Modifications on Translation Initiation

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Two studies by Meyer et al. and Wang et al. demonstrate a role for m^6A modification of mRNA in stimulating translation initiation. These findings add to the growing number of diverse mechanisms for translation initiation in eukaryotes.

The control of translation initiation is a critical aspect of modulating protein production, particularly when rapid responses to extracellular cues are required, such as during neuronal stimulation or stress conditions (Sonenberg and Hinnebusch, 2007). Translation initiation requires the delivery of the small 40S ribosomal subunit to the mRNA. In eukaryotes, this is primarily achieved in a mechanism that begins with binding of the 5′ mRNA cap by the eIF4F complex, which recruits the 40S subunit pre-bound to a multifactor complex, including eIF3, eIF2, and the initiator tRNA (Figure 1A). The ribosome then scans along the 5′ UTR to the AUG start codon, followed by joining of the large ribosomal subunit, producing a translation competent complex. In a second mechanism, specific mRNA structures referred to as internal ribosome entry sites (IRES) can recruit the 40S subunit either by binding to one of the initiation factors, which then recruits the 40S subunit, or by direct interaction with the 40S subunit, as in the case of CrPV IRES (Figure 1B) (Sonenberg and Hinnebusch, 2007). Two papers in this issue of *Cell* (Meyer et al., 2015; Wang et al., 2015) and a third study (Zhou et al., 2015) now argue that m^6A modifications in mRNA can promote translation initiation and suggest two possible mechanisms by which such RNA modifications can lead to ribosome recruitment (Figures 1C and 1D).

Convincing evidence that m^6A modifications can stimulate translation comes from the observations that uncapped m^6A-containing mRNAs are much more efficiently translated in cell-free extracts than unmodified mRNAs, and m^6A-modified mRNAs assemble translation initiation complexes in reconstituted systems in the absence of the eIF4F complex, unlike unmodified mRNAs (Meyer et al., 2015). Strikingly, a single m^6A in the 5′ UTR is sufficient to boost cap-independent translation both in extracts and when mRNAs are introduced into cells by transfection (Meyer et al., 2015; Zhou et al., 2015). Evidence that m^6A modifications promote translation in vivo is that depletion of the METTL3 m^6A methyltransferase reduces ribosome occupancy for mRNAs with 5′ UTR m^6A modification sites (Meyer et al., 2015), and on mRNAs that are bound by YTHDF1, an m^6A-binding protein (Wang et al., 2015). Moreover, for the Hsp70 mRNA, the extent of m^6A modification corresponds to the rate of protein production and polysome occupancy during heat shock (Meyer et al., 2015; Zhou et al., 2015). Finally, transfected mRNAs with a cap unable to stimulate translation are effectively translated under stress conditions if they contain a 5′ UTR m^6A modification (Zhou et al., 2015).

Meyer et al. (2015) provide three observations that m^6A stimulates cap-independent translation through interactions with eIF3, thereby leading to ribosome recruitment (Figure 1C). First, in a reconstituted system, eIF3 preferentially cross-links to RNA with m^6A modifications. Second, in vivo, eIF3-binding sites defined by cross-linking significantly overlap with m^6A modification sites in 5′ UTRs. Third, overexpression of the FTO demethylating enzyme reduces the association of 5′ UTR m^6A-modified mRNAs with eIF3. Interestingly, the authors demonstrate that eIF3 prefers to bind m^6A-modified mRNA when the modification is within the expected GAC sequence context. This may correlate with the observation that m^6A is not able to stimulate translation in all 5′ UTRs, demonstrating the importance of context (Zhou et al., 2015). However, whether this observation is due to differences in eIF3 interactions has not been determined.

In contrast, several observations lead Wang et al. (2015) to suggest that m^6A modifications in the 3′ UTR, and possibly the coding region, may enhance translation by binding the C-terminal domain of the YTHDF1 m^6A-binding protein, which then recruits the translation initiation complex through its N-terminal domain (Figure 1D). First, knockdown of YTHDF1 leads to reduced ribosome occupancy on mRNAs bound by YTHDF1. Second, tethering the N-terminal domain of YTHDF1 to an mRNA leads to some increase in translation. Finally, YTHDF1 co-purifies with a large number of proteins, including eIF3 in a RNase-resistant manner, suggesting that the interaction with eIF3 allows YTHDF1 to promote translation of m^6A modified mRNA (Wang et al., 2015). Interestingly, Meyer et al. (2015) do not see changes in translation profiles in YTHDF1 knockdown cells when examining 5′ UTR, 3′ UTR, or all m^6A-modified mRNAs, suggesting that YTHDF1 effect on translation would be limited to a subset of m^6A-modified mRNAs.

A number of questions remain. Do these two proposed mechanisms for m^6A stimulation of translation cooperate or compete in different contexts? How does the growing number of m^6A-binding proteins (YTHDF1, YTHDF2, eIF3, etc.) recognize specific binding sites? eIF3 interacts preferentially with m^6A modifications found in the 5′ UTR, but these are a minority of such modifications in the transcriptome. What other protein factors or local mRNA features define an
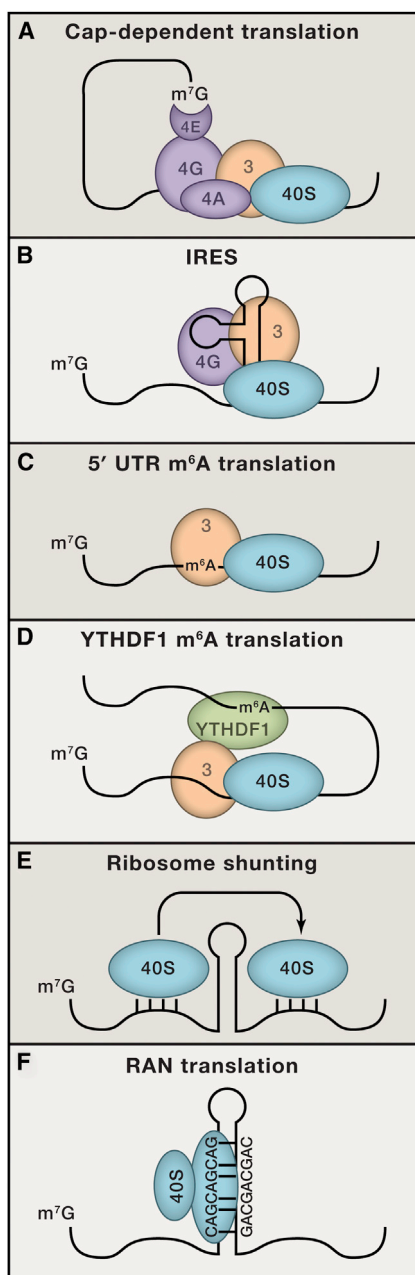
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Figure 1. Mechanisms of Translation Initiation in Eukaryotes

(A) Cap-dependent translation initiation. eIF4F complex binds the 5′ cap of mRNA and then recruits the 40S ribosomal subunit pre-bound to a multifactor complex, including eIF3, eIF2, and the initiator tRNA, to start translation initiation.

(B) IRES-stimulated translation initiation. Some mRNAs contain specific IRES structures that recruit the 40S subunit either indirectly by binding to one of the initiation factors.

(C) 5′ UTR m^{6}A-mediated translation initiation. Translation initiation is stimulated by m^{6}A modification of mRNA 5′ UTR via direct recruitment of eIF3.

(D) YTHDF1-mediated translation initiation. Translation initiation is stimulated by m^{6}A modification of the 3′ UTR of mRNA through recruitment of

YTHDF1, which subsequently recruits the translation initiation complex.

(E) Ribosome shunting. Ribosomal RNA base pairs with mRNA leading to the translocation of 40S subunit from the cap region to internal start codons for initiation.

(F) Repeat-associated non-AUG (RAN) translation. Translation initiation can occur at disease-associated CAG repeats.

eIF3-binding site to prevent binding in other regions of the mRNA? How is the competition between m^{6}A-binding factors properly balanced? Finally, since methylation is reversible, like many chromatin modifications, it will be important to determine the mechanisms regulating the rates of methylation and demethylation of specific sites.

A broader point from these papers is that eukaryotic cells contain a growing diversity of mechanisms for translation initiation, which has implications for our understanding of the predicted proteome. In addition to canonical cap-dependent translation, IRES, and now m^{6}A modification-stimulated initiation, other mechanisms exist (Figures 1E and 1F). For example, ribosome shunting involves the translocation of 40S ribosomes from the cap region to internal sites for initiation, which can lead to the use of internal AUGs, and/or the skipping of 5′ UTR RNA structures that would otherwise inhibit translation (Figure 1E) (Chappell et al., 2006). A mechanism by which ribosomes might be recruited to mRNAs independent of the cap is suggested by the binding of eIF3 to stem loops in specific 5′ UTRs, which can result in either stimulation or inhibition of translation (Lee et al., 2015). Ribosome profiling studies have also identified translation initiation sites at near-cognate start codons, suggesting that the start site, as well as the initiation complex, is malleable (de Klerk and 't Hoen, 2015). A striking example of an unexpected mode of translation is seen in the case of repeat-associated non-AUG (RAN) translation, which occurs at disease-associated CAG repeats (Figure 1F) (Zu et al., 2011). Although the mechanism of RAN translation is not known, a reasonable prediction is that cells use that same non-AUG-dependent mode of translation in some context.

One has to anticipate that cells use additional yet-to-be-discovered mecha-

nisms to recruit ribosomes to mRNAs. For example, is has been suggested that some mRNAs recruit eukaryotic ribosomes by direct base pairing to rRNAs, similar to the bacterial mechanism of initiation in which the Shine-Dalgarno sequence 5′ of the start codon base pairs to the small ribosomal subunit (Deforges et al., 2015). Moreover, one speculates that evolution is likely to have chanced upon sequence-specific RNA-binding proteins that interact with eIF3 or other initiation factors to recruit the 40S subunit in a cap-independent manner. Finally, it remains possible that other mRNA base modifications will also stimulate translation initiation in some context.

The growing diversity of translation initiation mechanisms allows the cell to preferentially control the translating population of mRNAs under different conditions. For example, cap-dependent translation is inhibited when the TOR pathway is inactive, such as under nutrient deprivation or stress. However, to survive such conditions, the cell must produce stress-response proteins, which can be done by utilizing cap-independent mechanisms of initiation. Consistent with this view, Meyer et al. (2015) observe that Hsp70 translation is stimulated via m^{6}A during heat shock, when cap-dependent translation is inhibited. They also analyze m^{6}A modification across the genome under heat and UV stress and find that m^{6}A modifications specifically increase in the 5′ UTR during stress. The increase in m^{6}A 5′ UTR modifications during heat shock may be due to nuclear import of YTHDF2 during heat stress, which allows it to compete with the demethylase FTO (Zhou et al., 2015). Importantly, many known variations of translation initiation have been identified under conditions considered non-standard, such as during development or under stress. As shown by Meyer et al. (2015) for m^{6}A modifications, these variations of translation initiation may be functional during normal growth conditions but are likely more active during conditions in which inhibition of cap-dependent translation allows alternative mechanisms to be more competitive. Thus, studies of translation mechanisms in non-traditional cellular conditions may reveal an even broader set of translation initiation mechanisms.

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