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Preview

A splicing factor's unexpected detour to the mitochondrial surface

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In this issue of *Molecular Cell*, Garcia et al.[1] reveal an unexpected role for the splicing factor U2AF in repressing translation and influencing the localization of nuclear-encoded mitochondrial mRNAs to the outer mitochondrial membrane.

Mitochondria depend on the import of nearly 1,500 nuclear-encoded proteins to carry out their essential functions; yet, how the mRNAs and proteins destined for the outer mitochondrial membrane (OMM) are targeted there remains a central unresolved question in cell biology and mitochondrial biogenesis.[2] In particular, the factors that localize mammalian nuclear-encoded mitochondrial (NE-mt) mRNAs to the OMM for the translation and eventual mitochondrial import of mitochondrial proteins are not well understood.[3] In contrast, molecular targeting to the endoplasmic reticulum is well characterized, with the signal recognition particle playing a critical role in co-translational targeting of proteins.[4]

Much progress has been made in our understanding of the post-translational recruitment of proteins to mitochondria, mediated by chaperones that keep the synthesized proteins in an unfolded state.[2] However, cytosolic ribosomes are found on the mitochondrial surface, and recent evidence has emerged revealing that co-translational targeting occurs as well for 20% of mitochondrially destined proteins.[5,6] Additionally, some mRNAs are localized to the OMM in a ribosome-independent manner, presumably through RNA-binding proteins (RBPs) that bind and recruit the mRNA.[7] Candidate RBPs have been proposed in this mechanism, yet direct evidence for regulating mRNA localization to the OMM exists only for a single factor and only for mRNAs with short coding sequences.[5,8] Therefore, how the timing and targeting of NE-mt mRNAs to the OMM are regulated, and by what factors, has remained a critical open question.

In this issue, Garcia et al.[1] explore these questions surrounding how mRNAs and proteins are targeted to the OMM and uncover an unlikely player: the heterodimeric U2AF1/U2AF2 splicing factors (referred to as U2AF here). They reveal an exciting and unexpected role for U2AF in the localization and translation of NE-mt mRNAs. Using a diverse set of tools, including biochemical assays, sequencing approaches, and advanced microscopy, Garcia et al.[1] provide compelling evidence that splicing factors mediate NE-mt mRNA and mitochondrial protein targeting to the OMM.

The authors previously demonstrated that U2AF interacts with hundreds of cytoplasmic mRNAs to repress their translation.[9] Here, they show that U2AF binding favors NE-mt mRNAs. Strikingly, binding occurs directly downstream of the encoded mitochondrial targeting sequence near the 5′ ends of NE-mt mRNAs. Notably, NE-mt mRNAs bound by U2AF were also found to be co-associated with eukaryotic translation initiation factor 3 (eIF3) complex subunits, suggesting these mRNAs are simultaneously regulated by U2AF and translation machinery.

Consistent with ribosome stalling and translation repression at sites of U2AF binding, the authors reveal an accumulation of back-to-back ribosomes (disomes) upstream of U2AF binding sites using ribosome profiling. Reporter assays both *in vitro* and in live cells confirm that U2AF-mediated translational repression of NE-mt mRNAs depends on intact binding sites and their proximity to the start codon, operating in a sequence- and position-dependent manner. Additionally, they demonstrate that the U2AF1 S34F mutation, which is recurrent in myelodysplastic syndromes (MDS), alleviates the block on translation, leading to a substantial increase in cytosolic translation and inefficient targeting of proteins to mitochondria.

Garcia et al.[1] also show that U2AF is found on the mitochondrial surface and associates with the outer membrane translocase subunits TOMM20/22 as well as translation machinery, including members of the eIF3 complex. Moreover, the S34F mutant that derepresses translation also reduces localization of NE-mt mRNAs to the mitochondrial surface. Thus, these data indicate that U2AF-mediated translational repression is coupled to the localization of both mRNAs and their encoded proteins to the OMM.

The authors' analysis of the MDS-associated S34F mutation underscores the clinical relevance of the findings. In both isogenic cells carrying S34F and primary MDS patient samples, the authors observe metabolic remodeling with an increased reliance on mitochondrial respiration. Consistent with the loss of U2AF-mediated repression, primary MDS patient samples also show increased basal translation and reduced NE-mt mRNA localization to mitochondria. These results raise the intriguing possibility that mitochondrial dysfunction in MDS stems not from canonical splicing defects but from the loss of U2AF's role in cytosolic gene regulation.

Thus, Garcia et al.[1] put forth a model in which U2AF functions as a subcellular navigator, shuttling from the nucleus to the

cytoplasm, where it represses translation of NE-mt mRNAs and influences their localization to the OMM. This study raises important mechanistic questions. In particular, how does U2AF mediate translational repression? What is the precise sequence of events linking U2AF-mediated translational repression of NE-mt mRNAs to their recruitment to the mitochondrial surface? Does translational repression serve to stall mitochondrial protein synthesis until proper localization to the mitochondrial surface has been achieved?

With a mammalian splicing factor shown to regulate NE-mt mRNA and protein localization to the OMM, additional RBPs and splicing factors may emerge as co-regulators with U2AF or regulators of a subset of NE-mt mRNAs. Indeed, SRSF proteins (serine/arginine-rich splicing factors) are enriched on the mitochondrial surface, including SRSF4, SRSF6, and SRSF9, suggesting that SRSF proteins may also play important roles in mitochondrial mRNA targeting.[1] A more complete mechanistic understanding of how targeting to mitochondria occurs may be within reach as unexpected players from the splicing machinery emerge as regulators at the mitochondrial surface.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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