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RESEARCH HIGHLIGHT

LncRNA-encoded peptides: More than translational noise?

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Long non-coding RNAs (lncRNAs) belong to the ever-increasing number of transcripts that are thought not to encode proteins. A recent study has now identified a small polypeptide encoded by the lncRNA LINC00961 that inhibits amino acid-induced mTORC1 activation in skeletal muscle.

LncRNAs are non-coding RNAs with a transcript length > 200 nucleotides. They are transcribed by RNA polymerase II, capped, spliced, and polyadenylated. They are expressed in a tissue- and development-specific manner and have been shown to directly regulate a large variety of functions [1]. LncRNAs can also code for short open reading frames (ORFs), which were considered, until very recently, not to be translated. However, recent studies in *Drosophila melanogaster* and zebrafish have uncovered functionally relevant "hidden polypeptides" encoded by lncRNAs [2-4]. Furthermore, in mammalian skeletal muscle, the recently identified, lncRNA-encoded micropeptides myoregulin and DWORF regulate Ca^{2+} handling by directly modulating the activity of the calcium pump SERCA [5, 6]. Despite increasing evidence of the important functional role of lncRNAs, our understanding of the biology and the potential of such small molecules remains in its infancy. In a recent study, Matsumoto and colleagues [7] uncovered a new "hidden polypeptide", termed "small regulatory polypeptide of amino acid response" (SPAR), which suppresses activation of the mammalian (or mechanistic) target of rapamycin complex 1 (mTORC1) in response to amino acids.

Activation of mTORC1 (Figure 1) is controlled by several mechanisms, including growth factors (through the PI3K/Akt pathway), energy levels (via AMP-dependent kinase, AMPK) and the availability of amino acids [8]. Of those, availability of amino acids, in particular of leucine, arginine and glutamine, is dominant for mTORC1 activation. Through its key downstream targets, ribosomal protein S6 kinase 1 (S6K1) and the eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1), mTORC1 promotes protein translation and lipid and nucleotide synthesis. Simultaneously, mTORC1 inhibits the unc-51-like autophagy activating kinase 1 (Ulk1), thereby inhibiting autophagy induction. Amino acids activate mTORC1, mediating its translocation to the lysosome. At the lysosomal membrane, the vacuolar H^+-ATPase (v-ATPase), best known for controlling acidification of the lysosomal lumen, interacts with the pentameric protein complex Ragulator and serves as an anchoring scaffold for the Rag GTPases. High levels of amino acids weaken the interaction between the v-ATPase and Ragulator, thereby activating the heterodimeric protein Rag by loading RagA/B with GTP and hydrolyzing RagC/D-GTP. The now active RagA/B-RagC/D heterodimers then recruit mTORC1 from the cytoplasm to the lysosome where it becomes fully activated by Rheb-GTP, the downstream target of growth factor signaling [8].

This regulatory network has now been extended by the work of Matsumoto *et al.* who demonstrate that SPAR, a polypeptide translated from lncRNA LINC00961, inhibits amino acid-mediated mTORC1 activation at the lysosomal membrane [7]. SPAR was discovered by proteomics aimed at identifying polypeptides encoded by lncRNAs. LINC00961 is conserved across species and is most highly expressed in lung, heart and skeletal muscle. It codes for three putative ORFs, but only one of them generates the 90 amino acid-long SPAR polypeptide. SPAR harbors an N-terminal transmembrane domain and binds to all four subunits of the v-ATPase complex at the lysosomal membrane. Knockdown and overexpression approaches revealed that SPAR inhibits the recruitment and subsequent activation of mTORC1 at the lysosome by binding to the v-ATPase complex.

The function of SPAR *in vivo* was approached with the generation of mice unable to synthesize SPAR but still expressing its lncRNA by removing the translation initiation codon. Surprisingly, despite pronounced activation of mTORC1 in skeletal muscle, *SPAR*$^{-/-}$ mice showed no obvious phenotype. Only challenging the mice by injection of cardiotoxin into muscle, revealed differences between *SPAR*$^{-/-}$ and littermate controls. Cardiotoxin destroys muscle fibers, which is followed by muscle regeneration. This paradigm was chosen as (1) muscle regeneration is accompanied by increased mTORC1 signaling, (2) the mTORC1 inhibitor rapamycin delays muscle regeneration and (3) supplementation of leucine, which activates mTORC1, accelerates injury-induced muscle regeneration [9, 10]. Indeed, *SPAR*$^{-/-}$ muscles exhibited an increased regenerative capacity due to accelerated stem cell proliferation, differentiation and myofiber maturation.

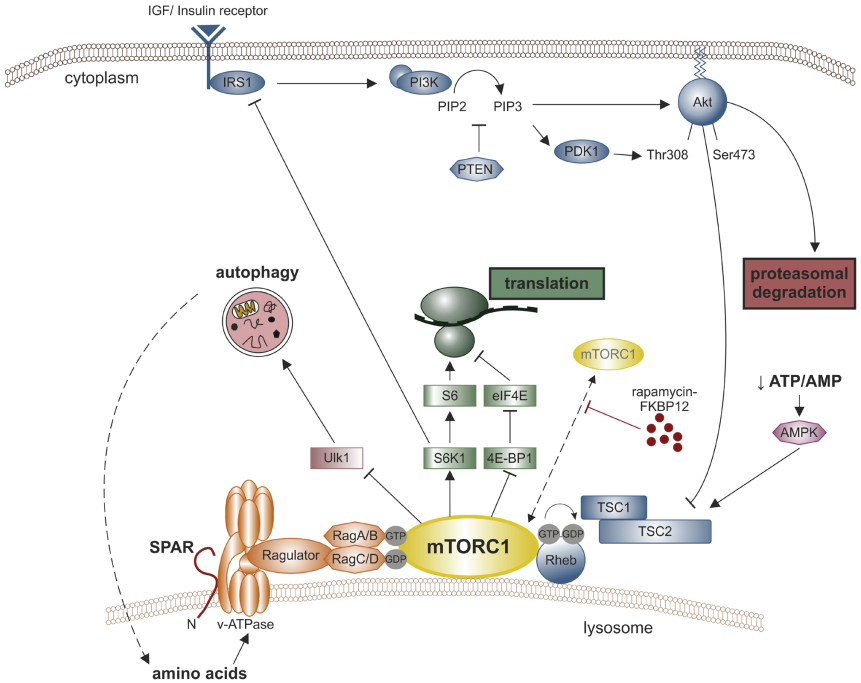

Figure 1 mTORC1 controls cell growth and is regulated by the lncRNA-encoded polypeptide SPAR. mTORC1 signaling induces protein translation through S6K1/S6 and 4E-BP1/eIF4E. The negative feedback from S6K1 to IRS1 reduces PI3K/Akt signaling, thereby promoting proteasomal degradation. mTORC1 is stimulated by growth factors that induce the PI3K/Akt pathway and thereby inhibit the TSC1/TSC2 complex which functions as a GTPase-activating protein for Rheb. GTP-bound Rheb recruits mTORC1 from the cytoplasm to the lysosome. Activation of mTORC1 is also achieved by free amino acids that weaken the interaction between the v-ATPase and Ragulator, consequently transforming the Rag proteins into their active form. SPAR, a polypeptide encoded by lncRNA LINC00961, directly binds to v-ATPase and blunts mTORC1 activation by amino acids. Other regulators of mTORC1 include AMPK that is activated upon low cellular energy levels (reduced ATP/AMP ratio) and stabilizes the TSC1/TSC2 complex, thereby increasing mTORC1 inhibition.

This effect was abrogated in the *SPAR*$^{-/-}$ mice when fed a leucine-free diet, consistent with the idea that the inhibitory effect of SPAR towards mTORC1 is specific for the amino acid-sensing arm (see Figure 1).

In summary, the findings of Matsumoto and colleagues define an additional level of mTORC1 control through the conserved lncRNA-encoded polypeptide SPAR. This study elegantly highlights that "hidden polypeptides" encoded by lncRNAs are not just "translational noise" but serve important biological functions. Interestingly, the SPAR-encoding lncRNA LINC00961 is expressed in a tissue- and age-specific manner, suggesting that the control of mTORC1 signaling is strongly context- and tissue-dependent. Understanding the mechanism of how expression of protein-coding lncRNAs is regulated might give further insights into the role of such polypeptides.

The discovery that SPAR modulates the amino acid-dependent activation of mTORC1 and the correlation with muscle regeneration are interesting aspects that need further investigation. Even though the pharmacological mTORC1 inhibitor rapamycin prevents muscle fiber restoration following severe muscle damage [9], the exact mechanisms of how mTORC1 regulates muscle regeneration are yet to be identified. As regenerative capacity of skeletal muscle declines during physiological aging and mTORC1-controlled autophagy appears to be an important aspect of muscle stem cell aging [11], it would be interesting to investigate the regulation of LINC00961 during aging and to test whether depletion of SPAR in aged mice has a similar beneficial effect on muscle regeneration as in young mice. Moreover, sustained activation of mTORC1 in skeletal muscle by depletion of the upstream inhibitor TSC1, causes a late-onset myopathy [12]. In light of these findings, it will also be important to study the long-term effects of SPAR inhibition *in vivo* and to investigate to what extent mTORC1 hyperactivation may be beneficial in clinical and therapeutic strategies.

Nathalie Rion[1],
Markus A Rüegg[1]

[1]*Biozentrum, University of Basel, Basel, Switzerland*
Correspondence: Markus A Rüegg
E-mail: markus-a.ruegg@unibas.ch

References

1 Quinn JJ, Chang HY. *Nat Rev Genet* 2016; **17**:47-62.
2 Pauli A, Norris ML, Valen E, *et al. Science* 2014; **343**:1248636.
3 Magny EG, Pueyo JI, Pearl FM, *et al. Science* 2013; **341**:1116-1120.
4 Kondo T, Plaza S, Zanet J, *et al. Science* 2010; **329**:336-339.
5 Anderson DM, Anderson KM, Chang CL, *et al. Cell* 2015; **160**:595-606.
6 Nelson BR, Makarewich CA, Anderson DM, *et al. Science* 2016; **351**:271-275.
7 Matsumoto A, Pasut A, Matsumoto M, *et al. Nature* 2017; **541**:228-232.
8 Shimobayashi M, Hall MN. *Cell Res* 2016; **26**:7-20.
9 Miyabara EH, Conte TC, Silva MT, *et al. Muscle Nerve* 2010; **42**:778-787.
10 Pereira MG, Silva MT, da Cunha FM, *et al. Exp Gerontol* 2015; **72**:269-277.
11 Sousa-Victor P, Munoz-Canoves P. *Mol Aspects Med* 2016; **50**:109-117.
12 Castets P, Lin S, Rion N, *et al. Cell Metab* 2013; **17**:731-744.