

Coordination of gene expression between organellar and nuclear genomes

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Abstract

Following the acquisition of chloroplasts and mitochondria by eukaryotic cells during endosymbiotic evolution, most of the genes in these organelles were either lost or transferred to the nucleus. Encoding organelle-destined proteins in the nucleus allows for host control of the organelle. In return, organelles send signals to the nucleus to coordinate nuclear and organellar activities. In photosynthetic eukaryotes, additional interactions exist between mitochondria and chloroplasts. Here we review recent advances in elucidating the intracellular signalling pathways that coordinate gene expression between organelles and the nucleus, with a focus on photosynthetic plants.

It is widely accepted that chloroplasts and mitochondria descended from free-living bacterial ancestors¹. Phylogenetic, biochemical, and structural analyses suggest that mitochondria descended from an α -proteobacterium-like ancestor that invaded or was engulfed by an archaeal-like host 1.5 billion years ago or earlier, and that primary chloroplasts descended from a cyanobacterium-like ancestor that invaded or was engulfed by a mitochondrion-possessing eukaryote between 1.5 and 1.2 billion years ago¹.

In most cases these organelles have retained a genome, but present-day organelle genomes are severely reduced and encode few proteins (from 3 in the *Plasmodium falciparum* mitochondrion² to 209 in the *Porphyra purpurea* chloroplast³) (BOX 1). On the other hand, proteomics and genome analyses of protein-localization sequences estimate that organelles might contain up to several thousand different proteins^{4,5}. This estimate is within the range of the number of predicted protein-encoding genes in current-day cyanobacteria⁵ and α -

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DATABASES

Entrez Gene: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene> *EXECUTER 1* | *flu* | *GUN1* | *petA* | *Rox1*

UniProtKB: <http://ca.expasy.org/sprot> ABI4 | BACH1 | Hap1 | MCA1 | TCA1

FURTHER INFORMATION

Joanne Chory's homepage: http://www.salk.edu/faculty/faculty_details.php?id=12

SUPPLEMENTARY INFORMATION

See online article: S1 (table)

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proteobacteria⁶. As these numbers imply, most proteins (93–99%) that are found in organelles are encoded in the nucleus, synthesized in the cytoplasm and then imported into the organelles.

Having organelle proteins encoded on two or more separate and compartmentalized genomes requires coordinated expression to produce the correct concentrations of organelle proteins and to maintain organelle function. These genome-coordinating mechanisms include both anterograde (nucleus to organelle) and retrograde (organelle to nucleus) signals (FIG. 1). Anterograde mechanisms coordinate gene expression in organelles in response to endogenous and environmental stimuli that are perceived by the nucleus. Retrograde mechanisms transmit signals that originate in the organelles to regulate nuclear gene expression, which can then alter anterograde control. In photosynthetic organisms this regulation is even more complex owing to cross-talk between mitochondria and chloroplasts. Tight coordination between the nucleus and organelles is crucial to the survival of eukaryotic cells: not only are chloroplasts and mitochondria of great bioenergetic importance, but they synthesize many different cellular metabolites, including amino acids, lipids, nucleotides, vitamins and porphyrins.

This Review summarizes recent progress on anterograde and retrograde signalling mechanisms between mitochondria or chloroplasts and the nucleus, with an emphasis on chloroplast-to-nucleus communication. During the past few years, there have been significant advances in uncovering specific signals from chloroplasts and their mechanisms of action. The availability of genome sequences has accelerated forward genetic analysis and allowed for the use of reverse genetics techniques to uncover signalling mechanisms and their protein components in model organisms such as the green alga *Chlamydomonas reinhardtii* and the flowering plant *Arabidopsis thaliana*. In addition, both transcriptomics and proteomics have provided insight into the effects that retrograde and anterograde signals have on nuclear and organellar gene expression, respectively. As well as being a fundamental feature of eukaryotic cell physiology, correct coordination of activities between the nucleus and organelles has recently been implicated in fertility in plants⁷, ageing in yeast⁸ and tumorigenesis in mammals⁹. Thus, it is becoming clear that retrograde and anterograde signalling mechanisms have broad roles within the cell and diverse roles between eukaryotic kingdoms.

Challenges associated with separate genomes

The presence of organelles that contain their own genomes presents the cell with several challenges for regulating gene expression and for controlling organelle protein levels. An individual cell can contain multiple organelles, each containing several copies of their genome; as a result there can be up to a 1:5,000 disparity between the copy number of nuclear and organellar genomes in metazoans¹⁰ and plants¹¹ that can fluctuate according to cell type, age and growth conditions¹¹. Because many organellar genes encode subunits for protein complexes that are largely encoded in the nucleus (BOX 1), regulation is required to ensure the correct amount of protein is available for assembly. Furthermore, organelles do not encode any known autonomous regulators of gene expression and must rely on the

nucleus for such proteins. These types of mechanisms are achieved through anterograde control, as discussed below.

In addition to developmental changes, organelles experience shifting environmental conditions, including rapid redox changes, oxidative damage and changes in nutrient availability. For the most part, they are not genetically equipped to manage these changes because the relevant genes are in the nucleus. Organelles must therefore be able to emit different signals that the nucleus can interpret and respond to by altering gene expression. This type of regulatory mechanism has been achieved through retrograde signalling, which is discussed in a later section.

Anterograde signalling

By and large, the nucleus and nuclear-encoded proteins control organelle function and proteome composition. The concentrations of many nuclear-encoded organelle proteins are efficiently regulated by nuclear transcription¹². In other cases, nuclear-encoded proteins regulate post-translational events such as protein transport, the assembly of large multi-subunit complexes (that is, photosynthetic and respiratory complexes) and the regulation of enzyme activities (for example, tetrapyrrole biosynthesis)^{13,14}. Organelle development, division and, in plastids, differentiation into chloroplasts, amyloplasts and chromoplasts¹⁵ is also largely, if not completely, dependent on nuclear-encoded proteins. Nuclear-encoded proteins also primarily control organelle gene expression. Because nuclear control of organelle function is such a broad subject, this Review only covers how it pertains to the coordination of nuclear and organelle genomes, and is limited to the anterograde mechanisms that control organelle gene expression (FIG. 2).

Transcriptional regulation

The regulation of organelle gene expression mostly occurs through post-transcriptional mechanisms and involves nuclear-encoded proteins. However, some degree of transcriptional regulation does occur in both mitochondria and chloroplasts^{16–19}, but all known organelle transcription factors are nuclearencoded proteins in yeast (mtTFB), mammals (TFB1M, TFB2M and the termination factor family²⁰) and plants²¹, with the exception of the plastid-encoded RNA polymerase (PEP) in plant chloroplasts.

Mitochondria use a nuclear-encoded RNA polymerase (NEP), chloroplasts use PEP, and chloroplasts of higher plants use both. The reason for having two polymerases is unclear as most chloroplast genes have promoters that are recognized by both enzymes²². In general, PEP transcribes photosynthesis-related genes and it has been proposed that chloroplast development in higher plants proceeds by switching from NEP- to PEP-dominated transcription²² (possibly through the inhibition of NEP activity by PEP-transcribed tRNA^{Glu} (REF. 23)), which, along with post-transcriptional mechanisms¹⁸, leads to differential transcript accumulation during primary leaf and chloroplast development²⁴. This switch, which is probably used for large developmental switches rather than subtle regulatory changes¹⁷, also leads to a general increase of transcription, perhaps as a mechanism to increase photosynthesis-related protein accumulation²⁴.

Furthermore, nuclear-encoded sigma factors (*A. thaliana* encodes six, SIG1–SIG6) regulate PEP activity to express different sets of genes²⁵. Again, these mechanisms of nuclear control appear to be used more on the developmental scale, as different sigma factors are important during different stages of *A. thaliana* growth²⁵. However, SIG5 does appear to be involved in the response to blue light²⁶ and stress²⁷, which might allow the nucleus to relay a specific stimulus to the chloroplast through transcriptional control.

Although transcriptional control and activities vary in organelles, they do not correlate much with protein levels in yeast²⁸ or plant mitochondria²⁹ or chloroplasts^{30,31}. Transcript levels might increase in some situations^{31,32} to ‘prime’ the organelle, but ultimately the post-transcriptional and post-translational mechanisms that are controlled by the nucleus determine the protein levels.

Post-transcriptional regulatory proteins

Most anterograde control of organellar gene expression is post-transcriptional^{13,17,19}. The processes that are involved, which include transcript maturation (for example, splicing, processing and editing) and translation, are controlled by nuclear-encoded proteins^{13,17,33}. Genetic analysis of mutants defective in organellar functions has revealed many nuclear-encoded post-transcriptional regulators of organelle gene expression (ROGEs). Often, ROGEs are dedicated to the proper expression of only one specific organellar gene. ROGEs fall into two principal classes of regulation: those that are required for the proper maturation and/or stabilization of organellar transcripts^{33,34} and those that affect the translation (translational initiation, elongation) of organellar transcripts^{13,33} (FIG. 2c). It is likely that ROGE proteins act as adaptors; they bind specific mRNA transcripts and recruit translation machinery or RNA metabolism enzymes. In most, if not all, cases, ROGE proteins are either pioneer proteins that are not conserved among eukaryotes, or they belong to protein families that are identified only by their sequence motifs that are involved in protein–protein interactions (tetratricopeptide repeat motifs)³⁵ or protein–RNA interactions (pentatricopeptide repeat (PPR) motifs)^{34,36} (BOX 2; [Supplementary information S1](#) (table)).

Although most of the research on ROGE proteins has suggested that they might enable the nucleus to control organelle gene expression, our understanding of their physiological roles is limited. Some ROGE proteins might be constantly present in organelles to control the assembly-dependent regulatory process named control by epistasy of synthesis (FIG. 2d). This mechanism, for which several cases have been reported^{33,37,38}, involves negative feedback by the unassembled protein on the translation of its own mRNA. In the best-studied example, in *C. reinhardtii* chloroplasts, the ROGE protein translation factor TCA1 (a non-conserved pioneer protein required for *petA* translation)³³ binds the exposed C-terminus of unassembled cytochrome *f*. When *petA*-encoded cytochrome *f* is assembled with the cytochrome *b₆*-cytochrome subunit IV complex, its C-terminus is unexposed and TCA1 is able to activate translation of *petA* mRNA.

The nuclear-controlled fluctuation of ROGE protein levels in response to environmental stimuli can directly regulate protein abundance in organelles. In *C. reinhardtii* chloroplasts, expression of the *petA* gene is controlled by the abundance of TCA1 (REF. 33) and another

ROGE protein: RNA stability factor MCA1, a PPR protein required for the stable accumulation of the *petA* transcript³⁹. Nitrogen starvation conditions led to the nuclear downregulation of both MCA1 and TCA1, resulting in a decreased accumulation of *petA* transcripts and its protein product.

A similar situation exists in *Saccharomyces cerevisiae*. Here, translation of the mitochondrial-encoded cytochrome *c* oxidase subunit III gene (*coxIII*) is regulated by Pet494 (REF. 28), a nuclear-encoded ROGE pioneer protein, the accumulation of which is regulated by the nucleus in response to oxygen availability and type of carbon source⁴⁰. The availability of ROGE proteins for organelle gene expression can be regulated by other mechanisms that are controlled by the state of the organelle. For example, the translation of photosystem II protein D1 (PsbA) in *C. reinhardtii* chloroplasts is regulated by an already present ROGE protein that responds to the redox state of the organelle³⁷.

It is unclear why so many organelle-encoded proteins are controlled by separate nuclear-encoded proteins (many, if not most, of the respiratory and photosynthesis complex proteins in *S. cerevisiae* and *C. reinhardtii*, respectively, are regulated by ROGE proteins^{13,38}), but it might indicate that intricate control of multi-subunit complexes is necessary for protein assembly or function. The lack of conservation among the ROGE proteins (most of the proteins that are involved in the regulation of yeast mitochondrial cytochrome *c* oxidase (*COX*) genes are absent in higher eukaryotes¹³) and the extra reliance on PPR proteins in plants (BOX 2) suggests that this type of regulation is evolving to suit the specific needs of a particular organelle in a particular species. This is surprising considering the sequence conservation of organellar genes, and it might reflect the differences in organellar transcriptional and translational mechanisms among species. For instance, most of the translational activators of the mitochondrial *COX* genes in yeast recognize the 5' UTR¹³. Mitochondrial transcripts in humans lack 5' UTRs and the existence of specific translational activators has not been confirmed¹³.

Post-translational regulation in mitochondria

Studies in both plant⁴¹ and yeast⁴² mitochondria have suggested that the rapid degradation of the excess number of unassembled mitochondrial-encoded proteins and the regulation of multi-subunit protein assembly are conserved mechanisms of post-translational regulation in these and other organelles. Given that there is little evidence of translational regulation in plant mitochondria¹⁷, such an anterograde mechanism could explain how the nucleus coordinates gene expression in these organelles (FIG. 2b).

It was recently shown that sucrose-starved *A. thaliana* cells had a decreased level of mitochondrial ribosomes and respiration protein complexes: nuclear (not mitochondrial) transcripts for these protein complexes became limiting²⁹. The levels of mitochondrial-encoded proteins and their assembly with nuclear-encoded proteins must be adjusted accordingly by post-translational mechanisms to control the correct concentrations of mitochondria-encoded protein complexes compared with nuclear-encoded protein complexes. It is expected that under sugar-starvation conditions, protein assembly is regulated by specific nuclearencoded chaperones, and that excess unassembled mitochondrial subunits are degraded by specific nuclear-encoded proteases.

In yeast, mitochondrial transcripts and protein levels fluctuate during glucose repression and derepression, but protein levels can be controlled, at least in part, by limiting the amount of ROGE proteins¹³. Therefore, as is the case in plants, the modulation of yeast respiration according to sugar availability is under the control of the nucleus and uses a mitochondrial system that is limited by nuclear-encoded proteins^{28,32}.

There is no universal mode of anterograde control of organellar gene expression, but certain themes have emerged. Organelle genome copy number fluctuates but it contributes little to gene expression^{18,43}, whereas transcriptional regulation with nuclear-encoded transcription factors leads to global rather than subtle changes in transcript accumulation. In general, however, post-transcriptional mechanisms dominate by using specific ROGE proteins to regulate gene expression, by the turnover of excess unassembled proteins and by the control of protein assembly. Nuclear control of organelle function can therefore be separated from any fluctuation in genome copy number and in mRNA transcript abundance that is caused by variable growth rates or nutrient availability³⁰.

Retrograde signalling

Retrograde mechanisms have evolved to communicate the functional and developmental state of organelles to the nucleus, which can then modulate anterograde control and cellular metabolism accordingly (FIG. 3). These signals are used for genome coordination, instructing adaptive responses to fluctuating or stressful environmental conditions, or to inform the nucleus of cellular stress. Although retrograde pathways appear to be used by virtually all eukaryotic organisms, there is little conservation between these signals as they vary according to organelle type, species and purpose. Like anterograde signalling, this might indicate that the relationship between organelles and the nucleus is continually evolving to suit an organism's needs.

Mitochondrial retrograde signalling: yeast and plants

The physiological state of mitochondria can reflect their degree of energy production or O₂ availability, or levels of reactive oxygen species (ROS). These metabolites allow mitochondria to inform the nucleus of these fluctuating conditions, and induce adaptive measures. Not surprisingly, nuclear genes that are related to respiration, peroxisomal biogenesis and oxidative-stress responses are regulated at the transcriptional level in response to the physiological state of mitochondria^{8,9,44}. Mitochondrial retrograde signalling might also be involved in other processes, including ageing and tumorigenesis in yeast and animals, respectively; (BOX 3).

Organelle retrograde signalling was first studied in *S. cerevisiae* mitochondria, where work has focused on the retrograde signals resulting from mitochondrial dysfunction (BOX 3) and the haem signalling pathway. Because haem biosynthesis in the mitochondrion is oxygen dependent, cellular haem concentrations can reflect oxygen availability⁴⁵. By interacting directly with the haem activator protein (**Hap1**) in the nucleus, haem switches the cell between aerobic and anaerobic growth through transcriptional regulation⁴⁶ (FIG. 3g). Under aerobic conditions, haem is available to bind Hap1, which then activates the transcription of many aerobic genes, including those required for respiration and controlling oxidative

damage, and the repressor of hypoxic genes (*Rox1*)^{45,47} by direct DNA binding. Under low-oxygen conditions, when haem is not synthesized, Hap1 acts as a repressor and represses at least three genes involved in ergosterol biosynthesis⁴⁸.

It is unclear whether other phyla share this mechanism of oxygen sensing, as Hap1 is not conserved outside of fungi. In mammals and humans, the activity of basic leucine zipper transcription factor 1 (*BACH1*) is regulated by haem⁴⁹. Because *BACH1* regulates genes that are involved in haem metabolism and the oxidative stress response⁴⁹, this might be an analogous system to yeast in which haem concentrations inform the nucleus of cellular oxygen concentrations or mitochondrial function. Chloroplasts use structurally related tetrapyrrole molecules of the chlorophyll biosynthetic pathway for chloroplast retrograde signalling; however, it is unknown whether a mechanism common to yeast haem signalling will be used.

In plants, mitochondrial retrograde signalling has been implicated in ROS signalling, O₂ sensing, heat shock, pathogen sensing and programmed cell death⁴⁴. Retrograde signals from dysfunctional mitochondria might also be the cause of at least some types of cytoplasmic male sterility (CMS) in flowering plants (BOX 4). No protein component of any plant mitochondrial retrograde signalling pathway has been identified, although Ca²⁺ signalling, protein kinases and nuclear transcription factors are predicted to be involved. The best-studied retrograde signal(s) in plant mitochondria involve increased expression of the nuclear-encoded alternative oxidase (AOX) as an adaptive response to recover from mitochondrial electron transport chain (mtETC) inhibition⁴⁴ (FIG. 3e). AOX reduces the production of ROS⁵⁰, and *A. thaliana mitochondrial retrograde regulation deficient* (*mrd*) mutants (the identities of which are unknown) that are unable to induce AOX after mtETC inhibition exhibit ROS-like cellular damage⁵¹. There might be several signals for AOX induction as these mutants do not all respond equally to different mechanisms of mtETC inhibition⁵¹, but these separate signals appear to lead to different elements within the same 93 bp promoter region of AOX⁵².

Signals from chloroplasts

The first evidence that signals from chloroplasts regulate nuclear gene expression came from studies on *albostrians* mutants of barley, which contain undifferentiated plastids that lack ribosomes⁵³. Cells with these albino plastids did not accumulate nuclear-encoded photosynthetic mRNA transcripts^{54,55}. This retrograde signal from chloroplasts, originally termed ‘the plastid factor’⁵⁶, alters the transcriptional regulation of nuclear genes and is quite complex. Although no chloroplast retrograde pathway is well-understood mechanistically, several signals have been reported to trigger retrograde signalling from chloroplasts, including accumulation of Mg–protoporphyrin IX (Mg–proto, the first unique intermediate of chlorophyll biosynthesis), redox signalling, and signals that are generated by inhibiting plastid gene expression or by accumulating various ROS.

The best-characterized chloroplast retrograde signalling pathway involves tetrapyrrole intermediates of the chlorophyll biosynthetic pathway. In *C. reinhardtii*, Mg–proto and its methyl ester, Mg–proto–Me, can substitute light to induce the transcription of at least four nuclear genes: *HEMA*⁵⁷ (the gene encoding glutamyl-tRNA reductase, which catalyses the

first step of tetrapyrrole biosynthesis in plants) and three heat shock protein 70 genes⁵⁸. Because these chlorophyll intermediates are chloroplast specific, using them as retrograde signals — instead of haem (as used in yeast), which can be synthesized in both plant chloroplasts and mitochondria⁵⁹ — informs the nucleus as to their origin.

Higher plants use Mg-proto as a chloroplast signalling molecule, but in a different way. In *A. thaliana*, undeveloped chloroplasts that have suffered photo-oxidative damage, owing to the lack of protective carotenoids, accumulate Mg-proto, which leads to the repression of nuclear genes that encode photosynthesis-related proteins⁶⁰ (FIG. 3c). Mutants that block this retrograde signalling have been isolated (*genomes uncoupled* or *gun* mutants⁶¹), and all but *gun1* (REF. 62) mutants (*GUN1* encodes a chloroplast-localized PPR protein⁶², see BOX 2; [Supplementary information S1](#) (table)) interfere with chlorophyll metabolism and affect Mg-proto accumulation^{60,63,64}. This role for Mg-proto is also corroborated by work with barley Mg-proto biosynthetic mutants⁶⁵, transgenic tobacco lines with altered expression of the Mg-proto methyl transferase gene (*CHLM*)⁶⁶, and *A. thaliana* *CHLM* knockout lines⁶⁷.

Work in tobacco has suggested that Mg-proto can have the opposite effect on nuclear gene expression. Transgenic plants with lowered levels of Mg-proto synthesis have repressed nuclear photosynthetic genes⁶⁸. Unlike the work with barley and *A. thaliana*, these studies were performed using adult plants with relatively developed chloroplasts. This might mean that the Mg-proto signal in higher plants as described above is used to coordinate genome expression in young seedlings during organelle development or during severe stress conditions, as opposed to throughout development under normal conditions⁶⁰. In developed chloroplasts, the accumulation of chlorophyll intermediates might have different effects (for example, to modulate levels of photosynthetic machinery in response to available chlorophyll).

Using *in vivo* visualization techniques, Mg-proto has been reported to exit the cytoplasm, where presumably it interacts with unidentified signalling factors⁶⁹ (FIG. 3c). This mechanism would probably require active transport of Mg-proto, although no transporters have been identified. Because the Mg-proto signal is dependent on the expression of *GUN1* (REF. 62), *GUN1* or a *GUN1*-dependent chloroplast protein (discussed below) might be necessary for this export. However, these studies did not analyse Mg-proto export in *gun1* mutant cells, nor have they demonstrated that Mg-proto export is required for nuclear gene repression. Alternatively, as discussed below, the Mg-proto signal might converge with other retrograde pathways inside the nucleus in a *GUN1*-dependent fashion and involve an unidentified signal that exits the chloroplast (FIG. 3c).

Different *cis*-acting elements in the promoters of nuclear genes have been identified in *C. reinhardtii* (the plastid response element⁷⁰) and *A. thaliana* (the CUF1 element of the *G-box*⁶⁰) that respond to the Mg-proto-induced signal(s). Both elements are also used for light signalling in these organisms and, at least in *C. reinhardtii*, Mg-proto might be mediating the light activation of genes harbouring these elements⁷⁰. In higher plants, the repressive effect of Mg-proto suggests that it antagonizes light activation, possibly by activating a transcriptional repressor that recognizes proximal or overlapping sequences of the CUF1 element (that is, abscisic acid insensitive 4 ([AB14](#)), see below)⁷¹. Because not all Mg-

protoresponsive nuclear promoters contain the CUF1 element, other *cis*-acting factors are probably involved in receiving the Mg–proto signal in *A. thaliana*.

A second retrograde signalling pathway (called the plastid gene expression (PGE) pathway) was discovered using inhibitors of chloroplast translation, which resulted in the repression of nuclear photosynthesis genes⁷². This pathway seems to be used to coordinate genomes during organelle development, as it is only active in young seedlings. In *A. thaliana*, the nuclear mutations *prp111* (chloroplast ribosome large subunit 11 (REF. 73)) and *prors1* (chloroplast and mitochondrial prolyl-tRNA synthetase 1 (REF. 74)) negatively affect translation in the chloroplast and also lead to similar light-independent repressive effects on nuclear genes. The repression of nuclear genes in *prp111* mutant plants was dependent on an additional mutation, *mrp111* (mitochondrial ribosomal protein L11), impairing mitochondrial translation, suggesting that both organelles send retrograde signals when protein synthesis is impaired, and that their signals are synergistic⁷⁴ (FIG. 3d).

Of the *A. thaliana gun* mutants mentioned above, only *gun1* affects the PGE pathway. GUN1 functions downstream of Mg–proto accumulation, as concluded by a recent microarray experiment (covering ~75% of *A. thaliana* genes) that showed that *gun1* and *gun5* mutants (the latter being defective in Mg–proto synthesis) misexpress similar sets of genes in cells with photobleached plastids⁶². This evidence supports a model in which Mg–proto and PGE signalling pathways converge within the chloroplast and that GUN1 is involved after the convergence point (FIG. 3c,d). Because Mg–proto accumulation does not affect the PGE signalling pathway⁶², the converged retrograde signal probably does not involve the export of Mg–proto from the chloroplast. As such, this model could be in conflict with the model of Mg–proto export from the chloroplast⁶⁹.

The nuclear-localized AP2-type transcription factor ABI4, which was originally characterized for its involvement in hormone (abscisic acid) signalling, also acts downstream of both Mg–proto and PGE signalling, where it can bind a CCAC motif (often found within close proximity or overlapping CUF1 elements) upstream of light-responsive genes and repress transcription⁶². A converged pathway would allow the chloroplast to integrate multiple retrograde signals to regulate similar genes in the nucleus.

The biochemical function of the GUN1 protein remains elusive, but its implicated role in other signalling events — such as circadian-clock regulation⁷⁵, de-etiolation and greening⁷⁶, and, along with AB14, sugar and redox signalling⁶² — suggests that it is used to integrate multiple retrograde signalling pathways within the chloroplast. The identity of GUN1 as a PPR protein suggests that it might be required for the expression (or repression) of one specific chloroplast gene, the product of which (GUN 1-dependent chloroplast protein) might be a plastid factor that interacts with Mg–proto and that is involved with multiple retrograde signals (FIG. 3c).

Reactive oxygen species as signals

Exposure to both biotic and abiotic stresses can lead to the increased accumulation of ROS, which cause irreversible oxidative damage to cells. ROS that are generated in chloroplasts, or the damage that they cause, act as retrograde signals to inform the nucleus to increase

antioxidant enzyme production and to adjust the photosynthetic machinery for more efficient light harvesting^{77,78}.

ROS production often involves multiple reactive species such as singlet oxygen ($^1\text{O}_2$) and H_2O_2 in multiple locations, but a breakthrough in understanding ROS signalling in chloroplasts came from using the *A. thaliana fluorescent in blue light (flu)* mutant⁷⁹, which over-accumulates the photo-excitabile chlorophyll precursor, protochlorophyllide, in the dark. A subsequent shift from dark to light leads to the rapid accumulation of $^1\text{O}_2$ in the chloroplast, followed by growth arrest and cell death. The nuclear transcriptional response is distinct from that when chloroplast H_2O_2 accumulates because of the application of the herbicide paraquat, which suggests that separate retrograde signalling pathways respond to $^1\text{O}_2$ and H_2O_2 accumulation in the chloroplast⁸⁰ (FIG. 3a). $^1\text{O}_2$ has a shorter half-life than H_2O_2 , but is now suggested to be able to travel over short distances (250 μM *in vivo*) and across membranes⁸¹, which introduces the possibility of $^1\text{O}_2$ acting outside of the chloroplast. Nonetheless, a mutational suppressor screen identified the chloroplast-localized protein EXECUTER 1 as being involved in the $^1\text{O}_2$ signal⁸² and mutations in *EXECUTER 1* allow *flu* mutants to be resistant to $^1\text{O}_2$ stress. Both growth inhibition and cell death were avoided in the double *flu/executer 1* mutant, suggesting that these phenotypes are genetically programmed responses to $^1\text{O}_2$ rather than the direct consequence of oxidative damage. $^1\text{O}_2$ accumulation in the *flu* mutant affects ~1,400 nuclear genes, but only ~200 of those encode chloroplast-localized proteins⁷⁷. Unlike the PGE–GUN signalling pathway, the ROS signalling pathway might be primarily used for stress signalling rather than genome coordination. Further work has shown that $^1\text{O}_2$ signalling involves at least one other chloroplast protein, EXECUTER 2 (REF. 77), and that there is an interaction with the H_2O_2 signalling pathway⁸³ (FIG. 3a).

Redox signals

As light intensity fluctuates, the chloroplast modulates the expression of photosynthesis-related genes to maximize photosynthesis and decrease ROS production. The redox state of the photosynthetic electron transport chain (PET), which is the link between photosynthetic light reactions and metabolism, fluctuates owing to varying light intensity and quality, and has been shown by microarray analyses to exert retrograde control of nuclear gene expression of photosynthetic genes in *A. thaliana*⁸⁴. Two main sources of retrograde signals have been proposed that involve assembled PET in developed chloroplasts; the redox state of the plastoquinone pool⁸⁵ and the photosystem I (PSI) acceptor site⁸⁶ (FIG. 3b).

Unlike ROS signalling, which is used as a stress response, the redox signal appears to be used to modulate metabolism in response to fluctuating light conditions⁸⁴. Mechanistically, little is known about the redox retrograde signal(s). However, genetic analyses in *A. thaliana* have identified components in chloroplast redox signalling including STN7 (REF. 87), a dual-function thylakoid protein kinase that is required for state transitions and photosynthetic acclimation, and five *redox imbalanced (rimb)* mutants, which have uncoupled transcriptional control of the nuclear-encoded chloroplast antioxidant enzyme 2-cys peroxiredoxin from the redox state of the PSI acceptor site⁸⁸.

The nuclear response to chloroplast retrograde signals

To understand how the nucleus integrates various retrograde signals from the chloroplast, two studies analysed the accumulation of over 3,000 nuclear gene transcripts of *A. thaliana*, most of them encoding chloroplast-localized proteins, under 101 different genetic and environmental conditions^{89,90}. This work led to the conclusion that, together, these retrograde signalling pathways result in several layers of transcriptional regulation of nuclear genes (that is, up, down or mixed response). Overall, these chloroplast-protein-encoding genes comprise 23 ‘regulons’ of co-expressed, but physically dispersed, sets of genes⁹⁰. The existence of a ‘master switch’ was proposed because most of these regulons are predominantly repressed or induced in over half of the conditions. The involvement of GUN 1 and AB14 in several retrograde pathways suggests that they could be part of this regulatory system in plants⁶².

Mitochondrion-chloroplast cross-talk

In plant cells, chloroplasts and mitochondria have complex metabolic interdependencies⁹¹. For instance, photosynthesis can use mitochondrial products (that is, CO₂ and ATP) and it provides compounds for mitochondrial respiration (that is, O₂ and malate). Mitochondria dissipate redox equivalents from the chloroplast, which protects the chloroplast from photoinhibition. The chloroplast provides haem precursors to the mitochondria⁹² and metabolic compounds that are involved in photorespiration, such as serine and glycine, are also exchanged through the peroxisome⁹¹. However, there is no evidence of direct signalling between chloroplasts and mitochondria, although molecules such as active oxygen species and ascorbate, and mitochondrially produced nitric oxide, have been suggested as candidate signalling molecules⁹¹. Additionally, the exchange of redox equivalents could potentially alter organelles’ redox environments and affect gene expression.

The existence of cross-talk signalling mechanisms has mostly been surmised from genetic studies. Mutations affecting glycine decarboxylase^{93,94} and NADH dehydrogenase^{95,96} activities in mitochondria led to impaired photorespiration and reduced photosynthesis, respectively. In the other direction, mutations in the *C. reinhardtii* chloroplast have been shown to act as mitochondrial mutant suppressors, suggesting that tRNA exchange occurs between the organelles⁹⁷.

Other cases suggest that chloroplast–mitochondrion cross-talk involves a retrograde signal from one of the organelles that modulates the anterograde control of the other. In *C. reinhardtii*, activation of the cytochrome respiratory pathway in mitochondria leads to an increase in expression of photosynthesis-related genes in the nucleus⁹⁸. In mutant barley cells that lack chloroplast ribosomes, the nuclear-encoded mitochondrial RNA polymerase is upregulated, resulting in increased mitochondrial transcription⁹⁹. The redox state of the plastoquinone pool in chloroplasts affects transcription of the mitochondrial RNA polymerase in the nucleus⁸⁴. The maize *non-chromosomal stripe6 (ncs6)* mutant has a dysfunctional mtETC that leads to a decrease in both chloroplast- and nuclear-encoded subunits of PSI¹⁰⁰. It remains to be determined whether mitochondria are signalling directly to the chloroplast or indirectly to the nucleus to affect anterograde mechanisms. Simultaneous retrograde signals from both organelles (such as the gene expression pathway)

might also interact to affect nuclear gene transcription (FIG. 3d), although it remains to be revealed how this or any of these signals are perceived⁷⁴.

Conclusions

A major challenge to understanding the coordination of nuclear and organelle genomes will be not only to identify proteins that are involved in organelle gene expression, but to determine how their functions or levels are controlled by the nucleus and to place anterograde signalling into a larger context. Because organelle gene expression appears to occur mostly at the post-transcriptional level, the measurement of protein levels rather than transcript levels will be necessary to understand the effect of anterograde control. Our understanding of retrograde signal mechanisms remains limited, in part because the identity of many of the proteins involved is not known, particularly on the ‘receiving side’ in the cytoplasm or the nucleus. This might be due to protein redundancy, to the essentiality of these signalling factors or to overlapping retrograde signals masking the effects of mutations. Innovative genetic or biochemical screens in model organisms should help to identify more of the proteins and signals in these pathways. Other than being a central and important feature of eukaryotic cell physiology, the increasing implication of genome coordination in fertility, ageing and tumorigenesis will produce exciting future research in this field.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Our work on genome coordination is supported by grants from the US Department of Energy (J. C), and the Howard Hughes Medical Institute (J. C). J. W. is supported by the Ruth L. Kirschstein National Research Service Awards postdoctoral fellowship from the National Institutes of Health. The authors wish to thank S. S. Orchard, G. H. Anderson, A. Nott, and J. M. Pérez-Ruiz for their reading and discussion of the manuscript.

Glossary

Amyloplasts	A non-pigmented heterotrophic type of plastid that is used for starch storage, mostly found in underground tissues such as roots and tubers. When the plant requires energy amyloplasts can convert the stored starch into usable sugars
Chromoplasts	Plastids that are dedicated to pigment synthesis and storage. Chromoplast colours vary according to the type of pigment they contain, and they are responsible for the distinctive colours of fruits and of flower petals
Pioneer protein	A protein that has no known homologue in other species. Therefore, pioneer proteins often lack any identifiable sequence motifs other than those that are used for protein localization

De-etiolation	In plants, de-etiolation is the irreversible process of shifting from a heterotrophic dark-grown (etiolated) lifestyle to a phototrophic light-grown lifestyle. De-etiolation involves both morphological and physiological changes that are needed to acclimatize to light and to harvest its energy
Greening	The shift of non-green plant seedlings grown in the dark (etiolated) to a green colour as a result of chloroplast biogenesis and chlorophyll accumulation during the process of de-etiolation

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BOX 1**Why maintain an organellar genome?**

The gene composition of organellar genomes varies between species, but they usually consist of genes encoding part of the translation machinery: 70S ribosome subunits, a variable set of tRNA genes (that can be complete, incomplete or even absent) and a small (~2–4) and incomplete set of rRNA genes¹⁰¹. Most sequenced chloroplasts from photosynthetic organisms contain a core constituency of ~44 protein-encoding genes encoding subunits for transcription (bacterial-like plastid-encoded RNA polymerase), photosynthesis (photosystem I, photosystem II, cytochrome *b₆f* complex and the coupling ATPase) and the large subunit for ribulose-1,5-bisphosphate carboxylase/oxygenase¹⁰². Mitochondrial genomes encode protein subunits for respiration (cytochrome *c* biogenesis and complexes I–IV)⁴.

The transfer of organellar genes to the nucleus is presumed to be advantageous — it allows simpler gene coordination and reduces the risk of mutations from free-radical by-products of electron transfer reactions¹⁰³. Maintaining a separate genetic system in an organelle creates logistical challenges for the cell, as discussed in the main text, and imposes a high energetic cost; for example, in *Saccharomyces cerevisiae* ~25% of the mitochondrial proteome is dedicated to maintaining and expressing the 19 mitochondrial proteins¹⁰⁴. It is therefore unclear why the genes mentioned above are still found in organelle genomes. It seems reasonable to conclude that the complete transfer of organellar genes to the nucleus is evolutionarily difficult and is occurring slowly, or that there is an advantage to maintaining separate genomes. Either way, there needs to be communication between the nucleus and the organelle (retrograde and anterograde, as discussed in the main text) to coordinate the separate genomes.

Two principal and non-exclusive hypotheses (reviewed in REF. 103) have tried to explain this mystery. In the first hypothesis, proteins that are involved in photosynthesis and respiration cannot be efficiently transported from the cytoplasm to the organelle, because they might be too hydrophobic or they are toxic if allowed to accumulate in the cytoplasm¹⁰⁵. Alternatively, the efficient assembly of multi-subunit complexes might require on-site synthesis¹⁰⁶. The second hypothesis states that organellar proteins that are involved in redox reactions are encoded in the organelles to enable a rapid regulatory response to changes in the redox state of an organelle¹⁰³. This allows a single organelle to rapidly change the concentration of a particular protein without having to send a signal to the nucleus, which, in a cell with multiple chloroplasts and/or mitochondria, would have no way of responding only to that individual organelle. This hypothesis is supported, for example, by the transcriptional regulation of chloroplast photosystem protein-encoding genes by the redox state of the chloroplast in mustard plants¹⁰⁷. However, neither of these two hypotheses explains the retention of chloroplast genomes in non-photosynthetic organisms; other reasons must therefore exist for the incomplete transfer of organelle genes¹⁰¹.

BOX 2**Pentatricopeptide-repeat proteins**

Genome sequencing has revealed the existence of large families of proteins, the functions of which have not been assigned or validated experimentally. One such large group of proteins, the pentatricopeptide repeat (PPR)-containing proteins, are found in most eukaryotes and are important in organelle communication and development³⁴.

The PPR motif is a degenerate 35-amino-acid sequence similar to the tetratricopeptide repeat (TPR) motif that is involved in protein-protein interactions³⁶. Like TPR motifs, PPR motifs are predicted to form a pair of anti-parallel helices¹⁰⁸. It is suggested that tandem arrays of these motifs form a superhelical structure containing a groove that is lined with positively charged residues that allows for binding to nucleic acids in a sequence-specific manner³⁶. Whereas all known PPR proteins are nuclear-encoded, most are predicted to be targeted to chloroplasts or mitochondria, and most of the ~30 genetically characterized PPR proteins are proposed to affect post-transcriptional gene regulation in organelles by interacting with one specific RNA or a small set of RNA molecules (FIG. 2c; [Supplementary information S1](#) (table)). Studies in fungi, plants, protists, insects and humans have strongly implicated PPR proteins in processes such as RNA editing^{109,110}, transcript processing (splicing)^{111–113}, transcript stabilization^{33,39,112,113}, transcript translation^{111,114} or some combination of these functions^{111,114}. Although several PPR proteins bind RNA in vitro^{110,115}, they have not been shown to catalyse any RNA processing. Instead, PPR proteins might act as adaptors, recognizing specific RNA molecules and facilitating interactions with processing enzymes.

In plants, PPR proteins have a larger role in organelle communication than in other eukaryotes. Whereas animal genomes encode fewer than ten PPR proteins, plant genomes encode hundreds¹¹⁶. Most plant PPR proteins are predicted to be localized to mitochondria, indicating that the presence of chloroplasts cannot fully explain the reason that plants have so many PPR proteins¹¹⁶. Most of the cytoplasmic male sterility (CMS) *restorers of fertility* (*Rf*) genes encode PPR proteins^{71,13,116} (FIG. 2a; [Supplementary information S1](#) (table)), suggesting that CMS increases any selective pressure for PPR diversity in plants (BOX 4).

Although redundancy is common in large gene families in plants¹¹⁷, no redundancy in PPR proteins has been demonstrated. In fact, many PPR loss-of-function mutants have strong and/or lethal phenotypes, suggesting they have important and non-redundant roles in plant organelle physiology¹¹⁶. The severe phenotypes caused by some PPR loss-of-function mutants might be due to pleiotropic effects that are caused by the loss of a specific organelle gene product. Alternatively, PPR proteins might have direct roles in plant development and signalling, perhaps by regulating developmentally significant RNAs or by being involved in an unidentified developmental process. This interpretation is supported by the PPR protein GENOMES UNCOUPLED 1 (*GUN1*), which functions in many retrograde signalling pathways in chloroplasts⁶². Whichever is the case, the

study of PPR proteins should see some fascinating developments into the understanding of this large group of proteins and their involvement in genome coordination.

BOX 3**Retrograde signalling: yeast, mammals and tumorigenesis**

The budding yeast, *Saccharomyces cerevisiae*, is an ideal system for understanding mitochondrial retrograde signalling; as yeast do not require mitochondria to ferment sugars it is possible to propagate cells with severe mitochondrial defects. Most of the work on the retrograde pathway has focused on the induction of the nuclear-encoded peroxisomal citrate synthase (Cit2) as an adaptive response to reduced respiration caused by mitochondrial dysfunction^{8,9}. In addition to increasing Cit2, which allows yeast to use acetate and ethanol as sole carbon and energy sources, the retrograde pathway induces α -ketoglutarate production in mitochondria to ensure glutamate pools meet the demand for nitrogen^{8,9}. It is unclear whether these signals are conserved in other phyla, because the regulators of these pathways have been identified only in yeast⁸.

These signals have also been implicated with ageing in yeast: the loss of mitochondrial DNA (mtDNA) extends the life span of some strains and is dependent on the retrograde positive regulator Rtg2 (REF. 118). Rtg2 might reduce the formation of extrachromosomal ribosomal DNA circles, which decrease the life span of yeast¹¹⁹. Mutations that affect ubiquinone biosyntheses, and thus mitochondrial function, increase the life spans of both *Caenorhabditis elegans*¹²⁰ and mice¹²¹, suggesting a conserved role of retrograde signalling and ageing across kingdoms. Although the decrease of reactive oxygen species might be involved, it is unclear how mitochondrial retrograde signals influence ageing in any species.

In mammalian cells, mitochondrial dysfunction also leads to retrograde signals that influence nuclear transcription. The signal involves increased Ca^{2+} levels in the cytoplasm, possibly owing to a change in the mitochondrial membrane potential or a reduced efflux of Ca^{2+} into storage organelles that is due to the decrease in ATP production⁹. The subsequent activation of cytosolic calcineurin and Ca^{2+} -dependent kinases might then start a signalling cascade that induces transcription factors including nuclear factor-kappa B, nuclear factor of activated T-cells and activating transcription factor^{9,122}.

Recently, a link between retrograde signalling and tumorigenesis has been suggested. Inducing the retrograde response by depleting mitochondria of their DNA, or by using inhibitors to block respiration, increases the invasive behaviour of otherwise non-tumorigenic rhabdomyoblasts¹²³, of human lung carcinomas^{123,124} and of human osteosarcomas¹²⁵, possibly by increasing Ca^{2+} levels followed by induction of calcineurin and Ca^{2+} -dependent kinases^{123,124}. Furthermore, retrograde signals that are elicited in response to mitochondrial dysfunction increase the resistance to apoptosis and the levels of anti-apoptotic proteins^{122,126}, and reduce the levels of pro-apoptotic proteins¹²⁶. Proteomic analyses of mtDNA-depleted rat myocyte¹²⁷ and human osteosarcoma¹²⁵ cells have detected altered levels of cell-cycle and apoptosis-related proteins, which is often observed in tumour cells. Because resistance to apoptosis is a hallmark of cancer cells at advanced stages of tumour progression, future work in this

field should determine whether mitochondrial dysfunction and the activation of retrograde signalling are contributing factors in cancer.

BOX 4**Cytoplasmic male sterility in plants**

In plants, cytoplasmic male sterility (CMS) is a maternally inherited phenotype that is characterized by the inability to produce fertile pollen^{7,128}. CMS has been characterized in over 150 plant species and is associated with aberrant mitochondrial genes (hence the maternal inheritance). The ~14 characterized mitochondrial genes that determine CMS are chimeric ORFs that often encode part of the ATP synthase gene fused to unique sequences. These fused genes are usually gain-of-function alleles that can lead to mitochondrial dysfunction, without altering the general phenotype of the plant. Instead, CMS lines are usually specifically defective in producing fertile pollen — which can be caused by abnormal pollen development, degeneration of male reproductive organs (stamens) or their components, or homeotic changes (stamens converted to non-reproductive petals or other organs)^{7,128}.

The exact causes of CMS are unknown, but in cases in which there are homeotic changes, retrograde signals from dysfunctional mitochondria might lead to transcriptional changes in nuclear genes that control organ identity and patterning¹²⁸ (FIG. 3f). Recent studies have associated CMS phenotypes with decreased expression of nuclear genes that encode class-B function MADS-box transcription factors (that is, *APETALA3*, *GLOBOSA*, *DEFICIENS*) that control flower morphology^{7,128,130}. This could be caused by a reduction of mitochondrial ATP that prevents the proteolysis of negative regulators of class-B gene expression, or by an unidentified mechanism¹²⁹. Other types of CMS might also be caused by mitochondrial retrograde signals. A microarray analysis of a CMS line of rice that produces morphologically normal but infertile pollen revealed ~140 differentially regulated nuclear genes in mature anther cells, but not in leaf, root or stem cells when compared with fertile lines^{44,131}.

CMS phenotypes can be reversed by nuclear *restorers of fertility* (*Rf*) genes that reduce the accumulation of the aberrant mitochondrial CMS protein^{7,34} (FIG. 2A). The one exception is *Rf2*, from Texas-type CMS maize, that encodes a mitochondrial-localized aldehyde dehydrogenase and restores fertility by an unknown mechanism¹³². All other cloned *Rf* genes encode mitochondrial-localized pentatricopeptide repeat (PPR) proteins (BOX 2, [Supplementary information S1](#) (table)), most of which act dominantly. There is no conserved mechanism to reduce production of the CMS protein, but PPR proteins probably act post-transcriptionally on CMS mRNA transcripts by promoting endonucleolytic cleavage^{113,133}, editing¹¹³ and destabilization^{113,134}, or by reducing translation^{113,134} ([Supplementary information S1](#) (table)).

In some circumstances, CMS can be advantageous as it allows female plants to avoid investing resources in pollen production and might also lead to reactive oxygen species resistance owing to retrograde-signalled stress responses. The diversity in *Rf* alleles, however, suggests that there is strong selective pressure to restore fertility, and this interaction between the two genomes has been dubbed the ‘intragenomic arms race’¹³⁵.

This cross-talk between the mitochondria and nucleus in CMS lines should open opportunities to understand both anterograde and retrograde signalling in plants.

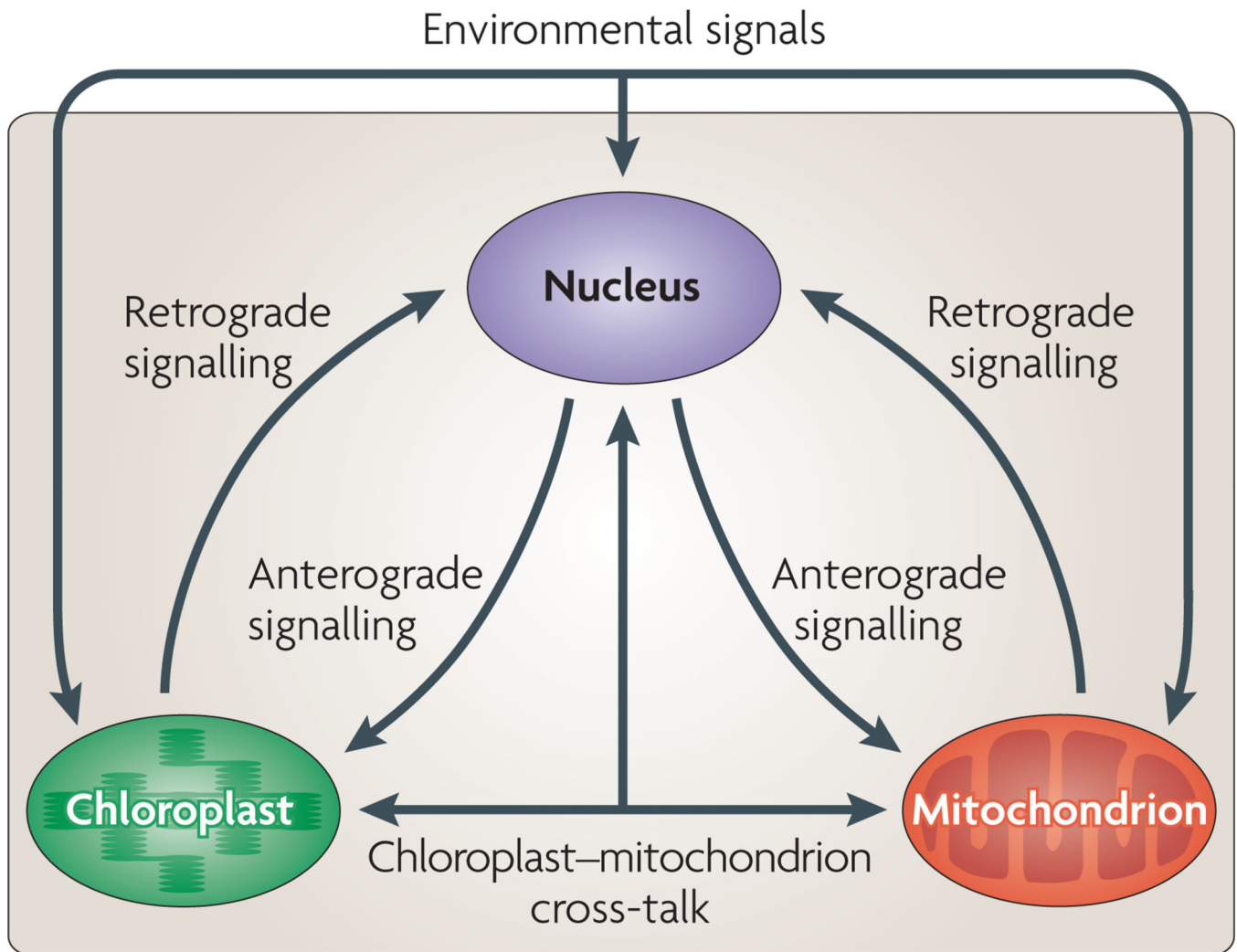


Figure 1. An overview of genome co-ordination between the nucleus and intracellular organelles
 The diagram depicts communication between the nucleus, chloroplast and mitochondrion. Details of anterograde signalling and retrograde signalling between the nucleus and the organelles, and of chloroplast-mitochondrion cross-talk are discussed in the main text. Environmental signals such as stress, oxygen or nutrient availability, light intensity or quality, developmental cues, and hormones affect the expression of nuclear genes that encode organellar proteins. This process will, in turn, affect organelle function and gene expression through anterograde mechanisms. Chloroplasts and mitochondria are also able to sense certain environmental conditions and stimuli that can affect their functional activities, for example, light intensity or quality (chloroplasts) and O₂ availability (mitochondria). Using retrograde signals, organelles communicate these received stimuli and their functional status to the nucleus, which leads to nuclear gene regulation.

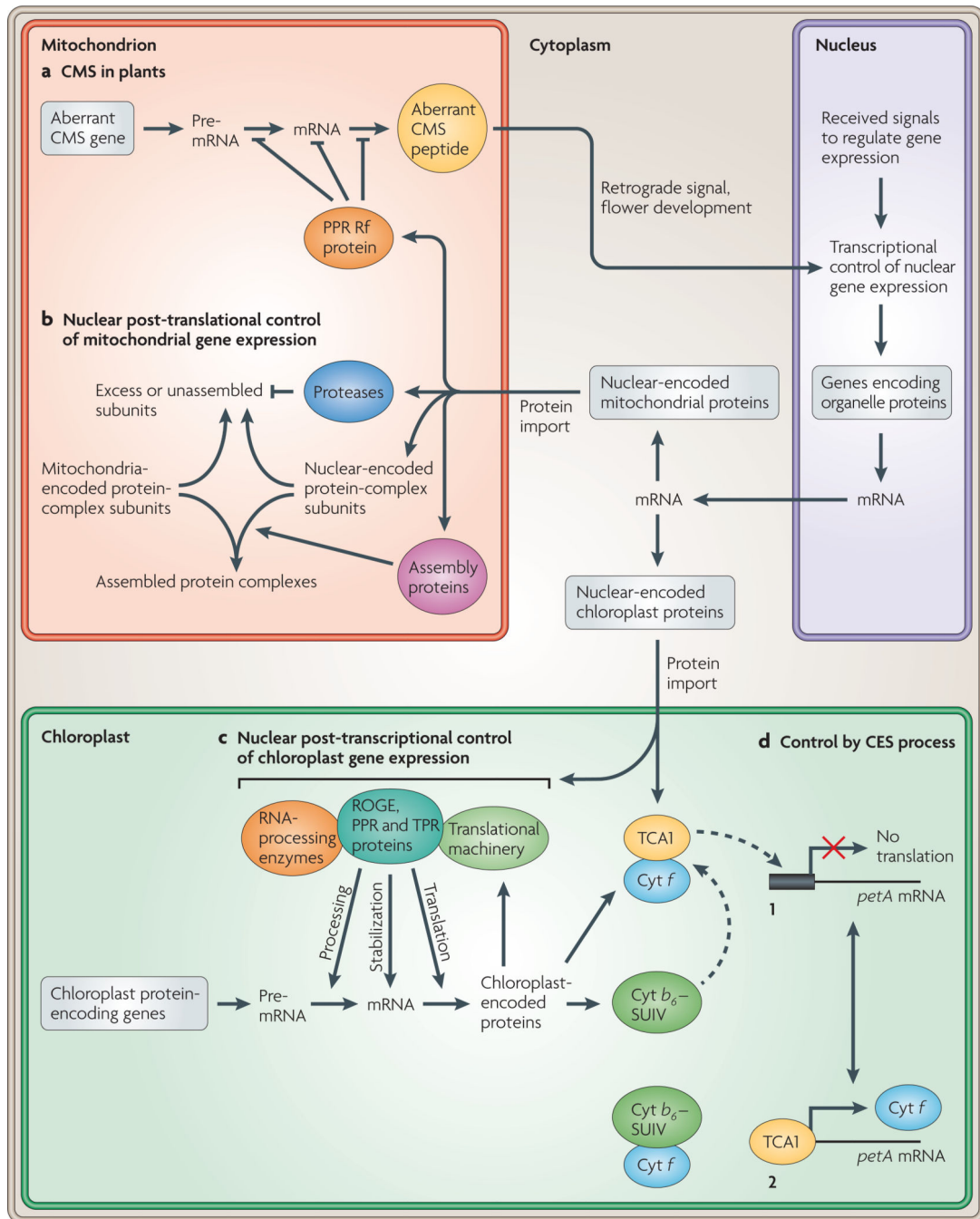


Figure 2. Nuclear anterograde control of organelle gene expression

A generalized model of the coordination of organelle gene expression through nuclear anterograde control in eukaryotes. Several processes are highlighted. **a** | In flowering plants, restorers of fertility (Rf) proteins reverse the cytoplasmic male sterility (CMS) phenotype by reducing the expression of the aberrant mitochondrial gene using post-transcriptional mechanisms, **b** | Post-translational control of mitochondrial gene expression using nuclear-encoded proteases and assembly proteins, **c** | Post-transcriptional control of chloroplast gene expression using nuclear-encoded regulators of organelle gene expression (ROGE) proteins

that target specific RNA transcripts, **d** | Control by epistasy of synthesis (CES) is an autoregulatory process in which unassembled organelle proteins repress their own translation. The autoregulation of the *petA* gene encoding the cytochrome *f* subunit (Cyt *f*) of the cytochrome *b₆f* complex in *Chlamydomonas reinhardtii* is used as an example of the CES process. In scenario 1, unassembled Cyt *f* binds the ROGE protein translation factor TCA1, and translation of the *petA* mRNA is inhibited. In scenario 2, the presence of cognate protein subunits cytochrome *b₆* (Cyt *b₆*) and cytochrome subunit IV (SUIV) assemble with Cyt *f*. TCA1 is now able to bind *petA* mRNA and activate its translation. A discussion of which proteins are organelle encoded can be found in BOX 1. A more complete model of retrograde signalling than that highlighted in panel **a** can be viewed in FIG. 3. Proteins and protein complexes are designated as ovals. PPR pentatricopeptide repeat; TPR, tetratricopeptide repeat.

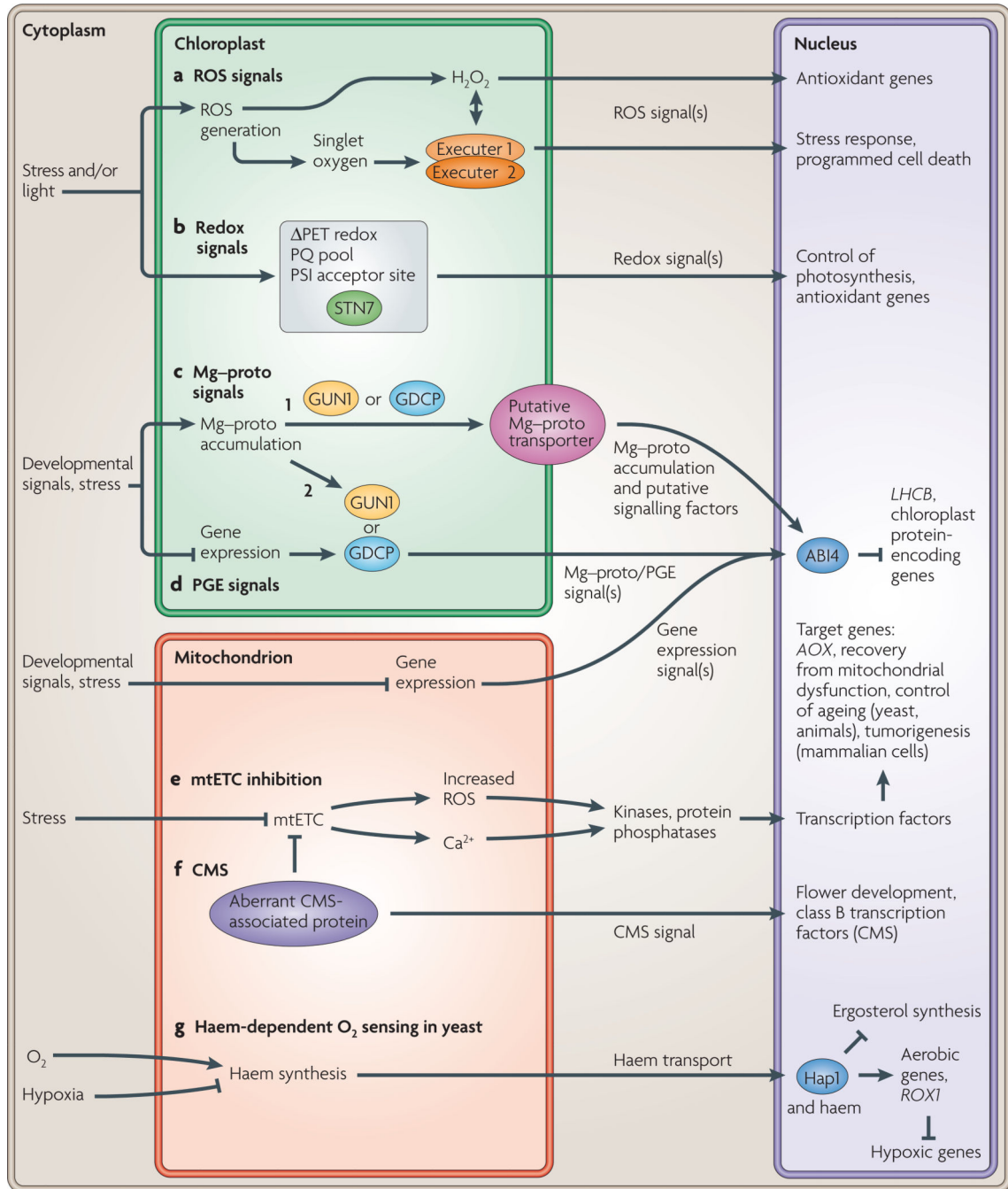


Figure 3. Retrograde signalling pathways and chloroplast-mitochondrion cross-talk in higher plant cells

This figure depicts chloroplast-to-nucleus and mitochondrion-to-nucleus retrograde signalling pathways in the higher plant cell (and in yeast and animals where noted). Seven different pathways are highlighted, **a** | The use of chloroplast-generated reactive oxygen species (ROS) to induce nuclear gene transcription, **b** | Control of nuclear gene regulation by the redox state of the photosynthetic electron transport chain (PET), **c** | Chloroplast Mg-protoporphyrin IX (Mg-proto) accumulation, **d** | Inhibition of plastid gene expression

(PGE). **c** and **d** lead to the repression of nuclear-encoded chloroplast protein genes. Signals from inhibited mitochondrial gene expression act synergistically with the PGE pathway. Two putative Mg-proto signalling pathways are depicted: in pathway 1, GENOMES UNCOUPLED 1 (GUN1) or a putative GUN1-dependent chloroplast protein (GDCP) facilitate the export of Mg-proto from the chloroplast where it interacts with cytoplasmic signalling factors; in pathway 2, GUN1 or GDCP sense Mg-proto accumulation and other retrograde signals within the chloroplast and send an unidentified signal to the nucleus to control transcription of chloroplast protein-encoding genes, **e** | Mitochondrial electron transport chain (mtETC) dysfunction leads to transcriptional changes in the nucleus in several phyla, **f** | An aberrant mitochondrial protein leads to cytoplasmic male sterility (CMS) by affecting nuclear gene expression, **g** | Mitochondrial haem synthesis as a cellular sensor for O₂ availability in yeast. Proteins that are known to be involved in these pathways are designated as ovals. ABI4, abscisic acid insensitive 4; *AOX*, gene encoding the mitochondrial alternative oxidase; HAP1, haem activation protein; *LHCB*, gene encoding photosystem II chlorophyll a/b-binding protein; PQ, plastoquinone; PSI, photosystem I; ROX1, repressor of hypoxic genes; STN7, a thylakoid protein kinase.