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Spotlight

A Versatile eIF3d in Translational Control of Stress Adaptation

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Lamper et al. (2020) reported that eIF3d-mediated cap-dependent translation is subject to regulation by phosphorylation during chronic glucose deprivation, providing a mechanism underlying selective translation of stress genes essential for cell survival.

A remarkable feature of all living organisms is the ability to sense fluctuations of environmental cues and respond to adverse conditions by adjusting cellular activities. Protein synthesis consumes a lion's share of cellular energy. Not surprisingly, many stress conditions suppress global protein synthesis as part of the stress response. However, translation of certain mRNAs needs to be maintained or even upregulated to sustain vital functions. Given the diverse types of stressors, how stress-specific mRNAs are selected for translation has been an area of great interest. Even for the same type of stress, acute and chronic stress conditions induce different translational regulation. Stress adaptation is essential for cells to restore cellular homeostasis, but the underlying mechanisms remain incompletely understood. Lamper et al., 2020 reported that eIF3d mediates a unique translational program in mammalian cells during cellular adaptation to prolonged glucose starvation.

In eukaryotic cells, mRNA translation typically begins with the binding of eIF4F to the 7-methylguanylate (m^7G) cap found on the 5′ end of mRNAs. eIF4F is a heterotrimeric complex consisting of eIF4E (cap-binding), eIF4G (scaffold), and eIF4A (RNA helicase) (Hershey et al., 2019). Under the normal growth condition, activated mTORC1 phosphorylates eIF4E-binding proteins (4E-BPs), permitting cap-dependent mRNA translation. A multitude of stresses inhibit mTORC1 activity, resulting in hypophosphorylated 4E-BPs that sequester eIF4E (Shu et al., 2020). Upon mTORC1 inhibition, however, overall translation is reduced only by ~60% (An et al., 2020). This does not necessarily mean that the remaining 40% of translation solely relies on cap-independent mechanisms. Notably, most mRNAs are still capped when the canonical eIF4F is inhibited. Although cap-dependent translation is widely believed to be driven through eIF4F, alternative mechanisms likely exist to mediate cap-dependent mRNA translation in the absence of functional eIF4F.

Indeed, a previous study uncovered eIF3d as another mRNA cap-binding protein (Lee et al., 2016). eIF3d is a subunit of eIF3, the largest and most complex initiation factor that binds to the solvent-exposed side of the 40S ribosome. eIF3 is involved in nearly every step of translation initiation, including ribosome loading, scanning, and start codon selection (Valášek et al., 2017). Surprisingly, eIF3 has been shown to regulate protein synthesis in a selective and mRNA-specific manner (Lee et al., 2015). It appears that the cap-binding activity of eIF3d defines the role of eIF3 in general versus specific mRNA translation. Importantly, eIF3d-targeted mRNAs, as exemplified by *Jun*, possess a stem loop structure in 5′ UTR to block canonical eIF4F binding. However, whether the eIF3d-mediated cap-dependent translation is subject to regulation was unclear until now (Figure 1).

The first clue came from the observation that chronic glucose deprivation increased translation of *Jun*. Lamper et al. (2020) measured the cap-binding activity of endogenous eIF3d by separating the cap-binding domain from the entire eIF3 complex. This is possible because eIF3d contains a natural HIV-1 protease cleavage site. Remarkably, glucose starvation led to an increased cap-binding activity of eIF3d by 10-fold. Since phosphorylation is a common mechanism in stress signaling pathways, the authors confirmed nutrient-dependent phosphorylation of eIF3d. Using biochemical and genetic approaches, they not only mapped the phosphorylation sites but also identified CK2 as the responsible kinase for eIF3d phosphorylation. During chronic glucose limitation, the inhibited CK2 leads to dephosphorylation of eIF3d. The subsequent increased cap-binding and enhanced *Jun* translation indicate a tight regulation of eIF3d-specialized mRNA translation via phosphorylation. Notably, CK2 has a broad range of phosphorylation substrates including eIF3b, whose functional coordination with eIF3d merits further investigation.

Apparently, *Jun* is not the only one mRNA preferentially bound by eIF3d. However, it is challenging to determine the scope of eIF3d-targeted mRNAs under metabolic stress because eIF3d is an integral subunit of the eIF3 complex. Lamper et al. (2020) developed an elegant approach by introducing a TEV protease cleavage site proximal to the cap-binding domain of eIF3d. After removing capped mRNAs undergoing canonical translation, mRNAs with the 5′ end cap protected by eIF3d were enriched followed by deep sequencing. A total of 668 transcripts were uncovered from glucose deprived HEK293T cells, suggesting a broad spectrum of eIF3d-programmed translation. Gene ontology analysis revealed that eIF3d-specific

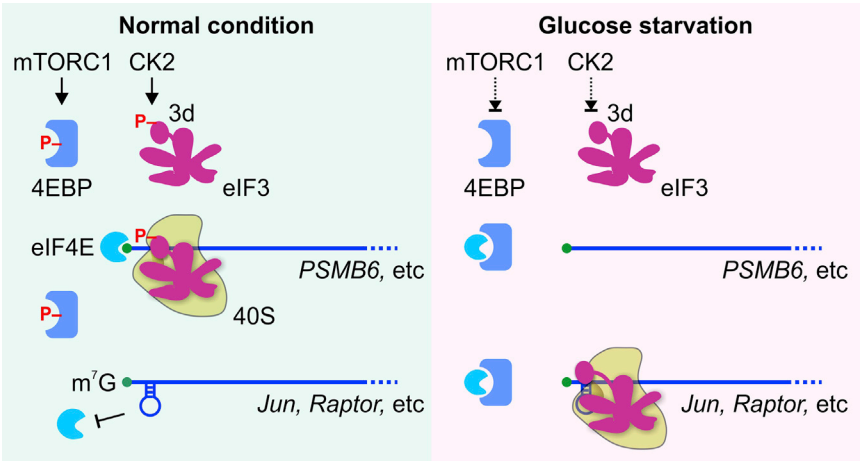

Figure 1. eIF3d Acts as a Translational Switch between Canonical and Noncanonical Cap-Dependent Translation

Under the normal growth condition, mTORC1 phosphorylates 4EBP, permitting eIF4E-mediated cap-dependent translation. CK2 phosphorylates eIF3d, thereby inhibiting its cap-binding activity. In response to chronic glucose deprivation, hypophosphorylated 4EBP prevents cap-dependent translation, contributing to suppression of global protein synthesis. However, dephosphorylated eIF3d gains the cap-binding activity, facilitating selective translation of mRNAs such as *Jun* and *Raptor*. This translational reprogramming is essential in metabolic stress adaptation.

mRNAs are enriched in cell metabolism and signaling pathways. One interesting target is *Raptor*, which encodes an essential component of mTORC1. Like *Jun*, *Raptor* also bears conserved secondary structures in 5′ UTR as evidenced by SHAPE, an RNA structure analysis. Importantly, disruption of the stem loop structure of *Raptor* abolished eIF3d cap binding.

The eIF3d-mediated translational control of *Raptor* is quite interesting because mTORC1 essentially controls the canonical eIF4F-mediated cap-dependent translation. Although a transient inhibition of mTORC1 might be beneficial for cells under acute stress, prolonged stress requires cellular adaptation via mTORC1 reactivation. This phenomenon was initially documented during chronic endoplasmic reticulum stress (Guan et al., 2017). It is conceivable that eIF3d acts as a switch via phosphorylation, permitting restoration of eIF4F-dependent canonical translation. This translational reprogramming corresponds to the transition from acute stress response to chronic stress adaptation. Failure of such translational switch, as is the case by introducing phosphomimetic eIF3d, causes cell death under chronic glucose deprivation.

The discovery of eIF3d cap-binding activity and stress-induced regulation expands the functional diversity of eIF3. Composed of 13 subunits, the 800-kDa eIF3 complex has long been viewed as a static entity by recruiting the 40S ribosome to mRNA via interaction with eIF4F. Accumulating evidence suggests that eIF3 also participates in non-canonical translation mediated by the internal ribosome entry site (IRES) as well as m^6A (Meyer et al., 2015). The remarkable feature of eIF3d also reshapes our current concept of cap-dependent translation. It is clear that cap-dependent mRNA translation is no longer equated with the canonical eIF4F. The deployment of eIF3d in cap-dependent translation enables cells to reprogram their translatome, such that proteins that confer adaptive benefits are preferentially synthesized. As this new paradigm of translatome remodeling emerges, a number of outstanding questions remain. For instance, the eIF3d-specialized mRNAs require presence of conserved stem loop structures in 5′ UTR. It is unclear how those structures block the canonical eIF4F binding. Structural studies revealed the cap-binding domain of eIF3d (Lee et al., 2016), but how eIF3d recognizes the stem loop remains obscure.

Another fundamental question is how cells reprogram translation in response to distinct stimuli. It appears that the phosphorylation-regulated eIF3d-mediated translation is specific to glucose deprivation but not serum or glutamine starvation. Does the cell have other types of switches to produce stimulus-specific translatome? Upon hypoxia, cells use different eIF4F variants to reprogram the translational output (Uniacke et al., 2012). Blocking canonical eIF4F might be a common theme to redirect mRNAs into specific translation pathways. Future studies will be required to uncover those pathways that enables the critical remodeling of the cellular proteome depending on environmental and physiological conditions of the cell. Given the multiple initiation factors, diverse RNA-binding proteins, and rich sequence elements, it is likely that their interactions represent a critical regulatory nexus that determines the translational priorities of mRNAs.

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