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REVIEW

Localization of Nuclear-Encoded mRNAs to Mitochondria Outer Surface

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Abstract—The diverse functions of mitochondria depend on hundreds of different proteins. The vast majority of these proteins is encoded in the nucleus, translated in the cytosol, and must be imported into the organelle. Import was shown to occur after complete synthesis of the protein, with the assistance of cytosolic chaperones that maintain it in an unfolded state and target it to the mitochondrial translocase of the outer membrane (TOM complex). Recent studies, however, identified many mRNAs encoding mitochondrial proteins near the outer membrane of mitochondria. Translation studies suggest that many of these mRNAs are translated locally, presumably allowing cotranslational import into mitochondria. Herein we review these data and discuss its relevance for local protein synthesis. We also suggest alternative roles for mRNA localization to mitochondria. Finally, we suggest future research directions, including revealing the significance of localization to mitochondria physiology and the molecular players that regulate it.

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The localization of mRNA molecules to specific cellular loci is a major regulatory mechanism to control protein synthesis both spatially and temporally. The accumulation of transcripts at a site where their protein products are required enables an immediate response to dynamic changes within the cell, and a rapid, on-site protein synthesis. mRNA localization and local protein synthesis reduce the probability of undesired protein activity outside its target site. Energy considerations present yet another advantage of this mechanism: the transport of multiple copies of a translated protein accompanied by their chaperones to their action site is a highly energy-consuming process. On the other hand, the transport of a single mRNA molecule that is capable of producing multiple copies of the protein on site can yield the same results for a lower energy cost [1, 2].

Sites of mRNA accumulation throughout the cell can have a regulatory role, afar from the protein activity site. For example, mRNA processing bodies (P-bodies) are messenger-ribonucleoproteins (mRNPs) complexes where mRNAs undergo a quality control process, and are decided for either degradation, silencing, or translation [3, 4]. Related to P-bodies are stress granules – mRNPs that assemble upon different stress signals and inhibit mRNA translation. These complexes are reversible, and dissociate when stress signals decay, releasing translation inhibition and restoring the translation process.

FUNCTIONAL IMPORTANCE OF mRNA LOCALIZATION

The functional importance of mRNA localization is most apparent in developmental systems, where a maternal mRNA is deposited into specific sites in the oocyte. Such localized mRNA will be translated locally during embryogenesis, thereby inducing local developmental processes [5, 6]. One of the most studied model systems in embryonal development is the fruit fly *Drosophila melanogaster*. In a comprehensive study of mRNA localization during embryonal development, thousands of mRNAs were shown to be asymmetrically distributed throughout the embryo [7]. Detailed studies throughout

Abbreviations: (c)ER, (cortical) endoplasmic reticulum; GFP, green fluorescent protein; mRNA, messenger RNA; MTS, mitochondrial targeting sequence; P-bodies, mRNA processing bodies; RBPs, RNA-binding proteins; SRP, signal recognition particle; TOM complex, translocase of the outer membrane (of mitochondria); 3′-UTR, 3′-untranslated region.

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the years revealed a clear impact on the fly development if mRNA localization is hampered. For example, mislocalization of *oskar* mRNA to the anterior of the embryo leads to formation of germ cells and abdominal structures instead of the head [8, 9]. Impact on embryonal development is also well studied in other organisms, such as *Xenopus laevis* [10], *Caenorhabditis elegans* [11], and *Danio rerio* [12]. In the budding yeast, *Saccharomyces cerevisiae*, localization of Ash1 mRNA to the daughter cell maintains synthesis of Ash1 protein at this site, and thereby prevent mating type switching in the daughter cell [13].

In all the aforementioned examples, localization of mRNA serves to induce local synthesis of a protein, thereby inducing local phenotypes. As discussed above, mRNAs can also localize to P-bodies or stress granules, which serve as depositories of untranslated messages. Additionally, mRNAs were shown to localize to certain domains for their proper inheritance. For example, localization of a subset of mRNAs to the cortical endoplasmic reticulum (cER) in yeast is important for their delivery, attached with the cER, to the daughter cell [14-16].

GENERAL MECHANISMS FOR mRNA LOCALIZATION

Targeting of an mRNA to its proper destination can occur either before its translation, by mechanisms that utilize mRNA sequences and specific RNA binding proteins (RBPs), or after its translation initiation, by mechanisms that utilize sequences of the encoded protein and chaperones that recognize them. In the former mechanism, the mRNA is bound by RBPs in the nucleus soon after its transcription and is escorted to its proper destination while kept in a translationally inactive state. Transport utilizes the cellular cytoskeleton and specific motor proteins [17]. Translation inhibition is usually relieved at the target site where the protein is synthesized [1, 18]. A well-studied example of such mechanism is the targeting of β-actin mRNA to the growing edge of fibroblasts. In this system, the RNA targeting motif ("zip-code"), its associated proteins, and the factors that mediate transport and release from translation inhibition are characterized. Furthermore, the dynamics of the process are resolved to their very fine details (reviewed in [19, 20]). The alternative mechanism, in which translation is not excluded and frequently necessary, is also extensively studied. Best described is the targeting mechanism to the ER, in which the mRNA is translated in the cytosol, and once a signal peptide emerges out of the ribosome it associates with the Signal Recognition Particle (SRP). SRP, while associated with the mRNA–ribosome–nascent peptide complex, interacts with its receptor on the ER membrane, thus mediating proper targeting. Once associated at the ER, the SRP dissociates from the complex allowing insertion of the emerging peptide through the translocon. Thus, translation is necessary for ER localization, and sequences from the encoded protein govern proper targeting. However, recent studies suggest that mRNAs can also be directed to the ER in a translation- and SRP-independent manner. RNA elements, usually from the 3′-UTR (3′-untranslated region), are bound by RBPs and mediate association with the ER [21-25]. This indicates that these two mechanisms are not mutually exclusive, and a mRNA can be targeted by seemingly redundant pathways.

mRNA LOCALIZATION TO MITOCHONDRIA

A growing body of evidence, primarily obtained in studies of yeast mitochondria, reveals localization of many different mRNAs to the proximity of the mitochondrial outer membrane [26]. These mRNAs, which are transcribed in the nucleus and transported to the mitochondria vicinity, usually encode mitochondrial proteins. This suggests a local translation of mitochondrial proteins, which may be linked to cotranslational import of the protein. Various experimental evidences (elaborated below) support this notion. However, mRNA localization to mitochondria does not necessarily imply its translation, and other interpretations are also possible. For example, the association of mRNA with the mitochondria may serve for depository purposes or for coordinated transport with mitochondria. We discuss these putative possibilities below.

The commonly used approach for studying mRNA localization to mitochondria is based on cell fractionation by differential centrifugation followed by RNA extraction from the mitochondrial fraction. Northern analysis of 12 transcripts encoding yeast mitochondrial proteins revealed differential association of these transcripts; some were found almost exclusively in the mitochondrial fraction, others were absent from this fraction and the rest distributed for varying degrees between mitochondrial and cytosolic fractions [27]. Later studies [28-30] applied genome-wide assays to such fractions and identified a large repertoire of mRNAs associated with mitochondria. Association differed among mRNAs, and those that were highly associated could be characterized as being longer and/or originate from endosymbiont genome [31]. In all the cases mentioned above, RNA was extracted as a fraction of mitochondria-associated polysomes. Indeed, pretreatment of the mitochondrial fraction with polysome-dissociating agents such as EDTA or puromycin resulted in decreased mRNA levels, indicating that association of mRNA to mitochondria is mediated at least in part by translating ribosomes [27, 32].

A visual evidence for mRNA localization to mitochondria was provided by *in situ* hybridization of the α- and β-subunits of F1-ATPase in rat liver. Transmission

electron microscopy analysis of the samples revealed a randomly distributed signal of α-F1-ATPase. However, the β-F1-ATPase signal appeared as clusters, frequently associated with mitochondria [33]. More recent [34] fluorescent *in situ* hybridization (FISH) assays of yeast cells revealed few additional examples of mRNAs that decorate the mitochondria outer membrane and *in vivo* imaging methodology [35] further expanded the list of mitochondria-associated transcripts. These methodologies too, revealed that different mRNAs are localized to a different extent, hence suggesting multiple modes of localization.

MECHANISMS OF LOCALIZATION TO MITOCHONDRIA

As indicated above, RNA can be targeted in a translation dependent or independent manner. Both modes appear applicable in targeting mRNA to the mitochondria, and are not mutually exclusive. Analyses of mRNA sequences that are important for targeting revealed that both coding regions and untranslated regions (in particular the mitochondrial targeting sequence (MTS) and the 3′-UTR, respectively) contribute to localization. The yeast *ATM1* mRNA was shown to localize to mitochondria vicinity by either its MTS sequence or its 3′-UTR [27, 36]. *In vivo* imaging of yeast revealed a reduction in *OXA1* and *ATP2* mRNA localization upon 3′-UTR deletion and upon mutating the translation start codon. Deletion of both elements further decreased localization [35]. The role of MTS and 3′-UTR was also demonstrated by fusing either of them to GFP (green fluorescent protein) [32]. Each exhibited increased association with mitochondria compared to the transcript carrying GFP alone. Moreover, attaching both elements to GFP further increased association. These data suggest that multiple localization pathways operate on a particular transcript and the necessity of each pathway differ among mRNAs.

The MTS and 3′-UTR are bound by protein factors that mediate proper localization. For the MTS, which must be translated to confer a targeting effect, protein receptors are necessary. Tom20, a protein receptor associated with the TOM complex (mitochondrial translocase of the outer membrane) was shown to affect mRNA localization through interaction with the incoming MTS [32]. Deletion of other TOM components such as Tom70 also affects mRNA localization, further supporting a role for the imported protein in localization [35, 37]. Chaperones that interact with the emerging peptide can also support localization. The yeast Hsp70-member Ssa1 supports localization of many mRNAs encoding hydrophobic domains through interaction with the synthesized protein and with Tom70 on the mitochondrial outer membrane [37]. Another example is the Nascent chain Associated Complex (NAC), which was shown to support ribosome-association with mitochondria and *in vitro* cotranslational import [38-40]. The impact of NAC is exerted through interaction with OM14, a small protein located in the mitochondrial outer membrane [40].

Proteins that bind the 3′-UTR may exert their role independently of a translational process. The yeast Puf3 protein is the only RBP that was shown to affect mRNA localization to mitochondria through the 3′-UTR [36, 41]. Its binding motif is well-defined [42], and a repertoire of mRNAs that are mislocalized upon its deletion was identified [36]. Puf3 is associated with the mitochondria outer membrane [41], thereby anchoring the mRNA while it is translated [26]. Puf3 is also known to be involved in regulation of mRNA stability [43, 44], which may suggest a role in coordinating localization with degradation [45]. Interestingly, deletion of either Puf3 or Tom20 confers only marginal effect on yeast growth under respiratory conditions, yet deletion of both proteins completely inhibits growth [32]. This again emphasizes the existence of redundant pathways for targeting mRNAs to mitochondria.

PHYSIOLOGICAL SIGNIFICANCE OF mRNA LOCALIZATION TO MITOCHONDRIA

The most obvious role for mRNA localization to mitochondria is to enhance protein import into the organelle. *In vitro* assays employing purified mitochondria and ribosome-nascent chain complexes revealed that import can efficiently occur cotranslationally [46]. Further *in vivo* assays, utilizing a fusion of mitochondrial protein to mouse dihydrofolate reductase (DHFR) suggested cotranslational import of this protein, since its import was not inhibited by a known post-translational import inhibitor of DHFR (methotrexate) [47]. Electron microscopy analysis of cycloheximide-treated yeast cells showed attachment of ribosomes to mitochondria outer membrane [48, 49]. The number of mitochondria-bound ribosomes was found to depend on the metabolic state of the cells, with approximately four times more ribosomes bound to log-phase compared to stationary or starved yeast mitochondria [50]. This suggests enhanced translation near the mitochondria during logarithmic growth, presumably to introduce more proteins to the organelle. Protein targeting studies utilizing MTS fused to the N-terminus of GFP that is also fused to an ER-targeting signal at its C-terminus, revealed exclusive localization to mitochondria, suggesting that the protein was synthesized in proximity to mitochondria [51]. Moreover, the absence of GFP from the ER might indicate that the N-terminus of the protein was already inserted into mitochondria when the C-terminus was translated, thus supporting not only localized translation, but also cotranslational import.

Concomitant translation and import is of particular advantage to membrane proteins that contain hydropho-

bic domains. Cotranslational import minimizes chances of aggregation in the cytoplasmic environment. Consistent with that, isolation of mitochondria-associated ribosomes and analysis of their mRNAs revealed that most mRNAs of the mitochondrial inner membrane are translated in proximity to the mitochondria [52].

Cytosolic proteins that need to undergo a maturation step in mitochondria also utilize a translation-coupled import process [53]. For example, fumarase is first translated into mitochondria, and after cleavage of its MTS, it is back-translocated and completes its translation in the cytosol [54, 55]. Proteins with dual localization, such as fumarase, appear to comprise a large fraction of the cell proteome [56], thus suggesting that translation near mitochondria is of importance for cytosolic processes too.

The importance of mRNA localization to mitochondria for local translation is well established. It might however have other roles that do not involve translation. As indicated above, Puf3 protein is known to have a role in mRNA stability. Interestingly, Puf3 is inactivated upon changes in carbon source, leading to reduced degradation of mRNAs that encode for mitochondrial proteins [44]. Since no significant changes in localization of Puf3 protein were apparent [44], it may suggest that under certain growth conditions Puf3 mediates degradation of specific mRNAs near the mitochondria. Whether degradation rates differ spatially in the cell is yet to be determined. Nevertheless, these results suggest novel means by which gene expression is spatially regulated.

Long distance mRNA trafficking may be another utilization of mRNA localization to mitochondria. mRNAs are known to travel long distances (for example, in neuronal cells) by utilizing the cytoskeleton and motor proteins [1, 57]. However, mRNA can travel as a cargo on cellular organelles [16]; an exemplary case is long-distance travel of mRNAs in the plant pathogen *Ustilago maydis*. Here, mRNAs are associated with endosomes, which transverse distances of tens of micrometers along the hyphae [58]. One can envision a similar role to mitochondria, shuttling its associated mRNAs throughout the cells. Such a shuttling role may not necessarily be confined to mRNAs that encode mitochondria proteins. Other mRNAs may piggyback ride on the traveling mitochondria to approach distant sites.

FUTURE DIRECTIONS

Three major questions are yet to be answered regarding mRNA localization to mitochondria. First, what is the extent of this phenomenon? Most data thus far is derived from studies in yeast, and reports in other organisms are scarce. Studies in *Drosophila melanogaster* identified mRNA localization to mitochondria for a few inner membrane genes [59] and revealed a role for the RBP Clueless in ribosome association [60]. VDAC3 mRNAs were shown to be localized to *Arabidopsis thaliana* mitochondria through elements in their 3′-UTR [61]. In spite of these emerging data, comprehensive characterization of the repertoire of localized mRNAs in different organisms is yet to be performed. Furthermore, the *cis*- and *trans*-factors that mediate this process are largely unknown.

A second intriguing question is the physiological significance of this phenomenon. While impact on protein import at the molecular level was described in a few cases [40, 47], the breadth of this role is not known. As indicated above, redundant pathways mediate mRNA localization, making the contribution of each pathway not easily discerned. Moreover, protein-targeting pathways act to ensure transport of the synthesized protein to the mitochondria even when the mRNA is mislocalized. Thus, experimental dissection of the role of each targeting pathway is challenging. For example, we were able to see a relevant growth defect in yeast only upon deletion of both Puf3 and Tom20 [32]. To overcome this complexity, high resolution and high throughput methodologies must be applied. These will allow detailed screening of many different conditions and many mutants to identify the unique conditions in which the impact of each mechanism is most apparent.

A third major question in this field is concerned with the transport mechanisms. Studies in yeast defined two general modes: one that utilizes RBPs and noncoding RNA sequences, and a second that utilizes the translated protein and chaperones that interact with it. Yet, the fine details of these schemes are not known. The involvement of cytoskeleton and motor proteins, mitochondrial acceptors, ribosome-associated complexes, and RBPs other than Puf3 is largely unknown. One should also note the limited generalizations that can be made from yeast cells. Their relatively small size and high mitochondria abundance may permit simple transport mechanisms that rely on passive diffusion and capture. Such transport is unsuitable to larger or polar cells. Thus, future studies in human cells may reveal novel transport pathways and molecular players that target mRNAs to distant mitochondria.

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REFERENCES

1. Holt, C. E., and Bullock, S. L. (2009) Subcellular mRNA localization in animal cells and why it matters, *Science*, **326**, 1212-1216.

2. Holt, C. E., and Schuman, E. M. (2013) The central dogma decentralized: new perspectives on RNA function and local translation in neurons, *Neuron*, **80**, 648-657.
3. Anderson, P., and Kedersha, N. (2009) RNA granules: post-transcriptional and epigenetic modulators of gene expression, *Nat. Rev. Mol. Cell Biol.*, **10**, 430-436.
4. Decker, C. J., and Parker, R. (2012) P-bodies and stress granules: possible roles in the control of translation and mRNA degradation, *Cold Spring Harb. Perspect. Biol.*, **4**, a012286.
5. Zhou, Y., and King, M. L. (2004) Sending RNAs into the future: RNA localization and germ cell fate, *IUBMB Life*, **56**, 19-27.
6. Cody, N. A., Iampietro, C., and Lecuyer, E. (2013) The many functions of mRNA localization during normal development and disease: from pillar to post, *Wiley Interdiscip. Rev. Dev. Biol.*, **2**, 781-796.
7. Lecuyer, E., Yoshida, H., Parthasarathy, N., Alm, C., Babak, T., Cerovina, T., Hughes, T. R., Tomancak, P., and Krause, H. M. (2007) Global analysis of mRNA localization reveals a prominent role in organizing cellular architecture and function, *Cell*, **131**, 174-187.
8. Ephrussi, A., and Lehmann, R. (1992) Induction of germ cell formation by oskar, *Nature*, **358**, 387-392.
9. Lehmann, R., and Nusslein-Volhard, C. (1986) Abdominal segmentation, pole cell formation, and embryonic polarity require the localized activity of oskar, a maternal gene in *Drosophila*, *Cell*, **47**, 141-152.
10. Kloc, M., Bilinski, S., Chan, A. P., Allen, L. H., Zearfoss, N. R., and Etkin, L. D. (2001) RNA localization and germ cell determination in *Xenopus*, *Int. Rev. Cytol.*, **203**, 63-91.
11. Evans, T. C., and Hunter, C. P. (2005) Translational control of maternal RNAs, *WormBook*, **10**, 1-11.
12. Medioni, C., Mowry, K., and Besse, F. (2012) Principles and roles of mRNA localization in animal development, *Development*, **139**, 3263-3276.
13. Singer-Kruger, B., and Jansen, R. P. (2014) Here, there, everywhere. mRNA localization in budding yeast, *RNA Biol.*, **11**, 1031-1039.
14. Aronov, S., Gelin-Licht, R., Zipor, G., Haim, L., Safran, E., and Gerst, J. E. (2007) mRNAs encoding polarity and exocytosis factors are cotransported with the cortical endoplasmic reticulum to the incipient bud in *Saccharomyces cerevisiae*, *Mol. Cell. Biol.*, **27**, 3441-3455.
15. Fundakowski, J., Hermesh, O., and Jansen, R. P. (2012) Localization of a subset of yeast mRNAs depends on inheritance of endoplasmic reticulum, *Traffic*, **13**, 1642-1652.
16. Jansen, R. P., Niessing, D., Baumann, S., and Feldbrugge, M. (2014) mRNA transport meets membrane traffic, *Trends Genet.*, **30**, 408-417.
17. Bullock, S. L. (2011) Messengers, motors and mysteries: sorting of eukaryotic mRNAs by cytoskeletal transport, *Biochem. Soc. Trans.*, **39**, 1161-1165.
18. Besse, F., and Ephrussi, A. (2008) Translational control of localized mRNAs: restricting protein synthesis in space and time, *Nat. Rev. Mol. Cell Biol.*, **9**, 971-980.
19. Buxbaum, A. R., Haimovich, G., and Singer, R. H. (2015) In the right place at the right time: visualizing and understanding mRNA localization, *Nat. Rev. Mol. Cell Biol.*, **16**, 95-109.
20. Eliscovich, C., Buxbaum, A. R., Katz, Z. B., and Singer, R. H. (2013) mRNA on the move: the road to its biological destiny, *J. Biol. Chem.*, **288**, 20361-20368.
21. Loya, A., Pnueli, L., Yosefzon, Y., Wexler, Y., Ziv-Ukelson, M., and Arava, Y. (2008) The 3′-UTR mediates the cellular localization of an mRNA encoding a short plasma membrane protein, *RNA*, **14**, 1352-1365.
22. Pyhtila, B., Zheng, T., Lager, P. J., Keene, J. D., Reedy, M. C., and Nicchitta, C. V. (2008) Signal sequence- and translation-independent mRNA localization to the endoplasmic reticulum, *RNA*, **14**, 445-453.
23. Yosefzon, Y., Koh, Y. Y., Chritton, J. J., Lande, A., Leibovich, L., Barziv, L., Petzold, C., Yakhini, Z., Mandel-Gutfreund, Y., Wickens, M., and Arava, Y. (2011) Divergent RNA binding specificity of yeast Puf2p, *RNA*, **17**, 1479-1488.
24. Cui, X. A., Zhang, H., and Palazzo, A. F. (2012) p180 promotes the ribosome-independent localization of a subset of mRNA to the endoplasmic reticulum, *PLoS Biol.*, **10**, e1001336.
25. Kraut-Cohen, J., Afanasieva, E., Haim-Vilmovsky, L., Slobodin, B., Yosef, I., Bibi, E., and Gerst, J. E. (2013) Translation- and SRP-independent mRNA targeting to the endoplasmic reticulum in the yeast *Saccharomyces cerevisiae*, *Mol. Biol. Cell*, **24**, 3069-3084.
26. Lesnik, C., Golani-Armon, A., and Arava, Y. (2015) Localized translation near the mitochondrial outer membrane: an update, *RNA Biol.*, **12**, 801-809.
27. Corral-Debrinski, M., Blugeon, C., and Jacq, C. (2000) In yeast, the 3′-untranslated region or the presequence of ATM1 is required for the exclusive localization of its mRNA to the vicinity of mitochondria, *Mol. Cell. Biol.*, **20**, 7881-7892.
28. Marc, P., Margeot, A., Devaux, F., Blugeon, C., Corral-Debrinski, M., and Jacq, C. (2002) Genome-wide analysis of mRNAs targeted to yeast mitochondria, *EMBO Rep.*, **3**, 159-164.
29. Sylvestre, J., Vialette, S., Corral Debrinski, M., and Jacq, C. (2003) Long mRNAs coding for yeast mitochondrial proteins of prokaryotic origin preferentially localize to the vicinity of mitochondria, *Genome Biol.*, **4**, R44.
30. Eliyahu, E., Melamed, D., and Arava, Y. (2011) Genome-wide analysis of RNA extracted from isolated mitochondria, *Methods Mol. Biol.*, **714**, 287-299.
31. Karlberg, O., Canback, B., Kurland, C. G., and Andersson, S. G. (2000) The dual origin of the yeast mitochondrial proteome, *Yeast*, **17**, 170-187.
32. Eliyahu, E., Pnueli, L., Melamed, D., Scherrer, T., Gerber, A. P., Pines, O., Rapaport, D., and Arava, Y. (2010) Tom20 mediates localization of mRNAs to mitochondria in a translation-dependent manner, *Mol. Cell. Biol.*, **30**, 284-294.
33. Egea, G., Izquierdo, J. M., Ricart, J., San Martin, C., and Cuezva, J. M. (1997) mRNA encoding the beta-subunit of the mitochondrial F1-ATPase complex is a localized mRNA in rat hepatocytes, *Biochem. J.*, **322** (Pt. 2), 557-565.
34. Garcia, M., Darzacq, X., Delaveau, T., Jourdren, L., Singer, R. H., and Jacq, C. (2007) Mitochondria-associated yeast mRNAs and the biogenesis of molecular complexes, *Mol. Biol. Cell*, **18**, 362-368.
35. Gadir, N., Haim-Vilmovsky, L., Kraut-Cohen, J., and Gerst, J. E. (2011) Localization of mRNAs coding for mitochondrial proteins in the yeast *Saccharomyces cerevisiae*, *RNA*, **17**, 1551-1565.

36. Saint-Georges, Y., Garcia, M., Delaveau, T., Jourdren, L., Le Crom, S., Lemoine, S., Tanty, V., Devaux, F., and Jacq, C. (2008) Yeast mitochondrial biogenesis: a role for the PUF RNA-binding protein Puf3p in mRNA localization, *PLoS One*, **3**, e2293.
37. Eliyahu, E., Lesnik, C., and Arava, Y. (2012) The protein chaperone Ssa1 affects mRNA localization to the mitochondria, *FEBS Lett.*, **586**, 64-69.
38. Funfschilling, U., and Rospert, S. (1999) Nascent polypeptide-associated complex stimulates protein import into yeast mitochondria, *Mol. Biol. Cell*, **10**, 3289-3299.
39. George, R., Beddoe, T., Landl, K., and Lithgow, T. (1998) The yeast nascent polypeptide-associated complex initiates protein targeting to mitochondria *in vivo*, *Proc. Natl. Acad. Sci. USA*, **95**, 2296-2301.
40. Lesnik, C., Cohen, Y., Atir-Lande, A., Schuldiner, M., and Arava, Y. (2014) OM14 is a mitochondrial receptor for cytosolic ribosomes that supports co-translational import into mitochondria, *Nature Commun.*, **5**, 5711.
41. Garcia-Rodriguez, L. J., Gay, A. C., and Pon, L. A. (2007) Puf3p, a Pumilio family RNA binding protein, localizes to mitochondria and regulates mitochondrial biogenesis and motility in budding yeast, *J. Cell Biol.*, **176**, 197-207.
42. Gerber, A. P., Herschlag, D., and Brown, P. O. (2004) Extensive association of functionally and cytotopically related mRNAs with Puf family RNA-binding proteins in yeast, *PLoS Biol.*, **2**, E79.
43. Olivas, W., and Parker, R. (2000) The Puf3 protein is a transcript-specific regulator of mRNA degradation in yeast, *EMBO J.*, **19**, 6602-6611.
44. Miller, M. A., Russo, J., Fischer, A. D., Lopez Leban, F. A., and Olivas, W. M. (2014) Carbon source-dependent alteration of Puf3p activity mediates rapid changes in the stabilities of mRNAs involved in mitochondrial function, *Nucleic Acids Res.*, **42**, 3954-3970.
45. Walters, R., and Parker, R. (2014) Quality control: Is there quality control of localized mRNAs? *J. Cell Biol.*, **204**, 863-868.
46. Fujiki, M., and Verner, K. (1991) Coupling of protein synthesis and mitochondrial import in a homologous yeast *in vitro* system, *J. Biol. Chem.*, **266**, 6841-6847.
47. Fujiki, M., and Verner, K. (1993) Coupling of cytosolic protein synthesis and mitochondrial protein import in yeast. Evidence for cotranslational import *in vivo*, *J. Biol. Chem.*, **268**, 1914-1920.
48. Kellems, R. E., Allison, V. F., and Butow, R. A. (1974) Cytoplasmic type 80S ribosomes associated with yeast mitochondria. II. Evidence for the association of cytoplasmic ribosomes with the outer mitochondrial membrane *in situ*, *J. Biol. Chem.*, **249**, 3297-3303.
49. Kellems, R. E., Allison, V. F., and Butow, R. A. (1975) Cytoplasmic type 80S ribosomes associated with yeast mitochondria. IV. Attachment of ribosomes to the outer membrane of isolated mitochondria, *J. Cell Biol.*, **65**, 1-14.
50. Kellems, R. E., and Butow, R. A. (1974) Cytoplasmic type 80S ribosomes associated with yeast mitochondria. 3. Changes in the amount of bound ribosomes in response to changes in metabolic state, *J. Biol. Chem.*, **249**, 3304-3310.
51. Ni, L., Heard, T. S., and Weiner, H. (1999) *In vivo* mitochondrial import. A comparison of leader sequence charge and structural relationships with the *in vitro* model resulting in evidence for co-translational import, *J. Biol. Chem.*, **274**, 12685-12691.
52. Williams, C. C., Jan, C. H., and Weissman, J. S. (2014) Targeting and plasticity of mitochondrial proteins revealed by proximity-specific ribosome profiling, *Science*, **346**, 748-751.
53. Regev-Rudzki, N., and Pines, O. (2007) Eclipsed distribution: a phenomenon of dual targeting of protein and its significance, *BioEssays*, **29**, 772-782.
54. Knox, C., Sass, E., Neupert, W., and Pines, O. (1998) Import into mitochondria, folding and retrograde movement of fumarase in yeast, *J. Biol. Chem.*, **273**, 25587-25593.
55. Yogev, O., Karniely, S., and Pines, O. (2007) Translation-coupled translocation of yeast fumarase into mitochondria *in vivo*, *J. Biol. Chem.*, **282**, 29222-29229.
56. Ben-Menachem, R., Tal, M., Shadur, T., and Pines, O. (2011) A third of the yeast mitochondrial proteome is dual localized: a question of evolution, *Proteomics*, **11**, 4468-4476.
57. Jung, H., Yoon, B. C., and Holt, C. E. (2012) Axonal mRNA localization and local protein synthesis in nervous system assembly, maintenance and repair, *Nature Rev. Neurosci.*, **13**, 308-324.
58. Haag, C., Steuten, B., and Feldbrugge, M. (2015) Membrane-coupled mRNA trafficking in fungi, *Annu. Rev. Microbiol.*, **69**, 265-281.
59. Gehrke, S., Wu, Z., Klinkenberg, M., Sun, Y., Auburger, G., Guo, S., and Lu, B. (2015) PINK1 and Parkin control localized translation of respiratory chain component mRNAs on mitochondria outer membrane, *Cell Metab.*, **21**, 95-108.
60. Sen, A., and Cox, R. T. (2016) Clueless is a conserved ribonucleoprotein that binds the ribosome at the mitochondrial outer membrane, *Biol. Open*, **5**, 195-203.
61. Michaud, M., Ubrig, E., Filleur, S., Erhardt, M., Ephritikhine, G., Marechal-Drouard, L., and Duchene, A. M. (2014) Differential targeting of VDAC3 mRNA isoforms influences mitochondria morphology, *Proc. Natl. Acad. Sci. USA*, **111**, 8991-8996.