We are IntechOpen, the world's leading publisher of Open Access books
Built by scientists, for scientists

6,900
Open access books available

186,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Viral Modulation of Host Translation and Implications for Vaccine Development

Abhijeet Bakre and Ralph A. Tripp

Abstract

Translation of mRNAs into protein is an essential mechanism of regulating gene expression—and a step exploited by viruses for their own propagation. In this article, we review mechanisms that govern translation and provide an overview of the translation machinery, discuss some of the components involved in this process, and discuss how viruses modulate host translational controls and implications in vaccine design.

Keywords: translation, transfer RNAs, transfer RNA fragments, tRNAs, viruses

1. Introduction

The central dogma of molecular biology is that data are organized by DNA, mRNA, and protein and that this information is translated during transcription leading to the execution of cellular programs via proteins, which are fundamental to the functioning of a cell. A vast body of literature has added to our understanding of the molecular interplay during translation; however, it is far from comprehensive as (1) biological systems are complex where there is little correlation between the sizes of an organism, its genome size, and the number of protein coding/noncoding genes; (2) biological systems respond acutely to changes in the environment or upon infection with a pathogen; (3) all biological systems are in a state of continuous evolution as they learn from new stimuli and adapt accordingly; (4) posttranslational modifications are normally required for assembly into molecular complexes/proteins to elicit a function; and (5) many proteins are multifunctional across different pathways. We begin this article with a succinct overview of the main components involved in protein translation and the translation process itself and then consider the multiple roles transfer RNAs (tRNAs) have during translation in virus-infected cells and how viruses modify tRNA expression and

function. We conclude with a discussion of how understanding the mechanisms by which viruses modulate host translation pathway can aid in an effective vaccine design.

Protein synthesis is a multistep process involving various error-checking mechanisms. For example, genes are transcribed in the nucleus, and mature messenger RNAs (mRNAs) are exported into the cytoplasm as ribonucleoprotein particles, and immediately they are associated with ribosomes (either free in the cytoplasm or bound to endoplasmic reticulum) for initiation of translation. In eukaryotes, ribosomes consist of two subunits, a small 40S (Svedberg) and a large 60S, which together form 80S macromolecular ribonucleoprotein complexes of ribosomal RNA and ribosomal proteins [1]. The 40S subunit scans the mRNA until it recognizes the first codon (triplet AUG) at which point the first amino acid (a.a) methionine (Met) which is bound to its cognate transfer RNA (tRNA) with the UAC anticodon enters and binds to the AUG codon via sequence complementarity. The 60S subunit binds to this complex forming two distinct pockets, the peptidyl (P) site containing the Met-tRNA and an amino-acyl (A) site where the next aa-tRNA comes in. The chain initiator Met from the P site is transferred to the a.a. at the A site with the formation of a peptide bond, and the empty tRNA at A site is released. The 80S ribosome scans the next codon and the dipeptide-tRNA complex moves to the P site, the next aa-tRNA is brought in and peptide chain elongation continues until the ribosome reads the special codon (stop codon) that signals chain ending. When stop codons are read, the peptide chain from the tRNA and the ribosome is released [2]. Typically, each mRNA is processed by multiple ribosomes simultaneously as polysome complexes [3]. Native peptides so formed may need substantial posttranslational modifications before they are transported to their cellular niche and become functional. Mistranslated peptides are degraded by a variety of proteolytic mechanisms and components are recycled. Some mRNAs are long-lived in the host cytoplasm, while others are rapidly degraded following protein synthesis [4].

2. Principal components of translation

2.1. Ribosomes

Both prokaryotic and eukaryotic ribosomes are macromolecular complexes consisting of ribosomal RNAs (rRNAs) and ribosomal proteins. Ribosomes are separated for structural and related studies using isopycnic ultracentrifugation [5] where eukaryotic ribosomes typically pellet at 80 Svedberg units of sedimentation and are referred to as 80S ribosomes though they consist of the smaller 40S and the larger 60S subunit [6–11]. The complete ribosome is 4.3 MDa where the larger 60S subunit contains 28S rRNA, 5S rRNA, 5.8S rRNA, and 47 distinct ribosomal proteins, while the smaller 40S contains a single 18S rRNA and 33 distinct ribosomal proteins [12]. Mammalian ribosomes contain all the sites necessary for interaction with the components of the translation machinery such as eukaryotic initiation factor 1 [13]. Structural studies have identified conserved cores in mammalian ribosomes as well as proteins that are unique to the human ribosome [14]. The main features of the ribosome involved in translation include the amino-acyl (A) site where aa-tRNAs bind, the P site where peptide bond formation occurs, and the E site where uncharged tRNAs exit the ribosome (**Figure 1**). Ribosomal RNAs are also posttranscriptionally modified at multiple positions and these modifications

are essential for proper folding and function [15, 16]. Typical rRNA modifications are catalyzed by small nucleolar RNAs (snoRNAs) and include 2'-O ribose methylation and pseudouridylation, which is a very abundant posttranscriptionally modified nucleotide in various stable RNAs of all organisms. These specific bases in the rRNA stabilize rRNA structure and function. Ribose modifications are guided by C/D box snoRNAs, while pseudouridylation modifications are regulated by H/ACA box snoRNAs [17–24].
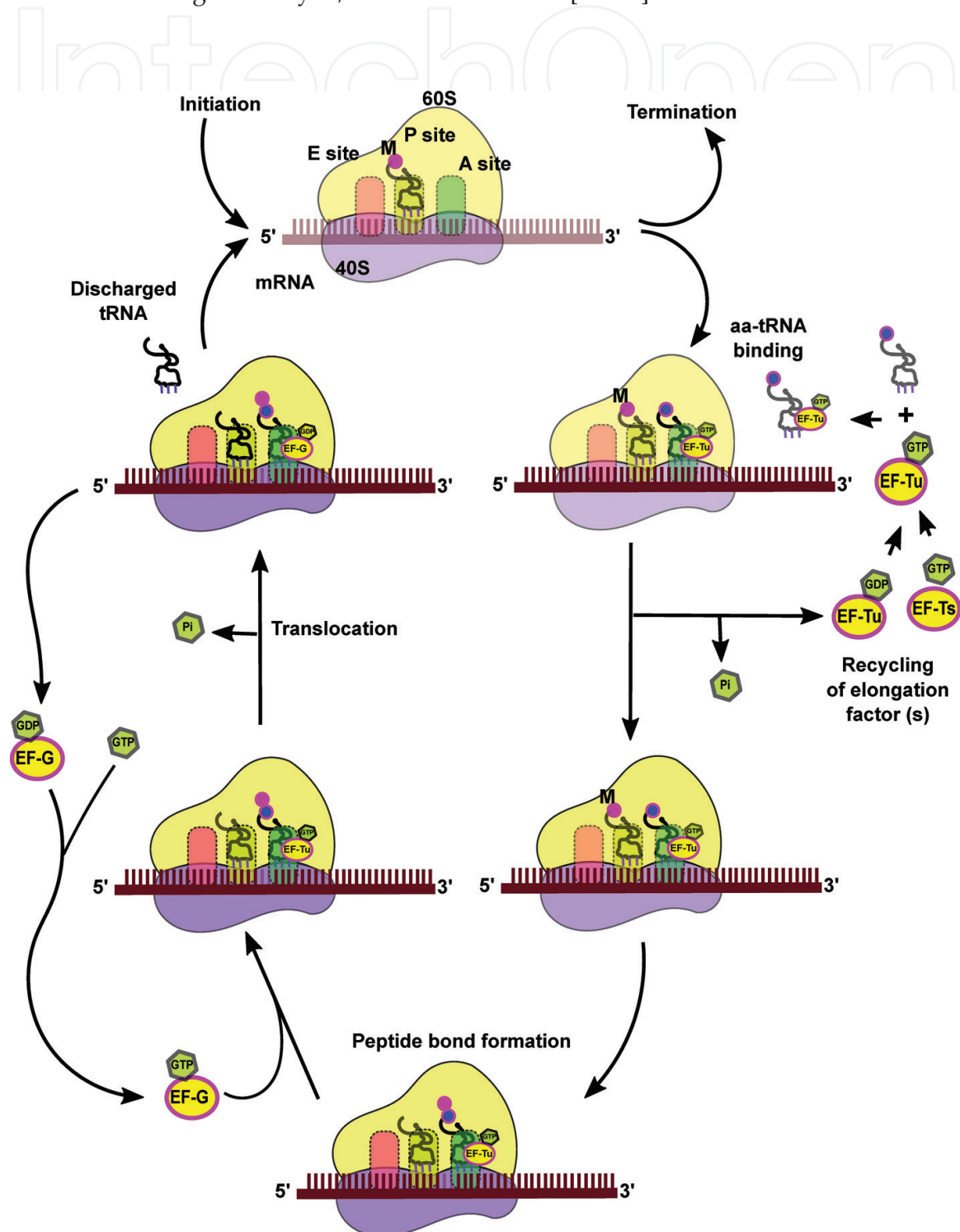

Figure 1. Overview of host translation.

2.2. Messenger RNA (mRNA)

The human genome is 3.4 billion base pair long and encodes ~32,000 protein-coding genes with a median gene size of ~1 kb containing 7 exons [25]. Protein-coding genes are transcribed by RNA polymerase II, and primary transcripts are spliced to remove introns to generate mature mRNAs, which are polyadenylated by a poly A polymerase at the 3′ end while the 5′ end carries a specific 7-methyl guanosine (m7G) modification that stimulates canonical translation initiation [26]. Mature mRNAs associate with several RNA-binding proteins and exit the nucleus as ribonucleoprotein complexes, which then associate with ribosomes to initiate translation. Multiple factors such as number of transcripts, half-life of the mRNA, etc. determine the level to which a particular mRNA is translated. Housekeeping mRNAs have long half-lives, while transcription factors and inducible genes constitute the bulk of mRNAs with short half-lives in concordance with their transient roles.

2.3. Transfer RNAs

The human genome encodes 610 tRNA genes [25] that are interspersed throughout the nuclear genome and can be classified into 51 anticodon families targeting the 64 codons. Significant intraspecies [26] and interspecies [25] copy number variation has been previously demonstrated and may extend to the tissue or cellular level. Approximately 50% of the nuclear tRNA genes are transcribed. The standard 20 a.a are decoded by 597 different tRNAs, and 3 tRNAs encode selenocysteine, where incorporation of selenocysteine into the growing peptide chain occurs by a unique suppressor tRNA and a stop codon. Moreover, 2 tRNAs have potential suppressor function, and 6 tRNAs have unknown a.a. that they carry. Additionally, the mitochondrial genome encodes 22 mitochondrial tRNAs (mtRNAs) [27]. Nuclear tRNAs are encoded by intronic or intergenic tRNA genes that are transcribed by RNA polymerase III in conjunction with transcription factors TBP, BDP1, BRF1, TFIIIB, and TFIIIC in a 3D spatially distinct region in the nucleus termed the nucleolus.

The prototypical tRNA genes consist of a 5′-UTR and signature A and B box motif [28, 29], followed downstream by a stretch of U residues that signal transcript termination. tRNA genes can be located within introns of protein coding genes where they are cotranscribed with their encoding genes. For all intergenic tRNAs, transcription is a concerted process initiating with binding of transcription factor TFIIIC to the A and B box region, recruiting TFIIIB upstream, and culminating in recruitment of RNA Pol III. Primary transcript is next processed by RNAse P- and RNAse Z-mediated removal of the 5′ leader and the 3′ trailer sequence, where tRNA nucleotidyl transferase mediates addition of the 3′-CCA trinucleotides [30–32]. Several posttranscriptional modifications on the tRNA are followed by coupling of the tRNA with the cognate a.a., a process mediated by aminoacyl tRNA synthetases. The process of tRNA charging involves recognition of several modifications on the tRNA body especially N73 near the CCA motif at the 3′ end [33]. Aberrant primary tRNA transcripts are recycled through a nonsense-mediated decay pathway involving degradation of their 3′ ends. Additionally, mature tRNAs lacking modifications are degraded via a 5′ exonucleolytic cleavage. Eukaryotic cells encode for 20 distinct tRNA synthetases for each of the 20 standard a.a. It remains unclear if amino acylation is restricted to the nucleus or also occurs in the cytoplasm. Mitochondrial tRNAs (mtRNAs) that are encoded on the circular mitochondrial genome between the rRNA and mRNA genes [27] are transcribed by

the mitochondrial RNA polymerase in conjunction with transcription factors Tfam and mtTFB from the bidirectional promoters on the circular mitochondrial genome.

Both cytosolic and mtRNAs are posttranscriptionally modified [34], though nuclear tRNAs [35] can have additional modifications presumably due to the mechanisms of action for nuclear tRNAs and the bacterial origin of mitochondrial tRNAs [27, 36, 37]. These modifications have at least three important functions: (1) modifications affecting the anticodon loop, which alter translation efficiency; (2) modifications to the tRNA body affecting tRNA secondary structure; and (3) modifications at other positions that determine aminoacyl transferase recognition and amino acid loading on the CCA motif [38]. More than 100 diverse modifications have been reported for nuclear tRNAs, while mtRNAs exhibit about 16 conserved posttranscriptional modified nucleosides [39]. The nature and role of tRNA modifications are beyond the scope of this review, but they have an essential role in tRNA function both canonical and noncanonical functions. Specifically, modifications in the anticodon loop affect tRNA translational function and increase translational accuracy by preventing translational frameshifting. Posttranscriptional modifications to tRNAs considerably increase tRNA complexity since the presence/absence of certain modifications can affect tRNA function, and it is estimated that the major tRNA modifications can lead to 8192 possible different species of tRNAs for each tRNA. Most mature nuclear tRNA molecules are ~76–93 nts, while mtRNAs are 57–73 nts. Nuclear tRNAs exhibit an evolutionarily conserved cloverleaf secondary structure across pro- and eukaryotic kingdoms consisting of four arms designated the acceptor arm, dihydrouridine arm, anticodon stem loop, and the TψC arm (ψ representing pseudouridine). The 3' end of all tRNA molecules terminates in a CCA sequence, the 2' or 3'-OH of the terminal adenosine being the site of aminoacyl-tRNA addition. In 3-D, tRNA molecules assume an L-shaped structure where the TψC arm stacks on the acceptor stem to form a 12 bp acceptor-TψC minihelix flanking the anticoding stem loop. mtRNAs can be structurally classified into three classes [40]: (1) class I mtRNAs (e.g., tRNA$^{Ser(UCN)}$), which contain a short and an extended anticodon stem [40], (2) class II mtRNAs lack the canonical D- and T-loop interaction and have variable lengths and are stabilized via an interaction between the D-stem and the extra loop [41, 42], and (3) class III mtRNAs (e.g., mtRNA$^{Ser(AGY)}$) lack the D-loop and do not exhibit the classical cloverleaf structure [43, 44].

2.4. Wobble-hypothesis and associated implications on translation

The specificity of the codon: anticodon interaction is crucial for incorporation of the correct amino acid into the growing peptide chain and determines the composition of the proteome [45–47], rate of a.a misincorporation [48–52], and ultimately protein folding [53, 54]. However, the standard genetic code is degenerate (i.e., more than one codon can specify the same amino acid). For example, six different codons can specify the a.a. lysine (K); tRNA^{Lys} is thus able to bind to six different codons for K in any given mRNA. This is because the ribosome can determine if the interactions between the first two bases of the anticodon on the tRNA and the corresponding complements on the mRNA are of Watson-Crick-type, but cannot distinguish if the third base interaction is perfectly complementary. Nuclear magnetic resonance (NMR) studies with anticodon stem loops of the smaller 40S unit of *E. coli* tRNA^{Lys} have clearly shown three modifications in this region, a N6-threonylcarbamoyladenosine (t^{6}A) modification at position 37, a 5-methylaminomethyl-2-thiouridine (S,mnm^{5}s^{2}U) modification at position 34, and a pseudouridine at position 39, which

force the dynamic loop structure to assume an open U-turn structure that perfectly fits the ribosomal decoding center [55]. Ribosomal profiling studies have shown that wobble positions slow the rates of protein translation [56]. Controlling the rate of translation via wobble base pairing has important implications: (1) utilizing infrequent tRNAs that are expressed only under particular stimuli, (2) allowing for stable and correct folding of the protein, and (3) allowing information for regulation of translation rate to be hard-coded in the mRNA [57, 58].

Recent studies have shown that in cellular organelles that do not encode all the tRNAs necessary to read the genetic code, a single tRNA species containing a U in the wobble position in the anticodon can read fourfold degenerate codon, a phenomenon described as superwobbling [58]. The superwobbling allows codons to be decoded not only by tRNAs containing a perfectly complementary or wobble 3rd base but also by tRNAs that employ superwobbling allowing for smaller genomes [58, 59].

2.5. Alternative functions of tRNAs

In addition to their normal function in protein synthesis, tRNAs acutely respond to cellular and environmental stresses. Cells with different proteomic profiles also exhibit diversity of tRNA iso-acceptor types, i.e., tRNAs with different anticodons but same a.a. tRNA expression, post-transcriptional modifications, and abundance (both copy numbers and expression) typically reflect the cellular state of tRNAs that code for the most abundant codons and are found in high copy numbers. tRNA expression levels in a particular cell type reflect the codon bias of that cell and indicate the proliferation status of a cell type, a feature that supports the proposition that tRNA gene expression is modulated in response to the host cell needs. The ribosomal tempo is thus regulated by abundance and diversity of the tRNA pool available during translation.

tRNAs are cleaved during cellular stress [60] and in immune response to infection generating specific tRNA fragments (tRFs) that contain the 5′ (5′tRFs) or the 3′ (3′tRFs) ends of the parent tRNA molecule (**Figure 2**). The most known tRFs are nuclear in origin though a few tRFs have been shown to originate from plastid genomes [61] or mitochondria [62]. tRFs have also been reported to originate from the pre-tRNA moiety instead of the mature tRNA molecule, and these are labeled as 3′-U tRFs since they match the 3′-trailer region of the precursor tRNA [63–65]. Many tRFs that result from cellular stress conditions consist of two 30–40 nt long fragments split across the anticodon loop and are referred to as tRNA-derived stress-induced RNAs (tiRNAs) [66–68]. tiRNAs reflect universal hallmarks of cellular stress across all kingdoms of life [69–75].

The level of parent tRNA molecules is maintained during tRF generation suggesting that tRF formation may be a mechanism to regulate translation via inhibition of initiation [76, 77]. Among tRFs, 5′-tRFs primarily function as signaling intermediates [78] and reduce translation [79] via induction of stress granule formation [80]. The complete biosynthesis of tRFs involves either degradation of pre-tRNA molecules via the TRAMP pathway in the nucleus [81–85] or via cytosolic degradation of mature tRNAs via the rapid tRNA decay (RTD) pathway. The TRAMP pathway consists of a polyadenylase Trf4 (topoisomerase 1-related 4), a RNA helicase Mtr4p (mRNA transport regulator 4 protein), and Air2 (arginine methyltransferase-interacting RING finger protein 2), which interacts with Rrp6, a 3′ exoribonuclease of the nuclear exosome. The RTD pathway involves methionine-requiring protein 22 (Met22) [86] and cytosolic 5′-3′ exonucleases such as ribonucleic acid trafficking protein 1 (Rat 1) [86], exoribonuclease 1 (Xrn1) [86, 87], endonucleases ELAC2 [65], Dicer [64, 88, 89], and angiogenin (ANG) [71]. Though the exact
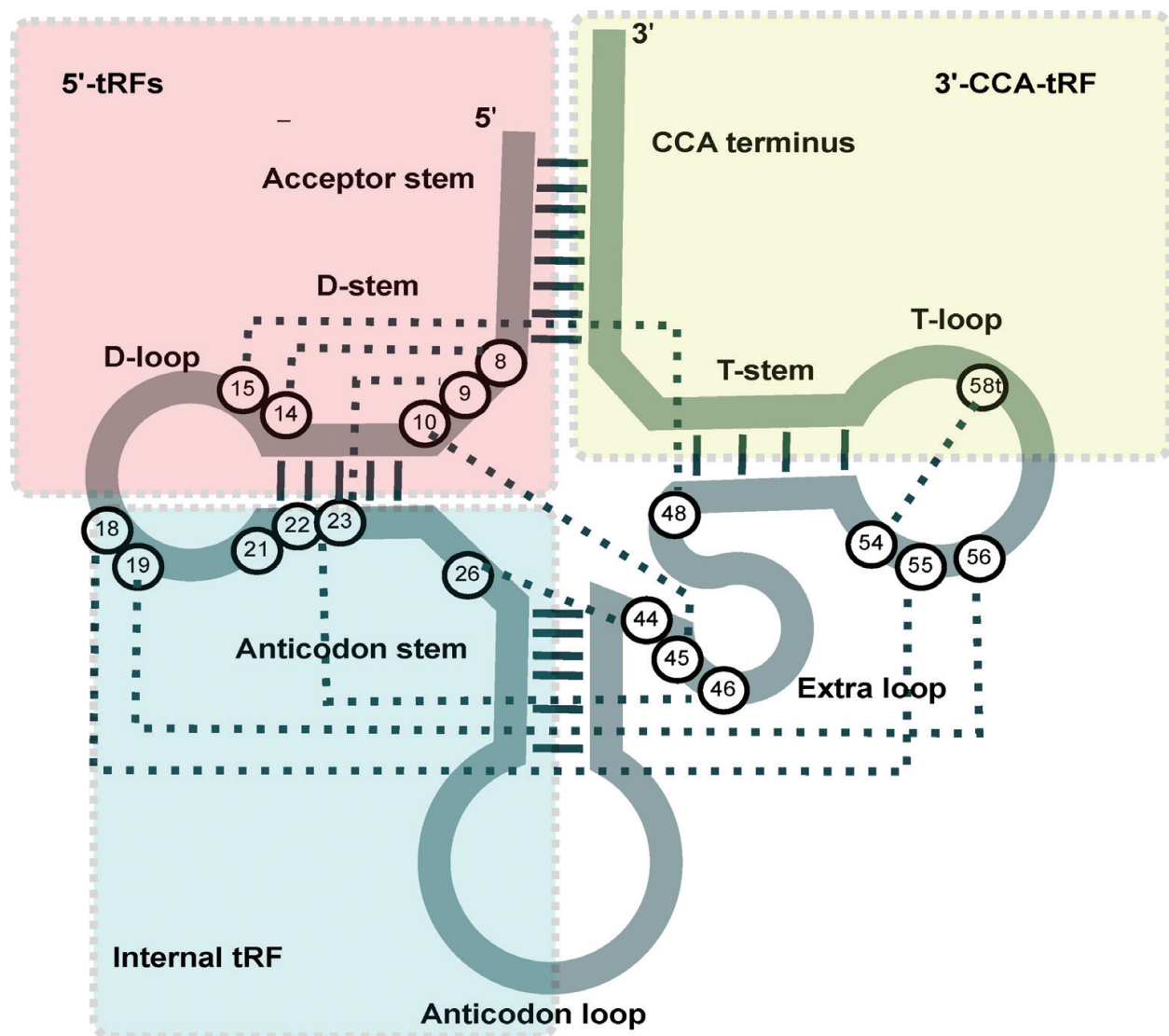
Figure 2. Transfer RNA structure and biogenesis of transfer RNA fragments (tRFs). The cloverleaf model of a canonical nuclear tRNA is shown. Bold lines indicate Watson-Crick base pairing in the tRNA stems while dotted lines indicate base pairing in the tertiary structure of the tRNA. Shaded areas indicate regions from where the 5′/ 3′ or internal tRFs are produced.

function of tRFs is not known, evidence indicates that tRFs can behave as siRNAs by degrading transcripts [90] and can regulate ribosomal loading and protein chain elongation [91]. Mechanisms of how tRFs are produced are most likely stimulus and species specific. Similarly, the functional roles of tRFs are yet to be elucidated (reviewed previously [92]). In yeast, tRFs are associated with starvation-induced vacuoles where they are degraded to provide phosphate and nitrogen [93]. tRFs also accumulate in plants during conditions of phosphate paucity [70]. Cleavage of the 3′ end CCA by angiogenin has been shown to reduce rate of protein translation [94], as well as initiation by competing with the eukaryotic initiation factor eIF4F.

2.6. How do tRNAs affect vaccine production and potentially efficacy?

Among the variety of stimuli host cells respond to, intracellular pathogens are a special case as many pathogens regulate host cell translation themselves. Viruses in particular regulate multiple facets of the host translation process since inhibition of host protein synthesis (1) makes

available crucial resources for translation of viral proteins, (2) reduces intracellular antiviral responses, and (3) reduces intercellular signaling helping viral spread in neighboring tissue. Immunization of a host with viral vaccine antigen can prevent viral modulation of the host translation machinery. Most viral antigens are considered "foreign" by the host cell—a feature tied to their codon usage that differs from the host.

The standard vertebrate genetic code contains 64 codons (61 coding for an amino acid and 3 stop codons); however, most eukaryotic proteins contain 20 standard amino acids, and thus, more than one codon can encode the same amino acid. Codons that specify the same amino acid are referred to as synonymous codons. Those that do not specify the same amino acid are termed nonsynonymous codons. However, most biological systems have evolved to preferentially utilize one or few codons for each amino acid during translation, a feature referred to as codon usage bias [95–98]. Thus, in an infected cell, viral and host proteins may be translated by very different collections of codons. Accumulating data show that many viruses evolve to adapt their codon usage to the host [99], and this can be specific for each virus or viral gene to regulate the tempo and pattern of expression. This raises a challenge in commercial vaccine production because rare codon usage can lead to low yield of the immunogen and increase production costs [100]. Secondly, while most host protein synthesis begins with an initiator codon (AUG) coding for methionine, viral genomes utilize multiple mechanisms of noncanonical translation such as internal ribosomal entry sites (IRES), ribosome shunting, leaky scanning of the viral open reading frame, non-AUG initiation, and reinitiation from AUG with frame shifts; read through translation and alternative stop; and carry on translation [101]. A detailed description of this is out of the scope of this examination, and it is important to understand how these mechanisms can be used to improve vaccine yield and/or efficacy.

A commonly employed strategy to improve vaccine yield is to optimize the codon usage pattern to overcome bias for the antigen in question [57, 102]. Codon usage bias is calculated by counting the number of time a particular codon is observed in a gene or set of genes. This can be extended to calculate the relative synonymous codon usage, which reflects the abundance of a particular codon relative to all other codons in the absence of a codon usage bias. By tabulating the most frequently used codons in the host genome and comparing to those used in the viral genome, it is possible to discern codon usage bias (CUB) for the virus. Immunogens in vaccines can then be expressed either in cells that overexpress the rare tRNA used by the viral protein to increase protein yield or engineered through molecular tools (site-directed mutagenesis, cloning, etc.) to utilize the most common host codons. This codon optimization strategy has been employed for developing a variety of vaccines [57, 103–140]. Codon optimization has been reported to reduce vaccine efficacy by increasing antigenicity and changing conformation of the native immunogen [141–145]. Codon optimization as a way to increase immunogen (vaccine) production suffers from the assumptions that: (1) rare codons limit rate of translation, (2) synonymous codons have redundant function, (3) replacing rare codons with high-frequency codons improves protein yield, and (4) sites of posttranslational modifications are preserved upon codon optimization. However, multiple studies have shown that these are not necessarily true and multiple other factors such as mRNA secondary structure [146] and posttranscriptional modifications on mRNAs [147] can alter rates of translation.

Conversely, incorporation of rare (nonpreferred) codons in viral genes used for antigen production can lead to decreased production of viral antigens and lead to attenuation. This codon deoptimization strategy has also been employed for a variety of viral vaccine candidates [148–163]. These studies have clearly shown attenuation of viral replication and improved immune responses. Further, it was recently shown that deoptimized live attenuated viral vaccines in case of respiratory syncytial virus (RSV) remain genetically stable if these changes in the genome are distributed throughout and not restricted to one viral gene or antigen [149]. Codon deoptimization strategies are still being explored for viral vaccine design; however, like codon optimization strategies, the rules for design of a safe and effective candidate are only partly recognized. Both optimization and deoptimization require extensive computational analysis, which needs to be followed up with measures of attenuation, antigenicity, and structural analysis of the antigen coupled with analysis of alternative peptides and proteins. An overview of codon optimization strategies currently used for viral antigens is shown in **Figure 3**.
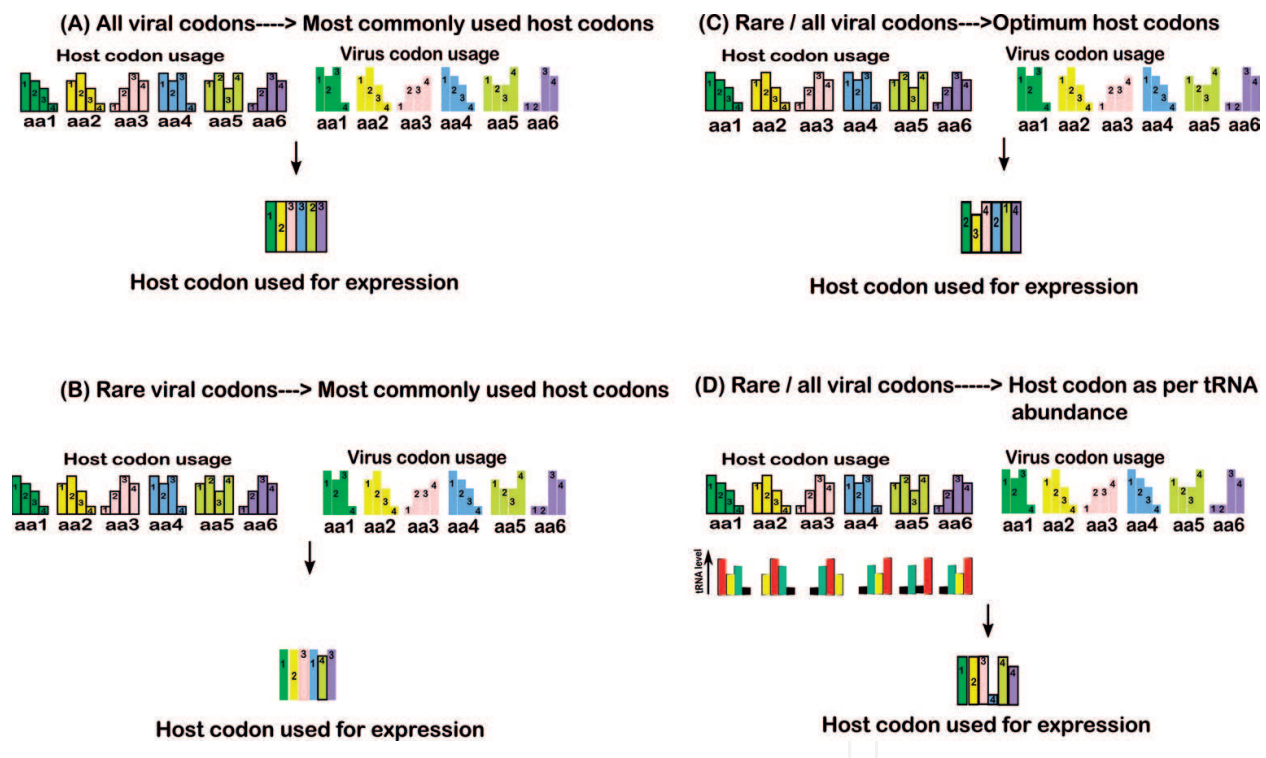

Figure 3. Strategies for codon optimization for viral gene expression. Schematic showing how poor translation of viral antigens owing to differential codon usage between viral and host genes can be overcome. In all panels, host/viral codon usage for six hypothetical amino acids (aa1–6) is shown using a color-coded histogram. Each bar represents a separate codon (1–4) used for that amino acid. Height of the bar is proportional to the frequency of that codon used. Host histograms are distinguished by bold outlines. (A) In this approach, the entire viral coding sequence is modified to reflect the most abundant codons used by the host. (B) In this strategy, only those viral codons that are rare in the host are mutated to the host codon. (C) In this approach, viral coding sequences are mutated to reflect optimum (not necessarily maximal) codon usage (D) This approach utilizes information on host transfer RNA (tRNA) expression to determine which codons in the viral coding sequence need to be mutated to the host. This strategy can include/exclude host codon usage bias. Host tRNA expression is depicted on a color scale with low (black) to red (high) expression.

3. Future directions

tRNAs and other molecules involved in host translation are an important target for disease intervention especially for intracellular viral pathogens, which are completely reliant on the host translation machinery for their successful replication and propagation in the host. However, mechanisms by which viruses and their hosts regulate translation are still being elucidated and this information is critical for development of novel interventions for both infectious and noninfectious diseases. Several vaccine production platforms use codon optimization strategies so that vaccine candidates mimic host codon usage and can be produced more efficiently with lower production costs. This results in selective usage of certain tRNAs to carry particular amino acids and to be recognized by the host cells. It is important that viral proteins can be synthesized preferentially over host proteins stimulating an immune response using these viral antigens and can be used to educate the host immunity to reduce or block damage due to subsequent infections. Inherently, every vaccine is foreign in nature for its host, which triggers an immune response. Prevalent vaccines used against infectious disease broadly fall into three categories: (1) those involving attenuated/killed pathogen, (2) subunit vaccines that contain one or more pathogen antigens (pathogen-derived or recombinant), and (3) recombinant plasmids that express one or more antigens as above. Additionally, vaccines are formulated considering delivery routes, speed of antigen release, need for adjuvants, and desired immune response. Irrespective of these criteria, the primary criterion that defines a vaccine is its antigenicity and it is important to understand mechanisms that regulate antigenicity of vaccine candidates to retain efficacy *in vivo*.

Author details

Abhijeet Bakre and Ralph A. Tripp*

*Address all correspondence to: ratripp@uga.edu

Department of Infectious Diseases, University of Georgia, USA

References

[1] Ramakrishnan V. Ribosome structure and the mechanism of translation. Cell. 2002;**108**(4): 557-572

[2] Frolova LY, Merkulova TI, Kisselev LL. Translation termination in eukaryotes: Polypeptide release factor eRF1 is composed of functionally and structurally distinct domains. RNA. 2000;**6**(3):381-390

[3] Wells JN, Bergendahl LT, Marsh JA. Co-translational assembly of protein complexes. Biochemical Society Transactions. 2015;**43**(6):1221-1226

[4] Schwanhausser B et al. Global quantification of mammalian gene expression control. Nature. 2011;**473**(7347):337-342

[5]   Nieuwenhuysen P, Slegers H. Purification of eukaryotic ribosomes by isopycnic cen-
trifugation in sucrose. Analytical Biochemistry. 1978;**89**(2):472-480

[6]   Yusupova G, Yusupov M. Crystal structure of eukaryotic ribosome and its complexes
with inhibitors. Philosophical Transactions of the Royal Society of London. Series B,
Biological Sciences. 2017;**372**(1716)

[7]   Yusupova G, Yusupov M. High-resolution structure of the eukaryotic 80S ribosome.
Annual Review of Biochemistry. 2014;**83**:467-486

[8]   Wilson DN, Doudna Cate JH. The structure and function of the eukaryotic ribosome.
Cold Spring Harbor Perspectives in Biology. 2012;**4**(5)

[9]   Ben-Shem A et al. The structure of the eukaryotic ribosome at 3.0 Å resolution. Science.
2011;**334**(6062):1524-1529

[10] Ben-Shem A et al. Crystal structure of the eukaryotic ribosome. Science. 2010;**330**(6008):
1203-1209

[11] Taylor DJ et al. Comprehensive molecular structure of the eukaryotic ribosome.
Structure. 2009;**17**(12):1591-1604

[12] Khatter H et al. Structure of the human 80S ribosome. Nature. 2015;**520**(7549):640-645

[13] Klinge S et al. Crystal structure of the eukaryotic 60S ribosomal subunit in complex with
initiation factor 6. Science. 2011;**334**(6058):941-948

[14] Anger AM et al. Structures of the human and drosophila 80S ribosome. Nature. 2013;
497(7447):80-85

[15] Roundtree IA et al. Dynamic RNA modifications in gene expression regulation. Cell.
2017;**169**(7):1187-1200

[16] Chow CS, Lamichhane TN, Mahto SK. Expanding the nucleotide repertoire of the ribo-
some with post-transcriptional modifications. ACS Chemical Biology. 2007;**2**(9):610-619

[17] Bachellerie JP, Cavaille J, Huttenhofer A. The expanding snoRNA world. Biochimie.
2002;**84**(8):775-790

[18] Dieci G, Preti M, Montanini B. Eukaryotic snoRNAs: A paradigm for gene expression
flexibility. Genomics. 2009;**94**(2):83-88

[19] Dupuis-Sandoval F, Poirier M, Scott MS. The emerging landscape of small nucleolar
RNAs in cell biology. Wiley Interdisciplinary Reviews. RNA. 2015;**6**(4):381-397

[20] Falaleeva M et al. C/D-box snoRNAs form methylating and non-methylating ribonucleo-
protein complexes: Old dogs show new tricks. BioEssays. 2017;**39**(6)

[21] Kiss T et al. Biogenesis and intranuclear trafficking of human box C/D and H/ACA
RNPs. Cold Spring Harbor Symposia on Quantitative Biology. 2006;**71**:407-417

[22] Maxwell ES, Fournier MJ. The small nucleolar RNAs. Annual Review of Biochemistry.
1995;**64**:897-934

[23] McMahon M, Contreras A, Ruggero D. Small RNAs with big implications: New insights into H/ACA snoRNA function and their role in human disease. Wiley Interdisciplinary Reviews. RNA. 2015;**6**(2):173-189

[24] Mleczko AM, Bakowska-Zywicka K. When small RNAs become smaller: Emerging functions of snoRNAs and their derivatives. Acta Biochimica Polonica. 2016;**63**(4):601-607

[25] Chan PP, Lowe TM. GtRNAdb 2.0: An expanded database of transfer RNA genes identified in complete and draft genomes. Nucleic Acids Research. 2016;**44**(D1):D184-D189

[26] Iben JR, Maraia RJ. tRNA gene copy number variation in humans. Gene. 2014;**536**(2): 376-384

[27] Suzuki T, Nagao A, Suzuki T. Human mitochondrial tRNAs: Biogenesis, function, structural aspects, and diseases. Annual Review of Genetics. 2011;**45**:299-329

[28] Green CJ. Transfer RNA gene organization and RNase P. Molecular Biology Reports. 1995;**22**(2-3):181-185

[29] Lassar AB, Martin PL, Roeder RG. Transcription of class III genes: Formation of preinitiation complexes. Science. 1983;**222**(4625):740-748

[30] Xiong Y, Steitz TA. Mechanism of transfer RNA maturation by CCA-adding enzyme without using an oligonucleotide template. Nature. 2004;**430**(7000):640-645

[31] Xiong Y, Steitz TA. A story with a good ending: tRNA 3′-end maturation by CCA-adding enzymes. Current Opinion in Structural Biology. 2006;**16**(1):12-17

[32] Lizano E et al. A comparative analysis of CCA-adding enzymes from human and E. coli: Differences in CCA addition and tRNA 3′-end repair. Biochimie. 2008;**90**(5):762-772

[33] Ibba M, Soll D. Aminoacyl-tRNA synthesis. Annual Review of Biochemistry. 2000;**69**: 617-650

[34] Cantara WA et al. The RNA modification database, RNAMDB: 2011 update. Nucleic Acids Research. 2011;**39**(Database issue):D195-D201

[35] Czerwoniec A et al. MODOMICS: A database of RNA modification pathways. 2008 update. Nucleic Acids Research. 2009;**37**(Database issue):D118-D121

[36] Lang BF, Gray MW, Burger G. Mitochondrial genome evolution and the origin of eukaryotes. Annual Review of Genetics. 1999;**33**:351-397

[37] Suzuki T, Suzuki T. A complete landscape of post-transcriptional modifications in mammalian mitochondrial tRNAs. Nucleic Acids Research. 2014;**42**(11):7346-7357

[38] Phizicky EM, Hopper AK. tRNA biology charges to the front. Genes & Development. 2010;**24**(17):1832-1860

[39] Machnicka MA et al. MODOMICS: A database of RNA modification pathways—2013 update. Nucleic Acids Research. 2013;**41**(Database issue):D262-D267

[40] Watanabe K. Unique features of animal mitochondrial translation systems. The non-universal genetic code, unusual features of the translational apparatus and their relevance to human mitochondrial diseases. Proceedings of the Japan Academy. Series B, Physical and Biological Sciences. 2010;**86**(1):11-39

[41] Wakita K et al. Higher-order structure of bovine mitochondrial tRNA(Phe) lacking the 'conserved' GG and T psi CG sequences as inferred by enzymatic and chemical probing. Nucleic Acids Research. 1994;**22**(3):347-353

[42] Messmer M et al. Tertiary network in mammalian mitochondrial tRNAAsp revealed by solution probing and phylogeny. Nucleic Acids Research. 2009;**37**(20):6881-6895

[43] Anderson S et al. Complete sequence of bovine mitochondrial DNA. Conserved features of the mammalian mitochondrial genome. Journal of Molecular Biology. 1982;**156**(4): 683-717

[44] Anderson S et al. Sequence and organization of the human mitochondrial genome. Nature. 1981;**290**(5806):457-465

[45] de Godoy LM et al. Comprehensive mass-spectrometry-based proteome quantification of haploid versus diploid yeast. Nature. 2008;**455**(7217):1251-1254

[46] Arava Y et al. Genome-wide analysis of mRNA translation profiles in Saccharomyces cerevisiae. Proceedings of the National Academy of Sciences of the United States of America. 2003;**100**(7):3889-3894

[47] Ingolia NT et al. Genome-wide analysis in vivo of translation with nucleotide resolution using ribosome profiling. Science. 2009;**324**(5924):218-223

[48] Drummond DA, Wilke CO. Mistranslation-induced protein misfolding as a dominant constraint on coding-sequence evolution. Cell. 2008;**134**(2):341-352

[49] Kramer EB, Farabaugh PJ. The frequency of translational misreading errors in E. coli is largely determined by tRNA competition. RNA. 2007;**13**(1):87-96

[50] Kramer EB et al. A comprehensive analysis of translational missense errors in the yeast Saccharomyces cerevisiae. RNA. 2010;**16**(9):1797-1808

[51] Kurland CG. Translational accuracy and the fitness of bacteria. Annual Review of Genetics. 1992;**26**:29-50

[52] Salas-Marco J, Bedwell DM. Discrimination between defects in elongation fidelity and termination efficiency provides mechanistic insights into translational readthrough. Journal of Molecular Biology. 2005;**348**(4):801-815

[53] Kaiser CM et al. The ribosome modulates nascent protein folding. Science. 2011;**334**(6063): 1723-1727

[54] Jacobson GN, Clark PL. Quality over quantity: Optimizing co-translational protein folding with non-'optimal' synonymous codons. Current Opinion in Structural Biology. 2016;**38**:102-110

[55] Kumar RK, Davis DR. Synthesis and studies on the effect of 2-thiouridine and 4-thiouridine on sugar conformation and RNA duplex stability. Nucleic Acids Research. 1997;**25**(6):1272-1280

[56] Stadler M, Fire A. Wobble base-pairing slows in vivo translation elongation in metazoans. RNA. 2011;**17**(12):2063-2073

[57] Mauro VP, Chappell SA. A critical analysis of codon optimization in human therapeutics. Trends in Molecular Medicine. 2014;**20**(11):604-613

[58] Rogalski M, Karcher D, Bock R. Superwobbling facilitates translation with reduced tRNA sets. Nature Structural & Molecular Biology. 2008;**15**(2):192-198

[59] Alkatib S et al. The contributions of wobbling and superwobbling to the reading of the genetic code. PLoS Genetics. 2012;**8**(11):e1003076

[60] Gebetsberger J, Polacek N. Slicing tRNAs to boost functional ncRNA diversity. RNA Biology. 2013;**10**(12):1798-1806

[61] Wang L et al. A novel class of heat-responsive small RNAs derived from the chloroplast genome of Chinese cabbage (Brassica rapa). BMC Genomics. 2011;**12**:289

[62] Burroughs AM et al. Deep-sequencing of human Argonaute-associated small RNAs provides insight into miRNA sorting and reveals Argonaute association with RNA fragments of diverse origin. RNA Biology. 2011;**8**(1):158-177

[63] Liao JY et al. Deep sequencing of human nuclear and cytoplasmic small RNAs reveals an unexpectedly complex subcellular distribution of miRNAs and tRNA 3′ trailers. PLoS One. 2010;**5**(5):e10563

[64] Haussecker D et al. Human tRNA-derived small RNAs in the global regulation of RNA silencing. RNA. 2010;**16**(4):673-695

[65] Lee YS et al. A novel class of small RNAs: tRNA-derived RNA fragments (tRFs). Genes & Development. 2009;**23**(22):2639-2649

[66] Raina M, Ibba M. tRNAs as regulators of biological processes. Frontiers in Genetics. 2014;**5**:171

[67] Ivanov P et al. G-quadruplex structures contribute to the neuroprotective effects of angiogenin-induced tRNA fragments. Proceedings of the National Academy of Sciences of the United States of America. 2014;**111**(51):18201-18206

[68] Anderson P, Ivanov P. tRNA fragments in human health and disease. FEBS Letters. 2014;**588**(23):4297-4304

[69] Haiser HJ et al. Developmentally regulated cleavage of tRNAs in the bacterium Streptomyces coelicolor. Nucleic Acids Research. 2008;**36**(3):732-741

[70] Hsieh LC et al. Abundance of tRNA-derived small RNAs in phosphate-starved Arabidopsis roots. Plant Signaling & Behavior. 2010;**5**(5):537-539

[71] Yamasaki S et al. Angiogenin cleaves tRNA and promotes stress-induced translational repression. The Journal of Cell Biology. 2009;**185**(1):35-42

[72] Dhahbi JM et al. 5′ tRNA halves are present as abundant complexes in serum, concentrated in blood cells, and modulated by aging and calorie restriction. BMC Genomics. 2013;**14**:298

[73] Ogawa T et al. A cytotoxic ribonuclease targeting specific transfer RNA anticodons. Science. 1999;**283**(5410):2097-2100

[74] Tomita K et al. A cytotoxic ribonuclease which specifically cleaves four isoaccepting arginine tRNAs at their anticodon loops. Proceedings of the National Academy of Sciences of the United States of America. 2000;**97**(15):8278-8283

[75] Lee SR, Collins K. Starvation-induced cleavage of the tRNA anticodon loop in Tetrahymena thermophila. The Journal of Biological Chemistry. 2005;**280**(52):42744-42749

[76] Thompson DM, Parker R. Stressing out over tRNA cleavage. Cell. 2009;**138**(2):215-219

[77] Saikia M et al. Genome-wide identification and quantitative analysis of cleaved tRNA fragments induced by cellular stress. The Journal of Biological Chemistry. 2012;**287**(51):42708-42725

[78] Zhang Y et al. Identification and characterization of an ancient class of small RNAs enriched in serum associating with active infection. Journal of Molecular Cell Biology. 2014;**6**(2):172-174

[79] Ivanov P et al. Angiogenin-induced tRNA fragments inhibit translation initiation. Molecular Cell. 2011;**43**(4):613-623

[80] Emara MM et al. Angiogenin-induced tRNA-derived stress-induced RNAs promote stress-induced stress granule assembly. The Journal of Biological Chemistry. 2010; **285**(14):10959-10968

[81] LaCava J et al. RNA degradation by the exosome is promoted by a nuclear polyadenylation complex. Cell. 2005;**121**(5):713-724

[82] Kadaba S et al. Nuclear surveillance and degradation of hypomodified initiator tRNAMet in S. cerevisiae. Genes & Development. 2004;**18**(11):1227-1240

[83] Anderson JT. RNA turnover: Unexpected consequences of being tailed. Current Biology. 2005;**15**(16):R635-R638

[84] Wang X et al. Degradation of hypomodified tRNA(iMet) in vivo involves RNA-dependent ATPase activity of the DExH helicase Mtr4p. RNA. 2008;**14**(1):107-116

[85] Maraia RJ, Lamichhane TN. 3′ processing of eukaryotic precursor tRNAs. Wiley Interdisciplinary Reviews. RNA. 2011;**2**(3):362-375

[86] Chernyakov I et al. Degradation of several hypomodified mature tRNA species in Saccharomyces cerevisiae is mediated by Met22 and the 5′-3′ exonucleases Rat1 and Xrn1. Genes & Development. 2008;**22**(10):1369-1380

[87] Watanabe K et al. Degradation of initiator tRNAMet by Xrn1/2 via its accumulation in the nucleus of heat-treated HeLa cells. Nucleic Acids Research. 2013;**41**(8):4671-4685

[88] Cole C et al. Filtering of deep sequencing data reveals the existence of abundant dicer-dependent small RNAs derived from tRNAs. RNA. 2009;**15**(12):2147-2160

[89] Babiarz JE et al. Mouse ES cells express endogenous shRNAs, siRNAs, and other microprocessor-independent, dicer-dependent small RNAs. Genes & Development. 2008;**22**(20):2773-2785

[90] Maute RL et al. tRNA-derived microRNA modulates proliferation and the DNA damage response and is down-regulated in B cell lymphoma. Proceedings of the National Academy of Sciences of the United States of America. 2013;**110**(4):1404-1409

[91] Sobala A, Hutvagner G. Small RNAs derived from the 5′ end of tRNA can inhibit protein translation in human cells. RNA Biology. 2013;**10**(4):553-563

[92] Megel C et al. Surveillance and cleavage of eukaryotic tRNAs. International Journal of Molecular Sciences. 2015;**16**(1):1873-1893

[93] Huang H et al. Bulk RNA degradation by nitrogen starvation-induced autophagy in yeast. The EMBO Journal. 2015;**34**(2):154-168

[94] Czech A et al. Reversible and rapid transfer-RNA deactivation as a mechanism of translational repression in stress. PLoS Genetics. 2013;**9**(8):e1003767

[95] Supek F. The code of silence: Widespread associations between synonymous codon biases and gene function. Journal of Molecular Evolution. 2016;**82**(1):65-73

[96] Behura SK, Severson DW. Codon usage bias: Causative factors, quantification methods and genome-wide patterns: With emphasis on insect genomes. Biological Reviews of the Cambridge Philosophical Society. 2013;**88**(1):49-61

[97] Novoa EM, Ribas de Pouplana L. Speeding with control: Codon usage, tRNAs, and ribosomes. Trends in Genetics. 2012;**28**(11):574-581

[98] Angov E. Codon usage: Nature's roadmap to expression and folding of proteins. Biotechnology Journal. 2011;**6**(6):650-659

[99] Barrai I et al. Oligonucleotide correlations between infector and host genomes hint at evolutionary relationships. Nucleic Acids Research. 1990;**18**(10):3021-3025

[100] Quax TE et al. Codon bias as a means to fine-tune gene expression. Molecular Cell. 2015;**59**(2):149-161

[101] Firth AE, Brierley I. Non-canonical translation in RNA viruses. The Journal of General Virology. 2012;**93**(Pt 7):1385-1409

[102] Kim CH, Oh Y, Lee TH. Codon optimization for high-level expression of human erythropoietin (EPO) in mammalian cells. Gene. 1997;**199**(1-2):293-301

[103] Lopes A et al. Codon-optimized P1A-encoding DNA vaccine: Toward a therapeutic vaccination against P815 mastocytoma. Molecular Therapy: Nucleic Acids. 2017;**8**:404-415

[104] Chen Q et al. Induction of humoral and cellular immune responses in mice by multiepitope vaccines composing of both T and B lymphocyte epitopes of MAGE-A3 which are recombined into HBcAg. Protein and Peptide Letters. 2017

[105] Wang D et al. Stop codon mutagenesis for homogenous expression of human papillomavirus L1 protein in Escherichia coli. Protein Expression and Purification. 2017;**133**: 110-120

[106] Stachyra A et al. Codon optimization of antigen coding sequences improves the immune potential of DNA vaccines against avian influenza virus H5N1 in mice and chickens. Virology Journal. 2016;**13**(1):143

[107] Yu TW et al. Novel GM-CSF-based vaccines: One small step in GM-CSF gene optimization, one giant leap for human vaccines. Human Vaccines & Immunotherapeutics. 2016;**12**(12):3020-3028

[108] Velazquez-Salinas L et al. Recoding structural glycoprotein E2 in classical swine fever virus (CSFV) produces complete virus attenuation in swine and protects infected animals against disease. Virology. 2016;**494**:178-189

[109] Bang HB et al. High-level production of human papillomavirus (HPV) type 16 L1 in Escherichia coli. Journal of Microbiology and Biotechnology. 2016;**26**(2):356-363

[110] Liang B et al. Enhanced neutralizing antibody response induced by respiratory syncytial virus prefusion F protein expressed by a vaccine candidate. Journal of Virology. 2015;**89**(18):9499-9510

[111] Datta D et al. Evaluation of the impact of codon optimization and N-linked glycosylation on functional immunogenicity of Pfs25 DNA vaccines delivered by in vivo electroporation in preclinical studies in mice. Clinical and Vaccine Immunology. 2015;**22**(9):1013-1019

[112] Zhang Y et al. Effects of the fusion design and immunization route on the immunogenicity of Ag85A-Mtb32 in adenoviral vectored tuberculosis vaccine. Human Vaccines & Immunotherapeutics. 2015;**11**(7):1803-1813

[113] Willet M et al. Preclinical development of inactivated rabies virus-based polyvalent vaccine against rabies and filoviruses. The Journal of Infectious Diseases. 2015;**212**(Suppl 2): S414-S424

[114] Liu Y et al. Expression, purification and identification of Pla a1 in a codon-optimized Platanus pollen allergen. Molecular Medicine Reports. 2015;**12**(2):2197-2202

[115] Lorenz FK et al. Codon optimization of the human papillomavirus E7 oncogene induces a CD8+ T cell response to a cryptic epitope not harbored by wild-type E7. PLoS One. 2015;**10**(3):e0121633

[116] Wang YY et al. Optimized codon usage enhances the expression and immunogenicity of DNA vaccine encoding Taenia solium oncosphere TSOL18 gene. Molecular Medicine Reports. 2015;**12**(1):281-288

[117] Williams JA. Improving DNA vaccine performance through vector design. Current Gene Therapy. 2014;**14**(3):170-189

[118] Fan X et al. Targeting the HA2 subunit of influenza A virus hemagglutinin via CD40L provides universal protection against diverse subtypes. Mucosal Immunology. 2015;**8**(1):211-220

[119] Cheng CY et al. Enhancing expression of the classical swine fever virus glycoprotein E2 in yeast and its application to a blocking ELISA. Journal of Biotechnology. 2014;**174**:1-6

[120] Hung YF et al. Recombinant production of the amino terminal cytoplasmic region of dengue virus non-structural protein 4A for structural studies. PLoS One. 2014;**9**(1):e86482

[121] Dutton JL et al. A novel DNA vaccine technology conveying protection against a lethal herpes simplex viral challenge in mice. PLoS One. 2013;**8**(10):e76407

[122] Spatz SJ et al. Expression of chicken parvovirus VP2 in chicken embryo fibroblasts requires codon optimization for production of naked DNA and vectored meleagrid herpesvirus type 1 vaccines. Virus Genes. 2013;**47**(2):259-267

[123] Gao J et al. Codon optimization of the rabbit hemorrhagic disease virus (RHDV) capsid gene leads to increased gene expression in Spodoptera frugiperda 9 (Sf9) cells. Journal of Veterinary Science. 2013;**14**(4):441-447

[124] Li K et al. Codon optimization and woodchuck hepatitis virus posttranscriptional regulatory element enhance the immune responses of DNA vaccines against infectious bursal disease virus in chickens. Virus Research. 2013;**175**(2):120-127

[125] Seo JY, Chung HJ, Kim TJ. Codon-optimized expression of fish iridovirus capsid protein in yeast and its application as an oral vaccine candidate. Journal of Fish Diseases. 2013;**36**(9):763-768

[126] Arora U et al. Virus-like particles displaying envelope domain III of dengue virus type 2 induce virus-specific antibody response in mice. Vaccine. 2013;**31**(6):873-878

[127] Kim HJ, Kwag HL, Kim HJ. Codon optimization of the human papillomavirus type 58 L1 gene enhances the expression of soluble L1 protein in Saccharomyces cerevisiae. Biotechnology Letters. 2013;**35**(3):413-421

[128] Tu Y et al. High-level expression and immunogenicity of a porcine circovirus type 2 capsid protein through codon optimization in Pichia pastoris. Applied Microbiology and Biotechnology. 2013;**97**(7):2867-2875

[129] Shin TH et al. Induction of robust cellular immunity against HPV6 and HPV11 in mice by DNA vaccine encoding for E6/E7 antigen. Human Vaccines & Immunotherapeutics. 2012;**8**(4):470-478

[130] Zhao KN, Chen J. Codon usage roles in human papillomavirus. Reviews in Medical Virology. 2011;**21**(6):397-411

[131] Mani I et al. Codon optimization of the major antigen encoding genes of diverse strains of influenza A virus. Interdisciplinary Sciences. 2011;**3**(1):36-42

[132] Laguia-Becher M et al. Effect of codon optimization and subcellular targeting on Toxoplasma gondii antigen SAG1 expression in tobacco leaves to use in subcutaneous and oral immunization in mice. BMC Biotechnology. 2010;**10**:52

[133] Zhu Y et al. Synergistic enhancement of immunogenicity and protection in mice against Schistosoma japonicum with codon optimization and electroporation delivery of SjTPI DNA vaccines. Vaccine. 2010;**28**(32):5347-5355

[134] Tenbusch M et al. Codon-optimization of the hemagglutinin gene from the novel swine origin H1N1 influenza virus has differential effects on CD4(+) T-cell responses and immune effector mechanisms following DNA electroporation in mice. Vaccine. 2010;**28**(19):3273-3277

[135] Siegismund CS et al. Enhanced T- and B-cell responses to simian immunodeficiency virus (SIV)agm, SIVmac and human immunodeficiency virus type 1 Gag DNA immunization and identification of novel T-cell epitopes in mice via codon optimization. The Journal of General Virology. 2009;**90**(Pt 10):2513-2518

[136] Ahn I, Jeong BJ, Son HS. Comparative study of synonymous codon usage variations between the nucleocapsid and spike genes of coronavirus, and C-type lectin domain genes of human and mouse. Experimental & Molecular Medicine. 2009;**41**(10):746-756

[137] Richardson JS et al. Enhanced protection against Ebola virus mediated by an improved adenovirus-based vaccine. PLoS One. 2009;**4**(4):e5308

[138] Muthumani K et al. Immunogenicity of novel consensus-based DNA vaccines against Chikungunya virus. Vaccine. 2008;**26**(40):5128-5134

[139] Ternette N et al. Immunogenicity and efficacy of codon optimized DNA vaccines encoding the F-protein of respiratory syncytial virus. Vaccine. 2007;**25**(41):7271-7279

[140] Zakhartchouk AN et al. Optimization of a DNA vaccine against SARS. DNA and Cell Biology. 2007;**26**(10):721-726

[141] Tsai CJ et al. Synonymous mutations and ribosome stalling can lead to altered folding pathways and distinct minima. Journal of Molecular Biology. 2008;**383**(2):281-291

[142] Spencer PS et al. Silent substitutions predictably alter translation elongation rates and protein folding efficiencies. Journal of Molecular Biology. 2012;**422**(3):328-335

[143] Zhou JH et al. The effects of the synonymous codon usage and tRNA abundance on protein folding of the 3C protease of foot-and-mouth disease virus. Infection, Genetics and Evolution. 2013;**16**:270-274

[144] Zhang F et al. Differential arginylation of actin isoforms is regulated by coding sequence-dependent degradation. Science. 2010;**329**(5998):1534-1537

[145] Katsnelson A. Breaking the silence. Nature Medicine. 2011;**17**(12):1536-1538

[146] Chen C et al. Dynamics of translation by single ribosomes through mRNA secondary structures. Nature Structural & Molecular Biology. 2013;**20**(5):582-588

[147] Irimia M et al. Evolutionarily conserved A-to-I editing increases protein stability of the alternative splicing factor Nova1. RNA Biology. 2012;**9**(1):12-21

[148] Jack BR et al. Reduced protein expression in a virus attenuated by codon deoptimization. G3 (Bethesda). 2017;**7**(9):2957-2968

[149] Le Nouen C et al. Genetic stability of genome-scale deoptimized RNA virus vaccine candidates under selective pressure. Proceedings of the National Academy of Sciences of the United States of America. 2017;**114**(3):E386-E395

[150] Stobart CC et al. A live RSV vaccine with engineered thermostability is immunogenic in cotton rats despite high attenuation. Nature Communications. 2016;**7**:13916

[151] Cheng BY et al. Development of live-attenuated arenavirus vaccines based on codon deoptimization of the viral glycoprotein. Virology. 2017;**501**:35-46

[152] Butt AM et al. Evolution of codon usage in Zika virus genomes is host and vector specific. Emerging Microbes & Infections. 2016;**5**(10):e107

[153] Rostad CA et al. A recombinant respiratory syncytial virus vaccine candidate attenuated by a low-fusion F protein is immunogenic and protective against challenge in cotton rats. Journal of Virology. 2016;**90**(16):7508-7518

[154] Diaz-San Segundo F et al. Synonymous deoptimization of foot-and-mouth disease virus causes attenuation in vivo while inducing a strong neutralizing antibody response. Journal of Virology. 2015;**90**(3):1298-1310

[155] Baker SF, Nogales A, Martinez-Sobrido L. Downregulating viral gene expression: Codon usage bias manipulation for the generation of novel influenza A virus vaccines. Future Virology. 2015;**10**(6):715-730

[156] Cheng BY et al. Development of live-attenuated arenavirus vaccines based on codon deoptimization. Journal of Virology. 2015;**89**(7):3523-3533

[157] Meng J et al. Refining the balance of attenuation and immunogenicity of respiratory syncytial virus by targeted codon deoptimization of virulence genes. MBio. 2014;**5**(5):e01704-14

[158] Le Nouen C et al. Attenuation of human respiratory syncytial virus by genome-scale codon-pair deoptimization. Proceedings of the National Academy of Sciences of the United States of America. 2014;**111**(36):13169-13174

[159] Nogales A et al. Influenza A virus attenuation by codon deoptimization of the NS gene for vaccine development. Journal of Virology. 2014;**88**(18):10525-10540

[160] Ni YY et al. Computer-aided codon-pairs deoptimization of the major envelope GP5 gene attenuates porcine reproductive and respiratory syndrome virus. Virology. 2014;**450-451**:132-139

[161] Aragones L et al. Fine-tuning translation kinetics selection as the driving force of codon usage bias in the hepatitis A virus capsid. PLoS Pathogens. 2010;**6**(3):e1000797

[162] Mueller S et al. Reduction of the rate of poliovirus protein synthesis through large-scale codon deoptimization causes attenuation of viral virulence by lowering specific infectivity. Journal of Virology. 2006;**80**(19):9687-9696

[163] Burns CC et al. Modulation of poliovirus replicative fitness in HeLa cells by deoptimization of synonymous codon usage in the capsid region. Journal of Virology. 2006;**80**(7):3259-3272