tRNA biology has lately seen a revival with the discovery of tRNA cleavage products as mediators of stress responses. In this issue of The EMBO Journal, Blanco et al now report that tRNA methylation, by protecting from cleavage, is relevant for normal brain development. The versatility of tRNA is further emphasized by a recent study in Cell that uncovered differential expression of tRNAs as a means to accommodate codon usage bias to the needs in proliferating versus differentiating cells.

See also: S Blanco et al and H Gingold et al

First discovered in the 1950s as ‘soluble RNA’ that makes up 10% of the total cellular RNA, the principal function of tRNAs as ‘intermediates’ that are covalently attached to amino acids prior to their incorporation into polypeptides became clear as early as 1958. In 1965, yeast tRNAAla was the first nucleotide sequence ever to be deciphered, a historic achievement that took several years of work and involved the purification of sufficient amounts of a single tRNA, digestion with different endoribonucleases followed by chromatographic separation and identification of the tRNA fragments. The availability of tRNA sequences immediately led to the recognition of its cloverleaf secondary structure and the fundamental importance of the trinucleotide anticodon as the reader of the genetic code, which had been broken around the same time in the early 60s (reviewed in Raj-Bhandary & Kohrer, 2006). Due to its compact nature and stable folding, tRNA was also the first RNA to be crystallized, and its three-dimensional L-shaped structure was solved at a 3 Å resolution in 1974. After these seminal discoveries, most scientists closed the books on what was now understood as the key adapter molecule linking 20 different amino acids to the genetic code.

Recent discoveries, however, taught us unexpected lessons on regulatory functions of tRNAs: Their fragments turn out to mediate stress responses with critical impact on neurodevelopmental disorders. Moreover, there is growing evidence that relative tRNA abundance is actively regulated as to coordinate translation efficiency with gene expression profiles in a cell type-dependent manner.

In the past few years, the correlation between tRNA availability, codon usage, and translation efficiency has been studied in multiple prokaryotic and eukaryotic species. In general, codons recognized by abundant tRNAs are more efficiently translated—and isoacceptor tRNAs for frequently used codons can be up-regulated to ensure efficient translation (reviewed in Novoa & Ribas de Pouplana, 2012). Yet, codon bias varies between species and genes—and even within genes, there is a bias for rare codons at the beginning of the open reading frame (Tuller et al, 2010). Hence, cells combine the abundance of tRNAs with codon usage bias to optimize gene expression—or to intentionally restrict protein expression of specific genes: Translation of cell cycle regulators is limited by non-optimal codon usage, presumably aiding fidelity at specific cell cycle phases (Frenkel-Morgenstern et al, 2012). Following similar logic, differential tRNA expression has been described in different tissues after stress or in cancer (Pavon-Eternod et al, 2009).

Important novel insight comes from systematic analyses of tRNA expression, histone modification, and transcript profiles studied in hundreds of human samples (Gingold et al, 2014). Notably, the tRNA profiles strongly differ between those categorized as ‘proliferating’ (e.g., tumor) and ‘differentiating’ (e.g., normal) cells. The most striking finding is that the codon usage of genes induced during proliferation versus differentiation correlates with the changes in tRNA abundance. In molecular terms, the authors identified sequence motifs within the promoters and histone modification patterns unique to differentiation- or proliferation-associated tRNA genes. Hence, mRNAs expressed during proliferation and differentiation appear intimately coupled to the tRNAs needed for their optimal translation through coordinated transcriptional control (Gingold et al, 2014).

The discovery of tRNA–mRNA co-regulation and the existence of a translational code within the genetic code are intriguing: They raise the possibility that cells are able to sense the demand of tRNAs, adjust the transcription of more than a hundred tRNA genes accordingly, and thereby control differential protein synthesis. It would now be important to determine the impact of experimental perturbations of this co-regulation on the fate of proliferating and differentiating cells. The future identification of factors that discriminate between tRNA-promoter classes will be essential to gain mechanistic insight into tRNA–mRNA co-regulation. This might eventually open new directions for cancer therapy by means to direct proliferating (malignant) cells toward a differentiated (benign) phenotype.

Notably, tRNA abundance is not only determined by transcription, but also by targeted degradation (Fig 1). In many eukaryotes, tRNAs are cleaved in the anticodon loop under various stress conditions (reviewed in Thompson & Parker, 2009). In general, stress-induced cleavage affects a large number of tRNA species, but only a small fraction of each tRNA pool. As a
consequence, specific fragments can elicit stress responses and actively cause translational repression (see Ivanov et al., 2011), while the cellular pool of tRNAs does not become depleted.

In human cells, the endoribonuclease angiogenin (ANG) mediates tRNA cleavage, though its cleaving activity is normally suppressed by the RNase inhibitor RNH1 (Thompson & Parker, 2009). Cytosine-5 methylation presents an alternative way to prevent tRNA cleavage, and Dnmt2 was the first tRNA methyltransferase identified with such an activity in flies (Schaefer et al., 2009) and mice (Tuorto et al., 2012). A second tRNA methyltransferase, NSun2, methylates tRNAs at different cytosines: most frequently at the base of the variable loop on C48 and C49 (Fig 1) (Tuorto et al., 2012). Combined deletion of Dnmt2 and NSun2 in mice reveals strong synergy with pronounced growth retardation that correlates with reduced stability of hypomethylated tRNAs and a decline in global protein synthesis (Tuorto et al., 2012).

In this issue of The EMBO Journal, Michaela Frye and co-workers now report that deletion of NSun2 alone causes microcephaly and reduced spatial memory due to impaired development of the cortex, hippocampus, and striatum (Blanco et al., 2014). The authors further reveal reduced protein synthesis and synaptogenesis, with increased apoptosis in specific brain areas of NSun2−/− mice. Moreover, NSun2 methylates more than 75% of all tRNAs, and a specific subset of these tRNAs is prone to cleavage in the absence of NSun2. Studies in human keratinocytes confirm that loss of NSun2 elicits cellular stress responses that translate into apoptosis. Finally, the authors causally link the neurodevelopmental defects to tRNA cleavage by pharmacological ANG-inhibition, which rescues both brain size and neuronal apoptosis (Blanco et al., 2014).

Intriguing generalization for these results comes from the pathology observed in humans with a mutation in the CLP1 gene, who develop severe microcephaly and other neurological defects (Karaca et al., 2014; Schaffer et al., 2014). CLP1 is an RNA kinase required for ligation of tRNA exons after excision of an intron positioned in the anticodon loop of a small number of pre-tRNAs. CLP1-deficiency causes accumulation of non-ligated 5′ and 3′ tRNA exons, which are virtually identical to the 5′ and 3′ tRNA halves observed in the absence of tRNA methylation. Thus, toxic tRNA fragments and uncontrolled stress responses represent critical barriers for unperturbed brain development. It remains unclear why neurons seem more susceptible to tRNA perturbation than other cell types. Moreover, it will be important to determine whether general translation repression by tRNA fragments or subtle imbalances in protein homeostasis are at the base of the observed neuropathology.

Given that a large number of different isoacceptor tRNAs are expressed from >400 tRNA genes in mammals and that (across all species) >90 chemical modifications in addition to cytosine-5 methylation are known to occur on tRNAs, the studies discussed above offer only a glimpse on cell type-specific tRNA regulation and unexpected tRNA functions. We should be prepared for ‘more tricks from old dogs’.

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