Decoding ARE-mediated decay: is microRNA part of the equation?

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Messenger ribonucleic acids (mRNAs) containing adenine/uridine-rich elements (AREs) in their 3’ untranslated region are particularly labile, allowing for the regulation of expression for growth factors, oncogenes, and cytokines. The regulators, effectors, and location of ARE-mediated decay (AMD) have been investigated by many groups in recent years, and several links have been found between AMD and microRNA-mediated decay. We highlight these similarities, along with recent advances in the field of AMD, and also mention how there is still much left unknown surrounding this specialized mode of mRNA decay.

Introduction

One mechanism used by cells to prevent the overexpression of genes is to target their mRNA for decay. Although several destabilizing elements have been described to alter mRNA stability, the most widely studied is the adenine/uridine-rich element (ARE), which often includes a repeat of the AUUUA pentamer. AREs are found in the 3’ untranslated region of certain mRNAs and have been shown to severely decrease the stability of the mRNA in which they reside. The involvement of AREs in the decay of mRNA is described as ARE-mediated decay (AMD; Barreau et al., 2005). In recent years, many advances have been made in the field of AMD relating to its effectors, regulators, and location. The physiological significance of AREs and AMD has also been revealed through several studies (Gingerich et al., 2004) that underscore the importance of tightly regulating the AMD process.

Another form of mRNA degradation that has received much attention lately involves microRNAs (miRNAs). miRNAs are derived from short hairpin RNA fragments, which are processed through a specific pathway to yield oligomers complementary to specific messages. When these oligomers then interact with their targets, one of two outcomes is observed: either translation is repressed or the target mRNA molecule is sentenced to degradation (Filipowicz et al., 2008). Interestingly, recent studies have indicated that some players in the miRNA pathway may interact with and affect the fate of ARE-containing messages (Jing et al., 2005; Vasudevan et al., 2007). In this mini-review, we will highlight what is known regarding AMD, how the rapidly evolving miRNA field may tie in, and where recent work may lead. We will also mention areas of controversy that may complicate future directions.

Regulators and effectors: a roster for AMD

Given the fact that AMD allows control over protein expression, it is not surprising that the various ARE binding proteins (AUBPs) play entirely different roles in regulating the stability of ARE mRNAs (Bevilacqua et al., 2003). Some direct ARE mRNAs toward rapid decay by AMD (e.g. tristetraprolin [TTP]; Lai et al., 1999; Lykke-Andersen and Wagner, 2005), others increase the stability of their mRNA ligands (e.g. HuR; Brennan and Steitz, 2001), and still others may do both (e.g. AUF-1/hnRNP D; Barreau et al., 2005). A series of studies have also shown that certain AUBPs, such as TIA-1/TIAR and HuR, are capable of influencing mRNA translation (Barreau et al., 2005), and although it is not known if this activity of AUBPs is related to AMD, it certainly merits further attention.

Interestingly, most AUBPs have not yet been shown to be the direct executors of AMD but rather recruit and regulate effectors of this process (Table I, AUBPs). There exist only a limited number of known enzymes capable of degrading mRNA, and so it is not surprising that many of them have been linked to AMD (Table I, Degradation machineries; Chen et al., 2001; Gingerich et al., 2004; Parker and Sheth, 2007). The most prominent of these are the ribonucleases (RNases), of which there exist two types: exo- and endoribonucleases. The most common exoribonucleases, performing 3’ to 5’ degradation, exist in a large complex known as the exosome (Bousquet-Antonelli et al., 2000; Parker and Song, 2004). This complex, with various exonuclease subunits, also contains proteins that may be capable of binding directly to AREs. It was found that the subunits PM-Scl-75, OIP2, and RRP41 can specifically bind to AREs via their RNase PH domain (Mukherjee et al., 2002; Anderson et al., 2006).

Recent studies have demonstrated that in some cases, 5’ to 3’ mRNA decay is also significant (Stoecklkin et al., 2006). The major player responsible for this nonexosomal ribonuclease
Another class of endonucleases that has also received much attention lately is the Argonaute (AGO) proteins. These endonucleases are clearly linked to miRNA-mediated gene silencing, and growing evidence supports that this newfound pathway of gene expression regulation is somehow related to AMD.

**miRNA: the missing piece of the puzzle?**

miRNAs have been shown to influence gene expression both by modulating translation and by causing the degradation of target mRNAs, although it is uncertain if the latter of these effects is a consequence of the former (Filipowicz et al., 2008). miRNAs are typically found associated with various factors, which together form microRNPs (miRNPs). A core component of miRNPs is the AGO protein, which exists in various isoforms, some of which are capable of interfering with translation and of degrading mRNA by way of their endonuclease activity (Filipowicz et al., 2008; Wu and Belasco, 2008). It is intriguing to note that these two effects of miRNA mirror those linked to AUBPs, suggesting that perhaps the AMD and translational roles of AUBPs are mediated, or at least influenced, by miRNA.

AMD and miRNA-mediated decay involve some of the same players, such as the CCR4 deadenylase complex and the decapping enzymes Dcp1/2 (Behm-Ansment et al., 2006). Beyond this, a few important studies have actually shown interactions between the two processes. Jing et al. (2005) found that Dicer, a key player in the biogenesis of miRNAs, is a required component for the degradation via AMD of the ARE-containing message TNFα. They reported that miR16 targets a sequence located outside the 3′ untranslated regions of ARE mRNA (Yang et al., 2004a; Stoecklin et al., 2006), suggesting that perhaps the AMD and translational roles of AUBPs are mediated, or at least influenced, by miRNA.
Recent results from Vasudevan and Steitz (2007) further support this idea. They showed that a well-known AUBP, fragile X mental retardation–related protein 1 (FXR1), binds to the ARE of TNFα mRNA to promote translation during serum starvation in an AGO2-dependent manner (Vasudevan and Steitz, 2007). For translation of the ARE mRNA to increase, both FXR1 and AGO2 had to be present, showing that it is both the cellular environment and an interplay between AUBPs and miRNA factors that influence gene expression. A subsequent study demonstrated that this translational up-regulation depended on miR369-3 to bring FXR1 and AGO2 to the ARE and that miRNAs enable the transition between the repression and promotion of translation (Vasudevan et al., 2007). Collectively, these studies show that translation may be influenced by players associated with both AMD and miRNAs and that only through cooperation can the desired outcome be obtained.

If a known destabilizing AUBP, such as TTP, can assist the miRNA-mediated degradation of a target message, then it would be reasonable to speculate that a stabilizing AUBP, such as HuR, could interfere with miRNA binding. The idea of HuR interfering with the effects of miRNA is not unheard of. A recent study by Bhattacharyya et al. (2006) found that HuR was capable of rescuing translationally repressed mRNA, most likely by interfering with the association of miR122 with ARE mRNA. If TTP can assist a miRNA in carrying out decay, then having AUBPs either interfere with or support the translational effect of miRNA is just as likely. This supports the idea that RNA binding proteins and miRNAs may regulate each other’s effects by competing for binding or complementing the binding of one another (George and Tenenbaum, 2006). Recent results from Vasudevan and Steitz (2007) further support this idea. They showed that a well-known AUBP, fragile X mental retardation–related protein 1 (FXR1), binds to the ARE of TNFα mRNA to promote translation during serum starvation in an AGO2-dependent manner (Vasudevan and Steitz, 2007). For translation of the ARE mRNA to increase, both FXR1 and AGO2 had to be present, showing that it is both the cellular environment and an interplay between AUBPs and miRNA factors that influence gene expression. A subsequent study demonstrated that this translational up-regulation depended on miR369-3 to bring FXR1 and AGO2 to the ARE and that miRNAs enable the transition between the repression and promotion of translation (Vasudevan et al., 2007). Collectively, these studies show that translation may be influenced by players associated with both AMD and miRNAs and that only through cooperation can the desired outcome be obtained.

AMD and miRNA do more than share effectors of degradation. Their players have been shown to operate codependently, and the studies showing these relationships suggest that they work together in a variety of situations. Another commonality between these two involves where in the cell they carry out their roles, and, not surprisingly, uncertainty surrounds these details of AMD as well.

Figure 1. **Model for AMD.** Based on current literature, we propose three major pathways by which AMD is executed. In the first, AUBPs promoting degradation (e.g. TTP) may bind the ARE of the target mRNA and help recruit decapping enzymes such as Dcp1/2. After decapping, the 5’ to 3’ exoribonuclease Xrn1 may then carry out 5’ to 3’ decay. In the second, AUBPs may recruit endoribonucleases to internally cleave the target mRNA. Some data implicate miRNAs in this AUBP interaction, such as the miR16–AGO2–TTP complex (Jing et al., 2005). In the third, AUBPs may recruit deadenylases (such as PARN or CCR4) to remove the poly (A) tail from the 3’ terminus of the mRNA, and 3’ to 5’ degradation may then occur by way of the exosome. miRNPs complexes may also be involved in recruiting the machineries for this pathway. Stabilizing AUBPs, such as HuR, may be implicated in one or more of these pathways by competing with binding of destabilizing AUBPs or by preventing miRNA–mRNA interactions.
Common grounds

As mentioned earlier, the decapping enzyme complex has been linked to both AMD and miRNA function. It was noticed that these enzymes, along with several other factors that promote decapping, localized to cytoplasmic foci, and these have since been named processing bodies (PBs; Eulalio et al., 2007; Parker and Sheth, 2007). In addition, several mRNA degradation enzymes have been found to aggregate in another species of cytoplasmic granule, which specifically form under stressful conditions, named stress granules (SGs; Anderson and Kedersha, 2006). Both of these cytoplasmic bodies have raised interest because of their link to AMD and to miRNA-mediated effects, with the potential of being the specific loci where these processes are modulated.

PBs: into the lion’s den. The interaction between certain AUBPs and PB-associated proteins raised the possibility of a direct link between AMD and PBs. The most relevant of these binding proteins is TTP. TTP has been shown to interact with Dcp2 and other components of the decapping complex (Fenger-Grøn et al., 2005). TTP, as well as several AUBPs, has also been shown to associate with various other PB-associated factors, including Xrn1, CCR4 (Lykke-Andersen and Wagner, 2005; Hau et al., 2007), and the exosome (Chen et al., 2001), particularly the PM-Scl-75 subunit (Hau et al., 2007). These observations suggest that TTP and other AUBPs help recruit degradation factors to ARE mRNAs. This, and the result that exosome components can directly bind AREs (Mukherjee et al., 2002; Anderson and Kedersha, 2006), support the hypothesis of van Hoof and Kedersha (2006), that the exosome and, perhaps, other components of the decapping complex (Fenger-Grøn et al., 2007), particularly the exosome (Chen et al., 2001), particularly the PM-Scl-75 subunit (Hau et al., 2007). These observations have been named processing bodies (PBs; Eulalio et al., 2007; Parker and Sheth, 2007). Additionally, overexpressing TTP and BRF1 has been shown to cause stable interaction between PBs and SGs. These intriguing links between two cytoplasmic granules encourage speculation regarding their relationship. Is it possible that under stressful conditions, AUBP-bound mRNAs first localize to SGs and are then directed to PBs for decay? The PB–SG relationship has been further complicated after observations that the formation of PBs and SGs are independent (Kedersha et al., 2005). Moreover, some stresses that induce SG formation actually prevent both PB development and the decay of miRNA (Mazroui et al., 2007). These results make it difficult to develop an ambiguous model regarding the cellular location of AMD. Similar localization issues are also a problem in developing a unified model for miRNA-mediated repression (Filipowicz et al., 2008). When HuR rescues miRNA-repressed mRNA, it does so by causing the mRNA to leave PBs (Bhattacharyya et al., 2006). It was also reported that in the presence of miRNAs, AGO proteins are capable of dynamically associating with SGs, where these enzymes play a role in translation silencing but not in message decay (Leung et al., 2006). Although the compositions and proposed functions of PBs and SGs may differ, there is much evidence that they are both involved in both AMD and in miRNA-mediated repression.

Unsolved mysteries

As advances in the field of mRNA decay are made, it is apparent that AUBPs are a crucial component of AMD and that modification of AUBPs may regulate ARE mRNA decay (Stoecklin et al., 2004). At the same time, the localization of AMD-linked players is of great importance, and another potential complication in constructing a model for AMD is the possibility that the granules discussed are more complex than they appear. With the observations that the formation of SGs can be initiated in an eIF2α phosphorylation-dependent and -independent manner, it was proposed that different types of SGs may exist (Anderson and Kedersha, 2006; Mazroui et al., 2006). If true, then it is reasonable to hypothesize that different SGs can direct messages differently. This theory actually supports the various ways that SGs and PBs have been shown to interact. If there are various classes of SGs, then it would be reasonable to suspect that some support PB formation and AMD, whereas others promote...
alternative functions. What are taken to be a type of SG at this time could ultimately be shown to exist primarily for the purpose of reinitiating translation of mRNAs. Ultimately, SGs may be implicated in the balance a cell mediates between survival and death after stress, and differing granule classes and interactions may transiently exist as the cell gauges its fate (Mazroui et al., 2007). Similarly, although PBs and GW bodies were considered to be the same entity after their discovery, this conclusion may have been premature, as they may ultimately be distinct subsets of cytoplasmic bodies. It has been observed that GW bodies disappear with cell cycle arrest (Eulalio et al., 2007), whereas PBs remain (Vasudevan and Steitz, 2007). Moreover, AMD-linked players, such as the FXR1 and AGO2 members of the miRNA pathway, were originally thought to be components of PBs but have been shown to colocalize to GW bodies rather than Dcp1-containing cytoplasmic foci (Vasudevan and Steitz, 2007). This may explain why PBs could be seen in yeast even though they do not have an analogue of GW182 (Ding and Han, 2007). Even the recently proposed exosome granules, which may serve as a major site for AMD, could be distinct from PBs (Lin et al., 2007). Another important consideration is whether ARE mRNAs are brought to these preexisting cytoplasmic granules or whether a concentration of AMD-targeted mRNA, bound to its various factors, is necessary for the formation of SGs or PBs.

These discrepancies invite further investigation into the localization of AMD. Meanwhile, the relationship between AMD and miRNA-mediated decay warrants attention. Not only are there similarities among the processes but the colocalization of certain players strongly supports an underlying coordination. Ultimately, the details surrounding AMD, that is, the players involved in mediating such decay, the mechanism, the timing, the localization, and its regulation, leave much mystery regarding this process. Investigation into AMD has so far demonstrated quite well the validity of the adage that the more one knows, the more one learns is left unknown. 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