The Journal of Nutrition
Commentary

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Leucine-Induced Upregulation of Terminal Oligopyrimidine mRNA Translation in Skeletal Muscle: Just the Tip of the Iceberg?

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Of the 20 naturally occurring amino acids, leucine has been shown to be particularly effective in stimulating protein synthesis in skeletal muscle (1, 2). Indeed, studies performed >40 y ago showed that leucine was as effective as a complete mixture of amino acids in stimulating protein synthesis in the gastrocnemius muscle of perfused rat hindlimb preparations (2). Subsequent studies confirmed the results of the earlier in vitro work and showed a selective effect of oral leucine administration in stimulating protein synthesis in rat skeletal muscle in vivo [e.g., (3)]. Notably, in the study by Anthony et al. (3), the ability of leucine to stimulate protein synthesis was blunted by treatment with rapamycin before oral leucine administration. Rapamycin is a highly selective, allosteric inhibitor of the protein kinase called mechanistic target of rapamycin (mTOR) when it is in a complex with a protein referred to as regulatory associated protein of mTOR (Raptor) (4). The complex of mTOR and Raptor [along with several other proteins including mammalian lethal with SEC13 protein 8 (mLST8)] is referred to as mTOR complex 1 (mTORC1). The activation of mTORC1 upregulates protein synthesis through phosphorylation of downstream targets, including the eukaryotic initiation factor (EIF) 4E binding proteins (4EBPs) 1 and 2 and the 70-kDa ribosomal protein S6 kinase (p70S6K1). Activated p70S6K1 subsequently phosphorylates other proteins involved in mRNA translation, including EIF4B and programmed cell death 4 (PDCD4). Phosphorylation of PDCD4 by p70S6K1 causes it to be released from EIF4A, allowing the initiation factor to bind to EIF4G to form the active EIF4F complex (5). Together, phosphorylation of 4EBP1/2, EIF4B, and PDCD4 promotes the binding of the 7-methyl-GTP (m^7GTP) cap located at the 5′-end of the mRNA to the 40S ribosomal subunit. Because most mRNAs are translated in a cap-dependent manner (6), it might be expected that the inhibition of mTORC1 would lead to a reduction in translation of a large number of mRNAs. However, recent studies that used ribosome profiling (also known as Riboseq analysis) of cells in culture have shown that inhibition of mTORC1 by using either rapamycin or newer inhibitors that target the ATP binding site on mTOR [e.g., TOR inhibitor 1 (Torin 1)] leads to the preferential repression of translation of a relatively small number (i.e., a few hundred) of mRNAs (7, 8). In ribosome profile analysis, RNA is digested by using a single-stranded ribonuclease and 80S ribosomes are isolated. The short mRNA oligonucleotides that were protected from digestion because they were "buried" within the ribosome [i.e., ribosome-protected fragments (RPFs)] are then subjected to RNA deep sequencing (9, 10). The resulting RPF data yield information on not only the relative number of ribosomes bound to, and thus likely translating, a given mRNA but also the distribution of ribosomes along the message. Ribosome profile analysis of cells treated with mTORC1 inhibitors showed that the majority of the translationally repressed mRNAs have a unique cluster of nucleotides consisting of an uninterrupted stretch of pyrimidine residues that is located immediately after the m^7GTP cap [referred to as a terminal oligopyrimidine (TOP) tract] or a few nucleotides downstream from the cap [referred to as TOP-like mRNAs or those with a "pyrimidine-rich translational element" (PRTE)] (7, 8).

On the basis of the results from studies that used ribosome profiling of cells in culture treated with mTORC1 inhibitors, it might be expected that most of the mRNAs that are translationally upregulated in response to leucine-induced activation of mTORC1 in muscle would belong to the TOP or TOP-like family of mRNAs. However, in rats, rapamycin treatment only partially blocks the leucine-induced stimulation of muscle protein synthesis (3). Moreover, although leucine undoubtedly acts in part to directly stimulate protein synthesis by activating mTORC1 in muscle, it is likely that in vivo it also has indirect effects (e.g., by promoting insulin secretion). Leucine is a potent insulin secretagogue (11), and studies that used diabetic rats have shown that, although the effect is blunted compared with nondiabetic rats, leucine is able to stimulate muscle protein synthesis in the muscle of diabetic rats in the absence of changes in mTORC1 activation as assessed by altered phosphorylation of 4EBP1 and p70S6K1 (12). In addition, mTORC1-independent regulation of EIF2B is an important contributor to the leucine-induced stimulation of protein synthesis in L6 myoblasts (13). Thus, it might be expected that, in muscle, leucine could regulate the translation of not only TOP and TOP-like mRNAs but of other mRNAs as well.

In the current issue of the Journal, Drummond et al. (14) extend previous work by using ribosome profiling in cells in culture and use the technique to globally assess changes in translation of mRNAs that occur in the mouse quadriceps muscle 30 min after oral leucine administration. By using this approach, they identified 90 coding mRNAs with significant RPF changes. Not surprisingly, many of the translationally

Work in the author's laboratory referenced herein was supported by NIH grants DK13499 and DK15658.
Author disclosures: SRK, no conflicts of interest.
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Abbreviations used: EIF, eukaryotic initiation factor; mTOR, mechanistic target of rapamycin; mTORC1, mechanistic target of rapamycin complex 1; PDCD4, programmed cell death 4; p70S6K1, 70-kDa ribosomal protein S6 kinase 1; RPF, ribosome-protected fragment; TOP, terminal oligopyrimidine; 4EBP, eukaryotic initiation factor 4E–binding protein; 5′-UTR, 5′-untranslated region.

Manuscript received June 15, 2017. Initial review completed July 13, 2017. Revision accepted July 14, 2017.
First published online August 2, 2017; doi: https://doi.org/10.3945/jn.117.256289.

upregulated mRNAs are classified as either TOP or TOP-like, and most of those encode proteins with functions related to protein synthesis. However, a few mRNAs that lack TOP or TOP-like motifs were also identified as being translationally upregulated, and these include transcription factors and proteins involved in glucose metabolism and muscle contraction. The authors also showed that the 5′-untranslated regions (5′-UTRs) of the mRNAs that were translationally upregulated after leucine administration were shorter and had less secondary structure than mRNAs that were not upregulated. The extensive overlap in mRNAs identified by Drummond et al. (14) and those identified in studies that used mTORC1 inhibitors (7, 8) suggests that leucine acts primarily through mTORC1 to enhance translation of TOP mRNAs in skeletal muscle. Together with previous work that showed that mTORC1 upregulates ribosomal DNA transcription (15), the increase in translation of TOP mRNAs encoding ribosomal proteins and translation initiation and elongation factors in response to leucine treatment likely enhances the capacity of the muscle to synthesize protein.

Although the work of Drummond et al. (14) represents an important achievement, a number of questions remain. For example, although TOP and TOP-like mRNAs are the primary mTORC1 targets as assessed by ribosome profiling (7, 8), other methods, such as polysome profiling [e.g., (16)] or nano-cap analysis of gene expression (nanoCAGE) (17), suggest that mTORC1 targets a much broader array of mRNAs, including ones with long, highly structured 5′-UTRs. Indeed, mTORC1 would be expected to enhance the translation of mRNAs with highly structured 5′-UTRs by promoting the recruitment of the EIF4A helicase to the EIF4F complex to unwind the secondary structure in the 5′-UTR. The differences in the mRNAs identified by the ribosome and polysome profiling methods have been attributed to analytical and technical biases inherent to the ribosome profiling method (for an excellent review of the differences between ribosome and polysome profiling, see reference 18). For example, ribosome profiling shows a bias toward mRNAs that are abundant and that exhibit large shifts in ribosome occupation that are characteristic of the TOP mRNAs and reduced sensitivity toward mRNAs that are expressed at low levels and exhibit lesser shifts (e.g., the cyclin mRNAs that typically have longer, more structured 5′-UTRs). Consequently, it will be important to confirm, and possibly extend, the results of the study by Drummond et al. with the use of an alternative method to determine whether or not leucine modulates the translation of mRNAs in addition to those with TOP and TOP-like motifs. The technical limitations of ribosome profiling analysis by no means lessen the impact of the current work, which serves as a starting point for subsequent investigations into this important area of research.

Acknowledgments

The sole author had responsibility for all parts of the manuscript.

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