confers an evolutionary advantage to location of their genes within the organelle?

The second problem is the apparent diseconomy of extranuclear genetic systems. For maintenance and expression, organelle genomes require nuclear-encoded proteins and RNAs that together use more genetic information than is contained in the organelle genomes themselves. "The reason for such a costly arrangement is not clear. We cannot think of compelling reasons why proteins made in mitochondria and chloroplasts should be made there rather than in the cytosol" (Alberts *et al.*, 1989).

Here a solution to these problems is proposed. Chloroplasts and mitochondria are primarily bioenergetic organelles, compartments in which electrochemistry (Moser *et al.*, 1992) and chemiosmotic coupling (Mitchell, 1979) are sequestered from the cytosol. Because of this function, the chloroplast thylakoid, the Rubisco catalytic subunit and the mitochondrial inner membrane are in an energetic sense the boundary of the cell with its environment—they are the cell's front line in its interaction with light, carbon dioxide and oxygen. Since balanced operation of electron transport, assimilation, and substrate oxidation must be strictly maintained in chloroplasts and mitochondria, I propose that the symbiont ancestors of chloroplasts and mitochondria carried into the host cell not only the components of photosynthesis and respiration but also the regulatory systems that place synthesis of key components under the regulatory control of redox potential.

Both bacterial and bioenergetic organelle genomes are present in multiple copies, and it is therefore reasonable to assume that individual genes may exist for some evolutionary time in both the nucleus and the proto-organelle or symbiont, perhaps in some cases diverging to give distinct isoenzymes located in specific cell compartments. Each symbiont gene's nuclear copy or copies must be retained for long enough for it to become modified by addition of sequences for targeting proteins that enable the protein precursor to return to the organelle. Prior to the addition of targeting presequences compatible with an import and processing machinery, the proto-organelle gene copies may continue to function. However, after the addition of the correct presequences to the nuclear gene copies, intracellular selection will arise for each gene concerned. In the majority of cases, expression of the nuclear gene copy is sufficient, and the organelle copy becomes redundant and is lost. In a majority of cases, however, some distinctive requirement cannot be satisfied by the nuclear copy,

even granted a targeting, import and processing machinery that gives a mature protein identical to that synthesized in the organelle. I propose control by redox sensors and redox response regulators as the distinctive requirement favouring survival of the organelle-encoded gene copy, as depicted in Fig. 2. Redox repressors resembling FNR may also provide the necessary redox control.

Redox control of gene expression may be indispensable for certain genes of chloroplasts and mitochondria for at least two reasons, as follows.

(i) The signalling pathway is as short and simple as possible. Chloroplasts and mitochondria contain the sites at which light, oxygen and carbon dioxide are utilized. Some of these sites of utilization are subject to abrupt changes in redox potential when the physical environment changes. Continued synthesis of inappropriate components in new environments is disadvantageous, as is failure to synthesize components whose operation will restore redox poise.

(ii) No host cell redox sensor could be coupled as closely to electron transport as are those already operating within the organelle. As components of the electron transport chains whose composition they regulate, prokaryotic redox sensors should be expected to interact in a precise way with neighbouring membrane proteins. Only in unchanging redox environments would selective pressure be removed for long enough for new redox regulatory mechanisms to be brought into place, and, by definition, in such environments there was no selection pressure to maintain them.

I therefore propose that the common feature of organelle-encoded proteins is that they directly determine redox poise, and that they function safely together only within ranges of redox potential that are liable to be exceeded by environmental change. Since the complete nucleotide sequences of some chloroplast (Ohyama *et al.*, 1986; Shinozaki *et al.*, 1986; Hirasuka *et al.*, 1989) and mitochondrial (Anderson *et al.*, 1981; Oda *et al.*, 1992) genomes are known, it is possible to compare this prediction with reality. Major chloroplast-encoded proteins are listed in Table 1. Major mitochondrially encoded proteins are listed in Table 2.

An alternative proposal for proteins encoded within the mitochondrion is that their hydrophobic domains prohibit their import, as precursors, by cytosolic processing and targeting mechanisms (von Heijne, 1986). In the case of mitochondria, one could imagine instead that the requirement for redox control of synthesis, here proposed, is the primary reason for the retention of the mitochondrial genome, and that the amino-terminal hydrophobic domains of mitochondrially encoded proteins merely reflect their roles as intrinsic proteins of the mitochondrial inner membrane. In addition, hydrophobicity does not explain the contents of the chloroplast genome, since there are numerous counterexamples: the Rubisco large subunit is chloroplast-encoded but is not a membrane protein (Ellis, 1981), while polypeptides of the light-harvesting chlorophyll-binding complexes (Green *et al.*, 1991) are intrinsic thylakoid proteins which are nuclear-encoded. Furthermore, the problem of susceptibility to arrest *en route* through a membrane boundary has evidently been solved for precursors of plastocyanin and polypeptides of the oxygen-evolving complex of photosystem II, since both are nuclear-encoded and function in the thylakoid lumen (Smeekens *et al.*, 1990). It is difficult to see why this solution

should not also have been available to proteins of the mitochondrial inner membrane.

The question of the nuclear location of some but not all genes for chloroplast and mitochondrial components has also been considered independently of the endosymbiont theory. Bogorad (1975) made several suggestions, including the idea, elaborated for mitochondria by von Heijne (1986), that a component may be encoded and synthesized within the organelle because it is untransportable. Another proposal (Bogorad, 1975) is that organelle-encoded polypeptides may be necessary to lock into the organelle the nuclear-encoded polypeptides with which they interact. Subsequent work indicates that protein import into chloroplasts and mitochondria is likely to have evolved from protein export or secretion pathways (Hartl & Neupert, 1990; Robinson, 1990), and organelle protein import is now generally considered to be irreversible (see, for example, Ellis, 1983). Thus the need for "lock-in" devices (Bogorad, 1975) is no longer a significant problem.

Redox Roles and Redox Sensitivity of Chloroplast and Mitochondrially Encoded Proteins

Apart from certain components of organelle genetic systems themselves, the major gene products of chloroplasts (Ohyama *et al.*, 1986; Shinozaki *et al.*, 1986; Hiratsuka *et al.*, 1989) (Table 1) and mitochondria (Anderson *et al.*, 1981; Oda *et al.*, 1992) (Table 2) have functions which directly link the physical environment to electron transport and redox poise. I suggest that the distinguishing feature of these components is that they connect their electron transport chains with substrates which are sources and sinks for energy and electrons—they are in the bioenergetic "front line". Organelle-encoded proteins are located at the bioenergetic or assimilatory interface

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FIG. 2. The proposed sequence of events leading to distribution of copies of the symbiont's genes between the nucleus of the host cell and the cytoplasmic organelle to which the symbiont gives rise. (a) Genes *A*, *B* and *C* are located only in the genome of the symbiont. They are transcribed and translated into proteins A, B and C entirely within the symbiont. Transcription of gene *A* is regulated by redox potential by means of its redox sensor, which is in intimate contact with protein A in the bioenergetic membrane. The redox response regulator binds to the symbiont genome upstream of the gene *A* promoter. (See Fig. 1.) (b) Genes *A*, *B* and *C* are located in both organelle and nuclear genomes. Any and all genes from the symbiont genome (*A*, *B* and *C*) are copied to the nucleus of the host cell, and there targeting sequences are added which enable the protein to return, as a precursor, to the symbiont, now functionally a cytoplasmic organelle of the host cell. Hence any organelle protein may be synthesized both *in situ* and, in precursor form, in the cytosol of the host. (c) Gene *A* is confined to the organelle genome; genes *B* and *C* are confined to the nuclear genome. For the majority of organelle-encoded proteins (represented by B and C), nuclear encoding, cytosolic synthesis and import and processing of precursors together provide an effective alternative to synthesis *in situ*, and the organelle copies of these genes are lost. However, transcription of a minority of organelle genes (represented by *A*) must be closely coupled to redox potential at specific sites in the bioenergetic membrane. For these genes, the nuclear gene copy continues to be expressed in inappropriate environments, and the nuclear gene product continues to be synthesized even when its function is damaging to the cell. The organelle gene copy remains under redox control, and has a selective advantage. The nuclear gene copy is therefore lost, and a stable devision of genetic responsibility is reached in which the organelle retains a genome encoding those of its proteins whose synthesis must respond rapidly to alteration in the physical environment of the cell.
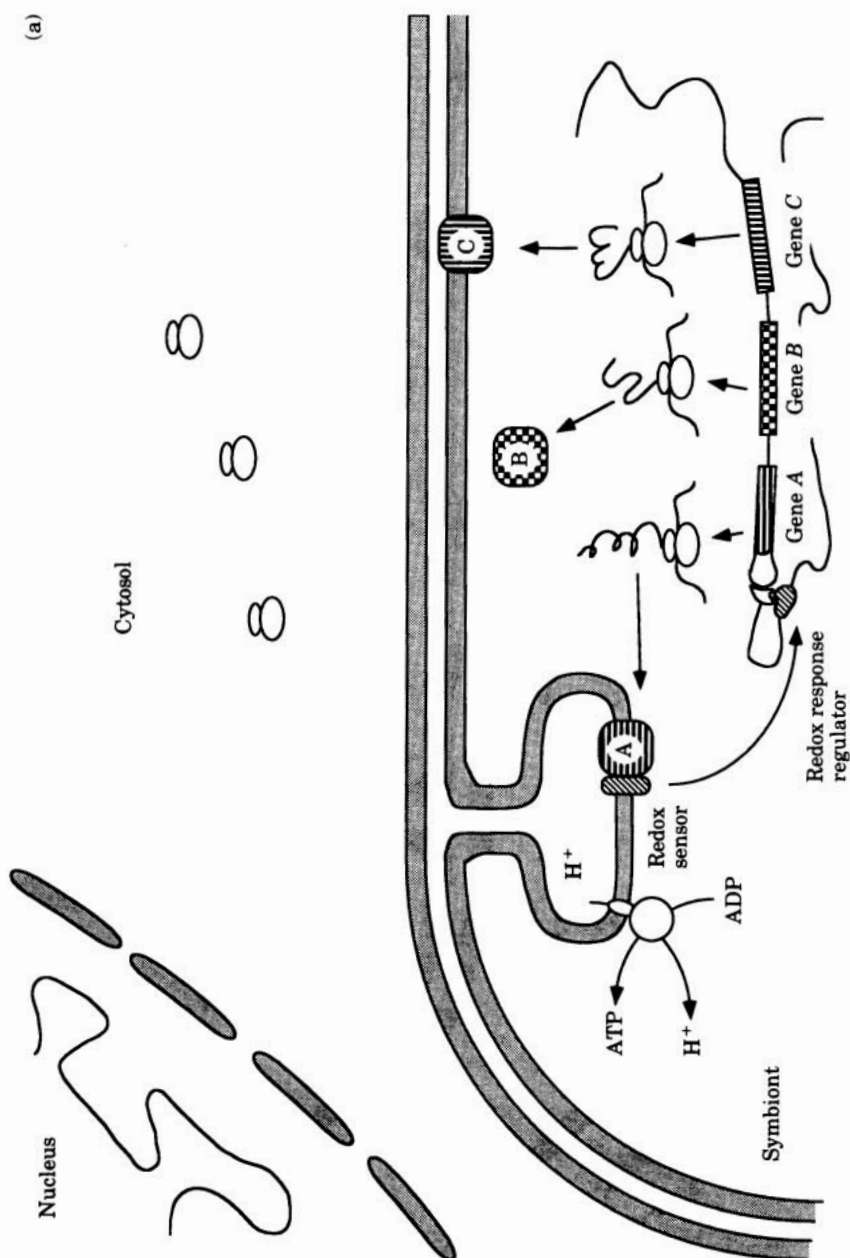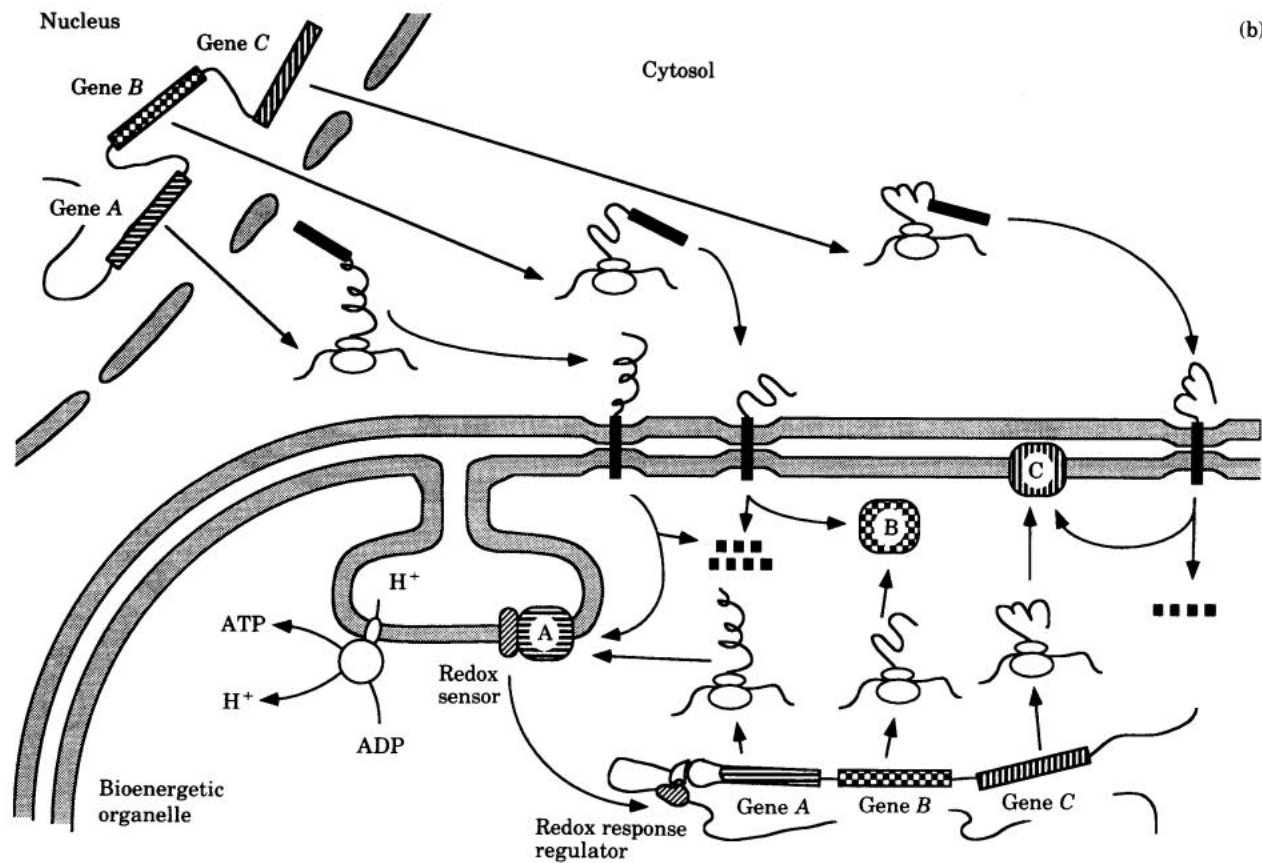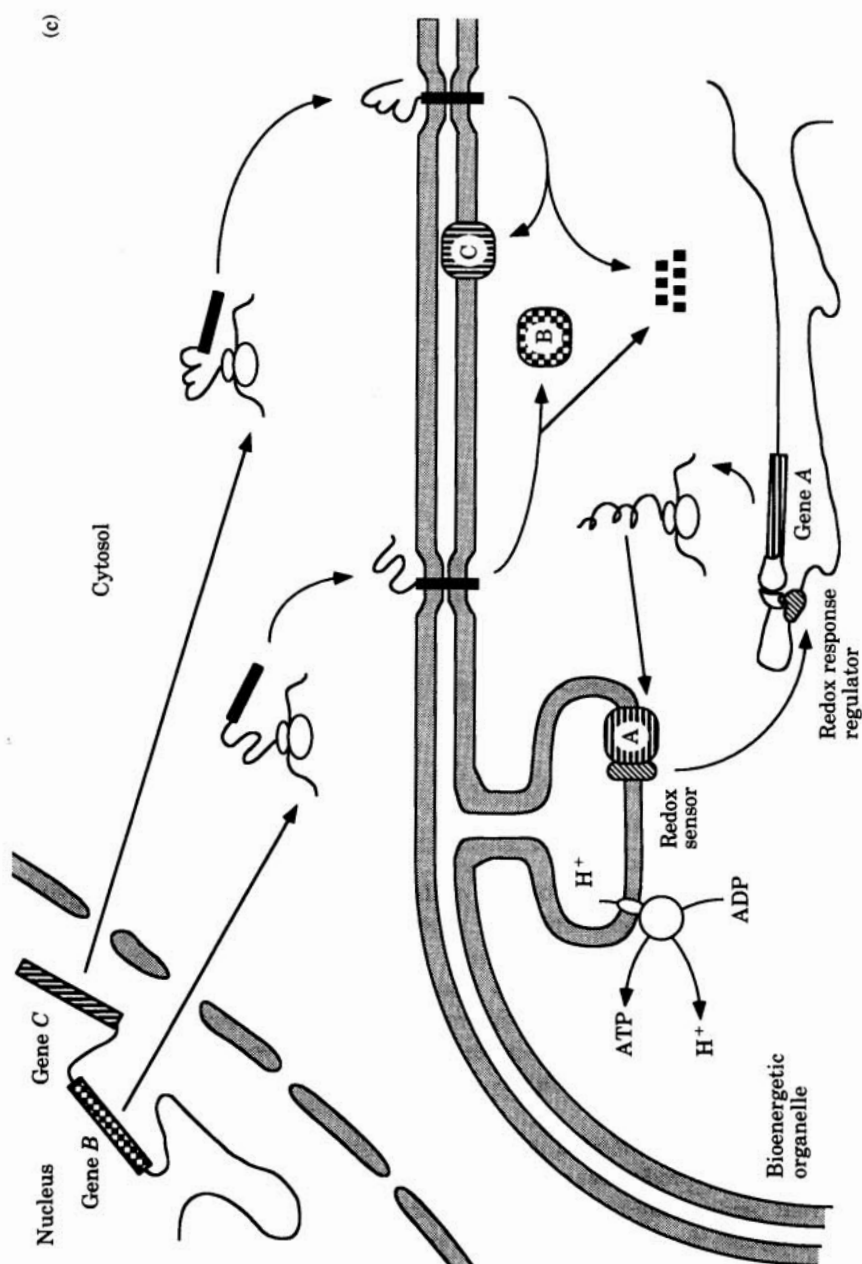
FIG. 2.

FIG. 2—continued.

FIG. 2 —continued.

TABLE 1

Major chloroplast genes and gene products. (Ohyama et al., 1986; Shinozaki et al., 1986; Hiratsuka et al., 1989) and their functions. Em_7 values are taken from Cramer & Knaff (1990)

Gene	Protein	Standard redox potential Em_7 (mV)	Substrate
	Photosystem II		
psbA	D1 of PS II reaction centre	$+1{,}170$ (P$^+$/P) -800 (P/P*)	Light
psbD	D2 of PS II reaction centre	$+1{,}170$ (P$^+$/P) -800 (P/P*)	Light
psbB, psbC	CP47 and CP43 of PS II	—	Light
	Energy coupling		
petA	Cytochrome f	$+350$	Pmf (ΔpH)
petB	Cytochrome b_6	-50	Pmf (ΔpH)
atpA, B, E	ATP synthase CF$_1\alpha$, β, ε		ΔpH, ATP
atpF, H, I	ATP synthase CF$_0$I, III, IV	—	ΔpH, ATP
	Photosystem I		
psaA	A1 of PS I reaction centre	$-1{,}250$ (P/P*) $+500$ (P$^+$/P)	Light
psaB	A2 of PS I reaction centre	$-1{,}250$ (P/P*) $+500$ (P$^+$/P)	Light
	Respiration: cyclic electron transport		
ndhA-F	NADH dehydrogenase subunits 1–5	-324	Respiratory substrates?
	CO$_2$ fixation		
rbcL	Rubisco large subunit	—	Carbon dioxide

of the cell; they are the first components of the cell to experience a changing light or redox environment, and their functions become rate-limiting or destructive if their synthesis cannot then respond. In contrast, nuclear-encoded components of chloroplast and mitochondrial genomes do not take such key roles, even where they function in light-harvesting or electron transport. I propose further that the minor variations in the location of genes between different eukaryotic groups depend upon whether their products play such a key role in the normal redox environment of the cell.

In general, organelle-encoded proteins function at primary points of energy transduction between redox reactions and other physical factors. These may include light, external oxidants and reductants, the proton motive force, and assimilation of CO_2. In particular, both respiratory and photosynthetic electron transport chains contain organelle-encoded proteins at their extremes of Em (Tables 1 and 2).

The requirement for an irreducible core of components subject to redox control may disappear when the organelle ceases to perform its primary bioenergetic role.

TABLE 2

*Major mitochondrial genes and gene products. (Anderson et al., 1981; Oda et al., 1992)
and their functions. Em_7 values are taken from Cramer & Knaff (1990).*

Gene	Protein	Standard redox potential Em_7 (mV)	Substrate
	Electron transport		
nad1–7	Seven subunits of NADH dehydrogenase	−324	Respiratory substrates
cox1–3	Cytochrome c oxidase subunits I, II and III	+815	Oxygen
	Energy coupling		
atp6	ATP synthase F_1 subunit 6	—	Proton motive force, ATP
cob	Cytochrome b	−50	Proton motive force

Examples of this may be the chloroplast genomes of parasitic plants (Morden *et al.*, 1991) and heterotrophic eukaryotes dependent exclusively on fermentation (Vossbrinck *et al.*, 1987). It is also possible to imagine that organelles such as glyoxysomes and peroxisomes have an endosymbiotic origin (Cavalier-Smith, 1987b) but have completely relinquished genetic control to the nucleus in the absence of a requirement for redox control of gene expression.

CHLOROPLASTS

The reaction centre core proteins of both photosystem I and photosystem II are chloroplast-encoded (Table 1). These are the primary generators of high and low redox potentials. Photosystem II is highly susceptible to light-induced damage—"photoinhibition"—which occurs when electron transport is restricted on either acceptor or donor sides, or both (Barber & Andersson, 1992; Prásil *et al.*, 1992). Photoinhibition of photosystem II results from utilization of inappropriate acceptors, notably oxygen (Richter *et al.*, 1990), when the acceptor pool becomes reduced (Richter *et al.*, 1990; Barber & Andersson, 1992; Prásil *et al.*, 1992), and of inappropriate donors, especially chlorophyll, when electron donation is impaired (Callahan *et al.*, 1986; Theg *et al.*, 1986; Prásil *et al.*, 1992). In the latter case the high redox potentials generated by P680/P680$^+$ ($Em_7 = +1170$ mV) and required for water oxidation ($Em_7 = +815$ mV) are necessary for non-cyclic electron transport and autotrophic growth, but difficult to contain if electron donation from water is impaired, which may itself be a consequence of damage induced on the acceptor side. Breakdown of the D1 protein of the photosystem II reaction centre occurs during photoinhibition (Kyle *et al.*, 1984; Barber & Andersson, 1992; Prásil *et al.*, 1992). D1, like the L subunit of the purple bacteria reaction centre (Deisenhofer *et al.*, 1985), is involved in binding and stabilization of both secondary acceptors and donors

(Mattoo *et al.*, 1989; Erickson & Rochaix, 1992). D1 is therefore a particularly vulnerable component of the chloroplast. Changes in light intensity in relation to the total capacity of the cell for utilization of the products of photoelectrochemistry can be amplified in a destructive cycle of positive feedback: damage to D1 induces changes of redox potential, themselves causing damage to D1.

The *psbA* genes encoding D1 (Rutherford, 1989) are therefore obvious candidates for redox control of gene expression. Multiple redox regulatory components might be involved, with an FNR-like high potential redox repressor monitoring the redox potential of the donor side of PS II, and a medium-potential (quinone?) redox sensor on the acceptor side. Photoinhibitory damage to D1 is repaired by means of a costly cycle of breakdown and resynthesis (Prásil *et al.*, 1992), in which the entire genetic system of the mature chloroplast appears to function largely to provide for resynthesis of this single polypeptide. D1 breakdown and photoinhibition occur in cyanobacteria (Vonshak *et al.*, 1988). D1 therefore seems an excellent example of a protein whose gene must throughout evolution have been continually subject to on-site redox control. The loss of *psbA* to the nucleus would greatly retard and weaken the coupling of its expression to transient changes in light intensity and redox potential.

Similar considerations apply to photosystem I, which generates extremely low redox potentials (for P700/P700* $Em_7 = -1250$ mV). The chloroplast-encoded reaction centre polypeptides of PS I (Scheller & Møller, 1990) might therefore be subject to low-potential redox control, perhaps by Fe-S sensors and specific response regulators operating at the promoter region of *psaA* and *psaB* genes. However, both photosystem I and chloroplast-encoded components of the intermediary electron transport chain such as cyt f and cyt b_6 are connected in series with photosystem II, and they therefore play a role in determining the redox potential of photosystem II, as may the CF_1-CF_0 coupling ATP synthase, indirectly through photosynthetic control (West & Wiskich, 1968). It is possible that the chloroplast genome retains an integrated system of redox control of gene expression, with different redox sensors operating at distinct potentials to control redox response regulators of genes encoding proteins which function at other potentials, and remote from at least some of the sensors governing their synthesis.

Chloroplast genomes also encode NADH dehydrogenases (Ohyama *et al.*, 1986; Shinozaki *et al.*, 1986; Hiratsuka *et al.*, 1989). These may function at early stages of chloroplast development, to provide respiratory generation of a proton motive force for ATP synthesis, protein synthesis, and protein import. *ndh* genes are also expressed in mature chloroplasts (Matsubayashi *et al.*, 1987), where they may function in chlororespiration (Bennoun, 1982) or as links between the acceptor site of photosystem I and the cyt b_6/f complex in proton-motive cyclic electron transport which may provide ATP for CO_2 fixation and other assimilatory reactions. The importance of redox poise for cyclic photophosphorylation has long been recognized (Whatley, 1963; Grant & Whatley, 1967), and can be demonstrated by addition of catalytic concentrations of oxidant or reductant to overcome inhibition by over-reduction or overoxidation in chloroplasts (Whatley, 1963; Grant & Whatley, 1967; Arnon & Chain, 1975; Ziem-Hanck & Heber, 1980; Allen, 1983; Heber & Walker, 1992) and photosynthetic bacteria (McEwan *et al.*, 1985). Redox control of

expression of chloroplast *ndh* genes may therefore be indispensable for maintaining photophosphorylation. The location of *ndh* genes in the chloroplast for *in situ* redox control of synthesis of NADH dehydrogenases may thus be a further example of the advantage of a genetic system sequestered in order to provide the shortest possible loop of feedback control. In *E. coli*, transcription of *ndh* genes is under the control of the high-potential redox regulator, FNR (Spiro *et al.*, 1989).

The chloroplast-encoded Rubisco large subunit does not itself participate in electron transfer. However, the primary carboxylation step of CO_2 assimilation may, under a variety of circumstances, become the limiting factor for utilization of electrons from the photosynthetic chain by triose phosphate dehydrogenase (Walker *et al.*, 1986). The activity of the Rubisco large subunit may therefore have immediate effects on the redox potential of components of the photosynthetic electron transport chain, including, for example, D1. It is therefore possible that the Rubisco large subunit is retained as a chloroplast-encoded protein in order that its synthesis can be under redox control. The nuclear-encoded Rubisco small subunit does not have a direct catalytic role, and its synthesis may therefore have no direct requirement for redox control. The hypothesis of redox control of gene expression is therefore one in which the division of function between the Rubisco large and small subunits may be consistent with their different sites of synthesis.

MITOCHONDRIA

Mitochondrial genomes (Anderson *et al.*, 1981; Oda *et al.*, 1992) encode components operating at each end of the respiratory electron transport chain, namely subunits of NADH dehydrogenase and cytochrome *c* oxidase (Table 2). Whereas redox control in chloroplasts may be required primarily to balance both light energy conversion between the photosystems and electron transport with assimilatory substrate, oxygen concentration is the environmental factor most obviously requiring control of gene expression in mitochondria.

Transition between anoxyia and aerobic metabolism is a normal course of events in all aerobic chemotrophs. Even in multicellular organisms, intracellular oxygen concentration can vary over a wide range, as physiological factors maintaining oxygen supply reach the limit of their range of control, as seen in the transition from oxidative phosphorylation to lactate fermentation in skeletal muscle. Cytochrome *c* oxidase is unnecessary under anaerobic conditions, so there is an advantage in anaerobic repression of its genes. However, on introduction of oxygen, there is a large energetic gain in switching to aerobic respiration, so a rapid response will confer selective advantage. In addition to energetic considerations, in the absence of a terminal oxidase capable of complete reduction of oxygen to water, oxygen may be reduced univalently to the superoxide anion radical by a wide variety of mitochondrial components, including iron sulphur centres, flavins, and semiquinones (Flohe *et al.*, 1977). The Em_7 of the O_2/O_2^- couple is $-330\,mV$, which means that superoxide production is thermodynamically favourable at the normal operating potential of NADH dehydrogenase ($Em_7 = -324\,mV$). Although superoxide may not itself be the primary reactant, it is now generally agreed that its production has a wide variety

of cytotoxic effects (Michelson *et al.*, 1977). Similar considerations apply to the photosynthetic chain, where univalent oxygen reduction can proceed at high rates in the presence of both artificial (Allen, 1977) and native (Allen, 1975, 1977) cofactors, and in both photosystems I and II (Allen, 1977).

The electrochemistry of cytochrome c oxidase requires a specific protein environment for safe four-electron reduction of oxygen to water (Babcock & Wikström, 1992). The composition of cytochrome c oxidase may therefore be redox-regulated in response to changes in oxygen concentration and in the redox potential generated by respiratory substrates. As in the photosynthetic chain, one might expect high-potential redox sensors at the level of cytochrome oxidase, and low-potential sensors at NADH dehydrogenase, each exerting redox control over response regulators which control synthesis of components of a number of respiratory complexes. The mitochondrially encoded cytochrome b and subunits of the F_1-F_0 ATP synthase may be subject to redox-regulated synthesis in response to respiratory control (Chance & Williams, 1955), though pmf sensors and response regulators are clearly an additional possibility.

Redox Sensors and Response Regulators may themselves be Encoded in the Nucleus

During the period of transition between endosymbiont and bioenergetic organelle (Fig. 2), the genes encoding the sensors and response regulators themselves need not have been locked in the organelle, since there is no obvious obstacle to their expression in the nucleus for cytosolic translation and uptake, in precursor form, by the organelle. Indeed, it is possible to imagine that a nuclear location for the redox regulatory genes conferred selective advantage, since nuclear-encoded redox sensors and response regulators might then be made subject to additional control (however, see Cavalier-Smith, 1987*b*). Nuclear transcription factors could determine organelle composition in response to cytosolic or extracellular signals. In multicellular organisms, positional information may determine organelle composition in pathways of cell differentiation.

It follows that the elements of redox control of organelle gene expression will themselves be nuclear-encoded. Though of prokaryotic origin, and related to existing bacterial two-component and other redox regulatory systems, the components regulating organelle gene expression and providing a necessary condition for organelle genomes may themselves be encoded in the nucleus, synthesized in the cytosol, and imported into chloroplasts and mitochondria by the same mechanisms which operate for the majority of organelle components.

Predictions

BACTERIAL REDOX SENSORS AND REDOX RESPONSE REGULATORS

Apart from those already known and described in section 2, further redox sensors may be found whose redox properties and prosthetic groups permit their operation at specific points in electron transport chains. Potentiometric redox titration of

expression of reporter genes (Unden *et al.*, 1990) may reveal structural genes under control of redox sensors at different potentials. The high-potential redox control factor FNR probably functions as both redox sensor and redox response regulator (Spiro *et al.*, 1989; Guest *et al.*, 1990; Unden *et al.*, 1990; Spiro & Guest, 1991). This may turn out to be typical of soluble control factors, or those operating at high potential. Although the arguments advanced here primarily concern transcriptional control of gene expression, redox control of translation or of messenger processing and stability may also be important.

In cyanobacteria, studies using electron donors, acceptors and inhibitors have demonstrated redox control of phosphorylation of a number of polypeptides (Harrison *et al.*, 1991), and these are candidates for redox sensors. Redox control of protein phosphorylation is also a property of a range of polypeptides in purple non-sulphur bacteria (Holmes & Allen, 1988).

CHLOROPLAST REDOX SENSORS AND REDOX RESPONSE REGULATORS

Redox control of response at the post-translational level is well established for chloroplasts (Allen, 1992*a, b*). The best-characterized substrate is the nuclear-encoded light-harvesting complex, LHC II (Allen, 1992*a*). Phosphorylation of LHC II is regulated by a protein kinase whose activity has an Em_7 of approximately $+80$ mV, becoming activated as plastoquinone is reduced (Allen *et al.*, 1981). Upon phosphorylation, a proportion of LHC II moves from photosystem II to photo-system I, thereby correcting the inequality of excitation energy distribution that caused plastoquinone to become reduced (Allen, 1992*b*). A number of other chloro-plast polypeptides are phosphorylated by a redox-controlled kinase or kinases (Allen, 1992*b*). The redox-sensitive LHC II kinase is a nuclear-encoded protein of 64 kDa in the mature form (Gal *et al.*, 1990, 1992). It is autophosphorylated in a reaction that is also under redox control. A 47 amino acid segment of the LHC II kinase precursor (Hind, G., personal communication) shows 28% sequence identity with the EnvZ osmotic pressure sensor of *E. coli* (Comeau *et al.*, 1985) and *Salmonella typhimurium* (Liljeström *et al.*, 1988) (Allen, J. F., unpublished), suggesting a relation with bacterial two-component sensors. It is therefore possible that the LHC II kinase functions to control chloroplast gene expression in addition to its regulation of phosphorylation of LHC II. It has been suggested that plastoquinone-redox control of chloroplast *psa* and *psb* gene expression serves to control photo-system stoichiometry, acting to maintain balanced utilization of light energy by photosystem I and photosystem II (Allen, 1992*b*). Parallel control of post-transla-tional modification and of transcription is a feature of the Ntr system which acts on glutamate synthetase to provide a nitrogen regulatory mechanism in *E. coli* (Ninfa & Magasanik, 1986; Ronson *et al.*, 1987; Magasanik, 1989; Saier *et al.*, 1990; Stock *et al.*, 1990). The LHC II kinase is therefore a candidate for a medium-potential ($Em_7 = +80$ mV), quinone redox sensor.

The chloroplast genome has promoter regions with similarities to those encoun-tered in Gram-negative bacteria, including cyanobacteria (Bülow & Link, 1988; Gruissem *et al.*, 1988; Bogorad, 1991), indicating the possibility of control of initia-

tion of transcription by regulator proteins of bacterial type (Bülow & Link, 1988), though these are likely to be predominantly nuclear-encoded (Rochaix, 1987). In particular, "−10" and "−35" sequences resembling *E. coli* regulatory sequences are found in the chloroplast genome, upstream of the presumed sites of initiation of transcription of *psbA*, *atpB*, *atpE* and *rbcL* (Table 1) (Ohyama *et al.*, 1986; Hiratsuka *et al.*, 1989).

MITOCHONDRIAL REDOX SENSORS AND REDOX RESPONSE REGULATORS

Redox control is one of a number of factors controlling phosphorylation of mitochondrial pyruvate dehydrogenase (Kerbey *et al.*, 1979). In plant mitochondria, protein phosphorylation has been demonstrated for a number of substrates, but their identities and the factors controlling phosphorylation are unresolved (Sommarin *et al.*, 1990). As in the case of chloroplasts, factors implicated in control of mitochondrial transcription are themselves nuclear-encoded (Rochaix, 1987; Melis, 1990).

GENERAL PROPERTIES OF REDOX CONTROL OF GENE EXPRESSION

In laboratory growth conditions, mutations affecting redox sensors and response regulators might be difficult to locate unless a suitable screening technique can be devised. Inability to control gene expression in response to redox potential should be expected to reduce the efficiency of energy conversion, and impair quantum yield in photosynthetic organisms. Mutants deficient in light-harvesting complexes show an inability to control photosystem stoichiometry in both eukaryotes and cyanobacteria (Melis, 1990), suggesting that regulation of gene expression may be affected by redox poise (Fujita *et al.*, 1987; Allen, 1992b).

The most promising route for identification of redox sensors and response regulators is probably through mutagenesis and screening. In the absence of a direct means of screening for adaptation-deficient mutants, use of reporter genes is most likely to yield evidence for redox response regulators operating on promoter regions of key structural genes. Many redox sensors may already be known: in chloroplasts and cyanobacteria, many membrane proteins are phosphorylated and redox-active, but have no obvious role in electron transport (Allen & Harrison, 1990; Allen, 1992b). Indeed, the ideal redox sensor might have no direct role in linear electron flow, existing instead as a side-arm of the chain whose sensing function need not then compete with the structural requirements of optimized electron transport. It should also be noted that the Em of a redox sensor may be quite different from that of certain of the proteins whose synthesis it controls, as seen in FNR [$Em_7 = +400$ mV (Unden *et al.*, 1990)] control of transcription of *ndh* genes (Spiro *et al.*, 1989) encoding NADH dehydrogenase ($Em_7 = -324$ mV).

As regards the proposed function of chloroplast and mitochondrial genomes, the hypothesis put forward here predicts the existence of a range of redox-active sensors and DNA-binding redox response regulators, all encoded in the nucleus. These should be identifiable by screening nuclear genomic libraries with probes constructed from consensus sequences of bacterial redox regulatory components. Inactivation

and complementation of such nuclear genes by means of directed mutagenesis or antisense RNA would provide a means of testing their predicted roles in redox control of gene expression and in maintenance of redox poise.

IMPLICATIONS FOR EVOLUTION AND GENE TRANSFER WITHIN EUKARYOTIC CELLS

A requirement for redox control of gene expression is proposed here as an explanation of the retention of chloroplast and mitochondrial genomes in evolution. However, this requirement is not one confined to a hypothetical environment in the remote past, but is likely still to operate today. The hypothesis therefore provides a plausible selective pressure serving to eliminate nuclear-encoded copies of genes for whose expression redox control is advantageous to the whole organism, and favouring the survival of the corresponding organelle-encoded copies. If this hypothesis is correct, it removes the need to suppose that the current distribution of genes within the three genetic compartments of the eukaryotic cell is a "frozen accident", or relic of a chance pattern of distribution that occurred in a common ancestor of all eukaryotes. It also provides an alternative to the view that gene transfer between compartments is so infrequent that the loss of chloroplast and mitochondrial genes to the nucleus is still underway, but so slow as to be yet incomplete.

Thus a further, general implication of the proposal put forward here is that the copying of genes in any direction between chloroplasts, mitochondria and nucleus may be a relatively frequent event (Brennicke, 1992), with the constancy of the observed distribution of genes being merely a result of the action of natural selection in maintaining a favoured equilibrium. The detailed distribution of individual genes in different eukaryotic groups may differ slightly according to other metabolic and physiological properties which may themselves affect the equilibrium position. For example, it should be possible to provide a functional explanation for the absence of NADH dehydrogenase genes from yeast mitochondria, and for differences in site of synthesis of ATP synthase subunits between yeast and *Neurospora* (Darnell *et al.*, 1990). It should also be possible to predict the contents of the chloroplast and mitochondrial genomes of metabolically specialized organisms such as parasites (Morden *et al.*, 1991). This general, evolutionary implication could also be testable by determining the direction of redistribution of genes in eukaryotes that can be grown for a large enough number of generations in environments with specified redox potentials.

In summary, the hypothesis of redox sensors and response regulators in bacterial electron transport and in function and maintenance of organelle genomes appears to be consistent with current knowledge on transcriptional control mechanisms, and to make sense of the otherwise puzzling distribution of genes between the eukaryotic nucleus and bioenergetic organelles. The predictions of this hypothesis extend into a number of specialist fields, and suggest many experiments by which it may be tested. It is hoped that the hypothesis will act as a useful stimulus to research.

REFERENCES

ALBERTS, B., BRAY, D., LEWIS, J., RAFF, M., ROBERTS, K. & WATSON, J. D. (1989). *Molecular Biology of the Cell*, 2nd Edn. New York: Garland Publishing.

ALLEN, J. F. (1975). A two-step mechanism for the photosynthetic reduction of oxygen by ferredoxin. *Biochem. Biophys. Res. Commun.* **66**, 36–43.

ALLEN, J. F. (1977). Superoxide and photosynthetic reduction of oxygen. *Superoxide and Superoxide Dismutases* (Michelson, A. M., McCord, J. M. & Fridovich, I., eds) pp. 417–436. New York: Academic Press.

ALLEN, J. F. (1983). Regulation of photosynthetic phosphorylation. *Crit. Rev. Plant Sci.* **1**, 1–22.

ALLEN, J. F. (1992a). How does protein phosphorylation regulate photosynthesis? *Trends Biochem. Sci.* **17**, 12–17.

ALLEN, J. F. (1992b). Protein phosphorylation in regulation of photosynthesis. *Biochim. Biophys. Acta.* **1098**, 275–335.

ALLEN, J. F., BENNETT, J., STEINBACK, K. E. & ARNTZEN, C. J. (1981). Chloroplast protein phosphorylation couples plastoquinone redox state to distribution of excitation energy between photosystems. *Nature, Lond.* **291**, 25–29.

ALLEN, J. F. & HARRISON, M. A. (1990). Phosphorylation of membrane proteins in control of excitation energy transfer. *Molecular Biology of Membrane-Bound Complexes in Phototrophic Bacteria* (Drews, G. & Dawes, E. A., eds) pp. 291–298. New York: Plenum Press.

ANDERSON, S., BANKIER, A. T., BARRELL, B. G., DE BRUIJN, M. H. L., COULSON, A. R., DROUIN, J., EPERON, I. C., NIERLICH, D. P., ROE, B. A., SANGER, F., SCHREIER, P. H., SMITH, A. J. H., STADEN, R. & YOUNG, I. G. (1981). Sequence and organization of the human mitochondrial genome. *Nature, Lond.* **290**, 457–465.

AOYAMA, T. & ATSUHIRO, O. (1990). A common mechanism of transcriptional activation by the three positive regulators, VirG, PhoB and OmpR. *FEBS Lett.* **263**, 1–4.

ARNON, D. I. & CHAIN, R. K. (1975). Regulation of ferredoxin-catalyzed photosynthetic phosphorylations. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4961–4965.

BABCOCK, G. T. & WIKSTRÖM, M. (1992). Oxygen activation and the conservation of energy in cell respiration. *Nature, Lond.* **356**, 301–308.

BARBER, J. & ANDERSSON, B. (1992). Too much of a good thing: light can be bad for photosynthesis. *Trends Biochem. Sci.* **17**, 61–66.

BENNOUN, P. (1982). Evidence for a respiratory chain in the chloroplast. *Proc. natn. Acad. Sci. U.S.A.* **79**, 4352–4356.

BOGORAD, L. (1975). Evolution of organelles and eukaryotic genomes. *Science* **188**, 891–898.

BOGORAD, L. (1991). Replication and transcription of plastid DNA. *Cell Culture and Somatic Cell Genetics of Plants* (Bogorad, L. & Vasil, I. K., eds) pp. 93–124. New York: Academic Press.

BORST, P., GRIVELL, L. A. & GROOT, G. S. P. (1984). Organelle DNA. *Trends Biochem. Sci.* **9**, 128–130.

BRENNICKE, A. (1992). Gene translocation between organelles. *Curr. Biol.* **2**, 46–47.

BÜLOW, S. & LINK, G. (1988). Sigma-like activity from mustard (*Sinapis alba* L.) chloroplasts conferring DNA-binding and transcription specificity to *E. coli* core RNA polymerase. *Plant molec. Biol.* **10**, 349–357.

CALLAHAN, F. E., BECKER, D. W. & CHENIAE, G. M. (1986). Studies on the photoactivation of the water-oxidizing enzyme. *Plant. Physiol.* **82**, 261–269.

CAVALIER-SMITH, T. (1981). The origin and early evolution of the eukaryotic cell. *Symp. Soc. Gen. Microbiol.* **32**, 33–84.

CAVALIER-SMITH, T. (1987a). The origin of eukaryote and archaebacterial cells. *Ann. New York Acad. Sci.* **503**, 17–54.

CAVALIER-SMITH, T. (1987b). The simultaneous symbiotic origin of mitochondria, chloroplasts, and microbodies. *Ann. N.Y. Acad. Sci.* **503**, 55–71.

CHANCE, B. & WILLIAMS, G. R. (1955). Respiratory enzymes in oxidative phosphorylation: III. The steady state. *J. Biol. Chem.* **217**, 409–427.

COMEAU, D. E., IKENAKA, K., TSUNG, K. & INOUYE, M. (1985). Primary characterization of the protein products of the *Escherichia coli ompB* locus: structure and regulation of synthesis of the OmpR and EnvZ proteins. *J. Bacteriol.* **164**, 578–584.

CRAMER, W. A. & KNAFF, D. B. (1990). *Energy Transduction in Biological Membranes. A Textbook of Bioenergetics.* New York: Springer-Verlag.

DARNELL, J., LODISH, H. & BALTIMORE, D. (1990). *Molecular Cell Biology*, 2nd Edn. New York: Scientific American Books.

DEISENHOFER, J., EPP, O., MIKI, K., HUBER, R. & MICHEL, H. (1985). Structure of the protein subunits in

the photosynthetic reaction centre of *Rhodopseudomonas viridis* at 3 Å resolution. *Nature, Lond.* **318**, 618–624.

ELLIS, R. J. (1981). Chloroplast proteins: synthesis, transport, and assembly. *Annu. Rev. Plant Physiol.* **32**, 111–137.

ELLIS, R. J. (1983). Chloroplast protein synthesis: principles and problems. *Subcellular Biochemistry* (Roodyn, D. B., ed.). New York: Plenum Publishing Corporation.

ERICKSON, J. M. & ROCHAIX, J.-D. (1992). The molecular biology of photosystem II. *The Photosystems: Structure, Function and Molecular Biology* (Barber, J., ed.) pp. 101–177. Amsterdam: Elsevier Science Publishers.

FEE, J. A. (1991). Regulation of *sod* genes in *Eschericia coli*: relevance to superoxide dismutase function. *Mol. Microbiol.* **5**, 2599–2610.

FLOHE, L., LOSCHEN, G., AZZI, A. & RICHTER, C. (1977). Superoxide radicals in mitochondria. *Superoxide and Superoxide Dismutases* (Michelson, A. M., McCord, J. M. & Fridovich, I., eds) pp. 323–334. New York: Academic Press.

FUJITA, Y., MURAKAMI, A. & OHKI, K. (1987). Regulation of photosystem composition in the cyanobacterial photosynthetic system: the regulation occurs in response to the redox state of the electron pool located between the two photosystems. *Plant Cell Physiol.* **28**, 283–292.

GAL, A., HAUSKA, G., HERRMANN, R. & OHAD, I. (1990). Interaction between LHCII kinase and cytochrome b_6/f: *in vitro* control of kinase activity. *J. Biol. Chem.* **265**, 19742–19749.

GAL, A., HERRMANN, R. G., LOTTSPEICH, F. & OHAD, I. (1992). Phosphorylation of cytochrome b6 by the LHC II kinase associated with the cytochrome complex. *FEBS Lett.* **298**, 33–35.

GILLES-GONZALEZ, M. A., DITTA, G. S. & HELINSKI, D. R. (1991). A haemoprotein with kinase activity encoded by the oxygen sensor of *Rhizobium meliloti*. *Nature, Lond.* **350**, 170–172.

GRANT, B. R. & WHATLEY, F. R. (1967). Some factors affecting the onset of cyclic photophosphorylation. *Biochemistry of Chloroplasts* (Goodwin, T. W., ed.) pp. 505–521. New York: Academic Press.

GREEN, B. R., PICHERSKY, E. & KLOPPSTECH, K. (1991). Chlorophyll *a/b*-binding proteins: an extended family. *Trends Biochem. Sci.* **16**, 181–186.

GRUISSEM, W., BARKAN, A., DENG, X. & STERN, D. (1988). Transcriptional and post-transcriptional control of plastid mRNA levels in higher plants. *Trends Genet.* **4**, 258–263.

GUEST, J. R. (1992). Oxygen-regulated gene expression in *Escherichia coli*. *J. Gen. Microbiol.* **138**, 2253–2263.

GUEST, J. R., GREEN, J., SPIRO, S., PRODROMOU, C. & SHARROCKS, A. (1990). Regulation of gene expression by oxygen in *Escherichia coli*. *The Molecular Basis of Bacterial Metabolism* (Hauska, G. & Thauer, R., eds) pp. 134–145. Berlin: Springer-Verlag.

HARRISON, S. C. (1991). A structural taxonomy of DNA-binding domains. *Nature, Lond.* **353**, 715–719.

HARRISON, M. A., TSINOREMAS, N. F. & ALLEN, J. F. (1991). Cyanobacterial thylakoid membrane proteins are reversibly phosphorylated under plastoquinone-reducing conditions *in vitro*. *FEBS Lett.* **282**, 295–299.

HARTL, F.-U. & NEUPERT, W. (1990). Protein sorting to mitochondria: evolutionary conservations of folding and assembly. *Science* **247**, 930–938.

HEBER, U. & WALKER, D. (1992). Concerning a dual function of coupled cyclic electron transport in leaves. *Plant Physiol.* **100**, 1621–1626.

HIRATSUKA, J., SHIMADA, H., WHITTIER, R., ISHIBASHI, T., SAKAMOTO, M., MORI, M., KONDO, C., HONJI, Y., SUN, C.-R., MENG, B.-Y., LI, Y.-Q., KANNO, A., NISHIZAWA, Y., HIRAI, A., SHINOZAKI, K. & MASAHIRO, S. (1989). The complete sequence of the rice (*Oryza sativa*) chloroplast genome: intermolecular recombination between distinct tRNA genes accounts for a major plastid DNA inversion during the evolution of the cereals. *Mol. Gen. Genet.* **217**, 185–194.

HOLMES, N. G. & ALLEN, J. F. (1988). Protein phosphorylation in chromatophores from *Rhodospirillum rubrum*. *Biochim. Biophys. Acta.* **935**, 72–78.

IUCHI, S., CAMERON, D. C. & LIN, E. C. C. (1989). A second global regulator gene (*arcB*) mediating repression of enzymes in aerobic pathways of *Escherichia coli*. *J. Bacteriol.* **171**, 868–873.

IUCHI, S., CHEPURI, V., FU, H.-A., GENNIS, R. B. & LIN, E. C. C. (1990). Requirement for terminal cytochromes in generation of the aerobic signal for the *arc* regulatory system in *Escherichia coli*: study utilizing deletions and *lac* fusions of *cyo* and *cyd*. *J. Bacteriol.* **172**, 6020–6025.

IUCHI, S. & LIN, E. C. C. (1988). *arcA(dye)*, a global regulatory gene in *Escherichia coli* mediating repression of enzymes in aerobic pathways. *Proc. natn. Acad. Sci. U.S.A.* **85**, 1888–1892.

IUCHI, S., MATSUDA, Z., FUJIWARA, T. & LIN, E. C. C. (1990). The *arcB* gene of *Escherichia coli* encodes a sensor-regulator protein for anaerobic repression of the *arc* modulation. *Mol. Microbiol.* **4**, 715–727.

KERBEY, A. L., RADCLIFFE, P. M., RANDLE, P. J. & SUGDEN, P. H. (1979). Regulation of kinase reactions in pig heart pyruvate dehydrogenase complex. *Biochem. J.* **181**, 427–433.

KLUG, G., GAD'ON, N., JOCK, S. & NARRO, M. L. (1991). Light and oxygen effects share a common regulatory DNA sequence in *Rhodobacter capsulatus*. *Mol. Microbiol.* **5**, 1235–1239.

KRANZ, R. G., PACE, V. M. & CALDICOTT, I. M. (1990). Inactivation, sequence, and *lacZ* fusion analysis of a regulatory locus required for repression of nitrogen fixation genes in *Rhodobacter capsulatus*. *J. Bacteriol.* **172**, 53–62.

KYLE, D. J., OHAD, I. & ARNTZEN, J. (1984). Membrane protein damage and repair: selective loss of a quinone-protein function in chloroplast membranes. *Proc. natn. Acad. Sci. U.S.A.* **81**, 4070–4074.

LILJESTRÖM, P., LAAMANEN, I. & PALVA, E. T. (1988). Structure and expression of the *omp B* operon, the regulatory locus for the outer membrane porin regulon in *Salmonella typhimurium* LT-2. *J. molec. Biol.* **201**, 663–673.

MAGASANIK, B. (1989). Regulation of transcription of the *gln*ALG operon of *Escherichia coli* by protein phosphorylation. *Biochimie* **71**, 1005–1012.

MARGULIS, L. (1981). *Symbiosis in Cell Evolution*. New York: W. H. Freeman & Co.

MATSUBAYASHI, T., WAKASUGI, T., SHINOZAKI, K., YAMAGUCHI-SHINOZAKI, K., ZAITA, N., HIDAKA, T., MENG, B. Y., OHTO, C., TANAKA, M., KATO, A., MARUYAMA, T. & SUGUIRA, M. (1987). Six chloroplast genes (*ndhA-F*) homologous to human mitochondrial genes encoding components of the respiratory chain NADH dehydrogenase are actively expressed: determination of the splice sites in *ndhA* and *ndhB* pre-mRNAs. *Mol. Gen. Genet.* **210**, 385–393.

MATTOO, A. K., MARDER, J. B. & EDELMAN, M. (1989). Dynamics of the photosystem II reaction center. *Cell* **56**, 241–246.

MCEWAN, A. G., COTTON, N. P. J., FERGUSON, S. J. & JACKSON, J. B. (1985). The role of auxiliary oxidants in the maintenance of a balanced redox poise for photosynthesis in bacteria. *Biochim. Biophys. Acta.* **810**, 140–147.

MELIS, A. (1990). Adaption of photosystem stoichiometry in oxygen-evolving thylakoid membranes. *Current Research in Photosynthesis* (Baltscheffsky, M., ed.) pp. 291–298. Dordrecht: Kluwer.

MICHELSON, A. M., MCCORD, J. M. & FRIDOVICH, I. (1977). *Superoxide and Superoxide Dismutases*. New York: Academic Press.

MITCHELL, P. (1979). Compartmentation and communication in living systems: ligand conduction: a general catalytic principle in chemical, osmotic, and chemiosmotic reaction systems. *Eur. J. Biochem.* **95**, 1–20.

MORDEN, C. W., WOLFE, K. H., DEPAMPHILIS, C. W. & PALMER, J. D. (1991). Plastid translation and transcription genes in a non-photosynthetic plant: intact, missing and pseudo genes. *EMBO J.* **10**, 3281–3288.

MOSER, C. C., KESKE, J. M., WARNCKE, K., FARID, R. S. & DUTTON, P. L. (1992). Nature of biological electron transfer. *Nature, Lond.* **355**, 796–802.

NINFA, A. J. & MAGASANIK, B. (1986). Covalent modification of the *glnG* product, NR$_I$, by the *glnL* product, NR$_{II}$, regulates the transcription of the *glnALG* operon in *Escherichia coli*. *Proc. natn. Acad. Sci. U.S.A.* **83**, 5909–5913.

ODA, K., YAMATO, K., OHTA, E., NAKAMURA, Y., TAKEMURA, M., NOZATO, N., AKASHI, K., KANEGAE, T., OGURA, Y., KOHCHI, T. & OHYAMA, K. (1992). Complete nucleotide sequence of the mitochondrial DNA from a liverwort, *Marchantia polymorpha*. *Plant Mol. Biol. Reporter.* **10**, 105–163.

OHYAMA, K., FUKUZAWA, H., KOHCHI, T., SHIRAI, H., SANO, T., SANO, S., UMESONO, K., SHIKI, Y., TAKEUCHI, M., CHANG, Z., AOTA, S., INOKUCHI, H. & OZEKI, H. (1986). Chloroplast gene organization deduced from complete sequence of liverwort *Marchantia polymorpha* chloroplast DNA. *Nature, Lond.* **322**, 572–574.

PRÁSIL, O., ADIR, N. & OHAD, I. (1992). Dynamics of photosystem II: mechanism of photoinhibition and recovery processes. *The Photosystems: Structure, Function and Molecular Biology* (Barber, J., ed.) pp. 295–348. Amsterdam: Elsevier Science Publishers.

RICHTER, M., RÜHLE, W. & WILD, A. (1990). Studies on the mechanism of photosystem II photoinhibition II. The involvement of toxic oxygen species. *Photosynth. Res.* **24**, 237–243.

ROBINSON, C. (1990). Targeting of proteins to chloroplasts and mitochondria. *Plant Genetic Engineering* (Grierson, D., ed.) pp. 179–198. Glasgow: Blackie Press.

ROCHAIX, J.-D. (1987). Molecular genetics of chloroplasts and mitochondria in the unicellular green alga *Chlamydomonas*. *FEMS Microbiol. Rev.* **46**, 13–34.

RONSON, C. W., NIXON, B. T. & AUSUBEL, F. M. (1987). Conserved domains in bacterial regulatory proteins that respond to environmental stimuli. *Cell* **49**, 579–581.

RUTHERFORD, A. W. (1989). Photosystem II, the watersplitting enzyme. *Trends Biochem. Sci.* **14**, 227–232.

SAGAN, L. (1967). On the origin of mitosing cells. *J. theor. Biol.* **14**, 225–275.

SAIER, M. H., JR, LONG-FEI, W. & REIZER, J. (1990). Regulation of bacterial physiological processes by three types of protein phosphorylating systems. *Trends Biochem. Sci.* **15**, 391–395.

SCHELLER, H. V. & MØLLER, B. L. (1990). Photosystem I polypeptides. *Physiol. Plant.* **78**, 484–494.

SGANGA, M. W. & BAUER, C. E. (1992). Regulatory factors controlling photosynthetic reaction center and light-harvesting gene expression in *Rhodobacter capsulatus*. *Cell* **68**, 945–954.

SHINOZAKI, K., OHME, M., TANAKA, M., WAKASUGI, T., HAYASHIDA, N., MATSUBAYASHI, T., ZAITA, N., CHUNWONGSE, J., OBOKATA, J., YAMAGUCHI-SHINOZAKI, K., OHTO, C., TORAZAWA, K., MENG, B. Y., SUGITA, M., DENO, H., KAMOGASHIRA, T., YAMADA, K., KUSUDA, J., TAKAIWA, F., KATO, A., TOHDOH, N., SHIMADA, H. & SUGIURA, M. (1986). The complete nucleotide sequence of the tobacco chloroplast genome: its gene organization and expression. *EMBO J.* **5**, 2043–2049.

SMEEKENS, S., WEISBEEK, P. & ROBINSON, C. (1990). Protein transport into and within chloroplasts. *Trends Biochem. Sci.* **15**, 73–76.

SOMMARIN, M., PETIT, P. X. & MØLLER, I. M. (1990). Endogenous protein phosphorylation in purified plant mitochondria. *Biochim. Biophys. Acta.* **1052**, 195–203.

SPIRO, S. & GUEST, J. R. (1991). Adaptive responses to oxygen limitation in *Escherichia coli*. *Trends Biochem. Sci.* **16**, 310–314.

SPIRO, S., ROBERTS, R. E. & GUEST, J. R. (1989). FNR-dependent repression of the *ndh* gene of *Escherichia coli* and metal ion requirement for FNR-regulated gene expression. *Mol. Microbiol.* **3**, 601–608.

STOCK, J. B., NINFA, A. J. & STOCK, A. M. (1989). Protein phosphorylation and regulation of adaptive responses in bacteria. *Microbiol. Rev.* **53**, 450–490.

STOCK, J. B., STOCK, A. M. & MOTTONEN, J. M. (1990). Signal transduction in bacteria. *Nature, Lond.* **344**, 395–400.

TAREMI, S. S. & MARRS, B. L. (1990). Regulation of gene expression by oxygen: phototrophic bacteria. *The Molecular Basis of Bacterial Metabolism* (Hauska, G. & Thauer, R., eds) pp. 146–151. Berlin: Springer-Verlag.

THEG, S. M., FILAR, L. J. & DILLEY, R. A. (1986). Photoinactivation of chloroplasts already inhibited on the oxidizing side of photosystem II. *Biochim. Biophys. Acta* **849**, 104–111.

TOKITO, M. K. & DALDAL, F. (1992). *petR* gene of *R. capsulatus*. *Mol. Microbiol.* **5**, 2599–2610.

TSINOREMAS, N. F., CASTETS, A. M., HARRISON, M. A., ALLEN, J. F. & TANDEAU DEMARSAC, N. (1991). Photosynthetic electron transport controls nitrogen assimilation in cyanobacteria by means of post-translational modification of the *glnB* gene product. *Proc. natn. Acad. Sci. U.S.A.* **88**, 4565–4569.

UNDEN, G., TRAGESER, M. & DUCHENE, A. (1990). Effect of positive redox potentials (> +400 mV) on the expression of anaerobic respiratory enzymes in *Escherichia coli*. *Mol. Microbiol.* **4**, 315–319.

VON HEIJNE, G. (1986). Why mitochondria need a genome. *FEBS Lett.* **198**, 1–4.

VONSHAK, A., GUY, R., POPLAWSKY, R. & OHAD, I. (1988). Photoinhibition and its recovery in two strains of the cyanobacterium *Spirulina platensis*. *Plant Cell Physiol.* **29**, 721–726.

VOSSBRINCK, C. R., MADDOX, J. V., FRIEDMAN, S., DEBRUNNER-VOSSBRINCK, B. A. & WOESE, C. R. (1987). Ribosomal RNA sequence suggests microsporidia are extremely ancient eukaryotes. *Nature, Lond.* **326**, 411–414.

WALKER, D. A., LEEGOOD, R. C. & SIVAK, M. N. (1986). Ribulose bisphosphate carboxylase-oxygenase: its role in photosynthesis. *Phil. Trans. R. Soc. Lond.* **B313**, 305–324.

WEST, K. R. & WISKICH, J. T. (1968). Photosynthetic control by isolated pea chloroplasts. *Biochem. J.* **109**, 527–532.

WHATLEY, F. R. (1963). Some effects of oxygen in photosynthesis by chloroplast preparations. *Photosynthetic Mechanisms of Green Plants.* 243–251. Washington D.C.: National Academy of Sciences, National Research Council, Washington, D.C.

WHATLEY, J. M., JOHN, P. & WHATLEY, F. R. (1979). From extracellular to intracellular: the establishment of mitochondria and chloroplasts. *Proc. R. Soc. Lond.* **B204**, 165–187.

ZIEN-HANCK, U. & HEBER, U. (1980). Oxygen requirement of photosynthetic CO_2 assimilation. *Biochim. Biophys. Acta* **591**, 266–274.