Title: Retrograde signals from endosymbiotic organelles: a common control principle in eukaryotic cells

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Abstract

Endosymbiotic organelles of eukaryotic cells, the plastids, including chloroplasts, and mitochondria, are highly integrated into cellular signalling networks. In both heterotrophic and autotrophic organisms, plastids and/or mitochondria require extensive organelle-to-nucleus communication in order to establish a coordinated expression of their own genomes with the nuclear genome that encodes the majority of the components of these organelles. This goal is achieved by the use of a variety of signals that inform the cell nucleus about the number and developmental status of the organelles and their reaction to changing external environments. Such signals have been identified in both photosynthetic and non-photosynthetic eukaryotes (known as retrograde signalling and retrograde response, respectively) and, therefore, appear to be universal mechanisms acting in eukaryotes of all Kingdoms. In particular, chloroplasts and mitochondria both harbour crucial redox reactions that are the basis of eukaryotic life and are, therefore, particularly susceptible to stress from the environment, which they signal to the rest of the cell. These signals are crucial for cell survival, life span and environmental adjustment, and regulate quality control and targeted degradation of dysfunctional organelles, metabolic adjustments, developmental signalling as well as induction of apoptosis. The functional similarities between retrograde signaling pathways in autotrophic and non-autotrophic organisms are striking, suggesting the existence of common principles in signalling mechanisms or similarities in their evolution. Here, we provide a survey for the newcomers to this field of research and discuss the importance of retrograde signaling in the context of eukaryotic evolution. Furthermore, we discuss commonalities and differences in retrograde signaling mechanisms and propose retrograde signaling as a general signaling mechanism in eukaryotic cells that will be also of interest for the specialist.

Keywords

Eukaryotic cell, plastids, mitochondria, intracellular communication, signalling, metabolites, chloroplasts

Introduction

Life on Earth can be subdivided into three different major domains, the bacteria, the archaea and the eukarya (Woese et al. 1990). Typically, only the latter (maybe with the exception of some actinomycetes) form complex, multicellular organisms since they posses a number of specific structural and functional properties that are not found in the other two domains. One of the most important features of the eukarya is their high degree of intra-cellular compartmentalization giving rise to a number of membrane-bound structures and organelles with specific biochemical activities. The most prominent is the cell nucleus that is not present in bacteria and archaea. Therefore, these two are often summarized as prokaryotes while the eukarya are distinguished from them as eukaryotes by having a "true nucleus“, the literal meaning of the term (Woese et al. 1990).

In prokaryotes the genomic DNA is localized within the cytoplasm together with all other soluble cell components and these are all enclosed by a plasma membrane. In eukaryotes the DNA is surrounded by a double-membrane forming the nucleus and separating the genetic material from the rest of the cell. Pores in the nuclear envelope membrane allow an exchange of molecules and a controlled transcription of genes within the nucleus followed by an export of RNAs into the cytoplasm where translation takes place. By this means, transcription and translation can be spatially and temporally separated providing additional levels of regulation in gene expression that are not present in prokaryotes (Meier et al. 2017).

The internal compartmentalization in eukaryotic cells not only separates transcription and translation, but also provides opportunities to separate specific biochemical pathways within the same cell, therefore providing a number of advantages in eukaryotic metabolism when compared to prokaryotes. This includes the avoidance of futile cycles, the localized concentration of certain metabolites or the separation of subcellular environments with different pH or ion concentrations. The latter has an important impact on energy metabolism allowing for ATP production through membrane-based proton-gradients in both mitochondria and chloroplasts (Diekmann and Pereira-Leal 2013). All of these advantages were highly beneficial for further evolution of eukaryotic organisms leading finally to multicellular organisms.

Biochemical compartments of eukaryotic cells

Besides the nucleus, all eukaryotic cells possess a set of subcellular membrane-bound compartments that allow for metabolic specialization in eukaryotic cells. These include the endoplasmic reticulum (ER) with the associated Golgi apparatus and vesicles, the peroxisomes, the mitochondria and, in some evolutionary lines, the plastids (Diekmann and Pereira-Leal 2013). The ER is a complex, single-membrane delimited structure that is directly connected to the nuclear envelope membrane and can either be associated with ribosomes (rough ER) or not (smooth ER). The ER provides a specific compartment for protein formation and maturation, and gives rise to an evolutionarily novel intracellular trafficking pathway, vesicular transport. The latter allows the passage of vesicle-enclosed components between the Golgi apparatus, endosomes and the plasma membrane. Both the ER and Golgi represent very dynamic compartments supporting secretion and absorption properties of cells. Peroxisomes, in contrast, are small, relatively stable organelles with particular redox-related activities often used or involved in detoxification of reactive oxygen species (ROS) or other metabolic compounds (Cohen et al. 2018).

Mitochondria and chloroplasts are the energy converting organelles of eukaryotic cells with chloroplasts being restricted to photosynthetically active eukaryotes (Allen 2015). In this context it is important to note that eukaryotic organisms, like prokaryotes, can be metabolically subdivided into autotrophic and heterotrophic organisms. Autotrophic eukaryotes can directly assimilate CO_2 through photosynthesis in chloroplasts while heterotrophic eukaryotes need to feed on organic carbon sources by respiration in mitochondria. All autotrophic eukaryotes, therefore, carry chloroplasts. These are, however, only one form of plastid (Pyke 2007). Plastids are morphologically heterogenous and also appear in a number of non-photosynthetic forms. The inverse conclusion that all heterotrophic eukaryotes do not carry plastids, thus cannot be drawn and, in fact, is not true (for more details see below).

Mitochondria and plastids are special among all eukaryotic cell structures in that they were not generated by evolution of the cell itself, but instead they were acquired by endosymbiosis. Endosymbiosis is the engulfment and functional as well as structural integration of an independent unicellular organism into another cell. This evolutionary concept was denied for many years by many scientists using classical observational methodologies, but modern molecular biology provided unequivocal evidence for its occurence (Archibald 2015). Typical features of this endosymbiotic ancestry for both mitochondria and plastids are the double-membrane envelope with a eukaryotic-like lipid composition of the outer membrane and a prokaryotic-like composition on the inner; the presence of an organellar genome; and the existence of a corresponding gene expression machinery including bacterial-type 70S ribosomes. Furthermore, both organelles multiply by fission and their inheritance is by random distribution during division of the host cell, often in an uni-parental manner (Pyke 2010; Archibald 2015).

Endosymbiotic ancestry and the requirement for organelle-to-nucleus communication

According to generally accepted hypotheses the endosymbiotic event leading to mitochondria took place first. An α-proteobacteria-like organism was integrated into a more complex host cell. However, it is still debated when this took place, which species of bacteria was integrated and the nature of the host (Archibald 2015; Martin et al. 2015; Imachi et al. 2020; Martijn et al. 2018). The aquisition of chloroplasts, in contrast, is better understood as it took place after the establishment of mitochondriated cells. It is estimated that a cyanobacteria-like ancestor was integrated into a eukaryotic host cell around 1.5 - 1.2 billion years ago (Parfrey et al. 2011) and this led to the first photosynthesizing eukaryotes (Fig. 1).

Both endosymbiotic events were not immediate but gradual and took place over a very long time scale accompanied by successive rounds of horizontal gene transfer from the endosymbiont to the host cell (Fig. 1). This process largely contributed to the evolutionary integration of the endosymbiont into the cellular structure of the eukaryotes leading to the contemporary phylae (Timmis et al. 2004). Genome sequencing and modern bioinformatic analyses have contributed a great deal to our understanding of these complex evolutionary processes as they can track the relationships between genomes of species as well as of endosymbiotic organelles (Martin et al. 2002).

While quite precise models exist that describe the horizontal gene transfer during evolution, much less is known about the regulatory consequences of this transfer for the establishment

of functional organelles. As already mentioned, organelles carry their own genetic material and are able to express it. It has long been debated why organelles retained their genomes and did not lose all of their genes and different explanations have been proposed (Björkholm et al. 2015). The two hypotheses that provide the best explanations to date to explain the existence of organellar genomes are i) the need for rapid control by redox signals from the respective electron transport chains (ETCs) (Allen 2017) and ii) that the high hydrophobicity of membrane-located ETC components would generate problems for effective organelle targeting when encoded in the nucleus (von Heijne 1986). In fact, it has been proposed that hydrophobic membrane proteins would be targeted to the ER instead of mitochondria (Björkholm et al. 2015).

Typical mitochondrial genomes of heterotrophs such as mammals are quite small (~ 16 kb) while those of plants are less reduced (see Table 1). Chloroplast genomes of vascular plants are around 150 kb in size and are highly conserved in gene arrangement and number (approx. 120 genes) (Table 1). By contrast, proteomic analyses have revealed that mitochondria and plastids contain thousands of different proteins and that the corresponding composition is highly variable depending not only on environmental conditions or tissue context, but also on the species (Lee et al. 2012; Prokisch et al. 2004; Szklarczyk and Huynen 2009; von Zychlinski et al. 2005; Zybailov et al. 2008; Ferro et al. 2010; Calvo et al. 2016). In conclusion, contemporary organelles have lost the majority of their ancient coding capacity (most likely thousands of genes) and need to import the vast majority of their proteins from the cytosol *via* specialized import machineries (Chacinska et al. 2009; Jarvis and Lopez-Juez 2013). As a consequence it was believed that the nucleus dominates the protein content of the organelles in that cell. This regulation principle is called “anterograde signalling“ (Woodson and Chory 2008). However, the imported nuclear-encoded proteins typically represent structural organellar components that do not exert direct signalling functions. A better term might therefore be “anterograde control“.

Once imported, most cytosolic proteins need to be assembled into protein complexes together with organelle-encoded proteins. One recurrent pattern in organelles of all species is the observation that all major protein complexes of the ETCs contain subunits encoded in both nuclear and organellar genomes. Since the organellar genomes in their general structure are polyclonal and dozens to hundreds of organelles exist per cell, a 10,000 fold excess in coding capacity for organellar genes can easily exist in the same cell. For the coordinated expression of nuclear and organellar encoded subunits, therefore, there must be some type of communication or signaling from the organelles to the nucleus which informs the nucleus about the structural and functional state of the organelles and its internal protein complexes. Pioneering work confirming the existence of such signals from mitochondria was performed in yeast, while signals from plastids were discovered in barley (Jazwinski 2014; Börner 2017). This type of regulation from organelles was later termed “retrograde signalling“, “retrograde regulation“ or “retrograde response“ (Woodson and Chory 2008). Recent research has uncovered numerous retrograde signalling pathways that report the current status of organelles to the nucleus under a variety of conditions and developmental situations. These signals inform the nucleus about the functional requirements of the organelles and also adjust nuclear gene expression for the highly divergent gene copy numbers between the nuclear and organellar genomes. For the establishment of true organelles during endosymbiosis, the generation of a network of retrograde signals thus became indispensable for evolution of the endosymbiont (Fig. 1). For integration into the host

cell the establishment of retrograde signals must be regarded as equally important as horizontal gene transfer from the endosymbiont to the cell nucleus.

Classes of retrograde signals from organelles

For plastids, the original idea of a single plastid signal (or plastid factor) has been modified substantially over the last two decades and it is now widely accepted that a whole array of signals exists (Pfannschmidt 2010). Currently five major signal classes can be distinguished: 1) signals that originate from plastid gene expression (Chan et al. 2016; de Souza et al. 2016), 2) signals mediated by the tetrapyrrole biosynthesis pathway (Terry and Smith 2013), 3) signals that depend on redox changes of components in the photosynthetic electron transport as well as coupled redox buffer systems and reactive oxygen species (ROS) (Pfalz et al. 2012; Exposito-Rodriguez et al. 2017b), 4) metabolic signals from disturbed or unbalanced plastid metabolism mediated by changes in the accumulation of 3´-phosphoadenosine 5´-phosphate (Estavillo et al. 2011), 2-C-methyl-D-erythritol 2,4-cyclodiphosphate (Xiao et al. 2012), ß-cyclocitral (a ROS-dependent oxidation product; Ramel et al. 2012) or apocarotenoids (Cazzonelli et al. 2020) and 5) signals mediated by dual-localized proteins acting in both the plastids and the nucleus (Krause et al. 2012). Most of these signals become active under defined conditions depending on the developmental stage of the plastid, the tissue context it is in or the environmental conditions the organism is exposed to. Some act simultaneously or together and a functional classification of these signals is not easy as quite diverse molecular mechanisms for their respective signalling functions have been described or suggested. For plastidial signals a categorization into *biogenic*, *operational* and *degradational* signals (Pfannschmidt and Munne-Bosch 2013; Pogson et al. 2008) has been now widely accepted as they align well with the respective developmental and/or functional state of the plastid. The group of *biogenic signals* typically comprises signals sent from plastids that are undergoing biogenesis and has only been studied in the context of chloroplast development. These signals adjust nuclear gene expression to meet the requirements for the establishment of novel organelles within growing or multiplying cells and organisms. In contrast, the group of *operational signals* represents signals that are sent from fully developed, functional plastids in response to changes in their immediate environment. Here, plastids display a sensor function that informs the cell nucleus about environmental influences that impact on the metabolism of the organelle. Retrograde operational signals then trigger appropriate cellular responses that re-balance organellar and wider cellular metabolism. Finally, *degradational signals* are retrograde signals sent from plastids that have become degraded or destroyed in response to external or internal stresses. These signals manage the controlled destruction of plastids (e.g. by autophagy) and the corresponding resource allocation of free compounds such as amino acids, lipids and so on. By this means retrograde signals provide an appropriate management of organelle function in the cell.

Signals from mitochondria are known as “retrograde response“ or “retrograde regulation“ and have been well characterized in animals and fungi. Despite a great variability in the signalling pathways between animal classes (e.g. mammals, worms, insects) it is possible to clearly distinguish three classes of signals: 1) signals emitted under energetic stress, 2) signals involving Ca^{2+}-dependent responses, and 3) signals mediated by ROS under a number of stresses (Quiros et al. 2016). Mitochondria show less variability in morphology than plastids but they display a remarkable flexibility in their metabolic activity. Retrograde signals from

mitochondria, therefore, are involved in a high number of reactions that affect cellular homeostasis and trigger appropriate adjustments to various stresses e.g. metabolic imbalances, energetic limitations, oxidative stress or disturbance of mitochondrial biogenesis and quality control. In heterotrophs, these stresses have a strong impact on mitochondrial to nucleus (mito-nuclear) feedback, the integrated stress response and life span regulation, as well as on extracellular communication (Quiros et al. 2016). In plants, we are only just beginning to understand the retrograde response, but considerable progress has been made in recent years (see below) and many new reports suggest a similarly important role as that observed in heterotrophs.

Retrograde signalling from plastids

In multicellular organisms such as plants, chloroplasts can be found in all green tissues where they perform photosynthesis. This is the most common form of plastid, but there are a great variety of other non-photosynthetic plastid types that are associated with other functions. Roots and other non-photosynthetic tissues contain colour-less amyloplasts that store starch, in fruits or flowers yellow or orange/red chromoplasts synthesize carotenoids to provide tissues with attractive colours and in seeds elaioplasts perform lipid storage. However, none of these forms are fixed and plastids can even switch between different forms depending on external conditions (Pyke 2007). All of these plastid types develop from a non-differentiated precursor, the proplastid, that is found in meristems and in seeds. In most plant species it is inherited by the egg cell. Thus, mutants with plastid defects often show a maternal inheritance. Nevertheless, despite their different appearance, the plastid genome qualitatively is the same in all cases and their respective function depends only on the protein composition determined by the anterograde control pathways that are activated by the respective tissue context (Lopez-Juez and Pyke 2005). It should be noted, however, that not much is known about the gene copy number in different plastid types and quantitative effects cannot be excluded (Morley and Nielsen 2016).

Retrograde signalling from plastids during chloroplast biogenesis (biogenic signalling) – One of the major plastid transitions takes place when plant seedlings move from darkness to light. During this critical time, photoreceptor-mediated signalling drives the change from a skotomorphogenic to a photomorphogenic growth strategy including the rapid development of chloroplasts from etioplasts (another plastid type) or proplastids. Chloroplast development is mediated by internal changes following light-dependent conversion of the chlorophyll precursor protochlorophyllide to chlorophyllide and by the induction of thousands of nuclear genes required for the assembly of the photosynthetic apparatus and to enable other chloroplast functions (Jarvis and López-Juez 2013; Pogson et al. 2015). As discussed earlier, nuclear control of chloroplast development is mediated via anterograde signalling. However, as with any assembly process, information needs to be fed back about the status of what is being built. In this case, information about the state of chloroplast development is signalled to the nucleus to modulate this transcriptional response. While we currently know rather little about how this signal is produced and transmitted, its existence can be demonstrated by looking at expression of nuclear-encoded transcripts in mutants that affect chloroplast development or when development is blocked by inhibitors. For example, the commonly used inhibitors Norflurazon (NF), that blocks carotenoid biosynthesis resulting in chloroplast photobleaching, or lincomycin (Lin), a plastid translation inhibitor, both result in inhibition of about 1000 nuclear genes (Koussevitzky et al. 2007; Woodson et al. 2013; Page

et al. 2017). To date this work has focused predominantly on the model plant Arabidopsis, but the article by Duan et al in this issue has started to investigate this system in the model monocot rice (Duan et al. 2020). Differences observed between species may help us identify the key conserved features of this response. The genes down-regulated after chloroplast damage not only encode proteins destined for the developing chloroplast, but also for many others functions consistent with the integration of the chloroplast into cellular metabolism and more broadly in different cell types and tissues (Larkin 2014). In this issue, one example of such regulation is addressed by Richter et al. who described regulation of anthocyanin synthesis by retrograde signalling (Richter et al. 2020). In addition to significant transcriptional regulation, there is also evidence that retrograde signals affect gene expression post-transcriptionally (Wu et al. 2019b) including though mRNA splicing (Petrillo et al. 2014) and regulation of protein abundance (Tokumaru et al. 2017).

The physiological interpretation of the effects of inhibitor treatments is still a subject of debate. While it might be expected that developing chloroplasts are always in communication with the nucleus and the loss of nuclear gene expression represents the reduction in a positive retrograde signal (Terry and Smith 2013), some researchers interpret the treatment as triggering an inhibitory signal. To some extent this has been resolved genetically by the identification of mutants in which the nuclear transcriptional response is uncoupled from chloroplast status. These *genomes uncoupled* (*gun*) mutants were identified based on an increase in expression of *LHCB1.2* following treatment with NF (Susek et al. 1993). In total six *gun* mutants have been identified and these provide significant insight into what might be happening. Five of the mutants *gun2-gun6* have mutations in genes encoding proteins associated with tetrapyrrole synthesis (Larkin et al. 2003; Woodson et al. 2011; Mochizuki et al. 2001). This includes the phytochrome chromophore synthesis (heme-degradation) proteins, heme oxygenase (GUN2) and phytochromobilin synthase (GUN3), as well as GUN4 and GUN5 (H subunit of Mg-chelatase) required for efficient synthesis of Mg-protoporophyrin IX, a chlorophyll precursor. The *gun6* mutant results in the increased expression of ferrochelatase 1 (FC1), an enzyme that mediates the synthesis of heme. Crucially, the *gun6* mutant is dominant leading to the hypothesis that the synthesis of heme by FC1 is required for the production of a positive retrograde signal that promotes expression of photosynthesis-associated nuclear genes (PhANGS) (Woodson et al. 2011), with the other *gun* mutations serving to promote heme accumulation less directly. This is still the dominant hypothesis for the role of tetrapyrroles in retrograde signalling and no data have yet been published that disprove it. Nevertheless, supporting evidence has been limited to date. Now in this issue Page et al demonstrate that overexpression of FC1 in the chloroplast alone can result in a *gun* mutant phenotype (Page et al. 2020), supporting the role for FC1-synthesized heme in chloroplast-to-nucleus retrograde signalling. How heme might regulate gene expression in plants is largely unknown but two contributions in this issue also address this question. Shimizu et al describe a proteomics study to identify heme-binding proteins in *Arabidopsis* and the alga *Cyanidioschyzon merolae* that could contribute to heme transport or signalling (Shimizu et al. 2020). One suggestion for proteins that could be involved comes from Sylvestre-Gonon et al. who hypothesize a role for tau glutathione transferases in tetrapyrrole metabolism and retrograde signalling in plants (Sylvestre-Gonon et al. 2020).

While a role for tetrapyrroles is well established (see also (Larkin 2016)), perhaps the key to understanding retrograde signalling from the chloroplast is understanding the function of the rather enigmatic protein GUN1. The *gun1* mutant shows the strongest phenotype of any of the *gun* mutants (based on less reduction of nuclear gene expression after NF treatment

when compared to WT under identical conditions) and while the protein affected, a chloroplast-localized, pentatricopeptide-repeat protein, has been known for sometime (Koussevitzky et al. 2007), there are still numerous hypotheses for the function of the GUN1 protein. Recent evidence has centred on a role for GUN1 in chloroplast protein homeostasis (Colombo et al. 2016; Llamas et al. 2017; Marino et al. 2019), which may explain why only *gun1* of the *gun* mutants has elevated nuclear gene expression after Lin treatment as well as NF treatment. How it does this is an open question with recent studies suggesting a range of specific functions. One interesting proposal is that GUN1 functions in chloroplast protein import through its interaction with the chloroplast chaperone cpHSC70-1 (Wu et al. 2019a). In this model, the resulting accumulation of chloroplast pre-proteins in the cytosol in the *gun1* mutant enhances nuclear gene expression resulting in the *gun* phenotype (Wu et al. 2019a). While the promotion of gene expression in this system is perhaps surprising, there are clear similarities with the mitochondrial proteostatic responses discussed later. Another proposed role for GUN1 in protein homeostasis is in chloroplast gene editing (Zhao et al. 2019b), which has been shown to be altered under many conditions affecting retrograde signalling (Kakizaki et al. 2012). Two contributions to this issue further explore the role of GUN1 within the context of plastid protein synthesis and homeostasis. Tadini et al review the literature on the link between chloroplast transcription and retrograde signalling, focusing in particular on regulation of the nuclear-encoded (NEP) and plastid-encoded (PEP) plastid RNA polymerases (Tadini et al. 2020). They also discuss the role of GUN1 in this process and in chloroplast protein homeostasis more broadly. The study by Loudya et al directly addresses many of the issues raised (Loudya et al. 2020). They investigate the *cue8* mutant, which has defects in chloroplast transcription and retrograde signalling that are partially dependent on GUN1 and demonstrate a "retro-anterograde" correction that leads to elevated expression of NEP-dependent genes (Loudya et al. 2020). While GUN1 therefore is strongly linked to various aspects of plastid protein synthesis, perhaps the most intriguing of the recent results on GUN1 is the observation that it can also alter tetrapyrrole metabolism (Shimizu et al. 2019). The mechanism is not well understood but may involve interaction with tetrapyrrole enzymes (Tadini et al. 2016) as well as direct tetrapyrrole-binding (Shimizu et al. 2019). One possibility is that GUN1 provides a link between key chloroplast processes required for chloroplast protein synthesis and regulation of the tetrapyrrole pathway where it may function to directly read out the FC1-dependent heme signal (Shimizu et al. 2019).

And what about the other three classes of retrograde signals – do they also have a role in chloroplast biogenesis? ROS production is mostly associated with the photosynthetic apparatus which is not yet mature in a developing seedling. However, singlet oxygen ($^1O_2$) derived from chlorophyll biosynthesis intermediates has been shown to inhibit expression of tetrapyrrole pathway (in particular) and other photosynthetic genes following mis-regulation of chlorophyll synthesis (Page et al. 2017). Certainly the developing chloroplast will have started to synthesize a range of metabolites that may be important. One recent example is the identification of a cis-carotene derived apocarotenoid that is required for etioplast and chloroplast development (Cazzonelli et al. 2020).

Finally, proteins dually localised to the chloroplast and nucleus seem to be critical for normal chloroplast biogenesis. Pioneering work characterized Whirly1 as a nuclear-encoded protein that localizes in the plastid nucleoid but is also able to translocate from its plastid location to the nucleus (Krupinska et al. 2014; Krause et al. 2012; Isemer et al. 2012). Such a dual localization was then reported or predicted for more proteins (Krause and Krupinska 2009) suggesting the mechanistic possibility for retrograde control through proteins released from

the plastid. An independent screen for novel regulators in phytochrome regulation identified HEMERA (Chen et al. 2010), a protein also known as the PEP subunit pTAC12/PAP5 (Pfalz et al. 2006; Steiner et al. 2011). The HEMERA protein can also localise to the nucleus where it directly interacts with Phytochrome-Interacting Factor (PIF) proteins to regulate transcription of PhANGs (Qiu et al. 2015). During light-induced chloroplast biogenesis the PEP complex is reorganized by the association of 12 additional nuclear-encoded proteins, the PEP-associated proteins (PAPs) (Pfannschmidt and Link 1994; Steiner et al. 2011). Genetic inactivation of any PAP leads to albinism indicating the importance of these proteins for chloroplast biogenesis (Pfalz and Pfannschmidt 2013). Recent studies identified two further proteins, RCB and NCP, as important regulators of PEP restructuring. Both appear to be dual-localized and to interact with the phytochrome signalling system (Yoo et al. 2019; Yang et al. 2019). As the sequences of half of the 12 PAPs conatin predicted nuclear localization signals (Pfannschmidt et al. 2015), even more of the PAP proteins may function in this way. The potential role of these dual-localised proteins in retrograde signalling is reviewed in detail in this issue by (Krupinska et al. 2020) and (Tadini et al. 2020).

Operational signalling from plastids - Operational signals from plastids are typically connected to the functionality of chloroplasts (Pogson et al. 2008). The dominant process in this plastid type is photosynthesis, the process that generates carbohydrates from ambient CO_2, H_2O and sunlight. In photosynthesis the light-driven electron transport from water to $NADP^+$ (the light reaction) in the thylakoid membrane of chloroplasts is functionally coupled to the chemical reactions in the stroma (the dark or carbon reaction), in which carbohydrates are generated by consumption of ambient CO_2, ATP and $NADPH_2$. Because of the functional coupling of both reactions, photosynthesis becomes very sensitive to changes in illumination, temperature or availability of water and CO_2 (*via* the opening of stomata). Variations in one or several of these environmental factors translates into imbalances of photosynthetic electron transport leading to changes in the reduction/oxidation (redox) state of the involved components. A prominent example for this type of regulation is the redox control initiated at the plastoquinone pool, the electron carrier that connects photosystem (PS) II with the cytochrome b_6f ($Cytb_6f$) complex. Its redox state acts as a major regulator of PS genes in both the plastid and nucleus, triggering a fine-tuning of photosystem stoichiometry and antenna protein synthesis in response not only to light-quality gradients found in dense plant populations (Dietzel et al. 2008), but also to fluctuating light intensity conditions or sudden high light stress. Aspects of this complex topic have been discussed in a contribution to this issue (Gollan and Aro 2020).

Photosynthetic organisms often perceive strong gradients in light intensity which show both high spatial and temporal dynamics. Very strong illumination can exceed the photosynthetic capacity of organisms and, therefore, lead to a situation in which excess excitation energy must be dissipated. This is achieved by removal of absorbed energy as heat via non-photochemical quenching or by transferring excess electrons to oxygen generating ROS that are detoxified by redox buffer systems such as the glutathione/ascorbate systems, peroxidases or glutaredoxins (Dietz and Pfannschmidt 2011). The protein components in these systems are encoded in the nucleus and are additionally up-regulated in response to increasing stress. The accumulation of ROS can also occur under low light when the ambient temperature is low, or when the Calvin cycle is inefficient because of substrate limitations. In all these cases the accumulation of ROS serves as an important stress signal that triggers several acclimation responses in the nucleus. There has been much debate about the specificity of such a signaling system as well as the spatio-temporal distribution of ROS

signals and a lot of current research in this field is still focussed on these questions. However, since the first proposal of plastid redox signals acting as retrograde signals (Pfannschmidt et al. 2003) the field has progressed a lot and far more detailed working hypotheses have now been developed (Leister 2019) including one in a contribution to this issue (Unal et al. 2020).

Uncharged ROS like hydrogen peroxide (H_2O_2) are able to pass through membranes and, therefore, may leave the chloroplast in order to activate cytosolic signalling pathways under conditions of excessive accumulation. It was shown that chloroplast-generated H_2O_2 is linked to cytosolic MAP kinase cascades that activate corresponding compensatory responses in the nucleus (Dietz et al. 2016). However, most ROS exhibit very short half-lives preventing a direct signalling function by long-range diffusion (Apel and Hirt 2004). Instead, ROS like $^1O_2$ or superoxide initiate signalling pathways that are mediated by oxidized compounds such as β–cyclocitral (Ramel et al. 2012). In addition, specific metabolites have been identified that accumulate under stress such as the dinucleotide 3´-phosphoadenosine-5´-phosphate (PAP) (Estavillo et al. 2011) and the isoprenoid precursor methyl erythritol cyclopyrophosphate (MEcPP) (Xiao et al. 2012). Furthermore, an apocarotenoid-dependent signal was recently identified that is required for proper plastid development (Cazzonelli et al. 2020). All these metabolites are much more stable than ROS and could be actively transported over the chloroplast envelope, diffuse through the cytosol and induce appropriate responses in the nucleus. Since they are small molecules they may even diffuse through plasmodesmata into neighbouring cells. One contribution to this issue deals with the interesting aspect of retrograde control in cell-to-cell signalling (Ganusova et al. 2020).

In addition, a number of specific signalling proteins such as EXECUTER 1 and 2 (Lee et al. 2007) were identified that relay $^1O_2$ signals towards the outside of the plastid (*via* a still unknown mechanism) inducing stress responses or even cell death (Kim et al. 2012). Novel data strongly suggest that the β–cyclocitral and EXECUTER-dependent pathways act independently leading to two separate signalling pathways although starting from the same ROS (Kim 2020). However, it appears that the two pathways orginate at different sites of $^1O_2$ formation (PSII reaction center *versus* grana margins) indicating that the intracellular location of ROS formation is an important determinant for the signalling pathway used. Interestingly, the biogenic $^1O_2$ signal described earlier is also partially dependent on EXECUTER proteins although presumably originating at a different plastid location entirely (Page et al. 2017). Intracellular location is also important for another novel potential pathway for ROS signalling that was recently proposed that involves the close proximity of the nuclear envelope with extensions of plastids called stromules (Exposito-Rodriguez et al. 2017a). This close proximity provides the opportunity for direct transfer of ROS into the nucleus without passage throught the cytosol. Details of this novel pathway are discussed in this issue (Mullineaux et al. 2020).

ROS are also involved in retrograde signaling from mitochondria (see below). Since both organelles operate simultaneously within the same cell one needs to ask how specificity in this signalling is achieved (Wang et al. 2020). In energy metabolism a tight functional interaction between plastids and mitochondria developed during the course of evolution. It can therefore be assumed that such interaction developed also in the context of retrograde signalling. Recent results have provided the first evidence that retrograde signals from chloroplasts and mitochondria indeed act in a coordinated or mutual manner for reciprocal regulation of status or gene expression (Dietzel et al. 2015; Cui et al. 2019; Shapiguzov et al.

2020). In light of these new results it should be remembered that mitochondrial and plastid gene expression systems are both under initial control of nuclear-encoded phage-type RNA polymerases that trigger all primary gene expression events in both organelles (Liere et al. 2011). A mutual control of organellar gene expression by retrograde signals from the respective other organelle, therefore, can be easily established. Nevertheless, both the functional and the evolutionary contexts are far from being understood and many aspects of the mutual regulation of mitochondria and chloroplasts remain to be explored.

Degradational signalling from plastids - Natural senescence in plants is known to cause slow chlorosis accompanied by a targeted degradation of chloroplasts. This helps the plant to recover nitrogen, lipids and amino acids mostly from chlorophyll, the highly abundant enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (known as RuBisCO) and the thylakoid membranes (Krupinska et al. 2012). Similar processes occur in response to drought stress, while in a hypersensitive response to pathogen attack resource allocation is not possible because of the rapidity of the response that sacrifices tissue resources for the sake of isolation and separation of the pathogen (Mur et al. 2008). In the light of global environmental change and the expected (and already experienced) extended drought phases in many countries, control of chloroplast degradation becomes an interesting target for improving plant drought and stress tolerance (Gregersen et al. 2013). It has been demonstrated that stabilizing the life span of chloroplasts under drought stress improves the ability of plants to recover upon re-hydration, thus expanding the vegetative growth phase and biomass production (Kusaba et al. 2013). Delaying or down-regulating degradational signals that induce resource allocation in response to drought may help to achieve these goals. In this context it is interesting to note that stressed chloroplasts are able to send a $^1O_2$-generated signal that activates ubiquitin-mediated chloroplast degradation representing a potential mechanism for avoidance of oxidative stress through ROS-overproducing chloroplasts (Woodson et al. 2015). Targeted degradation of damaged chloroplasts, however, provides a means to reallocate valuable resources during acute stress. Future research will provide more insights into this complex response.

Species with alternative plastid types - The evolution of plastids did not follow a strict vertical route but is complicated by a number of horizontal endosymbiotic events. After the unique primary endosymbiosis event, the plastid-bearing evolutionary line split into glaucophytes, red algae and green algae (Figure 1). From the latter the land plants emerged around 450-500 million years ago (Reyes-Prieto et al. 2007). Within the red and the green lineage, organisms exist that contain plastids with three, four or even five envelope membranes. Morphological and molecular analyses have identified such organisms as representatives of secondary and tertiary endosymbiotic events having integrated eukaryotes that already possessed plastids (Stoebe and Maier 2002). We only can speculate on the establishment of retrograde signals in secondary and tertiary plastids, but since such organisms exist we must assume that specific molecular signalling pathways evolved that coordinate the multiplicity of their intracellular genomes. This is of special interest as a number of pathogenic parasites within the phylum apicomplexa possess secondary, non-photosynthetic but essential plastids, among them *Plasmodium sp*. and *Toxoplasma gondii* which cause severe diseases such as malaria and toxoplasmosis (McFadden and Yeh 2017). In addition, many species in this phylum carry only a single mitochondrion with a single nucleoid. Its division is strictly coupled to the cell cycle of the parasite (Voleman and Dolezal 2019) and proper timely expression of mitochondria-located, nuclear-encoded proteins is absolutely essential for the survival of these cells. Understanding retrograde expression control of the imported

proteomes of both the apicoplast and the mitochondrion may pave new avenues to identify potential drug targets for fighting such parasites (Mallo et al. 2018).

Retrograde signalling from mitochondria in heterotrophs

In heterotrophic organisms, retrograde signals from mitochondria are one of the ways in which mitochondria and the nucleus communicate (Quiros et al. 2016; Jazwinski 2014; da Cunha et al. 2015). These signals are mainly activated upon stress and their diversity increases in parallel to the organismal complexity due to the tissue specialisation that occurs in multicellular organisms. In unicellular organisms, such as yeast, retrograde signals are mainly associated with the rewiring and adaptation of metabolism to the source of nutrients present in the environment; however, in multicellular organisms there is a great variety of retrograde signals that operate adapting cellular functions to each tissue and environment. Therefore, retrograde signals observed in neurons of mammals can be different to those observed in muscle or liver cells, and even different from those observed in the neurons of other organisms. Despite the great variety of responses that have been identified in heterotrophic organisms, the canonical retrograde signals can be classified in three main categories depending on the stressor that triggers its activation: energetic stress response, calcium-dependent response, and ROS-dependent response (Quiros et al. 2016; da Cunha et al. 2015; Jazwinski 2014). The aim of all retrograde signals is to activate a set of nuclear genes that alleviate the cellular stress originating in the mitochondria. These genes can modify cellular metabolism from oxidative to glycolytic, activate alternative pathways to generate ATP in the cell, promote mitochondrial biogenesis and quality control mechanisms, regulate calcium metabolism by stimulating its transport and storage, or activate antioxidant responses (Quiros et al. 2016; Arnould et al. 2015). The activation of retrograde responses depends on a series of mediators that are either released from mitochondria or originated in the cytosol as a consequence of the dysfunctional mitochondria. These include mainly ions, such as calcium, ROS, or metabolites, such as AMP, NAD^+, or tricarboxylic acid (TCA) cycle intermediates (Bohovych and Khalimonchuk 2016). Of note is that many of the metabolites of the TCA cycle are also required for regulating epigenetic marks in the nucleus, participating therefore in the regulation of mitochondrial protein synthesis and cellular adaptations in homeostasis and stress (Matilainen et al. 2017).

In addition to the classical retrograde signals, there are other forms of mitonuclear communication activated upon stress that also have a retrograde signalling component (Quiros et al. 2016). These signalling pathways are mainly triggered by proteotoxic stress and their output can be a specific proteostatic response or a general response which not only repairs the protein defects but also regulates metabolism to adapt to the cellular requirements. There are multiple responses associated with proteostatic stress, most of them described in yeast and worms. The translation of these responses to more complex organisms, such as flies or mammals, is sometimes difficult due to the higher complexity and tissue specialisation, and they may differ from those of lower organisms. In yeast, several responses have been identified upon proteotoxic stress. For instance, when mitochondrial protein import is decreased or blocked, it results in an accumulation of misfolded or unimported mitochondrial-targeted proteins, a phenomenon called mitochondrial precursor overaccumulation stress (mPOS). This stress condition activates a particular response, the unfolded protein response (UPR), by the mistargeting of proteins (UPR^{am}), which alleviate the stress by decreasing the rate of cytosolic protein synthesis and by activating proteasomal

degradation in the cytosol (Wang and Chen 2015; Wrobel et al. 2015). In addition, the accumulation of precursor proteins at the mitochondrial surface due to impaired mitochondrial protein import activates a similar response in yeast, defined as mitoCPR (mitochondrial compromised protein import response). This response is mediated by the expression of Cis1 which binds to the import protein Tom70 and recruits the ATPase Msp1, promoting the clearance of stalled proteins from the import channels and targeting them for degradation by the proteasome (Weidberg and Amon 2018). Both UPR^{am} and mitoCPR require the coordination of mitochondria and nucleus to activate a cross-compartmental response involving proteasomal degradation. The importance of interconnection and communication between compartments in yeast has also been highlighted with the discovery of the mechanism called MAGIC (mitochondria as guardian in cytosol) (Ruan et al. 2017). This stress response, which may also exist in human cells, leads to the degradation of cytosolic protein aggregates in the mitochondria. Thus, after heat-shock stress, cytosolic and mitochondrial protein aggregates interact with the mitochondrial import complex and can enter the mitochondria to be degraded by proteases (Ruan et al. 2017).

In multicellular organisms, the main mitochondrial stress responses are the mitochondrial unfolded protein response (UPR^{mt}) and the mitochondrial integrated stress response (ISR), both being conserved between nematodes, flies, and mammals, but with some specific properties in each organismic group. The UPR^{mt} is most well studied in worms and was first described as a proteostatic response; however, nowadays, it is known that its function goes beyond that and should be considered as a general stress response (Shpilka and Haynes 2018). The UPR^{mt} is regulated in worms by the mitochondrion-targeted transcription factor ATFS-1, which upon mitochondrial stress is partly retained in the cytosol and translocated to the nucleus where it activates the expression of several nuclear-encoded mitochondrial genes that participate in quality control, such as proteases and chaperones, mitochondrial dynamics, mitochondrial transport, glycolysis, and antioxidant detoxification (Nargund et al. 2015). In mammals, it has been proposed that the activation of the UPR^{mt} is mediated by ATF5, which acts as an orthologue of ATFS-1 (Fiorese et al. 2016). However, it has been demonstrated that the main stress response in mammals and flies is the ISR (Quiros et al. 2017; Bao et al. 2016; Khan et al. 2017; Hunt et al. 2019; Celardo et al. 2017; Guo et al. 2020; Fessler et al. 2020). Mitochondrial stress can activate the ISR through any of four kinases, PERK, GCN2, PKR or HRI (Balsa et al. 2019; Guo et al. 2019; Michel et al. 2015; Kim and Sieburth 2018), which in turn phosphorylate the translation initiation factor eIF2α. Phosphorylation of eIF2α decreases general cytosolic translation but, at the same time, favours the translation of a certain set of genes though upstream open reading frames, including *ATF4*, *ATF5* or *CHOP* (Pakos-Zebrucka et al. 2016). ATF4 acts as the main mediator of mitochondrial stress in mammals and flies, promoting the transcription of a specific set of genes that mainly reprogram cellular metabolism to adapt to the stress conditions. Depending on the stressor, the cellular context and the timing, different outputs may be promoted including remodelling of one-carbon metabolism; activation of metabolic cytokines, such as FGF21 and GDF15; stimulation of an antioxidant response; or even enhanced mitochondrial respiration through promoting super complex assembly (Quiros et al. 2017; Bao et al. 2016; Khan et al. 2017; Hunt et al. 2019; Celardo et al. 2017). Regulation of these processes occurs in temporal stages, as observed in a model of mitochondrial myopathy, in which it is regulated by the autocrine and endocrine effects of FGF21 (Forsstrom et al. 2019).

All forms of retrograde signalling are needed for a proper maintenance of the homeostasis of heterotrophic organisms. However, long-term activation or an exacerbation of the response can also be deleterious; consequently, in some contexts, an inhibition of the activation can help cells, tissues and organisms to recover from the stress and even promote longevity, in a so-called mitohormetic fashion (Barcena et al. 2018). Mitohormesis defines a concept in which activation of mitochondrial metabolism induces formation of cellular free radicals that in turn activate cellular defence reactions against ROS. As an example, this issue features studies describing retrograde signals in heterotrophic organisms in two important contexts: the nervous system and their connections with different neurological diseases (Granat et al. 2020) and their function regulating proteostasis and longevity (Molenaars et al. 2020).

Retrograde signalling from mitochondria in autotrophs

Although mito-nuclear signalling had been described for many decades in heterotrophic organisms (Butow and Avadhani 2004), mechanistic insight into plant retrograde signalling, here referred as mitochondrial retrograde regulation (MRR) (Rhoads and Subbaiah 2007), has only been obtained in the last 10 years. Plant MRR was originally described in the context of induction of alternative oxidase trancripts in response to mitochondrial inhibition (Maxwell et al. 1999). With the advent of microarray technology, it was realised that a much wider set of genes responded to mitochondrial dysfunction, for instance triggered by chemical inhibition of mitochondrial enzymes (Clifton et al. 2005) or by mutation of important mitochondrial proteins (Meyer et al. 2009). Using a range of screening methods, various transcription factors were discovered that could influence the expression of MRR marker genes, mainly using *Arabidopsis thaliana* as a model system. Firstly, ABscisic acid Insensitive 4 (ABI4) was shown to keep *ALTERNATIVE OXIDASE 1a (AOX1a)* expression in a repressed state (Giraud et al. 2009). Several WRKY transcription factors (mainly WRKY15, WKRY40 and WKRY63) were then implicated in co-regulating nuclear stress responsive transcripts that encode mitochondrial and chloroplast proteins (Vanderauwera et al. 2012; Van Aken et al. 2016a; Van Aken et al. 2013). Also MYB29 was shown to have a repressive role on MRR responses (Zhang et al. 2017), influencing the interplay of phytohormones such as ethylene, jasmonic acid, salicylic acid and ROS.

Probably the most significant breakthrough was made with the discovery of a class of transmembrane-domain containing NAC transcription factors (ANAC013, ANAC016, ANAC017, ANAC053, ANAC078) by forward genetic and DNA-binding screens (De Clercq et al. 2013; Ng et al. 2013b). Further studies have established that ANAC017 has the largest contribution in responses to both chemical (Van Aken et al. 2016a; Ng et al. 2013b) and genetic (Van Aken et al. 2016b; Shapiguzov et al. 2019; Merendino et al. 2020) mitochondrial inhibition, and is thus currently considered as a master regulator of plant MRR. Interestingly, this group of ANAC transcription factors have been shown to be attached to the ER and can be remobilised, most likely by proteolysis, to the nucleus (De Clercq et al. 2013; Ng et al. 2013b).

This central location in the cell may allow the ANAC transcription factors such as ANAC017 to regulate responses to a variety of cellular stresses, including chloroplast stress (Van Aken

et al. 2016a). An overlap between PAP-regulated and ANAC017-regulated genes was observed, providing more evidence for a convergence of mitochondrial and chloroplast retrograde signalling (Van Aken and Pogson 2017). The enzyme producing PAP (called SAL1) is also dual-targeted to chloroplasts and mitochondria (Estavillo et al. 2011). In fact, many regulators of plant MRR have been implicated in chloroplast retrograde signalling, including ABI4, WRKY40 and CDKE1 (Shang et al. 2010; Koussevitzky et al. 2007; Blanco et al. 2014; Ng et al. 2013a) although recent evidence has ruled out a role for ABI4 in chloroplast biogenic signalling (Kacprzak et al. 2019). This interaction between plant mitochondrial and chloroplast retrograde signalling has been explored in detail in a review in this issue (Wang et al. 2020). The ANAC017-induced genes are thought to help plants cope with oxidative stresses originating from the chloroplast, for instance during inhibition with methyl viologen (Van Aken et al. 2016a; De Clercq et al. 2013). Recently, the Arabidopsis Radical-induced Cell Death 1 (RCD1) protein was shown to bind ANAC017 and ANAC013 transcription factors and is thought to keep them in an inactive state, thereby repressing MRR responses (Shapiguzov et al. 2019). In this issue, Shapiguzov and colleagues further explore how plants respond to methylviologen, and show that hypoxia can reduce electron transfer from PSI to oxygen (the Mehler reaction) specifically in *rcd1* mutants, but not in wild-type (WT) plants (Shapiguzov et al. 2020). As *rcd1* plants show a constitutively high level of ANAC017-target genes, the authors could confirm that the effect of hypoxia on the Mehler reaction could be mimicked by preincubation of WT plants with antimycin A or by overexpression of *ANAC013*, which both switch on the MRR pathway. The effect could not be directly attributed to alternative oxidases, indicating that other MRR target genes are responsible for this effect. A very large overlap in target genes triggered by mitochondrial dysfunction and low oxygen treatment have been observed (Wagner et al. 2018). This suggests that response to low oxygen, for instance during flooding or germination, may depend at least in part on mitochondrial plasticity controlled by ANAC017 (Wagner et al. 2019; Wagner et al. 2018). Very recently, the protective role of ANAC017 in flooding tolerance was experimentally confirmed (Meng et al. 2020). Conversely, ANAC017 may have a growth limiting effect when expressed or active at high levels (Meng et al. 2019). ANAC017 has also been implicated in other processes such as cell wall synthesis (Hu et al. 2016) and senescence (Kim et al. 2018; Meng et al. 2019). Although so far the retrograde signalling-related roles of ANACs have been studied mostly in Arabidopsis, it is likely that this plant-specific MRR signaling pathway (NACs are a plant-specific protein family) has evolved with plant colonisation of land (Lama et al. 2019). It will thus be of significant interest to study whether similar MRR pathways are conserved throughout the plant kingdom. The computational models suggesting that MRR appeared in conjunction with the colonisation of land are in line with the observation that plant MRR is of importance for resistance to e.g. flooding, a condition that is uniquely associated with terrestrial growth. Similarly, the evolution of PAP-related chloroplast retrograde signalling appears to be associated with land colonization and dehydration tolerance (Zhao et al. 2019a).

Besides *AOX1a*, ANAC017 is thought to co-regulate up to 200 genes (or even more during e.g. antimycin A (AA) treatment), many of which are involved in non-mitochondrial cellular processes (Van Aken et al. 2016b), for example genes affecting hormone balances that could control how a plant prioritises between growth and defence. Indeed, it appears that auxin signalling and MRR are antagonistic pathways (Ivanova et al. 2014; Kerchev et al. 2014). Interestingly, a role for ethylene in plant MRR is emerging, with ethylene being produced during mitochondrial dysfunction (Christians and Larsen 2007) and boosting MRR responses. In this issue, Merendino et al. found that when Arabidopsis mutants with

mitochondrial defects were germinated in the dark, morphological changes were observed that are reminiscent of etiolated seedlings exposed to high ethylene concentrations (the triple response), including extreme apical hook formation (Merendino et al. 2020). This is in line with previous findings that *atphb3* mitochondrial mutants show increased sensitivity to ethylene (Christians and Larsen 2007). Merendino and colleagues further showed that this extreme ethylene response is dependent on the activity of AOX1a. Furthermore, increased *AOX1a* transcript levels and activity was found to be largely regulated by ANAC017, so it appears that ANAC017 plays an important part in this exaggerated ethylene sensitivity by regulating *AOX1a* levels (Merendino et al. 2020). As mitochondria are of key importance during germination, where seedlings would often have to penetrate through soil deprived of light and perhaps sufficient oxygen, it makes sense that there are response mechanisms in place that adjust plant growth to mitochondrial activity during seed germination. The apical hook is thought to protect meristems from physical damage inflicted during soil emergence. Exactly why AOX1a activity would be so crucial for this process, and how AOX1a controls a downstream response affecting apical hook angle is currently unclear.

The role of ethylene was studied more directly in another study in this issue (Kacprzak et al. 2020). It was shown that ethylene boosts MRR to antimycin A in Arabidopsis, while blocking of ethylene signalling partially represses MRR. However, ethylene signalling components like EIN2 or the kinase MPK6 do not seem to be required for MRR, indicating that ethylene is not necessary for ANAC017-dependent MRR, but can promote it. At this stage it remains difficult to draw clear conclusions on the interaction of MRR and ethylene signalling, but it appears that mitochondrial defects could cause the plants to produce more ethylene. When grown in the dark, this increased ethylene production likely contributes to the triple response-like effects observed by Merendino and co-workers, mediated by AOX1a, and requiring ANAC017 for its full induction (Merendino et al. 2020). The ethylene appears to further boost ANAC017-dependent MRR, suggesting there is a weak positive feedback loop occuring. More research will be needed to clarify the link between MRR and ethylene.

Another area that is just at the beginning of being explored in plants is the UPR^{mt}. Two recent studies showed that treatments used in heterotrophic systems to induce UPR^{mt} (e.g. doxycycline) also trigger transcriptomic and physiological responses in plants (Wang and Auwerx 2017; Moullan et al. 2015), demonstrating an interaction with a wide range of phytohormone signalling pathways such as jasmonic acid, auxin and ethylene. A wide range of transcription factor classes were suggested to play a potential role. In this issue, Kacprzak et al. showed that there is a significant overlap between the transcriptomic responses that trigger UPR^{mt} and antimycin A-induced MRR (Kacprzak et al. 2020). The genes in common appeared to belong to the ANAC017-regulon, so it was found that indeed ANAC017 plays a key role in mediating transcriptional responses and physiological resistance to a wide range of inhibitors that are used to induce UPR^{mt} in non-plant systems, including doxycycline, MitoBlock-6, carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP) and chloramphenicol. These treatments did not induce chloroplast UPR marker genes, demonstrating that mostly mitochondria were affected. ANAC017 loss-of-function mutants showed increased susceptibility when grown on UPR^{mt}-inducing media, while *ANAC017* overexpression lines were more resistant. Another study in this issue performed whole genome transcriptome studies on knock-down lines of another *Arabidopsis* mitochondrial ribosomal protein, RPS10 (Adamowicz-Skrzypkowska et al. 2020), which is also interesting as a model for UPR^{mt} in plants. Here the ANAC017 regulon was strongly induced, and the analysis showed that the only commonly differentially-regulated genes between doxycycline

treatment, *mrpl1* and *rps10* mutants were well-known antimycin A-induced ANAC017 target genes (e.g. *NDB4* and *At12Cys-2*). It thus appears that the classical MRR pathway and the newly discovered UPR^{mt} response in plants are likely identical, and are to a large extent under control of ANAC017.

Despite many decades of work in chloroplast retrograde signalling, many of the key signalling intermediates are still unknown. This appears to be the case also for plant MRR, as we still have very little insight into how mitochondrial dysfunction results in proteolytic activation of e.g. ANAC017 on the ER membrane, except that it may be cleaved by rhomboid-type proteases (Ng et al. 2013b). A significant overlap between hydrogen peroxide and antimycin A-induced signalling suggests that ROS could play an important role as signalling intermediates. However, compounds such as the mitochondrial and cytosolic aconitase inhibitor monofluoroacetate are not thought to induce a significant level of ROS responses, but are still capable of inducing MRR-targets in plants. Thus, what exactly is being sensed in a dysfunctional mitochondrion, and how this signal is transduced out of the mitochondria is an important outstanding question in the field.

Despite, the dominance of ANAC017 in the current literature of plant MRR, it is very likely that other mito-nuclear signalling pathways also exist. For instance, most mitochondrial function mutants in *Arabidopsis* have specific transcriptomic responses outside of the ANAC017 target genes (Van Aken et al. 2016b; Van Aken and Whelan 2012; Meyer et al. 2009; Schwarzlander et al. 2012). ANAC017 also affected approximately 35% of the transcriptomic response to AA (Ng et al 2013b), suggesting that other signalling pathways were activated. Interestingly, a study in this issue provides evidence using chemical and genetic approaches that simultaneous inhibition of Complex IV and alternative oxidase results in a down-regulation of chloroplast transcription (Adamowicz-Skrzypkowska et al. 2020). This inhibition was achieved by silencing of *rps10* (mitoribosomal subunit 10) and simultaneous mutation of dual-targeted organellar RNA polymerase *rpotmp* and *aox1a*, or by specific chemical inhibition using KCN and salicylhydroxamic acid. Although the ANAC017 pathway is also activated under these conditions, the down-regulation of chloroplast transcription appears to be independent of ANAC017 and may operate at least in part *via* down-regulation of nuclear-encoded components of the chloroplast transcriptional machinery. This implies the existence of an unknown signalling pathway that connects mito-nuclear signalling with chloroplast function. Again, this effect could be observed during low oxygen stress, which would indeed simultaneously inhibit Complex IV and alternative oxidase, providing more evidence that plant MRR is particularly relevant during hypoxia.

Common principles in retrograde control from organelles

The previous sections have demonstrated the multiplicity of mechanisms that are used by retrograde signalling pathways from both organelles. At the molecular level these pathways may exhibit numerous species-specific as well as condition-specific differences. Nevertheless, because of their evolutionary and functional relatedness some conserved regulatory paradigms can be identified for both organelles that appear within the same biological context and follow similar or even identical rules (Fig. 2).

Retrograde signals in biogenesis, redox homeostasis and stress conditions - Mitochondria and chloroplasts are the energy-converting organelles of eukaryotic cells and work closely

together either directly, as in autotrophs, or indirectly *via* the food chain in heterotrophs. The photosynthesis-driven carbon reduction of chloroplasts and the corresponding carbon oxidation in mitochondria, thus, provide the essential energetic cycle that drives eukaryotic life on Earth. Imbalances and disturbances in the ETCs of both organelles are typically reflected by changes in the redox state of electron transport components and/or increasing amounts of ROS and in both cases these trigger a number of stress responses that aim to counter-balance adverse influences from the environment or metabolism in order to re-establish redox homeostasis. For both organelles, therefore, a strong coordination in the expression of components of their ETCs is of vital importance.

Interestingly, for both organelles the vast majority of ETC components is encoded in the nucleus and their expression is under the control of only a few key regulators. In mitochondria (of heterotrophs) expression of nuclear genes for mitochondrial complexes involved in oxidative phosphorylation, mitochondrial gene expression and protein import is predominantly under control of nuclear respiratory factor 1 (NRF1) and the purin-rich repeat GA-binding protein α (GABPα) (Dhar et al. 2008; Virbasius and Scarpulla 1994; Gleyzer et al. 2005; Blesa et al. 2007). A similar situation is found for plastids where PhANGs are under control of Golden2-like 1 and 2 (GLK1 and GLK2), two key transcription factors required for building the photosynthesis apparatus (Waters and Langdale 2009; Waters et al. 2009). These key regulators are targets for retrograde signals from mitochondria or plastids, respectively allowing the coordinated expression of ETC components according to the needs of the respective organelles. These pathways are activated in particular when biogenesis of new plastids and mitochondria is required. This is typical in developing tissues (meristems in plants) of young organisms that are growing.

Furthermore, retrograde signalling pathways are activated under particular environmental conditions that generate an imbalance in the ETCs. By this means the redox state of the components involved in the electron transport may serve as a signal that activates and initiates corresponding molecular responses. Thus, the functioning of the ETC in both organelles acts as an environmental sensor that triggers compensatory cellular responses. Conditions that induce an ETC dysfunction or excessive electron flow through ETCs result in formation of ROS or oxidized metabolites or compounds. Both organelles induce a whole array of responses that mainly focus on stress compensation and maintenance of redox homeostasis. However, when the stress reaches a level that cannot be compensated for, cells may induce cell death in order to ensure the survival of the organism. For mitochondria in heterotrophs a number of different scenarios are known in which the organelle initiates a subsequent cell death (apoptosis), usually through the release of cytochrome c (Bock and Tait 2020). In autotrophs the scenario is more complex since besides mitochondria also plastids play an important role in cell death intiation (see above) (Van Aken and Van Breusegem 2015).

Proteostasis and maintenance of metabolism – Besides their role in energy metabolism. mitochondria and plastids have critical functions in the catabolic and anabolic reactions of most biosynthetic pathways of eukaryotic cells. Both organelles, therefore, represent metabolic hubs in primary and secondary metabolism. Since the organellar genomes encode almost exclusively components of ETCs and the gene expression machineries, virtually all enzymes for biosynthetic pathways must be imported from the cytosol and assembled in the matrix and stroma, respectively (Jarvis and Lopez-Juez 2013; Zhang and Xu 2016). In the context of a balanced metabolism in organelles, a striking similarity in retrograde control

appears to be the various types of UPR that resolve proteotoxic stresses by removing unassembled, unfolded, unimported or damaged proteins. The UPR^{mt} of heterotrophs is already well understood and is known to maintain various important cellular parameters including matrix homeostasis/proteostasis to balance the metabolism. It is only recently that a corresponding plastid UPR (UPR^{cp}) could be identified (Rochaix and Ramundo 2018; Llamas et al. 2017; Ramundo et al. 2014). As in mitochondria, unbalanced or repressed protein production induces a proteotoxic stress that sends retrograde signals to the nucleus to enhance the expression of a number of chaperones and proteases. Indeed, recent evidence has suggested that UPR^{cp} may be important for biogenic signalling mediated by GUN1 (Wu et al., 2019). Here GUN1 is implicated in a role in chloroplast protein import, but how this links in with the other known functions of GUN1 is still unknown (see earlier discussion). Interestingly, severe chloroplast stress resulting in $^1O_2$ production has also identified a possible additional route for removal of damaged organelles, in this case through a ubiquitin-mediated system that acts independently of autophagy (Woodson et al. 2015).

The nature of retrograde signals

Retrograde signalling from mitochondria and plastids displays many commonalities not only in the physiological and developmental context in which it is acting, but also in the physical nature of signalling molecules that actually pass through the envelopes of the two types of organelles. Despite manifold species-specific differences one can identify four classes of signals used by all organelles.

Calcium ions – In mitochondria of heterotrophic organisms Ca^{2+}-driven retrograde signalling is well established and studied. Release of Ca^{2+} ions from mitochondria in response to various stressors is known to affect the cytosolic Ca^{2+} concentration and to constitute a trigger for the expression of enzymes involved in re-establishment of Ca^{2+} balance, carbohydrate metabolism and cell proliferation (Quiros et al. 2016). In mitochondria of autotrophs this is still under investigation, but an involvement in retrograde signalling is very likely (Wagner et al. 2016). In plastids recent studies have uncovered that plastidial Ca^{2+}-metabolism is tightly linked to the cytosolic Ca^{2+} balance *via* the plastid localized calcium sensing (CAS) protein. Apparently, plastid Ca^{2+} release is required for a number of cellular responses that control photosynthetic efficiency and stress acclimation (Kmiecik et al. 2016; Kudla et al. 2018).

ROS and oxidized metabolites - As discussed above, energy conversion is one central function of plastids and mitochondria. Imbalances or dysfunctions in their ETCs result in both organelles in the formation of ROS and other oxidized compounds that provide either the trigger or the signal itself for retrograde control. The redox chemistry that is involved displays many commonalities between both organelles and ROS represent a dominant class of retrograde signals in both cases as they aim to achieve redox homeostasis.

Organelle-specific metabolites – Plastids and mitochondria are essential metabolic hubs of eukaryotic cells and contribute to many anabolic and catabolic reactions of the cell. The well-known exchange of substrates and products across the envelope generates genuine and natural retrograde signals that couple organellar and cytosolic functions *via* envelope-localized transporters as discussed by a contribution to this issue (Unal et al. 2020). Metabolite fluxes have been known for a long time to be important for metabolic homeostasis

and stress responses in eukaryotic cells. How far changes in specific metabolites and changes in metabolite signatures (representing combinations of fluxes of several metabolites) serve as signals is still under investigation. Another interesting example is the tetrapyrrole heme that is synthesized in plastids or, for yeast and mammals, in mitochondria (although intermediate steps occur in the cytoplasm). As discussed earlier heme is a major candidate as the signalling molecule for biogenic retrograde signalling and is well established as a regulator of gene expression, including for mitochondrial proteins, in yeast and mammals (Terry and Smith, 2013).

Dual-localized proteins – Nuclear-encoded proteins that target organelles and re-locate to the nucleus under specific conditions are a fascinating new field of research in retrograde signalling. This type of protein occurs in both mitochondria and plastids and are discussed in two contributions to this issue. While in mitochondria the mode of retrograde re-location is well investigated, it is still under extensive debate in plastids and further research is required to resolve apparent contradictions (Tadini et al. 2020; Krupinska et al. 2020). Nevertheless, redirection of plastid proteins to the nucleus that potentially act as transcription factors provides a very direct mechanism for retrograde control that does not require further mediators.

Conclusions

Present plastids and mitochondria are essential compartments of eukaryotic cells and make a major contribution to their structural and functional properties. Despite many species-specific differences in specific properties that have appeared during evolution, a number of common paradigms can be clearly identified and which contributed to the establishment of multicellular organisms. Retrograde signals from the two types of organelles play an important role in cellular responses to developmental and environmental influences. Across all eukaryotic evolutionary lines a number of common principles can be identified which most likely represent the common evolutionary constraints imposed regardless of specific effects on the evolution of single species. Establishment of retrograde signalling pathways, therefore, appears to be located at the root of eukaryotic cell evolution.

Figure legends

Fig. 1: Establishment of retrograde control during evolution of eukaryotes. The diagram depicts the major steps in the evolutionary aquisition of mitochondria and plastids by eukaryotes. Rectangles represent endosymbiotic host cell or eukaryotic host cell, respectively. Ovals represent bacterial ancestors and resulting endosymbionts as well as final plastids. Final mitochondria are represented by small icons with brown outer and blue inner membrane. DNA of endosymbionts is represented by blue circles and gene transfer to the nucleus by black arrows. Reduction of coding capacity is indicated by reduced size of DNA-representing circles. Continuous light-blue arrows: Anterograde signalling. Broken light blue arrows: Retrograde signalling. Large connecting arrows in grey, green and red represent the evolutionary lines. Yellow circles represent the respective import machineries. Note that retrograde signalling from mitochondria was likely to be established when plastids evolved. Basic principles should have been conserved in the different evolutionary lines. Further evolution of mitochondrial retrograde signals in autotrophs, however, was probably

influenced by the presence of plastids (mutual arrow, interaction?). In heterotrophs other influences such as multicellularity and tissue context may have generated different evolutionary constraints. In rhodophyta (pink box) and chlorophyta (green box) mitochondria, plastids and the nucleus developed triangular signalling networks.

Fig. 2: Common principles in retrograde signalling of eukaryotic organelles. The diagram depicts four major biological triggers (indicated in left panel) in which mitochondria and chloroplasts (represented by head-to-head orange and green ovals as a combined symbol) initiate retrograde signal classes with high similarity in signal identity (pink triangle), gene target and cellular response (depicted in right panel). Organelles detect and integrate external or cellular triggers (yellow arrows, input integration), produce the corresponding retrograde signal(s) (pink arrow) that is/are detected by the nucleus where the information is finally integrated to initiate a corresponding response (blue arrows, output integration). ETC: electron transport chain; ROS: reactive oxygen species; OXPHOS: oxidative phosphorylation; UPR: unfolded protein response.

References

Adamowicz-Skrzypkowska A, Kwaśniak-Owczarek M, Van Aken O, Kazmierczak U, Janska H (2020) Joint inhibition of mitochondrial complex IV and AOX by genetic or chemical means represses chloroplast transcription in Arabidopsis. Philos Trans R Soc Lond B Biol Sci

Allen JF (2015) Why chloroplasts and mitochondria retain their own genomes and genetic systems: Colocation for redox regulation of gene expression. Proc Natl Acad Sci USA 112:10231-10238

Allen JF (2017) The CoRR hypothesis for genes in organelles. J Theor Biol 434:50-57. doi:10.1016/j.jtbi.2017.04.008

Apel K, Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol 55:373-399

Archibald JM (2015) Endosymbiosis and Eukaryotic Cell Evolution. Curr Biol 25 (19):R911-921. doi:10.1016/j.cub.2015.07.055

Arnould T, Michel S, Renard P (2015) Mitochondria Retrograde Signaling and the UPR mt: Where Are We in Mammals? Int J Mol Sci 16 (8):18224-18251. doi:10.3390/ijms160818224

Balsa E, Soustek MS, Thomas A, Cogliati S, Garcia-Poyatos C, Martin-Garcia E, Jedrychowski M, Gygi SP, Enriquez JA, Puigserver P (2019) ER and Nutrient Stress Promote Assembly of Respiratory Chain Supercomplexes through the PERK-eIF2alpha Axis. Mol Cell 74 (5):877-890 e876. doi:10.1016/j.molcel.2019.03.031

Bao XR, Ong SE, Goldberger O, Peng J, Sharma R, Thompson DA, Vafai SB, Cox AG, Marutani E, Ichinose F, Goessling W, Regev A, Carr SA, Clish CB, Mootha VK (2016) Mitochondrial dysfunction remodels one-carbon metabolism in human cells. Elife 5. doi:10.7554/eLife.10575

Barcena C, Mayoral P, Quiros PM (2018) Mitohormesis, an Antiaging Paradigm. Int Rev Cell Mol Biol 340:35-77. doi:10.1016/bs.ircmb.2018.05.002

Björkholm P, Harish A, Hagström E, Ernst AM, Andersson SGE (2015) Mitochondrial genomes are retained by selective constraints on protein targeting. 112:10154-10161

Blanco NE, Guinea-Diaz M, Whelan J, Strand A (2014) Interaction between plastid and mitochondrial retrograde signalling pathways during changes to plastid redox status. Philos Trans R Soc Lond B Biol Sci 369 (1640):20130231. doi:10.1098/rstb.2013.0231

Blesa JR, Prieto-Ruiz JA, Hernandez JM, Hernandez-Yago J (2007) NRF-2 transcription factor is required for human TOMM20 gene expression. Gene 391 (1-2):198-208. doi:10.1016/j.gene.2006.12.024

Bock FJ, Tait SWG (2020) Mitochondria as multifaceted regulators of cell death. Nat Rev Mol Cell Biol 21:85-100

Bohovych I, Khalimonchuk O (2016) Sending Out an SOS: Mitochondria as a Signaling Hub. Front Cell Dev Biol 4:109. doi:10.3389/fcell.2016.00109

Börner T (2017) The discovery of plastid-to-nucleus retrograde signaling - a personal perspective. Protoplasma 254:1845-1855

Butow RA, Avadhani NG (2004) Mitochondrial Signaling: The Retrograde Response. Molecular Cell 14 (1):1-15

Calvo SE, Clauser KR, Mootha VK (2016) MitoCarta2.0: an updated inventory of mammalian mitochondrial proteins. Nucleic Acids Res 44 (D1):D1251-1257. doi:10.1093/nar/gkv1003

Cazzonelli CI, Hou X, Alagoz Y, Rivers J, Dhami N, Lee J, Marri S, Pogson BJ (2020) A cis-carotene derived apocarotenoid regulates etioplast and chloroplast development. Elife 9. doi:10.7554/eLife.45310

Celardo I, Lehmann S, Costa AC, Loh SH, Miguel Martins L (2017) dATF4 regulation of mitochondrial folate-mediated one-carbon metabolism is neuroprotective. Cell Death Differ 24 (4):638-648. doi:10.1038/cdd.2016.158

Chacinska A, Koehler CM, Milenkovic D, Lithgow T, Pfanner N (2009) Importing mitochondrial proteins: machineries and mechanisms. Cell 138 (4):628-644. doi:10.1016/j.cell.2009.08.005

Chan KX, Phua SY, Crisp P, McQuinn R, Pogson BJ (2016) Learning the Languages of the Chloroplast: Retrograde Signaling and Beyond. Annu Rev Plant Biol 67:25-53. doi:10.1146/annurev-arplant-043015-111854

Chen M, Galvao RM, Li M, Burger B, Bugea J, Bolado J, Chory J (2010) Arabidopsis HEMERA/pTAC12 initiates photomorphogenesis by phytochromes. Cell 141 (7):1230-1240. doi:10.1016/j.cell.2010.05.007

Christians MJ, Larsen PB (2007) Mutational loss of the prohibitin AtPHB3 results in an extreme constitutive ethylene response phenotype coupled with partial loss of ethylene-inducible gene expression in Arabidopsis seedlings. J Exp Bot 58:2237–2248

Clifton R, Lister R, Parker KL, Sappl PG, Elhafez D, Millar AH, Day DA, Whelan J (2005) Stress-induced co-expression of alternative respiratory chain components in Arabidopsis thaliana. Plant Mol Biol 58 (2):193-212. doi:10.1007/s11103-005-5514-7

Cohen S, Valm AM, Lippincott-Schwartz J (2018) Interacting organelles. Curr Opin Cell Biol 53:84-91

Colombo M, Tadini L, Peracchio C, Ferrari R, Pesaresi P (2016) GUN1, a Jack-Of-All-Trades in Chloroplast Protein Homeostasis and Signaling. Front Plant Sci 7:1427. doi:10.3389/fpls.2016.01427

Cui F, Brosche M, Shapiguzov A, He XQ, Vainonen JP, Leppala J, Trotta A, Kangasjarvi S, Salojarvi J, Kangasjarvi J, Overmyer K (2019) Interaction of methyl viologen-induced chloroplast and mitochondrial signalling in Arabidopsis. Free Radic Biol Med 134:555-566. doi:10.1016/j.freeradbiomed.2019.02.006

da Cunha FM, Torelli NQ, Kowaltowski AJ (2015) Mitochondrial Retrograde Signaling: Triggers, Pathways, and Outcomes. Oxid Med Cell Longev 2015:482582. doi:10.1155/2015/482582

De Clercq I, Vermeirssen V, Van Aken O, Vandepoele K, Murcha MW, Law SR, Inze A, Ng S, Ivanova A, Rombaut D, van de Cotte B, Jaspers P, Van de Peer Y, Kangasjarvi J, Whelan J, Van Breusegem F (2013) The membrane-bound NAC transcription factor ANAC013 functions in mitochondrial retrograde regulation of the oxidative stress response in Arabidopsis. Plant Cell 25 (9):3472-3490. doi:10.1105/tpc.113.117168

de Souza A, Wang JZ, Dehesh K (2016) Retrograde Signals: Integrators of Interorganellar Communication and Orchestrators of Plant Development. Annu Rev Plant Biol. doi:10.1146/annurev-arplant-042916-041007

Dhar SS, Ongwijitwat S, Wong-Riley MT (2008) Nuclear respiratory factor 1 regulates all ten nuclear-encoded subunits of cytochrome c oxidase in neurons. J Biol Chem 283 (6):3120-3129. doi:10.1074/jbc.M707587200

Diekmann Y, Pereira-Leal JB (2013) Evolution of intracellular compartmentalization. Biochem J 449:319-331

Dietz K-J, Turkan I, Krieger-Liszkay A (2016) Redox- and reactive oxygen species dependent signaling into and out of the photosynthesizing chloroplast. Plant Physiol 171:1541-1550

Dietz KJ, Pfannschmidt T (2011) Novel regulators in photosynthetic redox control of plant metabolism and gene expression. Plant Physiol 155 (4):1477-1485. doi:10.1104/pp.110.170043

Dietzel L, Brautigam K, Pfannschmidt T (2008) Photosynthetic acclimation: state transitions and adjustment of photosystem stoichiometry--functional relationships between short-term and long-term light quality acclimation in plants. FEBS J 275 (6):1080-1088. doi:10.1111/j.1742-4658.2008.06264.x

Dietzel L, Glasser C, Liebers M, Hiekel S, Courtois F, Czarnecki O, Schlicke H, Zubo Y, Borner T, Mayer K, Grimm B, Pfannschmidt T (2015) Identification of Early Nuclear Target Genes of Plastidial Redox Signals that Trigger the Long-Term Response of Arabidopsis to Light Quality Shifts. Mol Plant 8 (8):1237-1252. doi:10.1016/j.molp.2015.03.004

Duan L, Ruiz-Sola MA, Couso A, Veciana N, Monte E (2020) Red and blue light differentially impact retrograde signaling and photoprotection in rice. Philos Trans R Soc Lond B Biol Sci

Estavillo GM, Crisp PA, Pornsiriwong W, Wirtz M, Collinge D, Carrie C, Giraud E, Whelan J, David P, Javot H, Brearley C, Hell R, Marin E, Pogson BJ (2011) Evidence for a SAL1-PAP chloroplast retrograde pathway that functions in drought and high light signaling in Arabidopsis. Plant Cell 23 (11):3992-4012. doi:10.1105/tpc.111.091033

Exposito-Rodriguez M, Laissue P, Yvon-Durocher G, Smirnoff N, Mullineaux P (2017a) Photosynthesis-dependent H2O2 transfer from chloroplasts to nuclei provides a high-light signalling mechanism. Nat Commun 8:49

Exposito-Rodriguez M, Laissue PP, Yvon-Durocher G, Smirnoff N, Mullineaux PM (2017b) Photosynthesis-dependent H2O2 transfer from chloroplasts to nuclei provides a high-light signalling mechanism. Nat Commun 8 (1):49. doi:10.1038/s41467-017-00074-w

Ferro M, Brugiere S, Salvi D, Seigneurin-Berny D, Court M, Moyet L, Ramus C, Miras S, Mellal M, Le Gall S, Kieffer-Jaquinod S, Bruley C, Garin J, Joyard J, Masselon C, Rolland N (2010) AT_CHLORO, a comprehensive chloroplast proteome database with subplastidial localization and curated information on envelope proteins. Mol Cell Proteomics 9 (6):1063-1084. doi:10.1074/mcp.M900325-MCP200

Fessler E, Eckl E-M, Schmitt S, Mancilla IA, Meyer-Bender MF, Hanf M, Philippou-Massier J, Krebs S, Zischka H, Jae LT (2020) A pathway coordinated by DELE1 relays mitochondrial stress to the cytosol. Nature. doi:doi.org/10.1038/s41586-020-2076-4

Fiorese CJ, Schulz AM, Lin YF, Rosin N, Pellegrino MW, Haynes CM (2016) The Transcription Factor ATF5 Mediates a Mammalian Mitochondrial UPR. Curr Biol 26 (15):2037-2043. doi:10.1016/j.cub.2016.06.002

Forsstrom S, Jackson CB, Carroll CJ, Kuronen M, Pirinen E, Pradhan S, Marmyleva A, Auranen M, Kleine IM, Khan NA, Roivainen A, Marjamaki P, Liljenback H, Wang L, Battersby BJ, Richter U, Velagapudi V, Nikkanen J, Euro L, Suomalainen A (2019) Fibroblast Growth Factor 21 Drives Dynamics of Local and Systemic Stress Responses in Mitochondrial Myopathy with mtDNA Deletions. Cell Metab 30 (6):1040-1054 e1047. doi:10.1016/j.cmet.2019.08.019

Ganusova E, Reagan B, Fernandez J, Azim M, Sankoh A, Freeman K, McCray T, Patterson K, Kim C (2020) Chloroplast-to-nucleus retrograde signalling controls intercellular trafficking via plasmodesmata formation. Philos Trans R Soc Lond B Biol Sci

Giraud E, Van Aken O, Ho LH, Whelan J (2009) The transcription factor ABI4 is a regulator of mitochondrial retrograde expression of ALTERNATIVE OXIDASE1a. Plant Physiol 150 (3):1286-1296. doi:10.1104/pp.109.139782

Gleyzer N, Vercauteren K, Scarpulla RC (2005) Control of mitochondrial transcription specificity factors (TFB1M and TFB2M) by nuclear respiratory factors (NRF-1 and NRF-2) and PGC-1 family coactivators. Mol Cell Biol 25 (4):1354-1366. doi:10.1128/MCB.25.4.1354-1366.2005

Gollan P, Aro E-M (2020) Photosynthetic signalling during high light stress and recovery; targets and dynamics. Philos Trans R Soc Lond B Biol Sci

Granat L, Hunt RJ, Bateman JM (2020) Mitochondrial retrograde signalling in neurological disease. Philos Trans R Soc Lond B Biol Sci

Gregersen PL, Culetic A, Boschian L, Krupinska K (2013) Plant senescence and crop productivity. Plant Mol Biol 82 (6):603-622. doi:10.1007/s11103-013-0013-8

Guo X, Aviles G, Liu Y, Tian R, Unger BA, Lin Y-HT, Wiita AP, Xu K, Correia MA, Kampmann M (2019) Mitochondrial dysfunction is signaled to the integrated stress response by OMA1, DELE1 and HRI. bioRxiv:715896. doi:10.1101/715896

Guo X, Aviles G, Liu Y, Tian R, Unger BA, Lin YF, Wiita AP, Xu D, Correia MA, Kampmann M (2020) Mitochondrial stress is relayed to the cytosol by an OMA1-DELE-HRI pathway. Nature. doi:doi.org/10.1038/s41586-020-2078-2

Hu Z, Vanderhaeghen R, Cools T, Wang Y, De Clercq I, Leroux O, Nguyen L, Belt K, Millar AH, Audenaert D, Hilson P, Small ID, Mouille G, Vernhettes S, Van Breusegem F, Whelan J, Hofte H, De Veylder L (2016) Mitochondrial Defects Confer Tolerance against Cellulose Deficiency. Plant Cell. doi:10.1105/tpc.16.00540

Hunt RJ, Granat L, McElroy GS, Ranganathan R, Chandel NS, Bateman JM (2019) Mitochondrial stress causes neuronal dysfunction via an ATF4-dependent increase in L-2-hydroxyglutarate. J Cell Biol 218 (12):4007-4016. doi:10.1083/jcb.201904148

Imachi H, Nobu MK, Nakahara N, Morono Y, Ogawara M, Takaki Y, Takano Y, Uematsu K, Ikuta T, Ito M, Matsui Y, Miyazaki M, Murata K, Saito Y, Sakai S, Song C, Tasumi E, Yamanaka Y, Yamaguchi T, Kamagata Y, Tamaki Y, Takai K (2020) Isolation of an archeon at the prokaryote-eukaryote interface. Nature 577:519-525

Isemer R, Mulisch M, Schafer A, Kirchner S, Koop HU, Krupinska K (2012) Recombinant Whirly1 translocates from transplastomic chloroplasts to the nucleus. FEBS Lett 586 (1):85-88. doi:10.1016/j.febslet.2011.11.029

Ivanova A, Law SR, Narsai R, Duncan O, Lee JH, Zhang B, Van Aken O, Radomiljac JD, van der Merwe M, Yi K, Whelan J (2014) A Functional Antagonistic Relationship between Auxin and Mitochondrial Retrograde Signaling Regulates Alternative Oxidase1a Expression in Arabidopsis. Plant Physiol 165 (3):1233-1254. doi:10.1104/pp.114.237495

Jarvis P, Lopez-Juez E (2013) Biogenesis and homeostasis of chloroplasts and other plastids. Nat Rev Mol Cell Biol 14 (12):787-802. doi:10.1038/nrm3702

Jarvis P, López-Juez E (2013) Biogenesis and homeostasis of chloroplasts and other plastids. Nature Reviews Molecular Cell Biology 14:787. doi:10.1038/nrm3702

Jazwinski SM (2014) The retrograde response - a conserved compensatory reaction to damage from within and from without. Prog Mol Biol Transl Sci 127:133-154

Kacprzak S, Dahlquist A, Van Aken O (2020) The transcription factor ANAC017 is a key regulator of mitochondrial proteotoxic stress responses in plants. Philos Trans R Soc Lond B Biol Sci

Kacprzak SM, Mochizuki N, Naranjo B, Xu D, Leister D, Kleine T, Okamoto H, Terry MJ (2019) Plastid-to-Nucleus Retrograde Signalling during Chloroplast Biogenesis Does Not Require ABI4. Plant Physiol 179 (1):18-23. doi:10.1104/pp.18.01047

Kakizaki T, Yazu F, Nakayama K, Ito-Inaba Y, Inaba T (2012) Plastid signalling under multiple conditions is accompanied by a common defect in RNA editing in plastids. J Exp Bot 63 (1):251-260. doi:10.1093/jxb/err257

Kerchev PI, De Clercq I, Denecker J, Muhlenbock P, Kumpf R, Nguyen L, Audenaert D, Dejonghe W, Van Breusegem F (2014) Mitochondrial perturbation negatively affects auxin signaling. Mol Plant 7 (7):1138-1150. doi:10.1093/mp/ssu071

Khan NA, Nikkanen J, Yatsuga S, Jackson C, Wang L, Pradhan S, Kivela R, Pessia A, Velagapudi V, Suomalainen A (2017) mTORC1 Regulates Mitochondrial Integrated Stress Response and

Mitochondrial Myopathy Progression. Cell Metab 26 (2):419-428 e415. doi:10.1016/j.cmet.2017.07.007
Kim C (2020) ROS-driven oxidative modification: Its impact on chloroplast-nucleus communication. Front Plant Sci 10:1729
Kim C, Meskauskiene R, Zhang S, Lee KP, Lakshmanan Ashok M, Blajeckka K, Herrfurth C, Feussner I, Apel K (2012) Chloroplasts of Arabidopsis are the source and a primary target of a plant-specific programmed cell death signaling pathway. Plant Cell 24:3026-3039
Kim HJ, Park J-H, Kim J, Kim JJ, Hong S, Kim J, Kim JH, Woo HR, Hyeon C, Lim PO, Nam HG, Hwang D (2018) Time-evolving genetic networks reveal a NAC troika that negatively regulates leaf senescence in Arabidopsis. Proc Natl Acad Sci USA 115:E4930-E4939
Kim S, Sieburth D (2018) Sphingosine Kinase Activates the Mitochondrial Unfolded Protein Response and Is Targeted to Mitochondria by Stress. Cell Rep 24 (11):2932-2945 e2934. doi:10.1016/j.celrep.2018.08.037
Kmiecik P, Leonardelli M, Teige M (2016) Novel connections in plant organellar signalling link different stress responses and signalling pathways. J Exp Bot 67:3793-3807
Koussevitzky S, Nott A, Mockler TC, Hong F, Sachetto-Martins G, Surpin M, Lim J, Mittler R, Chory J (2007) Signals from chloroplasts converge to regulate nuclear gene expression. Science 316 (5825):715-719. doi:10.1126/science. 1140516
Krause K, Krupinska K (2009) Nuclear regulators with a second home in organelles. Trends Plant Sci 14 (4):194-199. doi:10.1016/j.tplants.2009.01.005
Krause K, Oetke S, Krupinska K (2012) Dual targeting and retrograde translocation: regulators of plant nuclear gene expression can be sequestered by plastids. Int J Mol Sci 13 (9):11085-11101. doi:10.3390/ijms130911085
Krupinska K, Blanco N, Oetke S, Zottini M (2020) Genome communication in plants mediated by organelle-nucleus located proteins. Philos Trans R Soc Lond B Biol Sci
Krupinska K, Mulisch M, Hollmann J, Tokarz K, Zschiesche W, Kage H, Humbeck K, Bilger W (2012) An alternative strategy of dismantling of the chloroplasts during leaf senescence observed in a high-yield variety of barley. Physiol Plant 144 (2):189-200. doi:10.1111/j.1399-3054.2011.01545.x
Krupinska K, Oetke S, Desel C, Mulisch M, Schafer A, Hollmann J, Kumlehn J, Hensel G (2014) WHIRLY1 is a major organizer of chloroplast nucleoids. Front Plant Sci 5:432. doi:10.3389/fpls.2014.00432
Kudla J, Becker D, Grill E, Hedrich R, Hippler M, Kummer U, Parniske M, Romeis T, Schumacher K (2018) Advances and current challenges in calcium signalling. New Phytologist 218414-431
Kusaba M, Tanaka A, Tanaka R (2013) Stay-green plants: what do they tell us about the molecular mechanism of leaf senescence. Photosynth Res 117:221-234
Lama S, Broda M, Abbas Z, Vaneechoutte D, Belt K, Sall T, Vandepoele K, Van Aken O (2019) Neofunctionalization of Mitochondrial Proteins and Incorporation into Signaling Networks in Plants. Mol Biol Evol 36 (5):974-989. doi:10.1093/molbev/msz031
Larkin RM (2014) Influence of plastids on light signalling and development. Philos Trans R Soc Lond B Biol Sci 369:20130232
Larkin RM (2016) Tetrapyrrole Signaling in Plants. Front Plant Sci 7:1586. doi:10.3389/fpls.2016.01586
Larkin RM, Alonso JM, Ecker JR, Chory J (2003) GUN4, a regulator of chlorophyll synthesis and intracellular signaling. Science 299 (5608):902-906. doi:10.1126/science.1079978
Lee CP, Eubel H, Solheim C, Millar AH (2012) Mitochondrial Proteome Heterogeneity between Tissues from the Vegetative and Reproductive Stages of Arabidopsis thaliana Development. J Proteome Res. doi:10.1021/pr3001157
Lee KP, Kim C, Landgraf F, Apel K (2007) EXECUTER1- and EXECUTER2-dependent transfer of stress-related signals from the plastid to the nucleus of Arabidopsis thaliana. Proc Natl Acad Sci U S A 104 (24):10270-10275. doi:10.1073/pnas.0702061104

Leister D (2019) Piecing the puzzle together: The central role of reactive oxygen species and redox hubs in chloroplast retrograde signaling. Antioxid Redox Signal 30:1206-1219

Liere K, Weihe A, Borner T (2011) The transcription machineries of plant mitochondria and chloroplasts: Composition, function, and regulation. J Plant Physiol 168 (12):1345-1360. doi:10.1016/j.jplph.2011.01.005

Llamas E, Pulido P, Rodriguez-Concepcion M (2017) Interference with plastome gene expression and Clp protease activity in Arabidopsis triggers a chloroplast unfolded protein response to restore protein homeostasis. PLoS Genet 13 (9):e1007022. doi:10.1371/journal.pgen.1007022

Lopez-Juez E, Pyke KA (2005) Plastids unleashed: their development and their integration in plant development. Int J Dev Biol 49 (5-6):557-577. doi:10.1387/ijdb.051997el

Loudya N, Okunola T, He J, Jarvis P, Lopez-Juet E (2020) Retrograde signalling in a virescent mutant triggers an anterograde delay of chloroplast biogenesis that requires GUN1 and is essential for survival. Philos Trans R Soc Lond B Biol Sci

Mallo N, Fellows J, Johnson C, Sheiner L (2018) Protein import into the endosymbiotic organelles of apicomplexan parasites. Genes 9:412-431

Marino G, Naranjo B, Wang J, Penzler JF, Kleine T, Leister D (2019) Relationship of GUN1 to FUG1 in chloroplast protein homeostasis. Plant J 99 (3):521-535. doi:10.1111/tpj.14342

Martijn J, Vosseberg J, Guy L, Offre P, Ettema TJG (2018) Deep mitochndrial origin outside the sampled alphaproteobacteria. Nature 557:101-105

Martin W, Rujan T, Richly E, Hansen A, Cornelsen S, Lins T, Leister D, Stoebe B, Hasegawa M, Penny D (2002) Evolutionary analysis of Arabidopsis, cyanobacterial, and chloroplast genomes reveals plastid phylogeny and thousands of cyanobacterial genes in the nucleus. Proc Natl Acad Sci U S A 99 (19):12246-12251. doi:10.1073/pnas.182432999

Martin WF, Garg S, Zimorski V (2015) Endosymbiotic theories for eukaryote origin. Philos Trans R Soc Lond B Biol Sci 370 (1678):20140330. doi:10.1098/rstb.2014.0330

Matilainen O, Quiros PM, Auwerx J (2017) Mitochondria and Epigenetics - Crosstalk in Homeostasis and Stress. Trends Cell Biol 27 (6):453-463. doi:10.1016/j.tcb.2017.02.004

Maxwell DP, Wang Y, McIntosh L (1999) The alternative oxidase lowers mitochondrial reactive oxygen production in plant cells. Proc Natl Acad Sci U S A 96 (14):8271-8276

McFadden GI, Yeh E (2017) The apicoplast: now you see it, now you don´t. Int J Parasitol 47:137-144

Meier I, Richards EJ, Evans DE (2017) Cell biology of the plant nucleus. Annu Rev Plant Biol 68:139-172

Meng X, Li L, De Clercq I, Narsai R, Xu Y, Hartmann A, Lozano Claros D, Custovic E, Lewsey MG, Whelan J, Berkowitz O (2019) ANAC017 Coordinates Organellar Functions and Stress Responses by Reprogramming Retrograde Signaling. Plant Physiol 180:634-653

Meng X, Li L, Narsai R, De Clercq I, Whelan J, Berkowitz O (2020) Mitochndrial signalling is critical for acclimation and adaptation to flooding in Arabidopsis thaliana. Plant J. doi:10.1111/tpj.14724

Merendino L, Courtois F, Grübler B, Bastien O, Straetmanns V, Chevalier F, Lerbs-Mache S, Lurin C, Pfannschmidt T (2020) Retrograde signals from mitochondria reprogram skoto-morphogenesis in 1 Arabidopsis thaliana via Alternative Oxidase 1a. Philos Trans R Soc Lond B Biol Sci

Meyer EH, Tomaz T, Carroll AJ, Estavillo G, Delannoy E, Tanz SK, Small ID, Pogson BJ, Millar AH (2009) Remodeled respiration in ndufs4 with low phosphorylation efficiency suppresses Arabidopsis germination and growth and alters control of metabolism at night. Plant Physiol 151 (2):603-619. doi:10.1104/pp.109.141770

Michel S, Canonne M, Arnould T, Renard P (2015) Inhibition of mitochondrial genome expression triggers the activation of CHOP-10 by a cell signaling dependent on the integrated stress response but not the mitochondrial unfolded protein response. Mitochondrion 21:58-68. doi:10.1016/j.mito.2015.01.005

Mochizuki N, Brusslan JA, Larkin R, Nagatani A, Chory J (2001) Arabidopsis genomes uncoupled 5 (GUN5) mutant reveals the involvement of Mg-chelatase H subunit in plastid-to-nucleus signal transduction. Proc Natl Acad Sci U S A 98 (4):2053-2058. doi:10.1073/pnas.98.4.2053

Molenaars M, Daniels E, Meurs A, Janssens G, Houtkooper R (2020) Mitochondrial cross-compartmental signaling to maintain proteostasis and longevity. Philos Trans R Soc Lond B Biol Sci

Morley SA, Nielsen BL (2016) Chloroplast DNA copy number changes during plant development in organelle DNA polymerase mutants. Front Plant Sci 7:57

Moullan N, Mouchiroud L, Wang X, Ryu D, Williams EG, Mottis A, Jovaisaite V, Frochaux MV, Quiros PM, Deplancke B, Houtkooper RH, Auwerx J (2015) Tetracyclines Disturb Mitochondrial Function across Eukaryotic Models: A Call for Caution in Biomedical Research. Cell Rep 10 (10):1681-1691. doi:10.1016/j.celrep.2015.02.034

Mullineaux P, Exposito-Rodriguez M, Laissue P, Smirnoff N, Park E (2020) Spatial chloroplast-to-nucleus signalling involving plastid-nuclear complexes and stromules. Philos Trans R Soc Lond B Biol Sci

Mur L, Kenton P, Lloyd AJ, Ougham H, Prats E (2008) The hypersensitive response; the centenary is upon us but how much do we know? J Exp Bot 59:501-520

Nargund AM, Fiorese CJ, Pellegrino MW, Deng P, Haynes CM (2015) Mitochondrial and nuclear accumulation of the transcription factor ATFS-1 promotes OXPHOS recovery during the UPR(mt). Mol Cell 58 (1):123-133. doi:10.1016/j.molcel.2015.02.008

Ng S, Giraud E, Duncan O, Law SR, Wang Y, Xu L, Narsai R, Carrie C, Walker H, Day DA, Blanco NE, Strand A, Whelan J, Ivanova A (2013a) Cyclin-dependent kinase E1 (CDKE1) provides a cellular switch in plants between growth and stress responses. J Biol Chem 288 (5):3449-3459. doi:10.1074/jbc.M112.416727

Ng S, Ivanova A, Duncan O, Law SR, Van Aken O, De Clercq I, Wang Y, Carrie C, Xu L, Kmiec B, Walker H, Van Breusegem F, Whelan J, Giraud E (2013b) A membrane-bound NAC transcription factor, ANAC017, mediates mitochondrial retrograde signaling in Arabidopsis. Plant Cell 25 (9):3450-3471. doi:10.1105/tpc.113.113985

Page M, Allison S, Terry MJ (2020) Overexpression of chloroplast-targeted ferrochelatase 1 results in a genomes uncoupled chloroplast-to-nucleus retrograde signalling phenotype. Philos Trans R Soc Lond B Biol Sci

Page MT, McCormac AC, Smith AG, Terry MJ (2017) Singlet oxygen initiates a plastid signal controlling photosynthetic gene expression. New Phytol 213 (3):1168-1180. doi:10.1111/nph.14223

Pakos-Zebrucka K, Koryga I, Mnich K, Ljujic M, Samali A, Gorman AM (2016) The integrated stress response. EMBO Rep 17 (10):1374-1395. doi:10.15252/embr.201642195

Parfrey LW, Lahr DJ, Knoll AH, Katz LA (2011) Estimating the timing of early eukaryotic diversification with multigene molecular clocks. Proc Natl Acad Sci U S A 108 (33):13624-13629. doi:10.1073/pnas.1110633108

Petrillo E, Godoy Herz MA, Fuchs A, Reifer D, Fuller J, Yanovsky MJ, Simpson C, Brown JW, Barta A, Kalyna M, Kornblihtt AR (2014) A chloroplast retrograde signal regulates nuclear alternative splicing. Science 344 (6182):427-430. doi:10.1126/science.1250322

Pfalz J, Liebers M, Hirth M, Grubler B, Holtzegel U, Schroter Y, Dietzel L, Pfannschmidt T (2012) Environmental control of plant nuclear gene expression by chloroplast redox signals. Front Plant Sci 3:257. doi:10.3389/fpls.2012.00257

Pfalz J, Liere K, Kandlbinder A, Dietz KJ, Oelmuller R (2006) pTAC2, -6, and -12 are components of the transcriptionally active plastid chromosome that are required for plastid gene expression. Plant Cell 18 (1):176-197. doi:10.1105/tpc.105.036392

Pfalz J, Pfannschmidt T (2013) Essential nucleoid proteins in early chloroplast development. Trends Plant Sci 18 (4):186-194. doi:10.1016/j.tplants.2012.11.003

Pfannschmidt T (2010) Plastidial retrograde signalling--a true "plastid factor" or just metabolite signatures? Trends Plant Sci 15 (8):427-435. doi:10.1016/j.tplants.2010.05.009

Pfannschmidt T, Blanvillain R, Merendino L, Courtois F, Chevalier F, Liebers M, Grubler B, Hommel E, Lerbs-Mache S (2015) Plastid RNA polymerases: orchestration of enzymes with different evolutionary origins controls chloroplast biogenesis during the plant life cycle. J Exp Bot 66:6957-6973. doi:10.1093/jxb/erv415

Pfannschmidt T, Link G (1994) Separation of two classes of plastid DNA-dependent RNA polymerases that are differentially expressed in mustard (Sinapis alba L.) seedlings. Plant Mol Biol 25 (1):69-81

Pfannschmidt T, Munne-Bosch S (2013) Plastidial signaling during the plant life cycle. . In: Biswal B, Krupinska K (eds) Plastid development in leaves during growth and senescence., vol 36. Advances in Photosynthesis and Respiration. Springer, Dordrecht. doi:10.1007/978-94-007-5724-0_22

Pfannschmidt T, Schutze K, Fey V, Sherameti I, Oelmuller R (2003) Chloroplast redox control of nuclear gene expression--a new class of plastid signals in interorganellar communication. Antioxid Redox Signal 5 (1):95-101. doi:10.1089/152308603321223586

Pogson BJ, Ganguly D, Albrecht-Borth V (2015) Insights into chloroplast biogenesis and development. Biochim Biophys Acta 1847 (9):1017-1024. doi:10.1016/j.bbabio.2015.02.003

Pogson BJ, Woo NS, Forster B, Small ID (2008) Plastid signalling to the nucleus and beyond. Trends Plant Sci 13 (11):602-609. doi:10.1016/j.tplants.2008.08.008

Prokisch H, Scharfe C, Camp DG, 2nd, Xiao W, David L, Andreoli C, Monroe ME, Moore RJ, Gritsenko MA, Kozany C, Hixson KK, Mottaz HM, Zischka H, Ueffing M, Herman ZS, Davis RW, Meitinger T, Oefner PJ, Smith RD, Steinmetz LM (2004) Integrative analysis of the mitochondrial proteome in yeast. PLoS Biol 2 (6):e160. doi:10.1371/journal.pbio.0020160

Pyke K (2007) Plastid biogenesis and differentiation. In: Bock R (ed) Cell and Molecular Biology of Plastids, vol 19. Springer-Verlag, Berlin, pp 1-28

Pyke KA (2010) Plastid division. AoB Plants 2010:plq016. doi:10.1093/aobpla/plq016

Qiu Y, Li M, Pasoreck EK, Long L, Shi Y, Galvao RM, Chou CL, Wang H, Sun AY, Zhang YC, Jiang A, Chen M (2015) HEMERA Couples the Proteolysis and Transcriptional Activity of PHYTOCHROME INTERACTING FACTORs in Arabidopsis Photomorphogenesis. Plant Cell 27 (5):1409-1427. doi:10.1105/tpc.114.136093

Quiros PM, Mottis A, Auwerx J (2016) Mitonuclear communication in homeostasis and stress. Nat Rev Mol Cell Biol 17 (4):213-226. doi:10.1038/nrm.2016.23

Quiros PM, Prado MA, Zamboni N, D'Amico D, Williams RW, Finley D, Gygi SP, Auwerx J (2017) Multi-omics analysis identifies ATF4 as a key regulator of the mitochondrial stress response in mammals. J Cell Biol 216 (7):2027-2045. doi:10.1083/jcb.201702058

Ramel F, Birtic S, Ginies C, Soubigou-Taconnat L, Triantaphylides C, Havaux M (2012) Carotenoid oxidation products are stress signals that mediate gene responses to singlet oxygen in plants. Proc Natl Acad Sci U S A 109 (14):5535-5540. doi:10.1073/pnas.1115982109

Ramundo S, Casero D, Muhlhaus T, Hemme D, Sommer F, Crevecoeur M, Rahire M, Schroda M, Rusch J, Goodenough U, Pellegrini M, Perez-Perez ME, Crespo JL, Schaad O, Civic N, Rochaix JD (2014) Conditional Depletion of the Chlamydomonas Chloroplast ClpP Protease Activates Nuclear Genes Involved in Autophagy and Plastid Protein Quality Control. Plant Cell 26 (5):2201-2222. doi:10.1105/tpc.114.124842

Reyes-Prieto A, Weber AP, Bhattacharya D (2007) The origin and establishment of the plastid in algae and plants. Annu Rev Genet 41:147-168. doi:10.1146/annurev.genet.41.110306.130134

Rhoads DM, Subbaiah CC (2007) Mitochondrial retrograde regulation in plants. Mitochondrion 7:177-194

Richter A, Tohge T, Fernie AR, Grimm B (2020) The genomes uncoupled dependent signaling pathway coordinates plastid biogenesis with the synthesis of anthocyanins. Philos Trans R Soc Lond B Biol Sci

Rochaix JD, Ramundo S (2018) Chloroplast signaling and quality control. Essays Biochem 62 (1):13-20. doi:10.1042/EBC20170048

Ruan L, Zhou C, Jin E, Kucharavy A, Zhang Y, Wen Z, Florens L, Li R (2017) Cytosolic proteostasis through importing of misfolded proteins into mitochondria. Nature 543 (7645):443-446. doi:10.1038/nature21695

Schwarzlander M, Konig AC, Sweetlove LJ, Finkemeier I (2012) The impact of impaired mitochondrial function on retrograde signalling: a meta-analysis of transcriptomic responses. J Exp Bot 63 (4):1735-1750. doi:10.1093/jxb/err374

Shang Y, Yan L, Liu ZQ, Cao Z, Mei C, Xin Q, Wu FQ, Wang XF, Du SY, Jiang T, Zhang XF, Zhao R, Sun HL, Liu R, Yu YT, Zhang DP (2010) The Mg-chelatase H subunit of Arabidopsis antagonizes a group of WRKY transcription repressors to relieve ABA-responsive genes of inhibition. Plant Cell 22 (6):1909-1935. doi:10.1105/tpc.110.073874

Shapiguzov A, Nikkanen L, Fitzpatrick D, Vainonen JP, Gossens R, Alseekh S, Aarabi F, Tiwari A, Blokhina O, Panzarová K, Benedikty Z, Tyystjärvi E, Fernie AR, Trtílek M, Aro E-M, Rintamäki E, Kangasjärvi J (2020) Dissecting the interaction of photosynthetic electron transfer with mitochondrial signalling and hypoxic response in the Arabidopsis rcd1 mutant. . Philos Trans R Soc Lond B Biol Sci

Shapiguzov A, Vainonen JP, Hunter K, Tossavainen H, Tiwari A, Jarvi S, Hellman M, Aarabi F, Alseekh S, Wybouw B, Van Der Kelen K, Nikkanen L, Krasensky-Wrzaczek J, Sipari N, Keinanen M, Tyystjarvi E, Rintamaki E, De Rybel B, Salojarvi J, Van Breusegem F, Fernie AR, Brosche M, Permi P, Aro EM, Wrzaczek M, Kangasjarvi J (2019) Arabidopsis RCD1 coordinates chloroplast and mitochondrial functions through interaction with ANAC transcription factors. Elife 8. doi:10.7554/eLife.43284

Shimizu T, Kacprzak SM, Mochizuki N, Nagatani A, Watanabe S, Shimada T, Tanaka K, Hayashi Y, Arai M, Leister D, Okamoto H, Terry MJ, Masuda T (2019) The retrograde signaling protein GUN1 regulates tetrapyrrole biosynthesis. Proc Natl Acad Sci U S A 116 (49):24900-24906. doi:10.1073/pnas.1911251116

Shimizu T, Yasuda R, Mukai Y, Shimada T, Imamura S, Tanaka K, Watanabe S, Masuda T (2020) Proteomic analysis of heme-binding protein from Arabidopsis thaliana and Cyanidioschyzon merolae. Philos Trans R Soc Lond B Biol Sci

Shpilka T, Haynes CM (2018) The mitochondrial UPR: mechanisms, physiological functions and implications in ageing. Nat Rev Mol Cell Biol 19 (2):109-120. doi:10.1038/nrm.2017.110

Steiner S, Schroter Y, Pfalz J, Pfannschmidt T (2011) Identification of essential subunits in the plastid-encoded RNA polymerase complex reveals building blocks for proper plastid development. Plant Physiol 157 (3):1043-1055. doi:10.1104/pp.111.184515

Stoebe B, Maier UG (2002) One, two, three: nature's tool box for building plastids. Protoplasma 219 (3-4):123-130. doi:10.1007/s007090200013

Susek RE, Ausubel FM, Chory J (1993) Signal transduction mutants of Arabidopsis uncouple nuclear CAB and RBCS gene expression from chloroplast development. Cell 74:787-799

Sylvestre-Gonon E, Schwartz M, Girardet M, Hecker A, Rouhier N (2020) Is there a role for tau glutathione transferases in tetrapyrrole metabolism and retrograde signaling in plants? Philos Trans R Soc Lond B Biol Sci

Szklarczyk R, Huynen MA (2009) Expansion of the human mitochondrial proteome by intra- and inter-compartmental protein duplication. Genome Biol 10 (11):R135. doi:10.1186/gb-2009-10-11-r135

Tadini L, Jeran N, Peracchio C, Masiero S, Colombo M, Pesaresi P (2020) The plastid transcription machinery and its coordination with the expression of nuclear genome: PEP-NEP and the GUN1-mediated retrograde communication. Philos Trans R Soc Lond B Biol Sci

Tadini L, Pesaresi P, Kleine T, Rossi F, Guljamow A, Sommer F, Muhlhaus T, Schroda M, Masiero S, Pribil M, Rothbart M, Hedtke B, Grimm B, Leister D (2016) GUN1 Controls Accumulation of the Plastid Ribosomal Protein S1 at the Protein Level and Interacts with Proteins Involved in Plastid Protein Homeostasis. Plant Physiol 170 (3):1817-1830. doi:10.1104/pp.15.02033

Terry MJ, Smith AG (2013) A model for tetrapyrrole synthesis as the primary mechanism for plastid-to-nucleus signaling during chloroplast biogenesis. Frontiers in Plant Sciences 4:14. doi:10.3389/fpls.2013.00014

Timmis JN, Ayliffe MA, Huang CY, Martin W (2004) Endosymbiotic gene transfer: organelle genomes forge eukaryotic chromosomes. Nat Rev Genet 5 (2):123-135. doi:10.1038/nrg1271

Tokumaru M, Adachi F, Toda M, Ito-Inaba Y, Yazu F, Hirosawa Y, Sakakibara Y, Suiko M, Kakizaki T, Inaba T (2017) Ubiquitin-Proteasome Dependent Regulation of the GOLDEN2-LIKE 1 Transcription Factor in Response to Plastid Signals. Plant Physiol 173 (1):524-535. doi:10.1104/pp.16.01546

Unal D, Garcia-Caparros P, Kumar V, Dietz K-J (2020) Chloroplast-associated molecular patterns (ChAMP) as concept for fine-tuned operational retrograde signalling. Philos Trans R Soc Lond B Biol Sci

Van Aken O, De Clercq I, Ivanova A, Law SR, Van Breusegem F, Millar AH, Whelan J (2016a) Mitochondrial and Chloroplast Stress Responses Are Modulated in Distinct Touch and Chemical Inhibition Phases. Plant Physiol 171 (3):2150-2165. doi:10.1104/pp.16.00273

Van Aken O, Ford E, Lister R, Huang S, Millar AH (2016b) Retrograde signalling caused by heritable mitochondrial dysfunction is partially mediated by ANAC017 and improves plant performance. Plant J 88 (4):542-558. doi:10.1111/tpj.13276

Van Aken O, Pogson BJ (2017) Convergence of mitochondrial and chloroplastic ANAC017/PAP-dependent retrograde signalling pathways and suppression of programmed cell death. Cell Death Differ 24 (6):955-960. doi:10.1038/cdd.2017.68

Van Aken O, Van Breusegem F (2015) Licensed to kill: mitochondria, chloroplasts, and cell death. Trends in Plant Science 20 (11):754-766

Van Aken O, Whelan J (2012) Comparison of transcriptional changes to chloroplast and mitochondrial perturbations reveals common and specific responses in Arabidopsis. Front Plant Sci 3:281. doi:10.3389/fpls.2012.00281

Van Aken O, Zhang B, Law S, Narsai R, Whelan J (2013) AtWRKY40 and AtWRKY63 modulate the expression of stress-responsive nuclear genes encoding mitochondrial and chloroplast proteins. Plant Physiol 162 (1):254-271. doi:10.1104/pp.113.215996

Vanderauwera S, Vandenbroucke K, Inze A, van de Cotte B, Muhlenbock P, De Rycke R, Naouar N, Van Gaever T, Van Montagu MC, Van Breusegem F (2012) AtWRKY15 perturbation abolishes the mitochondrial stress response that steers osmotic stress tolerance in Arabidopsis. Proc Natl Acad Sci U S A 109 (49):20113-20118. doi:10.1073/pnas.1217516109

Virbasius JV, Scarpulla RC (1994) Activation of the human mitochondrial transcription factor A gene by nuclear respiratory factors: a potential regulatory link between nuclear and mitochondrial gene expression in organelle biogenesis. Proc Natl Acad Sci U S A 91 (4):1309-1313. doi:10.1073/pnas.91.4.1309

Voleman L, Dolezal P (2019) Mitochondrial dynamics in parasitic protists. PLoS Pathog 15:e1008008

von Heijne G (1986) Why mitochondria need a genome. FEBS Lett 198:1-4

von Zychlinski A, Kleffmann T, Krishnamurthy N, Sjolander K, Baginsky S, Gruissem W (2005) Proteome analysis of the rice etioplast: metabolic and regulatory networks and novel protein functions. Mol Cell Proteomics 4 (8):1072-1084. doi:10.1074/mcp.M500018-MCP200

Wagner S, De Bortoli S, Schwarzländer M, Szabo I (2016) Regulation of mitochondrial calcium in plants versus animals. J Exp Bot 67:3809-3829

Wagner S, Steinbeck J, Fuchs P, Lichtenauer S, Elsasser M, Schippers JHM, Nietzel T, Ruberti C, Van Aken O, Meyer AJ, Van Dongen JT, Schmidt RR, Schwarzlander M (2019) Multiparametric real-time sensing of cytosolic physiology links hypoxia responses to mitochondrial electron transport. New Phytol 224 (4):1668-1684. doi:10.1111/nph.16093

Wagner S, Van Aken O, Elsasser M, Schwarzlander M (2018) Mitochondrial Energy Signaling and Its Role in the Low-Oxygen Stress Response of Plants. Plant Physiol 176 (2):1156-1170. doi:10.1104/pp.17.01387

Wang X, Auwerx J (2017) Systems Phytohormone Responses to Mitochondrial Proteotoxic Stress. Molecular cell 68 (3):540-551 e545. doi:10.1016/j.molcel.2017.10.006
Wang X, Chen XJ (2015) A cytosolic network suppressing mitochondria-mediated proteostatic stress and cell death. Nature 524 (7566):481-484. doi:10.1038/nature14859
Wang Y, Selinski J, Mao C, Zhu Y, Berkowitz O, Whelan J (2020) Linking mitochondrial and chloroplast retrograde signaling in plants. Philos Trans R Soc Lond B Biol Sci
Waters MT, Langdale JA (2009) The making of a chloroplast. EMBO J 28:2861-2873
Waters MT, Wang P, Korkaric M, Capper RG, Saunders NJ, Langdale JA (2009) GLK transcription factors coordinate expression of the photosynthetic apparatus in Arabidopsis. Plant Cell 21 (4):1109-1128. doi:10.1105/tpc.108.065250
Weidberg H, Amon A (2018) MitoCPR-A surveillance pathway that protects mitochondria in response to protein import stress. Science 360 (6385). doi:10.1126/science.aan4146
Woese CR, Kandler O, Wheelis ML (1990) Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. Proc Natl Acad Sci U S A 87 (12):4576-4579. doi:10.1073/pnas.87.12.4576
Woodson JD, Chory J (2008) Coordination of gene expression between organellar and nuclear genomes. Nat Rev Genet 9 (5):383-395. doi:10.1038/nrg2348
Woodson JD, Joens MS, Sinson AB, Gilkerson J, Salome PA, Weigel D, Fitzpatrick JA, Chory J (2015) Ubiquitin facilitates a quality-control pathway that removes damaged chloroplasts. Science 350 (6259):450-454. doi:10.1126/science.aac7444
Woodson JD, Perez-Ruiz JM, Chory J (2011) Heme synthesis by plastid ferrochelatase I regulates nuclear gene expression in plants. Curr Biol 21 (10):897-903. doi:10.1016/j.cub.2011.04.004
Woodson JD, Perez-Ruiz JM, Schmitz RJ, Ecker JR, Chory J (2013) Sigma factor-mediated plastid retrograde signals control nuclear gene expression. Plant J 73 (1):1-13. doi:10.1111/tpj.12011
Wrobel L, Topf U, Bragoszewski P, Wiese S, Sztolsztener ME, Oeljeklaus S, Varabyova A, Lirski M, Chroscicki P, Mroczek S, Januszewicz E, Dziembowski A, Koblowska M, Warscheid B, Chacinska A (2015) Mistargeted mitochondrial proteins activate a proteostatic response in the cytosol. Nature 524 (7566):485-488. doi:10.1038/nature14951
Wu GZ, Meyer EH, Richter AS, Schuster M, Ling Q, Schottler MA, Walther D, Zoschke R, Grimm B, Jarvis RP, Bock R (2019a) Control of retrograde signalling by protein import and cytosolic folding stress. Nat Plants 5:525-538
Wu GZ, Meyer EH, Wu S, Bock R (2019b) Extensive post-transcriptional regulation of nuclear gene expression by plastid retrograde signals. Plant Physiol. doi:doi.org/10.1104/pp.19.00421
Xiao Y, Savchenko T, Baidoo EE, Chehab WE, Hayden DM, Tolstikov V, Corwin JA, Kliebenstein DJ, Keasling JD, Dehesh K (2012) Retrograde signaling by the plastidial metabolite MEcPP regulates expression of nuclear stress-response genes. Cell 149 (7):1525-1535. doi:10.1016/j.cell.2012.04.038
Yang EJ, Yoo CY, Liu J, Wang H, Cao J, Li FW, Pryer KM, Sun TP, Weigel D, Zhou P, Chen M (2019) NCP activates chloroplast transcription by controlling phytochrome-dependent dual nuclear and plastidial switches. Nat Commun 10 (1):2630. doi:10.1038/s41467-019-10517-1
Yoo CY, Pasoreck EK, Wang H, Cao J, Blaha GM, Weigel D, Chen M (2019) Phytochrome activates the plastid-encoded RNA polymerase for chloroplast biogenesis via nucleus-to-plastid signaling. Nat Commun 10 (1):2629. doi:10.1038/s41467-019-10518-0
Zhang X, Ivanova A, Vandepoele K, Radomiljac J, Van de Velde J, Berkowitz O, Willems P, Xu Y, Ng S, Van Aken O, Duncan O, Zhang B, Storme V, Chan KX, Vaneechoutte D, Pogson BJ, Van Breusegem F, Whelan J, De Clercq I (2017) The Transcription Factor MYB29 Is a Regulator of ALTERNATIVE OXIDASE1a. Plant Physiol 173 (3):1824-1843. doi:10.1104/pp.16.01494
Zhang Y, Xu H (2016) Translational regulation of mitochondrial biogenesis. Biochem Soc Trans 44:1717-1724
Zhao C, Wang Y, Chan KX, Marchant DB, Franks PJ, Randall D, Tee EE, Chen G, Ramesh S, Phua SY, Zhang B, Hills A, Dai F, Xue D, Gilliham M, Tyerman S, Nevo E, Wu F, Zhang G, Wong GK-S, Leebens-Mack JH, Melkonian M, Blatt MR, Soltis PS, Soltis DE, Pogson BJ, Chen Z-H (2019a)

Evolution of chloroplast retrograde signaling facilitates green plant adaptation to land. Proc Natl Acad Sci U S A 116:5015-5020

Zhao X, Huang J, Chory J (2019b) GUN1 interacts with MORF2 to regulate plastid RNA editing during retrograde signaling. Proc Natl Acad Sci U S A 116 (20):10162-10167. doi:10.1073/pnas.1820426116

Zybailov B, Rutschow H, Friso G, Rudella A, Emanuelsson O, Sun Q, van Wijk KJ (2008) Sorting signals, N-terminal modifications and abundance of the chloroplast proteome. PLoS One 3 (4):e1994. doi:10.1371/journal.pone.0001994

Table 1: Overview of genome and proteome sizes of eukaryotic organelles

Species	*Arabidopsis thaliana*		*Plasmodium*		*Saccharomyces cerevisiae*	*Drosophila melanogaster*	*Homo sapiens*
Organelle	Chloroplast	Mitochondrion	Apicoplast	Mitochondrion	Mitochondrion	Mitochondrion	Mitochondrion
Genome size, structure	154.5 kb, circular, up to 100 copies	367 kb Linear and circular	~35 kb	~ 6kb, circular	86 kb, circular	19.5 kb, circular	16.5 kb, circular
Coding capacity	157 genes (88 cod; 69 nc)	164 genes (122 cod; 42 nc)	~68 genes	~37 genes	55 genes (28 cod; 27 nc)	37 genes (13 cod; 24 nc)	37 genes (13 cod; 24 nc)
Proteome	2500-3000 proteins	>2000 proteins	~551 proteins	~246 proteins	1000-3500 proteins	>1000 proteins	>1000 proteins

Table 1: Main genomic and proteomic characteristics of mitochondria and plastids in different organisms. Genome size and coding capacity data were extracted from Ensembl (ensembl.org). For *Plasmodium*, all data were extracted from PlasmoDB (plasmodb.org). cod: coding genes; nc: non-coding genes.

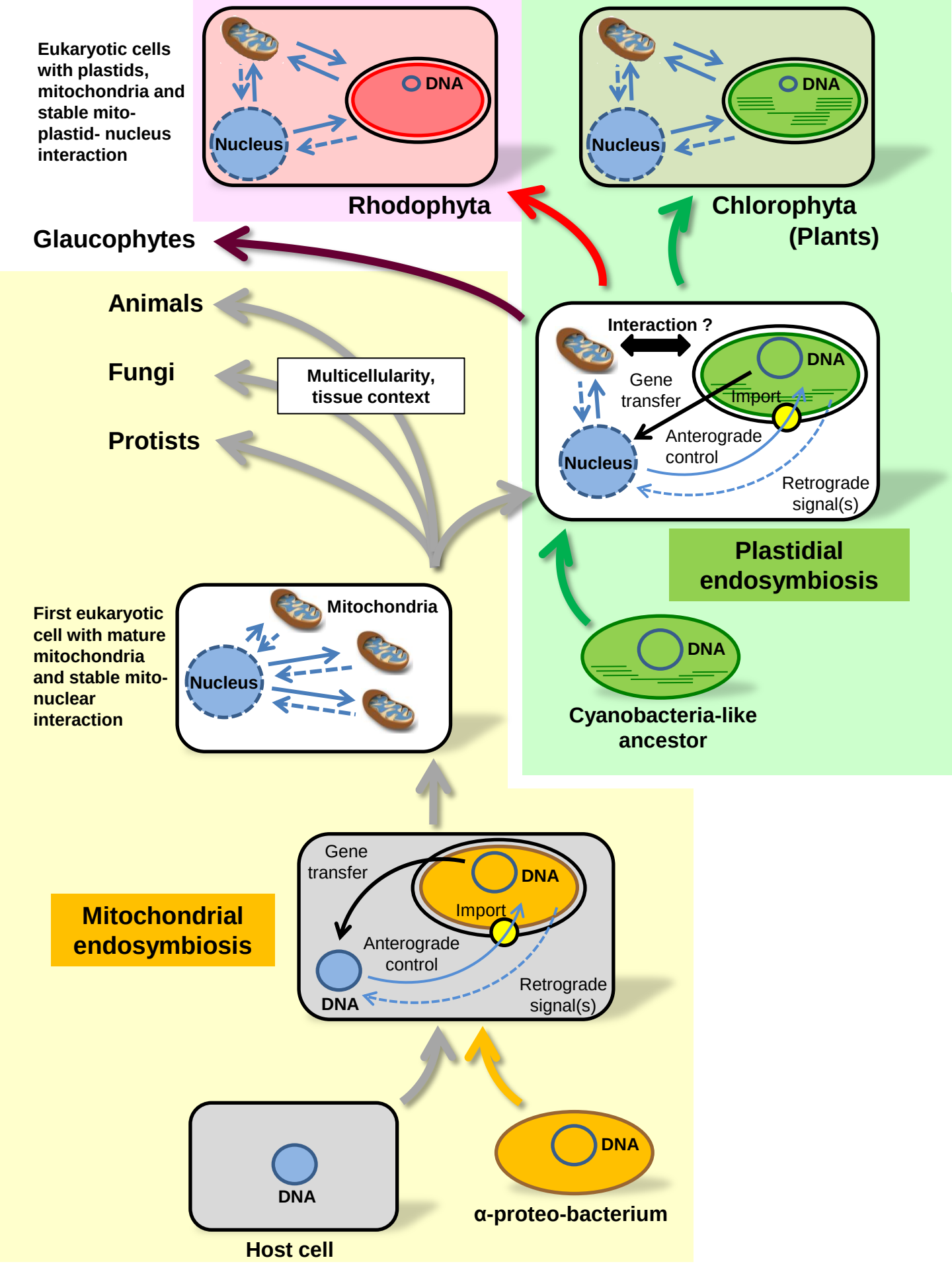
Eukaryotic cells with plastids, mitochondria and stable mito-plastid- nucleus interaction
DNA
Nucleus
Rhodophyta
DNA
Nucleus
Chlorophyta (Plants)
Glaucophytes
Animals
Fungi
Protists
Multicellularity, tissue context
Interaction ?
DNA
Gene transfer
Import
Nucleus
Anterograde control
Retrograde signal(s)
Plastidial endosymbiosis
First eukaryotic cell with mature mitochondria and stable mito-nuclear interaction
Mitochondria
Nucleus
DNA
Cyanobacteria-like ancestor
Mitochondrial endosymbiosis
Gene transfer
DNA
Import
Anterograde control
DNA
Retrograde signal(s)
DNA
Host cell
DNA
α-proteo-bacterium

Fig. 1: Establishment of retrograde control during evolution of eukaryotes. The diagramme depicts the major steps in the evolutionary aquisition of mitochondria and plastids by eukaryotes. Rectangles with round corners represent endosymbiotic host cell or eukaryotic host cell, respectively. Ovals represent bacterial ancestors and resulting endosymbionts as well as final plastids. Final mitochondria are represented by small icons with brown outer and blue inner membrane. DNA of endosymbionts is represented by blue circles, gene transfer to the nucleus by black arrows. Reduction of coding capacity is indicated by reduced size of DNA-representing circles. Continuous light-blue arrows: Anterograde signalling. Broken light blue arrows: Retrograde signalling. Large connecting arrows in grey, green and red represent the evolutionary lines. Yellow circles represent the respective import machineries. Note that retrograde signalling from mitochondria was likely already established when plastids evolved. Basic principles should have been conserved in the different evolutionary lines. Further evolution of mitochondrial retrograde signals in autotrophs, however, was probably influenced by the presence of plastids (mutual arrow, interaction?). In heterotrophs other influences such as multicellularity and tissue context may have generated different evolutionary constraints. In rhodophyta (pink box) and chlorophyta (green box) mitochondria, plastids and the nucleus developed triangular signalling networks.

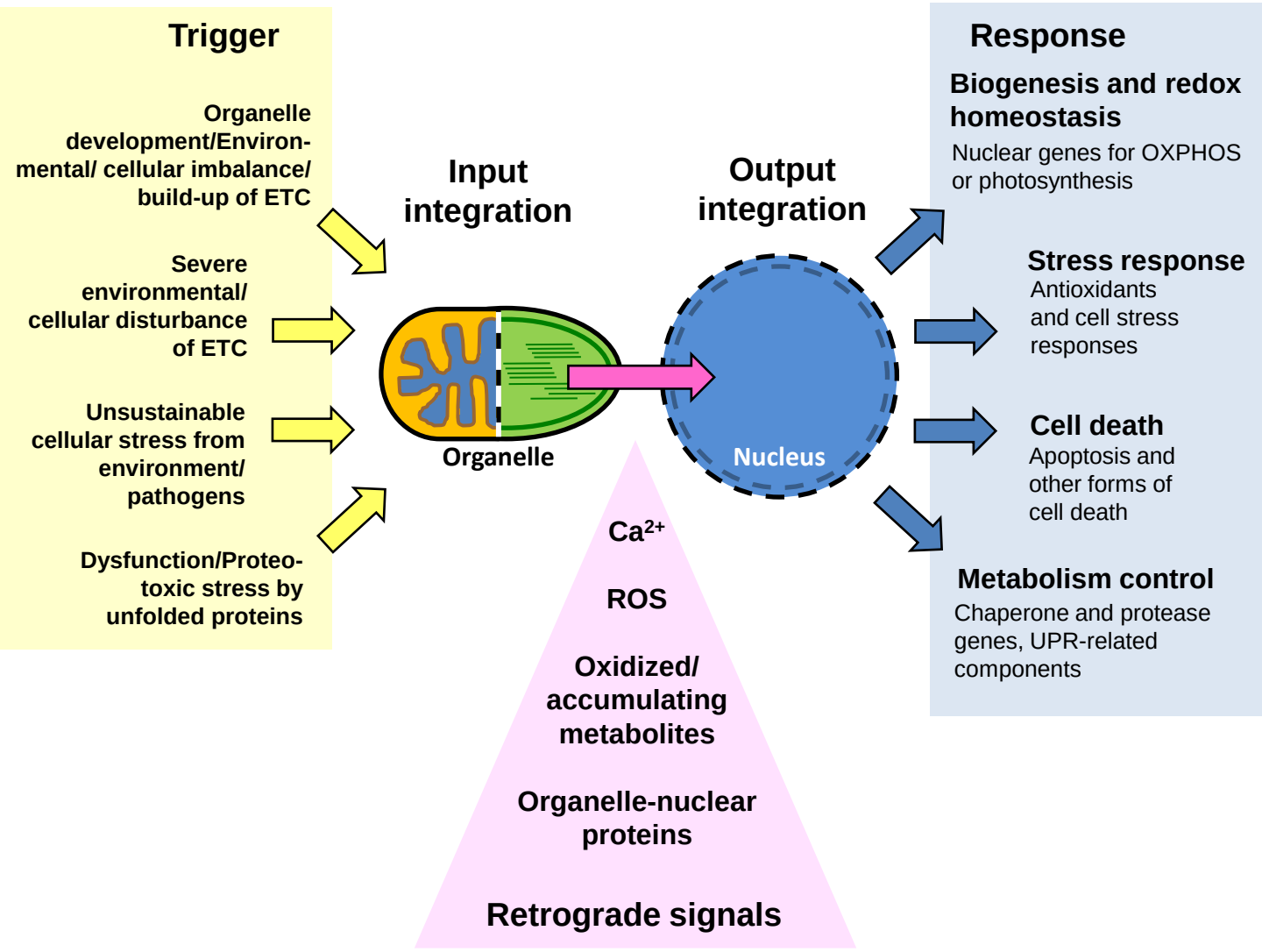

Fig. 2: Common principles in retrograde signalling of eukaryotic organelles. The diagramme depicts four major biological triggers (indicated in left panel) in which mitochondria and chloroplasts (represented by head-to-head orange and green ovals) initiate retrograde signal classes with high similarity in signal identity (pink triangel), gene target and cellular response (depicted in right panel). Organelles detect and integrate external or cellular triggers (yellow arrows, input integration), produce the corresponding retrograde signal(s) (pink arrow) that is/are detected by the nucleus where the information is finally integrated to initiate a corresponding response (blue arrows, output integration). ETC: electron transport chain; ROS: reactive oxygen species; OXPHOS: oxidative phosphorylation; UPR: unfolded protein response.