Why translation counts for mitochondria – retrograde signalling links mitochondrial protein synthesis to mitochondrial biogenesis and cell proliferation

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Summary

Organelle biosynthesis is a key requirement for cell growth and division. The regulation of mitochondrial biosynthesis exhibits additional layers of complexity compared with that of other organelles because they contain their own genome and dedicated ribosomes. Maintaining these components requires gene expression to be coordinated between the nucleo-cytoplasmic compartment and mitochondria in order to monitor organelle homeostasis and to integrate the responses to the physiological and developmental demands of the cell. Surprisingly, the parameters that are used to monitor or count mitochondrial abundance are not known, nor are the signalling pathways. Inhibiting the translation on mito-ribosomes genetically or with antibiotics can impair cell proliferation and has been attributed to defects in aerobic energy metabolism, even though proliferating cells rely primarily on glycolysis to fuel their metabolic demands. However, a recent study indicates that mitochondrial translational stress and the rescue mechanisms that relieve this stress cause the defect in cell proliferation and occur before any impairment of oxidative phosphorylation. Therefore, the process of mitochondrial translation in itself appears to be an important checkpoint for the monitoring of mitochondrial homeostasis and might have a role in establishing mitochondrial abundance within a cell. This hypothesis article will explore the evidence supporting a role for mito-ribosomes and translation in a mitochondria-counting mechanism.

Key words: Cell proliferation, Mitochondria, Ribosome, Translation

Introduction

The complexities of mitochondrial biogenesis

Mitochondria are double-membrane organelles of endosymbiotic origin that are estimated to comprise ~1100 proteins (Pagliarini et al., 2008); they have two features that distinguish them from other organelles: their own genome and exclusive ribosomes. Both require tight genetic regulation by the nucleus for maintenance and function, which is essential for the synthesis of a small subset of proteins that are destined for assembly into the mitochondrial respiratory chain complexes and ATP synthase (Fox, 2012). Collectively, these complexes are essential for generating the electrochemical gradient that is required to couple ATP production to oxidation and sustain the redox balance.

Mitochondria cannot be generated *de novo*, so mitochondrial biogenesis is essential for establishing the cellular abundance of the organelle and modulating this level in response to physiological stimuli (Rafelski et al., 2012). Given that mitochondria are widely known for their role in aerobic energy metabolism, a predominant perspective of mitochondrial biogenesis in the literature concerns the regulation of this energy supply (Kelly and Scarpulla, 2004). The transcriptional coactivator peroxisome proliferator-activated receptor (PPAR) γ coactivator 1α (PPARGC1A) is a well-known player in this process; however, it appears to be dispensable for establishing the basal mitochondrial abundance in a cell. Genetic ablation of *Ppargc1a* does not impair cell proliferation during development (Lin et al., 2004) or the formation of the female germline (Selesniemi et al., 2011). These are two examples where mitochondrial abundance is tightly regulated, suggesting the existence of alternative regulatory mechanisms and a greater genetic complexity for mitochondrial biogenesis. Unfortunately, little is known about these alternative mechanisms, despite their central importance for mitochondrial biology.

In vertebrates, mitochondrial DNA (mtDNA) is a small circular genome of ~16 kb that encodes 13 polypeptides, two ribosomal (r)RNAs and 22 transfer (t)RNAs (Falkenberg et al., 2007). This genome is uniparentally inherited, passing down the female germline without homologous recombination. All of the factors required for maintenance and transcription of this genome are encoded within the nucleus; they are thus synthesized on cytoplasmic ribosomes and imported into mitochondria. Evidence from a variety of different experimental approaches suggests that the mitochondrial genome is compacted into a highly ordered structure known as the nucleoid (Bogenhagen, 2012; Brown et al., 2011; Kaufman et al., 2007; Kukat et al., 2011). Most of the genome is coding, with a strand bias towards the GC-heavy strand, but it also comprises a short non-coding region that contains important sequence elements for its replication and transcription (Falkenberg et al., 2007). Transcription produces polycistronic messages from both strands that are subject to post-transcriptional processing (Falkenberg et al., 2007). The mature rRNAs are assembled into mitochondrial ribosomes, whereas the leaderless and

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Box 1. Mitochondrial protein synthesis

In mammals, translation of the 13 mitochondrially encoded polypeptides occurs on a dedicated set of mitochondrial ribosomes that have mechanistic features that are more similar to those of prokaryotes than to cytoplasmic ribosomes (Christian and Spremulli, 2012; Koc et al., 2010). Mitochondrial translation initiation requires a formylated tRNA-methionine. As a result, nascent proteins contain a formyl methionine at the N-termini. In most eukaryotes, but not *S. cerevisiae* and *C. elegans*, mitochondria have a N-terminal methionine excision (NME) pathway to remove the formyl group and starter methionine (Giglione et al., 2003; Serero et al., 2003). These functions are catalysed by peptide deformylase and a mitochondrial methionine amino peptidase. In mammals, only one of the 13 polypeptides is deformylated and missing the starter methionine (Walker et al., 2009). At present the basis for this molecular discrimination is unknown. The class 1 release factor MtRF1l terminates translation at the standard UAA and UAG stop codons, which are either encoded within the gene or result from frameshifting at so-called 'hungry' codons (Soleimanpour-Lichaei et al., 2007; Temperley et al., 2010). Hungry codons can arise when the abundance of an aminoacylated-tRNA are limiting or in short supply, which can stall ribosomes. Three other class 1 release factors (ICT1, C12ORF65 and MtRF1) can be found in mammalian mitochondria; they are important for translation but have no codon specificity (Antonicka et al., 2010; Richter et al., 2010; Soleimanpour-Lichaei et al., 2007). All contain the conserved GGQ motif that is required for catalysing the cleavage of the ester bond of a peptidyl-tRNA, but the precise function of these three factors in translation termination is not entirely clear, although it has been proposed that they are involved in the rescue of stalled ribosomes (Chrzanowska-Lightowlers et al., 2011; Huynen et al., 2012). Very little is known about the factors and assembly steps for mitochondrial ribosomes, and combined with the inability to reconstitute a functional *in vitro* translation system, this has hampered our detailed molecular understanding of mitochondrial protein synthesis.

uncapped mRNAs are translated on mitochondrial ribosomes (Christian and Spremulli, 2012).

The composition of mitochondrial ribosomes is unique among the three domains of life because they are predominantly protein rich (67%), in contrast to bacterial and cytoplasmic ribosomes that contain more RNA (Koc et al., 2010). Mitochondrial ribosomes contain only two rRNA species and ~80 proteins, the latter of which are encoded in the nucleus along with all the factors that coordinate their assembly and function (Box 1). These proteins must be translated on cytoplasmic ribosomes and imported into the mitochondrial matrix. The function of the mitochondrial ribosomes is restricted to the translation of only 13 extremely hydrophobic proteins that are subunits of the much larger respiratory chain complexes (Fox, 2012).

An additional layer of complexity to mitochondrial biogenesis is the movement and morphology of mitochondria, which can be cell specific (Westermann, 2010). Typically, in cultured mammalian cells, the organelle forms a dynamic reticulum that is in close contact with the endoplasmic reticulum (ER) (Friedman et al., 2011; Kornmann et al., 2009) and is regulated by a core machinery of dynamin-related GTPases that are conserved across taxa (Hoppins et al., 2007). The shape of mitochondria is sensitive to stress; it can be either fragmented or hyperfused depending upon the conditions (Frank et al., 2001; Gomes et al., 2011; Rambold et al., 2011; Tondera et al., 2009). Because nucleated cells always contain mitochondria, the final assembly of the mitochondrial respiratory chain complexes requires tight regulatory control of multiple processes that occur in three compartments: the mitochondria, cytoplasm and nucleus. In this Hypothesis, we will explore the link between translation stress on mitochondrial ribosomes and disruptions in cell proliferation. We hypothesize that mitochondrial protein synthesis is integrated into a cellular circuit that monitors organelle homeostasis independently of aerobic energy metabolism.

The importance of mitochondrial translation to cell proliferation

Successful coordination of mitochondrial biogenesis requires the establishment of a circuit (Kelly and Scarpulla, 2004) with two major components: anterograde and retrograde regulation (Fig. 1). Anterograde regulation modulates the organelle in response to a genetic program that is initiated by transcription factors and activated by external and internal stimuli. In contrast, retrograde regulation monitors mitochondrial function and signals this information back to the nucleus to be integrated into the relevant genetic network. Such a circuit would allow the cell to count or monitor mitochondrial abundance in a manner that is possibly analogous to quorum sensing in bacteria (Turovskiy et al., 2007). Any disruptions in this circuit should activate a mitochondrial checkpoint, enabling the cell to adjust to the stress and respond to the problem. In the case of dividing cells, a disruption in the circuit would activate an acute checkpoint that impairs proliferation because nucleated cells are never without any mitochondria. However, which mitochondrial parameters are monitored as a reliable indicator of homeostasis in proliferating cells? Furthermore, it is unclear how many circuits are required to monitor mitochondrial

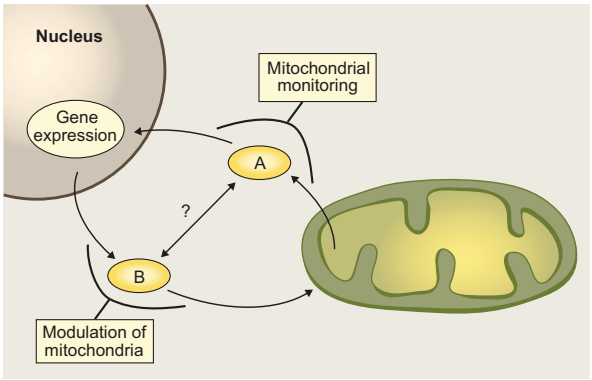

Fig. 1. Schematic overview of a potential mitochondrial intracellular circuit. Integrating mitochondrial function within the cell depends upon on anterograde and retrograde signalling. The anterograde regulation (modulation of mitochondria) integrates signals from external and internal stimuli at the nucleus to activate a genetic programme that adjusts mitochondrial parameters accordingly. The retrograde regulation monitors the health or functional state of the mitochondria and signals back to the nucleus (mitochondrial monitoring). Cytoplasmic nodes (A and B) could act as the rheostats that integrate anterograde and retrograde signals in this circuit and adjust the system accordingly. There is potential for crosstalk between the two cytoplasmic nodes, which might allow for more rapid regulation on the organelle (indicated by the question mark).

homeostasis and to successfully coordinate mitochondrial biogenesis, and whether such circuits would have redundancy and/or hierarchy.

Invariably, mitochondrial dysfunction can impair assembly and function of the respiratory chain, but human mitochondrial diseases display a heterogeneous tissue-specificity and have variable age-of-onset and severity (Ylikallio and Suomalainen, 2012), suggesting that factors other than the loss of respiratory chain function account for the clinical manifestations. An important step in elucidating the pleiotropic effects of mitochondrial dysfunctions is to consider the specific molecular defects and the processes that are directly affected. Retrograde signalling responses arising from altered metabolism and Ca^{2+} dynamics in mitochondria have been reviewed elsewhere (Butow and Avadhani, 2004) and will not be discussed further. Instead, our goal is to critically discuss the link between mitochondrial dysfunction and cellular proliferation to propose a testable hypothesis as an underlying mechanism.

The proliferation of cultured mammalian cells can be impaired following disruption of mitochondrial function (Dennerlein et al., 2010; Escobar-Alvarez et al., 2010; Richter et al., 2010; Richter et al., 2013; Rorbach et al., 2008; Skrtić et al., 2011; Soleimanpour-Lichaei et al., 2007; Uchiumi et al., 2010). In some cases, these proliferation defects were attributed to dysfunctional mitochondrial respiration (Escobar-Alvarez et al., 2010; Skrtić et al., 2011). However, this interpretation is at odds with data from a vast scientific literature that demonstrate that glycolysis fuels the metabolic demands of cellular proliferation (reviewed by Hanahan and Weinberg, 2011). Moreover, these proliferation defects persist even when the culture medium is supplemented with uridine (Richter et al., 2013), which can bypass the dependency of the mitochondrial respiratory chain function on dihydroorotate dehydrogenase, which is required for *de novo* pyrimidine biosynthesis (Rawls et al., 2000). Therefore, loss of the respiratory chain is simply a downstream consequence of mitochondrial dysfunction and not the biological driver that activates the checkpoint in this model system.

We recently addressed this question and demonstrated that mitochondrial translational stress impaired cell proliferation, and not the loss of respiratory chain complexes (Richter et al., 2013). In this study, we showed that treatment with the antibiotic actinonin induced stalling of mitochondrial translation elongation, which, in turn, activated a pathway to rescue dysfunctional mitochondrial ribosomes. The activation of this rescue pathway then impaired cell proliferation. Prolonged activation of this mitochondrial ribosome rescue pathway ultimately lead to the degradation of the pool of mitochondrial ribosomes and mitochondrial mRNAs. A question that emerged was whether these findings are unique to actinonin, or whether they point to a broader paradigm that could be used to develop hypotheses linking a mitochondrial checkpoint(s) to cell proliferation. Surprisingly, disruptions in protein synthesis on mitochondrial ribosomes emerge as a common link to impaired cell proliferation.

It has been shown previously that disrupting mitochondrial translation in cultured mammalian cells, either genetically or with antibiotics, can impair cell proliferation (Dennerlein et al., 2010; Escobar-Alvarez et al., 2010; Richter et al., 2010; Rorbach et al., 2008; Skrtić et al., 2011; Soleimanpour-Lichaei et al., 2007; Uchiumi et al., 2010); notably these proliferation defects occur when the cells are cultured in glucose. Typically, cultured cells from patients with mitochondrial disorders are not reported to have proliferation defects when they are grown in glucose medium and the patients themselves do not manifest symptoms that are compatible with proliferation defects, such as dysmorphy or abnormal development (Ylikallio and Suomalainen, 2012). However, patients with defects in the assembly of the mitochondrial small ribosomal subunit display clinical features (Miller et al., 2004; Smits et al., 2011) that could be interpreted as mild proliferation defects during development. The molecular basis for these developmental defects has not been tested but could be due to a decrease in the initiation rate of mitochondrial translation because the small ribosomal subunit is limiting.

This raises the question as to why mitochondrial translation is special and what specifically could be monitored. Using proliferation defects as a readout of altered translation is not straightforward, as illustrated by the following two genetic and two pharmacological examples. First, mitochondrial release factor 1-like (MTRF1L) is the *bone fide* release factor for translation termination; its knockdown by small interfering (si)RNA produces no detectable effect in the *de novo* synthesis of the 13 mitochondrial proteins, but induces a proliferation defect in glucose medium and increased mitochondrial stress (Soleimanpour-Lichaei et al., 2007). The synthesis of mitochondrial translation products in cells is typically assayed in a 1-hour pulse radiolabelling experiment where cytoplasmic translation is simultaneously inhibited (Chomyn, 1996). Second, ICT1 is another mitochondrial protein that is homologous to class 1 release factors, but it has no codon specificity and is important for the assembly of the large ribosomal subunit (Richter et al., 2010). Knockdown of ICT1 produces a dramatic decrease in translation elongation (as measured by pulse experiments) and a proliferation defect in glucose medium (Richter et al., 2010). Third, chloramphenicol is a well-known antibiotic that inhibits both prokaryotic and mitochondrial function by occupying the A site of the ribosome (Bulkley et al., 2010; Koc et al., 2010), which systematically blocks all translation elongation. We found that after 48 hours of administration, there was no proliferation defect in glucose, even though we could not detect any translation or mitochondrially encoded subunits (Richter et al., 2013). It has been shown previously that only much longer incubations with chloramphenicol (greater than 10 days) result in impaired proliferation (Bunn et al., 1974). Finally, treatment with actinonin, which binds to mitochondrial peptide deformylase (PDF) (Box 1) (Fieulaine et al., 2011) and traps it on mitochondrial ribosomes, stalls translation elongation and impairs cell proliferation in as little as 12 hours (Richter et al., 2013). These examples demonstrate that the reduction in the rate of translation elongation in itself, at least as measured in a 1-hour pulse radiolabelling experiment, cannot account for the acute proliferation defects and emphasise that more in-depth studies into the complexity of ribosomal function are required to elucidate the molecular basis of these phenotypes.

An alternative fate for mitochondrially synthesised proteins and implications for organelle homeostasis

The classical view of mitochondrial translation is understandably that its purpose is the generation of polypeptide chains that are destined for assembly into the respiratory chain complexes (Fox, 2012). However, mitochondrial polypeptides can have an alternative fate as peptides that are exported from the

organelle. In mammals, pioneering work in the 1980s and 1990s from the laboratories of Kirsten Fischer Lindahl and Robert Rich demonstrated that mitochondrially encoded polypeptides were presented as self-antigens on a specific MHC class Ib receptor to prime the adaptive immune system for bacterial infection in rodents (Loveland et al., 1990; Shawar et al., 1991). These peptides were derived from *de novo* protein synthesis on mitochondrial ribosomes and were not the result of protein turnover (Han et al., 1989) to be delivered to the endoplasmic reticulum through a Tap1- or Tap2-dependent process (Chun et al., 2001). Has the system evolved specifically in vertebrates to achieve immunological function or is it an important cellular pathway co-opted through the evolutionary fight against pathogens? Looking across taxa from the eukaryotic tree suggests that mitochondrial peptide export is a conserved process, and, as discussed below, it has been implicated in mitochondrial biogenesis, stress responses, and immunity, although in most cases it remains to be established whether these peptides have a direct regulatory role.

Work from the laboratory of Thomas Langer has shown that, in the budding yeast *Saccharomyces cerevisiae*, the peptides exported from the mitochondria are predominantly derived from two mitochondrial proteins, and that this export is dependent upon a specific inner membrane ABC transporter and proteases (Augustin et al., 2005; Young et al., 2001). Unlike the situation reported so far in mammals, the peptides were derived from both nuclear and mitochondrially encoded proteins. This peptide export is not the result of insufficient mitochondrial protease activity or overloading of the quality control system because genetic ablation of the transporter can lead to complete degradation of the peptides in the matrix space (Young et al., 2001). Loss of this peptide export leads to a significant upregulation of genes involved in mitochondrial translation, suggesting the existence of a regulon response to peptide export (Arnold et al., 2006). Moreover, antibiotics, such as chloramphenicol, can also induce this regulon independently from growth under respiring or fermenting conditions. The exact signalling function of these peptides and the responsive transcription factors remain unknown, but they clearly fulfil the requirement for a circuit that is important for translation homeostasis on mitochondrial ribosomes (see Figs 1, 2).

Recent work from Cole Haynes and David Ron in *C. elegans* built upon those yeast studies and work from the Hoogenraad laboratory to demonstrate how mitochondrial peptide export can serve as an indicator of protein stress within the organelle (Haynes et al., 2010; Nargund et al., 2012; Zhao et al., 2002). Burdens on the mitochondrial quality control mechanisms can trigger an increased efflux of mitochondrial peptides, which activate a gene expression profile that is compatible with an unfolded protein response (UPR). In these studies, none of the identified peptides were derived from the 13 mitochondrially encoded proteins, but from nuclear-encoded proteins (Haynes et al., 2010). This pathway also depends upon an ABC transporter in the inner membrane, proteases and the translocation of the bZip transcription factor ATFS-1 from the mitochondria to the nucleus to activate this UPR (Haynes et al., 2010; Nargund et al., 2012). Loss of the key members of this pathway has no deleterious consequences to the worms unless they are faced with protein toxicity or stress within the organelle. The relocalisation of ATFS-1 as a means to monitor mitochondria is particularly intriguing from a mechanistic point of view. ATFS-1 contains

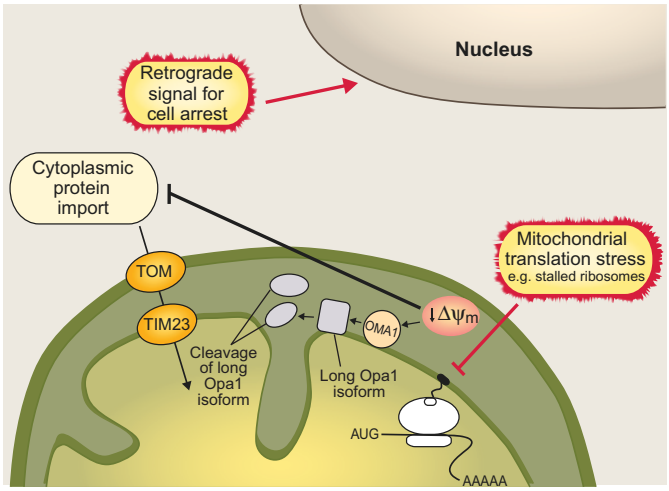

Fig. 2. Proposed mechanism for retrograde signalling that links mitochondrial translation stress to cell proliferation. The stalling of translation elongation on mitochondrial ribosomes (either pharmacologically or because of mtDNA mutations) is a significant stress that can disrupt protein synthesis and impair cell proliferation. All 13 of the mitochondrially synthesised proteins are very hydrophobic, so they require co-translational insertion into the inner membrane. Stalled mitochondrial ribosomes would generate stress on the inner membrane that leads to the loss of mitochondrial membrane potential ($\Delta\Psi_m$). This scenario has two consequences: fragmentation of the mitochondrial reticulum and reduced cytoplasmic protein import. A loss of $\Delta\Psi_m$ activates the Oma1 protease, which cleaves the long membrane-anchored Opa1 isoforms required for inner membrane fusion. For the sake of clarity, the mitochondrial fragmentation has been omitted from this diagram. It has been proposed that cytoplasmic protein import is the key parameter that is monitored in this circuit, thereby linking mitochondrial translation stress to cell proliferation. Disrupting this process triggers a signal to the nucleus to activate a genetic programme for growth arrest. A protein containing both a mitochondrial and a nuclear localisation motif would be an ideal candidate to act as a direct sensor in this retrograde signalling.

two localisation signals, a classical N-terminal mitochondrial-targeting sequence and a nuclear localisation signal (NLS) in the C-terminal part of the protein. Under normal conditions, ATFS-1 localises to the mitochondria but is not stable and is rapidly degraded within the matrix. Upon protein stress within the matrix, import of cytoplasmically translated proteins into the organelle through the dedicated protein import machinery located in the outer and inner mitochondrial membrane (Fox, 2012) is shut down. Thus, ATFS-1 translocates to the nucleus through its NLS where it acts as a transcription factor for upregulating the mitochondrial UPR (Nargund et al., 2012). There appears to be no orthologue of ATFS-1 in vertebrates, so the conservation of this mechanism outside of nematodes is in question.

The molecular evidence for a regulatory role of mitochondrial peptide export is far from established, but there are precedents for protein synthesis and peptide export regulating growth and metabolism. In eukaryotes, translational output from cytoplasmic ribosomes is actively monitored for cell growth (Jorgensen et al., 2004; Rudra and Warner, 2004). Furthermore, in bacteria, the regulation of growth, metabolism, and quorum sensing relies on monitoring of protein synthesis and peptide export (Perego, 1997; Turovskiy et al., 2007). It is tempting to speculate that mitochondrial peptide export could generate both a constitutive and inducible cytoplasmic pool of mitochondrially derived

content that could be actively monitored as a parameter indicative of organelle homeostasis (Fig. 3). Therefore, alterations in this cytoplasmic pool of mitochondrial peptides in abundance or composition would feed into a corresponding regulatory genetic network. Such a mechanism would fulfil the requirements of a full circuit to monitor the organelle (see Fig. 1). Clearly more experiments are required to substantiate such a regulatory mechanism.

In our study on actinonin-induced growth arrest, two observations stand out that do not support a role for monitoring mitochondrial peptide export (Richter et al., 2013). After 48 hours of chloramphenicol treatment, which would prevent *de novo* protein synthesis on mitochondrial ribosomes, there was no proliferation defect compared with that in controls. Moreover, the gene expression profile following actinonin treatment was not enriched for mitochondrial proteins that are involved in translation, and thus fails to fulfil the requirements of a circuit (Fig. 3). Therefore, our data suggest the existence of another checkpoint that is monitored for mitochondrial translation.

A link between mitochondrial translation stress and cell proliferation

Ribosome stalling can induce the activation of rescue pathways as part of the co-translational mRNA surveillance mechanisms that have been best described on cytoplasmic and bacterial ribosomes (Graille and Séraphin, 2012; Shoemaker and Green, 2012). To initiate these rescue pathways requires access to the ribosomal A-site, to which release factors can bind and catalyze the cleavage of the ester bond of the polypeptidyl-tRNA that is stuck in the ribosomal P-site. The data from treatments of cells with actinonin suggest that an organelle ribosome quality control pathway is initiated when mitochondrial ribosomes stall and that this is linked to the proliferation defect (Richter et al., 2013). The strongest evidence for such a potential mechanism comes from the suppression of all actinonin effects when the cells are co-treated with chloramphenicol. Under these conditions, chloramphenicol could occupy the A-site of mitochondrial ribosomes, thereby preventing access to mitochondrial release factors (see Box 1) and rescue of the stalling. However, an alternative explanation, which is not necessarily mutually exclusive, could involve the generation of abnormal translation products during mitochondrial ribosomal stalling that are inserted into the inner membrane. Chloramphenicol would simply stop their production and thereby eliminate the organelle stress signal. Further experiments that manipulate the abundance of the putative mitochondrial release factors (see Box 1) are required to resolve this issue and to determine whether abnormal polypeptides are generated by mitochondrial ribosomes during stalling.

Another important observation with regard to mitochondrial translation stress following actinonin treatment was the fragmentation of the mitochondrial reticulum that occurred in tandem with the ribosome stalling (Richter et al., 2013). The dynamin-related GTPase Opa1 is an essential factor for the fusion of the inner membrane and for maintaining mitochondrial cristae (Meeusen et al., 2006); it is proteolytically processed, both

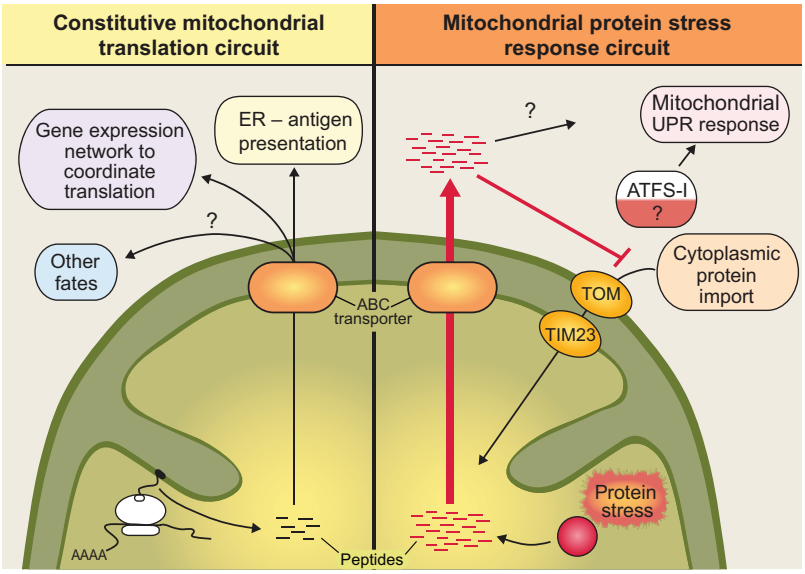

Fig. 3. Proposed mechanism for retrograde signalling that links mitochondrial peptide export to the monitoring of organelle homeostasis. Mitochondrial peptide export could be regulated at two levels, constitutively and in response to stress, and these processes might not be mutually exclusive. The left-hand panel represents the potential constitutive regulation, where *de novo* synthesised mitochondrial proteins are proteolytically processed following their release from the mitochondrial ribosome and are exported by an inner membrane ABC transporter. Mitochondrial peptide synthesis is monitored in order to regulate the genetic network that coordinates mitochondrial translation. In addition, these peptides can also be imported into the endoplasmic reticulum (ER) for antigen presentation, and might have other fates. The right-hand panel represents the stress-induced regulation of mitochondrial protein export. This export is initiated by an excessive burden on the mitochondrial quality control mechanisms that generate increased levels of peptides that are then exported from the mitochondria into the cytoplasm by an ABC transporter. It is unknown what exactly monitors the peptides or whether they have any regulatory function. Their efflux blocks cytoplasmic protein import, and, at least in *C. elegans*, the bZIP transcription factor ATFS-1 has been shown to translocate to the nucleus to initiate a genetic network for the mitochondrial unfolded protein response (UPR), thus acting as such as sensor. ATFS-1 has only been identified in nematodes, so the conversation of this mechanism in other taxa is unknown. To reflect this uncertainty the factor is shown half in red and with a question mark.

constitutively and in response to organelle stress (Westermann, 2010). Oma1 is a mitochondrial protease that can be activated by stress, such as loss of membrane potential, which leads to the processing of the long Opa1 isoforms and mitochondrial fragmentation (Ehses et al., 2009; Head et al., 2009; Quirós et al., 2012). We found that stalling mitochondrial translation with actinonin led to the processing of the long Opa1 isoforms, an effect that could be suppressed by co-treatment with chloramphenicol. Although the membrane potential was never directly measured, we found that total respiration was higher with actinonin, a finding consistent with uncoupling (Richter et al., 2013). Because mitochondrially translated proteins are hydrophobic, they require co-translational insertion into the membrane (Fox, 2012); hence, the interface between the inner membrane and mitochondrial ribosomes is important. Mitochondrial membrane potential could be disrupted during mitochondrial ribosome stalling if abnormal translation products are inserted into the inner membrane, or following the rescue of the stalled ribosome, which would disrupt the interface between the ribosome and membrane. Because the chloramphenicol-mediated suppression we observed is consistent with both possible interpretations, it is important to resolve the molecular basis for this effect. Moreover, the overall relevance of mitochondrial fragmentation and Opa1 processing also needs to be addressed to establish whether it is simply a by-product of mitochondrial translational stress, or an integral factor of retrograde signalling.

So, is the loss of mitochondrial membrane potential (uncoupling) the stress signal that has been induced by actinonin, and what is the impact on the organelle? Uncoupling has been shown to inhibit cell proliferation in cultured cells, leading to arrest in G2-M (Koczor et al., 2009). A well-known response to uncoupling is loss of cytoplasmic protein import into the matrix through the inner membrane Tim23 complex (Fox, 2012). Interestingly, one of the few mitochondrial genes that are essential in the facultative anaerobic yeast *S. cerevisiae* is Tom40, the highly conserved β-barrel protein that forms a channel in the outer membrane and is essential for protein import (Baker et al., 1990; Fox, 2012). If mitochondrial translation stress generated uncoupling, then a protein with two localisation motifs (mitochondrial and nuclear) could relocate to the nucleus when protein import into the organelle is blocked and could transduce a mitochondrial distress signal to the nucleus. One of the consequences of disrupting protein import into mitochondria is the acute quality control that is required on the organelle surface to handle the cytoplasmic ribosomes, partially translated proteins and accumulated mRNA. Given that 99% of the mitochondrial proteome needs to be delivered from the cytoplasm into the organelle (Pagliarini et al., 2008), arrested protein import poses a considerable challenge to cytoplasmic and mitochondrial quality control pathways. Moreover, data from yeast suggest that proteins that are stuck in the outer mitochondrial membrane translocation machinery because of stalled cytoplasmic ribosomes are deleterious to the cell and impair growth (Izawa et al., 2012). Therefore, we hypothesise that the actinonin-induced mitochondrial translation stress disrupts the monitoring of mitochondrial protein import, thereby triggering a checkpoint for cell proliferation (Fig. 2). The robustness of this pathway for monitoring mitochondrial homeostasis can be determined by testing other mitochondrial translation factors that have been implicated in proliferation defects.

There is one potential weakness in this model with respect to proliferation in mammalian cells and that is the ability to chemically generate rho zero cells in aneuploid cell lines (King and Attardi, 1989); these rho zero cells are devoid of the mitochondrial genome, so they do not have mitochondrial ribosomes or protein synthesis, but maintain a membrane potential for protein import. It should be pointed out that the use of intercalating drugs such as ethidium bromide to deplete the mitochondrial genome has only been accomplished in a small number of aneuploid cell lines, and even then it is only a rare event. Nonetheless, these cells can still proliferate in the absence of mtDNA. However, the nuclear instability of these cell lines combined with the use of a mutagenic agent, such as ethidium bromide, could have selected for those cells in which the factors that are integral to these circuit checkpoints have been lost. Generating a rho zero state genetically in immortalised, but not transformed, murine embryonic fibroblasts leads to a strong and robust proliferation defect (Richter et al., 2013), suggesting that losing the mitochondrial translation apparatus has severe consequences to the cell.

An important prediction of this model is that any source of elevated translation stress within mitochondria should trigger the activation of this pathway. There is evidence that mtDNA mutations, such as deletions and point mutations, can induce proliferation defects in the haematopoietic system and stem cells (Ahlqvist et al., 2012; Larsson et al., 1990). These mtDNA mutations could potentially generate mRNAs that interfere with mitochondrial translation and stall ribosomes (Graille and Séraphin, 2012). To experimentally validate this interpretation requires more detailed studies into the role of mutations in mitochondrial DNA and RNA for inducing translation stress.

Conclusions

We hypothesise that there are at least three retrograde signalling events that feed into circuits coupling mitochondrial translation to cell proliferation and that these are not necessarily mutually exclusive (Figs 2, 3). The first and most dominant signalling is triggered by mitochondrial ribosome stalling and the resulting translation stress that ultimately fragments mitochondria and blocks protein import, which, in turn, is relayed back to the nucleus (Fig. 2). The importance of the mitochondrial fragmentation in this response needs to be tested, i.e. whether it is essential or just a by-product of translation stress. The second retrograde signalling event relies upon constitutive mitochondrial peptide export into the cytoplasm for coordinating a genetic network that regulates mitochondrial translation (Fig. 3). Long-term disruption of this retrograde signalling, at least in mammalian cells, will impair proliferation. The last signalling event potentially combines elements of the first two with respect to cell proliferation (Fig. 3). An increase in the abundance of mitochondrial peptides in the cytoplasm could potentially block mitochondrial protein import and trigger a retrograde signal to the nucleus, resulting in the downregulation of gene expression. Further studies aimed at elucidating in particular the molecular details of mitochondrial ribosomal function during stress will be key to unravelling the link between mitochondrial translation and cellular proliferation, and to test the validity of the hypotheses proposed here.

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