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Review

The RNPs of eukaryotic translation control

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ABSTRACT

Protein synthesis is the essential cellular process of translating the genetic code into the major structural and functional biomolecule of the cell: the protein. Eukaryotic translation is a dynamic molecular process choreographed by translation ribonucleoprotein (trRNP) complexes that assemble upon a messenger RNA (mRNA) and regulate its interaction with the bimolecular catalyst of this event, the ribosome. From their transcription, mRNAs assemble within dynamic RNA-protein structures that regulate their stability, processing, localization, and ultimately their translation. Ribonucleoprotein (RNP) complex is a term used to broadly define these RNA-protein assemblies. trRNP complexes specifically relate to dynamic mRNA-protein structures that coordinate the translation process. trRNPs are at the core of eukaryotic translation control. They are endowed with the ability to regulate the localization, conformation, and activation of mRNAs, all features that regulate engagement with the ribosome and generation of protein product. This review provides a comprehensive analysis of the trRNPs of eukaryotic translation control. Mechanisms known for regulating eukaryotic trRNP activity will be discussed with reference to their significance in cell biology. The importance of distinct trRNPs in selective translation control will be highlighted with a specific focus on the DExH/D-box RNA helicase trRNPs and those of unique RNA binding proteins. The outcome is an enhanced understanding and appreciation for the role of RNP biology in the regulation of protein synthesis.

KEYWORDS: protein synthesis, eukaryotic translation control, translation RNP (trRNP), CBC trRNP, eIF4E trRNP, DExH/D-box RNA helicases

INTRODUCTION

Eukaryotic translation occurs in three mechanistic stages: initiation, elongation, and termination. Each stage is coordinated by a set of translation factors, which are RNA binding and/or scaffolding proteins that assemble upon an mRNA and regulate its engagement with the ribosome. These RNA-protein complexes give rise to the trRNPs of eukaryotic translation control. The integration of distinct RNA binding proteins, such as DExH/D-box RNA helicases, into this process creates selective trRNPs important for targeted protein synthesis.

Initiation trRNPs

Initiation is the rate-limiting step of protein synthesis with numerous regulatory mechanisms identified to control its activation and efficiency. The defining principle of eukaryotic translation initiation is a cap-dependent scanning mechanism whereby the ribosome binds to the 5' terminus of an mRNA and proceeds along the transcript, inspecting base-by-base, for an appropriate start codon to initiate polypeptide synthesis [1]. This process requires at least nine initiation factors (eIFs) and begins with the activation of an mRNA and its association with the 43S pre-initiation ribosome complex [1] (Figure 1).

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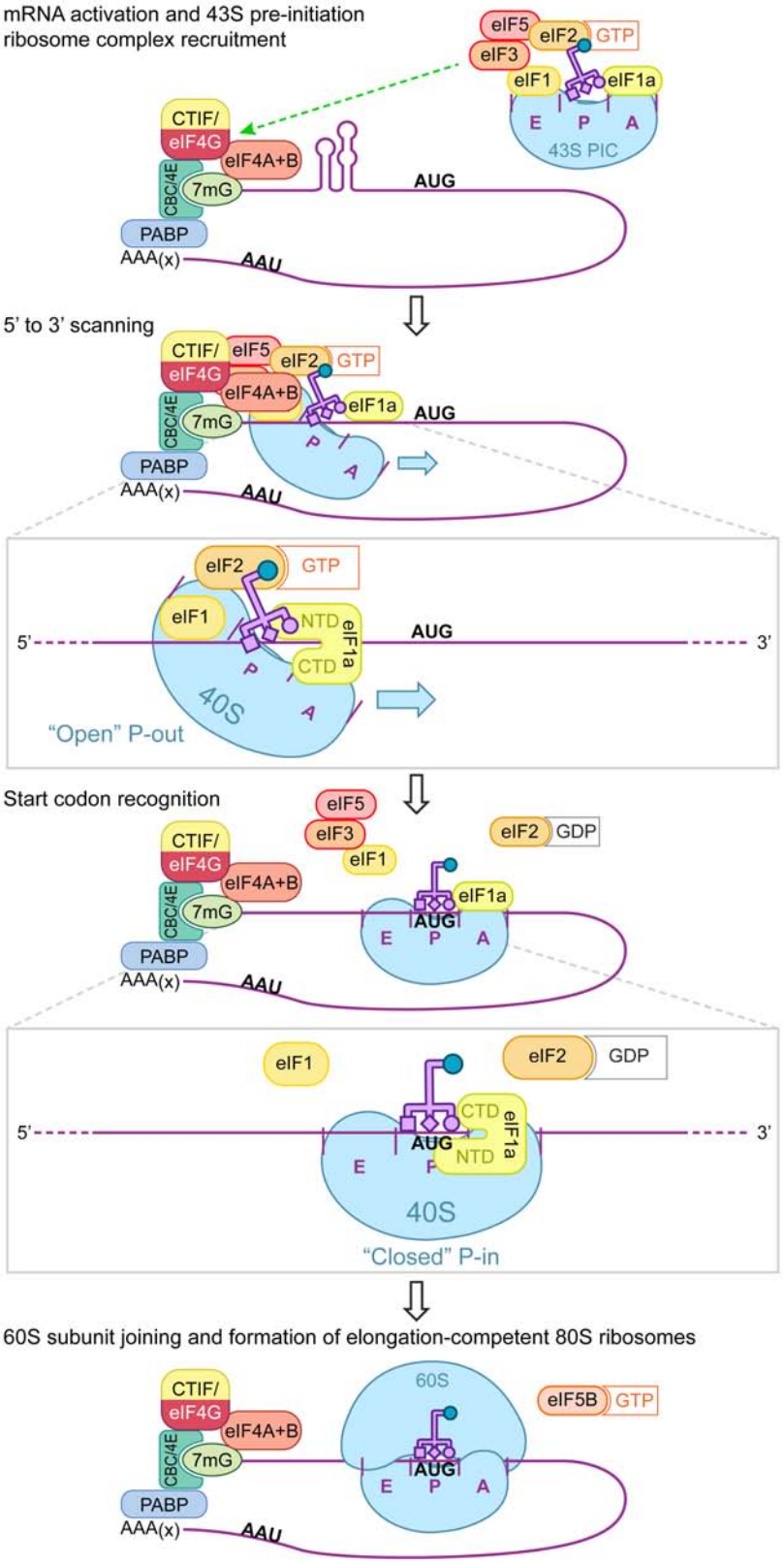
mRNA activation and 43S pre-initiation ribosome complex recruitment
eIF5
eIF2
GTP
eIF3
eIF1
eIF1a
E
P
A
43S PIC
CTIF/
eIF4G
eIF4A+B
CBC/4E
7mG
AUG
PABP
AAA(x)
AAU
5' to 3' scanning
eIF1a
Start codon recognition
eIF2
GDP
5'
3'
NTD
CTD
40S
"Open" P-out
"Closed" P-in
60S subunit joining and formation of elongation-competent 80S ribosomes
60S
eIF5B

Figure 1

Eukaryotic mRNAs are defined by a 5' 7-methyl guanosine cap, which is bound co-transcriptionally and throughout the lifespan of an mRNA by a cap-binding protein. The cap-binding protein, CBP80/20 or eIF4E, and its associated scaffolding factor, CTIF and/or eIF4G, generate a trRNP that primes an mRNA for translation (discussed further in section 'The CBC and eIF4E trRNPs'). This activation encompasses circularization of the mRNA via an interaction between the 5' scaffolding factor and the 3'-bound poly-A binding protein (PABP) to result in recruitment of the 43S pre-initiation ribosome complex to the 5' terminus of a transcript [2, 3] (Figure 1). The 43S pre-initiation ribosome complex consists of the small (40S) ribosomal subunit, initiator methionyl-tRNA, and five core initiation factors (eIF1, 1A, 2, 3 and 5), each with a distinct role in the recruitment and scanning process [1]. Interactions between eIF3 of the 43S pre-initiation ribosome complex and the 5' mRNA-bound scaffolding factor, CTIF and/or eIF4G, drive the association of the 43S pre-initiation ribosome complex with the activated mRNA [2-4] (Figure 1).

Once associated with a target mRNA, the 43S pre-initiation ribosome complex begins the scanning process in search of an appropriate start codon to initiate polypeptide synthesis. eIFs 1 and 1A are critical for facilitating this process. The positioning of eIFs 1 and 1A within the 43S pre-initiation ribosome complex confers structural arrangements that stabilize an 'open' conformation of the mRNA entry channel latch within the 40S ribosomal subunit [1-3] (Figure 1). This places the 40S ribosomal subunit and associated initiator methionyl-tRNA in a conformation that is conducive for movement along an mRNA and for base-by-base inspection [1-3]. Appropriate anticodon-codon base pairing between the initiator methionyl-tRNA and a start AUG triggers a conformational rearrangement in the 43S pre-initiation ribosome complex that causes the mRNA entry channel latch to close, stopping the scanning process [1-3] (Figure 1). This repositions the initiator methionyl-tRNA and 40S ribosomal subunit in a favorable context for joining of the large (60S) ribosomal subunit and formation of elongation-competent 80S ribosomes [1-3].

Interactions between mRNA sequence, eIF2 and the 18S rRNA facilitate favorable anticodon-codon base pairing that halts ribosome scanning [1-3]. Contacts between eIF2 and the 18S rRNA with distinct purine nucleotides at positions -3 and +1 relative to the adenine nucleotide within an identified AUG codon defines an optimal sequence context to end ribosome scanning and initiate polypeptide synthesis [1-3]. These associations induce conformational changes within the 43S pre-initiation ribosome complex that tighten the interaction of eIF1A with the 40S ribosomal subunit, displacing eIF1 and resulting in a 'closed' conformation of the mRNA entry channel latch with the initiator methionyl-tRNA oriented in an inward, locked position primed for subsequent peptide bond formation [1-3]. Ejection of eIF1 releases an inhibitory block on both ribosome conformation and eIF5-regulated eIF2 GTPase

Legend to Figure 1. Schematic of canonical eukaryotic translation initiation. Canonical eukaryotic translation initiation is characterized by a cap-dependent scanning mechanism whereby the ribosome binds to the 5' terminus of an mRNA and proceeds along the transcript, inspecting base-by-base, for an appropriate start codon to initiate polypeptide synthesis. This process begins with the activation of an mRNA and its association with the 43S pre-initiation ribosome complex (43S PIC). Interactions among the 5' associated scaffolding factor, CTIF or eIF4G, and the 3'-bound poly-A-binding protein (PABP) circularizes an mRNA to generate a translation ribonucleoprotein (trRNP) complex that engages with the 43S PIC. This occurs via stimulated interactions between the scaffolding factor and eIF3. 5' to 3' scanning follows, as the 43S PIC searches for an optimal start codon (AUG) to initiate polypeptide synthesis. Directed movement is facilitated by initiation factors eIF1 and eIF1A. Their positioning within the 43S PIC stabilizes an 'open' conformation of the mRNA entry channel latch and orients the initiator methionyl-tRNA in an outward position that is conducive for scanning. Start codon recognition occurs when an AUG is reached within an optimal sequence context and appropriate anticodon-codon base pairing occurs. This induces a conformational rearrangement in the 43S PIC that causes the mRNA entry channel latch to close and the scanning process to stop. Repositioning of eIF1A stimulates eIF1 release and rotation of the initiator methionyl-tRNA into an inward position that is favorable for 60S ribosomal subunit joining. Joining of the large (60S) ribosomal subunit is facilitated by eIF5B and results in the formation of an elongation-competent 80S ribosome primed for polypeptide synthesis.

activity that results in phosphate release, initiation factor dissociation and 60S ribosomal subunit joining [1-3].

Outstanding questions remain in regard to the mechanism(s) underlying net 5' to 3' movement of the 43S pre-initiation ribosome complex as well as the fate of the initial recruitment connections during the scanning process. Studies of dicistronic polypeptide production from eukaryotic viral transcripts indicate an oscillating 43S pre-initiation ribosome complex with both forward and reverse movements [5]. This fluctuating motion is critical to sequence surveillance and selection of an AUG for initiation of polypeptide synthesis [5]. Structural analyses support these molecular findings by presenting the ribosome as a 'processive Brownian motor' [5]. This model proposes that movement of the ribosome along a transcript is directed by interactions with the nearby environment, such as collisions with mRNA secondary structure or associations with protein cofactors. The conformational changes that result permit both forward and reverse movements of the ribosome that are intricate to the selection of an AUG for initiation of polypeptide synthesis. A sum of these localized fluctuations results in a net outcome of observed 5' to 3' directionality in ribosome scanning.

The initial attachment of a 43S pre-initiation ribosome complex to an activated mRNA requires interactions between eIF3 of the 43S pre-initiation ribosome complex and the 5'-bound scaffolding factor eIF4G or CTIF [2-4]. eIF4G also directly associates with the cap-binding protein eIF4E, regulating the stable association of eIF4E with the 5' cap [6]. The additional RNA binding activity of eIF4G facilitates this effect [6]. Furthermore, eIF4G directly associates with the 3' poly-A binding protein (PABP) to generate a circularized mRNA that is effective for 43S pre-initiation ribosome complex recruitment [2, 7]. Collectively, these eIF4G-driven associations link the 5' cap of a target mRNA with the 43S pre-initiation ribosome complex and are intricate to its recruitment and scanning activity. However, it remains to be determined whether these associations persist during ribosome scanning of the 5' terminus. If a connection remains between the 5' cap and 43S pre-initiation complex then the outcome supports a 'loop-out' effect of the 5' untranslated region whereby the scanned mRNA bulges as the ribosome continues forward [1]. This outcome would effectively allow only one scanning ribosome at a time [1]. Alternatively, the connections between the 43S pre-initiation ribosome complex and the 5' cap could be broken, allowing several ribosomes to translocate a 5' terminus simultaneously [1]. Advances in single molecule imaging technology and their application to understanding translation control will provide clarification on the fate of the initial recruitment connections during the scanning process.

The final step in the initiation stage of translation is joining of the large (60S) ribosomal subunit with the small (40S) ribosomal subunit at the start codon to generate an elongation-competent 80S ribosome (Figure 1). The translation initiation factor eIF5B mediates this effect. The presence of eIF5B and the 60S ribosomal subunit stimulates complete release of eIF2-GDP and eIF5 from the stopped 43S pre-initiation ribosome complex at the start AUG [2]. This dissociation allows for the interaction of eIF5B in a GTP-bound form with eIF1A, which remains temporarily associated with the 40S ribosomal subunit [2]. Binding of the 60S ribosomal subunit then follows, stimulating the GTPase activity of eIF5B [2]. The hydrolysis of GTP to GDP weakens the affinity of eIF5B for the ribosome, resulting in its release [2]. eIF1A subsequently dissociates to leave an 80S ribosome primed for elongation [2].

Several mechanistic aspects remain to be defined for this final stage of translation initiation. First, it is unknown how eIF5B and the 60S ribosome are recruited to a 43S pre-initiation ribosome complex [2]. Is eIF5B a component of this multifactor complex that then recruits the 60S ribosomal subunit when in an optimal conformation? Alternatively, does the recruitment of eIF5B to the 43S pre-initiation ribosome complex draw in the 60S ribosome or simply increase the likelihood of interactions between the small and large ribosomal subunits? Second, it is unclear as to what the molecular significance of the eIF5B-eIF1A interaction is in facilitating 60S ribosomal subunit joining [2]. Does the interaction of eIF5B with eIF1A stimulate conformational rearrangements in the 40S ribosomal subunit that favor appropriate 60S binding? Or

does an eIF5B-eIF1A association engage more in active recruitment? Ongoing structural studies are aimed at providing greater mechanistic understanding into the significance of eIF5B and eIF1A in the generation of elongation-competent 80S ribosomes.

DExH/D-box RNA helicase trRNPs

Intricate to mRNA activation and ribosome recruitment are DExH/D-box RNA helicases. DExH/D-box RNA helicases are both RNA binding proteins and enzymatic catalysts that couple the free energy of nucleotide triphosphate hydrolysis to RNA unwinding and/or RNP remodeling [8-11]. The outcome is dynamic structural rearrangements within the trRNP that regulate placement and then movement of the 43S pre-initiation ribosome complex along the 5' terminus of an mRNA [12].

Over fifty DExH/D-box RNA helicases have been identified with critical roles in post-transcriptional gene control. They are recognized by the presence of a core helicase domain within their protein structure that harbors nine conserved motifs involved in RNA binding, nucleotide triphosphate association, and its hydrolysis [8-11]. The DExH/D-box RNA helicases are identified by a distinct Asp-Glu-x-His/Asp (DExH/D) core within motif II, which lies within the center of the helicase domain and is critical for association with the β- and γ-phosphates of a nucleotide triphosphate [8-11]. Although conservation of sequence and function are critical for the classification of DExH/D-box RNA helicases, variations in the defining helicase core along with differences in flanking domains result in distinct trRNP association and function for each DExH/D-box RNA helicase (Table 1).

eIF4A (DDX2) is the DEAD-box RNA helicase most understood for regulating translation of eukaryotic mRNAs [12]. Functioning as an ATP-dependent RNA binding protein, eIF4A separates localized duplexed strands by coupling ATP-driven enzymatic changes to RNA unwinding [12].

Table 1. Known features of select DExH/D-box RNA helicase trRNPs.

	DDX2 (eIF4A)	**DDX48 (eIF4AIII)**	**DDX3**	**DHX29**	**RNA helicase A (RHA/DHX9)**
1. Cognate *cis*-acting mRNA element	$(CGG)_4$ motif/ G-quadruplex	Stable stem loop	Long, structured, and G-C rich	Long, structured, and G-C rich	Post-transcriptional control element (PCE)
2. Cap-dependent translation	Enhancer	Enhancer	Enhancer or repressor	Enhancer	Enhancer
CBC trRNP	+	+	-		To be determined
eIF4E trRNP	+	-	+	+	To be determined
Mechanism	mRNA unwinding to promote 43S PIC scanning	mRNA unwinding to promote 43S PIC scanning	mRNA unwinding and mRNP remodeling to facilitate 80S formation	mRNP remodeling to facilitate 48S formation	To be determined
3. IRES-mediated translation	Enhancer of type I and II		Enhancer of HCV (type III)	Enhancer of select type II; Interferes with select type III and type IV	Inferred not influential because PCE inactive

Its associations with eIF4G and the initiation factors eIF4B and eIF4H coordinate this activity near the 5' cap of an mRNA [12]. eIF4G, by virtue of its interaction with the cap-binding protein eIF4E and a target transcript, recruits eIF4A to the 5' terminus of an activated mRNA [1, 2]. The interaction of eIF4G with the helicase domain of eIF4A stabilizes eIF4A in a closed, RNA-bound conformation that stimulates its helicase activity [12]. This effect is also facilitated by eIF4B and eIF4H, which function to regulate the affinity of eIF4A for ATP and ADP during its ATPase-driven RNA remodeling as well as its association with a target mRNA [12]. eIF4B and eIF4H also have critical roles in preventing mRNA refolding and for directing the movement of the 43S pre-initiation ribosome complex in a 5' to 3' direction [12].

The necessity of eIF4A for translation initiation of cellular mRNAs harboring 5' secondary structure, which characterizes many eukaryotic transcripts, together with the impact of its mutation on global translation indicated eIF4A as a universal effector of protein synthesis [13]. This established eIF4A, together with eIF4G, as an integral member of the eIF4E trRNP complex regulating steady-state expression of the majority of cellular transcripts (discussed further in the section 'The CBC and eIF4E trRNPs'). However, a recent application of ribosome profiling to the study of eIF4A-dependent translation control revealed paradigm-shifting specificity to eIF4A-mediated protein synthesis [14].

Ribosome profiling is a recently introduced technique that has already revolutionized the study and understanding of eukaryotic translation control by allowing for an *in vivo* global characterization of translational landscapes, such as that of eIF4A-dependent translation control [15, 16]. By combining polysome profiling with nuclease footprinting and deep sequencing, this experimental approach provides high-resolution data on both ribosome abundance and positional occupancy along mRNAs in live cells [15, 16]. The outcome is two-fold: (1) the identification of mRNAs subjected to regulation within a translational landscape based upon a change in their number of ribosome footprints between control and experimental conditions and (2) a molecular basis for this effect based upon the accompanied accumulation in ribosome footprint reads at particular positions along the target mRNAs [15]. Distinct patterns that emerge from these results inform subsequent analyses that allow for identification of molecular signatures defining a particular translation landscape.

In the case of eIF4A, treatment of cells with silvestrol, a direct inhibitor of eIF4A helicase activity [17], revealed that eIF4A-sensitive transcripts are specifically defined by a 12 or 9-nucleotide $(CGG)_4$ motif within their 5' termini [14]. This $(CGG)_4$ motif forms a stable G-quadruplex secondary structure, sensitizing mRNAs to eIF4A helicase activity and identifying the eIF4A translational landscape [14]. This eIF4A translational landscape encompasses many prominent transcriptional regulators, including several super-enhancers [14], providing a molecular basis for the vast expression reprogramming observed upon mutation of eIF4A, even given its newly identified specificity in translation control. Thus, the significance of eIF4A in 43S pre-initiation ribosome complex recruitment and scanning is specific for a subset of mRNAs harboring its cognate *cis*-acting RNA element, the $(CGG)_4$ motif.

The repertoire of *cis*-acting structural elements found within the 5' termini of eukaryotic mRNAs is diverse, and extends beyond G-quadruplex structures to include various stem and internal loops, bulges, junctions and pseudoknots. The DExH/D-box RNA helicases eIF4AIII (DDX48), DDX3, DHX29, and RNA helicase A (RHA/DHX9) are intricate to the molecular basis by which these distinct *cis*-acting structural elements mediate 43S pre-initiation ribosome complex recruitment and scanning. Similar to eIF4A, the selectivity by which eIF4AIII, DDX3, DHX29 or RHA exert translational control is governed by distinct interactions between a defining *cis*-acting RNA element within the 5' termini of an mRNA and its cognate DExH/D-box RNA helicase. Together these select RNA-protein interactions generate distinct trRNPs important for targeted protein synthesis (Table 1). As discussed below, the discrete composition and architecture of each DExH/D-box RNA helicase trRNP confers targeted roles for eIF4AIII, DDX3, DHX29 and RHA in ribosome recruitment and scanning that extends beyond basic mRNA unwinding to encompass dynamic trRNP remodeling as well.

eIF4AIII is a known component of exon junction complexes, which are dynamic protein assemblies that organize upon the coding sequence of a mRNA with the conclusion of a splicing event to facilitate critical post-transcriptional activities. A recent study, however revealed the significance of eIF4AIII as a 5' interacting factor necessary for translation initiation of mRNAs harboring stable stem loop structures within their 5' UTRs [18]. The recruitment of eIF4AIII to the 5' terminus of a target transcript is facilitated by its direct interaction with the 5' scaffolding factor CTIF and is independent from its structural and functional integration within exon junction complexes [18]. The ATPase/helicase function of eIF4AIII is required for its stimulatory effect on translation initiation, indicating a role for this DEAD-box RNA helicase in resolving the stem-loop secondary structures to facilitate 43S pre-initiation ribosome complex association and scanning on target transcripts [18]. However, the mRNA features and/or protein cofactors that contribute to the specificity of the eIF4AIII trRNP in targeted 5'-cap-dependent translation control remain to be elucidated.

For DDX3, its functions in translation initiation are diverse and transcript-specific. In the case of cellular mRNAs with long and highly structured 5' UTRs, DDX3 is critical for translation activity via its helicase function and interaction with eIF4A and PABP of an activated mRNA as well as eIF2 and eIF3 of the 43S pre-initiation ribosome complex [19-21]. These identified cellular interactions indicate significance for DDX3 in functioning synergistically with eIF4A to resolve extensive secondary structure in the 5' terminus, allowing for ribosome association and scanning.

Similarly, DDX3 is necessary for resolving the complex secondary structure directly near the 5' cap of the human immunodeficiency virus type 1 (HIV-1) genomic RNA [22]. The outcome is productive translation and generation of the major viral structural protein Gag [22]. Distinctly, however, this requirement of DDX3 for targeted HIV-1 translation control is independent of its helicase activity [22]. Instead, it involves the association of DDX3 with eIF4G, PABP, and the viral protein Tat to generate a DDX3 trRNP that activates the target HIV-1 genomic RNA for 43S pre-initiation ribosome complex attachment and subsequent protein synthesis [22-24].

DDX3 also functions in late-stage initiation with targeted 80S ribosome formation [25]. Here DDX3 coordinates a protein bridge or conformational rearrangement within the 60S ribosomal subunit that facilitates its recruitment to and association with the 40S ribosomal subunit at the start AUG to generate an elongation-competent 80S ribosome [25]. This effect is critical for expression of hepatitis C virus in a manner that is independent of cap-dependent scanning activity [25].

Experimental data also indicate a role for select DDX3 trRNPs in inhibiting cellular cap-dependent initiation by functioning as a competitive eIF4E-binding protein and translational inhibitor [26]. DDX3 demonstrates a strong cellular affinity for eIF4E, which impedes its association with eIF4G [26]. The outcome is impaired eIF4E trRNP formation and repressed translation activity [26]. This effect is independent of DDX3's ATPase or helicase activity [26]. However, the cellular association of DDX3 with eIF4E and its significance for regulating protein synthesis remains controversial [21-23]. Likewise, the mRNA signatures and/or protein factors that dictate the specificity, both in composition and function, of the DDX3 trRNP in targeted translation control remain ambiguous.

The DExH-box RNA helicase DHX29 is critical for generating a trRNP that facilitates expression of cellular mRNAs harboring stable stem-loop structures within their 5' termini [27]. DHX29 associates with the 40S ribosomal subunit within a 43S pre-initiation complex [28]. It is positioned near the mRNA entry channel latch where it stimulates its opening to capture single-stranded bases that have been released from resolution of nearby stem-loop structure [27-29]. This activity of DHX29 in conjunction with the known roles of eIF4A in mRNA unwinding collaboratively facilitate association and scanning of the 43S pre-initiation ribosome complex along a target mRNA [27]. The outcome is ensured fidelity of base inspection and start codon recognition [27, 30]. Thus, DHX29 drives critical trRNP remodeling rather than traditional mRNA unwinding to facilitate efficient and appropriate translation initiation [28, 30]. The significance of this translation activity is seen in the silencing of DHX29 expression, which results in suppression of cellular translation by approximately fifty percent [31]. However, identification of the

molecular signatures, both mRNA sequence motifs and protein cofactors, which characterize the specific DHX29 trRNP and its translational landscape, have yet to be defined.

DHX9/RNA helicase A (RHA) is critical for facilitating the expression of retroviral transcripts, including the human pathogenic retroviruses HIV-1 and HTLV-1, as well as the cellular proto-oncogene junD [32-35]. Here, this DEIH-box helicase selectively associates with the 5' *cis*-acting post-transcriptional control element (PCE) that defines these target mRNAs [32-35]. The PCE is a G-C-rich, dual stem-loop structure that specifically regulates cap-dependent translation of harboring transcripts [33, 36-38]. Notably, the PCE functions as a positive *cis*-acting translational regulator [36]. This effect is mediated through its association with the *trans*-acting host factor RHA [32]. Suppression of cellular RHA expression reduces PCE translation activity, the association of target mRNAs with ribosomes, and subsequent protein production [32-34]. The outcome, as studied thus far, is impaired infectivity of HIV-1 progeny virions [34].

The role of RHA in cap-dependent translation control requires its association with the 5' terminal PCE as well as its ATPase activity, indicating canonical helicase function in its molecular basis of protein synthesis [34, 35]. However, RHA can also function in translation control independent of PCE binding. Here RHA is recruited to a target mRNA through association with a cognate RNA binding protein. Such is the case for regulated expression of the pluripotency factor Oct4 [39]. The RNA binding protein Lin28 recruits RHA to the Oct4 mRNA, which in turn facilitates ribosome association of the trRNP and subsequent protein expression [39]. A similar molecular basis for RHA-mediated translation control is critical for regulated expression of the type I collagen mRNAs [40]. The RNA binding protein La ribonucleoprotein domain family member 6 specifically recognizes and binds the defining 5' stem-loop structure of type I collagen mRNAs to bridge an association with RHA, stimulating translation activity [40]. Likewise, nuclear factor 90 bound to the 5' terminus of p53 recruits RHA to this target mRNA and facilitates its expression in response to DNA damage [41]. It is proposed that the recruitment of RHA to a target transcript, whether it be through direct PCE RNA binding or a protein bridge, facilitates trRNP remodeling in a manner that allows for 43S pre-initiation ribosome complex recruitment, scanning, and ultimately target protein production [32, 39, 40]. An unresolved issue, however, is a complete characterization of the RHA trRNP and how this informs its role in targeted translation control.

Collectively, the DExH/D-box RNA helicase family harbors several members with critical roles in 43S pre-initiation ribosome complex recruitment. Yet, as discussed, the functional significances for each DExH/D-box RNA helicase in this process are distinct (mRNA unwinding versus trRNP remodeling) to result in select trRNPs, like the RHA trRNP, that afford the cell novel and diverse mechanisms for controlling the initiation of protein synthesis. Irrespective, an outstanding question across all mechanisms of 43S pre-initiation ribosome complex recruitment is the manner by which the mRNA becomes positioned within the binding channel of the 40S ribosomal subunit and the location at which scanning-dependent base inspection begins [1]. Is the mRNA threaded through its binding channel in the 40S ribosomal subunit or does the 43S pre-initiation ribosome complex undergo direct positioning near the 5' terminus [1]? How close to the 5' cap does the 43S pre-initiation ribosome complex need to bind in order to engage appropriate scanning in the correct reading frame [1]? It is likely that the molecular bases for these effects are distinct for each class of transcripts and are mediated by the particular mode of action of the associated DExH/D-box RNA helicase.

IRES trRNPs

The cap-dependent scanning mechanism of translation initiation was identified as the defining feature for eukaryotic protein synthesis [42]. The study of RNA viruses, however, soon challenged this central canon with the observation of several alternative mechanisms for translation initiation. These include, but are not limited to, internal ribosome entry-site (IRES)-mediated translation initiation, ribosome shunt, ribosomal frameshifting, leaky scanning, non-AUG initiation and reinitiation [43]. Variations to the central theme of 5'-end-dependent translation were also soon realized amongst classes of cellular

transcripts. This was particularly observed during times of cell stress and division, resulting in non-canonical translation initiation becoming a hallmark feature of the cellular stress response and mitosis [44-47]. Recent application of ribosome profiling to the study of eukaryotic translation control has extended our realization of alternative initiation mechanisms in cells [15]. The data from these studies support pervasive engagement of non-AUG codons and upstream open reading frames in the steady-state regulation of cellular protein synthesis [15]. Thus, eukaryotic translation initiation encompasses a diverse array of mechanisms to regulate the start of protein synthesis and intricately control gene expression.

Internal ribosome entry-site (IRES)-mediated translation initiation is by far the most studied mechanism of alternative engagement into protein synthesis. An IRES is an RNA element capable of directly recruiting the small ribosomal subunit to a start codon without the need for interactions with the 5' cap or cap-associated factors [1, 43, 48]. Although pervasively observed to control the expression of many RNA viruses and stress-response transcripts, there is no unifying mechanism to describe IRES-mediated translation control [1, 43, 48]. This is because an IRES can only be identified experimentally due to the lack of sequence and structure conservation [48]. The gold-standard approach is a bicistronic reporter assay whereby a putative IRES element is cloned to regulate the expression of a second, internal cistron within a plasmid harboring two adjacent open reading frames (typically Renilla and firefly luciferase) [48]. Expression of the first cistron is cap-dependent whereas protein production from the second cistron is regulated by the putative IRES element [48]. A relative increase in second cistron protein production, when compared to a construct cloned with a random sequence in its place, indicates IRES activity [48]. A major caveat of this approach, however, is the challenge of discriminating true IRES activity from cryptic promoter functions [49]. Additionally, the manner by which a putative IRES sequence is cloned out from its original context and into the bicistronic reporter can have significant consequences on the classification of a particular RNA element as IRES or not [49]. Thus, in many instances, such as that for the translational regulation of HIV-1, IRES activity remains controversial.

Yet, the concept of end-independent translation initiation is still well founded with unifying themes observed across instances of accepted IRES activity. This is particularly evident for IRES-mediated translation control of RNA viruses. Type I and II IRESes are identified by their maintained use of eIF4G and eIF4A to bridge contacts with eIFs 2 and 3 of the 43S pre-initiation ribosome complex, which facilitates its recruitment to a target mRNA [1, 43]. Instead of associating with eIF4E at the 5' cap, as seen in canonical cap-dependent initiation, eIF4G and eIF4A in these instances associate with RNA elements within the IRES itself [1]. A type I IRES, as exemplified in poliovirus, is mechanistically distinguished from a type II, such as the encephalo-myocarditis virus IRES, by the fact that decent scanning of the 43S pre-initiation ribosome complex to the start codon is still involved upon mRNA association [1, 43]. On the contrary, type II IRESes exhibit recruitment of the 43S pre-initiation complex in close proximity to the start codon with minimal scanning observed [1, 43]. A third class of IRES, type III, is identified by the requirement of only eIFs 2, 3 and 5 to facilitate direct positioning of the 43S pre-initiation ribosome complex at the start codon [1, 43]. This is observed in the case of hepatitis C virus-mediated translation control [1, 43]. Type IV IRESes, like the cricket paralysis virus, interact with the ribosome independently of any eIF and initiate translation via a structural mimic of the initiator methionyl-tRNA conferred by the IRES itself [1, 43].

RNA binding proteins are also critical to the mechanisms by which IRESes initiate translation by binding directly to or nearby an IRES to regulate its efficiency [50, 51]. Known as IRES *trans*-acting factors (ITAFs), these RNA binding proteins are critical to the diversification observed in IRES activity, both in mechanism and occurrence [50, 51]. They can function in RNA conformation control or in RNP remodeling by affecting the stability of RNA secondary structure or serving as bridging cofactors with the ribosome, respectively [50, 51]. Of the ITAFs identified thus far, many are members of the heterogeneous nuclear ribonucleoprotein (hnRNP) family and include PTB (hnRNP1), hnRNPA1, hnRNPE2, hnRNPE1, hnRNPC and

hnRNPL [50, 51]. This significance is linked with the nuclear-cytoplasmic shuttling activity and additional post-transcriptional functions of hnRNPs, and appears distinct for each IRES target [51]. Thus, the formation of select trRNPs to regulate eukaryotic translation is fundamental to IRES-mediated initiation.

The importance of trRNPs in translation control is also seen for the other mechanisms of end-independent initiation. Ribosome shunt, ribosomal frameshifting, leaky scanning, non-AUG initiation and reinitiation are observed due to trRNP elements directing the ribosome to move in an alternative manner as their names imply [43]. Although mechanistically characterized by variations in RNA sequence and/or structure impacting ribosomal movement, it can be envisioned that distinct RNA binding proteins have critical roles in these processes as well.

Elongation trRNPs

Extension of a polypeptide chain to produce a full-length protein product occurs by three steps: amino acid incorporation, peptide bond formation and ribosome translocation. Two elongation factors (eEFs) coordinate these activities, the dynamics of which are influenced by associated RNA binding proteins. Thus, trRNP biology is fundamental to targeted translation control during the elongation stage of protein synthesis.

Polypeptide extension begins with the incorporation of a charged transfer RNA (tRNA) into the unoccupied 3' aminoacyl-site (A site) of an 80S ribosome poised to elongate from the start codon [52] (Figure 2). This activity is facilitated by the multi-subunit complex eEF1 in its GTP-bound form [52]. Anticodon-codon base pairing drives the identification of appropriate aminoacylated tRNA incorporation [52]. Only correct matches allow for conformational arrangements of the tRNA that are tolerated by the ribosome [52]. This recognition triggers GTP hydrolysis, eEF1 release, and full accommodation of the accepted aminoacyl-tRNA in the A site [52].

Next, the A-site aminoacyl-tRNA is linked to the P-site aminoacyl-tRNA, which harbors the initiator methionine, via peptide bond formation [52] (Figure 2). This reaction is catalyzed by the peptidyl transferase center of the large ribosomal subunit [52]. The peptidyl transferase center is a region of highly conserved ribosomal RNA that functions in orienting the acceptor stems of the A- and P-site tRNAs, which are the regions bound to the cognate amino acids, to drive the two-step reaction that results in peptide bond formation [53]. This amino acid linkage between the A- and P-site tRNAs triggers the ribosomal subunits to move in a ratchet-like motion that results in a hybrid tRNA state prior to complete translocation [52]. Here the acceptor stems of the A- and P-site tRNAs are positioned within their adjacent E- and P-sites, respectively, while their anticodon stem loops remain in their corresponding A- and P-sites [52]. Complete translocation of the tRNA-ribosome complex to the subsequent codon is driven by eEF2 association, GTP-hydrolysis and phosphate release [52] (Figure 2). This results in a deacylated tRNA (a tRNA without its charged amino acid or associated polypeptide chain) within the 5' E-site, a P-site tRNA with a dipeptide bound to its acceptor stem, and an unoccupied A-site that is primed for acceptance of the next aminoacyl-tRNA based upon anticodon-codon base pairing. Repetition of these three steps along an open reading frame grows the encoded polypeptide and occurs until a stop codon is reached, which triggers termination and release of the protein product.

Intricate to the control of translation elongation are select RNA binding proteins, which associate with target transcripts and form distinct trRNPs that regulate the dynamics of polypeptide synthesis. Four major elongation effectors have been identified and mechanistically studied. They are, the fragile X mental retardation protein (FMRP), heat shock protein 70 (Hsp70), pumilio and argonaute (PUF-Ago), and heterogenous ribonucleoprotein E1 (hnRNP E1) [54-58]. Although their targets and mechanisms for regulation are distinct, the unifying principle among all four effectors is that their formation of distinct trRNPs is intricate to the molecular basis by which they regulate the dynamics of polypeptide synthesis.

FMRP binds the mRNA coding sequence of pre- and postsynaptic transcripts and induces elongation pausing by competing with P-site tRNA binding [54, 55]. The result is impacted ribosome-tRNA dynamics that compromise the elongation process on target transcripts [54, 55]. This directed translation

control is critical for the spatiotemporal regulation of neuronal protein expression, which is essential for proper nervous system function [54, 55]. The specificity for FMRP-mediated control of translation elongation is driven by its affinity for particular RNA sequence elements, GAC, ACUG/U and A/UGGA, which have predicted G-quadruplex secondary structure [59]. Mutational studies indicate that the ability of FMRP to bind RNA is sufficient for its induced elongation pausing effect [54, 55].

Hsp70 is a critical molecular chaperone that facilitates protein production by associating with nascent polypeptide chains during the elongation process and coordinating their correct folding into a functional protein product [56]. This activity of Hsp70 involves its association with ribosomal proteins of the peptide exit tunnel, particularly the large ribosomal subunits RPL4 and RPL22, and elongation factor eEF1A [56]. These interactions function to facilitate efficient movement of the nascent polypeptide chain through the peptide exit tunnel and proper elongation kinetics [56]. Heat shock, however, alters the interaction of Hsp70 with the ribosome and eEF1A such that the peptide exit tunnel becomes constricted and the nascent polypeptide chain exposed in a manner that compromises elongation kinetics to cause global ribosome pausing at codon 65 of most mRNAs [56]. Codon 65 correlates with a sixty-five amino acid polypeptide, which is long enough to traverse the peptide exit tunnel and become exposed so that it is affected by altered interactions of Hsp70 during heat shock. The outcome is regulated translation that is fundamental to the heat shock response [56].

PUF-Ago and hnRNP E1 control translation elongation by regulated interactions with elongation factor eEF1A. eEF1A is the subunit of eEF1 that binds GTP and is responsible for delivering aminoacyl-tRNAs to the A site of the ribosome [60]. In the case of PUF-Ago, these RNA binding proteins associate with eEF1A in a manner that inhibits its GTPase activity [57]. The effect is stalled elongating ribosome complexes within the open reading frames of transcripts, hindering protein production [57]. The influence of PUF-Ago on eEF1A GTPase activity appears specific for elongating complexes that have traversed the mRNA to the point of nascent polypeptide chain emersion from the ribosomal exit tunnel [57]. The factors directing this specificity and its significance for mRNA-mediated translation control remain to be elucidated. hnRNP E1, on the other hand, regulates eEF1A function by binding to and inhibiting its dissociation from ribosomes [58]. The outcome is impaired ribosome translocation and protein production [58]. This effect is critical for regulated expression of epithelial-mesenchymal transition transcripts that are significant in development and cancer [58].

Termination trRNPs

The translation process concludes with termination of polypeptide elongation, nascent chain release, and ribosome recycling. Translation termination is triggered by the recognition of a stop codon (UAA, UAG or UGA) within the A-site of an elongating ribosome [61] (Figure 3). This recognition occurs in eukaryotes by the eukaryotic termination factor 1 (eRF1), which structurally exists as a tRNA mimic and associates with the stop codon-containing A site [61]. eRF1 harbors a conserved GGQ motif that positions within the peptidyl transferase center and induces conformational changes that allow access of a water molecule to this active site [61] (Figure 3). The result is breakage of the ester bond that holds the polypeptide chain to the P site tRNA, releasing the protein product [61]. This function of eRF1 is stimulated by its association with the second termination factor, eRF3 [61]. eRF3 is a GTPase whose affinity for GTP is increased by association with eRF1 [61]. eRF1 and eRF3 are found within stable complexes and their ribosome association triggers GTP hydrolysis, movement of the GGQ motif into the peptidyl transferase center, and eRF1-induced polypeptide release [61].

The ATP-binding cassette protein ABCE1 is responsible for ribosome recycling upon nascent chain release [61] (Figure 3). The retention of eRF1 on post-termination complexes recruits ABCE1 and stimulates its ATPase activity [61]. Hydrolysis of ATP induces a conformational switch in ABCE1 from a closed ATP-bound state to an open ADP-bound state [61]. This movement of ABCE1 causes the 60S ribosomal subunit to split away from its 40S counterpart [61]. Initiation factors eIF1, 1A and 3 facilitate subsequent deacylated tRNA and mRNA release from the 40S ribosomal

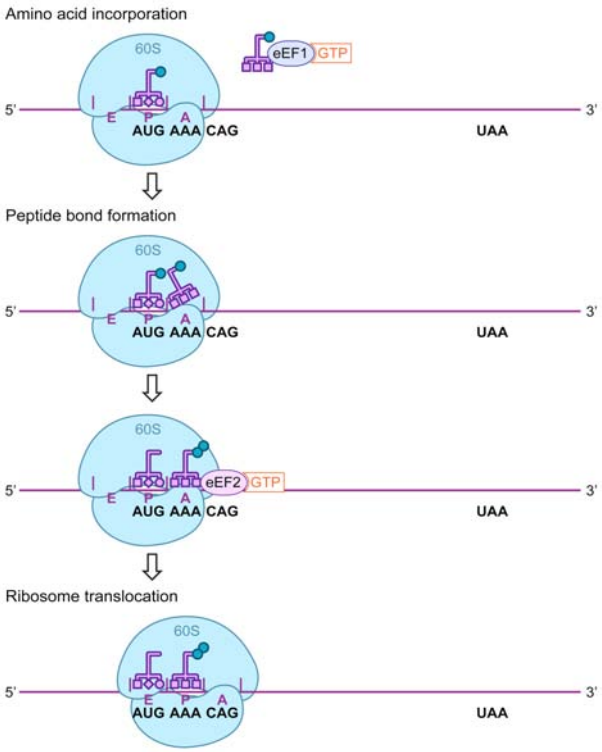

Figure 2. Schematic of canonical eukaryotic translation elongation. Canonical eukaryotic translation elongation proceeds in three steps: amino acid incorporation, peptide bond formation, and ribosome translocation. Upon formation of an elongation-competent 80S ribosome at the start codon (AUG), the elongation factor eEF1 facilitates association of a charged tRNA with an available codon in the adjacent, 3' A-site. Recognition of appropriate anticodon-codon base pairing stimulates GTP hydrolysis and release of eEF1. Peptide bond formation is subsequently catalyzed by the peptidyl transferase center of the large ribosomal subunit. This results in a polypeptide chain of n+1 positioned with the A site of the ribosome. Ribosomal translocation to the next 3' codon is stimulated by peptide bond formation and completed with the association of elongation factor eEF2 and GTP hydrolysis. Repetition of these three steps along an open reading frame grows the encoded polypeptide.

subunit via competitive binding interactions [61]. The outcome is recycling of all factors required for subsequent rounds of protein synthesis. Interactions between the 3' poly-A binding protein (PABP) and the 5' scaffolding factor eIF4G, which as previously discussed function to create a circularized mRNA, allow for efficient reinitiation and subsequent rounds of protein synthesis upon termination [1-3].

Two main exceptions to this mechanistic paradigm of eukaryotic translation termination are known. They occur during the phenomena of reinitiation and nonsense-mediated mRNA decay. Reinitiation is when the ribosome fails to dissociate from a transcript upon termination of polypeptide synthesis [1]. Instead it resumes scanning to a downstream

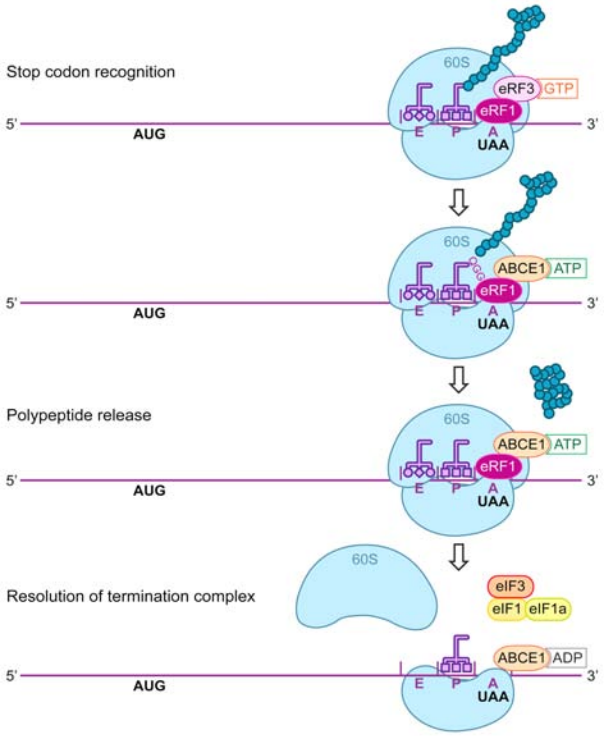

Figure 3. Schematic of canonical eukaryotic translation termination. Canonical eukaryotic translation termination is triggered by the recognition of a stop codon (e.g. UAA) within the A-site of an elongating ribosome. This recognition occurs by the eukaryotic translation termination factor eRF1 in complex with eukaryotic translation termination factor eRF3 and GTP. eRF1 harbors a conserved GGQ motif that positions within the peptidyl transferase center upon stop codon recognition and GTP hydrolysis. This movement of the GGQ motif into the peptidyl transferase center induces a conformational rearrangement in the large ribosomal subunit that causes hydrolysis of the ester bond linking the polypeptide chain to the P site tRNA, releasing the protein product. The ATP-binding cassette protein ABCE1 resolves the post-termination complex by splitting away the large (60S) ribosomal subunit from the now vacant 80S complex. Initiation factors eIF1, 1A and 3 facilitate subsequent separation and recycling of the deacylated tRNA, mRNA, and small (40S) ribosomal subunit.

AUG where a second initiation event is engaged [1]. This is observed among cellular transcripts that harbor short upstream open reading frames and is critical for their targeted translation control [61]. A principle of the eukaryotic scanning model of protein synthesis is that a 43S pre-initiation ribosome complex scans an mRNA until the first AUG is detected [61]. It will then engage in 80S formation and polypeptide production irrespective of the length of the open reading frame [61]. Consequently, for mRNAs that harbor several open reading frames, a competition arises between upstream initiation events and downstream polypeptide production [61]. Often it is only when translation activity is impaired, such as during cell stress, does compromised initiation at the upstream open reading frame allow for effective expression of the downstream protein product (see section below, 'Regulation of eukaryotic translation'). This results in a means for effective translation control, as the downstream open reading frame often encodes the functional cellular protein.

Reinitiation is also intricate to the molecular basis by which many infectious viral proteins are expressed [43, 61]. Although the mechanisms regulating reinitiation are diverse, a common theme is the role of distinct trRNPs in mediating this effect. Such is the case, for example, in the expression of critical structural proteins for mammalian caliciviruses, which are responsible for several life-threatening diseases in animals. An RNA element termed 'termination codon upstream ribosome-binding site' (TURBS) is critical to reinitiation and expression of infectious downstream viral protein products on caliciviral transcripts [61]. The TURBS consists of two motifs that harbor sequences of the 3' terminus of the upstream open reading frame and the region between the first stop and second start site [61]. These motifs are necessary for reinitiation events by functioning to capture 40S ribosomal subunits upon termination of upstream translation, effects that occur by the TURBS element forming a critical trRNP that associates with eIF3 and mimics 18S rRNA [61]. The outcome is effective expression of downstream viral protein products in measured amounts that are critical to caliciviral infection [61].

Nonsense-mediated mRNA decay is the essential cellular process of mRNA surveillance and quality control. It is responsible for the resolution of premature termination events and subsequent degradation of the effected mRNA so as to prevent production and accumulation of rogue protein products. Premature termination results from the recognition of premature termination codons by eRF1 and eRF3 [62]. Premature termination codons are stop codons that are positioned upstream of the normal stop codon within an open reading frame [62]. Although eRF1 and eRF3 are recruited to premature termination codons, the subsequent termination events of ABCE1 recruitment and ribosome recycling do not occur. Instead, an alternative trRNP is formed that halts the translation process, inhibits subsequent initiation events, and induces mRNA decay [62]. This alternative trRNP consists of a complex series of interactions between the cap-binding protein CBP80/20, a nearby exon junction complex, and recruited factors from the up-frameshift (UPF) and suppressor of morphogenetic effect on genitalia (SMG) protein families [62]. Collectively, these interactions and the progression in their association regulate trRNP dynamics that result in altered termination fates to control protein expression.

trRNPs in the regulation of eukaryotic translation

Global and targeted regulation of eukaryotic translation is observed at all three stages of protein synthesis. These mechanisms can be constitutive or induced upon changes in the cellular environment. In either case, distinct trRNP formation and activity is fundamental to each effect.

43S pre-initiation ribosome complex formation, its recruitment and scanning, and 60S ribosomal subunit joining are all examples of targetable steps for regulated gene expression at the stage of translation initiation. As previously discussed, DExH/D-box RNA helicases and the select trRNPs they create, are instrumental for coordinating 43S pre-initiation ribosome complex recruitment and scanning on target transcripts. Other trRNPs, formed by alternative RNA binding proteins, contribute a similar effect. Two classic examples are the regulated expression of ferritin mRNA in response to iron homeostasis and male-specific lethal-2 (msl-2) translation in *Drosophila* X-chromosome dosage compensation. During iron deprivation, the RNA

binding protein iron regulatory protein (IRP) binds with high affinity to its cognate *cis*-acting RNA element, the iron-responsive element (IRE), which defines the 5' terminus of ferritin mRNA [63]. The IRE is a stem-loop structure that when bound by IRP effectively impedes 43S pre-initiation ribosome complex association and expression of the ferritin mRNA [63, 64]. Elevated iron levels reduce the affinity of IRP for IRE, effectively lifting its block on translation initiation and allowing for expression of ferritin [63].

Inhibition of msl-2 expression in female flies is fundamental to dosage compensation and survival. This effect is mediated by the female-specific RNA binding protein Sex-lethal (SXL), which binds to distinct poly-uridine tracts in the 5' and 3' termini of msl-2 and inhibits translation [65]. The molecular basis for this effect is two-pronged. At the 3' terminus, SXL impairs 43S pre-initiation ribosome complex recruitment by associating with the 3'-bound poly-A binding protein (PABP) to interfere with dynamics at the 5' cap that allow for appropriate initiation [65, 66]. This coordination between 3' effectors and a 5' outcome is mediated by PABP-induced mRNA looping [66]. At the 5' terminus, SXL stalls scanning 43S pre-initiation ribosome complexes that were able to circumvent the 3'-mediated block, effectively reducing their affinity for msl-2 and causing their dissociation [65]. The outcome is repressed expression of msl-2 protein and effective dosage compensation.

Blockade of 60S ribosomal subunit joining is another effective mechanism employed by distinct trRNPs to coordinate targeted translation regulation. Such is the case for controlled expression of erythroid 15 lipoxygenase (LOX) mRNA during erythroid differentiation. LOX mRNA encodes a critical enzyme important for internal membrane reorganization during late stages of red blood cell maturation [67, 68]. Temporal restriction of its expression is mediated by the association of the RNA binding proteins hnRNP K and hnRNP E1 with the *cis*-acting differentiation control element (DICE) in the 3' terminus of the LOX mRNA [67, 68]. DICE is a repeated CU-rich motif that when bound by hnRNP K and hnRNP E1 inhibits 60S ribosomal subunit joining by interfering with initiation factor activity that mediates this effect [67, 68]. A similar molecular basis is seen in the spatiotemporal regulation of β-actin expression whereby the RNA binding protein Zipcode binding protein 1 (ZBP1) binds the 3' *cis*-acting zipcode RNA element in the β-actin mRNA and impedes 60S ribosomal subunit joining to effectively suppress translation activity [69]. This blockade is uplifted by phosphorylation of ZBP1, which reduces its affinity for the β-actin mRNA and allows translation to proceed [69].

Regulated elongation is the third effective means by which protein synthesis can be controlled. As previously discussed, there are known instances for targeted elongation regulation in which specific RNA binding proteins influence trRNP dynamics to control elongating ribosome activity. Global regulation of elongation is also observed and it occurs with the phosphorylation of the elongation factor eEF2. This post-translational modification interferes with eEF2-GTP complex formation, which inhibits the association of eEF2 with the ribosome and effectively impedes translocation [70].

Phosphorylation of eEF2 occurs by the eEF2 kinase [70]. Mammalian eEF2 kinase activity is controlled by the cell's central commander of translation: the mammalian target of rapamycin (mTOR) (Figure 4). mTOR is a serine/threonine kinase that directs protein synthesis in reflection of the cellular environment by acting upon signals received from almost all major cell-receptor signaling pathways. These include, but are not limited to the PI3K/AKT and Ras/ERK signaling cascades [71]. One downstream effector of mTOR is the ribosomal protein S6 kinase (S6K) (Figure 4). During normal growth conditions or in response to mitogenic stimuli, mTOR is activated and phosphorylates S6K [71]. S6K, in turn, phosphorylates the eEF2 kinase, which results in eEF2 kinase inactivation, eEF2-GTP association, and the promotion of translation elongation [70, 72]. However, when mTOR is inactivated by cellular stress the inhibitory block of S6K on eEF2 kinase activity is relieved, resulting in phosphorylation of eEF2 and impaired translation elongation activity [70, 72]. The outcome is suppression of global translation.

Regulated protein synthesis in response to the environment is also observed at the stage of translation initiation. Here mTOR and its downstream

effector S6K once again serve as a central line of command to influence trRNP dynamics and protein production (Figure 4). In this instance, mTOR-directed S6K activation targets the translation initiation factor eIF4B and the regulatory protein programmed cell death 4 (PDCD4) [71, 72]. Phosphorylation of eIF4B by S6K enhances eIF4B's stimulation of eIF4A helicase activity and protein production [71, 72]. Likewise, phosphorylation of PDCD4 results in eIF4A-mediated translational activation by causing its subsequent ubiquitylation and degradation [71, 72]. PDCD4 is an inhibitor of eIF4A helicase activity; thus, its removal relieves an inhibitory block on eIF4A-dependent translation initiation [71, 72]. The outcome of both effects is activation of eIF4A and effective translation initiation on target mRNAs.

Several additional downstream targets of S6K are also known and their activity has significant implications for regulated translation initiation (Figure 4). These S6K targets are, the 40S ribosomal subunit protein S6 (rpS6), the S6K1 Aly/REF-like target (SKAR), the cAMP-responsive element modulator τ (CREM τ), CBP80 of the CBP80/20 cap-binding protein complex, the proapoptotic protein BAD, insulin receptor substrate IRS (IRS), and mTOR itself [72]. However, in many of these studied instances, such as rpS6 and CBP80 phosphorylation, it remains controversial about the exact effects of this post-translational modification on their role in translation control and the influence of S6K in these outcomes [72].

A second line of command used by mTOR to control translation initiation activity is the eIF4E inhibitory protein, 4E-binding protein 1 (4E-BP1) [71, 72] (Figures 4 and 5). 4E-BP1 influences cap-dependent translation initiation by competing with eIF4G for eIF4E association [73-75]. A 4E-BP1:eIF4E interaction effectively disrupts cap-associated trRNP dynamics to suppress translation initiation [73-75]. The association of 4E-BP1 with eIF4E is regulated by its phosphorylation status. In response to mitogenic stimuli, activation of mTOR results in its hyperphosphorylation of 4E-BP1 [71, 76]. In this post-translational state, 4E-BP1 has reduced affinity for eIF4E [76]. This allows the eIF4E cap-binding protein to interact with eIF4G and promote cap-dependent translation [76]. On the contrary, stress-induced suppression of mTOR activity results in hypophosphorylation of 4E-BP1, a strong 4E-BP1:eIF4E association, and consequent suppression of eIF4E-dependent translation initiation [71, 76].

Besides directing mTOR-mediated translational control, the cellular environment influences protein synthesis by regulating the function of eIF2 in the earliest stage of initiation, 43S pre-initiation ribosome complex formation. The very initial association of an initiator methionyl-tRNA with a 40S ribosomal subunit is facilitated by eIF2 in its GTP-bound form [2, 3]. eIF2 consists of 3 subunits: α, β and γ [2, 3] (Figure 6). The α subunit together with β serves as a critical allosteric effector of direct GTP and initiator methionyl-tRNA binding to the γ subunit of eIF2 [2, 3]. GTP association occurs first and is rate-limiting for initiator methionyl-tRNA binding [2, 3]. Phosphorylation of the α subunit at serine residue 51 inhibits the exchange of GDP for GTP on eIF2, an effect mediated by the guanine nucleotide exchange factor eIF2B [2, 3] (Figure 6). The outcome is impaired efficiency of ternary complex formation (initiator methionyl-tRNA and eIF2 bound to the 40S ribosomal subunit), which results in reduced 43S pre-initiation ribosome complex formation, its recruitment to mRNAs, and subsequent translation [2, 3].

Four distinct protein kinases have been identified that phosphorylate eIF2α: haem-regulated inhibitor kinase (HRI), protein kinase R (PKR), PKR-like endoplasmic reticulum kinase (PERK), and general control non-derepressible-2 (GCN2) (Figure 6). Notably, their activation occurs in response to a variety of environmental stressors as the cell attempts to rapidly adjust to a change in its homeostasis: Iron deficiency and osmotic or heat shock activate HRI; double-stranded RNA triggers PKR activity; ER-stress and hypoxia activate PERK; amino-acid deprivation and UV irradiation stimulate GCN2 [77]. The outcome in all instances is phosphorylation of eIF2α and suppression of general translation, as the 43S pre-initiation complex is fundamental to protein synthesis. However, distinct transcripts like mammalian activating transcription-factor-4 (ATF4) and the yeast transcriptional activator GCN4 circumvent this block and exhibit efficient expression even in the advent of eIF2α phosphorylation. This targeted translation activity is specific for critical stress response proteins and

is fundamental to the cell's ability to regain homeostasis and avoid apoptosis and cell death.

The ability of specific mRNAs to engage in translation in the advent of eIF2α phosphorylation is dictated by their distinct trRNPs. In the case of ATF4, its 5' terminus is characterized by two upstream open reading frames [77]. In cellular states when eIF2α phosphorylation is low and ternary complex is abundant, upstream open reading frame 2 preferentially engages 43S pre-initiation ribosome complexes in recruitment and scanning over the downstream ATF4 open reading frame [77]. The outcome is inhibited expression of ATF4 [77]. However, in ER stress when misfolded proteins accumulate and activate PERK, phosphorylation of eIF2α causes the limiting pool of 43S pre-initiation ribosome complexes to scan through upstream open reading frame 2 and initiate translation at the downstream ATF4 open reading frame [77]. The result is efficient synthesis of ATF4, which is a major transcription factor that facilitates expression of genes critical to resolving ER stress [77]. A similar molecular basis explains the translational activation of yeast GCN4 in response to amino acid starvation [77].

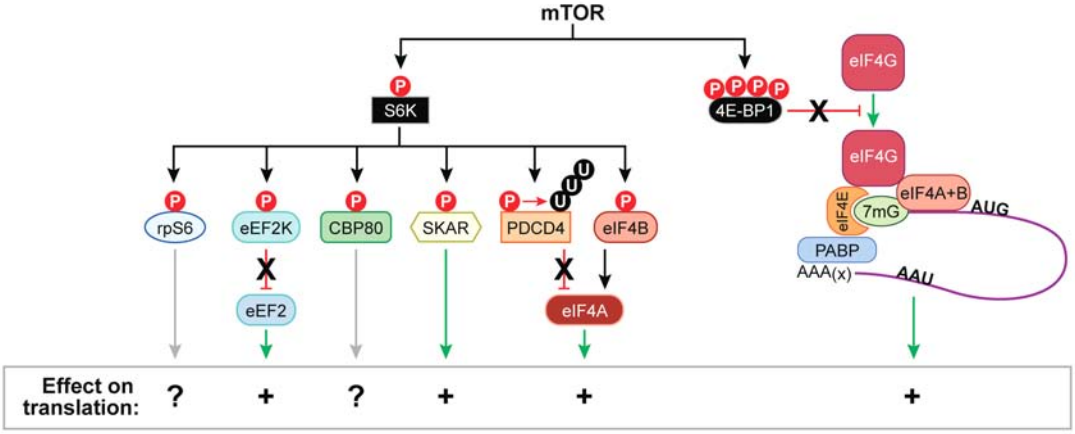

Figure 4

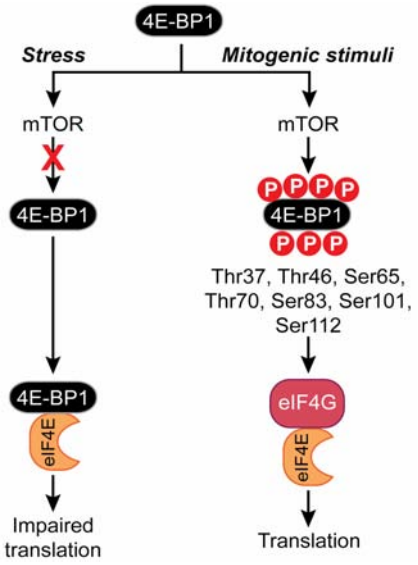

Figure 5

Distinct mechanisms of translation regulation, both global and specific, are also observed in virus-infected cells. Here a complex interplay between translation suppression and activation, and canonical and non-canonical protein synthesis is essential for viral replication and the host innate defense. Although the specific mechanisms characterizing this translational reprogramming event are diverse among each virus infection, the central theme of trRNP-mediated translation control is fundamental to explaining each observed outcome. Human immunodeficiency virus type 1 (HIV-1) infection is a model example to demonstrate how trRNP biology is at that core of translational reprogramming that characterizes viral infections.

A hallmark feature of HIV-1 infection is global suppression of host cell translation [78]. This effect is mediated by HIV-1-induced activation of 4E-BP1 [78]. 4E-BP1 is the eIF4E inhibitory protein that binds to the translation initiation factor eIF4E and disrupts associated trRNP dynamics to impede canonical cap-dependent protein synthesis. Since the eIF4E trRNP is responsible for the bulk of cellular steady-state protein synthesis (see below), the outcome is observed global suppression of host cell translation [78]. Yet during this effect, HIV-1 maintains expression of its critical structural proteins [78]. As an obligate parasite, HIV-1 requires the host cell translation machinery for expression of its encoded viral proteins. Furthermore, it does

Legend to Figure 4. Overview of mTOR-directed eukaryotic translation control. Mammalian target of rapamycin (mTOR) is a serine/threonine kinase that serves as the central commander of eukaryotic translation control. It directs protein synthesis in reflection of the cellular environment by acting upon two downstream effectors: ribosomal protein S6 kinase (S6K) and eIF4E binding protein 1 (4E-BP1). S6K targets several factors critical in the regulation of translation. These include, the 40S ribosomal subunit protein S6 (rpS6), eEF2 kinase (eEF2K), CBP80 of the CBP80/20 cap-binding complex, the S6K1 Aly/REF-like target (SKAR), regulatory protein programmed cell death 4 (PDCD4), and translation initiation factor eIF4B. The phosphorylation of these translation factors by mTOR-S6K signaling alters their function in a manner that facilitates translation activity. For eEF2K, its phosphorylation by S6K results in its inactivation. This allows elongation factor eEF2 to effectively associate with elongating ribosomes and facilitate their translocation. Similarly, the phosphorylation of PDCD4 results in its inactivation to facilitate steady-state protein synthesis. Here S6K-mediated phosphorylation of PDCD4 induces its ubiquitinylation and subsequent degradation. This removes PDCD4 as an inhibitor of eIF4A helicase activity, allowing translation initiation to proceed. Likewise, the phosphorylation of eIF4B by S6K also facilitates effective steady-state protein synthesis by stimulating eIF4A helicase activity. In this instance, phosphorylated eIF4B exhibits enhanced association for eIF4A, which effectively stimulates translation activity. The direct effects of mTOR/S6K-induced phosphorylation on rpS6 and CBP80 translation activity remain ambiguous; however, data support a stimulatory outcome on initiation. For the second line of command, mTOR-directed 4E-BP1 signaling controls translation by regulating trRNP dynamics. Here phosphorylation of 4E-BP1 by mTOR reduces its affinity for the eIF4E cap-binding protein. This allows eIF4E to effectively associate with the translation initiation factor eIF4G and create a trRNP that is productive for steady-state protein synthesis.

Legend to Figure 5. Regulation of 4E-BP1 phosphorylation events and its role in eukaryotic translation control. The eIF4E binding protein 1 (4E-BP1) is a critical effector of translation activity. Its function is dependent on upstream signaling events that converge on the regulation of its major commander, the mammalian target of rapamycin (mTOR). In the presence of mitogenic stimuli (e.g. growth factors), mTOR is activated and results in the hyperphosphorylation of 4E-BP1. The hyperphosphorylation of 4E-BP1 is a hierarchical event with phosphorylation at threonine (Thr) residues 37 and 46 priming for phosphorylation at Thr70, which then allows for phosphorylation at serine (Ser) residue 65. Phosphorylation at Ser65 is the critical effector post-translational modification that regulates the functional effects of 4E-BP1 in translation control. In its presence, 4E-BP1 exhibits reduced affinity for the eIF4E cap-binding protein. This allows eIF4E to associate with the translation initiation factor and scaffolding protein eIF4G. An eIF4E-eIF4G association allows for effective trRNP formation that facilitates translation activity. In the advent of stress, however, mTOR activity is reduced to result in hypophosphorylation at Ser65. This change in the post-translational modification status of 4E-BP1 increases its affinity for eIF4E. A 4E-BP1-eIF4E association effectively inhibits an interaction between eIF4E and eIF4G. This results in impaired trRNP formation and inhibited translation activity. It has also been shown that phosphorylation at Ser101 of 4E-BP1 regulates phosphorylation at Ser65 and that phosphorylation at Ser112 facilitates release of 4E-BP1 from eIF4E.

so in an arguably cap-dependent scanning manner [33, 34]. Yet, how is this possible with its suppression of eIF4E trRNP activity? The answer is in the ability of HIV-1 transcripts to engage within an alternative trRNP that harbors the CBP80/20 cap-binding protein complex (CBC) [78]. CBC functions in translation initiation like eIF4E, however, its prominence is during the pioneer rounds of translation (see below). Notably, CBC activity is independent of regulation by 4E-BP1 and is known to be functional during states of cell stress (see below). Thus, the CBC trRNP provides an alternative mechanism for HIV-1 viral protein expression that is cap-dependent but independent of eIF4E tRNP activity [78]. Therefore, the translation dynamics during HIV-1 infection demonstrate how distinct trRNP activity choreographs targeted protein synthesis.

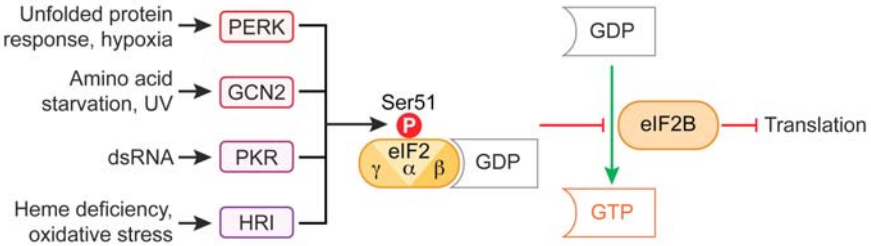

Figure 6

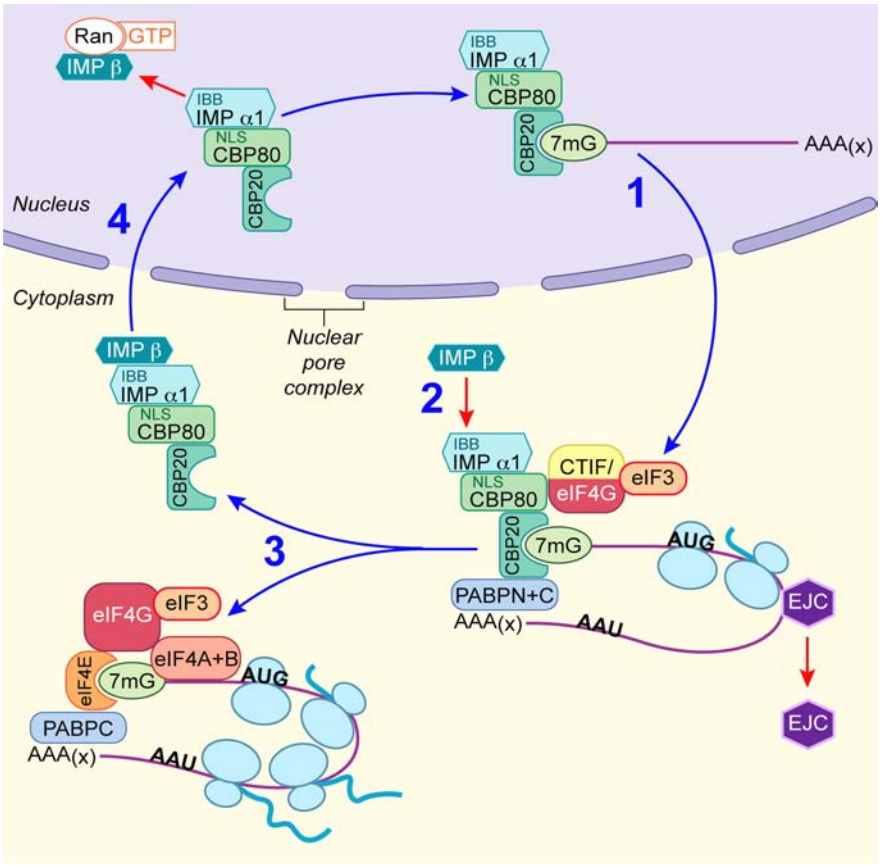

Figure 7

The CBC and EIF4E trRNPS

Two core trRNPs characterize general eukaryotic cap-dependent translation. These are, the CBC trRNP and the eIF4E trRNP. As previously discussed, initiation of eukaryotic translation is defined by a cap-dependent scanning mechanism whereby the ribosome binds to the 5' terminus of an mRNA and proceeds along the transcript, inspecting base-by-base, for an appropriate start codon to initiate polypeptide synthesis. This effect is directed by trRNP complexes, and in particular the defining cap-binding proteins, CBP80/20 (CBC) or eIF4E. Although CBC and eIF4E direct the interaction of an mRNA with the ribosome in a similar manner, the molecular basis by which each mediates this effect is distinct [79]. Consequently, the CBC trRNP and the eIF4E trRNP have distinct functional significances in the choreographing of eukaryotic translation (Figure 7).

Legend to Figure 6. Integration of stress response signals into the phosphorylation of eIF2α and its outcome on regulated eukaryotic protein synthesis. Integral to the function of translation initiation factor eIF2 is its ability to function as a GTPase. eIF2-GTP drives ternary complex formation and is necessary for 43S pre-initiation ribosome complex assembly, recruitment and scanning. Start codon recognition triggers GTP hydrolysis, an outcome that induces conformational rearrangements that halt the scanning process and engage 80S ribosome complex formation. The exchange of GDP for GTP on eIF2 is necessary for subsequent re-engagement of eIF2-driven translation initiation. This requires the guanine nucleotide exchange factor eIF2B, an effect that is highly regulated by the cellular environment. eIF2 consists of 3 subunits: α, β and γ. The α subunit is the critical allosteric effector of direct GTP binding. Phosphorylation of the α subunit at serine (Ser) residue 51 inhibits the exchange of GDP for GTP on eIF2. This effectively impedes eIF2-mediated initiation events, resulting in compromised translation activity. Phosphorylation of eIF2α occurs by four distinct kinases, each of which is stimulated by distinct cellular stressors. Haem-regulated inhibitor kinase (HRI) is activated by iron deficiency and osmotic or heat shock, protein kinase R (PKR) is stimulated by double-stranded RNA, PKR-like endoplasmic reticulum kinase (PERK) responds to ER-stress and hypoxia, and general control non-derepressible-2 (GCN2) is activated by amino-acid deprivation and UV irradiation. The outcome in all instances is phosphorylation of eIF2α, which impairs the exchange of GDP for GTP to result in suppression of general translation.

Legend to Figure 7. Model of CBC and eIF4E trRNP dynamics in the control of eukaryotic translation. Canonical eukaryotic cap-dependent translation is governed by a complex interplay between the CBC trRNP and the eIF4E trRNP. Beginning at transcription, the CBP80/20 cap-binding protein complex (CBC) directly binds the 5' 7-methyl-guanosine cap of a nascent transcript (1). This association is driven by the high affinity of CBC for 7-methyl-guanosine and its abundant nuclear localization. In this RNP association, CBC facilitates mRNA maturation and its nuclear export. Upon entry into the cytoplasm, CBC engages trRNP formation that facilitates the initial round(s) of translation (2). This involves the direct association of CBP80 with either CTIF or eIF4G, which provides a molecular bridge to eIF3 binding and 43S pre-initiation ribosome complex recruitment. This CBC trRNP also harbors nuclear and cytoplasmic poly-A binding protein (PABP) at its 3' terminus and exon junction complexes (EJC) along the open reading frame. These features are critical for the CBC trRNP in engaging translation activity by facilitating interactions between the target transcript and the 43S pre-initiation ribosome complex. Remodeling of a transcript from a CBC trRNP to an eIF4E trRNP follows the pioneer round(s) of translation and is critical for regulated steady-state protein synthesis (3). This trRNP remodeling is facilitated in two ways. At the 5' terminus, exchange of CBC for eIF4E is governed by a translation-independent mechanism whereby the strong affinity of CBP80 for the nuclear import factor importin α1 (IMP α1) drives its association with the nuclear pore-associated karyopherin importin β1 (IMP β1). This CBC-IMP α1-IMP β1 complex formation results in CBC destabilization from the 5' cap and its nuclear recycling. The association between CBC and IMP α1 is driven by the canonical nuclear localization sequence (NLS) found within the N-terminus of CBP80. IMP α1 recruits IMP β1 via its IMP β1-binding domain (IBB). Displacement of CBC from the 5' cap allows eIF4E to bind. eIF4E then draws stabilized associations with eIF4G that recruit eIF4A and complete formation of the eIF4E trRNP at the 5' terminus. Removal of exon junction complexes and the complete exchange of PABPN for PABPC occur in a translation-dependent manner, although the exact molecular mechanisms remain unknown. Re-engagement of CBC in post-transcriptional gene control is facilitated upon its nuclear re-entry and re-association with a nascent transcript (4). The maintained nuclear interaction between CBC and IMP α1 is believed to prime for subsequent trRNP remodeling upon cytoplasmic entry.

The CBC trRNP

CBC is a heterodimeric protein complex of two subunits: CBP80 and CBP20. CBP20 directly binds to the 7-methyl guanosine cap of eukaryotic mRNAs while CBP80 regulates this interaction [80]. The association of CBC with a 5' cap occurs co-transcriptionally when a nascent transcript emerges from the RNA polymerase II holoenzyme [81] (Figure 7). This affinity is facilitated by the abundant steady-state localization of CBC within the nucleus, an effect driven by a bipartite nuclear localization sequence within CBP80 and its association with the nuclear import factor, importin-α1 [82, 83]. Here CBC is critical for the major post-transcriptional events of 3' end processing and splicing that mature an mRNA [80, 84, 85]. CBC then remains bound to the 5' cap and facilitates mRNA nuclear export [81, 83, 85-87].

Once in the cytoplasm, CBC engages the trRNP activity that coordinates the so-called pioneer round(s) of translation [88, 89] (Figure 7). These encompass the initial interactions of a ribosome with an mRNA that generate protein products. This CBC trRNP is defined by CBC, bound to the 5' cap, and its direct association with the translation initiation factor eIF4G or CTIF [90, 91]. A CBC-eIF4G/CTIF interaction is important for generating a molecular bridge with the translation initiation factor eIF3, which recruits the 43S pre-initiation ribosome complex to the 5' terminus and initiates scanning [4, 90, 91].

Pioneer CBC trRNP complexes are also defined by the presence of exon junction complexes and the nuclear poly-A binding protein (PABPN) [89, 92] (Figure 7). Exon junction complexes are dynamic protein assemblies that organize upon the coding sequence of an mRNA with the conclusion of a splicing event and facilitate critical post-transcriptional activities. One of these post-transcriptional activities is to enhance the translation of spliced mRNAs by providing a molecular bridge with the 43S pre-initiation ribosome complex [93]. This occurs *via* a direct association between the EJC interacting factor PYM and the 40S ribosomal subunit [93]. PABPN is a 3' associated factor that is bound to the poly-A tail of mRNAs and functions in the earlier post-transcriptional event of 3' end processing [94].

The significance of the CBC trRNP, both in composition and function, is for the mRNA surveillance and quality control process of nonsense-mediated mRNA decay (NMD) [79, 89]. NMD is intricately linked to the pioneer rounds of translation whereby it assesses initial mRNA integrity for appropriate full-length protein production [62]. This activity involves the recognition and resolution of premature termination events so as to prevent production and accumulation of rogue protein products [62]. Critical interactions between CBP80 and the NMD effector up-frameshift protein 1 (UPF1) mediate this effect [95]. The associated exon junction complexes of the CBC trRNP are also necessary for coordinating recognition of premature termination events with subsequent translation inhibition and directed mRNA decay [62]. Thus, the long-standing model has been that the CBC trRNP is distinct for the pioneer round(s) of translation in order to identify targets of NMD.

Recent studies, however, have provided significance for the CBC trRNP beyond the pioneer round(s) of translation and NMD. Such is the case for the expression of antigenic peptides of the MHC class I pathway [96]. The ability to distinguish self from non-self is critical to appropriate immune function and recognition of invading pathogens. The MHC class I pathway functions in both T-cell education and activation of the immune system. Critical to this effect is the generation of antigenic peptides. Cap-dependent translation affords a molecular basis for regulated peptide expression. It was demonstrated that inhibition of eIF4E trRNP dynamics impaired the production of full-length protein products without consequence on the generation of antigenic peptides [96]. Furthermore, the temporal regulation of antigenic peptide production was shown to coincide with prominent CBC trRNP activity [96]. Thus, these findings indicate a role for the CBC trRNP in the innate immune response.

The CBC trRNP is also critical for the regulated expression of the core histone proteins [97]. Genome integrity is dependent upon the effective packaging of DNA into appropriate chromatin structure. This effect is mediated by the coordination of histone protein synthesis with DNA replication. Robust histone protein production is observed during the S phase of the cell cycle when DNA replication

occurs. Completion of S phase triggers the rapid degradation of histone mRNAs, which effectively inhibits their expression and coordinates histone production with DNA replication. The molecular basis by which histone translation is linked to its mRNA degradation is the CBC trRNP [97]. Histone mRNAs exhibit preferential association with the CBC trRNP during steady-state translation due to a direct interaction between CTIF and the identifying 3' stem-loop binding protein (SLBP) of histone mRNAs [97]. This SLBP-CTIF interaction generates a trRNP that facilitates efficient translation of histone mRNAs during S phase and primes for their rapid degradation upon the completion of DNA synthesis [98]. The rapid degradation of histone mRNAs is driven by S phase-dependent phosphorylation of UPF1 and its competition with CTIF for SLBP association [98]. The outcome is a dynamic rearrangement in the CBC trRNP that facilitates mRNA degradation [98]. Collectively, these findings indicate significance for the CBC trRNP in the molecular basis of genome integrity.

An additional function for the CBC trRNP beyond the pioneer round(s) of translation is cap-dependent gene expression of HIV-1 [78]. As previously discussed, a hallmark feature of HIV-1 infection is global suppression of host cell translation [78]. This effect is due to HIV-1-induced activation of 4E-BP1 and consequent suppression of eIF4E trRNP activity [78]. However, as an obligate parasite, HIV-1 requires host cell translation machinery for protein expression. Furthermore, it does so in an arguably cap-dependent manner [33, 34]. This seemingly paradoxical conundrum is resolved by the virus selectively engaging the CBC trRNP for expression of its critical structural proteins [78]. Unlike eIF4E, CBC is independent of regulation by 4E-BP1 (see below). Furthermore, regulation of CBC trRNP activity has yet to be identified (see below). Therefore, this finding implies significance for the CBC trRNP in maintained cap-dependent translation during cell stress.

The eIF4E trRNP

The eIF4E trRNP dynamically assembles upon an mRNA subsequent to the CBC trRNP and facilitates steady-state protein synthesis [79, 99] (Figure 7). The eIF4E trRNP is compositionally distinguished from the CBC trRNP by the presence of the eIF4E cap-binding protein bound to the 5' cap of an mRNA in place of CBC. eIF4E is selective for an association with eIF4G and does not exhibit an interaction with CTIF, making eIF4G another defining member of the eIF4E trRNP [91]. Furthermore, eIF4E selectively interacts with the DEAD-box RNA helicase eIF4A such that eIF4A is also a defining member of eIF4E trRNP [79]. The eIF4E trRNP is further distinguished from the CBC trRNP by its absence of associated exon junction complexes and its exclusive interaction with the cytoplasmic poly-A binding protein (PABPC) [79, 92].

These characteristic differences between the eIF4E trRNP and the CBC trRNP, both in composition and temporal association with an mRNA, are driven by the dynamic trRNP remodeling events that occur following the pioneer round(s) of translation (Figure 7). CBC has a high affinity for the nuclear import factor importin α1, an association that is driven by the classic nuclear localization sequence in CBP80 [83]. Notably, the interaction of CBC with importin α1 is resistant to high salt and observed throughout the nuclear-cytoplasmic shuttling activity of CBC [83]. Typical nuclear-cytoplasmic shuttling proteins only demonstrate a robust interaction with importin α1 in the cytoplasm, as their binding is rapidly dissociated upon nuclear import by the Ran-GTP gradient and interactions with the nuclear export factor exportin 2 (also known as CAS) [100]. The unique affinity of CBC for importin α1 primes CBC for an interaction with importin β1 in the cytoplasm [99]. Importin β1 is the critical karyopherin that drives importin α1-mediated nuclear import by serving as a molecular bridge between importin α1-cargo complexes and the nuclear pore complex [100]. A cytoplasmic CBC-importin α1-importin β1 interaction destabilizes CBC from the 5' cap, allowing eIF4E to bind and for CBC to recycle to the nucleus [83, 99]. Impaired importin α1-importin β1 dynamics reduces the exchange of CBC for eIF4E on mRNAs [99]. Additionally, direct cofactor interactions can retain CBC on target transcripts by functioning as a molecular clasp that latches CBC onto the 5' cap and prevents eIF4E association [97]. Notably, importin-driven CBC dissociation from the 5' cap is critical for the molecular basis of trRNP remodeling

as eIF4E exhibits a lower affinity for 7-methyl-guanosine relative to CBC [101]. Thus, affinity competition is not sufficient to drive eIF4E association with the 5' cap even with eIF4E's abundant cytoplasmic localization.

The binding of eIF4E to the 5' cap draws stabilized associations with eIF4G that recruits eIF4A and completes formation of the eIF4E trRNP at the 5' terminus. Removal of exon junction complexes and the exchange of PABPN for PABPC occur with the pioneer rounds of translation [99]. The exact remodel mechanisms governing these effects remain to be elucidated [99]. It is important to emphasize here a critical distinction between trRNP remodeling at the 5' terminus from that at the 3' terminus in the exchange of the CBC trRNP for the eIF4E trRNP. As previously discussed, exchange of CBC for eIF4E at the 5' cap is driven by a translation-independent association of CBC with the nuclear import factors importin α1 and importin β1 [99]. A CBC-importin α1-importin β1 complex formation destabilizes CBC from the 5' cap, allowing eIF4E to bind [99]. Remodeling at the 3' terminus, however, with the removal of exon junction complexes and the exchange of PABPN for PABPC is dependent upon active ribosome scanning and translation [99]. These mechanistic distinctions provide opportunities for a transcript to undergo retention of CBC at the 5' cap but exhibit efficient remodel at the 3' terminus. In most instances, however, complete trRNP exchange occurs and an mRNA becomes fully engaged within the eIF4E trRNP to undergo steady-state translation [99].

Regulation of the CBC and EIF4E trRNPs

Both the CBC trRNP and the eIF4E trRNP are subjected to regulation by changes in the core translation machinery. This includes phosphorylation of eIF2α. CBC and eIF4E each interact with eIF2 and eIF3, implying similar associations with the 43S pre-initiation ribosome complex [79]. Furthermore, introduction of a phosphomimetic mutant of eIF2α significantly impairs nonsense mediated mRNA decay to indicate that the pioneer round of translation requires functional eIF2α like steady-state protein synthesis [79].

A distinction is made between the regulation of the CBC trRNP and that of the eIF4E trRNP when 4E-BP1-mediated translation control is considered. As previously discussed, 4E-BP1 is a primary target and effector of mTOR activation. mTOR is the major cellular commander that translates extracellular stimuli into coordinated effects on protein synthesis. In an mTOR-induced hyper-phosphorylated state, 4E-BP1 exhibits reduced affinity for eIF4E [73-75]. This allows an association between eIF4E and eIF4G that facilitates a composed eIF4E trRNP to promote steady-state translation [73-75]. These trRNP dynamics are observed in response to mitogenic stimuli [71, 76]. However, in cell stress, such as amino acid deprivation or HIV-1 infection, mTOR activation is reduced [71, 78]. This results in hypophosphorylation of 4E-BP1 [71, 76, 78]. Hypophosphorylated 4E-BP1 exhibits a high affinity for eIF4E that competes with eIF4G for an association [76]. The outcome is a 4E-BP1 : eIF4E interaction that impairs eIF4E trRNP complex formation and results in suppression of eIF4E-mediated translation [76].

On the other hand, the CBC trRNP is insensitive to regulation by 4E-BP1 [78, 102, 103]. This results in its maintained translation activity during cell stress events like hypoxia, serum starvation, and HIV-1 infection, which evoke suppression of mTOR activity and 4E-BP1 activation [78, 102, 103]. In fact, no regulators of cytoplasmic CBC function have been identified. The nuclear activity of CBC in pre-mRNA splicing is sensitive to several extracellular stimuli such as growth factors and UV irradiation. However the mechanisms governing this effect and its significance for downstream CBC trRNP function remain to be elucidated [104]. Likewise, CBC was identified as a phosphorylation target of the ribosomal protein S6 kinase (S6K) [72]. Yet the actual occurrence of CBC phosphorylation in cells and its significance for regulating CBC trRNP activity remain ambiguous [72].

A distinction between CBC trRNP regulation and that of eIF4E trRNP control is significant for eukaryotic translation in three main ways. First, it affords the cell a mechanism to separate mRNA surveillance translation activities from that of steady-state protein synthesis. This allows for the maintenance of critical quality control functions in the advent of suppressed steady-state protein synthesis. Second, it provides two distinct molecular

bases for cap-dependent translation control. This feature is critical for enacting targeted protein synthesis, especially during states of cellular stress. Third, it presents an opportunity for multiple integration mechanisms of invading viral pathogens into the host translation process.

CONCLUSION

RNP biology is fundamental to eukaryotic protein synthesis. Deregulated trRNP activity is associated with cancer, neurological diseases and disorders, neurodegeneration, growth defects, and innate immune disorders [105-108]. Herein we provided a comprehensive analysis of the trRNPs regulating eukaryotic protein synthesis. We defined the core trRNPs directing each stage of the translation process and discussed their significance in facilitating polypeptide production. Notably, we identified distinct trRNPs with targeted activities in the regulation of translation. These included the specialized DExH/D-box RNA helicase trRNPs and select RNA binding proteins that govern protein synthesis at distinct stages of the translation process on targeted mRNAs. Furthermore, we discussed the molecular bases for the regulation of trRNP activity, particularly the role of cell stress in influencing trRNP dynamics and protein production. Critically, we highlighted the differing responses of each trRNP to cell stress and the significance of this distinction for novel mechanisms of translation control during the cellular stress response. Collectively, our analyses demonstrated the breadth and depth of trRNP biology in eukaryotic protein synthesis. Future studies connecting trRNP biology to the role of specialized ribosomes in targeted translation control are interesting to consider.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing financial interests.

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