

A Nuclear RNA Is Cut out for Translation

In this issue of *Cell*, Prasanth et al. (2005) provide evidence that an inosine-containing RNA that is normally retained in the nucleus is cleaved within its 3' untranslated region following cellular stress. It is then transported to the cytoplasm and translated into protein. These findings suggest that the nucleus may store RNAs destined for translation that then can be released, as needed, in response to specific cellular signals.

Repeat sequences are a ubiquitous feature of genomes. Some of the repetitive sequences in DNA are transcribed and are found in heterogeneous nuclear RNA (hnRNA) but rarely in cytoplasmic mRNA (Jelinek and Darnell, 1972). Although it has been largely forgotten that multiple repeats often occur within a single hnRNA—presumably to allow intramolecular pairing—a recent bioinformatics study reminds us that 4%–8% of the protein-coding genes of the human genome produce RNAs with complementary repeats in their untranslated regions (UTRs) and introns (Levanon et al., 2004). So why, after all these years, do we not know the function of double-stranded regions in nuclear RNA? Have we assumed that if these RNAs never reach the cytoplasm, they are of no consequence?

In their research article in this issue, Spector and colleagues (Prasanth et al., 2005) monitor the fate of a nuclear RNA with complementary repeats and show that it reaches the cytoplasm following cleavage in its 3' UTR. They identify the 8 kb transcript of the mouse *CAT2* (cationic amino acid transporter 2) gene—called *CAT2* transcribed nuclear RNA (CTN-RNA)—in interchromatin granules of mouse nuclei. Through the use of an alternate promoter and polyadenylation site, the mouse *CAT2* gene also yields a 4.2 kb transcript (*mCAT2* mRNA). CTN-RNA and *mCAT2* mRNA differ only in their 5' UTRs and the existence of an additional 4.5 kb of 3' UTR sequence unique to CTN-RNA (Figure 1A). The difference in the sequence and fate of these two RNAs forms the basis of the story.

Although both transcripts contain a “forward” short interspersed nucleotide element (SINE) repeat in their 3' UTR (brown), CTN-RNA contains three additional repeat elements in “reverse” orientation to allow base pairing (yellow) (Figures 1A and 1B). The shorter *mCAT2* mRNA goes to the cytoplasm in order to produce *mCAT2* protein (Figure 1B), a cell-surface receptor for L-arginine, which is used to synthesize nitric oxide in response to stress. In contrast, CTN-RNA is usually retained in the nucleus. However, Spector and colleagues show that, under stress conditions, the 3' UTR extension of CTN-RNA is removed by cleavage, which allows the RNA to enter the cytoplasm for translation (Figures 1B and 1C). These data raise the exciting possibility

that the nucleus contains a reservoir of translation-competent mRNAs that are released as needed.

The data presented by Prasanth et al. (2005) clearly establish that the extended 3' UTR of CTN-RNA is important for nuclear retention. Experiments using RNA fluorescence in situ hybridization show that full-length CTN-RNA localizes to the nucleus, whereas a construct encoding CTN-RNA without the 3' UTR produces a transcript that is exported. Fusion of the 3' UTR of CTN-RNA, but not that of *mCAT2* mRNA, to a reporter open reading frame prevents translation of the reporter.

But what features of the 3' UTR are important for the nuclear retention of CTN-RNA? One obvious feature is the inverted repeats that are likely to form regions of double-stranded RNA (dsRNA). Nuclear dsRNA is a target for RNA-editing enzymes known as adenosine deaminases that act on RNA (ADARs), which convert adenosine residues in dsRNA to inosine (Bass, 2002). Indeed, a sequence analysis indicates that the forward repeat and the reverse repeat 2 (see Figure 1, middle inverted repeat) have ADAR editing sites, confirming that these sequences are base paired in vivo. In contrast, editing is not observed in the *mCAT2* mRNA “forward” repeat, consistent with the fact that it does not have a complementary repeat to pair with.

Intriguingly, there is a precedent for nuclear retention through RNA editing. For example, ADARs target the dsRNA that forms between early and late transcripts of polyoma virus, leading to nuclear retention and down-regulation of early transcripts (Kumar and Carmichael, 1997). Similar experiments with transcripts designed to form dsRNA show that nuclear retention occurs by interaction with a protein complex containing p54^{nrb} (a multifunctional nuclear RNA binding protein), PSF (a splicing factor), and matrin 3 (a structural protein of the inner nuclear matrix) (Zhang and Carmichael, 2001). In support of the idea that RNA editing leads to the nuclear retention of CTN-RNA, Prasanth et al. (2005) show that p54^{nrb} colocalizes and coimmunoprecipitates with CTN-RNA.

Although inosine-mediated nuclear retention of CTN-RNA is an intriguing model, it has not yet been tested directly, which would require assessing CTN-RNA localization in cells lacking ADAR activity. A further caveat to the model presented by Prasanth et al. (2005) is that some RNAs containing inosine residues in codons clearly make it to the cytoplasm and are translated (for examples, see Bass, 2002). In addition, although Carmichael's work shows that inosine in single- or double-stranded RNA leads to nuclear retention of RNA (Zhang and Carmichael, 2001), it is unclear whether dsRNA, or the repeats themselves, in the absence of inosine, can lead to nuclear retention. Thus, it remains possible that retention of CTN-RNA in the nucleus results from the presence of inverted repeats and is not necessarily a consequence of RNA editing. In this regard, further dissection of CTN-RNA will be necessary to confirm that it is the repeats, rather than some other part of the 3' UTR, that are important for nuclear retention.

The *mCAT2* protein is important for the cellular stress

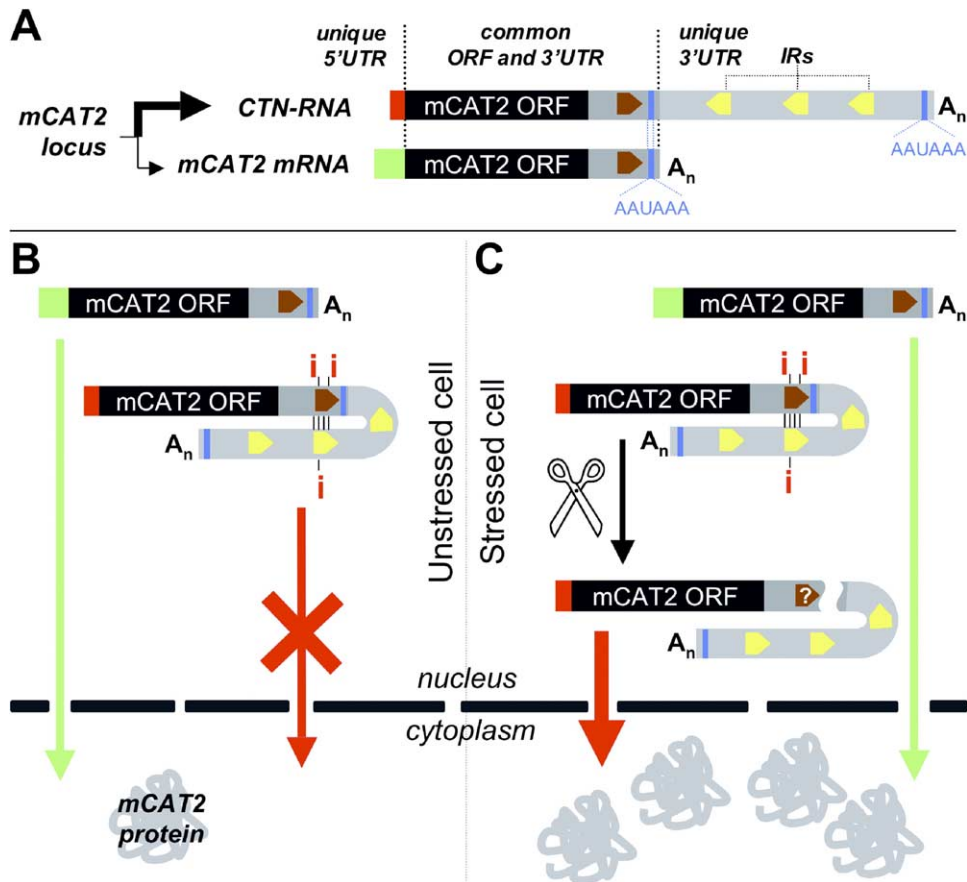


Figure 1. The Different Fates of mCAT2 Transcripts

(A) Two polyadenylated (A_n) transcripts of the *mCAT2* locus are shown. The more abundant CTN-RNA and the *mCAT2* mRNA differ only in their 5' UTRs (orange and green, respectively) and an extended 3' UTR unique to CTN-RNA. The proximal and distal polyadenylation sites are indicated (blue) as well as the forward (brown) and reverse inverted repeats (IR, yellow).

(B and C) In both unstressed (B) and stressed (C) cells, the *mCAT2* mRNA is exported to the cytoplasm and translated to yield mCAT2 protein (gray). In contrast, CTN-RNA is retained in the nucleus in unstressed cells but cleaved in stressed cells to yield a transcript that is exported and translated. Inosine residues from RNA editing are indicated (orange "i"); the question mark signifies that the cleavage site has not been mapped, so it is possible that the forward repeat is in the other fragment.

response, and so the authors wondered whether the more abundant CTN-RNA might enter the cytoplasm and become translated in times of stress. Indeed, in situ hybridization revealed a movement of nuclear RNA into the cytoplasm in response to stress. Furthermore, Northern blot analyses using probes unique to the 5' UTRs for each transcript showed that the increase in cytoplasmic transcript was due to an increase in a 4.2 kb RNA that contained the CTN-RNA 5' UTR. In addition, a reporter open reading frame fused to the CTN-RNA 3' UTR was only translated under stress conditions.

These data are entirely consistent with the authors' proposal that the stress-induced cytoplasmic transcript arises via posttranscriptional cleavage of nuclear-retained CTN-RNA (Figure 1C). Furthermore, using transcription inhibitors, the authors ruled out the possibility that the 4.2 kb CTN-RNA results from new transcription or cotranscriptional processes such as splicing. However, the exact site of cleavage within the common 3' UTR was not mapped. This information would have bol-

stered the evidence for cleavage and would have informed its mechanism.

Given that the long form of CTN-RNA retains the proximal poly(A) site (Figure 1A) and that cleavage at this site produces a 4.2 kb RNA, it is tempting to propose that stress induces the cleavage/polyadenylation machinery to revisit CTN-RNA. This model for "post-transcriptional cleavage/polyadenylation" requires the existence of a factor that blocks the use of the proximal polyadenylation site of CTN-RNA in unstressed cells. Upon exposure to stress, the factor would be degraded, allowing the cleavage/polyadenylation machinery access. This model would explain how the shorter CTN-RNA is generated, as well as how the cleaved RNA becomes polyadenylated and thus translationally competent.

To further characterize the function of CTN-RNA, the authors introduce antisense oligonucleotides into cells; surprisingly, not only are CTN-RNA levels decreased, but those of *mCAT2* mRNA are as well. The two antisense oligonucleotides used correspond to unique re-

gions of the 5' and 3' UTR of CTN-RNA and should not target *mCAT2* mRNA. Thus, the authors conclude that CTN-RNA is necessary for the stability of *mCAT2* mRNA. To delineate which region is required for stability, they introduce constructs that express different parts of CTN-RNA and find that loss of *mCAT2* mRNA is rescued by expression of the common 3' UTR sequence. The authors propose that when CTN-RNA is present at normal levels, it sequesters a factor that promotes degradation of *mCAT2* mRNA. When CTN-RNA levels are reduced by the antisense oligonucleotides, the factor is free to bind to *mCAT2* mRNA and promote its degradation. But if the sequences in CTN-RNA that bind and sequester the factor are the same as those in *mCAT2* mRNA, why is CTN-RNA not degraded as well?

The antisense data are the most perplexing data in the Spector paper. In addition, their biological relevance is questionable because it is unclear when CTN-RNA would naturally be absent—and if it is, why would *mCAT2* mRNA need to be degraded? With a bow to parsimony, it seems possible that the antisense effect does not signal an additional complexity in CTN-RNA regulation but occurs because it mimics what normally takes place during stress. For example, extending the posttranscriptional-cleavage/polyadenylation model, loss of CTN-RNA after antisense treatment might allow the factor that blocks its proximal polyadenylation site to act on the *mCAT2* mRNA instead, making the latter unstable. This model does not explain why the *mCAT2* mRNA cannot use the distal polyadenylation site when proximal polyadenylation is blocked, but a possibility is that the use of the distal site is promoter dependent.

The observation that CTN-RNA is cleaved and moves to the cytoplasm in response to stress is the most significant observation in the Spector paper, and one with far-reaching implications. If this is a general mechanism, it implies that nuclear retention, perhaps mediated by inverted repeats containing inosine, serves to store translatable RNAs for their release as needed. Thus, the data hint at an explanation for the existence of the mysterious repeats in hnRNA and suggest an answer to a conundrum in the ADAR field—if inosines promote nuclear retention, how are the scores of ADAR substrates translated (Levanon et al., 2004; Morse et al., 2002)? If the fate of CTN-RNA is a general one, the answer is that cleavage releases the inosine-containing part of the 3' UTR.

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Selected Reading

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