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, oncoproteins, 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 (Stoecklin et al., 2006). The major player responsible for this nonexosomal ribonuclease
activity is Xrn1 (Larimer and Stevens, 1990). Intriguingly, both Xrn1 and PM-Scl-75 have been shown to be essential for adequate AMD (Yang et al., 2004a; Stoecklin et al., 2006), suggesting that more than one pathway is being used by this process (Fig. 1). Regardless of the direction exonuclease cleavage occurs in, other factors, such as decapping enzymes and deadenylases, are also typically implicated, and these have also been shown to associate with AUBPs (Table I). Evidence has also pointed toward endonucleases being involved in cleaving ARE mRNA. GAP-SH3 binding protein and the erythroid cell–enriched endoribonuclease have actually been shown to target the 3′ untranslated regions of ARE mRNA (Wang and Kiledjian, 2000; Tourriere et al., 2001; Schoenberg, 2007), making them possible suspects in AMD.

Another class of endoribonucleases that has also received much attention lately is the Argonaute (AGO) proteins. These endoribonucleases 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-Ansmant 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 34-nt ARE region (Vasudevan and Steitz, 2007) that is needed for AMD of TNFα mRNA and that this miRNA indirectly associates with TTP through the AGO complex. The authors speculated that ARE recognition by TTP aids miR16 in binding to a target sequence and that the miR16-associated

### Table I. Key players of AMD and their link to miRNA

<table>
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<tr>
<th>AUBPs</th>
<th>Links to AMD</th>
<th>Links to miRNA</th>
<th>Major references</th>
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<tr>
<td>AUF-1/ hnRNP D</td>
<td>Various effects on ARE mRNA stability; associates with exosome components</td>
<td></td>
<td>Chen et al. (2001); Barreau et al. (2005)</td>
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<tr>
<td>BRF1</td>
<td>Destabilizes ARE mRNA; associates with many degradation enzymes</td>
<td></td>
<td>Lykke-Andersen and Wagner (2005)</td>
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<tr>
<td>HuR</td>
<td>Stabilizes ARE mRNA</td>
<td>Rescues miR122 translationally repressed ARE mRNA from PBs</td>
<td>Brennan and Steitz (2001); Bhattacharyya et al. (2006)</td>
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<tr>
<td>KSRP</td>
<td>Destabilizes ARE mRNA; associates with many degradation enzymes</td>
<td></td>
<td>Chen et al. (2001); Chou et al. (2006)</td>
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<tr>
<td>TTP</td>
<td>Destabilizes ARE mRNA; associates with many degradation enzymes</td>
<td>Binds miR16 and AGO2</td>
<td>Lai et al. (1999); Chen et al. (2001); Fengler-Gran et al. (2005); Ling et al. (2005); Lykke-Andersen and Wagner (2005); Hau et al. (2007)</td>
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Degradation machineries

| AGO2 | Associates with TTP | miRNA processing and many other roles | Ling et al. (2005) |
| CCR4 | Associates with TTP and BRF1 | Required for miRNA-mediated decay | Lykke-Andersen and Wagner (2005); Behm-Ansmant et al. (2006) |
| Decapping components | Associates with TTP and BRF1; required for AMD | Required for miRNA-mediated decay | Lykke-Andersen and Wagner (2005); Rehwinkel et al. (2005); Stoecklin et al. (2006); Behm-Ansmant et al. (2006); Lin et al. (2007) |
| Exosome | Certain subunits have been shown to associate with AREs directly and several subunits also bind to AUBPs; required for AMD | | Chen et al. (2001); Mukherjee et al. (2002); Lykke-Andersen and Wagner (2005); Anderson et al. (2006); Lin et al. (2007) |
| GW182 | | Required for miRNA-mediated decay | Rehwinkel et al. (2005); Filipowicz et al. (2008) |
| PARN | Associates with KSRP and is activated by TTP; required for AMD | | Lai et al. (2003); Chou et al. (2006); Lin et al. (2007) |
| Xrn1 | Associates with TTP and BRF1; required for AMD | | Lykke-Andersen and Wagner (2005); Stoecklin et al. (2006); Lin et al. (2007) |

This table lists the AUBPs (for a more thorough listing of the stabilizing and destabilizing roles of AUBPs, see Barreau et al. [2005]) and degradation enzymes that have been implicated in AMD. The links that these proteins have to AMD, as well as to miRNA-based processes, are highlighted, with select references (those underlined are in relation to miRNA and those not underlined are in relation to AMD).
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.

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.

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).

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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.
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., 2005).

AUBPs to interact with PB-associated decay enzymes, there is 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 pur-
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 similar aspects to the processes but the colocalization
of certain players strongly supports an underlying coordination.
Ultimately, the details surrounding AMD, that is, the players in-
volved 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. For a process linked to so
many evolving fields, there is little doubt that the existing facts
about AMD may drastically evolve, all the while bringing us

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