PERSPECTIVE

CARMing down the SINEs of anarchy: two paths to freedom from paraspeckle detention

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A subset of messenger RNAs (mRNAs) that contain inverted Alu elements in their 3′ untranslated region are inefficiently exported to the cytoplasm and retained in subnuclear bodies called paraspeckles. The arginine methyltransferase CARM1 (coactivator-associated arginine methyltransferase 1) promotes the nuclear export of these mRNAs by methylating the paraspeckle component p54^{nrb}, which reduces the binding of p54^{nrb} to the inverted Alu elements, and down-regulating synthesis of another paraspeckle component, the long noncoding RNA *NEAT1*, which inhibits paraspeckle formation.

To serve as templates for protein synthesis, messenger RNAs (mRNAs) must be exported from the nucleus in which they are synthesized to the cytoplasm, where their translation occurs. In many human cell types, however, a subset of mRNAs is retained within nuclei and therefore is only inefficiently translated. One feature common to a number of these retained mRNAs is a 3′ untranslated region (UTR) inverted repeat of *Alu* elements (IR*Alu*s) that forms a hairpin structure containing an imperfectly base-paired stem as long as 300 nucleotides. *Alu* elements are primate-specific short interspersed elements (SINEs). The degree to which different 3′ UTR IR*Alu*s-containing mRNAs (IR*Alu*s mRNAs) are retained within nuclei varies depending on the particular mRNA and the cell type in which it is expressed. In addition to the widely used process of alternative RNA 3′ end formation that can determine whether a transcript contains a 3′ UTR IR*Alu*s, regulatory mechanisms responsible for variability in the nuclear retention of IR*Alu*s mRNAs exist but are incompletely understood. In the March 15, 2015, issue of *Genes & Development*, Hu et al. (2015) revealed that the nuclear export of IR*Alu*s mRNAs that can enter nuclear paraspeckles is regulated by a multifunctional arginine methylase.

Paraspeckles are subnuclear sites of IR*Alu*s mRNA retention (Chen et al. 2008). These nonmembrane-delimited bodies are built around the long noncoding RNA *NEAT1* (which is essential for paraspeckle integrity) and contain multiple RNA-binding proteins that include non-POU domain-containing octamer binding (p54^{nrb} or NonO), p54^{nrb}-associated splicing factor proline/glutamine-rich (PSF or SFPQ), and paraspeckle component 1 (PSPC1) (Fox et al. 2002; Prasanth et al. 2005). In search of new substrates for the coactivator-associated arginine methyltransferase 1 (CARM1; also called PRMT4), Hu et al. (2015) used mass spectrometry and identified p54^{nrb} in acid-extracted histones from the nuclei of CARM1$^{-/-}$ mouse embryonic fibroblasts as a substrate for in vitro methylation by *Escherichia coli*-produced GST-tagged CARM1. Using protein immunostaining and RNA in situ hybridization, p54^{nrb} and CARM1 were subsequently shown to colocalize within HeLa cell nuclei largely to *NEAT1*-defined paraspeckles. CARM1-mediated methylation within and near the coiled-coil domain of p54^{nrb} reduced p54^{nrb} binding to dsRNAs in vitro and to 3′ UTR IR*Alu*s reporter and cellular mRNAs in HeLa cells. Additionally, replacing endogenous CARM1 with a catalytically inactive variant or replacing endogenous p54^{nrb} with a variant in which three sites of CARM1-mediated methylation were mutated from arginine to lysine enhanced the retention of 3′ UTR IR*Alu*s reporter and cellular mRNAs within nuclei.

Remarkably, a second path by which CARM1 mediates the retention of reporter and cellular IR*Alu*s mRNAs in paraspeckles was uncovered when the investigators pursued evidence from others that CARM1 associates with different classes of transcription factors. Assays of the abundance of *NEAT1* in CARM1 knockdown HeLa cells revealed abnormally more *NEAT1* and paraspeckles, and chromatin immunoprecipitations demonstrated that CARM1 is enriched at the *NEAT*1 promoter. Promoter-

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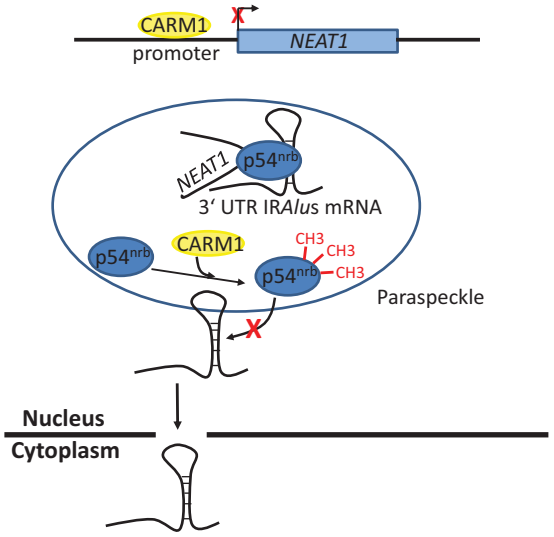

Figure 1. CARM1 promotes the nuclear export of 3′ UTR *IRAlu*s mRNAs by two mechanisms. The arginine methylase CARM1 enhances the nuclear export of a subset of 3′ UTR IR*Alu*s mRNAs that are retained in nuclear paraspeckles by methylating the coiled-coil domain of the paraspeckle component $p54^{nrb}$, which reduces the binding of $p54^{nrb}$ to 3′ UTR *IRAlu*s mRNAs. Additionally CARM1 binding to the promoter of the *NEAT1* gene somehow down-regulates synthesis of the paraspeckle long noncoding RNA *NEAT1,* which reduces paraspeckle formation.

bound CARM1 down-regulated *NEAT1* gene transcription so as to reduce the number of paraspeckles and thereby promote IR*Alu*s mRNA export to the cytoplasm. Thus, CARM1 "frees" IR*Alu*s mRNAs from paraspeckle "detention" via two paths: methylating $p54^{nrb}$ and down-regulating *NEAT1* gene transcription (Fig. 1). Stimulating *NEAT1* synthesis and paraspeckle formation by exposing HeLa cells to poly(I:C), which binds to toll-like receptor 3 and induces an antiviral response (Imamura et al. 2014), was accompanied by reduced CARM1 binding to the *NEAT1* promoter. In addition, poly(I:C) treatment reduced CARM1 localization to paraspeckles so as to decrease $p54^{nrb}$ methylation. As a consequence, poly(I:C) enhanced the nuclear retention of IR*Alu*s mRNA. The mechanism by which poly(I:C) influences cellular CARM1 localization and function remains to be studied.

Data presented by Hu et al. (2015) raise many interesting questions. As examples, what is the structural explanation for the finding that CARM1-mediated methylation of $p54^{nrb}$ inhibits $p54^{nrb}$ binding to dsRNA, and how does CARM1 down-regulate *NEAT1* synthesis? Another conundrum is why different transcripts with seemingly similar 3′ UTR IR*Alu*s manifest distinctly different subcellular localizations whereby some are efficiently retained in nuclear paraspeckles while others are largely cytoplasmic and either translationally active or translationally silent (Chen et al. 2008; Hundley and Bass 2010; Capshew et al. 2012; Fitzpatrick and Huang 2012; Elbarbary et al. 2013). Competition among the various dsRNA-binding proteins that differentially affect the metabolism of different IR*Alu*s mRNAs undoubtedly plays a role in regulating IR*Alu*s mRNAs on top of which might be layered yet to be discovered post-translational modifications that alter the affinities of dsRNA-binding proteins for IR*Alu*s. For example, it is already known that IR*Alu*s are major binding sites for the dsRNA-binding protein Staufen 1 (STAU1) (Elbarbary et al. 2013; Ricci et al. 2014). When STAU1 outcompetes $p54^{nrb}$ binding to one class of 3′ UTR IR*Alu*s, then nuclear retention of the affected IR*Alu*s mRNAs is inhibited, and their export to the cytoplasmic is enhanced (Elbarbary et al. 2013). In the cytoplasm, 3′ UTR IR*Alu*s have the potential to bind and activate another dsRNA-binding protein, the translational inhibitor protein kinase R (PKR), making 3′ UTR IR*Alu*s negative regulators of mRNA translation both in *cis* and in *trans* (Elbarbary et al. 2013; Kim et al. 2014). STAU1 also competes with PKR for binding to 3′ UTR IR*Alu*s so as to alleviate translational repression (Elbarbary et al. 2013). The segregation of nuclear-retained IR*Alu*s mRNAs away from cytoplasmic PKR during interphase complements the binding of STAU1 to preclude PKR binding to 3′ UTR IR*Alu*s that would otherwise inhibit cellular translation (Elbarbary et al. 2013; Kim et al. 2014). In contrast, dissolution of the nuclear membrane during mitosis removes the boundaries between nuclear-retained IR*Alu*s mRNAs and cytoplasmic PKR, resulting in PKR activation that is necessary for regulated mitosis (Kim et al. 2014).

In summary, genes harboring the potential to encode one or more mRNA isoforms that contain a 3′ UTR IR*Alu*s can be regulated by specialized mechanisms that depend on the efficiencies with which their product mRNAs are retained within the nucleus versus exported to the cytoplasm with or without the concomitant possibility of their translation. These efficiencies, which vary during mitosis and undoubtedly in other cellular states as a means of maintaining cellular homeostasis, are dictated by the potential binding of multiple proteins to 3′ UTR IR*Alu*s within both the nucleus and the cytoplasm. Binding is likely to be regulated by post-translational modifications beyond the CARM1-mediated methylation of $p54^{nrb}$ described by Hu et al. (2015). Also, while IR*Alu*s are known to be major sites of A-to-I editing and while A-to-I editing does not appear to regulate $p54^{nrb}$ or STAU1 binding to those IR*Alu*s studied (Elbarbary et al. 2013), editing may influence the binding of other IR*Alu*s factors. It is clear that there is still much to learn about how IR*Alu*s influence mRNA metabolism.

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