Origin and Evolution of Translation: A Unifying Perspective Across Time

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SUMMARY

Translation is a foundational biological process that decodes genetic information provided by an mRNA template. Over the past decade major advancements have been made towards understanding the origins and early evolution of translation. There remain two critical gaps: First, we lack a coherent view of how translation factors emerged and co-evolved to regulate cellular protein synthesis. Second, we know little about the evolutionary and environmental basis of variation and complexity of translation across the tree of life. Here we present a comprehensive survey of translation machinery diversity and similarity across bacteria, eukaryotes, and archaea with particular emphasis on the translation factors and ribosome. Finally, we interrogate translation at the sub-ribosomal, ribosomal and cellular scales and highlight research questions for the origin and early evolution of translation studies. The broad array of perspectives afforded by biological studies across the molecular to ecosystem levels may provide an opportunity to advance our understanding of the origins, complexity, and evolution of this fascinating machinery.

KEYWORDS: translation machinery, translation factors, origins of life, early life

INTRODUCTION

Translation is a universal information processing system present in all organisms across the tree of life. During translation, heritable genetic information in the form of messenger RNA is decoded to produce proteins, which themselves are responsible for carrying out the vast majority of molecular functions (**Figure 1**). Thought to have evolved ~3.5 billion years ago, translation machinery is referred to as a fossil, a machine that is frozen in time [1]. The core of the translation machinery and the genetic code it imparts are thought to have existed in their same basic forms with relatively minor modifications and elaborations over the billions of years since the time of the last universal common ancestor (LUCA) [2-4]. The emergence of translation as a means of genetically encoding protein functions may have been critical to the evolution of cellular life as a whole and remains a foundational property of all life on Earth [5]. While the core of the translation system remains highly conserved across the tree of life, elaboration on this core has occurred since the time of the LUCA, especially in the eukaryotic domain.

Here we discuss important aspects of the translation machinery with an emphasis on its origin and evolution. We present a synthesis of variations in translation across the three domains of life, and its complexity across biological scales. Our view centers on two gaps critical for understanding translation: the emergence and establishment of cellular life's earliest translation function and the evolutionary basis of variation of translation across the tree of life. In an attempt to bridge origin of translation studies with complex chemical system/future-looking origins of life work, we provide a unified framework of translation by outlining the processes that occur at sub-ribosomal, ribosomal and

cellular scales. Finally, we provide a summary of the outstanding questions and identify areas of inquiry that would benefit from approaches based on evolutionary biology, protein science, as well as complex systems studies to illuminate the origins and early evolution of translation.

Conceptual frameworks for reconstructing the earliest translation machinery

Fingerprints of ancient molecules exist in extant biology. Translation is likely both the most conserved biological system in evolution [6-8] and the oldest biological system that is retained in cells today. Yet, the process of translation does not leave any known isotopic or biomolecular trace in the ancient rock record, unlike other key metabolic uptake pathways that can be studied and dated through geological methods [9, 10] — there are, however, recent proposed efforts to use the action of translation to probe extant biological activity on a body of interest, for example [11].

Where the rock record falls short, extant genomes offer a way to reconstruct the history of translation. The "universality" of biological components – the features of life shared by organisms existing today – allows biologists to generate hypotheses regarding LUCA and early cells [12, 13]. Thus, the early evolution of translation prior to the LUCA can be reconstructed with greater confidence than most other biological systems. This so-called "*top-down"* approach to early life considers existing genetic systems and reconstructs the past biological states using genomic databases, *in silico* models and laboratory proxy studies [13, 14].

Complementary to the top-down approach, *bottom-up* research on the origins and early evolution of life approaches from the other end of the chronology: simulating potential

prebiotic chemistry or precursor life forms in laboratories or through computational models. This could involve *de novo* chemical synthesis of critical pathways [15], reconstituting RNA-replication systems inside primitive cells [16], generating polynucleotides that have some nascent functionality with each other and the environment [17, 18], or generating geochemical settings to replicate early Earth conditions to experimentally simulate origins of life processes [19].

According to the RNA World hypothesis, the current genetic system based on translation of nucleic acid polymers to form proteins was preceded by an RNA-based system in which RNA played both a genetic and functional role [20, 21]. A corollary to this hypothesis is that the translation machinery was distilled from a pre-existing network of functional RNAs interacting within a geochemical setting proximal to life's origins [22]. Despite the lack of a comprehensive theory about the pre-cellular era, we can assume that some fundamental processes predated translation: prebiotic entities could have manifested as networks of chemical reactions able to extract or channelize energy from different sources [23-27]. Such networks could have exhibited certain traits of complexity that would allow them to persist and evolve over time [28]. Following the prebiotic era, early cells represented by the LUCA are thought to have had a complex translation machinery like their extant counterparts [29, 30].

The biggest challenge in understanding the origin of translation is in the transition between a prebiotic or RNA-world era and the sophisticated translation system that appears to have been present by the time of the LUCA. A step-wise theory for the origin

of translation was proposed based on the possibility of autonomous self-replicating RNA molecules [2] (**Figure 2***, Stepwise evolution*). The self-replicating entities could have used embedded amino acids as cofactors that improved their own replication yield (*fitness*), in a selfish cooperation with other entities [31]. Later diversification would result in different components of translation, increasing the fitness of these entities. Thus, the origin of translation would be a case of incremental and continuous Darwinian evolution.

Analysis of the ribosome structure has provided an alternative step-wise model for the evolution of translation machinery via accretion [32] (**Figure 2**, *Accretion, "Onion Model"*). According to this model, the first emergent element would be a "Peptidyl Transferase Center" PTC that generated random peptides. Through time, the initial core would include other RNA regions and proteins binding to those regions, leading to new functionalities. The work from Bose *et al.* [33] has shown that the PTC can be isolated from the rest of the ribosome with some level of functionality, which could constitute a reasonable piece of evidence indicating a modular, ancient PTC. A similar model of accretion and hierarchical modularity builds on an evolutionary analysis of translation component traits [34] suggesting the emergence of translation in the translocation-related components [32, 34] (**Figure 2**, *Accretion & Hierarchical Modularity*).

Regardless of their specific details, these different models for translation origins all share the concept of early evolution of translation through exaptation: components that had a different, earlier function eventually generated a new structure that evolved and

diversified to generate what became the broader functions of the translation machinery [35]. Undoubtedly, much integrative work needs to be done to understand how translation machinery as-we-know-it evolved in the first place, and to understand how this machinery has persisted across billions of years. New insights into the emergence and establishment of the translation system can be gained by refreshing our look at translation, and by explicitly bridging its molecular, kinetic, and structural components with its evolutionary history.

TRANSLATION ACROSS THE TREE OF LIFE

Ribosomes are often considered to be largely unchanged molecular fossils [36], but in reality they exhibit a plethora of both conserved as well as functionally consequential divergent features across the tree of life [37]. Each ribosome is composed of small (SSU) and large subunits (LSU); the small subunit (30S in bacteria and archaea; 40S in eukaryotes) decodes the genetic code in mRNA while the large subunit (50S in bacteria and archaea; 60S in eukaryotes) catalyzes peptide bond formation at the peptidyl transfer center (PTC) [38]. This core set of components is shared by all living organisms and is crucial for understanding the origins of the ribosome (**Figure 3**, **Table 1**). On the other hand, ribosome structure and its interaction partners vary across the three domains of life, specifically with respect to the ribosome's size, abundances of ribosomal proteins (r-proteins), ratio of ribosomal RNAs (rRNAs) to r-proteins, and the number of steps required for assembly [39, 40] (**Figure 3**). Indeed, recent studies demonstrate how consequential some of these evolutionary signatures can be for translation function and ultimately for the cell.

Ribosomal expansion segments

A striking variation in the ribosome is seen in rRNAs which have insertions of sequence blocks that are referred as “expansion segments” [41-43]. The length and location of expansion segments vary within and between domains of life [44, 45] and the core rRNA sequence is conserved. For instance, bacterial and archaeal expansion segments (~50-250 base lengths) are shorter than those of eukaryotes (up to ~2400 base lengths) with a core, in addition to the ~4000 nucleotide long shared domain across all organisms (**Figure 3**) [43-46]. It is yet unknown whether the domain-specific rRNAs expansions are retained as a result of adaptation to domain-specific regulations [41], or through non-adaptive changes that were retained on the genomic architecture [47]. Some segments were shown to have potential roles for ribosome biogenesis [45], bridge formation between subunits [48], translation factor binding [49], or protein localization facilitation [50]. Based on the maintenance of ribosomal function in the face of these diverse elaborations, it is likely that these expansions may impact interactions of translation components with other cellular structures distinct for each lineage.

Translation factors

Translation factors are regulatory proteins that assist four mechanistically conserved steps of translation processes – initiation, elongation, termination, and recycling. The translation process as we know it cannot exist without these translation factors; however, different domains of life exhibit domain-specific properties and diversities of translation factors (**Figure 3**, **Table 1**) [51-53]. The general trend is that as the

174 complexity of the organism increases, the complexity of the translation factors, in terms
175 of the number of interaction partners, increases as well.

176

177 Initiation is one of the rate-limiting steps of translation that controls translation efficiency
178 and is a particularly interesting example of translation factor diversity [52, 54-56]. The
179 number of accessory proteins used in initiation across domains is usually accounted for
180 by the differences in mRNA structure [54]. Bacterial translation initiation involves only
181 three initiation factors (IFs) for the recruitment of mRNA while eukaryotic translation
182 initiation is much more complex and requires many IFs to recognize a matured mRNA
183 (**Figure 3**) [52]. Archaeal initiation is in between bacterial and eukaryotic initiation;
184 archaeal mRNA structure is similar to bacterial mRNAs and requires only a subset of
185 eukaryotic initiation factors (**Figure 3**) [57]. Beside the number of proteins, the structure
186 of the ortholog proteins show different characteristics. For example, bacterial IF2
187 includes an N-terminal domain that facilitates ribosomal subunit joining [58], but
188 archaeal IF5B lack this N-terminal domain. The evolutionary molecular function of the
189 IF2 N-terminal diversity in IF2 is not yet clearly known, but it is possible that N-terminal
190 evolution is related to ribosome specialization between bacteria and archaea by way of
191 impacting the tRNA recruitment and ribosome joining.

192

193 The elongation step is carried out by three elongation factors (EF-Tu, EF-Ts, and EF-G
194 in bacteria, which are homologs of a/eEF1A, a/eEF1B, a/eEF2 in archaea and
195 eukaryotes, respectively) (**Figure 3**). The functions, structures and sequence of
196 elongation factors are quite similar across the tree of life. Elongation factors, in

particular EF-Tu and EF-G proteins, have been subjected to evolutionary interrogation relatively more than other factors owing to their G-protein characteristics [59, 60]. Recent studies applied ancestral sequence reconstruction to reveal the evolution of elongation factors, more specifically EF-Tu. Phylogenetics and resurrection of EF-Tu suggested EF-Tu evolved from a generalist protein.- Unlike the extant counterpart, the ancestor can remarkably perform both in thermophilic and mesophilic ribosomes [61], and yet remains tightly coupled to the host translation machinery and physiology [61-65]. Intriguingly, while the evolutionary origin of IF2 is less well known, several paths have been proposed for the diversification of elongation and initiation factors from a single ancestor at the time of LUCA [59, 60, 66-68]. Recently, reconstruction of IF2/EF-Tu ancestry suggested that the common ancestor of IF2/EF-Tu exhibits characteristics of both IF2 and EF-Tu and evolved specialized functions after gene duplication [67]. The emergence of new translation factors by gene duplication from a less specialized ancestor indicates an early translation machinery with fewer, generalist parts [61].

What is revealed by surveying this remarkable molecular diversity is that the ribosome is not a frozen molecular machine. Its constituent proteins and RNAs, and the relationships between them, have been elaborated, tinkered, and altered in all domains of life across its full recorded history.

EVOLUTION OF TRANSLATION FACTOR PROTEINS AND NETWORKS

The translation process can be defined simply in terms of biomolecular inputs and outputs: an mRNA sequence is fed into the ribosome at one site, a sequence of amino

acids that correspond to the mRNA sequence is delivered, and a functional protein emerges at another site [69] (**Figure 1A**). Expanding upon this depiction with specific details of how each of these processes occurs quickly illustrates how tricky and convoluted translation actually is (**Figure 1B**). For example, many of the key components of the translation process occur in tandem, within and beyond the ribosome: the precise recognition and placement of tRNA complexes is carried out by inter- and intra-atomic forces that manifest at the level of interfaces deep within the ribosome [70]. At the same time, the assignment of an mRNA triplet codon to an amino acid is possible only by the proper loading of tRNA molecules in processes that occur via critical cyclic pathways outside the ribosome [71] (**Figure 1B**). The overall function of the ribosome is additionally regulated within physiological and cellular contexts through a myriad of epigenetic signals [72, 73], stress responses [74, 75] and transcriptional control [76-78]. Translation is thus an essential activity whose critical operative processes occur at various scales of operation in the cell.

A great deal of research into the origins of translation has centered upon the origins of the central structures of the ribosome [32-34, 36, 38, 43, 79-85]. The ribosome is obviously a hub at the level of cellular physiology, as all other translated proteins in a cell must originate there. Yet, many of the macromolecules interact with the broader translation machinery in direct and essential ways [86]. A comprehensive network depicting the proteins and RNAs that perform translation can simultaneously map protein-protein or protein-RNA interactions without omitting or oversimplifying interactions occurring at different scales (**Figure 4**). Critical mechanisms and

243 relationships that enable translation that differ across scales can be reduced to three
244 main process levels. **(i) Sub-ribosomal**: translation is a process resulting from a
245 network of atomic- and molecular-force interactions that correlate triplets of
246 ribonucleotides with distinct amino acids. The interactions that lead to this correlation
247 are distributed across the activities of dozens of enzymes and r-proteins, but a plethora
248 of correlative interactions occurs within the ribosome [32, 43, 80, 83]. **(ii) Ribosomal**:
249 translation is a process resulting from an assembled group of macromolecular
250 components capable of coordinating the initiation, elongation, folding and termination of
251 a protein sequence that can carry out a distinct chemical function (*e.g.,* a chemical
252 reaction, sensing process, *etc.*) in a cell [87, 88]. Finally, **(iii) Cellular**: translation is a
253 process of coordinating the activity of dozens of enzyme- and ribozyme-interaction
254 partners and energy transducer molecules to power the process of peptide
255 polymerization against ambient conditions generally favorable for polymer hydrolysis
256 [86, 89, 90].
257
258 Translation is thus a very complex process consisting of highly interconnected
259 components and it is impossible to understand the evolution of translation machinery
260 but studying a single component in isolation. The ancestral states of several translation
261 proteins reveal deep evolutionary histories of each component [61-63, 65, 67], but
262 further work is required to understand the co-evolution of translation factors and how
263 their interaction and emergence factored into the origin of translation. Thus, the top-
264 down ancestry studies would benefit from the single component level to a network level
265 ancestry construction. Recent developments in ancestral network reconstruction

methods as well as complex system studies [91, 92] may leverage the top-down approaches in the origin of studies in general.

In addition to exploring the evolution of ribosome function across multiple scales of the functional hierarchy, other questions remain about the origin and evolution of translation, including:

- How did life end up with a system that is so universally shared and remained conserved in mechanism over billions of years?
- Did the components of the genetic code and translation machinery evolve in a specific order, or did they evolve simultaneously and assemble around the protein synthesis function? In the case of the latter, how did they first interact?
- What are the environmental factors that constrain translation evolution?
- Is the modern translation system the most optimized of all possible versions or could it be evolved into be a more efficient and accurate machine?

FINAL REMARKS

Translation is far from a single component show. It is akin to a jazz ensemble orchestrated through various key players including translation factors, cofactors, nucleotide exchange proteins and others. Therefore, for a thorough understanding of the evolutionary history of translation we need to consider the entire machinery as a self-organizing molecular entity that arose naturally within a background generated by geochemical circumstances. Translation belies a varied and complicated set of

relationships: i) between different portions of the ribosome with itself, ii) between the ribosome and its enzyme and RNA-structured interaction partners – the broader 'translation machinery' – iii) between each of these and a supporting array of metabolic substrates and reactions, and iv) between the combined whole of feedbacks maintained by translation and metabolism in the constructed cellular environment. Understanding the rules and exceptions of translation's function and diversity and mapping the full range of molecular interactions that enable it to exist, may be a necessity for understanding the very origins of cellular systems.

ACKNOWLEDGEMENTS

We acknowledge the funding by the Wisconsin Alumni Research Foundation, the John Templeton Foundation Grant #61926 and the the Universidad Politécnica de Madrid (UPM) Margarita Salas Fellowship, founded by the "Unión Europea-NextGeneration EU" (code UP2021-035).

GLOSSARY

Molecular fossil: referred to distinctive molecular entities usually remained conserved thus shared by all organisms.

Ribosomal core ("core"): the parts of ribosomal RNAs and ribosomal proteins which are universally shared by all domains of life.

Expansion segments: referred to the insertion of RNA segments into the ribosomal core.

Molecular dark age: The time interval when prebiotic chemistry starts to self-organize in a way to give rise to biological life.

Top-down: includes the analytic approaches to understand ancient life based on extant organism.

Bottom-up: the approach to synthesize life's building blocks from a chemistry-based environment.

Dark side of biology: referred by Carl Woese as the problem for complete understanding of gene expression processes which is missing understanding translation evolution.

Peptidyl Transferase Center (PTC): The region in the large ribosomal subunit that catalyzes the chemical reaction for peptide bind formation.

Translation factors: regulate proteins that catalyze translation reactions in each step.

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628 **FIGURES AND TABLES**

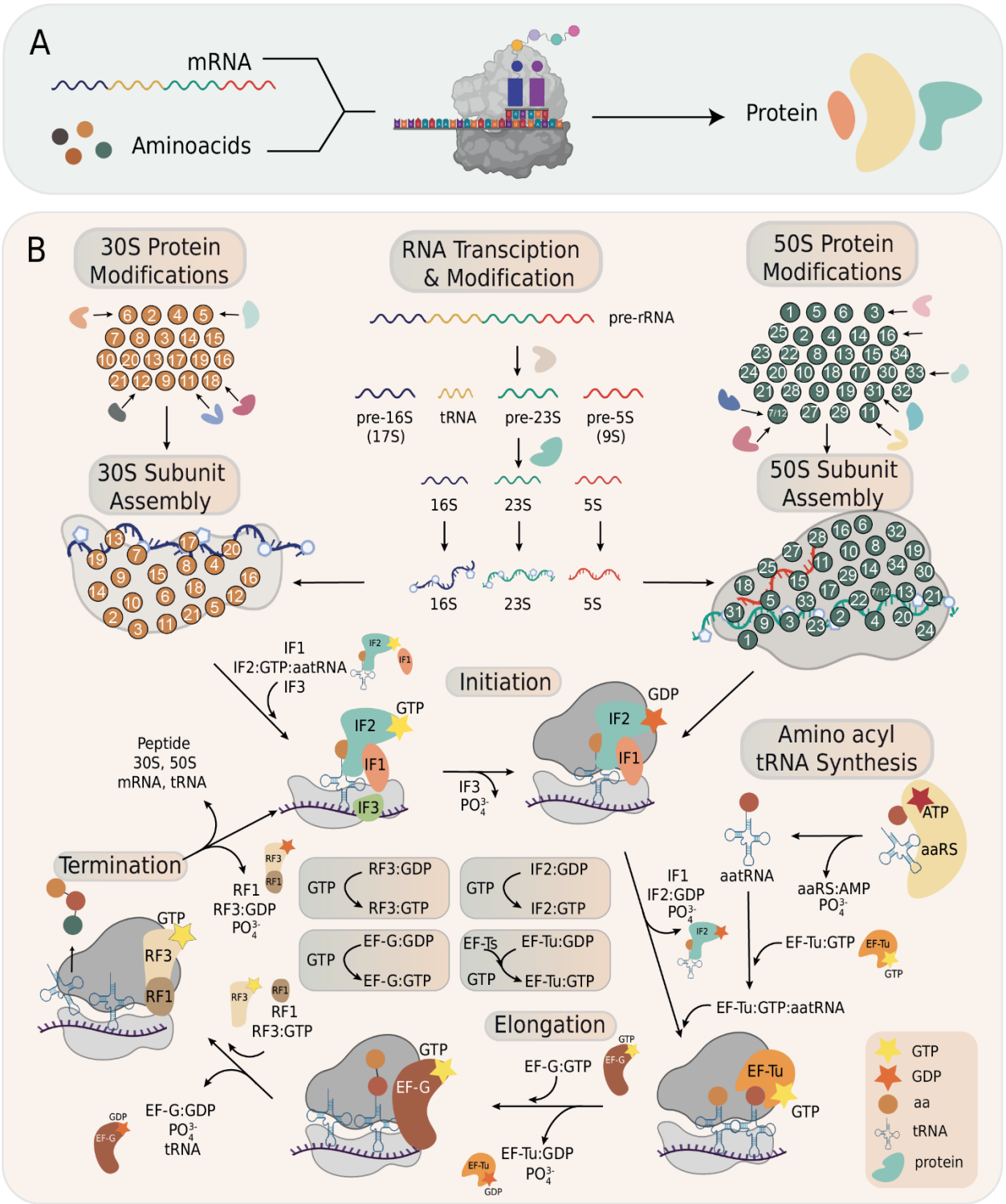

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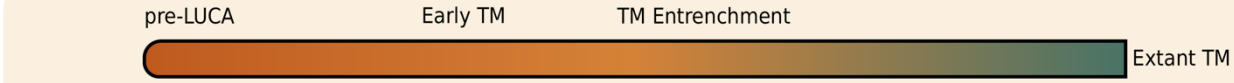
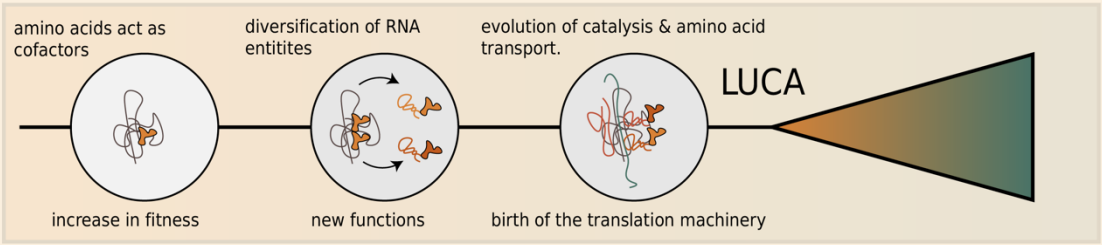
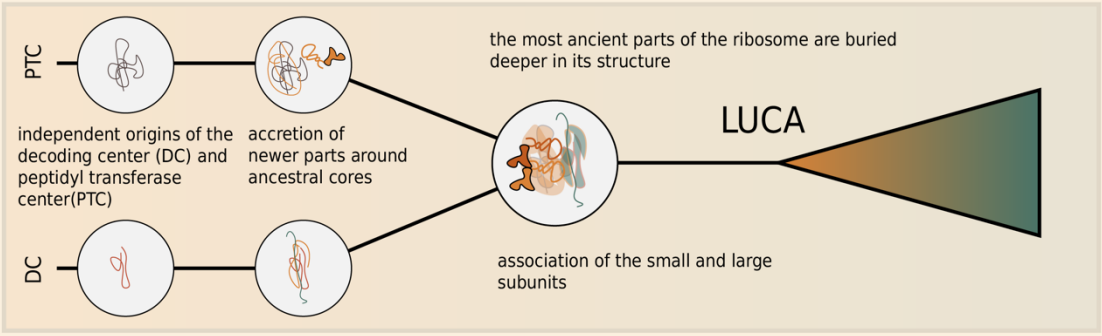
Figure 1 (A) Overview of translation depicts the ribosome decoding the information embedded in messenger RNA (mRNA) to build proteins. **(B)** A comprehensive view of translation in the bacterium, *Escherichia coli*. Beginning with the modification of the 30S (small subunit) and 50S (large subunit) ribosomal proteins, these proteins are built and modified by other proteins, and by the addition of ribosomal RNAs (rRNAs), which were transcribed and then processed and modified as shown. Amino-acylated tRNAs are prepared by aminoacyl tRNA synthetases (aaRS), which use energy from ATP hydrolysis to attach amino acids to the acceptor stem of the tRNA. Translation is initiated when IF1, IF2, and IF3 associate with the small subunit, mRNA, and initiator tRNA; the large ribosomal subunit then binds to the initiation complex. Elongation proceeds as elongator tRNAs are carried by EF-Tu and acylated tRNAs' anticodon sequences are matched to the codon sequences of the mRNA; amino acids are disassociated from the tRNAs and joined together by peptide bonds to form a growing polypeptide chain. The tRNAs and mRNAs are translocated on the ribosome by EF-G. When a stop codon is reached, RF1 and RF3 associate with the ribosome to release the mRNA, ribosomal subunits, and finished polypeptide.



Stepwise Evolution



Accretion (*"Onion-Model"*)



Accretion & Hierarchical Modularity

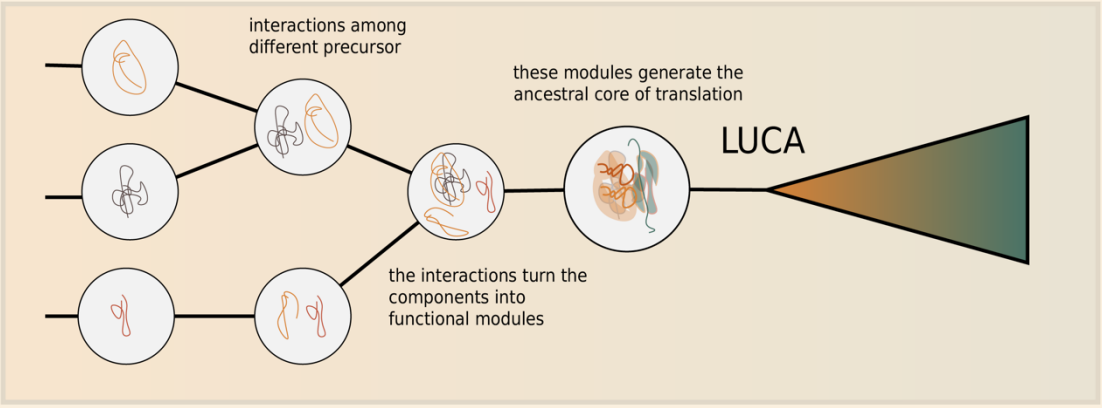


Figure 2 Proposed models on the emergence of the translation machinery. Upper panel: Stepwise evolution of the translation machinery. Amino acids and then smaller peptides provide a fitness benefit to RNA replicator entities. Later diversification enables the evolution of new specific functions (catalysis, transport of amino acids). Translation as we know it emerges following an early translocation mechanism. Medium panel: "Accretion Model" of the ribosome. Ancient RNA entities with independent origins perform different functions. Over time, newly evolved parts are layered onto the preexisting RNA entities, burying the earlier parts inside the ribosome. The association of the entities grown around the DC and PTC give place to the small and large ribosome subunits, and their association, to the ancestral ribosome. Lower panel: Accretion & Hierarchical modularity. Many different primordial RNA entities with independent origins interact with each other. Such interactions turn these entities into co-dependent functional modules that precede the extant translation machinery. DC= Decoding center. PTC=Peptidyl transfer center.

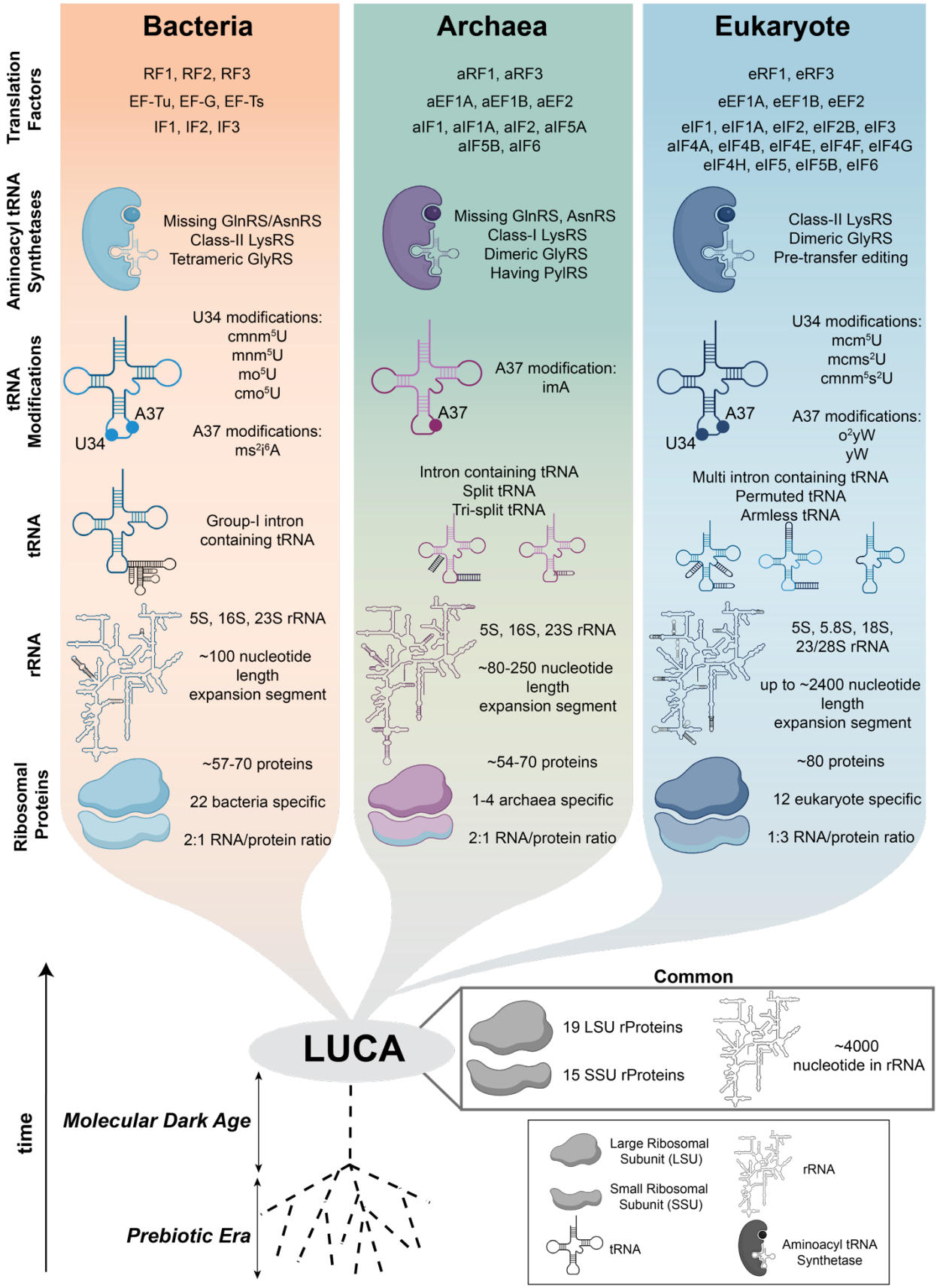

Bacteria
Archaea
Eukaryote
Translation Factors
RF1, RF2, RF3
EF-Tu, EF-G, EF-Ts
IF1, IF2, IF3
aRF1, aRF3
aEF1A, aEF1B, aEF2
aIF1, aIF1A, aIF2, aIF5A
aIF5B, aIF6
eRF1, eRF3
eEF1A, eEF1B, eEF2
eIF1, eIF1A, eIF2, eIF2B, eIF3
aIF4A, eIF4B, eIF4E, eIF4F, eIF4G
eIF4H, eIF5, eIF5B, eIF6
Aminoacyl tRNA Synthetases
Missing GlnRS/AsnRS
Class-II LysRS
Tetrameric GlyRS
Missing GlnRS, AsnRS
Class-I LysRS
Dimeric GlyRS
Having PylRS
Class-II LysRS
Dimeric GlyRS
Pre-transfer editing
tRNA Modifications
U34 modifications:
cmnm5U
mnm5U
mo5U
cmo5U
A37
U34
A37 modifications:
ms2i6A
A37 modification:
imA
A37
U34 modifications:
mcm5U
mcms2U
cmnm5s2U
A37
U34
A37 modifications:
o2yW
yW
tRNA
Group-I intron containing tRNA
Intron containing tRNA
Split tRNA
Tri-split tRNA
Multi intron containing tRNA
Permuted tRNA
Armless tRNA
rRNA
5S, 16S, 23S rRNA
~100 nucleotide length expansion segment
5S, 16S, 23S rRNA
~80-250 nucleotide length expansion segment
5S, 5.8S, 18S, 23/28S rRNA
up to ~2400 nucleotide length expansion segment
Ribosomal Proteins
~57-70 proteins
22 bacteria specific
2:1 RNA/protein ratio
~54-70 proteins
1-4 archaea specific
2:1 RNA/protein ratio
~80 proteins
12 eukaryote specific
1:3 RNA/protein ratio
Common
19 LSU rProteins
15 SSU rProteins
~4000 nucleotide in rRNA
LUCA
time
Molecular Dark Age
Prebiotic Era
Large Ribosomal Subunit (LSU)
Small Ribosomal Subunit (SSU)
tRNA
rRNA
Aminoacyl tRNA Synthetase

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Figure 3 An overview of variation of translation components across three domains of life. Bottom, the transition from prebiotic era to early translation. However, the transition from the prebiotic era to the last universal common ancestor (LUCA) is not well-understood. Translation in the LUCA possibly had some common components that are universal in all cells. After the LUCA, the organisms diverged to bacteria, archaea and eukaryotes which evolved domain-specific features and diversity in translation components.

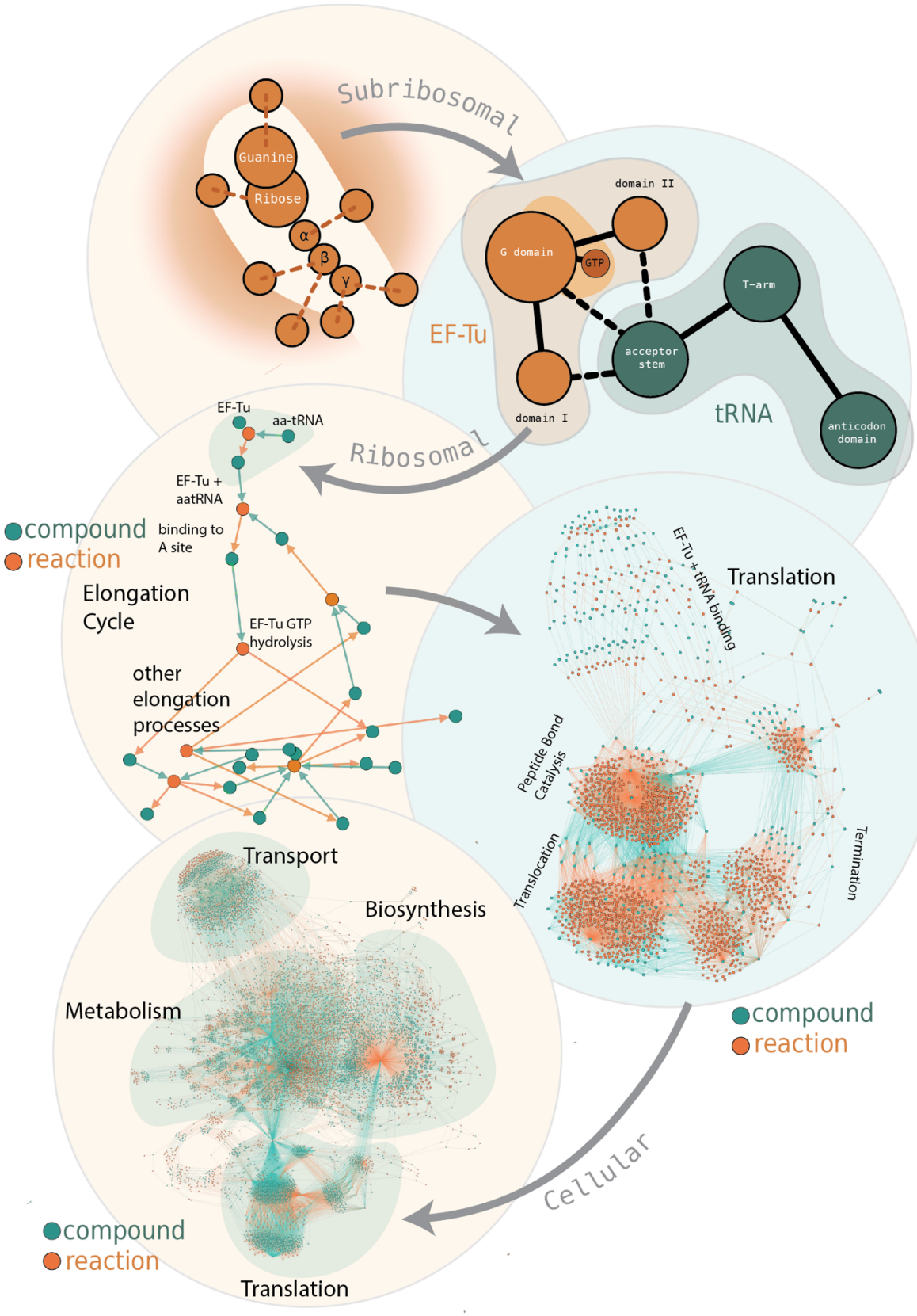
Subribosomal
Guanine
Ribose
α
β
γ
EF-Tu
domain II
G domain
GTP
T-arm
acceptor stem
domain I
tRNA
anticodon domain
Ribosomal
EF-Tu
aa-tRNA
EF-Tu + aatRNA
binding to A site
Elongation Cycle
EF-Tu GTP hydrolysis
other elongation processes
compound
reaction
Translation
EF-Tu + tRNA binding
Peptide Bond Catalysis
Translocation
Termination
compound
reaction
Transport
Biosynthesis
Metabolism
Cellular
compound
reaction
Translation

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Figure 4 A map of the functional relationships of translation across different scales of organization. *Sub-ribosomal* relationships are based on interatomic interactions that determine the specificity and conversion mechanisms between molecular complexes (in the case illustrated here, the recognition of GTP by a binding site within the EF-Tu protein). Groups of different interactions at the *Ribosomal* level leads to chemical reactions and assembly processes, as depicted here for the interaction between the acceptor loop of a tRNA and EF-Tu-GTP. Groups of components and their interactions create the physiological environment found within a cell by forming and maintaining dynamic energy transduction networks that guide specific inputs to yield specific outputs and waste products. Over time, different physiological components interact with each other and with perturbations from the external environment to create *cellular-level* changes of state such as growth, reproduction, or persistence.

691 **TABLE 1** The diversity of translation components across three domains of life.
692

	Bacteria	Archaea	Eukaryote
Ribosome	70S (SSU: 30S; LSU: 50S)	70S (SSU: 30S; LSU: 50S)	80S (SSU: 40S; LSU: 60S)
Ribosomal RNAs (rRNA) [a]	5S, 16S, 23S	5S, 16S, 23S	5S, 5.8S, 18S, 25/28S
Ribosomal Proteins (r-proteins)	~54-70 r-proteins 22 bacteria specific r-proteins	~54-70 r-proteins 1-4 archaea specific proteins	~80 r-proteins 12 specific proteins
	Extension segments at universal L2, L3, L4, S13 and S14 are variable across three domains of life		
Ribosome Modifications	Pseudouridinylation, methylation of hydroxyl groups and nucleotide bases	Methylation of ribose sugar methylation	Methylation of hydroxy and ribose sugar
tRNA Diversity	Group I intron containing tRNA genes in some bacteria Lack ANN anticodons	Intron containing, split, tri-split and permuted tRNA	Intron containing, permuted, nematode specific and armless tRNA Lack eight of GNN anticodons

[a] The diversity of "Expansion segments (ESs)" of rRNAs across three domains of life is given in the text.

tRNA Modifications	Carboxylaminomethyl, methylaminomethyl, methoxy and oxyacetic acid modifications at U34 position Methylthreonylcarba-moyl, and methylthioisopentenyl modifications at A37 position	Demethylwyosine modification at A37 position Archaeosine modifications at other positions	Methoxycarbonylmethyl, methoxycarbonylmethylthiol and carboxymethylaminomethylthiol modifications at U34 position Methylthiothreinylcarbamoyl, wybutosine and peroxywybutosine modifications at A37 position
Aminoacyl tRNA Synthetases (aaRS) Diversity	GlnRS is often missing Class II LysRS is present GlyRS is in tetramer form Some have PylRS Some lack editing domain use trans-editing and post-transfer editing	GlnRS is absent Class I LysRS is present GlyRS is in dimeric form Some have PylRS	Class II LysRS is present GlyRS is in dimeric form Predominantly uses pre-transfer editing
Initiation Factors	IF1 IF2 IF3	aIF1, aIF1A aIF2 aIF5A, B aIF6	eIF1, eIF1A eIF2, eIF2B eIF3 eIF4A, B, E, F, G, H eIF5, eIF5B eIF6
Elongation Factors	EF-Tu EF-Ts EF-G	aEF1A aEF1B aEF2	eEF1A eEF1B eEF2 (Some have EF3)

Termination Factors	RF1 RF2 RF3 RRF	aRF1 aRF3	eRF1 eRF3

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694

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