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The traffic jam: polyamine prevalence pauses protein production

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In this issue, Ivanov et al. (2018) demonstrate how cells sense metabolite levels and regulate production of their biosynthetic enzymes through the formation of "ribosome queues".

Main Text:

Cells must dynamically tailor their gene expression programs to their needs. Translation of mRNA into protein is the most energy-intensive step of gene expression and has the greatest potential for rapid changes in gene expression (Schwanhausser et al., 2011). Translation output of a coding sequence (CDS) is tuned by regulatory sequences within the associated untranslated regions (UTRs). For example, as the ribosome scans a 5' UTR it may encounter start codons prior to the correct start for the major protein encoded by that transcript. These upstream start codons can recruit scanning ribosomes, reducing the fraction that reaches the downstream, 'correct' start codon. Such start codons are often associated with a stop codon in the same frame, giving rise to a short upstream open reading frame (uORF) (Brar, 2016). In most cases, uORFs are inhibitory or neutral to the translation efficiency of the downstream CDS, since they prevent the initiating ribosome from reaching the CDS (Johnstone et al., 2016).

Both the act of initiating on an upstream start codon and the peptide encoded by a uORF can regulate translation. The prototype of the former is regulation of *GCN4* transcript in yeast. Under conditions of high nutrient availability, four uORFs within the *GCN4* 5'UTR engage ribosomes and downregulate Gcn4p production. However, upon starvation, phosphorylation of eIF2α results in "leaky" scanning of 40S ribosomes past the uORFs, resulting in increased initiation at the main ORF and higher Gcn4 production (Hinnebusch, 2005). An example of the latter is the arginine attenuator peptide (AAP), encoded by a uORF within the *CPA1* transcript in yeast. AAP inhibits downstream *CPA1* ORF translation in response to a surplus of arginine within the media (Gaba et al., 2001). Thus, cells use multiple modes of translational control by uORFs to achieve nutrient-dependent gene expression.

In this issue, Ivanov et al. describe feedback in polyamine dependent translational regulation. Polyamines are intracellular polycations that regulate gene expression and ion channel function. Polyamines also regulate enzymes involved in their own biosynthesis. In fact, translation of ornithine decarboxylase antizyme (OAZ), an inhibitor of polyamine biosynthesis (Figure 1A), is dependent on polyamine-induced translational frameshift (Matsufuji et al., 1996). Ivanov et al. demonstrate that AZIN1, a repressor of OAZ, is regulated by polyamines at the translational level (Figure 1A). Translation of a conserved PPW motif within a uORF in the *AZIN1* transcript stalls the elongating ribosome, which is typically resolved by eIF5A. However, polyamines compete with eIF5A for ribosome association. Therefore under conditions of high intracellular polyamines, the stall persists. This stall induces a "queue" of ribosomes stretching back to, and over, the putative start codon. The prolonged presence of ribosomes over the sub-optimal start codon in turn increases its usage and engagement of the ribosome with the uORF, leading to decreased translation of *AZIN1*. The uORF thus downregulates translation of the downstream CDS by engaging ribosomes itself, which is in turn caused by a sequence-induced stall, an elegant convergence of the two modes of uORF mediated translational regulation described earlier (Figure 1B).

While initiation is the dominant regulatory step of translational control, noncanonical modes of regulation during translation elongation are emerging. Recently, it was found that translational control of the *AMD1* gene (encoding adenosylmethionine decarboxylase 1 or AdoMetDC) is achieved by stochastic readthrough of the main stop codon. Ribosomes translating the 3' UTR then stall, creating a "queue" that eventually interrupts the translation of the *AMD1* coding sequence. In that case, the length of the queue was proposed to depend on the number of times that particular individual transcript had been translated, thereby serving as a molecular counter for the number of AdoMetDC synthesized (Yordanova et al., 2018). While the specifics of the mechanisms are different, the principle underlying the regulation of *AMD1* and *AZIN1* remains the same. In both cases, feedback from the amount of translation a gene has undergone (via the number of times a stochastic process occurs in the case of *AMD1*, or the final biochemical output of the pathway the gene is involved in, in the case of *AZIN1*), inhibits further translation of the message via formation of a queue of ribosomes.

Regulation of *AZIN1* by polyamine levels raises many interesting possibilities and questions. Firstly, how widespread is this mode of regulation? Polyproline induced stalls of the ribosome (typically resolved by eIF5A) are common (Gutierrez et al., 2013). Does resolution of these stalled ribosomes also suffer as a consequence of high intracellular polyamine concentrations? If so, what are the effects on regulatory UTRs, and also the usage of alternative start codons in coding genes? Does this produce proteins with variable N-termini from the exact same transcript, further diversifying the proteome? Conversely, if this is not a widespread phenomenon, how do ribosomes upon other transcripts escape the polyamine dependent occlusion of the eIF5A association site? Lastly, how do transcripts with ribosomes stalled in this manner avoid mRNA surveillance mechanisms such as no-go decay?

The mutual exclusivity of polyamines and eIF5A at the ribosome in *AZIN1* regulation raises the potential for another interesting regulatory layer. The ability of eIF5A to resolve ribosomal stalls is dependent on the post-translationally modified hypusine residue (Gutierrez et al., 2013). Interestingly, the addition of hypusine is dependent on spermidine, a polyamine (Park, 2006). Increased intracellular spermidine could then contribute to both increasing ribosomal clearance of the PPW motif via eIF5A hypusinylation, as well as decreasing it, by competing with eIF5A for the ribosome. A possible resolution to this apparent paradox might lie in the quantitative nature of enzymatic eIF5A hypusinylation (which occurs at low polyamine concentrations), and its relatively long half-life (~38 hours) (Cambridge et al., 2011). Nevertheless, further examination of the interplay between polyamine concentrations and eIF5A is likely to reveal additional regulatory information.

Finally, polyamines play a crucial role in cell growth and tumor proliferation. Polyamine metabolism is often dysregulated in cancer, suggesting its inhibition might yield therapeutic benefit. However, polyamine inhibition has shown limited efficacy (Murray-Stewart et al., 2016). Nevertheless, this study shows that there is still much to learn about the regulation of polyamine metabolism, which may expose new therapeutic targets. The study also shows that translational control still holds many secrets, just "queuing up" for another generation of scientists.

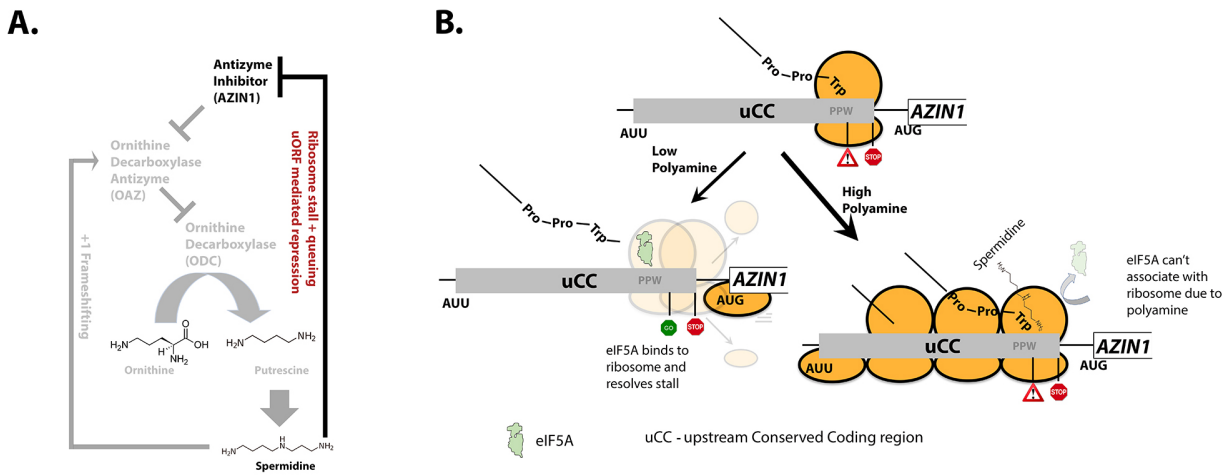

Figure 1: Polyamines regulate translation of their biosynthetic enzymes. A. Feedback regulation of genes involved in polyamine biosynthesis: Spermidine activates OAZ, a repressor of its biosynthesis, via translational +1 frameshifting; and represses AZIN1, an inhibitor of OAZ, via the formation of stall-induced ribosome queues. **B. Polyamine induced ribosomal queuing inhibits AZIN1 translation:** Polyamines compete with eIF5A for the ribosome, causing it to stall at the PPW sequence within the *AZIN1* uORF. This stall initiates a queue of ribosomal subunits, increased usage of the non-cognate start codon of the uORF, and decreased translation of *AZIN1*.

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