

Translation initiation: variations in the mechanism can be anticipated

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Abstract Translation initiation is a critical step in protein synthesis. Previously, two major mechanisms of initiation were considered as essential: prokaryotic, based on SD interaction; and eukaryotic, requiring cap structure and ribosomal scanning. Although discovered decades ago, cap-independent translation has recently been acknowledged as a widely spread mechanism in viruses, which may take place in some cellular mRNA translations. Moreover, it has become evident that translation can be initiated on the leaderless mRNA in all three domains of life. New findings demonstrate that other distinguishable types of initiation exist, including SD-independent in Bacteria and Archaea, and various modifications of 5' end-dependent and internal initiation mechanisms in Eukarya. Since translation initiation has developed through the loss, acquisition, and modification of functional elements, all of which have been elevated by competition with viral translation in a large number of organisms of different complexity, more variation in initiation mechanisms can be anticipated.

Keywords Translation initiation mechanism · mRNA · Ribosome · Initiation factor · Archaea · Bacteria · Eukarya · Virus · Evolution

Evolution of translation initiation: from simple to highly complex

Principles of protein synthesis are very similar across all three domains of life [1, 2], i.e., Archaea, Bacteria, and Eukarya [3, 4]. The genetic code, ribosomal RNA, and proteins are highly conserved in all organisms. Translation initiation is a key step for protein synthesis. In the majority of cases, it is a rate-limiting and most influential step of the process. A variable number of structural and functional elements is used by different organisms to improve the control of translation initiation, some of which provide an advantage over other competitors. It is most likely that for the above reasons translation initiation has diverged significantly among different groups of organisms and has adopted more individual mechanisms than other steps of translation.

Between prokaryotes (Archaea and Bacteria) and eukaryotes (Eukarya) there are some remarkable differences in RNA (rRNA and mRNA) and proteins (ribosomal and IFs), which are involved in the translation initiation. In prokaryotes, the small ribosomal subunit 30S, composed of ~1,500-nucleotide-long 16S rRNA and ~21 proteins, and the large ribosomal subunit, comprised of 3,000–3,200 nucleotides of rRNAs and 31–34 proteins, form a 70S ribosome. The eukaryotic counterparts, the 40S subunit (1,800–1,900-nucleotide-long 18S rRNA and ~32 proteins) and 60S subunit (3,600–5,000 nucleotides of rRNAs and 45–49 proteins) yield the 80S ribosome [5–7].

In prokaryotes, the 3' end of 16S rRNA is equipped with an anti-SD sequence that interacts with the Shine-Dalgarno

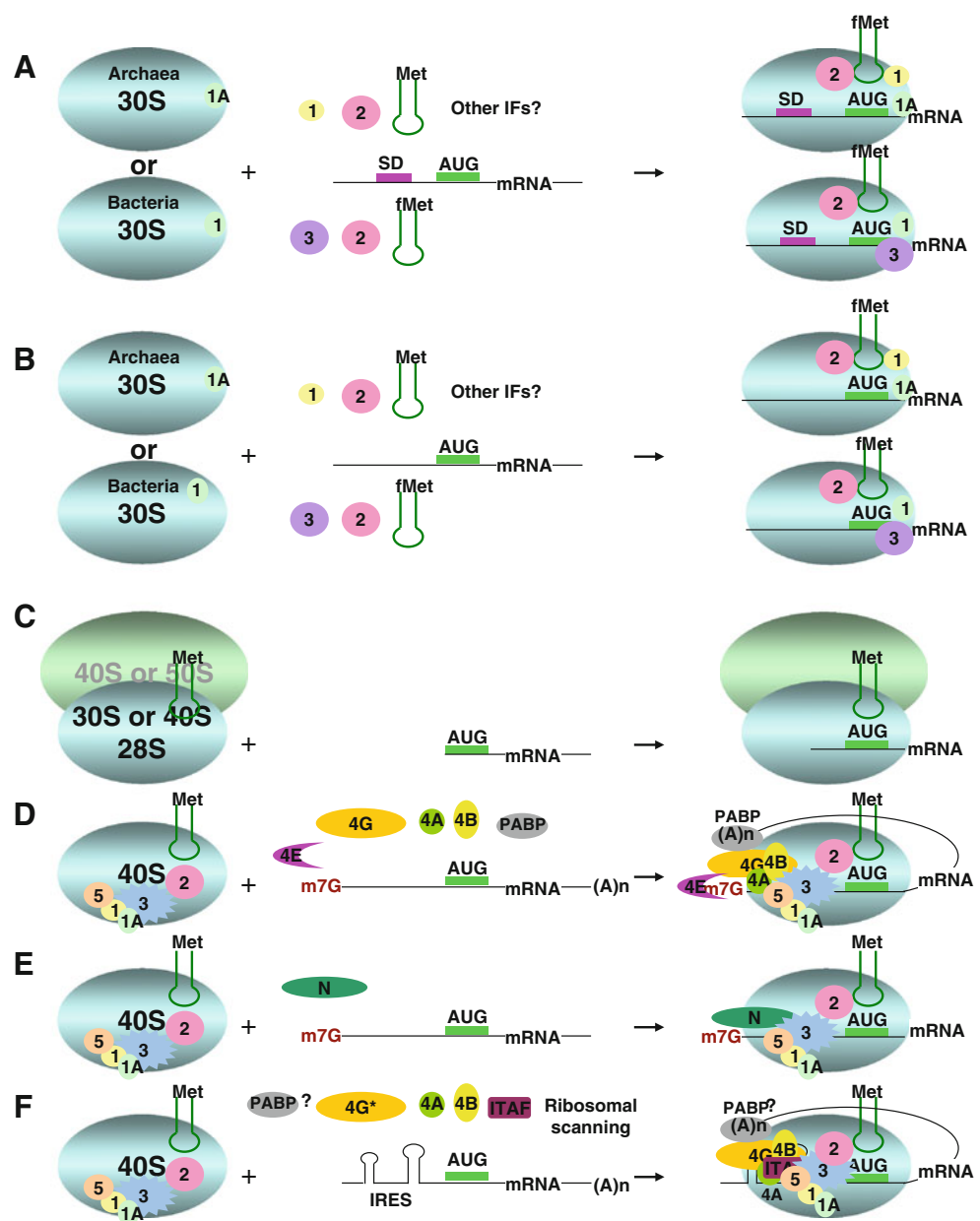
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(SD) sequence located at the 5' untranslated region (UTR) of the open reading frame (ORF), or initiation may involve ribosomal protein S1 that interacts with pyrimidine-rich sequences upstream of the SD region [2, 8–10]. These interactions directly stimulate formation of the initiation complex (Fig. 1a). More than one ORF can be transcribed into polycistronic mRNA, 5' and 3' ends of which are unmodified. In contrast, the majority of eukaryotes use the 'cap' at the 5' end of mRNA for the primary interaction during the initiation complex formation [11–13] (Fig. 1d). The mRNA is usually monocistronic with a capped 5' end and polyadenylated 3' end. These differences between prokaryotes and eukaryotes have most likely evolved for a number of reasons, e.g., (1) prokaryotic translation is

directly coupled to the transcription with translation initiation occurring instantly after the beginning of mRNA synthesis, and RNA is protected by ribosomes from nucleases, whereas eukaryotic mRNA has to be transported from the nucleus before being translated [14–16]; (2) in prokaryotes, a relatively large number of ribosomes translates the same mRNA transcript, and ribosomes do not necessarily have to be recycled through the mRNA circularization like in eukaryotes [14, 17]; (3) the relatively short living transcript is sufficient in protein production for a small and quickly dividing prokaryotic cell, while in eukaryotes, to allow mRNA transport from the nucleus to cytoplasm and to produce the necessary amount of protein for the larger and more competitive cellular environment,

Fig. 1 Comparison of key translation initiation mechanisms. Figure shows simplified diagrams of the ribosome recruitment process up to the stage of AUG recognition. Ribosomal subunit initiation factors and mRNA with key structural elements are depicted. The following translation initiation mechanisms are shown: **a** SD-dependent, **b** SD-independent; both (**a**, **b**) are common to Archaea and Bacteria; **c** a leaderless mechanism, which occurs in Archaea, Bacteria, Eukarya, and mitochondria and may be initiated by small ribosomal subunits or 70S and 80S ribosomes; **d** 5' end-dependent, which is represented as a 'canonical' cap-dependent mechanism; **e** 5' end-dependent mechanism found in *Hantavirus*, which uses a viral multifunctional translation factor that substitutes cap binding complex; **f** internal initiation, which is represented as an IRES-driven mechanism. Initiation factors eIF1, eIF1A, and eIF2 of Archaea are labeled as 1, 1A, and 2; IF1, IF2, and IF3 of Bacteria are 1, 2, and 3; eIF1, eIF1A, eIF2, eIF3, eIF4A, eIF4B, eIF4E, eIF4G, and eIF5 of Eukarya are 1, 1A, 2, 3, 4A, 4B, 4E, 4G, and 5, respectively



the transcript has to be stabilized through the capping of the 5' end and mRNA secondary structure [16, 18, 19]; and (4) translation initiation in prokaryotes is tightly regulated through the polycistronic gene arrangement (occasionally with overlapped ORFs) causing coupled translation and translation driven by reinitiation [2, 20, 21], and through the primary mRNA sequence, e.g., the 5'-UTR region, which, in addition to the SD sequence, may include a pyrimidine-rich region for interaction with the ribosomal protein S1, a mRNA sequence that forms a secondary structure, a repressor protein binding site, and/or a riboswitch that binds low molecular weight effectors [10, 22–27]. Although eukaryotes occasionally use strategies that depend on the mRNA sequence, e.g., reinitiation or secondary structure, translation initiation is significantly more dependent on other elements, e.g., initiation factors [2, 12, 19].

Prokaryotes use a relatively simple mechanism involving a small number of initiation factors (IFs) such as IF1, IF2, and IF3 for Bacteria [2, 28, 29], and aIF1, aIF1A, aIF2, aIF5B, and aIF6 for Archaea [30–32]. Eukaryotes have a significantly greater number of IFs (core factors eIF1, aIF1A, eIF2, eIF2B, eIF3, eIF4A, eIF4B, eIF4E, eIF4G, eIF4H, eIF5, and eIF5B; auxiliary factors eIF6, PABP and others [33]), some of which are large multimeric complexes [12, 33]. Although the sequence similarity between bacterial and eukaryal IFs is quite obscure, strong homologies are found between archaeal and bacterial, and between archaeal and eukaryal IFs [34]. Structural and, more importantly, functional comparisons demonstrate that at least a small subset of factors is shared among all three domains, e.g., IF1, IF2, and IF3 in Bacteria; eIF1A, eIF2, and eIF1 in Eukarya; and aIF1A, aIF2, and aIF1 in Archaea, respectively [31, 34, 35]. These three universal IFs can be regarded as ancestral that most likely originated from Archaea [31].

The evolution of translation initiation has likely occurred through the shift from an archaeal to bacterial mechanism involving the loss of some factors and the simultaneous acquisition of more robustly controlled elements (e.g., 16S rRNA anti-SD sequence and mRNA SD sequence [35], and to an eukaryal mechanism through the gradual addition of IFs (e.g., eIF3, eIF4E, eIF4G, eIF4B, eIF5, etc. [4]) mRNA structural elements (cap structure and Kozak sequence [36–38]) and with some possible degeneration of unnecessary relics (e.g., IRES [39]).

In this paper, currently known translation initiation mechanisms are reviewed, placing a special focus on diverse cases evolved by viruses. The distribution and variation of these mechanisms across three domains of life will be discussed. Special emphasis will be placed on IFs and mRNA structures involved in translation initiation. The concept that suggests that translation initiation has evolved from the simplest leaderless mechanism will be discussed

[30, 31], and that the initiation mechanism has developed both 'horizontally' across and 'vertically' among Archaea, Bacteria, and Eukarya will be hypothesized. Research on viral RNA translation supports the idea that evolution has stimulated the mimicry, acquisition, or loss of translation initiation elements among different organisms [39, 40].

The SD-dependent mechanism in Archaea and Bacteria

The SD-dependent translation in prokaryotes requires SD interaction and is controlled by a few IFs and mRNA structural elements that are clustered within and in near proximity to the ribosome binding site (RBS) [2, 8, 25, 29]. First, bacterial IF3 binds to the 30S ribosomal subunit triggering 70S ribosome dissociation and release of 30S–IF3 complex for the next round of translation [8, 28, 34]. Then, IF1, IF2, mRNA, and fMet-tRNA associate with the small ribosomal subunit in an unknown and possibly random order, forming a 30S initiation complex (Fig. 1a). In Archaea, aIF1A, aIF1, aIF2, and mRNA unformylated Met-tRNA associate with the 30S ribosomal subunit; however, the order of the binding events is still unclear [31]. Translation initiation is stimulated through RNA duplex formation with SD sequence base pairs with the variable subset of the anti-SD sequence GAUCCACCUCCUUA in the 3' end of the 16S rRNA [41]. This SD interaction attaches an mRNA to the 30S ribosomal subunit, allowing selection of the start codon by the ribosomal P site. SD interaction strength and the distance between the SD sequence and start codon affect the initiation efficiency [42, 43]. Interaction between S1 and the pyrimidine-rich mRNA that precedes the SD sequence is essential in gram-negative bacteria [10, 22, 43]. The presence of a mRNA secondary structure in the RBS is usually regarded as an inhibitory element [44, 45]. Some evidence shows that specific mRNA folding in the RBS can stimulate translation, e.g., by the accommodation of the SD sequence in the single-stranded RNA region [46] or through the spatial structure in which apical loops of stable hairpins might directly interact with the ribosomal mRNA track [47]. Accessibility of the RBS including the SD region and thus translation initiation may be controlled by a large number of other factors, including small metabolites, RNA binding proteins, antisense RNAs, and temperature (Table 1) [8, 48, 49].

SD-independent mechanisms in Archaea and Bacteria

Recent findings have demonstrated that the SD interaction may not be that essential for the translation initiation of leadered archaeal and bacterial mRNAs (Fig. 1b). Bioinformatics have revealed that SD interaction elements are

Table 1 Translation initiation mechanisms: their types, distribution, regulatory elements, and potential subdivisions

Type of mechanism	Distribution across domains	Key control elements	Potential subdivisions of mechanisms and comments	References
Shine-Dalgarno dependent	Archaea, Bacteria (cellular and viral)	SD sequence that interacts with 3'-end of 16S rRNA; SD length, distance between SD, RNA secondary structure, and type of start codon affects initiation efficiency	A. SD sequence dependent only	[2, 41, 50]
			B. Protein dependent (e.g., polypyrimidine sequence and S1 protein interaction)	[10, 47, 49, 132]
			C. Sequence-specific (upstream and downstream) mRNA element dependent	[2, 19, 49, 133]
Shine-Dalgarno independent	Archaea, some cases in Bacteria	SD sequence is absent; initiation efficiency is regulated by 5'-UTR sequence; start codon AUG	A. 5'-UTR sequence dependent only	[54]
			B. Protein dependent (e.g., polypyrimidine sequence and S1 protein interaction)	[10, 52, 132]
			C. Sequence-specific upstream and downstream mRNA element dependent (e.g., downstream box and Shine-Dalgarno-like interactions)	[19, 47, 133]
Leaderless	Archaea, some cases in Bacteria and Eukarya	70S and 80S ribosome binds directly to AUG; initiation factor dependent	A. "Truly" independent	[24, 55–58, 61, 62]
5' end-dependent	Eukarya (cellular and viral)	40S subunit is recruited on 5'-cap structure by cap binding complex of eIFs; subunit scans until the first AUG is reached	A. Cap structure dependent	[11, 13, 65]
			B. Protein dependent (variable set of IFs may be involved in the translation initiation; some extreme cases exist: cap binding complex eIF4 replaced by a single multifunctional protein in <i>Hantavirus</i>)	[12, 13, 33, 81]
			C. Sequence-specific 5'-UTR and 3'UTR mRNA element dependent (e.g., Kozak element, polyA tail, short uORF, etc.)	[2, 19, 38, 95, 134]
			D. Cap structure like dependent (cap function replaced by covalently linked viral protein to the 5' end of RNA)	[82–84]
Internal initiation	Eukarya (viral, some potential cellular cases)	IRES within the 5'-UTR; ITAFs promote recruitment of 40S subunit to the IRES; cap, polyA tail or both may be absent	A. IRES-driven	[85–88, 117–119]
			B. Protein dependent (variable set of IFs may be involved in the translation initiation; ITAFs)	[33, 85, 92, 135]
			C. Sequence-specific 5' end and 3' end mRNA element dependent (e.g., viral coat protein, NSP3, etc.)	[84, 124–126, 134]
			D. 5' end and 3' end mRNA secondary structure dependent (e.g., tRNA mimicry, 3'CITE, etc.)	[84, 137, 138]

significantly less conserved in Euryarchaea and Crenarchaea [50]. A large-scale analysis of completed prokaryotic genomes showed that a significant proportion of ORFs is leaderless or led by SD free 5'-UTRs [51]. These features of 5'-UTR are especially dominant in Archaea [52, 53]. In some cases of SD-independent translation, the initiation is mediated by ribosomal protein S1 [47, 52].

Recently, Hering et al. [54] experimentally characterized a new type of the SD-independent translation that occurs on the leadered mRNAs in Haloarchaea. This new initiation mechanism does not require a cap structure and has not led to the identification of the conserved 5'-UTR sequence. Further investigation has shown that it also does not involve a ribosome scanning as in Eukarya. Taken together, this newly discovered type of SD-independent translation and an overall high diversity of 5'-UTR of prokaryotic genes suggest that a variety of different mechanisms are employed in the process of initiation in prokaryotes, especially Archaea, and therefore more alternatives to the SD sequence-dependent mechanism can be anticipated (Table 1).

Leaderless mechanism in all three domains of life

For Archaea, Bacteria, and Eukarya, the translation initiation on leaderless transcripts is common in principle [55] (Fig. 1c). Until recently, leaderless mRNAs have been considered as a rare exception rather than a typically occurring type of transcript. Now, they are found in all three domains of life [24, 30, 56–58]. A high abundance of leaderless transcripts in Archaea [31, 56, 59] and a steadily increasing number of new examples in Bacteria and Eukarya [31, 57, 58], as well as their presence in eukaryotic organelle mitochondria [60], indicate that the leaderless translation initiation is a common phenomenon. Even more, Moll et al. [61] have suggested that the leaderless mRNAs may play a mediatory role in horizontal gene transfer at the translational level.

Several lines of evidence demonstrate that the leaderless initiation is significantly different from the other mechanisms as it can occur on mRNAs that have very short or no 5'-UTR and lack any substantial signals for the ribosomal recruitment except the one, the start codon (Table 1) [55]. Moreover, it can be driven by undissociated 70S or 80S ribosomes and in the absence of IFs [24, 56, 62]. Although the efficiency of initiation on leaderless transcripts is significantly lower than on the other type of transcripts, leaderless mRNA may have a structural advantage under distinct conditions, e.g., stress.

Since leaderless translation initiation occurs in Archaea, Bacteria, and Eukarya, as well as eukaryotic organelle mitochondria, this mechanism might be regarded as a

remnant of ancestral translation initiation and, because of its simplicity, could be considered to have an evolutionary origin [31]. This notion is also supported by the fact that 61S complex lacking a number of 30S proteins is found to be functionally effective in the translation of leaderless mRNA [63]. The irrelevance of the otherwise functionally important protein S1 (and some other proteins) to the leaderless translation suggests that it is likely these proteins were functionally linked to the ribosome later in evolution and that the leaderless translation evolved before domains of life diverged.

The 5' end-dependent mechanisms in eukarya

In eukaryotes, translation initiation is significantly more complex than in prokaryotes, involving many additional initiation factors and their isoforms [2, 12, 33, 64]. Until recently, it was thought that eukaryotic translation initiation mainly relies on a cap-dependent translation in which the formation of translation complex is initiated by the factor eIF4E that binds to the 7-methylguanosine cap structure at the 5' end of eukaryotic mRNA and tethers eIF4G [11, 65]. The latter, in the complex with other initiation factors eIF4A and eIF4B, mediates a recruitment of the 43S ribosomal subunit that is already associated with a number of IFs (eIF1, eIF1A, eIF2, eIF3, and eIF5) and initiator Met-tRNA, resulting in the 48S complex [12, 13, 33]. Poly(A)-binding protein (PABP) stimulates translation initiation by binding simultaneously to the 3' mRNA poly(A) tail and eIF4G, which triggers circularization of mRNA [17], which may facilitate translation reinitiation by the same mRNA translating ribosome [12], and enhances eIF4F binding to the cap [66]. Recently, it has been shown that poly(A)-binding protein-interacting protein 1 (Paip1) stabilizes PABP-eIF4G interaction [67]. Recruitment of ribosome is supported by the helicase activity (eIF4A and eIF4B) that unwinds a secondary structure at the 5' proximal region of mRNA [33, 68]. Subsequently, the small ribosomal subunit is enabled to scan along the mRNA transcript until the first start codon AUG is reached. Figure 1d represents a simplified scheme of translation initiation up to the stage of AUG recognition. The interaction of the initiator tRNA anticodon with AUG fixes the 43S at the start codon, allowing it to bind to the 60S ribosomal subunit, which completes a 80S complex. Now, it is becoming evident that eukaryotic ribosomes can also start translation from alternative start codons [69, 70].

Translation initiation factors eIF4E, eIF4G, and eIF4A, which form eIF4F, have been found to exist as different isoforms [13, 71]. For example, three eIF4Es have been identified in mammal *Mus musculus* (eIF4E-1, 4EHP, and eIF4E-3) [72] and plant *Arabidopsis thaliana* (eIF4E,

eIFiso4E, and nCBP) [73], and two in yeast *S. pombe* (eIF4E-1 and eIF4E-2) [74], while *Caenorhabditis elegans* contains five isoforms [75]. In the latter, a large proportion of mRNAs contains an unusual 2,2,7-trimethylguanosine cap structure. Three isoforms (IFE-1, IFE-2, and IFE-5) bind this type of cap, whereas the other two, IFE-3 and IFE-4, bind only the usual 7-methylguanosine cap structure. All three mammalian eIF4Es can bind to the 7-methylguanosine cap structure [72]. However, only eIF4E-1 interacts with both the initiation factor eIF4G and the eIF4E-binding proteins (4E-BPs) that repress translation. eIF4E-2 and eIF4E-3 have differentiated functions, since the second isoform interacts only with 4E-BPs, while the third only with eIF4G. It is thought that only one of the isoforms is accountable for 'steady-state' cap-dependent translation, whereas the others are required under specific environmental conditions, e.g., stress [74, 76]. Multiple isoforms have also been found for eIF4G (two different forms) and eIF4A [13].

Some eIF4E and eIF4G family members in complex with other proteins are involved in specialized functions, including translational repression of specific mRNAs, e.g., eIF4E in the complex with Maskin represses CPE-containing mRNA translation (for other examples, see [64, 71]). Factor eIF4E has also been implicated as a molecular target in translation repression by miRNA [77, 78]. PABP participates in mRNA turnover and stability [79, 80]. These findings demonstrate a high level of complexity in the translation initiation of eukaryotes (Table 1).

A significantly different 5' end-dependent mechanism, which does not use the cap binding complex, has been discovered in the Sin Nombre virus belonging to the *Hantavirus* genus of the *Bunyaviridae* family [81]. This minus-strand RNA virus possesses a N protein that simultaneously binds to the cap structure and a small ribosomal subunit. This single protein performs and can replace three biological functions that are normally associated with the cellular eIF4E, eIF4G, and eIF4A (Fig. 1e). More surprisingly, protein N is also associated with other functions in the virus life cycle, the replication and viral nucleic acid encapsulation. As this protein is relatively small, only 48 kDa in size, more detailed study on the initiation mechanism is required to understand how so many functions can be performed by a single macromolecule.

Other 5' end-dependent initiation mechanisms that have evolved in viruses are discussed below.

Internal initiation mechanisms in Eukarya

Presently, an increasing number of published findings demonstrate that a 5' end-independent translation involving

an internal initiation may function in parallel to the cap-dependent translation in eukaryotes. Moreover, this mechanism is widely exploited by viruses. Here, translation is initiated at the internal ribosomal entry site (IRES), which usually contains highly structured elements within the 5'-UTR of the transcript. Numerous studies of the viral IRES-based translation have shown that, although many canonical eIFs are involved in the initiation, a wide structural diversity and a high degree of variation in IF requirements exist [33, 82–84].

Currently, four types of IRES are distinguished [33, 85]. The schematic representation in Fig. 2b–e shows key elements and factors involved in the IRES-driven translation initiation. Here, a small ribosomal subunit is recruited in a few different ways that require a structured mRNA at the 5'-UTR and a distinct number of canonical eIFs except for eIF4E and the N-terminal domain of eIF4G to which eIF4E binds [33, 85–87]. Translation initiation driven by type 1 and type 2 IRESs can occur with the help of IRES transactivating factors (ITAFs) [82, 88, 89], the majority of which are characterized as RNA-binding proteins [90–92] and possess a translation initiation function that is not yet fully defined [93–95] (Fig. 2b, c). Type 3 IRES (hepatitis C virus-like) can directly attach 40S–eIF2–eIF3–eIF5 complex to the initiation codon without the requirement of eIF1, eIF1A, eIF4B, and eIF4F (Fig. 2c). Few proteins have been proposed to function as ITAFs in hepatitis C virus translation [83, 96, 97]. Type 4 IRES within the intergenic region of cricket paralysis virus (CrPV) mimics a tRNA directly recruiting 40S or 80S ribosome without initiation factors and initiator tRNA, and initiates translation at a non-AUG codon from the ribosomal A-site [98–101] (Fig. 2d). Other examples of internal initiation mechanisms in viruses are discussed in the following section.

More than 2 decades ago, IRES was found to be essential for the initiation of viral translation [102–104]. Now, there is much evidence that when the cap-dependent translation is compromised, e.g., during stress, mitosis, apoptosis, etc., the translation initiation in 10–15% of cellular transcripts occurs via cap-independent mechanisms, some of them potentially involving IRES [105]. However, unlike viral internal ribosome entry site elements, none of the proposed cellular IRESs have been convincingly verified [106].

So far, a widespread structural and functional diversity has been shown among the studied cases of cellular IRESs and noncanonical *trans*-activating factors involved in the IRES-dependent initiation [12]. IRES activity has been found in functionally different mRNA elements [107, 108]. The lack of a well-defined model and shortage of experimental evidence showing that IRES function can be reconstituted in vitro for any cellular ORF [12, 109] are

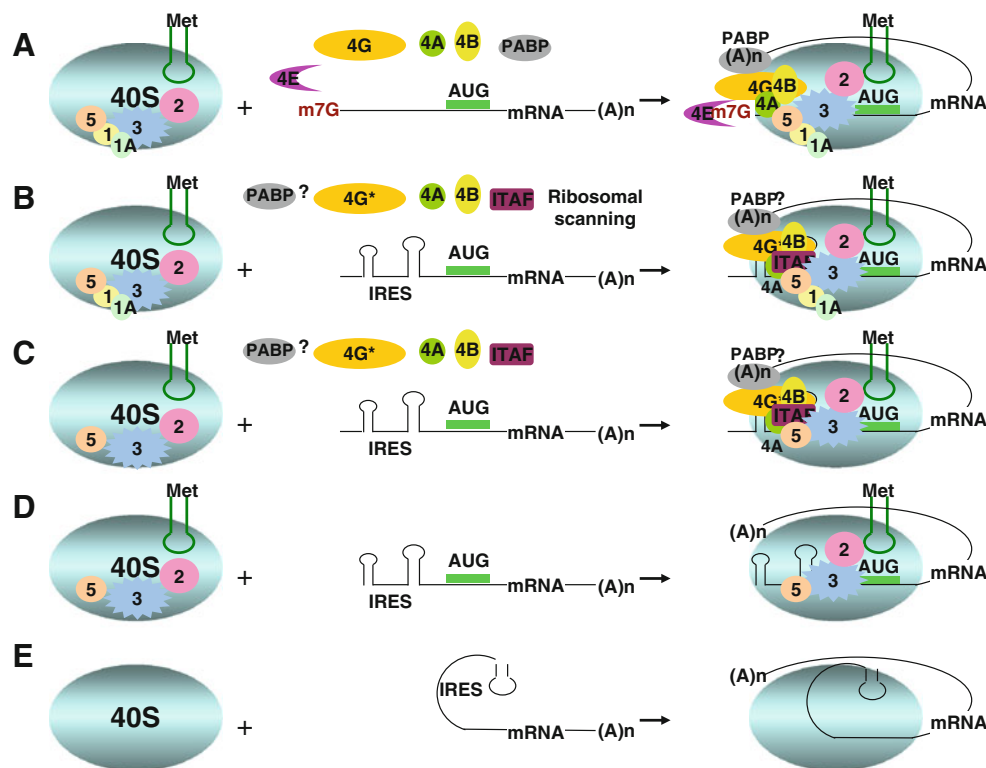


Fig. 2 Variations of IRES types in the viral translation initiation. The diagram is a schematic representation of the ribosomal subunits, IFs, and mRNA involved in the IRES-driven translation. Highlighted mechanisms are adopted from [33, 82]. Canonical initiation requires the 5' capped mRNA and all IFs (a), whereas IRES-driven initiation uses a broad variety of IRESs and different subsets of IFs (b–e). b, c are found in *Picornaviridae* and require ITAFs. b IRES-driven initiation involving the majority of canonical eIFs except eIF4E and ribosomal scanning that occurs prior to the start codon is reached (e.g., poliovirus (PV), classed as type 1 IRES by [33]). c The initiation

codon is located immediately after the 3' end of IRES. Here, ribosomal scanning does not occur, and eIF1 and eIF1A are not required [e.g., encephalomyocarditis virus (EMCV) [86, 87] (type 2)]. d Hepatitis C virus (HCV, *Hepacivirus* genus, *Flaviviridae*) IRES directly attaches Hepatitis C virus ribosomal subunit to the initiation codon, and limited eIFs are required (type 3). e Initiation in CrPV (*Cripavirus* genus, *Dicistroviridae*) that has adapted IRES, which mimics a met-tRNA, and translation initiation (type 4) does occur. All initiation factors are labeled as in Fig. 1. 4G*, truncated eIF4G

still preventing a detailed understanding of this molecular mechanism.

Viruses may drive the evolution of translation initiation

Viruses have adopted a huge variety of tools that enable them to propagate in the cellular environment. In order to overcome the cellular defenses and to prevail against cellular transcripts while competing for the translational machinery, viruses have evolved a number of factors and other modifications that provide an advantage for the translation of their mRNAs in the host cell [40, 110, 111]. The majority of cases are based on the modulation of cellular translation factors by viral activities, making them more efficient and selective towards the viral mRNA [112, 113]. Most commonly, viruses escape translation competition with the capped cellular mRNAs via IRES-driven translation [85, 88, 114]. IRES elements differ in size,

include various secondary structures, and require different sets of IFs (Fig. 2) for their function [105, 115, 116].

For example, plus-strand RNA viruses that lack the 5'-cap, 3'-poly(A) tail, or both use alternative strategies for translation initiation [117], which include features of 5' end-dependent and internal initiation mechanisms and are enhanced by virus-born proteins or RNA elements, e.g., viral coat protein (CP) [118], internal sequence (IS) [119], 3'-cap-independent translational enhancer (3'CITE) [120, 121], etc., all of which are adopted to recruit host translation apparatus. Structural variations of 5' and 3' UTRs in plus-strand virus RNA have been reviewed recently [122]. Common features and differences in the translation initiation of some plus-strand RNA viruses studied so far are summarized in Table 2 and illustrated in Figs. 2e and 3.

An interesting case is found in TEV and FCV viruses, where, in the absence of 7-methylguanosine cap structure, the viral genome-linked protein (VPg) is covalently bound to the 5'-end of RNA contributing to the replication and

Table 2 Translation initiation elements in plus-strand RNA viruses

Virus	eIF4E or eIF-iso4E	eIF4G or eIF-iso4G ^a	IRES	PABP	Met-tRNA	Cap structure	PolyA tail	Other 3' element	References
PV, EMCV	–	+	+	±	+	–	+		[33, 82–85, 115, 122]
TEV, FCV	±	+	+	+	+	VPg ^c	+		[117, 123, 124, 136]
AMV	+	+	–	CP ^b	+	+	–	TR ^d	[117, 118]
DENV	+	+	±	+	+	+	–	IS ^e	[115, 119, 122, 131]
TYMV	+	+	–	–	–	+	–	TLS ^f	[117, 137]
BYDV, TNV	+	+	–	–	+	–	–	3'CITE	[117, 120, 138]
CrPV	–	–	+	–	–	–	–	TLS ^f	[33, 82, 122]

^a In some cases truncated forms are involved in viral translation initiation [118]

^b Viral coat protein (CP)

^c Viral protein covalently linked to the 5' end of RNA

^d 3' Terminal RNA region that binds CP

^e Internal sequence at 3' end that binds PABP

^f mRNA forms a tRNA-like structure (TLS) at the 5' or 3' end

stability of the viral genome [123, 124]. In addition, VPg interacts (and has likely co-evolved) with eIF4E and eIFiso4E [123, 125], but its precise role in translation initiation has not yet been fully elucidated.

Double-stranded RNA viruses, rotaviruses, lack the 3' polyA tail [126, 127]. Instead, the 3' end contains a nucleotide motif that is recognized by the viral protein NSP3, which also binds eIF4G. Due to its higher affinity to eIF4G, NSP3 is capable of replacing PABP and stimulating rotavirus mRNA circularization and translation initiation [95, 128] in a similar fashion as AMV virus (Fig. 3d). Retroviruses (*Retroviridae* family) contain a 5'-capped end of genomic RNA and may also include IRES elements in both mRNA circularization 5'-UTR and the gene coding region, which allows them to use alternative mechanisms (5' end-dependent and internal initiation) during the course of cell infection [129].

Ultimately, the great variety of mechanisms available for the translation initiation of viruses suggests that larger diversity of mechanisms can be expected for Eukarya, or even Archaea and Bacteria. This is likely to be found in the specialized cellular mRNA translation systems [40]. Since the origin of viruses remains unclear and the vast majority of viral biodiversity has not yet been studied, it may be that some of viruses have developed principally distinct and unique translation initiation mechanisms.

Concluding remarks

Understanding of translation has recently accepted that the initiation mechanism has diverged in many more ways than was initially thought. New results show that Bacteria, Archaea, and Eukarya contain common or closely related

mechanisms, which have evolved from the same origin as proposed by Londei [30] and Hernandez [130], and that the leaderless mechanism can be regarded as the original ancestor of translation initiation that has evolved in Archaea [30, 31].

Current knowledge about translation does not fully appreciate the existing variety of initiation mechanisms as yet. An overwhelming proportion of the biodiversity of life still has not been essentially explored in all three domains of life, as only about 6,000 species have been sequenced out of the estimated 7 million species identified overall. Here, it is suggested that the variety of translation initiation mechanisms is more complex than has been elucidated so far. A large number of other mechanisms and their variations can be expected to be discovered in the future. Certainly a major research focus should be kept on viruses as they are important shuttles that are able to take and transfer genes from species to species. Viruses are most likely to inherit different types of mechanisms that may have existed earlier in evolution and are still present now. Although we cannot expect from the viruses to be able to retain multiple IFs that are involved in the translation initiation of cellular organisms, the study of their molecular principles will shed light on the origins and variations in the initiation mechanisms. Evidence of this is that the less controllable mechanisms (e.g., leaderless) or those requiring fewer mechanisms (e.g., IRES-driven) have evolved in the early stage of the development of translation initiation machinery [31, 39]. In the later stages of evolution, more complexity and initiation factors were incorporated into translation initiation, resulting in a much more controllable and robust mechanism such as a 5' end-dependent translation and SD-based initiation. This may suggest that several intermediate mechanisms among the leaderless and

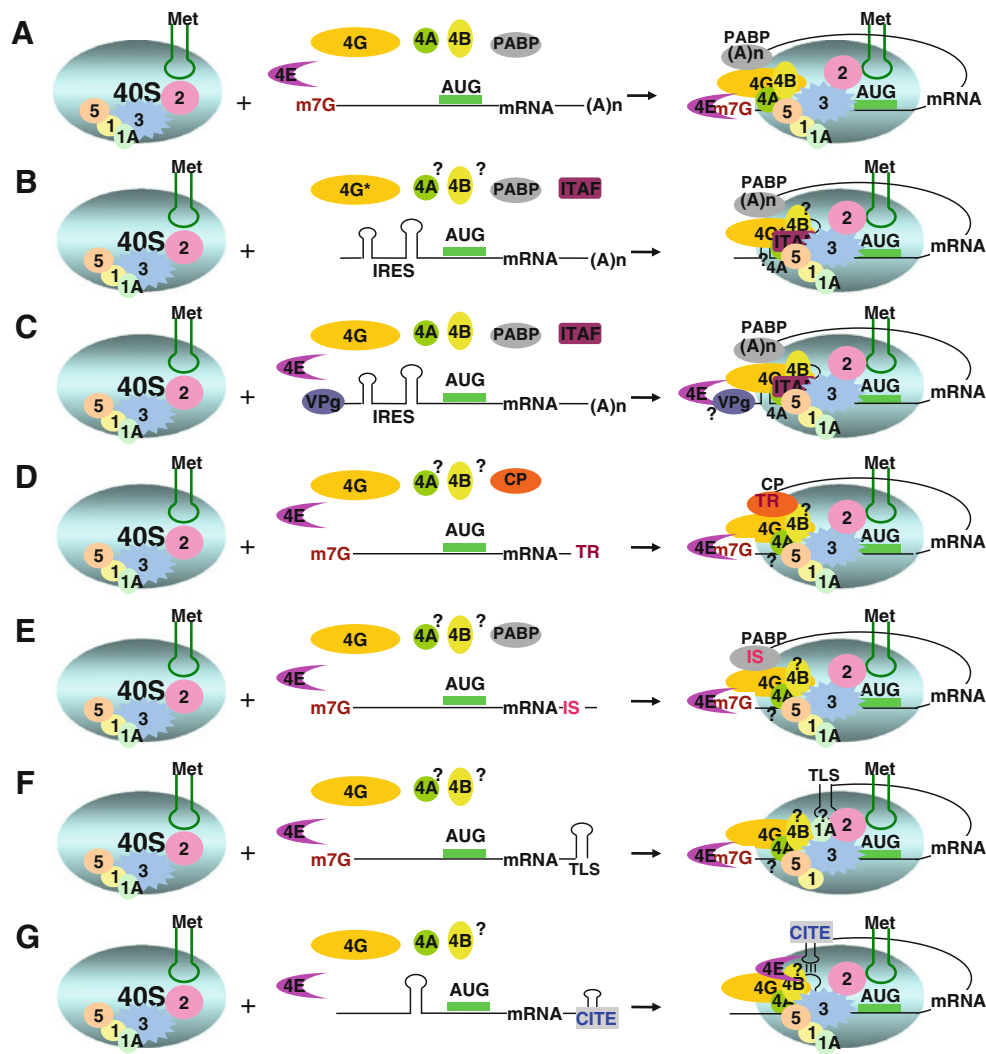


Fig. 3 Variations in the translation initiation mechanisms for plus-strand RNA viruses. The diagram depicts relatively well-established cases in virus families or genera, and does not represent in detail variations that occur inside the virus family or genus (not all factors are shown; question marks indicate unknown or uncertain details). **a** Initial 43S subunit recruitment step for the canonical cap-dependent translation. Diagrams (**b–g**) illustrate variations of initiation factors and structural elements that are involved in the initial initiation step for viral mRNAs that lack a 7-methylguanosine cap, poly(A) tail, or both: **b** translation is based on internal initiation via IRES (e.g., PV or EMCV); **c** initiation involves IRES and viral protein VPg that is covalently linked to the genomic RNA and interacts with eIF4E and eIFiso4E, but its role in translation initiation has not been fully understood [e.g., tobacco etch virus (TEV, *Potyviridae*) and feline calicivirus (FCV, *Caliciviridae*)]; **d** viral coat protein (CP) evicts the

PABP and stimulates translation by interacting with eIF4G and 3' terminal RNA region (TR) of the nonpolyadenylated viral RNA [e.g., alfalfa mosaic virus (AMV) from *Bromoviridae*]; **e** PABP stimulates translation by interacting with the internal sequence (IS) in the 3' end of the nonpolyadenylated RNA [e.g., dengue virus (DENV) from *Flavivirus* genus *Flaviviridae*]; **f** initiation involves the tRNA-like structure (TLS) in the 3' end of viral RNA [e.g., turnip yellow mosaic virus (TYMV) from *Tymovirus* genus (*Tymoviridae*)]; **g** noncapped and nonpolyadenylated RNA translation is initiated via 3'CITE [e.g., barley yellow dwarf virus (BYDV) from *Luteovirus* genus (*Luteoviridae*) and tobacco necrosis virus (TNV) from *Necrovirus* genus (*Tombusviridae*)]. Factors eIF4E or eIF-iso4E, and eIF4G or eIF-iso4G are shown under 4E and 4G, respectively. All other initiation factors are labeled as in Fig. 1

SD-dependent, 5' end-dependent, and internal initiation exist. Translation initiation has evolved under the influence of viral advantages and through the continuous exchange, loss, acquisition, and modification of functional elements among a large number of different complexity organisms. The existence of the vast diversity of viruses, with specific

adaptation and reproduction requirements for the different hosts, and their ability to infect more than one type of cell (organism) supports the idea of a larger array of possible intermediate stages and variations in the translation initiation. In addition to this, as has been shown in *Hantavirus* [81], TEV, and FCV [123, 124], and others [122, 129, 131],

considerably diverged extremes and modifications of the translation initiation can be anticipated in a higher variety and number.

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