On the importance of being tRNA

Konrad Ludwig Moritz Rudolph

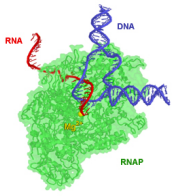
23rd July 2014

SUMMARY

The genetic code is an abstraction of how mRNA codons and tRNA anticodons molecularly interact during protein synthesis; the stability and regulation of this interaction remains largely unexplored. Here, we characterized the expression of mRNA and tRNA genes quantitatively at multiple time points in two developing mouse tissues. We discovered that mRNA codon pools are highly stable over development and simply reflect the genomic background; in contrast, precise regulation of tRNA gene families is required to create the corresponding tRNA transcriptome. The dynamic regulation of tRNA genes during development is controlled in order to generate a anticodon pool that closely corresponds to messenger RNAs. Thus, across development, the pools of mRNA codons and tRNA anticodons are invariant and highly correlated, revealing a stable molecular interaction interlocking transcription and translation.

1

INTRODUCTION



Polymerases are a large, strongly conserved enzyme complexes consisting of multiple proteins

Transcription of the mammalian genome is divided among multiple RNA polymerases (Pol), each transcribing a non-overlapping set of genes. Messenger RNAs (mRNAs) for protein-coding genes are synthesized by Pol II, while the genes encoding transfer RNAs (tRNAs) are transcribed by Pol III. The direct interaction of these transcripts produced by Pol II and Pol III is a vital step in the flow of genetic information, in which the triplet codons in mRNAs are selectively identified by their counterpart tRNA anticodons to direct protein synthesis. To explore the largely unknown regulatory mechanisms active at this mRNA–tRNA interface, we exploited the rapid and extensive changes in the transcriptome occurring among different developmental stages of mammalian organogenesis (Kyrmizi et al. 2006; Li et al. 2009; Kang et al. 2011; Lee et al. 2012; Liscovitch and Chechik 2013; Sunkin et al. 2013).

Conceptually, one possible mechanism to control protein abundance in developing tissues could be the deliberate mismatch of triplet codons in mRNAs and their corresponding tRNA anticodon isoacceptors (Brackley et al. 2011). In protozoa, this strategy is used to modulate the rate of translation of specific subsets of mRNAs containing a particular profile of codons (Horn 2008). Alternatively, if the large-scale changes in protein-coding transcriptomes result in a stable distribution of mRNA triplet codons, then deliberate changes in the population of tRNA anticodons could be used to fine-tune protein translation. Hypertranscription of tRNAs by Pol III has been observed in cancers (Winter et al., 2000; Pavon-Eternod et al., 2009; Pavon-Eternod et al., 2013), with recent work suggesting that differences in expression of specific tRNA genes may contribute to tumourigenesis by favoring translation of cancer-promoting mRNAs driving proliferation (Pavon-Eternod et al., 2009). It is unknown whether normal mammalian cells modulate tRNA gene expression to regulate information flow from mRNAs to protein synthesis.

2

RESULTS

2.1 MOUSE TISSUE DEVELOPMENT AS A MODEL SYSTEM TO STUDY MRNA AND TRNA GENE REGULATION

Organogenesis during mouse development is a well-understood process (McLin and Zorn 2006; Bruneau 2008; Zorn and Wells 2009; Si-Tayeb et al. 2010; Kang et al. 2011). For example, the molecular landscape within the liver is known to undergo radical changes during development in response to shifts in the liver's physiological functions during embryogenesis.. During early development, the embryonic liver is a haematopoetic organ; at birth, the neonatal liver becomes the primary metabolic and detoxification organ (Si-Tayeb et al. 2010); at weaning, further metabolic pathways are upregulated (Bohme et al. 1983; Girard et al. 1992). In the developing brain, coordinated gene expression changes in a heterogeneous collection of diverse cell types shape the functional specialization of specific regions in both embryonic and postnatal brains (Liscovitch and Chechik 2013; Sunkin et al. 2013).

To characterize changes in the mRNA and tRNA transcriptomes during development, we performed strand-specific, total RNA-seq, as well as ChIP-seq against Pol III in C57BL/6J mice in liver and brain at the following developmental stages: two embryonic (E15.5 and E18.5), two post-birth (P0.5 and P4) and immediately pre- and post-weaning (P22 and P29) stages (Fig. 1). For each experiment at each tissue and developmental stage, we performed two biological replicates that were highly correlated (Methods, Supplemental Fig. 1–3). This approach allowed us to quantify expression levels for protein-coding genes, as well as Pol III occupancy at every tRNA locus, which quantitatively captures the utilization of each tRNA gene (Barski et al. 2010; Moqtaderi et al. 2010; Oler et al. 2010; Kutter et al. 2011; Canella et al. 2012; Carriere et al. 2012; Renaud et al. 2014).

TO DO: Make it sound sexy

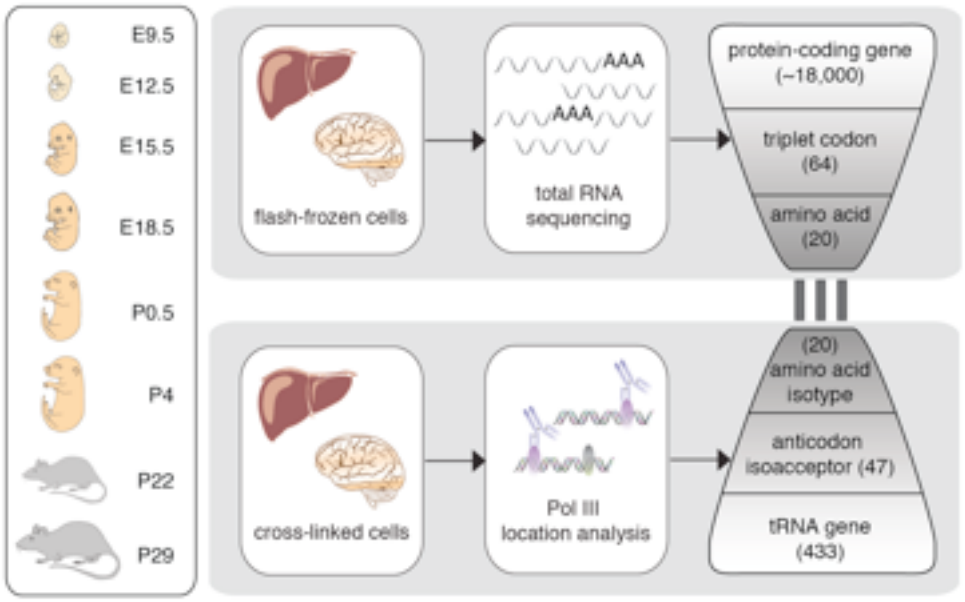


Figure 1: **Transcriptome-wide analysis of protein-coding and tRNA genes during mouse organ development.** Liver and brain tissues were isolated at eight mouse developmental stages. Tissue samples were flash-frozen for RNA-sequencing (RNA-seq) and cross-linked using formaldehyde to preserve protein–DNA interactions for ChIP-sequencing (ChIP-seq) of Pol III. Using the RNA-seq data we calculated from all expressed protein-coding genes the frequencies of each triplet codon for all 64 possible codons and 20 amino acids. Similarly, Pol III binding to tRNA genes in the mouse genome was collapsed into 47 anticodon isoacceptor families and 20 amino acid isotypes (Methods). The bars linking RNA- and ChIP-seq data represent the three-nucleotide interactions between codon and anticodon. Pol III occupancy was determined also in E9.5 (whole embryo) and E12.5 (head versus remaining body).

2.2 DYNAMIC CHANGES IN PROTEIN-CODING GENE EXPRESSION DURING MOUSE DEVELOPMENT

TO DO: explain this section properly

As expected, between stages we saw large-scale changes in the expression of protein-coding genes known to have different functions during liver and brain development (Li et al. 2009; Kang et al. 2011; Lee et al. 2012; Liscovitch and Chechik 2013). For instance, *ApoB*, which is the primary apolipoprotein carrying low-density lipoproteins, is steadily upregulated during development; in contrast, α-fetoprotein (*Afp*), the fetal version of serum albumin, is the primary circulatory carrier protein that over development is downregulated and replaced by its adult counterpart (Chen et al. 1997; Lee et al. 2012) (Fig. 2A, Supplemental Table 1). By performing matched RNA-seq experiments during brain development, we observed similar dynamics of gene expression rewiring at the neural transcription factor *Foxp2*, where transcription decreases steadily after birth, and at the neurotransmitter calmodulin (*Calm1*), where transcription increases after birth (Huang et al. 2011; Tsui et al. 2013) (Fig. 2B, Supplemental Table 2).

BIBLIOGRAPHY

Pavon-Eternod, Mariana et al. (2009). "tRNA over-expression in breast cancer and functional consequences". In: *Nucleic Acids Res* 37.21, pp. 7268–80. DOI: 10.1093/nar/gkp787.

Pavon-Eternod, Mariana et al. (2013). "Overexpression of initiator methionine tRNA leads to global reprogramming of tRNA expression and increased proliferation in human epithelial cells". In: *RNA* 19.4, pp. 461–466. DOI: 10.1261/rna.037507.112.

Winter, A G et al. (2000). "RNA polymerase III transcription factor TFIIIC2 is overexpressed in ovarian tumors". In: *Proc Natl Acad Sci U S A* 97.23, pp. 12619–24. DOI: 10.1073/pnas.230224097.