INSIGHTS

c-Myc steers translation in lymphoma

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Members of the MYC family of oncogenes are master regulators of mRNA translation. In this issue of *JEM*, Singh et al. (https://doi.org/10.1084/jem.20181726) demonstrate that c-Myc governs protein synthesis in lymphoma cells by interfering with SRSF1- and RBM42-mediated suppression of mRNA translation and by altering selection of translation initiation sites.

Aberrant mRNA translation is a common feature of cancer. Translational dysregulation in neoplasia commonly stems from the hyperactivation of oncogenic pathways and/or overexpression of the components of the translational machinery (Chu et al., 2016). Up-regulated protein synthesis and translational reprogramming are associated with increased proliferation and survival of cancer cells. Furthermore, perturbations in mRNA translation are linked to metastatic dissemination of the disease and unoptimal therapeutic responses. Members of the MYC family of transcription factors act as potent oncogenes, with c-Myc being one of the top 10 genes across a large number of cancer types showing increased copy numbers (Beroukhim et al., 2010). Accordingly, c-Myc is altered in ≤70% of cancers including Burkitt lymphoma, multiple myeloma, and malignancies of the breast, ovary, colon, and prostate (Dang, 2012). Tumorigenic activity of c-Myc appears to be critically dependent on its function in stimulating protein synthesis, a feature attributed to its ability to stimulate ribosome biogenesis by promoting the transcription of both ribosomal DNA and ribosomal protein genes, as well as cofactors required for ribosomal RNA processing and transport of ribosomal subunits (van Riggelen et al., 2010). Moreover, c-Myc stimulates transfer RNA (tRNA) synthesis (van Riggelen et al., 2010). Finally, c-Myc stimulates mRNA translation by increasing levels of various translation initiation factors including eIF4E, eIF2α, eIF4A1 and eIF4G1 (Chu et al., 2016).

In this issue of *JEM*, Singh et al. employ ribosome profiling to investigate the effects of c-Myc depletion on the translatome of lymphoma cells in cell culture (Singh et al., 2019). "Translatome" refers to the collection of mRNAs which are being actively translated on a transcriptome-wide scale. Singh et al. (2019) found that for most of the transcripts, c-Myc–dependent changes in ribosome association were paralleled by congruent alterations in mRNA abundance, consistent with the well-established role of c-Myc as a transcription factor. Of note, the experimental setup encompassed ~24-h depletion of c-Myc, which may have contributed to the relatively low contribution of translational changes which typically occur within the first 12 h after the stimulus. Nonetheless, translation of a subset of mRNAs was affected without concomitant alterations in mRNA levels. This subset of mRNAs was enriched for those encoding mitochondrial proteins, including several components of the electron transport chain (ETC). The authors propose that these effects are mediated by a hitherto elusive regulon that involves interactions between discrete cis elements in the 5′UTR present in a cohort of mRNAs encoding ETC components and the trans-acting RNA-binding proteins SRSF1 and RBM42. According to this model, c-Myc by yet-to-be-determined mechanism alleviates SRSF1- and RBM42-mediated translational repression of mRNAs encoding ETC components. In addition, modulation of c-Myc levels appears to be paralleled by changes in translation initiation site (TIS) selection. Surprisingly, whereas high c-Myc status was paralleled by increased usage of alternative TIS (ATIS) upstream of the annotated TIS, thereby


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creating longer open reading frames (ORFs), c-Myc depletion preferentially led to the usage of ATIS downstream of the annotated TIS, thus resulting in truncated versions of the corresponding proteins. Collectively, the authors identified translational de-repression of SRSF1- and RBM42-bound mRNAs and ATIS selection as two ostensibly independent modes of translational regulation by c-Myc in lymphoma cells which seem to implicate disparate mechanisms and appear to affect different mRNA subsets. Moreover, these two distinct modes of c-Myc–dependent translational control appear to exert nonredundant functional consequences. While the ability of c-Myc to stimulate translation by interfering with SRSF1 and RBM42 stimulates mitochondrial functions, ATIS selection appears to impact on immunosurveillance by altering CD19 receptor.

Protein synthesis is one of the most energy-consuming processes in the cell (Buttgereit and Brand, 1995). Singh et al. (2019) found that synthesis of nuclear-encoded components of the ETC complexes I/III/IV/V is regulated by c-Myc. This

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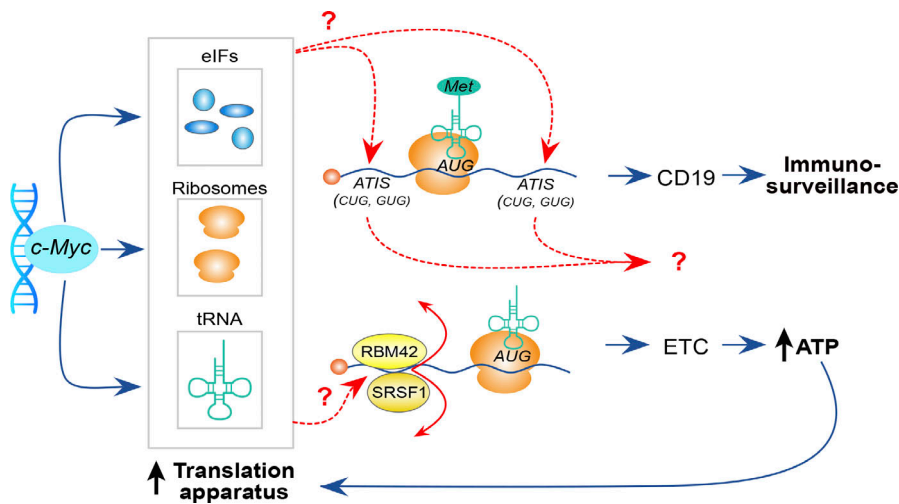
Potential mechanisms of translational regulation by c-Myc. c-Myc oncogene promotes mRNA translation by stimulating ribosome biogenesis, tRNA synthesis, and transcription of genes encoding translation factors. Singh et al. (2019) provide evidence suggesting that in lymphoma cells, c-Myc selectively controls translation of a subset of mRNAs which are repressed by SRSF1 and RBM42 and enriched for transcripts encoding components of ETC complexes. In addition, alterations in c-Myc levels appear to have a dramatic impact on selection of TISs, including in CD19 mRNA. This c-Myc–dependent translational reprogramming is thus anticipated to impact mitochondrial ATP production (which may help fuel translational machinery) and efficacy of immunotherapies. Future work is warranted to help consolidate these findings and, in particular, to establish the molecular underpinnings and physiological consequences of the observed phenomena. eIF, eukaryotic translation initiation factor; SRSF1, serine and arginine-rich splicing factor 1; RB42, RNA-binding motif protein 42; CD19, cluster of differentiation 19.

suggests that c-Myc may bolster mitochondrial ATP production to fuel increased energy demand of high protein synthesis activity in lymphoma cells. Intriguingly, translation of mRNAs encoding ETC complex components is also regulated via the mTOR/4E-BP/eIF4E axis (Morita et al., 2013). To this end, translational reprogramming via the mTOR/4E-BP/eIF4E axis up-regulates transcription factor A, mitochondrial (TFAM), as well as ETC components, thereby resulting in increased mitochondrial ATP production to compensate for high energy consumption by protein synthesis machinery (Morita et al., 2013). Since the activation of mTOR signaling and eIF4E up-regulation frequently parallel c-Myc overexpression in neoplasia (Pourdehnad et al., 2013), it would be of interest to determine whether the mechanisms engaged by c-Myc to control levels of ETC components also rely on the mTOR/eIF4E axis, or whether the stimulation of mitochondrial functions by c-Myc represents an additional mean to engage translation apparatus to bolster mitochondrial ATP production independent of the mTOR pathway. Importantly, it has been established that c-Myc stimulates transcription of the *EIF4E* gene, whereas eIF4E overexpression

protects rat embryonic fibroblasts from c-Myc–dependent apoptosis and accelerates tumorigenesis in vivo (Tan et al., 2000; Wendel et al., 2004). These findings suggest potential cross-talk between c-Myc- and mTOR/4E-BP/eIF4E-dependent mechanisms of translational reprogramming. Furthermore, these data imply that translational mechanisms play a major role in metabolic perturbations required to support neoplastic growth.

In addition to alleviating translational repression by SRSF1 and RBM42, Singh et al. (2019) provide evidence suggesting that modulation of c-Myc levels may have profound effects on TIS selection. For instance, under low c-Myc conditions, downstream ATIS appear to be massively favored over annotated TIS, whereby CUG and GUG start codons are preferred over their AUG counterparts. Notwithstanding these apparent pervasive effects of c-Myc on TIS selection, the conundrums that remain are related to the underpinning mechanisms and functional consequences of resulting ORF extensions and truncations. The mechanisms whereby c-Myc impacts the location of TIS (whether upstream or downstream of the annotated start codon) and usage of AUG versus non-

AUG start codons remain elusive. Several mechanisms contributing to the selection of TIS have been described. The sequence context (e.g., Kozak sequence, surrounding secondary structures) dictates start codon selection (Kearse and Wilusz, 2017). Moreover, initiation factors including eIF1, eIF1A, eIF2, eIF2A, eIF2D, as well as eIF5 and its mimics (e.g., 5MP1 and 2), have been implicated in the control of TIS selection and the stringency of AUG versus CUG, GUG, and UUG recognition (Kearse and Wilusz, 2017). In such context, it would be important to determine whether c-Myc alters expression and/or activity of these initiation factors and/or related proteins. In fact, a similar mechanism of ATIS selection has been proposed in a model of squamous cell carcinoma overexpressing the transcription factor SOX2 (Sendoel et al., 2017). This study found that SOX2-driven cancer cells may engage eIF2A to increase translation from upstream ORFs and redirect initiating ribosomes to use CUGs as start codons on cancer-related mRNAs.

In addition, usage of ATIS induced by alterations in c-Myc levels is expected to generate either longer or truncated protein products resulting respectively in N-terminal extensions or truncations. Interestingly, mRNAs that are subjected to c-Myc–dependent selection of ATIS appear to be enriched in those encoding RNA-binding proteins and a variety of post-transcriptional and translational regulators including SRSF1/2/6, PTBP1, eIF2AK, eIF5A, eIF2B5, eIF3, eIF4B, 4E-HP, and LARP1. This suggest that c-Myc–dependent alterations in TIS selection may alter post-transcriptional and in particular translational programs, thereby implying a multitude of plausible regulatory feedbacks, which, in coordination with well-established transcriptional programs, suggests that c-Myc engages complex gene expression networks to diversify cellular proteomes. This begs for additional proteomics-based experiments aiming to confirm the existence, the stoichiometry, and the consequences of the apparent c-Myc–dependent protein truncations and extensions. Accordingly, it remains to be established whether these effects are limited to lymphomas, or if they occur in other cancers characterized by c-Myc overexpression. Finally, functional consequences of c-Myc–dependent ATIS

selection remain largely obscure. To partially address this outstanding question, Singh et al. (2019) provide evidence suggesting that the truncation of the immune receptor CD19 in cells with low c-Myc impacts on CAR-T cell therapy. This is in line with recent studies whereby translational mechanisms have been shown to modulate PD-L1 and thus impact on immunosurveillance and efficacy of immune-checkpoint inhibitors (Cerezo et al., 2018; Xu et al., 2019).

In conclusion, although Myc family members have been extensively studied since their discovery almost four decades ago (Varmus, 1984), the understanding of the molecular mechanisms that underlie their oncogenic actions remains incomplete. Singh et al. (2019) provide new insights in potential mechanisms that underpin the role of c-Myc as translational regulator, which further corroborates the tenet that c-Myc acts a multifaceted conductor to orchestrate multiple levels of gene expression; thus enticing metabolic reprogramming and promoting neoplastic growth. Considering that Myc has been notoriously difficult to drug, these and similar findings hold the promise to provide the molecular basis for developing novel strategies to target MYC family members in the clinic.

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