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Non-coding RNAs: the dark side of nuclear–mitochondrial communication

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Abstract

Mitochondria are critical hubs for the integration of several key metabolic processes implicated in cell growth and survival. They originated from bacterial ancestors through endosymbiosis, following the transfer of more than 90% of their endosymbiont genome to the host cell nucleus. Over time, a mutually beneficial symbiotic relationship has been established, which relies on continuous and elaborate signaling mechanisms between this life-essential organelle and its host. The ability of mitochondria to signal their functional state and trigger compensatory and adaptive cellular responses has long been recognized, but the underlying molecular mechanisms involved have remained poorly understood. Recent evidence indicates that non-coding RNAs (ncRNAs) may contribute to the synchronization of a series of essential cellular and mitochondrial biological processes, acting as "messengers" between the nucleus and the mitochondria. Here, we discuss the emerging putative roles of ncRNAs in various bidirectional signaling pathways established between the host cell and its mitochondria, and how the dysregulation of these pathways may lead to aging-related diseases, including cancer, and offer new promising therapeutic avenues.

Keywords miRNA; mitochondria; non-coding RNA; retrograde signaling
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Mitochondria are multitasking stress sensors and integrators

Cells rely heavily on mitochondrial metabolic pathways for energy conversion and ATP production through oxidative phosphorylation (OXPHOS), for fatty acid and amino acids metabolism, control of the cellular redox status, and consequently for growth and survival. Changes in the environmental growth conditions experienced by a given cell can directly impact on the biology and function of mitochondria, which can in turn trigger adaptive and compensatory responses or—in the case of exposure to extremely unfavorable growth conditions—engage the intrinsic programmed cell death pathway. In addition, recent evidence indicates that mitochondria are also capable of sensing and integrating (stress) signals that modulate nuclear gene expression and overall protein homeostasis (Raimundo *et al*, 2012; Richter-Dennerlein *et al*, 2015; Fig 1).

Derived from a bacterial ancestor, mitochondria have their own circular genome, which in humans encodes 22 tRNAs, 2 rRNAs and 13 subunits of the OXPHOS machinery. Mitochondria, nevertheless, are largely dependent on nuclear DNA for their assembly and function (Richter-Dennerlein *et al*, 2015). In fact, 99% of the mitochondrial proteome is encoded by the nuclear genome and synthesized in the cytoplasm as precursor proteins that have to be imported into mitochondria (Schmidt *et al*, 2010). This is the case for all proteins necessary for replication, transcription, and translation of the mitochondrial genome and for several OXPHOS chain subunits. Mitochondria therefore rely on both mitochondria- and nuclear-encoded proteins to build a functional ATP-producing OXPHOS machinery and to generate essential metabolites. Critically, this also implies that proper assembly of the mitochondrial respiratory chain complexes depends on a fine-tuned equilibrium of the synthesis (and degradation) of proteins encoded in both compartments. Deregulation of coordinated transcription and/or translation of the nuclear or mitochondrial genome would thus ultimately perturb mitochondria biogenesis and function. The ratio between mitochondria- and nuclear-encoded proteins can therefore function as a particularly subtle sensor and integrator of (stress) signals that modulate mitochondria function and cell behavior.

Quality control process in the symbiosis

Monitoring and fine-tuning mitochondria copy number, status, morphology, and function are essential for growth and survival and therefore, unsurprisingly, under tight nuclear control (Fig 1). This so-called anterograde regulation can modulate mitochondrial activity and promote mitochondrial biogenesis, depending on the cellular needs.

Anterograde regulation depends on a limited number of transcription factors (NRF1 and 2; Scarpulla, 1997), a set of nuclear receptors (i.e., PPARs, ERRs), and their cofactors (PGC1α, PGC1β; Fan & Evans, 2015) that coordinately regulate all the nuclear-encoded mitochondrial proteome.

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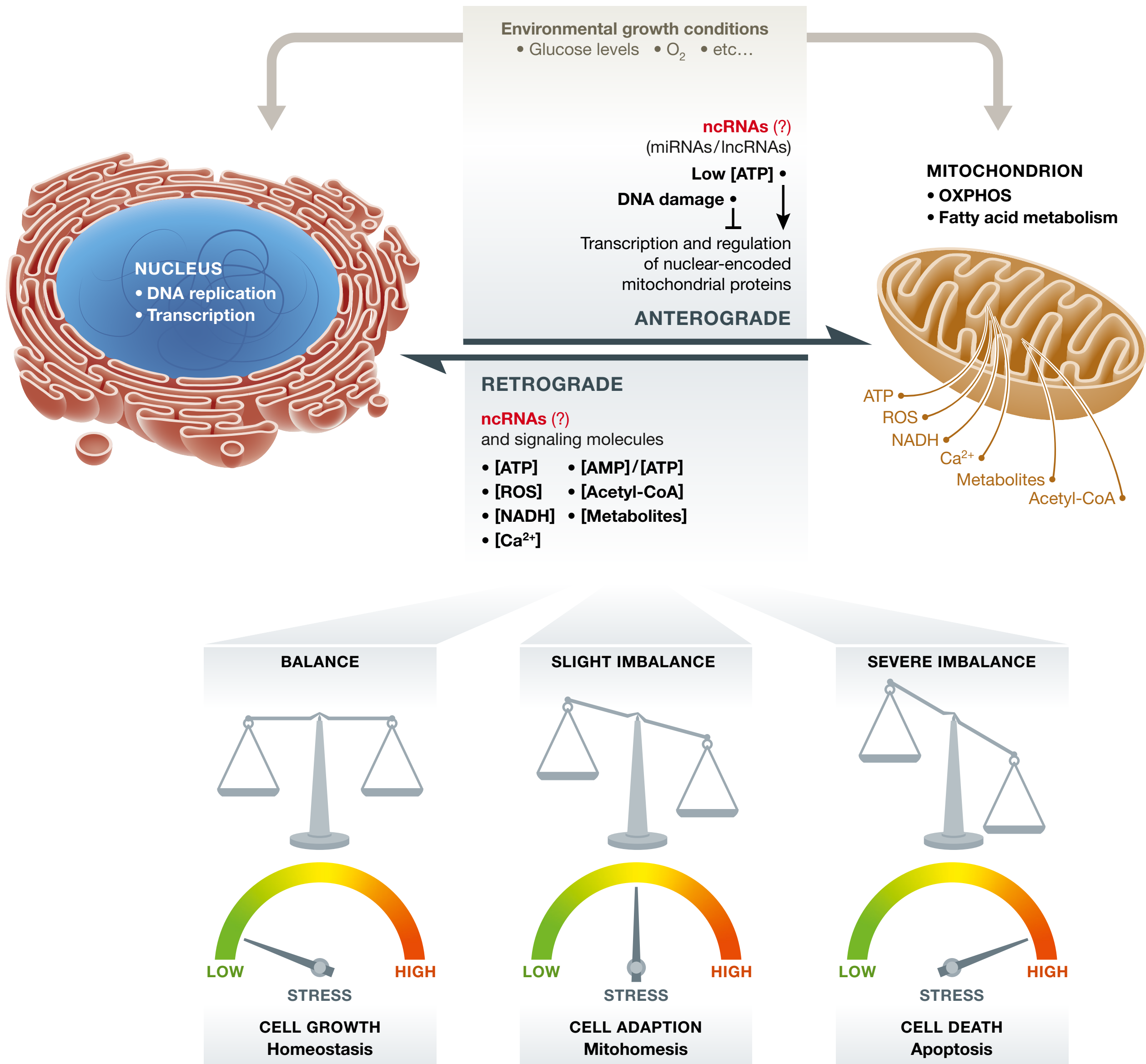

Figure 1. Bidirectional nuclear–mitochondrial communication.
Emerging evidence has highlighted numerous means through which mitochondria and the host nucleus communicate their reciprocal status through anterograde and retrograde signaling in order to adapt and coordinate their respective activities to the environmental growth conditions. Recent studies propose that ncRNAs might also play a role in this process.

These downstream effectors are regulated by upstream sensors that detect changes in metabolic conditions. For example, a decrease in ATP production is sensed by AMP-activated protein kinase (AMPK), which increases cellular NAD^+ levels and leads to activation of Sirtuin 1 (SIRT1). SIRT1 is a NAD^+-dependent deacetylase that positively regulates PGC1α, with subsequent activation of mitochondrial energy metabolism and biogenesis (Fulco & Sartorelli, 2008).

Anterograde signaling can also reduce mitochondrial metabolism in the presence of nuclear stress, such as DNA damage. Telomere dysfunctions in mice are associated with impaired mitochondrial biogenesis and function through the repression of PGC1α- and PGC1β-driven programs by p53 (Sahin *et al*, 2011). Activation of poly(ADP-ribose) polymerase 1 (PARP1) and PARP2 in response to DNA strand breaks likewise represses mitochondrial function by depleting cellular NAD^+ levels and consequently inhibiting the activity of SIRT1 (Bai & Cantó, 2012).

On the other hand, mitochondrial state and activity are constantly reported to the host cell and are capable of activating cellular pathways that trigger metabolic reprogramming, ultimately leading to cell adaptation or cell death. This process is known as retrograde signaling (Fig 1). Notably, cellular responses are not limited to nuclear-encoded mitochondrial transcripts but include global gene expression changes (Raimundo *et al*, 2012). In case of

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severe mitochondria damage, retrograde signaling can directly engage dramatic cytoplasmic stress responses in the absence of nuclear involvement (Richter-Dennerlein *et al*, 2015; Leucci *et al*, 2016), as seen with the intrinsic apoptotic program. Among the biological processes influenced by mitochondria retrograde signaling is epithelial-to-mesenchymal transition (EMT; Guha *et al*, 2014) and thereby chemoresistance (Esteves *et al*, 2014). In addition, dysfunctional mitochondria can give rise to deafness (Raimundo *et al*, 2012), aging-related processes including Alzheimer's disease (Cai & Tammineni, 2016), and several acquired and heritable neurological disorders. Interestingly, a whole class of mitochondria-related disorders seems to originate from mutations in genes affecting mitochondrial protein synthesis (De Silva *et al*, 2015).

Mitochondrial fitness is monitored on the basis of the metabolites that they produce, with these acting as signaling molecules to modulate the activity of key effector proteins such as AMPK, mTOR, HIF1α, sirtuins, and Ca^{2+}/calmodulin. These factors in turn engage the appropriate signaling pathways and adaptive cellular responses (Fig 1).

An example of this is acetyl CoA and *S*-adenosylmethionine, both substrates for acetylation and methylation of histones, that have a direct impact on chromatin and gene expression (Su *et al*, 2016). Calcium release from mitochondria stimulates NFAT and NF-kβ transcriptional programs (Biswas *et al*, 2003) and AMP/ATP imbalance activates catabolic metabolism, favoring fatty acids oxidation and glycogen synthesis (Kahn *et al*, 2005). Similarly, NAD^{+}/NADH levels promote mitochondria biogenesis and fatty acid oxidation through SIRT1 and SIRT3, while repressing ribosomal RNA (rRNA) transcription in the nucleolus (Chang & Guarente, 2014). High levels of NO, one of the reactive oxygen species (ROS) produced by mitochondria, were shown to simultaneously repress respiration and interfere with the maturation of rRNAs (Nisoli & Carruba, 2006).

Moreover, oxidative stress caused by accumulation of ROS is responsible, among other factors, for inactivation of mTOR, the master modulator of protein synthesis in the cytosol. Conversely, mTOR directly controls mitochondrial biogenesis and activity (Morita *et al*, 2015) and is therefore sitting at an important crossroads between anterograde and retrograde signaling. The key role played by mTOR in these processes is clearly illustrated by a recent paper from Gao *et al.* They reported that mutant mice with accelerated mitochondrial translation rates are sterile due to induction of apoptosis in the spermatocytes and spermatogonia compartments. In these compartments, mTOR activity and cytosolic protein synthesis are notably reduced, resulting in a dramatic imbalance between translation rates in the mitochondria and cytosol, thus leading to cell death (Gao *et al*, 2016).

Although the underlying molecular mechanisms remain unclear, interfering with mitochondrial protein synthesis using specific antibiotics can also engage retrograde responses (Skrtić *et al*, 2011; Richter *et al*, 2013). An increasing body of evidence indicates that in yeast this process is, at least partly, a consequence of mitochondrial membrane depolarization, which impairs mitochondrial protein import and affects mitochondrial functions. This leads to the accumulation of mis-targeted nuclear-encoded mitochondrial precursors in the cytoplasm (Wang & Chen, 2015; Wrobel *et al*, 2015) and to the induction of a mitochondrial precursor protein over-accumulation stress (mPOS) response. In yeast, mPOS was shown to cause a global reduction in cytoplasmic cap-dependent protein translation and an increase in proteasome-dependent cytosolic protein degradation. These data indicate that the functional state of mitochondria regulates cellular protein homeostasis (i.e. translation and degradation) and that—so far unknown—quality control mechanisms outside of mitochondria can be activated in response to defects inside this organelle.

Does the expanding non-coding RNA universe reach the mitochondria?

Large parts of the non-coding genome, which itself represents more than 98% of the total genome, is transcribed into various ncRNA species (Carninci *et al*, 2005), including the well-characterized rRNAs, tRNAs, snoRNAs, snRNAs, and the more recently identified microRNAs (miRNAs) and long non-coding RNAs (lncRNAs). According to the last GENCODE release (v25), the human genome contains more than 4,000 miRNA and 15,000 lncRNA genes.

Emerging evidence raises the possibility that several classes of ncRNAs may impact indirectly and/or directly on mitochondrial biology. Below we describe the different classes of ncRNAs that have been implicated as putative modulators of mitochondrial function.

miRNAs are 22-nucleotide-long, highly conserved ncRNAs that are linked to a large variety of (patho-)physiological processes including aging and cancer (Szafranski *et al*, 2015; Peng & Croce, 2016). They exert their canonical function in the cytoplasm, where they bind to the 3′-UTR of dozens to hundreds of mRNA targets and recruit the RNA-induced silencing complex (RISC), resulting in degradation and/or inhibition of translation. Several recent studies have also highlighted non-canonical miRNA functions outside the cytosol (Roberts, 2014). In the nucleus, for instance microRNAs may regulate chromatin state and/or transcription (Place *et al*, 2008) and abundance of specific ncRNAs (Leucci *et al*, 2013). Importantly, although controversial (see below), several studies raised the possibility that nuclear-encoded microRNAs could be imported inside mitochondria (Bandiera *et al*, 2011; Barrey *et al*, 2011; Zhang *et al*, 2014), where one of them was proposed to enhance mitochondrial translation (Zhang *et al*, 2014).

In addition, the human and mouse mitochondrial genome could encode small microRNA-like ncRNAs (Ro *et al*, 2013), a finding supported by the fact that these small RNAs are not expressed in cells devoid of mitochondrial DNA, such as the Roh0 cells. Given that some of these ncRNAs display tissue-specific expression (Ro *et al*, 2013), it will be of great interest to confirm their existence and understand their (patho)physiological functions.

LncRNAs are a large class of heterogeneous RNAs longer than 200 nucleotides and with low evolutionary conservation in comparison with miRNAs (Quinn & Chang, 2015). In general, lncRNAs share many features with messenger RNAs: They are often subjected to alternative splicing, 5′-end capping and 3′-end polyadenylation (Carninci *et al*, 2005; Quinn & Chang, 2015; Schlackow *et al*, 2017). Although many lncRNAs localize to the nucleus and regulate gene expression in *cis* or in *trans* by recruiting chromatin modifiers (Batista & Chang, 2013), others have been found in the cytoplasm where they can associate with ribosomes (van Heesch *et al*, 2014; Ruiz-Orera *et al*, 2014) and/or with specific

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organelles, including mitochondria (Cannon *et al*, 2015; Leucci *et al*, 2016). In addition, the genome of mitochondria may also encode several lncRNAs (Burzio *et al*, 2009).

Several studies have presented data supporting a putative role for ncRNAs as modulators of mitochondrial biology (for some examples, see Table 1). However, it is important to note that the presence of both the microRNA machinery and nuclear-encoded ncRNAs in the mitochondria remains highly controversial. This is in part due to the technical challenges of truly separating isolated and uncontaminated mitochondria. Mitochondria are tightly associated with other membrane vesicles such as the endoplasmic reticulum, the Golgi apparatus, or the endosomes. The use of inappropriate markers to assess purity of mitochondria or mitoplasts is often a critical confounding factor. Indeed, ER or other membrane components should be used as markers instead of soluble cytoplasmic or nuclear proteins. Additionally, in order to minimize the risk of contamination, mitoplasts—rather than partially purified mitochondria—should be analyzed when studying ncRNA effects in mitochondria. Moreover, mitoplasts should be subjected to RNase treatment before lysis. Unfortunately, these important controls have not always been done systematically, leaving the interpretation of many published studies complicated.

Whether mitochondria are able to produce lncRNAs that can, in turn, be exported to the cytosol is also a matter of debate. The human nuclear genome contains dozens of mitochondrial genome equivalents and may therefore be the template for mitochondrial-like transcripts (Tsuzuki *et al*, 1983; Yuan *et al*, 1999; Woischnik & Moraes, 2002). Unfortunately, much of the existing literature on putative mitochondrial ncRNAs has so far failed to demonstrate that these transcripts are specifically transcribed from mitochondrial DNA.

Despite these uncertainties, an increasing body of evidence indicates that ncRNAs are likely to play key roles in the coordination of a series of essential cellular and mitochondrial biological processes, by modulating specific bidirectional signaling pathways. While providing a critical assessment of the existing literature on this topic, we will describe examples of how both miRNAs and lncRNAs may be able to achieve this.

MicroRNA-dependent regulation of mitochondria biology

The term mitomiRs was proposed to describe miRNAs that are located inside mitochondria, irrespective of whether they are directly encoded by the mitochondrial genome or transcribed in the nucleus and imported to the organelle (Barrey *et al*, 2011; Bandiera *et al*, 2013). Intriguingly, most nuclear-encoded mitomiRs originate from loci that are either transcribed from mitochondrial gene clusters or are located close to mitochondrial genes. Given that the transcription of miRNA loci and their neighboring genes is often coregulated (Baskerville & Bartel, 2005), this preferential genomic localization raises the possibility that a functional link may exist between these miRNAs and mitochondrial biology.

Notably, mitomiRs appear to have an unusual size (between 17 and 25 nt as opposed to the average 22 nt for the "canonical" miRNAs) and unique thermodynamic features (i.e. minimum folding energy) that distinguish them from other "conventional" cytosolic miRNAs. It has thus been speculated that the genomic position and unique structural features of mitomiRs could promote their entry to mitochondria (Bandiera *et al*, 2011). As discussed above, however, firm evidence supporting mitochondrial import of nuclear-encoded miRs is still lacking.

In silico analysis revealed multiple putative mitomiR binding sites on the mitochondrial DNA (mtDNA) raising the possibility that mitomiRs could interact with mitochondrial-encoded transcripts (Barrey *et al*, 2011). In line with this possibility, the muscle-specific miRNA miR-1 was proposed to enhance protein synthesis of specific mitochondrial genome-encoded transcripts inside mitochondria, ultimately resulting in increased ATP production. It was suggested that the selective and sustained expression of miR-1 in the cardiac and skeletal muscle is a consequence of the particular high-energy demands in these tissues. Mechanistically, miR-1 was shown to

Table 1. ncRNAs and their function linked to their ability to modulate mitochondrial biology.

Name	Expression pattern	Target(s)	Function	References
miR-338	Brain	COX4	Regulation of axonal energy metabolism	Aschrafi *et al* (2012)
miR-210	Ubiquitous	COX10/ISCU	Cell adaptation and survival under hypoxic conditions	Chan *et al* (2009) and Chen *et al* (2010)
miR-1	Cardiac/skeletal muscle	IGF-1	Regulation of mitochondrial protein synthesis, metabolism and apoptosis	Yu *et al* (2008) and Zhang *et al* (2014)
miR-378	Cardiac	Caspase-3	Inhibition of apoptosis	Fang *et al* (2012)
miR-23a/b	Ubiquitous	Glutaminase	Tumor suppression	Gao *et al* (2009)
miR-30 family	Cardiac	p53	Inhibition of mitochondrial fission and apoptosis	Li *et al* (2010)
SncmtRNA	Proliferating cells	16S, chromatin	Promotion of cell proliferation	Burzio *et al* (2009) and Landerer *et al* (2011)
LIPCAR	Plasma, heart?	Unknown	Unknown	Kumarswamy *et al* (2014)
SAMMSON	Melanoma	p32	Regulate mitochondrial protein synthesis	Leucci *et al* (2016)
GAS5	Ubiquitous	mTOR?	Increase mitochondrial protein synthesis and mitochondrial activity	Meyuhas and Kahan (2015) and Mourtada-Maarabouni *et al* (2010)
linc-p21	Ubiquitous	HIF1α, VHL	Increase HIF1α stability, promotion of glycolysis	Yang *et al* (2014)

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specifically recruit the ND1 and COX1 mRNAs to mitochondrial ribosomes in an AGO2-dependent manner and using canonical seed-target base pairing (Zhang *et al*, 2014). Note, however, that the conclusion that miR-1 binds to these mitochondrial transcripts was solely based on AGO2 CLIP experiments and that direct evidence for this association is still lacking.

Several groups have reported that miRNAs can mediate translation activation in the cytosol under specific conditions (Henke *et al*, 2008; Ørom *et al*, 2008). This effect was dependent on miRNA recruitment to the 5′-end of target mRNAs and/or internal ribosome entry site (IRES; Iwasaki & Tomari, 2009). miR-1, however, did not show preference for the 5′-end of its mitochondrial target transcripts. Given the strong interaction between AGO2 and the 12S rRNA, the authors instead proposed a mechanism whereby AGO2 bridges the ribosome to the miR-1-bound mitochondrial mRNA, leading to increased translation (Zhang *et al*, 2014). In this model, miR-1 would act as a transcript-specific translational activator exerting the same function as protein-coding activators known from yeast (Herrmann *et al*, 2013), but absent in mammalian mitochondria. The presence of a stable miR1:mRNA complex in mitochondria could be explained by the absence of GW182, an AGO2 partner implicated in the scaffolding of the silencing apparatus. Based on these observations, it has been speculated that most, if not all, mitomiRs may be able to promote translation of mitochondrial transcripts through specific miR:mRNA base pairing (Zhang *et al*, 2014).

Although this is a potentially interesting and attractive hypothesis, it is important to emphasize that independent confirmation of the miR-1 findings is still lacking and that, to date, miR-1 is still the only example of a mitomiR reported to stimulate translation.

In a different set of experiments, pre-miR-let7b, pre-miR-302a, and their corresponding mature miRNAs were found in the mitochondria, as assessed by RT-qPCR and *in situ* hybridization (Barrey *et al*, 2011). These data raised the intriguing possibility that miRNA processing may also occur in the mitochondria. However, as discussed above, these data need to be taken with caution and this possibility needs to be further tested using a series of more sophisticated methodologies such as combined RNA FISH cryo-EM-based approaches (EM-ISH).

If the ability of mitomiRs to modulate mitochondrial translation were to be confirmed, one could envision a scenario in which a miRNA-mediated mechanism would uncouple the translation of mitochondrial proteins that belong to the same respiratory chain complex. Alterations in the stoichiometry of such complexes would then alter mitochondrial homeostasis and prime retrograde signaling.

In addition to mitomiRs, several canonical microRNAs have been shown to modulate mitochondrial functions through several mechanisms in the cytoplasm. Given that the best-characterized function of mitochondria is to produce energy, it is not surprising that the first miRNAs linked to mitochondria biology were found to indirectly or directly affect OXPHOS. One example is miR-338, a brain-specific miRNA, that reduces levels of the nuclear-encoded cytochrome *c* oxidase subunit IV (COX4) by binding to its 3′ UTR (Aschrafi *et al*, 2012). Targeting of COX4 by miR-338 affects not only energy metabolism but also intracellular ROS levels and thereby axonal growth. Another fascinating example is miR-210. This miRNA is significantly induced by hypoxia-inducible factor 1α (HIF1α) under hypoxic conditions and can directly repress OXPHOS by targeting the nuclear-encoded cytochrome *c* oxidase assembly protein (COX10) and the iron–sulfur cluster scaffold (ISCU). In this way, miR-210 contributes to the shift from OXPHOS toward glycolysis in anaerobic conditions (Chan *et al*, 2009; Chen *et al*, 2010).

In addition to OXPHOS, several miRNAs play pivotal roles in the modulation of the intrinsic apoptotic program. One example is miR-378—expressed in cardiac tissue—which inhibits the apoptotic program by downregulating caspase-3 protein levels (Fang *et al*, 2012). Moreover, in cardiac and skeletal muscles the aforementioned miR-1 is induced by high glucose stress to regulate cell viability. High miR-1 causes a drop in mitochondrial membrane potential and cytochrome *c* release by targeting insulin-like growth factor (IGF-1; Yu *et al*, 2008). In light of the previously described role of miR-1 in mitochondria (Zhang *et al*, 2014), it is tempting to speculate that the drop in membrane potential may also be a consequence of miR-1-mediated increase in mitochondrial translation without the necessary concomitant increase in cytosolic translation, thus resulting in an unbalanced OXPHOS chain. However, such a model awaits experimental testing.

Through their ability to modulate mitochondrial metabolism, several miRNAs have also been implicated in tumorigenesis, both as oncogenes and tumor suppressors. As an example, miR-23a and miR-23b are known to control expression of the mitochondrial glutaminase (Gao *et al*, 2009). Both miRNAs were found to be downregulated upon oncogenic activation, such as following c-Myc overexpression. The decrease in miRNA levels was accompanied by a significant increase in ATP synthesis through the TCA cycle and in the expression of the mitochondrial glutaminase. Oncogenic activation also resulted decreased ROS production, as glutamine is an essential substrate for glutathione synthesis. Interestingly, these metabolic changes were shown to contribute to Myc-dependent cell transformation, including sustained cell proliferation (Gao *et al*, 2009).

Other miRNAs, such as the miR-30 family, have been implicated in anterograde signaling by modulating mitochondrial dynamics. The miR-30 family is particularly abundant in cardiac tissues where it regulates fission and fusion via the p53-Drp1 axis (Li *et al*, 2010). Diverse members of the miR-30 family were found to target p53 and to decrease its abundance, leading to a concomitant reduction of Drp1, an essential regulator of mitochondrial dynamics. Interestingly, it has been suggested that this mechanism is particularly important in cardiac tissue in order to maintain p53 basal levels (which are particularly low in this tissue) and to promote mitochondrial fusion to support high-energy demands. Moreover, since mitochondrial fission is one of the first steps in the apoptotic pathway, it has been proposed that the miR-30 family, by regulating the p53-Drp1 axis, could increase the threshold required for apoptotic activation in differentiated post-mitotic cells such as cardiomyocytes. This would prevent pathophysiological disorders caused by the loss of such an irreplaceable cell type (Li *et al*, 2010). It would be interesting to assess whether similar mechanisms (mediated either by the miR-30 family or by other miRNAs) could be identified in other tissues that lack self-renewal capacity such as the brain and skeletal muscle cells.

Together, there is an increasing body of evidence indicating that canonical miRs, and possibly mitomiRs, modulate essential mitochondrial functions and thereby contribute directly or indirectly to mitochondria-derived signals that trigger adaptive cellular responses and/or nuclear-dependent reprograming of mitochondrial activities.

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Linking lncRNAs and mitochondrial biology

A regulatory role for lncRNAs in the biology of mitochondria has only recently been suggested based on the discoveries that: (i) lncRNAs may be encoded in the mitochondrial genome (Burzio *et al*, 2009; Rackham *et al*, 2011), and (ii) nuclear-encoded lncRNAs may be transported inside mitochondria (Mercer *et al*, 2011; Leucci *et al*, 2016; Noh *et al*, 2016). By analogy to the mitomiRs, we propose to refer to this particular class of lncRNAs as mitolncRNAs.

An example of a mitochondria-encoded RNA is SncmtRNA, a 2,374-kb-long chimeric transcript composed of 815 nucleotides of inverted repeats covalently bound to the mitochondrial 16S ribosomal RNA. SncmtRNA is preferentially expressed in highly proliferating normal and cancer cells, suggesting a possible implication in the control/maintenance of cell proliferation. Notably, normal proliferating cells also express two antisense transcripts of this particular lncRNA, both of which are downregulated in tumor cells (Burzio *et al*, 2009). Both sense and antisense transcripts of SncmtRNA can be found in the nucleus, indicating that they may directly participate in retrograde signaling (Landerer *et al*, 2011). However, to date the role of these antisense transcripts remains enigmatic and the possibility that they derive from mitochondrial sequences in the nuclear genome still remains to be ruled out.

More recently, Rackam *et al* identified three additional lncRNAs transcribed from the mitochondrial genome and processed by the nuclear-encoded mitochondrial RNase P protein 1 (MRPP1). Interestingly, these three lncRNAs exhibit distinct expression profiles in various tissues indicating that they may exert tissue-specific functions (Rackham *et al*, 2011). This possibility, however, still awaits experimental testing, ideally using *in vivo* conditional knockdown or knockout approaches.

The last known mitochondria-encoded lncRNA is another chimeric/fusion transcript formed between the 5′-end of *COX2* gene and 3′-end of *CYTB*. This transcript, named LIPCAR, was identified in plasma from patients with chronic heart failure (Kumarswamy *et al*, 2014). It remains unclear though, whether LIPCAR is actually an aberrant transcript generated specifically upon infarction. Moreover, considering that there is a fair amount of mitochondrial genome inserted in the nuclear genome, it needs to be excluded if this particular transcript could be derived from the nuclear genome.

Evidence is now emerging that several nuclear-encoded lncRNAs can be transported into mitochondria. We recently identified a lncRNA, referred to as *SAMMSON*, which predominantly localizes to the cytoplasm of human melanoblasts and melanoma cells (Leucci *et al*, 2016). Multiplex RNA FISH and cell fractionation experiments indicated that *SAMMSON* associates with mitochondria. Using RAP-MS we identified, among other putative *SAMMSON* interactors, the protein p32 (Leucci *et al*, 2016), a well-established regulator of mitoribosome assembly and mitochondrial protein synthesis (Fogal *et al*, 2010). Knockdown of *SAMMSON* in melanoma cells decreased mitochondrial targeting of p32 and caused mitochondrial protein synthesis defects, which ultimately triggered apoptotic cell death. Cell death following *SAMMSON* depletion could be partly rescued by overexpressing an exogenous form of p32 that contains a functional mitochondrial targeting sequence. These findings established a clear functional epistatic relationship between *SAMMSON* and p32. Consistently, the decrease in mitochondrial protein synthesis and cell viability seen with *SAMMSON* loss could be phenocopied upon knockdown of p32 (Fogal *et al*, 2010). Notably, these effects could be recapitulated by exposure of the melanoma cells to antibiotics such as chloramphenicol (unpublished results), which are well known for their ability to inhibit protein synthesis in mitochondria. We provided evidence that, similar to the results described in yeast (Wang & Chen, 2015; Wrobel *et al*, 2015), the mitochondrial protein synthesis defects observed upon *SAMMSON* depletion caused mitochondrial membrane depolarization and induction of mPOS. Ultimately, mPOS triggered an apoptotic response, which could be rescued by interfering with cytosolic CAP-dependent protein synthesis (Wang & Chen, 2015; Wrobel *et al*, 2015).

We are currently performing Cryo-EM coupled to FISH to assess whether or not *SAMMSON* itself is imported into the mitochondrial matrix, although mitochondrial localization is not required for the lncRNA to interfere with p32 localization. *SAMMSON* may favor p32 mitochondrial import by, for instance, interfering with p32 post-translational modifications or masking its nuclear localization signal. Together, we concluded that *SAMMSON* is required to increase mitochondrial protein synthesis in highly dividing melanoma cells, and thereby to keep the proper balance between cytosolic and mitochondrial protein homeostasis. Failure to do so causes the accumulation of nonfunctional and faulty proteins outside the mitochondria, and ultimately triggers a fatal mPOS response (Fig 2).

Notably, we also identified XRN2, an exonuclease necessary for rRNA maturation in the nucleus (Petfalski *et al*, 1998), as a *SAMMSON* interactor. Although XRN2 is known to directly bind other lncRNAs (Chu *et al*, 2015) and to be involved in the processing of such transcripts, this observation raises the interesting possibility that *SAMMSON* could modulate nuclear/nucleolar ribosome biogenesis and thereby participate in retrograde signaling.

In addition to functioning as direct signaling molecules, lncRNAs can also indirectly modulate essential mitochondrial functions by, for instance, regulating key metabolic signaling molecules or pathways, including AMPK (Essers *et al*, 2015), mTOR (Mourtada-Maarabouni *et al*, 2010), HIF1α (Yang *et al*, 2014), and sirtuins (Wang *et al*, 2014). GAS5, for instance, is a lncRNA containing a 5′-terminal oligo pyrimidine (TOP) stretch (Mourtada-Maarabouni *et al*, 2010). The 5′-TOP sequence is generally found in mRNAs that encode translation factors and that are under the control of mTOR (Meyuhas & Kahan, 2015). Interestingly, GAS5 induction promotes growth arrest upon various stress stimuli, including mTOR inhibition (Mourtada-Maarabouni *et al*, 2010). mTOR is known to regulate protein synthesis of nuclear-encoded mitochondrial genes such as *TFAM* and genes encoding components of the mitochondrial respiratory complexes I and V, as well as mitochondrial ribosomal proteins (Morita *et al*, 2015). Although the mechanistic details linking GAS5 and mTOR functions remain to be elucidated, these data raise the interesting possibility that GAS5 may modulate the ability of mTOR to coordinate protein synthesis and mitochondrial activities.

LncRNAs have also been implicated in hypoxia-inducible factor 1α (HIF1α) signaling. LncRNAs antisense of HIF1α have been described and associated with cancer development and vasculogenesis (Chen *et al*, 2015; Wang *et al*, 2015). In addition, the hypoxia-induced lncRNA linc-p21 was shown to disrupt the interaction between VHL and HIF1α, thereby impeding HIF1α ubiquitination

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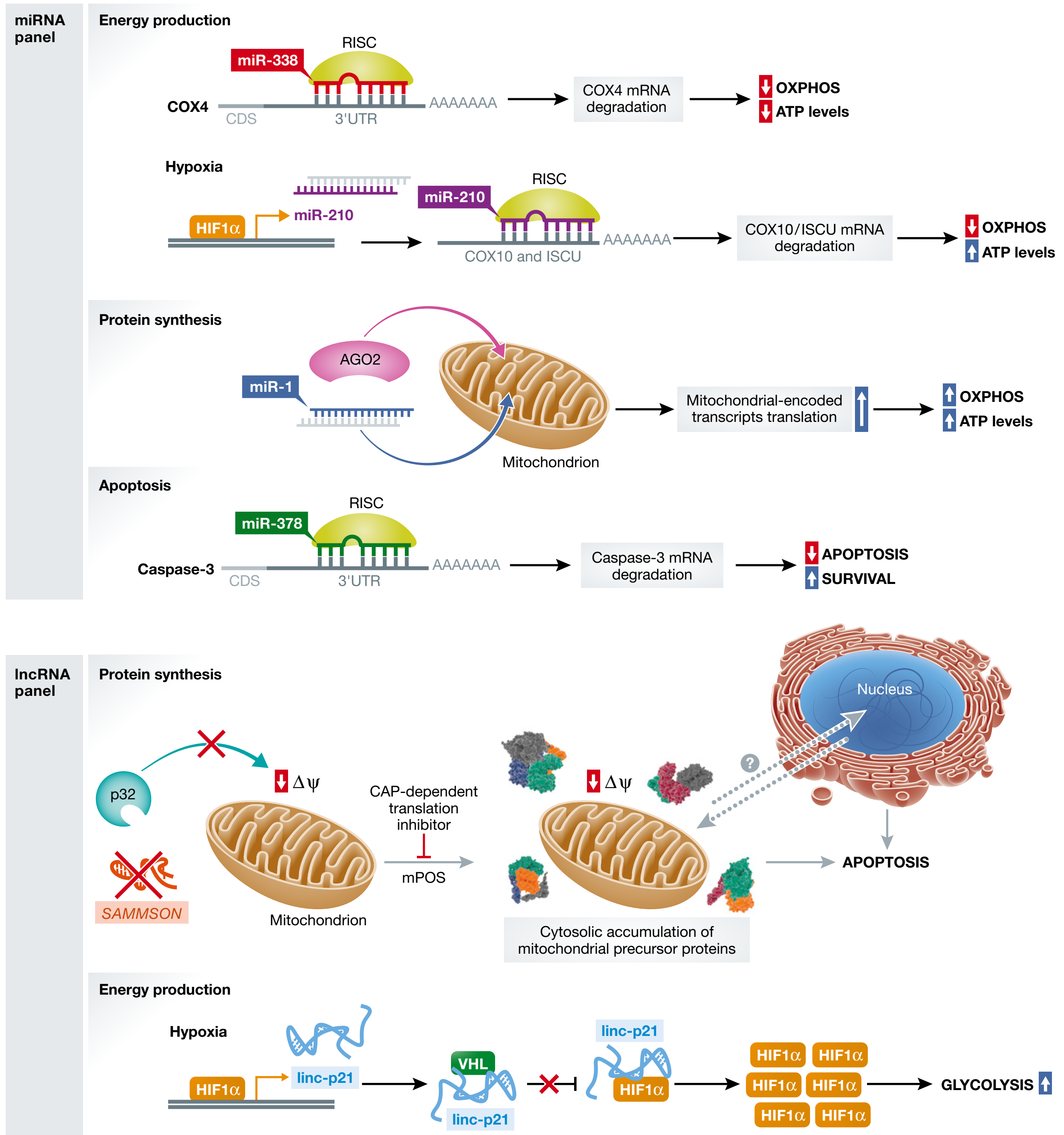

Figure 2. Examples of miRNAs and ncRNAs implicated in nuclear–mitochondrial communication.

and proteasomal degradation. As expected, an increase in HIF1α stability was associated with induction of the glycolytic pathway and metabolic reprogramming (Yang *et al*, 2014).

Although the mitochondrial lncRNA universe remains largely unexplored, these few examples highlight the enormous potential of these molecules as messengers in the communication between the nucleus and the mitochondria.

ncRNAs and the route to the mitochondrial matrix

It is well established that mitochondria import various nuclear-encoded non-coding RNA species, including tRNAs and the 5S ribosomal RNA, and that the latter is in fact the most abundant RNA in mitochondria (Beitzinger *et al*, 2007; Bian *et al*, 2010; Bandiera *et al*, 2011; Zhang *et al*, 2014; Cannon *et al*, 2015). Note that

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although the import of 5S RNA into the mitochondria is widely accepted, the functional relevance of this phenomenon remains unclear. The 5S RNA was previously reported to be part of the mitoribosome (Smirnov *et al*, 2011). However, cryo-EM studies by two independent groups have recently questioned this finding (Amunts *et al*, 2015; Greber *et al*, 2015). Regardless, the ability of 5S rRNA and tRNAs to be imported to mitochondria suggests that the same thing may happen for other ncRNA species.

In keeping with this possibility, hundreds of miRNAs have been suggested to localize to the mitochondrial matrix of mammalian cells (Lung *et al*, 2006; Bandiera *et al*, 2011; Mercer *et al*, 2011). Although of great potential interest, one still needs to be cautious about the interpretation of these data as—based on the methodologies used in most cases—it remains unclear to what extent the RNA samples analyzed contained cytosolic contaminants. Moreover, in at least one study the authors concluded that the RNAs are either not imported into this organelle or imported in very limited amount (Lung *et al*, 2006).

Part of the RISC machinery was also suggested to localize to the mitochondrial matrix (Bandiera *et al*, 2011; Zhang *et al*, 2014). Additional support for the presence of AGO2 inside mitochondria comes from profiling experiments of the AGO2-bound transcriptome that identified mitochondria-encoded transcripts (Beitzinger *et al*, 2007). This observation raises the possibility that a fraction of the AGO2 pool could localize to the mitochondrial matrix (Bian *et al*, 2010; Bandiera *et al*, 2013; Zhang *et al*, 2014). However, the possibility that mitochondrial transcripts are exported into the cytosol and eventually recruited to AGO2-containing complexes could also, at least partly, explain this finding. Moreover, these RNAs detected in AGO2-IPs could originate from transcription of nucleus-encoded mitochondrial pseudogenes.

Evidence that several nuclear-encoded lncRNAs, including the VL30 retro-element (Cannon *et al*, 2015), *RMRP* (Tarassov *et al*, 2007), and possibly *SAMMSON* (Leucci *et al*, 2016), may be imported into mitochondria has also been reported. Although still controversial for the technical reasons mentioned above, these observations lead to the next key question: How are these negatively charged ncRNA molecules transported to and inside the mitochondria?

Several ATP-dependent mechanisms that do not appear to rely on cytosolic proteins or protein import systems have been described (Tarassov *et al*, 2007). One example is the RNA import complex (RIC), described in *Leishmania,* that recognizes structural motifs in cytosolic tRNAs (Bhattacharyya *et al*, 2003). Although it is tempting to speculate on the existence of a similar complex in higher eukaryotes, no orthologues of components of the RIC complex have so far been identified in mammalian cells. In Wang *et al* (2010) reported that polynucleotide phosphorylase (PNPase), an enzyme that localizes to the mitochondrial intermembrane space (IMS) and is responsible for mitochondrial transcript processing, promotes the import of specific ncRNAs through the inner mitochondrial membrane into the mitochondrial matrix.

The well-accepted textbook view that RNA is never "naked" but that nascent RNA is immediately co-opted into ribonuclear particles raises the possibility that ncRNAs, including lncRNAs, may be able to sneak into the mitochondria making use of cargo proteins that carry mitochondrial targeting signals. This possibility is actually supported by a few recent reports. For example, the binding of 5S RNA to MRP-L18 causes an RNA conformational change that favors its binding to another cytoplasmic protein, rhodanese, which is ultimately responsible for carrying the 5S RNA inside the mitochondria (Smirnov *et al*, 2011).

Another recent example is the observation that the RNA component of the RNase MRP, the lncRNA RMRP, may be transported into the mitochondria by GRSF1 (Noh *et al*, 2016). GRSF1 is a well-established mitochondrial RNA-binding protein that localizes to RNA granules (Antonicka *et al*, 2013), punctate structures in the mitochondria containing RNA processing and mitoribosome assembly factors (Jourdain *et al*, 2016). It is important to note, however, that although the presence of lncRNA RMRP in mitoplasts was confirmed by independent studies (Topper *et al*, 1992; Li *et al*, 1994; Lu *et al*, 2010), its very limited abundance (less than 1 RMRP molecule per cell mitochondrial complement) has raised serious concerns about its actual presence in mitochondria and its possible impact on the biology of this organelle (Kiss & Filipowicz, 1992).

In conclusion, although the presence of ncRNAs, including lncRNAs, in the mitochondria has been reported by several studies, additional experiments are needed to firmly establish this possibility and, if eventually confirmed, there will be much to learn about the mechanisms underpinning the RNA shuttling.

Concluding remarks & opportunities for therapy

It has become increasingly clear that mitochondria are at the crossroads of many vital biosynthetic processes and that they exhibit critical functions that go much beyond aerobic oxidation. It is therefore not surprising that elaborate signaling mechanisms between host cell and mitochondria have evolved and that this symbiotic relationship constitutes a hub influencing life and death. This hub can be viewed as an ON–OFF switch that is activated depending on the environmental growth conditions. Mechanisms that keep the proper balance in cytoplasmic protein homeostasis seem particularly important in controlling this critical switch. For instance, various mechanisms that coordinate cytosolic ribosome biogenesis in the nucleus/nucleolus with mitochondrial protein synthesis have been uncovered. In fact, many of the signaling molecules known to participate in host–mitochondria interactions, such as SIRT1 or NO, have dual roles in both rRNA processing and/or maturation and the control of mitochondrial protein synthesis. Conversely, the malfunction of protein synthesis in mitochondria does not only lead to a decrease in the supply of mitochondrial products but also in the accumulation of mitochondrial precursors in the cytosol.

Recent findings have highlighted the importance of several ncRNAs in the control of both cytosolic and mitochondrial protein synthesis (Carrieri *et al*, 2012; Zhang *et al*, 2014; Essers *et al*, 2015). We therefore postulate that this emerging class of molecules participates in the control of the symbiotic relationship between the host and the mitochondria. Many ncRNAs have evolved to allow cells to cope with stress (Amaral *et al*, 2013), partly/possibly due to the fact that they are not translated and can therefore be mobilized rapidly in response to extracellular and intracellular stimuli. This is also an advantage for "messenger" molecules that need to rapidly relay vital information between the nucleus and mitochondria and vice versa.

Critically, deregulation of proper nuclear–mitochondria communication may contribute to the onset, course, and symptom severity

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of inherited mitochondrial diseases. In addition, given that proteotoxic stress and mitochondrial dysfunctions are often at the heart of aging-related diseases, such as neurodegeneration and cancer, the signaling pathways involved in nuclear–mitochondria crosstalks are also likely to be important players in these pathologies and thus potential targets for therapeutic interventions.

Notably, the function of microRNAs and lncRNAs can be modulated using clinically compatible drugs, such as antagomiRs or antisense oligonucleotides (ASOs). As an example, we have demonstrated that it is possible to efficiently target *SAMMSON in vitro* and in preclinical *in vivo* models such as patient-derived xenografts (Leucci *et al*, 2016). This study offers solid proof-of-concept evidence that interfering with cellular-mitochondria crosstalk mechanisms by targeting the specific ncRNAs may offer new promising avenues for cancer therapy. Importantly, more than a hundred of such drugs have been enrolled into various phase II and III clinical trials and several were recently approved by the FDA (Raal *et al*, 2010; Monteleone *et al*, 2015). Given the recent surge in optimism over RNA-targeting therapeutics and antisense drugs, such a therapeutic strategy may be rapidly amenable to the clinic. All in all, we conclude that a deeper understanding of the role of non-coding RNAs in anterograde and retrograde signaling may have rapid and important clinical implications.

Conflict of interest

The authors declare that they have no conflict of interest.

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