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Review

Cytoplasmic organelles on the road to mRNA decay☆

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ABSTRACT

Localization of both mRNAs and mRNA decay factors to internal membranes of eukaryotic cells provides a means of coordinately regulating mRNAs with common functions as well as coupling organelle function to mRNA turnover. The classic mechanism of mRNA localization to membranes is the signal sequence-dependent targeting of mRNAs encoding membrane and secreted proteins to the cytoplasmic surface of the endoplasmic reticulum. More recently, however, mRNAs encoding proteins with cytosolic or nuclear functions have been found associated with various organelles, in many cases through unknown mechanisms. Furthermore, there are several types of RNA granules, many of which are sites of mRNA degradation; these are frequently found associated with membrane-bound organelles such as endosomes and mitochondria. In this review we summarize recent findings that link organelle function and mRNA localization to mRNA decay. This article is part of a Special Issue entitled: RNA Decay mechanisms.

1. Introduction

The ability of mRNA decay pathways to rapidly change gene expression patterns, and thus allow cells to respond effectively to various stimuli, is now well established. How degradation is regulated for an individual mRNA is thought to rely strongly on the identity of proteins and small regulatory RNAs bound to *cis*-elements within the mRNA sequence. The localization of an mRNA within the cell is also important in determining its susceptibility to decay pathways; many mRNAs displaying distinct localization patterns in early embryos, for example, rely on mRNA decay to limit expression in other regions of the cell. That the *cis*-elements and bound factors important for localization often overlap with those governing decay rates and translation suggests that these effects represent an inter-related network of regulatory influences.

There are several ways in which organelles can affect mRNA decay. For example, nuclear-encoded proteins that function in membrane-bound organelles such as the ER and mitochondria often cross these membranes cotranslationally, leading to localization of certain mRNAs to intracellular membranes. This partitioning of mRNAs presents an opportunity for coregulation, by localizing specialized decay components to these same membranes, and at least in the case of the ER, cells appear to have taken advantage of this opportunity.

Secondly, components of major mRNA decay pathways appear to associate with intracellular membranes. For example, mammalian Argonaute 2, a key protein in small noncoding RNA-mediated silencing, was originally identified as a peripheral membrane protein associated with the ER and the Golgi apparatus [1]. Furthermore, ribonucleoprotein granules associated with mRNA decay and translational repression are often found associated with organelles. These granules include processing bodies (P-bodies), which are protein and RNA aggregates of 300–500 nm diameter that are devoid of membrane and constitutively present in mammalian cells [2]. P-bodies contain all of the major proteins involved in the 5′–3′ mRNA decay pathway, including the exonuclease Xrn1, the decapping enzyme Dcp2 and its cofactors Dcp1, Lsm1–7, Lsm16, Hedls, Lsm14A, Pat1b and Rck/p54. They also contain translational repressors, such as the above Lsm14A, Pat1b and Rck/p54, 4ET and CPEB1. Finally, they contain miRNA and associated proteins, including Ago1–4 and GW182 proteins. P-bodies are therefore thought to play a role in mRNA decay and repression, including RNA silencing. Their specific importance, however, may vary depending on cellular conditions, as the composition and abundance of P-bodies differ between cell types and stages of the cell cycle [3,4]. A second type of ribonucleoprotein granule, stress granules, assemble in response to treatments that repress translation, such as arsenite, heat shock, UV, or following infection by some RNA viruses. They contain translationally silent mRNAs, proteins of the pre-initiation complex, and translational repressors, including RISC [5]. Interestingly, both P-bodies and stress granules appear to associate with specific organelles, as described below, perhaps coupling organelle activity with specific mRNA decay pathways and/or providing a membrane platform for the organization of complexes and granules that regulate decay.

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Thirdly, the activity and function of certain organelles appear to be required for certain mRNA decay pathways. For example, both mitochondrial activity and the ESCRT proteins that mediate formation of multivesicular bodies are critical for efficient miRNA-mediated silencing. In this review we discuss the various links between mRNA decay and organelles.

2. The endoplasmic reticulum and mRNA degradation

Many mRNAs encoding secreted or membrane proteins are targeted to the cytosolic face of the ER membrane for cotranslational insertion into the ER. This process partitions mRNAs whose encoded proteins function cytosolically or extracellularly, and provides a mechanism for regulating the degradation of ER-associated mRNAs during ER stress, when the ER is overloaded with incoming proteins and is not able to fold and process them efficiently. ER stress can result from pathological conditions that compromise ER function, or from normal processes that place a high burden on the secretory pathway, such as the differentiation and maintenance of professional secretory cells [6,7]. A network of signaling pathways emanating from the ER membrane controls the response to ER stress and is termed the unfolded protein response (UPR). One of the main sensors of ER stress, Ire1, is a highly conserved ER membrane protein with a cytosolic ribonuclease activity that is activated during stress [8,9] (Fig. 1A). This nuclease cleaves at two specific sites in the mRNA encoding Hac1 (in *S. cerevisiae*) or Xbp1 (in other organisms), which initiates a cytosolic splicing event that allows translation of this very potent transcription factor [10,11]. Ire1 in metazoans has a much broader substrate range, mediating the degradation of many other mRNAs associated with the ER membrane through a pathway termed regulated Ire1-dependent decay, or RIDD [12,13]. In *Drosophila* cells, susceptibility to RIDD correlates strongly with the localization of mRNA substrates [14], and mutating the signal sequences of ER-targeted mRNAs disrupts their ability to be degraded by this pathway [12]. The relatively fewer targets in mammalian cells may additionally require a stem–loop similar to the sites in Xbp1 that are recognized and cleaved by Ire1 [13–16]. Although the details of substrate selection may vary between organisms, localization to the ER appears to be a prerequisite, consistent with a model whereby Ire1 directly cleaves mRNAs leading to their degradation. Indeed, at least in *Drosophila* cells, substrates appear to be cleaved by an endonuclease, as both 3′ and 5′ decay intermediates can be detected when the exonucleases responsible for degrading cap-less and polyA tail-less RNA fragments are depleted [12].

The advantage of degrading mRNAs based mainly on their localization patterns in this case is straightforward to rationalize. The ER during stress is overburdened with the task of folding proteins; thus, directly limiting the proteins entering the ER, by degrading mRNAs actively engaged in translation at the ER membrane, would relieve this stress. Metazoans also limit the ER burden through a parallel pathway that attenuates translation during stress, by activation of an ER membrane-bound kinase, Perk, which phosphorylates the translation initiation factor eIF2alpha [17,18]. The RIDD pathway may have the additional advantage of sensitizing the cell to the vast alterations in transcription that are induced by ER stress: by increasing the turnover of many mRNA associated with the ER membrane, Ire1 increases the accessibility of the translocation and early processing machinery to proteins whose messages are transcriptionally induced during the ER stress response.

A recent study indicates that the UPR also functionally interacts with components of the nonsense-mediated decay pathway (NMD), which degrades mRNAs containing premature termination codons [19]. Although Ire1 is an essential gene in vertebrates, *C. elegans* lacking Ire1 are able to survive unless they also contain deletions of other UPR signaling molecules [20,21]. Sakaki et al. [19] conducted a screen to identify genes required for survival and development of Ire1 knockout worms, and found among their ten best hits two components of the exosome complex, which is responsible for 3′ to 5′ mRNA degradation during various processes including NMD. Probing the NMD pathway more specifically revealed that defects in *smg-1*, *smg-4*, and *smg-6* also displayed synthetic effects with Ire1 and caused ER stress. Furthermore, SMG-6, responsible for cleaving mRNAs near the premature stop codon in NMD targets, was also induced by ER stress, required for survival during stress, and localized to the ER. As NMD can limit protein misfolding by degrading mRNAs that would give rise to truncated proteins, it is perhaps not surprising that this quality control pathway can prevent or minimize ER stress. Although the mechanism and importance of NMD factors localizing to the ER have yet to be characterized, their presence there suggests that the ER may provide a hub for NMD-mediated quality control.

The partitioning of ER-bound *vs.* cytosolic mRNAs has effects on regulation of translation as well as decay. Nicchitta and colleagues have found that when translation is physiologically attenuated, the ER becomes a privileged site of translation. For example, during both ER stress [22] and viral infection [23], mRNAs in the cytosol are translationally repressed whereas mRNAs bound to the ER membrane continue translation, though at a reduced rate. Furthermore, ER-associated mRNAs may be protected from sequestration in stress granules [24]. For at least one mRNA, this protection is mediated by the transmembrane domains of its encoded protein, P-glycoprotein, and is correlated with localization of the mRNA to the ER [24].

Together these observations indicate that association of an mRNA with the ER has profound effects on both its translation and its degradation. While in general these effects may serve to coregulate mRNAs encoding proteins entering the secretory pathway, accumulating evidence suggests that other mRNAs are bound to the ER and may be subjected to ER-specific regulation as well. Several large-scale studies in which mRNAs were fractionated based on their association with membrane-bound *vs.* free ribosomes found that specific mRNAs encoding cytosolic and nuclear proteins were reproducibly found in membrane-bound fractions [25–27]. These findings supported the existence of non signal sequence-dependent mechanisms for targeting mRNAs to the ER. More recently, it has been shown that certain mRNAs encoding proteins with traditional signal sequences can also be targeted to the ER independently, when the signal sequence is disabled or translation is blocked [28,29]. While the mechanism(s) governing localization in these cases have not been fully described, the membrane protein p180 appears to be important in anchoring both bulk mRNA and certain transcripts to the ER [30]. The reasons for ER localization of mRNAs encoding non-secretory proteins are not clear, but may include local translation of proteins peripherally associated with the ER. More speculatively, these localization patterns may reflect an advantage to the cell of regulating certain cytosolic mRNAs coordinately with those that bind the ER in a more traditional manner.

3. Multivesicular bodies as organizing centers for RNA silencing

Multivesicular bodies (MVBs) are intermediates in the endosomal pathways that transport material between the cell surface and the lysosome [31]. The inward budding of intraluminal vesicles that gives rise to MVBs allows for the storage of proteins and lipids that are then degraded when MVBs fuse with lysosomes, or released from the cell as exosomes (an unfortunate homonym of the exosome complex involved in RNA decay) when MVBs fuse with the plasma membrane (Fig. 1B). Many recent studies have revealed important roles for these organelles in cell signaling and membrane protein sorting and degradation. The ESCRT proteins that are responsible for MVB biogenesis are also critical for other processes that rely on membrane invagination, including cytokinesis and viral budding.

Recent observations suggest that MVBs play a key role in miRNA-mediated silencing. Two groups have observed that the P-body-

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