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Distinct mechanisms obviate the potentially toxic effects of inverted-repeat Alu elements on cellular RNA metabolism

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Abstract

RNA-binding proteins are shown in two independent studies to mediate distinct and beneficial effects to cells by binding to the extensive double-stranded RNA structures of inverted-repeat Alu elements. One study reports stress-induced export of ADAR1p110 to the cytoplasm where it binds inverted-repeat Alu elements so as to protect many mRNAs encoding anti-apoptotic proteins from degradation; the other demonstrates binding of the nuclear helicase DHX9 to inverted-repeat Alu elements embedded within RNAs to minimize defects in RNA processing.

Mammalian cells have evolved an innate immune, interferon-induced system that senses viral double-stranded RNAs (dsRNAs)¹. Activation of innate immunity induces antiviral gene expression, globally inhibits protein synthesis, degrades RNAs, and ultimately results in cell death. The transcriptional induction of antiviral genes is due to activation of toll-like receptors (TLRs), which recognize pathogen-associated molecular patterns^{2,3}, and the cytoplasmic helicases retinoic acid inducible gene I (RIG-I)^{4,5} and melanoma differentiation associated gene-5 (MDA5), which activate the mitochondrial antiviral signaling protein (MAVS)^{4,5}; the global inhibition of protein synthesis is mediated by activated protein kinase R (PKR)⁶; and the global decay of RNA degradation is mediated by activated 2′–5′-oligoadenylate synthetases, which produce 2′,5′-oligoadenylates that activate the nuclease RNase L⁷. How cells protect themselves from endogenous dsRNA structures that could potentially activate a toxic antiviral response remains a long-standing question. Two recent studies describe distinct mechanisms whereby human cells obviate the potentially deleterious immunostimulatory activities of endogenous dsRNAs and maintain cellular homeostasis^{8,9}.

In this issue of *Nature Structural & Molecular Biology*, Sakurai and co-workers report that the 110-kDa isoform of the adenosine deaminase acting on RNA 1 protein (ADAR1p110) suppresses apoptosis, a type of programmed cell death, when A172 human brain glioblastoma cells are UV-stressed⁹. ADAR proteins bind dsRNAs and convert adenosines to inosines in a process called A-to-I editing¹⁰ (Figure 1). Because the translational machinery reads inosines as guanosines, editing within coding regions results in recoding and protein diversification¹⁰. In human cells, most A-to-I editing occurs within Alu elements residing in non-coding regions, i.e. introns and 3′-untranslated regions (3′ UTRs)¹¹. ADARs target primarily inverted-repeat Alu elements (*IRAlus*) that form the imperfectly base-paired stems of stem-loop structures in order to destabilize them (Figure 1).

Expressing mCherry fusions of ADAR1 isoforms, Sakurai and co-workers confirmed previous reports that ADAR1p110 is localized almost exclusively in the nucleus, while ADAR1p150 is localized mainly in the cytoplasm. Exposing A172 cells to UV irradiation or heat shock induced the export of nuclear ADAR1p110 to the cytoplasm (Figure 2). Considering that the cellular localization of proteins can be altered by phosphorylation, and MAP kinase activating pathways are known to be upregulated in response to stress¹², tests of various constitutively activated kinases revealed that mitogen-activated protein kinase kinase 6 (MKK6) and its target p38 kinase were responsible for the UV-induced cytoplasmic relocalization of ADAR1p110. MKK6-mediated phosphorylation of p38 results in activated p38 transport to the nucleus where it phosphorylates mitogen- and stress-activated protein kinase 1 (MSK1) and MSK2, which the authors showed phosphorylates nuclear ADAR1p110, inducing its export to the cytoplasm. Consistent with this model, inhibiting p38 or the simultaneous knockdown of p38 substrates MSK1 and MSK2 blocked UV-induced relocalization of ADAR1p110 to the cytoplasm, and phos-tag western blotting demonstrated that phosphorylation of ADAR1p110 detected in stressed cells is abolished by either a p38 inhibitor or knockdown of MSK1 and MSK2. Mutating two threonine and three serine residues in FLAG-ADAR1p110 identified to be phosphorylated by mass spectrometry abolished UV-induced relocalization of ADAR1p110 to the cytoplasm, indicating that stress-induced phosphorylation of ADAR1p110 promotes its export to the cytoplasm. The export of nuclear ADAR1p110 is already known to be mediated by exportin-5¹³, and exportin-5 knockdown experiments and *in vitro*-binding assays demonstrated that phosphorylation induced the nuclear export of ADAR1p110 by enhancing its interaction with exportin-5.

Since *in vitro* assays revealed that ADAR1p110 phosphorylation did not alter its A-to-I editing or pre-miRNA processing activities, to address the biological significance of stress-induced cytoplasmic relocalization of ADAR1p110, the authors investigated the possibility that ADAR1p110 plays roles in apoptosis given that p38 signaling is known to promote cell death. Sakurai and co-workers showed that knockdown of ADARp110 increased UV-induced apoptosis in either A172 cells or U2OS osteocarcinoma cells, and expressing either wild-type or a A-to-I deamination-defective derivative of FLAG-ADAR1p110 reversed this effect. Thus, the authors concluded that the ability of ADARp110 to suppress stress-induced apoptosis is not attributable to its A-to-I editing activity, leaving the possibility that suppression is due to its RNA-binding activity.

Profiling global mRNA expression using RNA-seq identified several transcripts whose levels in UV-stressed A172 cells were significantly reduced upon ADAR1 knockdown. Most of these transcripts were found to harbor 3'UTR *IRA/us*. No significant upregulation in expression of interferon-induced genes was detected, indicating that the protective roles of ADAR1 are independent of innate immunity-activating dsRNA-sensing mechanisms. *IRA/us* have been previously reported to bind dsRNA-binding proteins in addition to ADARs, including the nuclear paraspeckle constituent p54nrb, nuclear and cytoplasmic Staufen 1 (STAU1), and cytoplasmic PKR¹⁴. By out-competing p54nrb binding, nuclear STAU1 binding to at least some 3'UTR *IRA/us* promotes the export of host mRNAs to the cytoplasm, and by outcompeting cytoplasmic PRK, cytoplasmic STAU1 promotes the translation of host mRNAs^{11,14}. Additionally, STAU1 binding to 3'UTR dsRNAs, including those formed by Alu elements, can promote STAU1-mediated mRNA decay (SMD)¹⁵.

Sakurai and co-workers found that STAU1 mediates the degradation of several 3'UTR *IRAlus*-containing mRNAs that encode anti-apoptotic proteins. Moreover, they found that the abrupt increase in the levels of cytoplasmic ADAR1p110 upon exposing cells to UV stress allowed ADAR1p110 to compete with STAU1 for binding to 3'UTR *IRAlus* so as to inhibit SMD, thereby explaining the observed anti-apoptotic effects of cytoplasmic ADAR1p110. Consistent with this, RNA-seq data demonstrated that simultaneous knockdown of STAU1 and ADAR1p110 restored the levels of hundreds of 3'UTR *IRAlus* mRNAs. Consistent with the dependence of SMD on the ATP-dependent helicase UPF1¹⁵, RT-qPCR analyses of select SMD targets revealed that UPF1 knockdown recapitulated STAU1 knockdown, providing evidence that stress-induced cytoplasmic relocalization of ADAR1p110 inhibits SMD rather than other functions of STAU1.

In the second study, Aktas. and co-workers aimed to define the role of the abundant nuclear helicase DHX9, which has a domain organization resembling that of ADAR and PKR. Using two different UV-crosslinking techniques – uvCLAP (UV-crosslinking and affinity purification) and FLASH (fast ligation of RNA after some sort of affinity purification for high-throughput sequencing) – Aktas and co-workers demonstrated that endogenous DHX9 as well as exogenously expressed FLAG-DHX9 in both human and mouse cells bind primarily intronic RNA, in particular intronic *Alu* elements in human cells (Figure 3) and the independently evolved B1 elements in mouse cells⁸. The genomic similarity search tool YASS revealed that DHX9-bound *Alu* elements have more potential to form *IRAlus* than do *Alu* elements that were not bound by DHX9, suggesting that DHX9 specifically targets *IRAlus*. DHX9 knockdown resulted in a global increase in the number of circular RNAs (circRNAs) produced by pre-mRNA back-splicing, an RNA processing defect resulting from the presence of *IRAlus* in flanking introns^{16,17} (Figure 3), indicating a protective role of DHX9.

To determine if DHX9 also influences 3'UTR *IRAlus*-containing transcripts, the authors showed that DHX9 enhances luciferase activity deriving from transcripts harboring 3'UTR *IRAlus* in a way that depends on its helicase activity. They concluded that DHX9 resolvase activity de-represses luciferase mRNA translation.

DHX9 knockdown was shown to generally disrupt normal RNA biogenesis, altering the splicing pattern of more than 9000 exons. DHX9 knockdown also upregulated expression of the gene encoding chemokine (C-C motif) ligand 25 (CCL25), which the authors attribute to defective pre-mRNA splicing and/or transcription termination of the DHX9-bound transcript encoding embryonic lethal, abnormal vision 1/human antigen R (ELAVL1) that is antisense to CCL25 RNA. Live-cell imaging revealed that DHX9 knockdown also resulted in giant multinucleated cells having small pleomorphic nuclei because chromosomes failed to align at metaphase. In explanation of this phenotype, 3'UTR *IRAlus*-containing mRNA encoding SPC24, a component of the Ndc80 complex that forms an essential part of the kinetochore, was downregulated due to the aberrant splicing. Expression of a splicing-independent and 3'UTR *IRAlus*-lacking allele of SPC24 did not require DHX9, indicating that *Alu* elements interfere with SPC24 gene expression in the absence of DHX9.

SILAC (stable isotope labelling with amino acids in cell culture)-based immunoprecipitations revealed that DHX9 interacts with ADAR1p150 even in the presence of RNase, suggesting that the interaction augments tolerance of cells to their many *IRA/Us*. Consistent with this suggestion, cells depleted for both DHX9 and ADAR accumulated dsRNAs and circRNAs. While DHX9-depleted cells manifested only a minor increase in editing, the appearance of J2 monoclonal antibody-positive speckles, which provide a measure of dsRNA structures, is consistent with the view that DHX9 functions as a dsRNA resolvase to prevent the various RNA processing defects that result from *IRA/Us*.

In conclusion, these two new studies uncover mechanisms by which ADARp110 and DHX9 allow mammalian cells to tolerate retrotransposon-derived dsRNAs, including human Alu and rodent B1 elements, allowing these elements to colonize genomes and thereby contribute to post-transcriptional gene control.

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Figure 1.

ADAR mediates A-to-I editing so as to destabilize cellular double-stranded RNA structures. Editing in human cells occurs largely within the > 1 million Alu elements, particularly within the double-stranded (dsRNA) structures formed by inverted-repeat Alu elements (*IRAlus*). Editing prevents recognition of endogenous dsRNAs as non-self, e.g. as infecting viruses, by inhibiting the binding of dsRNA sensors that include TLRs, RIG-I, MDA5 and PKR, which would otherwise trigger an innate immune response.

Figure 2.

Export of nuclear ADAR1p110 to the cytoplasm protects cells from apoptosis triggered by 3' UTR inverted-repeat Alu elements. Exposure of human cells to UV-irradiation, which is a form of stress, activates the MKK6-p38-MSK1/2 pathway. Ensuing phosphorylation of the largely nuclear ADAR1 isoform ADAR1p110 promotes its export to the cytoplasm, where it binds and stabilizes a number of 3' UTR inverted-repeat Alu element (*IRAlus*)-containing mRNAs that encode anti-apoptotic proteins. Binding confers stability by outcompeting STAU1 binding, thereby inhibiting STAU1-mediated mRNA decay (SMD).

Figure 3. The nuclear helicase DHX9 binds intronic inverted-repeat Alu elements to suppress defective RNA processing. Intronic inverted-repeat *Alu* elements (*IRAlus*) promote RNA back-splicing, resulting in defective RNA processing and circRNA production. To protect against this, DHX9 binds the intronic *IRAlus* within nuclei, acting as dsRNA resolvase to inhibit circRNA formation and promote productive RNA processing.