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Metabolic Influences on RNA Biology and Translation

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Abstract

Protein translation is one of the most energetically demanding processes for a cell to undertake. Changes in the nutrient environment may result in conditions that cannot support the rates of translation required for cell proliferation. As such, a cell must monitor its metabolic state to determine which mRNAs to translate into protein. How the various RNA species that participate in translation might relay information about metabolic state to regulate this process is not well understood. In this review, we discuss emerging examples of the influence of metabolism on aspects of RNA biology. We discuss how metabolic state impacts the localization and fate of different RNA species, as well as how nutrient cues can impact post-transcriptional modifications of RNA to regulate their functions in the control of translation.

INTRODUCTION

Translation is the most energy and nutrient-demanding process in actively growing cells. Studies over the years using the budding yeast *Saccharomyces cerevisiae* have offered insights into how nutrient cues can influence which mRNAs are made into protein. For a yeast cell, there can be ~2000 ribosomes generated every minute to support rapid cell growth. Such production has been estimated to require the majority of total transcription capacity just for rRNA synthesis, and the majority of RNA polymerase II transcription and mRNA splicing capacity for ribosomal protein mRNA synthesis (Warner, 1999). For a yeast cell growing in glucose, these demands are readily met and the synthesized mRNAs encoding these components are translated to support ribosome biogenesis and a rapid growth rate. However, upon glucose depletion, global translation is rapidly reduced within one minute (Ashe et al., 2000). The transcription of those genes involved in ribosome biogenesis is further repressed (Fingerman et al., 2003; Jorgensen et al., 2004; Marion et al., 2004), which in turn limits the synthesis of new proteins and energy consumption.

However, beyond transcriptional regulation, post-transcriptional regulation mechanisms are also utilized to regulate translational capacity and in the determination of which mRNAs should be translated. In fact, such post-transcriptional mechanisms are likely more immediate as they have the potential to facilitate a faster cellular response in the face of a dynamic nutrient environment.

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DECLARATION OF INTEREST

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How might a cell sense nutrient cues such as glucose to elicit such remodeling of its translational output? In prokaryotes, mRNA noncoding regions can form secondary structures called riboswitches that directly bind to key metabolites such as amino acids and coenzymes to mediate transcription and translation (Breaker, 2012). In this manner, prokaryotes can quickly respond to key nutrients and regulate gene expression via an RNA structural switch, without the need for protein factors. However, throughout all three kingdoms of life, only the thiamine pyrophosphate (TPP)-binding riboswitch is conserved (Breaker, 2012), but it is absent from yeast. Instead, metabolic sensing mechanisms may be mediated by protein factors and then transduced to RNAs. Translation involves at least three different types of RNAs: rRNA, tRNA and mRNA. Therefore, nutrient cues can act on each of these different RNA species in the regulation of mRNA translation. In this review, we explore the thesis that nutrients impinge on different aspects of RNA biology for the regulation of protein translation. We discuss how the nutrient and metabolic state can influence the localization of RNAs, their fates, as well as covalent modifications on the RNAs themselves.

Metabolic regulation of translation through modulation of RNA localization

Transcribed mRNAs are typically exported out of the nucleus for translation in the cytosol. However, the destination of these mRNAs can change under different nutrient environments. In response to glucose depletion, yeast cells reduce global translation (Ashe et al., 2000), repress the transcription of ribosomal biogenesis genes, and induce the transcription of those involved in survival and stress responses (Fingerman et al., 2003; Marion et al., 2004). How do cells ramp down global translation and where do those pre-existing mRNAs go? Might they be degraded? When particular RNA degradation enzymes and translation factors are tagged with fluorescent proteins, they appear as punctate foci within the cell called processing bodies (P-bodies) or stress granules (Hoyle et al., 2007; Teixeira et al., 2005). Such RNA-protein granules increase in size and number upon glucose starvation, suggesting pre-existing mRNAs might be sequestered and subjected to decay or storage (Buchan and Parker, 2009; Collier and Parker, 2005). Although studies reported the successful purification of P-bodies and stress granules (Jain et al., 2016; Teixeira et al., 2005), it is not yet clear what types of mRNAs might be preferentially retained in these granules. It is predictable that those mRNAs associated with growth functions might be preferentially directed to such granules upon glucose starvation, and stress-responsive mRNAs would be excluded from those granules to facilitate their translation (Figure 1). Thus, nutrient cues, such as glucose depletion, can trigger mRNA spatial sequestration, which might repress translation of particular mRNAs and activate translation of others.

Interestingly, it was suggested P-bodies might be capable of directly binding small molecule metabolites. Edc3p, thought to be a scaffold of P-bodies, was observed to bind NAD^+ or an NAD^+ -related ligand in vitro. Mutation of residues within this putative binding site affects P-body formation and RNA decay (Walters et al., 2014). However, the functional significance of this NAD^+ -binding capability remains unclear.

A second localization-based mechanism to regulate translation is through depleting the cytosolic tRNA pool. In yeast, the tRNA maturation process occurs in both the nucleus and

cytosol. Intron-containing tRNA intermediates must be spliced on the mitochondrial surface and then imported into the nucleus for maturation before final re-export (Hopper, 2013; Yoshihisa et al., 2003). In the presence of amino acids and glucose, the tRNA pool is distributed evenly between the nucleus and cytosol. Upon amino acid or glucose depletion, some tRNAs, particularly tRNA_{Tyr}, accumulate in the nucleus, which might contribute to decreased translational capacity (Whitney et al., 2007). This regulation is reversible, as amino acid or glucose repletion rapidly induces a more balanced tRNA nuclear-cytosol distribution.

Why the tRNA splicing machinery is tethered to the mitochondrial surface remains mysterious. Such an association might function as a means to link the maturation process to the metabolic status of mitochondria. Interestingly, relocating the tRNA splicing machinery from the mitochondria to nucleus does not impede tRNA splicing or maturation, but leads to a defect in “pre-rRNA” processing, as can be seen by the accumulation of 20S and other pre-rRNA intermediates (Dhungel and Hopper, 2012; Volta et al., 2005). How and why the nuclear localization of the tRNA splicing machinery results in a defect in pre-rRNA processing is unclear. It is possible that mitochondria might monitor the production of tRNA and rRNA, two essential factors for translation, to avoid imbalance of tRNA and rRNA pools. As mitochondria are the power houses of the cell and hubs for cellular metabolism, this phenomenon suggests an intricate interplay between metabolism, tRNA maturation, pre-rRNA processing, and ribosome biogenesis (Huang and Hopper, 2014; Whitney et al., 2007).

Amino acids and glucose are also able to influence mRNA fate through TORC1, a master regulator of cell growth. TORC1 prevents eIF2 α phosphorylation (Cherkasova and Hinnebusch, 2003) and promotes phosphorylation of ribosomal protein S6 kinase/ Sch9p (Saitoh et al., 2002; Urban et al., 2007) and 4E-BP/ Caf20p/ Eap1p (Brunn et al., 1997; Cosentino et al., 2000), which function to stimulate cap-dependent translation. These events alter global translational rates as well as which subsets of mRNAs are translated. We refer to reader to several comprehensive reviews on how TORC1 regulates mRNA translation (Laplane and Sabatini, 2009; Ma and Blenis, 2009; Wullschleger et al., 2006).

Metabolic regulation of translation through mRNA cis-acting elements

Nutrient cues not only regulate intracellular localization of various RNA species, but also control the translation of specific mRNAs to tune protein production. Some mRNAs have upstream open reading frames (uORF) to regulate translation of their coding sequence, while others share similar sequences that enable co-regulation by RNA-binding proteins. In this manner, the coordinated post-transcriptional regulation of mRNAs encoding proteins with similar functions can be achieved. In fact, mRNA transcripts encoding genes with similar functions might share a conserved sequence element, especially in the 3' untranslated region (3'UTR), thereby enabling co-regulation by sequence-specific RNA-binding proteins (Gerber et al., 2004; Keene, 2007). We illustrate several examples to show how these mechanisms are achieved, and how metabolic cues can subsequently direct these RNA-binding proteins to regulate the fate of their mRNA targets. Altering localization of mRNA

can of course also regulate their resulting fate and their ability to be translated (discussed below).

The mRNA encoding the GCN4 bZIP transcription factor contains uORFs to regulate its translation in response to amino acid and glucose availability. Gcn4 activates ~50 genes associated with cell growth, many of which are involved in amino acid biosynthesis. Its mRNA possesses four small uORFs that are actively translated in the presence of amino acids or glucose and function to negatively regulate translation of the main coding region (CDS) (Hinnebusch, 1984). The four uORFs of GCN4 compete for ribosome occupancy of the CDS. Upon amino acid or glucose depletion, GCN2 phosphorylates a translational elongation factor (eIF2 α) to globally repress translation including the GCN4 uORFs. Some ribosomes can bypass the uORFs to then translate the GCN4 CDS to induce amino acid synthesis and other stress responses (Figure 2). In mammalian systems, a similar mode of uORF regulation occurs with ATF4, the transcription factor directing the integrated stress response (Baird and Wek, 2012; Vattem and Wek, 2004). Beyond GCN4 and ATF4, ribosome profiling studies revealed an unexpected abundance of other uORFs in both yeast and human cells (Ingolia et al., 2014).

mRNAs encoding mitochondrial biogenesis proteins are co-regulated in a manner closely linked to metabolism. In yeast growing in glucose, mitochondrial biogenesis is repressed, but must be induced upon glucose depletion to enable energy production using alternative carbon sources such as ethanol or acetate through mitochondrial respiration. Yeast cells growing in glucose constitutively transcribe nuclear-encoded mitochondrial ribosomal mRNAs at a basal level (DeRisi et al., 1997). However, instead of sharing a common upstream activating sequence for transcription, those mRNAs all harbor a common sequence motif within their 3'UTRs (Gerber et al., 2004). Puf3p, a member of the PUmilio-FBF family of RNA-binding proteins (Zamore et al., 1997), can directly bind to this class of mRNA transcripts (Gerber et al., 2004). In glucose medium, Puf3p recruits the mRNA decay machinery to degrade these mRNAs, thereby preventing their translation (Figure 2) (Olivas and Parker, 2000; Tucker et al., 2002). However upon glucose depletion, these mRNAs are more stable (Foat et al., 2005), and Puf3p becomes highly phosphorylated and associates with ribosomes to promote translation of these mRNAs encoding mitochondrial proteins (Lee and Tu, 2015). The phosphorylation status of Puf3p is tied to glucose availability, thereby demonstrating how a nutrient cue can switch the fate of a class of mRNA transcripts between degradation and translation through post-translational modification of an RNA-binding protein. In this manner, yeast cells can jump-start mitochondrial biogenesis by activating the translation of pre-existing mRNAs, without having to rely on the de novo transcription of those genes. Consistent with this idea, mRNAs that encode proteins involved in the earlier stages of mitochondrial biogenesis tend to contain a Puf3p binding site in their 3'UTRs (Lelandais et al., 2009). Thus, those cells that have PUF3 at their disposal may show a fitness advantage, especially under environments with fluctuating glucose availability. Other PUF proteins might similarly be capable of switching their target mRNA transcripts between degradation and translation, perhaps also in response to metabolic cues.

In contrast to mitochondrial biogenesis genes, many growth-related genes are translationally repressed upon glucose starvation. Are these mRNAs simply degraded, or might they be

stored in a manner that enables reactivation? As discussed above, upon glucose depletion, global translation is decreased, and protein synthesis capacity is re-directed to those mRNAs encoding proteins involved in the stress response and alternative carbon source utilization. The discovery of P-bodies suggested that mRNAs can either be stored or perhaps degraded by the RNA degradation machinery within these structures (Figure 1). After a period of brief glucose starvation and followed by glucose repletion, P-bodies disassemble and it is believed that most paused mRNAs are “reactivated” (Ashe et al., 2000; Brengues et al., 2005). Again, these observations suggest that such dynamic changes in mRNA fate are downstream of a glucose cue. Although these studies were limited to examination of just a few genes, they proposed “a global reactivation” of all paused mRNAs. Subsequent genome-wide studies revealed that only ~400 mRNAs are reactivated with most of them encoding ribosomal proteins (Arribere et al., 2011). This poses the question of why cells selectively preserve ribosomal protein mRNAs. Because ribosomal protein mRNAs can account for ~50% of the total mRNA pool in a fast-growing cell (Warner, 1999), the production of those mRNAs is costly and they might be temporarily spared. Furthermore, these mRNAs might need to be translated immediately upon re-entry into growth. How then does a cell selectively reactivate ribosomal protein mRNAs? One possible explanation is that ribosomal protein mRNAs tend to possess long polyA tails that may help protect them from degradation and efficiently recruit ribosomes (Arribere et al., 2011). It is also possible that the splicing of ribosomal protein pre-mRNAs might somehow mark these mRNAs and distinguish them from other growth-related mRNAs. Collectively, these studies reveal that a cell might deploy additional mechanisms reliant on cis-acting elements within mRNAs to selectively reactivate those important to resume cell growth. These pathways are again subject to regulation by glucose or a glucose-dependent cue.

Metabolic regulation of translation through nucleotide modification

tRNAs are known to be decorated with a variety of nucleotide modifications (Bjork et al., 1987). In addition to tRNA, other RNA species such as rRNA and mRNA are also subjected to post-transcriptional nucleotide modifications (Cantara et al., 2011). These modifications have typically been thought to be constitutive. However, we discuss emerging evidence suggesting that some of these modifications are in fact dynamic and subject to regulation by important nutrient and metabolic cues.

The wobble uridine nucleotide (U34) can be thiolated in tRNA_{Lys} (UUU), tRNA_{Glu} (UUC) and tRNA_{Gln} (UUG). This thiolation modification replaces the 2' oxygen with sulfur in the uracil base and can occur in all three kingdoms of life. Thiolation enhances the ability of the uridine to base pair with “G”, in addition to “A”, at the 3rd codon position, thereby enhancing the ability of the ribosome to read these G-ending codons (Yarian et al., 2002). Notably, lysine, glutamate and glutamine each are specified by only two synonymous codons; thiolated tRNA_{Lys} (UUU), tRNA_{Glu} (UUC) and tRNA_{Gln} (UUG) can therefore facilitate translation of genes with either of these codons. However, these tRNAs are not constitutively thiolated in yeast, as upon switch from amino acid-replete medium to amino acid-deficient medium, the abundance of thiolated tRNAs in the cell decreases substantially (Laxman et al., 2013). tRNA thiolation could be restored by the addition of cysteine or methionine, but not other amino acids, suggesting that tRNA thiolation specifically reflects

sulfur amino acid availability, as cysteine and methionine can donate sulfur for the thiolation modification. Interestingly, GO analysis reveals lysine, glutamate and glutamine are especially enriched in those genes encoding proteins involved in translation and ribosome biogenesis. Therefore, ribosome biogenesis is subject to regulation by tRNA thiolation. As such, tRNA thiolation enables the translation of particular mRNAs to be regulated in tune with sulfur availability. Moreover, in the absence of tRNA thiolation, cells compensate by increasing the abundance of enzymes involved in methionine, cysteine and lysine biosynthesis. These findings collectively suggest methionine and cysteine can serve as signatures of amino acid status to regulate both amino acid synthesis and protein synthesis. Interestingly, uridine thiolation was also observed to enhance rates and fidelity of non-enzymatic RNA replication, suggesting a very ancient function for this conserved modification (Heuberger et al., 2015).

How might tRNA thiolation be linked to metabolic state? Cysteine is the donor of the sulfur utilized to modify uridine. The sulfur-containing amino acids (cysteine and methionine) are the most costly amino acids for a yeast cell to synthesize due to a large requirement of ATP and NADPH (Thomas and Surdin-Kerjan, 1997). As such, a high amount of sulfur-containing amino acids might represent a metabolically favorable environment, and tRNA uridine thiolation may relay a signal to boost translational capacity. In contrast, tRNA thiolation might be repressed in response to a limited supply of sulfur-containing amino acids (Laxman et al., 2013). Therefore, a cell must carefully balance sulfur consumption for tRNA thiolation with other metabolic requirements for sulfur that may be more important for survival and stress responses.

A nutrient-responsive queuosine (Q) modification on guanine (G) occurs at the wobble nucleotide position of tRNA^{Tyr}(GUA) in *Drosophila melanogaster*. The presence of queuine, the precursor of queuosine, promotes the queuosine modification and shifts the reading preference from C to U at mRNAs (Grosjean et al., 1978; Meier et al., 1985). Intriguingly, queuine, the precursor for queuosine modifications, can only be obtained from food or gut bacteria. The level of queuosine modification can change across development, perhaps as a means to influence the landscape of codon usage in different developmental stages in a manner linked to queuine availability (Zaborske et al., 2014).

Recently, the *N*⁶-methyladenosine (m⁶A) modification on mRNA has become a topic of intense interest after it was first observed 40 years ago (Desrosiers et al., 1974). With the discovery of m⁶A writers (Agarwala et al., 2012; Liu et al., 2014) and erasers (Jia et al., 2011), it has become clear that the m⁶A RNA modification is reversible (Fu et al., 2014). This modification has been proposed to regulate mRNA stability (Wang et al., 2014), translational efficiency (Wang et al., 2015), and influence RNA structure to regulate RNA-protein interactions (Liu et al., 2015). However, it remains to be determined whether particular m⁶A modifications are regulated by metabolism, for example by amounts of the methyl donor SAM. These findings also suggest the possibility that other RNA modifications are subjected to regulation. For example, pseudouridylation, an isomerization of the uridine nucleoside, occurs in many tRNAs, some rRNAs, ncRNAs, as well as mRNAs. Next-generation sequencing showed that mRNA pseudouridylation amounts fluctuate depending on the nutrient condition (Carlile et al., 2014; Lovejoy et al., 2014;

Schwartz et al., 2014), which can change the secondary structure of RNA (Charette and Gray, 2000) or alter base-pairing interactions (Karijovich and Yu, 2011). Such mRNA pseudouridylation is observed to occur on many ribosomal protein mRNAs, potentially influencing ribosome biogenesis and translational capacity. These environment-sensing RNA nucleotide modifications are summarized in Table 1.

In addition to cysteine, methionine and SAM, what other sentinel metabolites might reflect the metabolic state and influence RNA nucleotide modifications? Acetyl-CoA, situated at the intersection of many metabolic pathways, might represent one such metabolite through its ability to modulate not only protein acetylation but perhaps also RNA acetylation (Cai et al., 2011). Interestingly, the acetyltransferase for 18S rRNA and tRNA is conserved from yeast to humans, and essential for 18S rRNA maturation (Ito et al., 2014; Sharma et al., 2015). Intracellular acetyl-CoA levels fluctuate in a manner dependent on the growth state or nutrient environment, such as in log phase versus stationary phase, or in a fed versus starved state. When acetyl-CoA levels reach a certain threshold, the SAGA histone acetyltransferase complex induces transcription of growth-related genes, while mRNAs involved in stress response are rapidly degraded (Cai et al., 2011). Given the importance of acetyl-CoA in many cellular pathways (Shi and Tu, 2014; Shi and Tu, 2015), it is predictable that acetyl-CoA might also influence RNA biology. Beyond acetylation and thiolation modifications of RNA, more than a hundred other types of covalent modifications occur on RNA species (Cantara et al., 2011; Fu and He, 2012; Sharma and Lafontaine, 2015; Yi and Pan, 2011). While some of them are dynamic and responsive to nutrients (Preston et al., 2013), the functional roles of the majority of these modifications remain a mystery. Intriguingly, since many key metabolites and cofactors are required and consumed for these RNA modifications, (Helm and Alfonzo, 2014), they have the potential to be modulated by the metabolic state of the cell.

Conclusions and perspective

As organisms frequently encounter different types of nutritional stress, they have evolved cellular responses that are intricately intertwined with cellular metabolism. In this manner, they can carefully coordinate different pathways to regulate energy utilization to promote either survival or growth under different situations. However, the molecular dissection of mechanisms pertaining to mRNA transcription, processing, degradation, and translation have tended to have been conducted under highly nutrient-rich, growth-promoting conditions. It is therefore not surprising that under such conditions, a cell does not typically display nutritional stress responses, including tRNA nuclear accumulation, P-body formation and dynamic RNA modifications. This realization might partially explain why many RNA modifications are assumed to be static, but might in fact be dynamically regulated after examining them under particular nutrient limitations. Many RNA modifications are conserved throughout evolution, yet their functions are poorly understood. Given the abundance of some of these RNA species, (e.g. tRNAs, rRNAs and ribosomal protein mRNAs), their post-transcriptional regulations and modifications could be energetically costly. Perhaps their functions may be revealed upon their investigation under more challenging environments. In summary, we have briefly discussed here a few examples of the emerging links between metabolism and the RNA world. Future studies will undoubtedly

reveal additional examples by which metabolism exerts its influence on numerous aspects of RNA biology.

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Figure 1. Glucose depletion triggers global RNA localization changes

In the presence of glucose, growth genes are highly transcribed and the translational machinery is optimized to promote rapid cell growth. Upon glucose depletion, mRNAs encoding growth genes might be localized to processing bodies (P-bodies), and those mRNA are poorly polyadenylated. In addition, the cytosolic pool of aminoacyl-tRNAs decreases as a result of limited nuclear re-export, thereby global translation is repressed. However, specific stress genes are expressed and those mRNAs can access ribosomes and translation factors that are recycled from the growth mRNAs in P-bodies. In this manner, a cell can spare and redirect resources to accommodate adverse conditions. The yellow and green factors in the P-body denote RNA decapping and RNA degradation enzymes.

Figure 2. Nutrient depletion changes the fate of mRNAs

Under amino acid-replete conditions, eIF2α is dephosphorylated at Ser51 and ribosomes predominantly associate with GCN4 uORFs as ribosome scanning starts from the 5' end. The translation of the GCN4 CDS is thus limited due to poor accessibility to ribosomes. Upon amino acid starvation, Gcn2 is activated by uncharged tRNAs to phosphorylate Ser51 of eIF2α, thereby global translation is repressed and ribosomes no longer assemble well on the GCN4 uORFs. Under such conditions, GCN4 CDS has a higher likelihood of accessing the ribosome and Gcn4 is produced to elevate amino acid synthesis in the cell.

Puf3p is a sequence-specific RNA binding protein that can recognize conserved sequences in the 3'UTR of nuclear-encoded mitochondrial ribosomal mRNAs. In the presence of glucose, Puf3p is hypophosphorylated and recruits a deadenylase complex to degrade its bound mRNAs, thereby inhibiting mitochondrial biogenesis. Upon glucose depletion, highly phosphorylated Puf3p helps recruit ribosomes to its bound mRNAs to induce mitochondrial biogenesis for metabolizing non-fermentable carbon sources. As a result, the fate of pre-existing mRNAs can be switched to enable rapid adaptation to a glucose-limited environment. This kind of phosphorylation-mediated switch is observed in both amino acid- and glucose-sensing cascades.

Table 1

List of environment-sensing RNA nucleotide modifications discussed in this review.

TYPE	MODIFICATION	DONOR	EFFECT
mRNA	Methylation (m ⁶ A)	SAM	RNA stability, translational efficiency, and RNA-protein interaction
	Pseudouridylation (Ψ)	–	RNA structure and altered base-pairing
tRNA	Thiolation (mcm ⁵ s ² U)	Cysteine	Decoding synonymous codon
	Queuosine (Q)	Queuosine	Switching decoding preference from C to U